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Quality Seed Production, Nutritionally Enriched Variety Development and Potato Germplasm Conservation Through in Vitro and Ex Vitro Techniques

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QUALITY SEED PRODUCTION, NUTRITIONALLY ENRICHED VARIETY DEVELOPMENT AND POTATO GERMPLASM CONSERVATION THROUGH *IN VITRO* AND *EX VITRO* TECHNIQUES

THESIS SUBMITTED FOR THE DEGREE OF
DOCTOR OF PHILOSOPHY
IN THE INSTITUTE OF BIOLOGICAL SCIENCES
UNIVERSITY OF RAJSHAHI, BANGLADESH

BY
MD. REZAUL KARIM
B. Sc. (Hons.), M. Sc.

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DEPARTMENT OF BOTANY
RAJSHAHI UNIVERSITY
RAJSHAHI 6205
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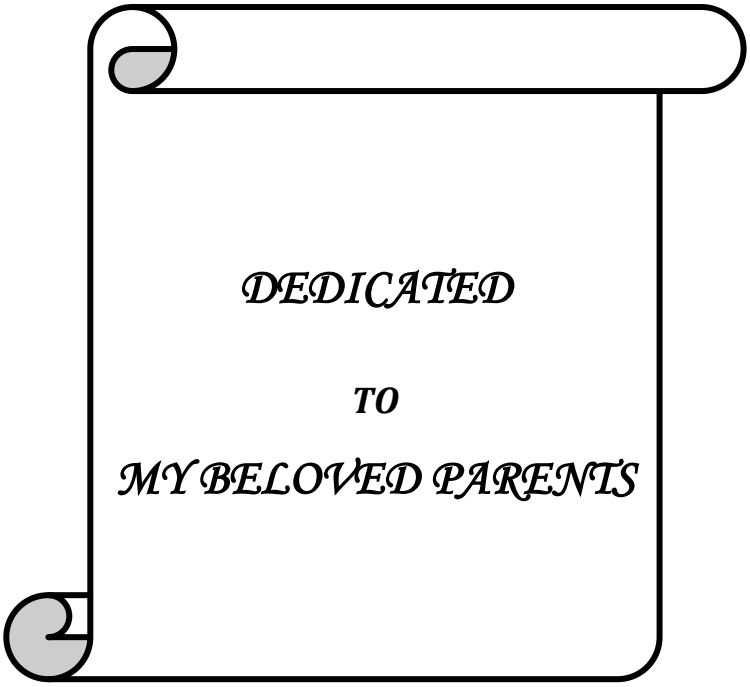
DEDICATED
TO
MY BELOVED PARENTS

DECLARATION

I hereby declare that the whole work submitted as a thesis entitled **"QUALITY SEED PRODUCTION, NUTRITIONALLY ENRICHED VARIETY DEVELOPMENT AND POTATO GERMPLASM CONSERVATION THROUGH *IN VITRO* AND *EX VITRO* TECHNIQUES"** in the Institute of Biological Sciences, Rajshahi University, Rajshahi, Bangladesh for the degree of Doctor of Philosophy is the results of my own investigation and was carried out under the supervision of Professor Dr. M. Monzur Hossain, Department of Botany, Rajshahi University, Rajshahi. The thesis has not already been submitted in the substance for any degree and has not been concurrently submitted in the candidature for any other degree.

December 2013

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CERTIFICATE

It is my pleasure to certify the thesis entitled **"QUALITY SEED PRODUCTION, NUTRITIONALLY ENRICHED VARIETY DEVELOPMENT AND POTATO GERMPLASM CONSERVATION THROUGH *IN VITRO* AND *EX VITRO* TECHNIQUES"** by Md. Rezaul Karim, Institute of Biological Sciences, Rajshahi University, Bangladesh for the degree of Doctor of Philosophy.

I hereby certify that (i) the candidate has fulfilled the residential requirements, (ii) the works embodied in the thesis were carried out by the candidate and (iii) the data, to the best of my knowledge are genuine and original. No part of the work has been submitted in the substance for any other degree.

Dr. M. Monzur Hossain
Professor and Supervisor

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December 2013 The Author

ABSTRACT

Potato is the 2nd most important crop after rice in Bangladesh where 147.9 million people live in a small 147570 square kilometer space. The cultivable land scarcity is the major limitation for the horizontal increase of food production for the future food security of the country in terms of yield and nutritional values. The potato is the most energy efficient crop in the world that can contribute to vertical increase in food production for insuring food security in Bangladesh. Keeping this notion in mind the present research work was undertaken with a view to improve potatoes for both quantitative and qualitative attributes. In the present study the following research issues were addressed: i) The improvement of tissue culture technology for the production of high quality seed potato; ii) The development of nutritionally enriched potato cultivars through selective biochemical property profiling; iii) The improvement of pro-vitamin A and iron (Fe) content in selected potato cultivar through the induction and evaluation of somaclonal variation and iv) the improvement of the *in vitro* conservation method for potato germplasm conservation.

For the production of high quality seed potato using tissue culture, apical meristems of 25-35 days old field grown plants were cultured onto filter paper bridge in liquid MS medium supplemented with different formulation of GA_3 and Kin. Among the various growth regulator formulations 0.5 mg/l GA_3 + 0.4 mg/l Kin was found the most effective medium formulation for the primary establishment of meristem culture. The cultured meristem tips were found to grew into healthy shoots. The meristm grown plants were found free of the presence of PVX, PVY and PLRV as reveled by ELISA test.

In order to improve the micropropagation method for virus free planting materials the meristem grown plantlets were cut into individual nodes and cultured separately onto MS0 medium. This experiment reveals that healthy shoot growth along with the highest numbers of culturable nodes was found when 3rd node was used as explant.

In order to optimize the requirement of organic components in MS medium the individual node cuttings of six potato cultivars was sub cultured onto different MS media formulations viz. M_1 (Thiamine HCl free MS medium), M_2 (Nicotinic acid free MS medium), M_3 (Pyridoxine HCl free MS medium), M_4 (Myo-inositol free MS medium), M_5 (Glycine free MS medium) and MS0. It is noticed that M_2 and M_3 media had no significant effect on micropropagation of six potato cultivars. Among the different cultivars, Asterix was found to be the most responsive cultivar followed by Diamant to micropropagation.

Single nodal cuttings of *in vitro* grown plantlets of six potato cultivars were cultured onto agar solidified MS medium supplemented with different concentrations of sucrose and different BA or Kin formulations for microtuber development. Single nodal cuttings were also cultured onto cotton based and cotton free MS liquid medium. Among the different treatments 8% sucrose with 10 mg/l BA or 3.5 mg/l Kin was found the most effective media formulations for microtuberizaton.

The plantlets (PL) and the microtubers (MT) of six potato cultivars were transplanted in to field in order to study their field performance. The performance of plantlet derived plants was significantly better than that of microtuber grown plants for various characters used as assessment parameters including tuber yield.

Field performance of minituber, breeder and foundation seed tubers of PL and MT groups were also studied. The tuber yield of the tissue culture derived plants was significantly higher than that of source seed propagated crop. Asterix was found to be superior to other cultivars in tuber weight.

Nutritional quality of field grown fresh potato tubers of 44 potato cultivars was studied. Among the cultivars, All Red possessed the highest concentration of iron (Fe) and pro-vitamin A (β-carotene); Indurkani was possessed of high concentrations of soluble protein, iron (Fe) and phenolic compounds; Lady Rosetta had highest Zn and Co; JPR was abundant in K and Cd respectively. The cultivars Baraka, Hagri, Jam Alu and Lal Pakri were rich in Ca, starch, vitamin C and Co, respectively. On other hand cultivars Banana, Chipita, Kenne, Blondy, Martin, Febula and Call White were abundant in total sugar, reducing sugar, Mn, Cu, Cr, Pb and As, respectively.

Callus was induced and plant was regenerated form the calli for the induction and evaluation of somaclonal variation with a view to increase pro-vitamin A and iron (Fe) content in selected potato cultivar. The higher shoot regeneration rate was found in MS medium supplemented with 1.0 mg/l BA + 0.1 mg/I GA_3. In the field evaluation of somaclones AR-01 line appeared to show significantly higher content of iron.

For *in vitro* germplasm conservation, single nodal explants of five potato cultivars were sub cultured onto MS medium supplemented with different sorbitol and mannitol formulations. In this experiment MS medium with 20 g/l sucrose + 40 g/l sorbitol was the most effective medium formulation where the survivability of plantlet was the longest.

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CHAPTER I

GENERAL INTRODUCTION

1.1. THE POTATO CROP: GLOBAL PERSPECTIVE

Potato is the world's fourth most economically important food crop, after wheat, rice and maize (Dowling, 1995; Nyende *et al.,* 2005 and FAO, 2009). The crop has great yield potential. Potato provides roughly half of the world's annual production of all root and tuber-based food, making it the leading non-cereal crop. It is a part of the diet of a half billion consumers in the developing countries (Ghislain *et at.,* 1999). It is grown as a major crop in countries with different climatological zones exists. Currently the crop is grown at a significant scale in about 163 countries (Anonymous, 2007) and covers yearly worldwide about 19.32 million ha (FAO, 2008).

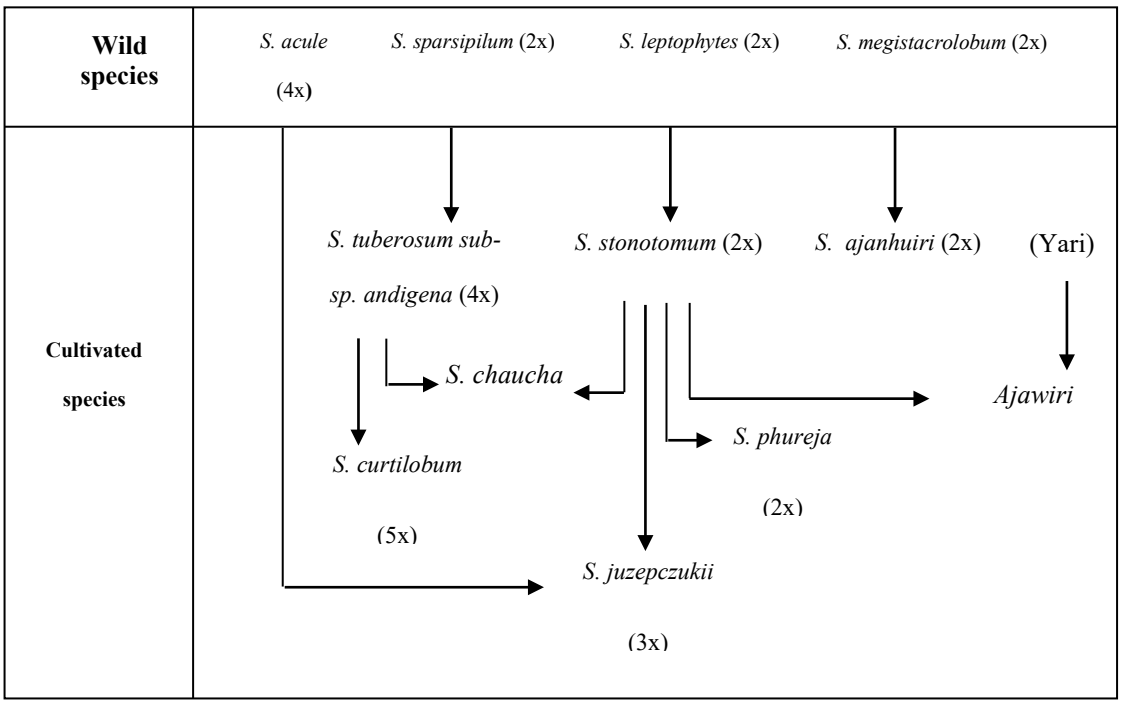
The crop is becoming less important in the developed world, but since the early 1960s the increase in area planted with potato in the developing countries has been greater than for any other major crop. Currently 25% of the world's area cropped with potato can be found in India and China (Maldonado *et al*., 1998), the two countries with the largest population. The potato has become an important staple food in parts of the world where there is a limited (but increasing) purchasing power, an increasing demand for food and an increasing pressure on scarce land.

There are large differences in yield per unit area among the different zones and countries in the world. This difference in yield potential is associated with differences in climate and availability of inputs, such as nutrients and crop protectants, quality of seed, and technological level. Despite this difference in yield per unit area, associated with differences in climate and input level, there is a clear and continuous shift in production from the developed countries to the developing countries. The area cropped with potato has shown significant increases in many developing countries, whereas it has declined in many developed countries. However, despite this trend the average tuber yield is still lower in developing countries (Africa, most of Asia, Latin America) than in the developed countries (Europe, North America, and parts of Asia, Oceania, and a few other

countries). The increase in potato area in developing countries has been possible due to major developments in the availability of adapted cultivars and adequate volumes of seed tubers from them.

1.2. THE ORIGIN AND DISTRIBUTION OF THE CULTIVATED POTATO

There are seven cultivated species of potato including; diploids (S. *ajanhuiri*, S. *phureja* and *S. stenotomum*), triploids (*S. chaucha*, *S. juzepczukii*), tetraploids (*S. tuberosum* sub-sp. *andigena*) and one pentaploid (*S. curtilobum*) (Struik and Wiersema, 1999). It is believed that four wild species, *S. leptophyes*, *S. megistacrolobum*, *S. sparsipilum* and *S. acaule* were involved in the creation of this group [**Figure. 1.1.** (Hawkes, 1992)]. By the mid-eighteenth century, the areas planted with potatoes had begun to increase and many peasant farmers came to depend on the crop because of its ease of cultivation, reasonable yields and good nutritional status. However, the sudden appearance of potato blight (*Phytophthora infestans*) caused severe losses in 1845 and 1846, bringing famine to Ireland and severe hardship to parts of mainland Europe (Turner and Evans, 1998). The famine was followed by a decline in the area under potato cultivation in Europe, with only Britain slightly increasing potato consumption from 88 kg in 1914 to 102 kg/head per year in 2000 (BCP, 2000).



Source: Bushra, 2002

Figure 1.1: Evolutionary relationships of cultivated potatoes and their ploidy levels

Until the middle of the last century, the number of introductions from South America was limited, so that the genetic base of most breeding programmes that developed commercial cultivars was relatively narrow. The need for more genetic variation in agricultural characters, including resistance to diseases and pests, has stimulated the use of genetic material from either wild or primitive species. As a result, most of the newer S. *tuberosum* cultivars now have gene combinations introgressed from other species (Struik and Wiersema, 1999).

1.3. AGRONOMIC FEATURES OF POTATO

The potato has a wide range of seasonal adaptability. It is a cool season vegetable crop and is moderately tolerant to frost. The young plants grow best at a temperature of 24°C. Later growth is favored at a temperature of 18 °C. Tuber production is the maximum at 20°C and at about 30°C the tuber production is totally stop. Tuber production is the maximum at 17°C to 20°C and decreases the production with rise in temperature. At about 30°C tuber production totally stops. Relative humidity is need above 50%. Photoperiod is need 14-16 hours.

It thrives in cool regions where there is sufficient moisture and fertile soil. The ideal soil for potato is well-drained, well aerated, deep and having a pH range 5.2 to 6.4. So, potato can be produced on a wide range of soils ranging from sandy loam, silt, loam and clay soil. Well-drained sandy loam and medium loam soils rich in humus are most suitable for potato. Alkaline or saline soil is not suitable for potato cultivation. The acidic conditions (pH 5.2 to 6.4) tend to limit scab disease (Sing, 1989). Application of proper doses of NPK is important for potato cultivation

1.4. ECONOMICALLY SIGNIFICANT DISEASES OF POTATO

The world potato production is hampered due to attack of a number pest and disease. The potato is known to be infected by as many as 175 diseases besides several physiological disorders (Weilman, 1972).The causal organisms of potato disease include bacteria, fungi, viruses, viriods, mycoplasma and nematodes (Khan *et al.,* 2003). Further damage can be inflicted by insects such as aphids, cutworms, Colorado potato beetles and leaf foliage; by stunting growth or reduced growth of foliage; by causing a breakdown in the transport of photosynthesis to the tubers; or by causing tubers to rot either during tuber

growth or during harvesting (Struik and Wiersema, 1999). As many as 25 important virus diseases have been reported that infect potato crop (Hooker, 1987). Among them, Potato leaf roll virus (PLRV), Potato virus Y (PVY), Potato virus M (PVM), Potato virus A (PVA) and Tobacco mosaic virus (TMV) are of immense importance in causing yield loss of potato all over the world (Singh, 1980; Hooker, 1987). In Bangladesh, at least seven viruses, namely PVY, PLRV, PVX, PVS, PVM, PVA and TMV infect potato (Ali and Khan, 1990). Out of seven viruses, PVY and PLRV have been found to be the two most important viruses affecting the yield in all the potato growing countries has been recognized as a severe menace of potato cultivation in all the potato growing regions of the world (Pushkarnath, 1976; Singh, 1980; Hooker, 1981; Bhandal and Naik, 1991). PVY is the second most important virus disease, which occur worldwide after PLRV (Anonymous, 2006). Both the viruses are tuber-borne resulting degeneration (Singh, 1980; Singh and Khurana, 1980; Hooker, 1987; Brunt *et al.*, 1990). In India, PVY and PLRV singly reduced potato yield up to 60-75% (Gupta *et al.,* 1985). Hoa *et al.* (1991) reported that moderate infection and severe infection due to PVY, respectively, caused 49% and 61% yield loss in the Philippines under low land field condition. Yield loss due to PVY raised up to 95% with severe infection in Bangladesh (Hossain and Ali, 1992). Different bacterial diseases such as common scab, soft rot and bacterial wilt; fungal diseases such as late blight, stem canker, powdery scab, verticillium wilt and early blight; golden nematode, root-knot nematode, lesion nematode, wireworms, colorado potato beetle and green peach aphid are also causes of significant yield losses of potato.

1.5. POTATO PRODUCTION IN BANGLADESH

Potato cultivation in Bangladesh began in the later part of the 19th century. The varieties grown in this country can be categorized into (i) modem potato varieties (MPV) and (ii) local potato varieties (LPV). The LPVs were introduced by European intruder during colonial rule in India subcontinent whereas, MPVs were imported from Europe and adopted in Bangladesh during 1970s by Bangladesh Agriculture Research Institute (BARI). The main difference between MPV and LPV is the composition of starch content and ploidy level (Hossain, 2001). LPVs are either diploid or triploid, contains higher proportion of amylopection but MPVs are tetraploid with less amylopection. High amylopection in LPVs make them sticky and tastier. In spite of low yield the LPVs are popular among the growers and consumers mainly because it contains higher percentage

of dry mater and exhibit good keeping quality under ordinary room temperature (Ahsan *et al*., 2003; Islam and Alsadon, 2003).

Potato is the second largest food crop in Bangladesh and occupying 2nd position after rice (FAO, 2011). In Bangladesh, average production of potato was very low (11.2 t ha^{-1}) up to 1990s. However, the production of potatoes has been increasing gradually due to increasing use of quality seed potato. In 2011-2012 crop session, the tuber yield was 19.071 MTs/ha and total potato production was 82, 05,470 MTs (BBS, 2012). According to the FAO report (FAO, 2011) Bangladesh was raised to the rank 6th just behind the Germany (**Figure 1.2**) in the scenario of world potato production.

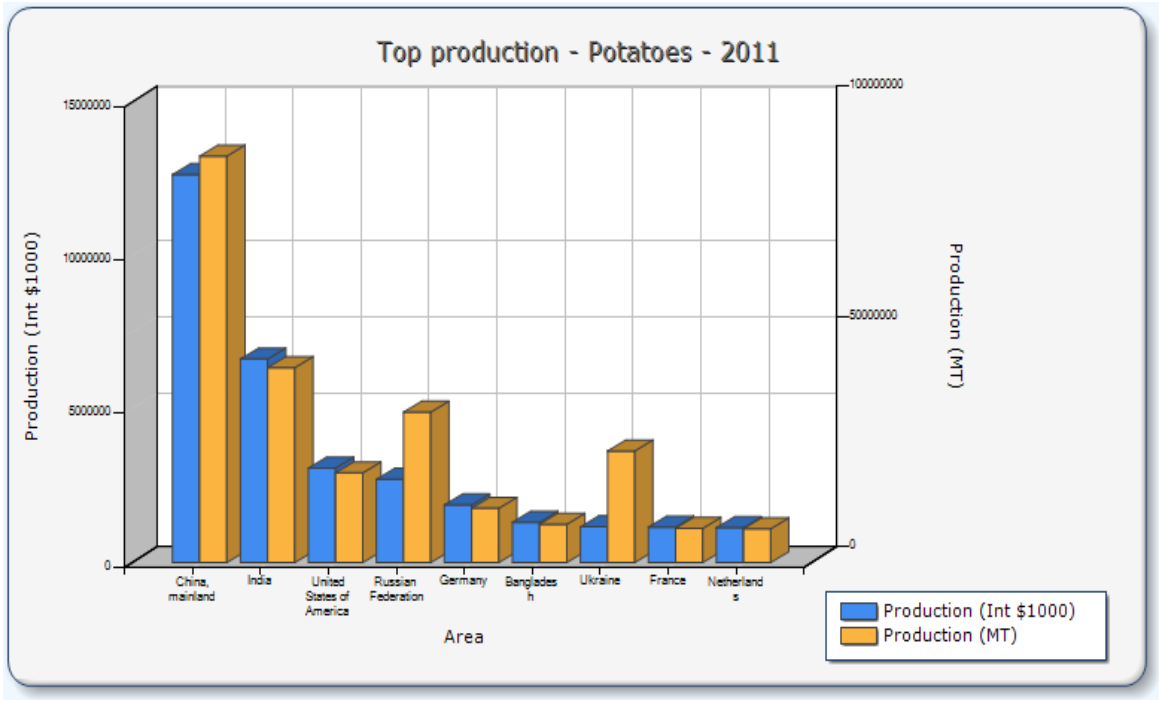


Figure 1.2: The ten highest potato producing countries in the world for the year of 2011. (Source: http://faostat.fao.org/site/339/default.aspx)

1.6. IMPORTANCE OF SEED TUBER QUALITY ON POTATO PRODUCTIVITY

Like most crops, the use of high quality planting material has of paramount on tuber yield. There is a direct link between the quality of the planting materials used and the productivity of potato. There are two types of planting materials commonly being used in commercial cultivation: (1) use of seed tubers and (2) the use of true potato seed (TPS; the very small botanical seed obtained from the berries) derived planting material, such

as transplants or seedling tubers. Although TPS is being used as source of planting materials in a few countries, it is illustrated the importance of using high quality seed tubers only. Seed tubers are special as planting material since they transfer soil- borne diseases, accumulate seed-borne diseases, are less storable than grain seeds and have a physiology affecting their use which is quite different from the physiology of grains.

The costs of producing high quality planting material are high, but seed of quality is a necessary investment both in low-input and high-input agriculture. In fact the quality of the seed potato tubers is the most important yield determining factor that can be influenced by the farmer and also the most important yield constraint in many potato growing countries.

Potato is distinguishable for following reasons from most other major world food crops as far as production of planting material is concerned:

i) Low multiplication rate: The multiplication rate of seed potato tubers is much lower than for example in cereals and other grain crops (**Table 1.1**). The multiplication rate based on seed weight is higher, about 10 - 12 for a seed potato crop and about 20 for a ware crop which means that a substantial proportion of the total potato area is devoted to the production of seed tubers. It has been reported that about 15% of the potato produced in the world is utilized for seed. It also means that it takes a long time to build up adequate amounts of commercial seed tubers. Therefore optimizing the production scheme of seed tubers can have a significant effect on land use and yield.

Table 1.1. Some figures for normal seed rates, yields and multiplication factors for various crops in Western Europe.

Crops	Seed rate (kg/ha)	Yield (t/ha)	Multiplication factor	Hectarage ratio of seed crop to food crop
Potato	2000	40	20	1:10
Wheat	160	8	50	1:30
Barley	120	6	50	1:40
Maize	30	9	300	1:100

Source: Hossain and Islam, 2013

ii) Vegetative planting materials: As a vegetative organ the seed potato tuber is prone to "seed degeneration", i.e. a decrease in the quality of the seed from continued propagation, mostly caused by a decrease in health status (e.g. caused by an increase in virus frequency and concentration). Many plants have internal infections of pathogens and these pathogens can easily be transferred to the next generation. Pathogens transferred from one generation to the next include viroids (e.g. potato spindle tuber viroid), viruses (e.g. potato leafroll virus), bacteria (e.g. *Erwinia* spp.), and fungi (e.g. *Rhizoctonfa solani*). Also insects (e.g. potato tuber moth), nematodes (e.g. golden nematode), weeds (e.g. weed stolons of *Sorghum halepense*, *Cyperus* spp., *Cynodon* spp.) and other organisms can be transferred by or with seed tubers.

iii) Dormancy in seed tubers: Seed tubers usually remain dormant for 1 - 15 weeks. Therefore, seed tubers must be stored for breaking dormancy before use. As an active organ seed tubers transpire, respire, lose water and dry weight and also show physiological development during storage. The physiological status of the seed tuber is of great importance to its vigour of growth and thereby to the potential yield of the crop grown from it. The physiological status of the seed tuber depends on its health status, size and age, conditions during production, storage duration and pre-treatment before planting. Storing tubers too long results in seed decline, i.e. a strong reduction in seed quality when the seed is getting older. Note the difference between seed degeneration and seed decline.

iv) The cost of seed tubers production: The costs of seed tubers are very high. In certain countries they may make up 50 - 70 % of the total costs of production of a commercial ware crop.

v) Techniques of potato seed production: A wide range of suitable techniques relating to the production of high quality seed potato tubers is available. The best set of techniques should be selected based on the agro-ecological conditions of seed and ware potato production and the size of the operations. However, seed production programmes have often lost their impetus because the managerial requirements are underestimated. This easily leads to failure in applying the right techniques, but especially to failure in applying effective procedures in handling, management, quality control and certification.

1.7. SEED POTATO PRODUCTION IN BANGLADESH

Seed potato production system in Bangladesh is not being stringently regulated. Any persons/agencies can produce seed potato and can sell to the farmers as "Truthfully Labeled Seed" without going through any inspection agencies. Seed potato produced through the seed certification agency (SCA) of Department of Agriculture Extension, Bangladesh can met only 15% of the national demands. The rest of the seed potatoes are coming from farmers own produce. In general, potato tubers propagated by vegetative methods (cloning), most commonly by harvesting and replanting the tubers. When the potatoes are harvested, the tubers, or new potatoes, are generally found attached to the plant on underground stolons. The tubers are either saved for eating or replanted. The tubers used for planting are known as "seed potatoes", as opposed to "potato seeds".

In Bangladesh seed potatoes are being produced through four different ways:

i) Conventional way of seed potato production: Conventionally seed potato tuber production in Bangladesh started from imported of Elite class seed potato from abroad by BADC (Bangladesh Agricultural Development Corporation) and other seed farms. Imported seed tubers were multiplied for two to three times by seed agencies and registered seed growers to produce certified seed and sold to the farmers. This method operates as a-"flush through" system starting each year with fresh true to type and healthy imported tubers which have been indexed for freedom from viruses.

ii) True potato seed (TPS) production: True potato seed (TPS) or botanical seed is being used as an alternative planting material of course in very limited scale in some parts of Bangladesh. Research on TPS at the CIP was started in 1977 whereas in Bangladesh it was initiated in 1980 by Tuber Crop Research Center (TCRC) of BARI in collaboration with the International Potato Center (CIP). During 1980's attention was given to produce open pollinated seeds, and during 1990's hybrid TPS were produced by crossing of selected lines under artificially created long days with the help of incandescent lights. TPS is used to grow commercial potato crop by two methods: (i) transplanting of seedlings and use of seedling tubers. In the former method, potato seedlings are raised in nursery beds and transplanted in the field at 4-5 leaf stage. Potato crop raised from seedlings is harvested at maturity. However, despite intense effort by TCRC and DAE (Department of Agriculture Extension) TPS technology is failed to get popularity among the potato growers in Bangladesh because, the seeds are not

genetically pure and exhibit high heterogeneity; the crop is late in maturity as compared to the crop grown from seed tubers; do not meet market demand and the technology is labour intensive. This process is useful for potato breeders, but it is of little value to today's commercial potato grower.

iii) Seed potato production by stem/sprout cuttings: BADC has been trying to produce virus free seed potato by using sprout cutting and stem cutting from imported seed tubers. It has also been claimed by BADC that the seed potato tubers produced by sprout cutting and stem cutting are similar to elite class (E-class) seed potato tubers imported from Holland in respect of PVY and PLRV infection. Incidence of PVY, PLRV and their mixed infection was low in sprout cutting tuber as compared to stem cutting and conventional tubers of E-class seed (Rahman *et al.*, 2009). Performance of seed tubers obtained from sprout cutting was better in respect of growth parameters, yield and yield contributing characters as compared to stem cutting and conventional tubers in a net house (Rahman *et al.*, 2009). Despite these merits, seed tuber production through stem cutting has failed to get popularity in the private sectors in Bangladesh.

iv) Seed potato production through tissue culture: Plant tissue culture is a specialized technology used for plant propagation. It operates on the principle of growing disease free plant tissues under sterile conditions in artificial plant growth medium. Through tissue culture, very large numbers of identical plantlets can be derived from one mother plantlet. This technology and the resulting plantlets now form the basis of many plant nursery and flower trade industries. Throughout the world, thousands of laboratories apply plant tissue-culture technology to crops, ornamental plants and endangered plant species. In the late 1970's, the technology for large scale tissue culture was refined for potato production (Hossain and Islam, 2013). Today, almost all seed potato production systems incorporate this technology in some way.

In Bangladesh, the seed potato production package including the establishment of low cost plant tissue culture lab and field management were initially transferred to the private sector company (RANTIC, Rainbow Tissue Culture Ltd., Rajshahi, Bangladesh), in 1996 (Hossain and Islam, 2013). With the active support of the researchers of Plant Breeding and Gene Engineering Laboratory, Department of Botany, Rajshahi University, Bangladesh RANTIC Ltd. was able to create exemplary success in using tissue culture

technology to seed potato production in Bangladesh. The success of RANTIC Ltd. inspired other organizations from private sector entrepreneurs as well as from GOs (BADC) and NGOs to invest capital to build tissue culture based commercial laboratories (Hossain and Islam, 2013). At present there are 30 plant tissue culture laboratories established in different parts of the country chiefly targeted for seed potato production. In addition about twelve thousands small farmers have been integrated with seed potato production by using disease indexed plantlets purchased from private companies (Hossain and Islam, 2013).

1.8. PROBLEMS OF SEED POTATO PRODUCTION IN BANGLADESH

In Bangladesh seed potato production is difficult due to prevalence of several limiting factors.

i) Land scarcity: As we mentioned earlier that Bangladesh is very densely populated and about 147.9 million people live in only 147570 sq kilometer area with more than 993 people/sq kilometers (BES, 2011). Out 147.9 million people 53% population are agrarian and somehow involved in agro based production system. As result lands are fragmented into small pieces. Average farm sizes are between 0.5 - 5 acres. Seed potato production requires isolated and large farm lands which are inexistence in Bangladesh. In reality most of the commercial potato growers cultivate potato on leased land. Moreover, most of the farm lands are occupied with multiple cropping patterns. As a result scarcity of suitable farm lands is the major constraint for high quality seed tuber production.

ii) Climatological barriers: In Bangladesh potatoes are being cultivated during winter season as a short duration crop. Winter season here is very short (November to February). The optimum plantation time for seed potato production starts from November 15 to 30. Seed potato production requires at least 70 day growth period. Therefore, tubers planted for seed potato production in mid November should be haulm killed in the first week of February. Moreover, there is a presence of strong vector (Aphids, *Myjuspersiki* sp.) pressure during late February. Aphids carrying viral diseases start to fly from India to Bangladesh during mid January and come intensify their infestation from mid February. Potatoes plants are exposed to higher density of vectors have greater chance become infect with viral diseases.

iii) Technology impediment: Seed potato production using tissue culture technology requires following infrastructural establishment: a. A tissue culture laboratory; b. green house; c. A numbers of relevant equipment and lab wares; d. A range of high quality chemicals; e. Trained man power

Establishment of a standard tissue culture laboratory equipped with quality lab gadgets and chemicals is costly and not affordable to entrepreneurs of LDC like Bangladesh. Moreover, establishment and maintenance of a green house in subtropical climate is even more difficult and expensive.

1.9. THE IMPORTANCE OF POTATO

The potato crop is very efficient in terms of production per unit time, per unit area and per unit input. It is grown not only for food, but also for animal feed, industrial uses and seed tuber production. Potato is a staple food in some countries and is used as a vegetable crop in many countries. As an industrial crop, potato is a raw material of various foods and confectioneries such as french fries, chips (crisps), bread, granules, flakes, puffs and rounds, patties etc. Potato can be also used for production of high quality starch, sugar (dextrose), alcohol, pectin, syrup and a by-product of high quality protein and fodder. It is the cheapest source of carbohydrates that is used as a supplementary diet to rice. The potato produces nearly twice the amount of calories per hectare than rice or wheat (Poehlman and Sleper, 1995; Poehlman and David, 2003). This crop is 83% more efficient than rice in producing protein (Khatun, 2004). Potatoes are also a good source of some minerals, at least 12 essential vitamins, and extremely high content of vitamin C comparable to other food crops (Struik and Wiersema, 1999).

1.10. NUTRITIONAL ASPECTS OF POTATO

Carbohydrate: The potato is the best known for its carbohydrate content. The predominant form of this carbohydrate is starch. They are very good natural sources of both soluble and insoluble fiber. In potato, sucrose is synthesized from carbohydrate in the foliage and transported to the tubers. The forms of sugar present in the tubers are sucrose, glucose and fructose. In mature tubers, these sugars remain in equilibrium with the starch. The sugar content of potato tubers is normally low, but varies greatly in storage under varying temperatures.

Protein: Potato protein has high nutritional value, ranging from 60 to 90. Rahman (1990) mentioned index values as high as 72 as the biological value of the amino acids of potato protein samples measured against an index value of 100 for a complete protein. The high nutritional value of protein in potatoes is evident when its composition is compared with that of whole wheat (Smith, 1968). Potato protein contains substantially greater amounts of all the essential amino acids except histidine, and the amount of lysene in potatoes is similar to that in most animal proteins.

Vitamins: The tubers are one of the richest sources of B-complex group of vitamins such as pyridoxine (vitamin B_6), thiamin, niacin, pantothenic acid and folates. Fresh potato along with its skin is good source of antioxidant vitamin; vitamin-C but the potato tubers contain either no or only trace amount of pro-vitamin A/β- carotene (Burton, 1989).

Minerals: The minerals present in potato tuber are potassium, phosphorus, magnesium, sulphur, chlorine, calcium, iron, silicon, zinc, boron, bromine, aluminium, sodium, manganese, iodine, fluorine, copper, cobalt, arsenic, lithium, molybdenum and *nickel (Rahman, 1990; CIP,1895). Some of these minerals are essential elements, or are catalytic agents in metabolic processes, while others happen to be present in the soil in which the crop has been grown (Rahman, 1990). Elements that may be found in larger quantities are potassium, phosphorus, magnesium, sulphur, chlorine and calcium. Calcium, phosphorus and iron are considered most important to the nutritional value of potato tubers although the potato tubers contain small amounts of iron.

1.11. POTATO IMPROVEMENT BY CONVENTIONAL METHODS

The use of the term “traditional” does not necessarily mean that the methods are static. Breeders are always looking for progressive tools and techniques (Plaisted *et al.,* 1994). Conventional breeding procedures, such as recurrent selection through the use of hybridization between commercial cultivars and unilateral and by lateral sexual polyploidation, can result in new cultivars with desirable characteristics, such as earliness and resistant to heat, frost, drought or disease (Poehlman and Sleper, 1995). However, certain aspects of the breeding process will remain relatively constant, such as the time consuming and often expensive field evaluation and replicated yield trial procedure, which are the foundation of a potato breeding programmes. All most all of the

well- known commercial cultivars of potato have been produced by traditional breeding methods.

1.12. APPLICATION OF TISSUE CULTURE FOR POTATO IMPROVEMENT

Plant biotechnology has been developed as a new technology to put forward as a potential way of propagation as well as increasing genetic variability for plant improvement. It is now important application in potato breeding and production of potato varieties. For many years tissue culture has been applied to improve potato production by means of micropropagation, pathogen elimination and germplasm conservation (Roca *et al.* 1978; 1979; Schilde *et al.* 1984; Islam and Alsadon, 2003).

Potato breeding is slow when compared to other major food crops. Therefore, it is not surprising that several applications of biotechnology have found a particular fit with the potato crop (Ghislain *et al*. 1999). Major techniques of potato tissue culture can be divided into two categories:

1. The propagation, storage and dissemination of virus-free plants.
2. The widening of genetic variability and the introduction of novel traits into potato by somaclonal and protoclonal variation, ploidy manipulations, embryo rescue, genetic transformation and somatic hybridization. While progress in the area of biotechnology provides new opportunities for potato breeding, most experts agree that biotechnology-based methods will always supplement, but not replace traditional breeding methods.

1.12.1. Micropropagation for Quality Seed Potato Production

Micropropagation is a tissue culture (*in vitro*) method used for rapid and true to type multiplication of plants on artificial nutrient media under controlled environment. It is the most commercially exploited area of plant tissue culture, having been widely used for production of quality planting material in vegetative propagated species. The most significant advantages offered by micropropagation are: (i) large numbers of disease-free propagules can be obtained from a single plant in a short period, (ii) propagation can be carried out throughout the year, and (iii) the propagating material can be accommodated in a small space. For the rapid multiplication of potato *in vitro*, three types of propagules are used (Lommen, 1995): i) Production of plants by nodal cuttings; ii) Meristem or shoot tip culture; iii) Production of *in vitro* tubers (microtubers). Micropropagation has

been successfully used in almost all potato seed producing countries to speed up initial stages of seed production. The process typically consists of: (i) production of virus-free potato plants using meristem culture (ii) micropropagation of virus-free plants, (iii) production of micro-and/or mini-tubers from micropropagated plants and (iv) growing healthy seed crop using minitubers as a planting material Mondal *et al.* (2012); Hossain *et al.* (2012).

Over the last three decades plant regeneration has progressively become possible from a variety of explants/specialized cells of plant species, including dicotyledonous as well as monocotyledonous species (Anthony, 1999). The application of tissue culture and rapid multiplication techniques in potato seed programmes have now become wide spread in both developed and developing countries. A tissue culture based seed potato production facility was established at TCRC of BARI, Joydebpur, Bangladesh with the help of DANIDA in mid 1980s. However, due to expensive nature of lab establishment and prolong and complicated seed multiplication phases, this establishment was failed to get appreciation and acceptance to seed potato growers in Bangladesh.

In order to overcome the problem with farm land scarcity, climatological barrier and technological hurdles a new approach for tissue culture based seed potato production system was developed during mid 1990s. Thanks to the researchers of Plant Breeding and Gene Engineering Laboratory of the Department of Botany, Rajshahi University, Bangladesh, for the development of low cost appropriate tissue culture based seed potato technology suitable for low impute entrepreneurs and small-scale farmers. They designed and developed low cost plant tissue culture laboratory with indigenously developed lab equipment and lab wares. They also successfully developed a new cost minimizing system for the production of minitubers/breeders seeds through transplanting plantlets directly to nursery bed covered with low cost vector protecting nylon nets. Minitubers are multiplied for two to three times in order to minimize the exposure of potato plants to viral vectors what they used call production early generation seed tubers for ware potato production. The outlines of the overall procedures for early generation seed potato production system illustrated in **Figure 1.3.**

Production of early generation seed tuber using this procedure has several advantages. In advanced countries the production time of certified seeds from nuclear seed is long (6 to

7 years). However, using this procedure certified seed potatoes can be produced from minitubers in three years that eventually reduces the time of exposures of seed plants to vector infested environment. Even many farmers in Bangladesh use second generation seed tubers (Foundation seeds) for the production of ware potato in order to ensure the highest crop yield through the reduction of vector threshold. Another important achievement is the integration of small scale farmers and low imputes entrepreneurs in the seed potato production system in Bangladesh. As mentioned before about scarcity of suitable farmlands and fragmented nature of the cultivated lands are the important impediments in seed potato production in Bangladesh. Therefore, direct involvement of farmers and entrepreneurs in the production of minitubers using plantlets purchased from private/public tissue culture labs is the best option to boost seed potato production in Bangladesh using small farm land in vector infested environments. Researchers of the Department of Botany, Rajshahi University have been playing key roles in popularizing this approach of seed potato production in Bangladesh. They have developed training manuals and have organized numerous training programs to train the small farmers and entrepreneurs about how to establish a low cost plant tissue culture laboratory and how to produce disease free planting materials.

Before the introduction of tissue culture based system Bangladesh was totally depended on imported basic seed potato tubers from abroad. But the scenario has been changed gradually after the introduction of tissue culture based production of disease free planting materials. Information says that about 80% of the seed potatoes are now being produced indigenously using minitubers produced from potato plantlets that are produced in the local tissue culture laboratories (Hossain and Islam, 2013). This success in seed potato sector has brought Bangladesh to 6th position among potato producing countries in the World ((Hossain and Islam, 2013). Now in Bangladesh many private farms, NGOs and GOs (such as BADC, RDA) apply plant tissue culture technology to seed potato production.

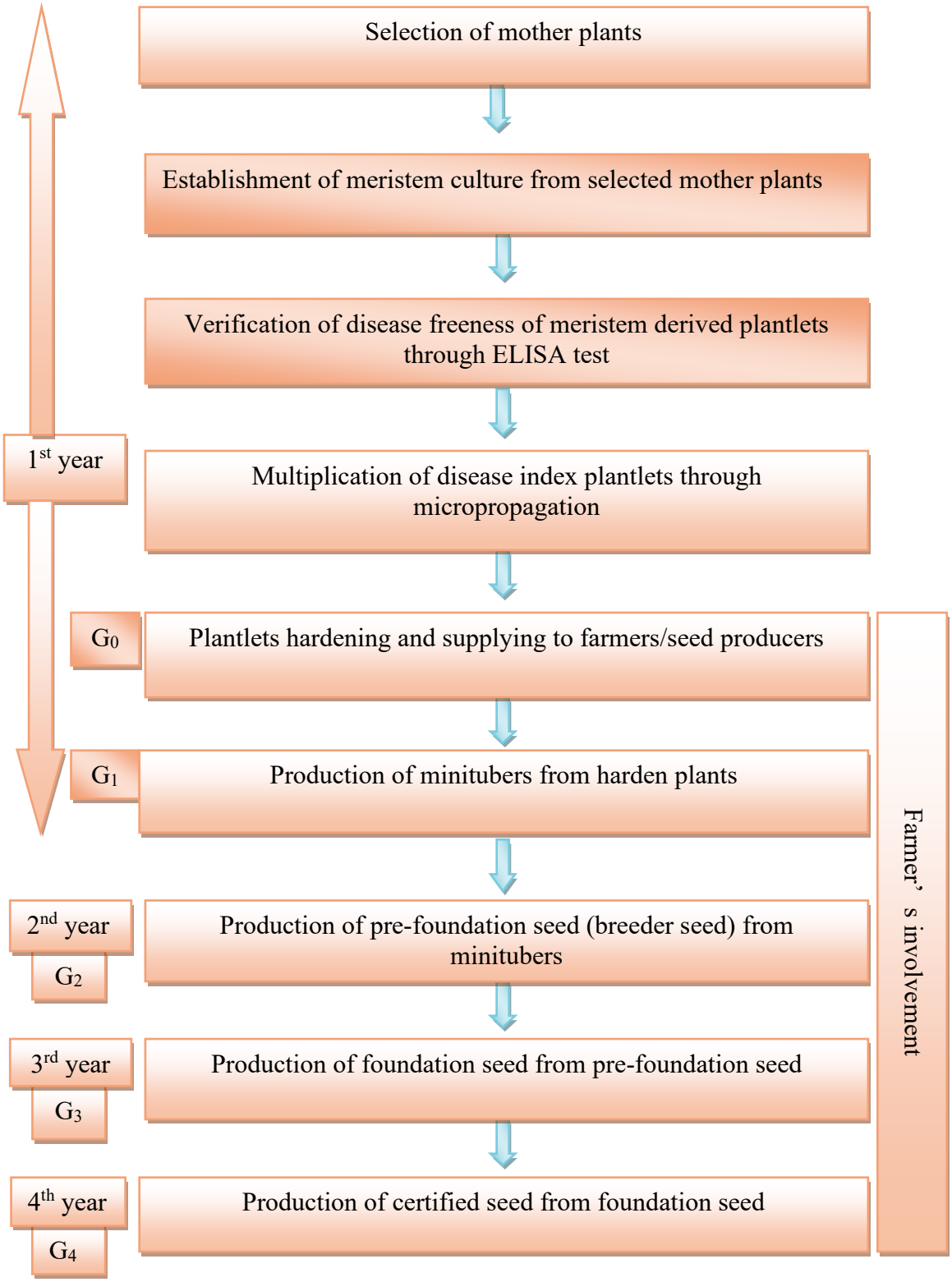


Figure 1.3: Step involved in the production of seed potato through tissue culture technology, currently being adapted by both public and private sector organizations in Bangladesh. (Source: Hossain and Islam, 2013)

1.12.2. *In Vitro* Germplasm Conservation

One way of conserving germplasm, an alternative to seed banks and especially to field collections of clonally propagated crops, is *in vitro* storage under slow-growth conditions (at low temperature and/or with growth-retarding compounds in the medium) or cryopreservation or as desiccated synthetic seed (Harry and Thorpe, 1991; Villalobos and Engelmann, 1995). The technologies are all directed towards reducing or stopping growth and metabolic activity. Techniques have been developed for a wide range of plants (Bajaj, 1991). Since 1975, CIP has contributed to developing tissue culture techniques for conserving germplasm of potato *(Solanum tuberosum)* (Roca, 1975), sweet potato (*Ipomoea batatas*) (Siguenas, 1987) and Andean root and tuber crops (Toledo *et al*., 1994). At CIP, these procedures are applied to potato and sweet potato with good results. There are well-established protocols for micro propagation of potato (Espinoza *et al*., 1992), meristem culture (Lizarraga *et al*., 1991), *in vitro* tuber induction (Estrada *et al*., 1986), medium-term storage (Golmirzaie and Toledo, 1998), and cryopreservation (Golmirzaie and Panta, 1997).

The protocol for medium-term *in vitro* conservation of the potato collection at CIP is as follows. Accessions are conserved in a conservation medium containing 4% sorbitol at a temperature of 6-8ºC, and light intensity of 1,000 lux. That extends the *in vitro* conservation of the potato collection for 2-4 years without subculture. The use of sorbitol as an osmoticum in applied to many crops without any physiological changes such as callus formation or vitrification. But these undesirable reactions are produced when the media contains mannitol, which can affect potato genetic stability (Harding, 1994). After several in *in vitro* culture, plantlets can recover normal growth after one to two subcultures in propagation media. This conservation method is one of the most efficient for managing a large number of potato accessions and the time interval between subcultures is longer than for other crops. At the central potato research institute (CPRI), Shimla, India more than 1,500 parental lines and potato varieties are maintained through *in vitro* conservation (Gopal *et al*., 2010). Crops can also minimize the growth rate in many crops (Dodds and Roberts, 1985; George and Sherrington, 1984).

The production of microtubers in *in vitro* culture as an alternative method for long-term conservation of potato cultivars has also been evaluated at CIP. Microtubers are

produced in 2 to 3 months and can be stored at 10ºC for up to 10 months after harvest (Estrada *et al*., 1986). The dormancy of these microtubers can be controlled by environmental changes (Estrada *et al.,* 1986; Tovar *et al.*, 1985). Alternatively, once the microtubers sprout, growth can be retarded, as with *in vitro* plants, for 2-4 years by storing them embedded in a conservation medium.

1.12.3. Somaclonal Variation

Morphological variation has often been observed in potato plants regenerated by *in vitro* techniques involving an intermediate callus phase. Variation in a number of horticultural traits and disease resistance has been reported among plants regenerated from potato protoplast (Thomas *et al.,* 1982). With the exception of sugarcane, no world crop rivals with potato as a model for improvement via somaclonal variation (Orton, 1984). The possible *in vitro* selection for tolerance to toxic agents, including toxins from pathogens, increased the interest to use tissue culture techniques for the application of reliable selection pressure in order to screen up crop plants for disease resistance, herbicide tolerance and stress tolerance (Lepoiver *et al.,* 1986).Van Herten *et al.* (1981) observed variation frequencies from 50.3% and 12.3% among plants regenerated from potato petiole and leaf calli respectively, in the absence of mutagenesis treatments, some of these variations included growth habit, maturity date, tuber characteristics and light requirements for flowering. Five hundred protoclones of Russet Burbank were treated with culture filtrates of *A. solani* (causal agent of early blight) and 800 with *P. infestans* (causal agent of late blight). Two to three percent plantlets derived from treated protoclones were found resistant to these pathogens (Meulemans *et al.,* 1987). Resistant to some other fungi (Marten and Strobel, 1987) and several herbicides tolerant potato plants were also obtained (Weller *et al.,* 1987). Somaclonal variations with useful properties have been produced in potato and such variants are resistant to early blight (*Alternaria solani*) and to multiple races of *Phytopthora infestans* (Khatun *et al*., 2003; Nasrin *et al.,* 2003). In several commercial varieties of potato, tissue culture induced variations were observed in a wide range of characters, such as plant morphology, tuber characteristics (Taylor *et al*., 1993), disease resistance (Sebastini *et al*., 1994), iso-enzymatic pattern, tuber proteins and chromosome number and structure (Pijnacker and Sree Ramulu, 1990). Tissue culture-induced variations, including morphological, cytological, biochemical and genetic/epigenetic alterations, have been frequently

reported in many plants. However, the mechanism underlying this so called somaclonal variation remains largely unclear (Peredo *et al*., 2006; Kaeppler *et al*., 2000). The most common factors affecting somaclonal variation are genotype, explant source, *in vitro* period and culture conditions (Bordallo *et al*., 2004). Unlike epigenetic changes, somaclonal variation which results from altered gene expression is usually irreversible (Karp 1995). The segregation pattern of mutations in the progeny is mostly Mendelian (Larkin *et al.,* 1984). The studies with many potato varieties have evidenced that meristem clones may differ in their agronomic traits and disease resistance. More than 600 meristem clones of 40 potato varieties have been studied for a long time period. The results showed that meristem clones differed: in yield, tuber weight, number of tubers/plant, starch content and disease resistance (Rosenberg *et al.,* 2004; Rosenberg *et al*., 2008; 2007). The meristem clones differed in yield, number and weight of tubers and late blight resistance (Rosenberg *et al.,* 2010). The selection of somaclonal variations appearing in the regenerated plants may be genetically stable and useful in crop improvement (Rai *et al*., 2011).

1.13. OBJECTIVES OF THE PRESENT STUDY

Present study has the following objectives:

1. Development of improved tissue culture technology for the production of high quality seed potato for some selected potato cultivars.
2. Determination of nutritional properties of selected potato cultivars and selection of nutritionally enriched cultivars.
3. Induction of somaclonal variation and selection of plants with higher pro-vitamin A and iron (Fe) contents.
4. Development of a suitable method for *in vitro* conservation of potato germplasm.

CHAPTER II

PRODUCTION AND FIELD EVALUATION OF SEED TUBERS OF SOME SELECTED POTATO CULTIVARS USING TISSUE CULTURE

2.1. INTRODUCTION

Potato *(Solanum tuberosum* L.) is one of the most important food crops of Bangladesh. The different varieties of potato were grown in this country. The yield level of these varieties in Bangladesh is low as compared to other leading potato growing countries of the world (Anonymous, 2007). Among the various factors responsible for low yield in Bangladesh, viral diseases play a great role because the potatoes are very much susceptible to viral diseases than almost any other major crop. If the mother potato plant becomes infected with a disease during the growing season, each of the new daughter tubers is likely to be infected as well. This is called primary infection; secondary infection occurs when the diseased daughter tubers are planted and infect the new plant, which acts as an inoculum for other plants in the field. If left unchecked, a disease can eventually spread through the potato crop, reducing yields or even destroying an entire crop. In vegetative propagated potato once systematically infected with a viral disease, the pathogen is passed from one vegetative generation to next. Khan (1981) reported that a single plant of potato variety has been infected with four to five viruses. So there is an urgent need to take up programs for the production of virus free potato tuber seed.

It is realized that tissue culture is the most modern and scientifically proven advance technology for virus free seed potato production (Kassanis, 1957) and it is one way of ensuring that potato plants are disease-free. There are reports that meristem tissue culture is a good technique for eliminating viruses (Morel and Martin, 1955) and it was the first biotechnological approach which was successfully adopted and applied to obtain virus free potato stocks (Mellor and Smith, 1977). This technique has become extremely important for potato as a major means of freeing cultivars of virus infection and increasing productivity. This *in vitro* meristem culture has appeared a new venture in obliging virus free potato tuber seed. Zhang *et al.* (1993) reported that 40% yield increase in potato using virus free tuber seed. The application of meristem culture to

eliminate virus infection in clonal plants and large scale production in potato has been discussed in many different publications (Edris *at al.,* 1996; Islam and Chowdhury, 1998; Struik and Wiersema, 1999; Zaman *at al*., 2001; Ahsan *et al.,* 2003; Hossain and Islam, 2013). In the Republic of Korea, use of virus free planting material produced through tissue culture has increased the national potato yield from 11.9 t/ha in 1980 to 20.3 t/ha in 1986 (Chung, 1989). Subsequently, an *in vitro* tuberization system was also established and became an integral component of the potato seed industry in the country (Choi *et al.,* 1994). With the technical support from the CIP, China established an industry scale microtuber production facility with a production capacity of 10 million microtubers/annum. To start with, this facility produced one million microtubers in 1988, which increased to 4 million in 1989 (Li *et al.,* 1991). Researchers in Taiwan have reported production of 36,000 microtubers from 1,200 culture flasks in a period of 4 months (Wang and Hu, 1982). After 3 field multiplications, these microtubers produced 1,800 t seed potato, which was enough for 2000 ha on a schedule of one-third rotation per year. In India, Naik (2005) reported the possibility of producing 264,500 basic seed tubers after one nursery bed and two field multiplications of microtubers produced from one *in vitro* plant. In Bangladesh, use of virus free planting material produced through tissue culture has increased the national potato yield from 9.4 t/ha in 1990 to 19.07 t/ha in 2012 (HBAS, 2007 and BBS, 2012). Thanks to the researchers of Plant Breeding and Gene Engineering Laboratory of the Department of Botany, Rajshahi University, Bangladesh, for the vital contribution to increase the national potato yield through designed and the development of low cost plant tissue culture laboratory with indigenously developed lab equipment and lab wares. They also successfully developed a new cost minimizing system for the production of minituber/breeder seed through transplanting plantlets directly to nursery bed covered with low cost vector protecting nylon nets (Hossain and Islam, 2013). Many researchers (Mondal, 2003; Hossain, 2005; Alam, 2012) of this laboratory were worked on virus free seed potato production using micropropagation. In Bangladesh, mainly three potato cultivars viz. Diamant, Cardinal and Granola are commonly used in potato tuber seed production using micropropagation although more potential potato cultivars also exist in this country. Quality seed potato production efficiency using micropropagation of other potential potato cultivars vs. Diamant, Cardinal and Granola has not yet been properly evaluated in Bangladesh. So it is needed to evaluate the performance of other potential potato cultivars vs. Diamant,

Cardinal and Granola for the screening of high yielding cultivars which contribute to increase the national tuber yield of potato.

On the other hand in spite of different merits, the micropropagation technique has certain demerits also which have limited the use and exploitation of this technique at industrial level. The major limitation is the higher cost of plant production. Hence, the most challenging aspect at present is to reduce the production cost, thereby improving the production efficiency (Anderson and Meagher, 1977; Sluis and Walker, 1985; Donnan, 1986; Levin and Vasil, 1989; Aitken-Christie, 1991). Therefore, to overcome this limitation, it is needed to develop an effective protocol for producing of virus free potato tuber seed applicable for Bangladesh.

2.1.1. Objectives

From the above point of view, this part of the research has following objectives:

1. To develop an effective meristem culture method for the production of virus indexed planting materials for some selected potato genotypes.
2. To develop a cost effective rapid micropropagation method for disease free *in vitro* grown plantlets.
3. To optimize the microtuber production protocol from the *in vitro* plantlets.
4. To evaluate the performance of *in vitro* grown planting materials in field condition.

2.2. MATERIALS AND METHODS

2.2.1. Materials

2.2.1.1. Plant materials

Shoot tips and nodal segments of selected potato cultivars viz. All Blue, All Red, Asterix, Diamant, JPR and Indurkani (diploid cultivar) were used for conducting different experiments in the present investigation. The shoot tips were collected from 25-35 days old plants grown in the potato germplasm conservation field, Department of Botany, University of Rajshahi, Bangladesh and they were used as explants for meristem isolation. On the other hand nodal segments were obtained from meristem derived plantlets which were used as explants for micropropagation with different experiments.

2.2.1.2. Chemicals

All chemical compounds including macro and micro-nutrients, organic and inorganic acids, sugar, agar, KOH, $HgCl_2$, 70% ethyl alcohol etc. used in the present study were the reagent grade products of either BDH, England or E-mark, Germany. The vitamins, amino acids and different growth regulators were the products of Sigma Chemical Co. USA. The chemical compounds used in the present investigation are given bellow:

i) MS basal nutrients: The following MS (Murashige and Skoog, 1962) basal nutrients **(Appendix 1)** were used in this investigation which content macronutrients, micronutrients, vitamins and amino acid for growth and development of plant.

Macronutrients: KNO_3, NH_4NO_3, KH_2PO_4, $CaCl_2.2H_2O$, $MgSO_4.7H_2O$

Micronutrients: $FeSO4.7H_2O$, $Na_2EDTA.\ 2H_2O$, $MnSO_4.4H_2O$, H_3BO_3, $ZnSO_4.7H_2O$, KI, $Na_2MoO4.2H_2O$, $CuSO_4.5H_2O$, $CoCl_2.6H_2O$

Vitamins: Thiamine HCl, Pyridoxine HCl, Nicotinic acid, Myo-inositol.

Amino acid: Glycine

ii) Plant growth regulators: The following plant growth regulators were used in the present study.

Cytokinins: 6-Benzyl adenine (BA); 6- Furfurylaminopurine or kinetin (Kin)

Gibberellin: Gibberellic $Acid_3$ (GA_3)

iii) Sterilant and surfactant: $HgCl_2$ was used as surface sterilizing agent and Tween-20 and Savlon (Antiseptic, ACI Pharma, Bangladesh) were used as detergent and surfactant.

iv) ELISA Kits: ELISA Kits were used for virus freeness testing of meristems derived plantlets. ELISA Kits were imported from Agdia England.

2.2.1.3. Equipments

The culture vessels such as test tubes (25 × 150 mm), bottles or flasks (55 × 140 mm or 50 × 110 mm), conical flasks (250 ml, 500 ml, 1000 ml), measuring cylinders, glass rods, beakers, pipette pumps, cotton plugs, rubber bands, filter paper, filter sterilizer (0.20 µm, single use filter unit), aluminum foils, parafilms, marker pen, spirit lamp, forceps, needles, scalpel, firebox, stereomicroscope, electronic balance, autoclave, pH meter, magnetic stirrer, laminar airflow machine, UV spectrophotometer etc. were also used in the present investigation.

2.2.2. Methods

The methods involved in the present investigation are described under the different subheads.

2.2.2.1. Preparation of culture media

In the first step of the preparation of MS culture medium, stock solutions were made. The various constituents of the MS medium were prepared into stock solutions for ready use during the preparation of the culture media for different experiments. As different constituents were required in different concentrations, stock solutions for macronutrients, micronutrients, plant growth regulators (PGRs), organic compounds (vitamins and amino acid) etc. were prepared separately.

I. Preparation of stock solutions

The following stock solutions were made before the preparation of MS culture medium:

Stock solution A: NH_4NO_3 33 g, KNO_3 38 g and KH_2PO_4 3.4 g were weighted accurately with the help of an electronic balance and dissolved in 200 ml of distilled

water. The final volume was made up to 400 ml (for 20 litres). This stock solution was stored in a refrigerator at 4°C for several weeks.

Stock solution B: $MgSO_4.7H_2O$ 7.4 g was weighted accurately with the help of an electronic balance and dissolved in 100 ml of distilled water. Then total volume was made up to 400 ml (for 20 litres) by further addition of remaining 300 ml of distilled water. Then poured into a bottle and stored in a refrigerator at 4°C for several weeks.

Stock solution C: $CaCl_2$. $2H_2O$ 8.8 g was weighted accurately with the help of an electronic balance and dissolved in 100 ml of distilled water. Then total volume was made up to 400 ml (for 20 litres) by further addition of remaining 300 ml of distilled water. Then poured into a bottle and stored in a refrigerator at 4°C for several weeks.

Stock solutions D: $FeSO_4.7H_2O$ 0.556 g and Na_2EDTA. $2H_2O$ 0.746 g were weighted accurately and dissolved separately in 175 ml of distilled water by heating and constant stirring. Then the two solutions were mixed and pH was adjusted to 5.5. The final volume was made up to 400 ml (for 20 litres) by further addition of remaining distilled water. Then poured into a bottle and stored in a refrigerator at 4°C.

Stock solutions E: $MnSO_4.4H_2O$ 0.446 g, H_3BO_3 0.124 g and $ZnSO_4.7H_2O$ 0.172 g were weighted accurately with the help of an electronic balance and dissolved in 200 ml of distilled water. The final volume was made up to 400 ml (for 20 litres). This stock solution was stored in a refrigerator at 4°C for several weeks.

Stock solutions F: KI 0.166 g, $CuSO_4.5H_2O$ 0.005 g, Na_2MoO4. $2H_2O$ 0.050 g and $CoCl_2.6H_2O$ 0.050 g were weighted accurately with the help of an electronic balance and dissolved in 200 ml of distilled water. Then the final volume made up to 400 ml and stored in a refrigerator at 4°C.

Stock solutions G: Myoinositol 5.0 g, Nicotinic acid 0.025 g, Pyridoxine-HCl 0.025 g, Thiamine-HCl 0.025 g and Glycine 0.100 g were weighted accurately with the help of an electronic balance and dissolved in distilled water. The final volume was made up to 100 ml. This stock solution was poured into a bottle and stored in a refrigerator at 0°C for several weeks.

Stock solutions of plant growth regulators (PGR_s): In adding to the inorganic and organic nutrients, it is generally necessary to add different growth regulators to the media for supporting suitable growth and development of tissue and organs (Bhojwani and Razdan, 1983). Stock solution of growth regulators was prepared separately. Details of the preparation method of stock solution are given in the **Appendix 2.** To prepare the stock solution of any plant growth regulators, 100 mg of the powdered growth regulators were taken in a clean 100 ml biker and then dissolved in 1 or 2 ml of respective solvent. The volume of the solution was then made up to 100 ml by adding distilled water. It was stored in a refrigerator in 4°C-6°C temperature for ready to use in time. Plant growth regulators were added separately to different media according to requirements. Stock solution of GA_3 should be filter sterilized.

II. Preparation of 1 litre MS medium

The following steps were done for the preparation of 1 litre MS medium:

i) Assembling of the MS medium components: For the preparation of 1 litre MS medium, 200 ml of distilled water was taken in a 1 litre volumetric flask and 20 ml of stock solution -1, 20 ml of stock solution -II, 20 ml of stock solution -III, 20 ml stock solution IV, 20 ml stock solution V, 2 ml stock solution -VI and 2 ml stock Solution -VII were added. After the addition of sugar solution made up to the 1 litre with distilled water and mixed well.

ii) Addition of sucrose: After the addition of all stock solution, 30 g/l sucrose was added and mixed well. For primary meristem culture sucrose was added at the rate of 25 g/l.

iii) Addition of plant growth regulators: Stock solutions of plant growth regulators were added in appropriate concentrations and combinations in above solutions and mixed well.

iv) pH of the medium: The pH of all culture media was adjusted to 5.8 using a digital (TOA, Japan) pH meter with the help of 0.1N HCl or 0.1N NaOH (where necessary) before addition of agar.

v) Addition of agar: After the adjustment of pH, agar 6-7 g/l was added. Then the medium was heated for 6-7 minutes in a microwave oven (Shimuju, Japan) to melt agar the completely. Agar was not added to liquid medium.

vi) Medium dispensing to culture vessels: The prepared melted medium was disposed into culture vessels such as test tubes or conical flasks or bottles, through separating funnel. The culture vessels were plugged with cotton plugs, wrapped with cheese cloth, which were inserted tightly at the mouth of culture vessels.

vii) Sterilization: Finally the culture vessels containing medium were autoclaved at 15 lb/sq inch (PSI) pressure and at the temperature of 121°C for 20 minutes to insure sterilization.

2.2.2.2. Culture techniques

The following culture techniques were applied in the present experiment for primary establishment of meristem culture, subculture and maintenance of culture.

Explants collection and preparation: Shoot tips of 25-35 days old field grown potato plants were used as a source of meristem. The shoot tips of potato were excised with the help of sharp blade and collected in a reagent bottle containing distilled water with few drops of savlon and few drops of tween-80 (Polyoxyethylene-80) and quickly brought to the laboratory. Then the explants were washed for 2 or 3 times with gradual change of sterile distilled water and were used in the experiment.

Surface sterilization of the explants: The explants were transferred to 250 ml sterilized conical flask. Surface sterilization was carried out by dipping the explants in 0.1% $HgCl_2$ solution with gentle shaking for 2-8 minutes followed by 3-5 times washing with sterile distilled water in front of running laminar airflow cabinet.

Inoculation technique: All inoculations and aseptic manipulations were carried out in a running laminar airflow cabinet. The cabinet was switched on for half an hour before used for cleaned with 70% ethyl alcohol to reduce the chances of contamination. All instruments like scalpels, needles, forceps, tiles, petridishes etc. were covered with aluminium foil paper and sterilized by steam sterilization method. During working time, these were again sterilized by 70% ethyl alcohol dip and flaming method inside the

inoculation chamber. To ensure complete aseptic condition both hands were also wiped by 70% ethyl alcohol.

Isolation of meristem and their culture: After sterilization, explants were laid on the sterile ceramic tiles using sterile forceps. Shoot tip was hold in one hand under the stereo-microscope with the help of a pair of forceps and the immature leaves and leaf primordial were snapped with slight pressure form the needle. Then the exposed meristem tips that appeared as shiny dome were gently isolates with a sharp blade. After deplugging of culture tubes and a single excised meristem tips (0.3 mm) were very carefully placed on the 'M' shaped filter paper bridge of the culture tubes containing liquid MS medium supplemented with different plant growth regulators for the primary establishment of meristems. The neck of the tubes were flamed with sprit lamp and then plugged. In each treatment 10 explants were inoculated. After inoculation, the culture tubes were labeled by glass marker pen, than the culture tubes were ready for incubation.

Meristem culture incubation: The inoculated culture tubes were incubated in a growth chamber providing a special culture environment. The tubes were placed on the shelves of a culture rack in the growth chamber. It may be mentioned specially that, all cultures were grown in the growth chamber illuminated by 40 watts white fluorescent tubes fitted at a distance of 40-30 cm from the culture shelves. The cultures were maintained at 22 ± 1°C with light intensity varied from 2000-3000 lux. The photoperiod was maintained generally 16 h light and 8 h dark. The culture tubes were checked daily to note the morphogenic responses of cultured meristem in different experiments conducted in the present experiment.

Subculture of primary established meristems: After 4 weeks of inoculation of meristems, the primary established meristems those showed morphogenic responses were removed aseptically from the culture tubes transferred into test tubes containing agar gelled MS medium supplemented with different plant growth regulators. During inoculation special care was taken that the explants must touch the medium equally and do not dip into the medium. After 30-35 days of culture initiation the primary established meristem formed shoot and root and well established to medium.

Virus indexing through ELISA test: The uses of various serological methods for rapid detection of plant viruses *in vitro* grown meristem derived plantlets are quite common

now a day in clinical plant virology. It is popularly known as serodiagnosis (Akanda *et al.,* 1991a, 1991b; Purciful and Hiebert, 1979; Van Regenmortel, 1978). Among the various serological methods so far developed, the double antibody sandwich enzyme linked immuno-sorbent assay (DAS-ELISA) is extensively applied for its various merits like high sensitivity, rapidness and reliability over the order methods as reported by Clark and Bar-Joseph, 1984. Different samples representing each symptom were tested by DAS-ELISA.

The ELISA test was performed by following steps:

1. Each of the polystyrene microtitre plates were coated with Y-globulin 2 µl/ml, 200 µl/well in 0.05 M carbonate buffer, pH 9.6 containing 0.02% sodium azide and incubated at 37°C for 3 hours in an incubator.

2. After then the microtitre plates were kept out of the incubator and washed 3 times with washing buffer. Washing buffer was made of phosphate buffer saline (PBS), pH 7.1 with few drops of T-20.

3. Sample plantlets (*in vitro* grown meristem derived plantlets) were taken in a polythene bag and were macerated well with the help of PBS and T-20 in 1:3 ratios. Then the mixture solution were kept on every grove of the polystyrene microtitre plates and kept in 4°C temperature for over night.

4. After 24 hours the microtitre plates were kept out of the refrigerator and the plates were washed for 3 times with PBS-T-20. Then every groves of the plate were block with the 1% carnation non-fat milk in 200 µl/well and kept in room temperature for 1 hour. It was again washed for 3 times with PBS-T-20.

5. The enzyme conjugated γ-globulin solution (200 µl/well) was added in the plate and the plate was then incubated at 37°C temperature for 4 hours. Then the plate was again washed for 3 times PBS-T-20.

6. An amount of 200 µl of substrate (P-nitro phenyl phosphate) 1 mg/l ml in 10% diethanolamine buffer containing 0.02% sodium azide (pH 9.8) per well was applied following incubation for 3 hours at 30°C. After 10-30 minutes reaction was held in microtitre plates and also in eppendrop tubes and positive reaction was visualized by naked eyes observing yellow colour development that proved the presence of specified

virus and the white or no colour proved the negative result that proved no virus in the sample plants.

Micropropagation of virus free plantlets: Well established healthy meristem derived plantlets length 4-5 cm was used as explants for micropropagation. Before mass micropropagation, all necessary materials were taken into laminar air flow cabinet. Then, the meristem derived plantlets were removed carefully from the culture tubes over a sterile paper tile using a pair of forceps. On the paper tile, the basal nodes from the stem were carefully removed and cut off single nodal segments. Each single nodal segment was bearing an undamaged auxiliary bud and generally it was 5-6 mm long. Basal nodes were not used as explants. Isolated shoot tips and nodal segments were transferred to 50 × 110 mm flasks containing agar solidified MS basal medium. The node cutting was placed in such away that it was in good contact with the surface of the medium but it was not pushed below the surface. Single node cuttings incubated at 22 ± 1°C temperatures with 16 h photoperiod/day. These plantlets were sub cultured as required at every 3 weeks.

Effect of different nodal positions of the explants on *in vitro* growth: In experiment, 5-6 cm long plantlets with at least four nodes were selected from six potato cultivars and they were very cautiously divided to shoot tip and single nodes. Four single node cuttings were taken from 1 through 4 of each stem from the top, and they were considered as treatment in this experiment. Node cuttings consisted of one leaf with its subtended bud and a minimal amount of stem required attaching the bud to the leaf. The leaf of 1st node (top node) cutting was very young. The 2nd and 3rd node (middle node) cuttings had fully expanded and matured leaf. The leaf of 4th node (basal node) cutting was old or very old. For cultivar Asterix, 3rd and 4th node cuttings (middle node) had fully expanded and matured leaf. Shoot tips consisted of very young and immature leaf. The shoot tips and single node cuttings were cultured in test tubes (150 × 25 mm) containing agar solidified MS basal medium and incubated under standard culture conditions. Data were collected on shoot and root induction after 21 days of inoculation.

Effect of different modified MS media formulations on *in vitro* growth: In this experiment, single nodal cuttings were sub cultured onto different modified MS media formulations viz. M_1 (Thiamine HCl free MS medium), M_2 (Nicotinic acid free MS medium), M_3 (Pyridoxine HCl free MS medium), M_4 (Myoinositol free MS medium) and

M_5 (Glycine free MS medium) media in search of *in vitro* growth response of potato. MS0 medium was used as a control medium and test tubes (25 × 150) were used as culture vessel. Basal nodes were not used as explants. The cultures were incubated under standard culture conditions. The experiment was repeated three times. In each treatment, at least 10 single nodal segments were cultured and data were collected on shoot and root induction after 21 days of inoculation.

Production of microtubers from the *in vitro* plantlets: For microtuberizaton, single nodal cuttings were sub cultured onto agar solidified MS medium supplemented with sucrose (4, 6, 8, 10 and 12%) either alone or in combination with BA (6-benzyl adenine) and kinetin (6-furfurylaminopurine) at varying concentrations. Single nodal cuttings were also cultured onto cotton free and cotton based MS liquid medium. Instead of agar, cotton was added to liquid medium before autoclaving. The cultures were incubated under standard culture conditions. The microtubers were carefully harvested in plastic trays and washed under running water to remove adhering constituents of the medium. The harvested microtubers treated with fungicide (mancozeb 78% WP at 0.2%) for 10 minutes and allow them to dry in the dark at 20ºC for 2 days. The dried microtubers were packed in perforated polyethylene bags and stored at 5ºC in a refrigerator for 4-5 months under dark for breaking the dormancy.

2.2.2.3. Data recording for in vitro culture and micropropagation

Data were collected on the following parameters and the methods of are given below:

i) Percentage of explants induced to develop shoot: Percentages of explants induced to develop adventitious shoots were calculated using following formula:

$$\%\ \text{of meristem explants showing growth rejuvenation} = \frac{\text{Number of explants form shoot}}{\text{Total number of cultured explants}} \times 100$$

ii) Shoot length: Shoot length was measured in cm after 21 days of culture.

iii) Nodes/shoot: Number of usable nodes/shoot was calculated after 21 days of culture.

iv) Roots/plantlet: Number of roots/plantlet was calculated after 21 days of culture.

v) Microtubers/shoot: The induced microtubers were counted after 70 days of culture.

vi) Average weight of microtubers: The induced microtubers were harvested aseptically after 70 days of culture and weighed in an electric balance.

2.2.2.4. Selection of experimental field

Research field of Akafuji Agro Technologies, Namo bhadra, Rajshahi was selected for the experimental field. The soil of the experimental field was sandy loam or loam and pH of the soil was 7.0.

2.2.2.5. Preparation of experimental field

The first condition of potato cultivation is the land should be plain enough. The land was ploughed well for four to five times by a power tiller or cow plough and debris were removed completely. During ploughing, the soil was pulverized with cow dung, manure, chemical fertilizer and sand mixed with soil according to need. Before transplantation, the bed was prepared after the field well pulverized. The bed was height 10-15 cm from the ground level.

2.2.2.6. Design of experimental field

The experiment was conducted with *in vitro* grown 6 potato cultivars. In the layout of the experimental field randomized block design was followed. The field was comprised an area of 610 × 590 cm having three replications. Each replication consisted of 6 beds. Each bed consisted of 2 rows and each row consisted of 10 plants. Row to row distance was 36 cm and plant to plant distance was 15 cm. The length of each bed was 150 cm long and breadth was 60 cm. The distance between two beds was 30 cm and boundary of the field was 40 cm.

2.2.2.7. Acclimatization of in vitro grown plantlets

When the micro propagated plantlets were 21 days old and 4-5 cm in height with good root system, they were ready for transplanting into seed beds. The plantlets grown inside the flask were brought out from the growth chamber. After deplaguing, the culture vessels were kept in the room temperature for 2-3 days to bring them in contact with normal temperature for acclimatization. During this period the physiological system of plant body will developed and the plantlets were more fresh and strong and ready for transplantation. After hardening the plantlets were taken out very carefully from the

culture vessels. Agar attached to the root was gently and carefully washed out with water. The plantlets were dipped in 0.1% Bavistin solution, a fungicide, (BASF Aktiengesellschaft, Germany) for 10-15 minutes to kill any microbes attached to the roots and transferred to Thump pots filled with vermicompost. Initially, the pots were placed under shady place and covered with polythene sheet to maintain high humidity around the juvenile plants. Finally they were transplanted in the field.

2.2.2.8. Transplantation of plantlets to field

The potted plants were transplanted on the raised seed beds keeping 15.24 cm space between two plants. The distance between two rows was 36 cm. Care was taken to avoid damage to the roots and to ensure good contact between roots and soil. The plants were kept in an environment with high relative humidity for the first few days following transplantation by covering the beds with polyethylene sheet and regular spraying of water in order to prevent viral vectors, the entire field was covered with a nylon nets. Interim care was taken by periodic irrigation, weeding, mulching and spraying insecticide. After plantation of *in vitro* grown plantlets, potatoes were collected from the field within 90-100 days. When haulms of potato plants started yellowing and falling on the ground, it was proper time for potato harvesting. Potato plants were haulm pulling before 8-10 days of potato harvesting. The data were recorded on different agronomical characteristics. The harvested minitubers were cold stored and used for growing seed crop in next season.

2.2.2.9. Planting of microtubers to field

After 4-5 months of storage, the microtubers were taken out for sprouting and field planting. The sprouted microtubers were directly planted by hand in the above mentioned seed beds. In each bed, it was parallel planted in the same distance with plantlets. The harvested minitubers were preserved in the cold storage for yield performance study.

2.2.2.10. Field performances of minitubers (G_1 seed), breeder (G_2 seed) foundation (G_3 seed) and source seed (SS) potato tubers

The minitubers (MNT), breeder seed (BS) and foundation seed (FS) potato originated from the plantlets (PL) and the microtubers (MT) derived crop were tested in a field trial. In the field trial, fertilizers were applied at 350-250-270-120-120-10.6-6.5 kg/ha of urea,

TSP, MP, gypsum, magnesium sulphate, zinc sulphate and boric acid/borax, respectively. Half of urea and full dose of all other fertilizers were applied at the time of planting. Other half of urea was applied as side dressing after 32 days of planting when first earthing up was done. During land preparation, cow dung was applied at 11 t/ha. In every crop season, seed tubers were planted on November 20 and seed tubers were preserved in the cold storage. Whole tubers were planted maintaining 55 cm row to row and 15 cm seed to seed distances. The experiment was laid out following randomized complete block design (RCBD) with four replications. The unit plot size was 5m × 5m. Intercultural operations, such as irrigation, weeding, mulching, and earthing up were done as and when necessary. Fungicide, Bavistin DF was applied at 0.2% to protect the crop from fungal diseases. Before haulm pulling, virus infected plants were identified studying symptoms of the diseases and confined using indicator plants (Hill, 1984). Ninety days after planting, the crop was harvested. Data on the incidence of viral diseases, tuber number and tuber yield/hill were recorded. Subsequently, the minituber, breeder seed and foundation seed tuber originated from the plantlets (PL) were designated as PL-MNT, PL-BS and PL-FS respectively. On the other hand the minituber, breeder seed and foundation seed tuber originated from the microtuber (MT) propagated crop were designated as MT-MNT, MT-BS and MT-FS respectively.

2.2.2.11. Data recording for field crop

10 plants were randomly selected from each replication/cultivar for collection of data on the following parameters:

a) Plant height; b) Number of stems/hill; c) Number of leaves/hill; d) 3rd leaf area/plant; e) Canopy coverage/hill; f) Fresh weigh of plants/hill; g) Dry weight of plants/hill; h) Number of tubers/hill and i) Tuber weight/hill. The data were collected after 65 days of planting in the field in respect of parameters for a - e number. For the rest of parameters, data were collected at harvest period.

2.2.2.12. Estimation of chlorophyll content

Chlorophyll content of the flag leaves was estimated according to the procedure reported by Mahadevan and Sridhar, 1982. Three discs each of 0.502 cm^2 area were taken from the different position of the flag leaves and taken their weight. The leaf discs were cut

using the lid of a 1.5 ml microfuge tube. Chlorophyll was extracted with 80% aqueous acetone using a 1.5 ml microfuge tubes and fine glass rod to grind the tissues. Then the suspension was centrifuged for 3 minutes. After centrifugation, the upper clean green solution was decanted into the volumetric vessel from the colorless residue and then made up to 10 ml with 80% acetone. The optical density (O.D) of this solution was determined against 80% acetone as blank using a Spectrophotometer at wavelengths of 645 nm (for chlorophyll-a) and 663 nm (for chlorophyll-b).The chlorophyll contents were calculated on fresh weight basis employing the following formula as described (Mahadevan and Sridhar, 1982) using the specific absorption coefficients for chlorophyll-a and chlorophyll-b at 645 and 663 nm in 80% acetone respectively.

$$\text{Total chlorophyll (mg/g)} = \frac{20.2A645 + 8.02A663}{1 \times 1000 \times w} \times v$$

Where, A_{645} and A_{663} are optical density at wavelengths of 645 and 663 nm respectively and v = volume of the solution e.g. 10 ml, W = weight of 3 leaf discs.

2.2.2.13. Techniques of data analysis

The collected data were analyzed using biometrical technique developed by Mather (1949) based on the mathematical models of Fisher *et al.* (1932) and those of Hyman (1958), Kempthorne (1957) and Allard (1960). The techniques used are described under the following heads.

Mean: Data on individual observation were added together and divided by the total number of observations and the mean was obtained as follow:

$$\text{Mean, } \bar{X} = \frac{\sum x_i}{N}$$

Where, $\bar{X}$ = Average number of observations

Σ = Summation

x_i = Individual reading recorded on each observation

N = Number of observations.

Standard error of mean (SE): Instead of taking one sample, several samples are taken to estimate the standard deviations of the deferent samples. The sample variations were measured by standard error (or standard error of mean) which was determined as follows:

$$SE = \frac{S}{\sqrt{N}}$$

$$\text{Where, } S = \sqrt{\frac{\sum X_1^2 - \frac{(\sum X_1)^2}{N}}{N-1}}$$

X_1 = Total number of observations

Σ = Summation

S = Standard deviation.

Test of least significant difference (LSD): To test the least significant difference between two means the following formula was used.

$$LSD = \sqrt{\frac{2 \times MSe}{n}} \times t \text{ at 5\% level of significant}$$

Here, MSe = Mean square of error

n = Number of observations

t = Value at 5% significant level from‘t’ table.

2.3. RESULTS

The present investigation was carried out for the production of high quality seed potato through meristem culture and subsequent micropropagation of six potato cultivars. Details of the results so far obtained from each of the experiments are described under the different heads.

2.3.1. Establishment of Primary Meristem Culture

The meristems isolated from shoot tips of 25-35 days old field grown plants of six different cultivars of potato were cultured onto filter paper bridges placed in culture tubes containing MS liquid medium supplemented with GA_3 alone or combination with Kin. Isolated meristems were also cultured onto MS0 medium as a control. The cultures were incubated in a plant growth chamber under 16 h/8 h light/dark cycle at 22 ± 1ºC. Data were recorded on percentage of explants resumed new growth after 30 days of culture incubation and length/meristem derived shoots after 45 days of culture incubation. The results of these experiments are discussed below.

2.3.1.1. Effect of GA_3 on the establishment of primary meristem culture

The isolated shoot apical meristems of six different cultivars were cultured onto filter paper bridges in liquid MS medium supplemented with 0.1, 0.25 and 0.5 mg/l filter sterilized GA_3. The results about the effect of different concentrations of GA_3 on morphogenic response of the cultured meristems and length of meristem derived shoots are summarized in **Table 2.1.** In general all cultured meristems did not resume new morphogenic growth. Resumption of new growth of the excised cultured meristems was influenced both by the GA_3 formulations and the genotypes of the potato cultivars. The cultured meristems commenced their new growth by increasing their size. The meristems those resumed new growth were continued their development along the central axis and eventually differentiated leaf and shoot (phytomer) **(Plate 2.1, Figure A-F)**. Among the three different GA_3 formulations 0.5 mg/l was found to be the most effective in resuming new growth and development of the explanted meristems. The highest length of meristem derived shoots was also noticed in this medium formulation. Root formation of the cultured tissue was not noticed in any of the treatments. Among the six potato cultivars, explanted meristems of cultivar Diamant was the most responsive to morphogenic growth and exhibited the highest shoot length when it was cultured onto media having 0.5 mg/l GA_3 formulation.

Table 2.1. Effect of different concentrations of GA_3 in MS liquid medium on the establishment of primary meristem culture. Ten isolated meristems were incubated for each treatment.

Name of cultivars	GA_3 (mg/l)	% explants resumed new growth	Shoot length (cm) of meristem derived plantlets (after 45 days of culture)
All Blue	0.1	20	2.34 ± 0.34
	0.25	30	2.88 ± 0.23
	0.5	40	3.14 ± 0.43
	control	20	1.92 ± 0.34
All Red	0.1	20	2.33 ± 0.22
	0.25	40	2.68 ± 0.44
	0.5	40	3.44 ± 0.35
	control	10	2.22 ± 0.36
Asterix	0.1	30	2.51 ± 0.54
	0.25	50	3.11 ± 0.33
	0.5	60	3.42 ± 0.21
	control	20	2.46 ± 0.32
Diamant	0.1	30	2.88 ± 0.34
	0.25	40	3.26 ± 0.22
	0.5	70	3.73 ± 0.18
	control	20	2.56 ± 0.22
JPR	0.1	20	1.76 ± 0.34
	0.25	20	2.34 ± 0.20
	0.5	30	2.92 ± 0.41
	control	10	1.58 ± 0.33
Indurkani	0.1	10	2.13 ± 0.41
	0.25	20	3.12 ± 0.32
	0.5	30	2.96 ± 0.29
	control	-	2.05 ± 0.35

2.3.1.2. Effect of Kin with GA_3 on the establishment of primary meristem culture

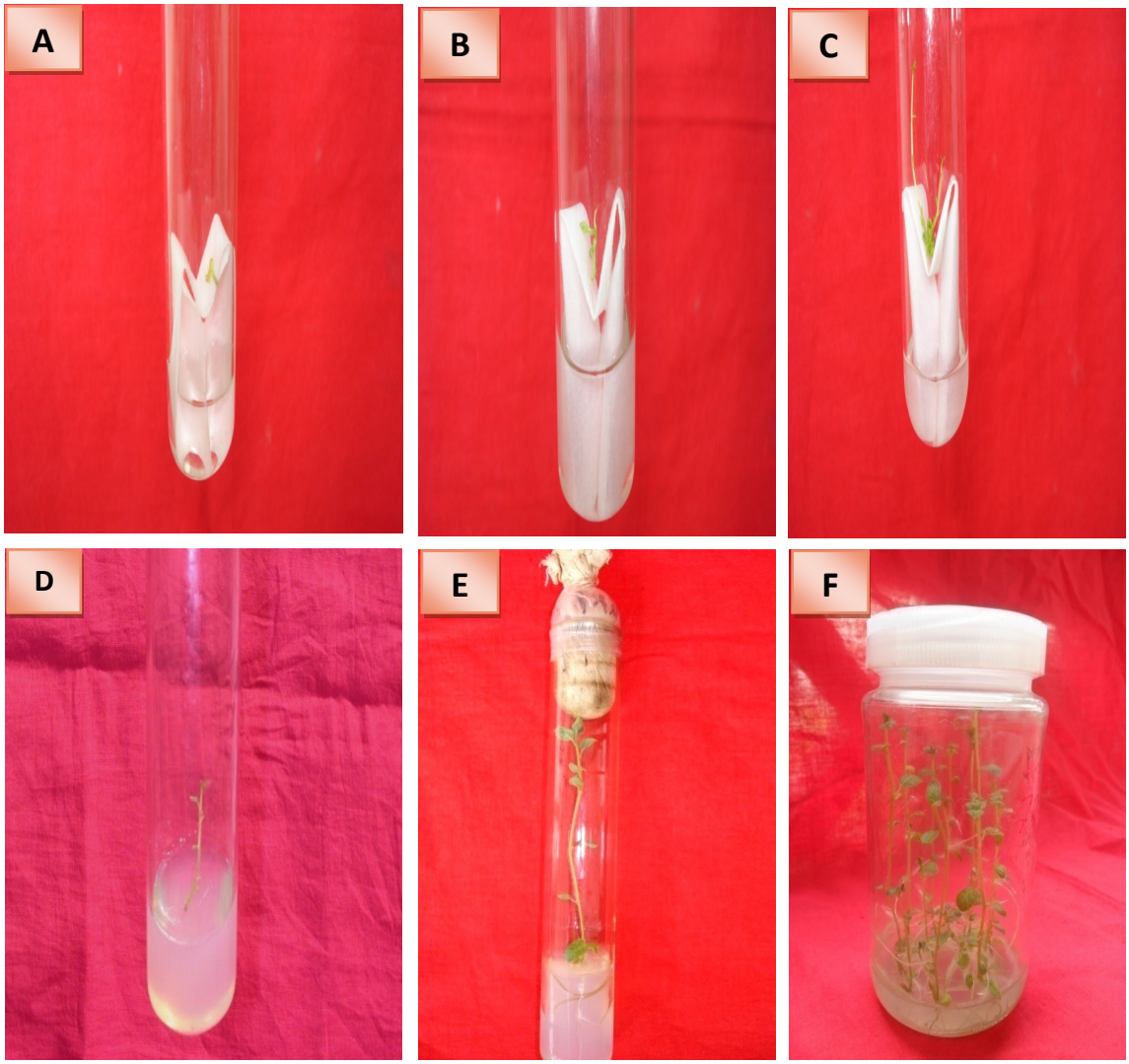
The isolated shoot apical meristems of six potato cultivars were cultured onto filter paper bridges in liquid MS medium supplemented with three concentrations (0.1, 0.2 and 0.4 mg/l) of Kin combination with 0.5 mg/l GA_3. The results about the effect of three concentrations of Kin combination with 0.5 mg/l GA_3 on morphogenic response of the cultured meristems and length of the meristem derived shoots are presented in **Table 2.2.**

Resumption of new growth of the excised cultured meristem was influenced both by the Kin + GA_3 formulations and by the genotypes of the potato cultivars. The cultured apical meristems commenced their new growth by increasing their size and formed phytomers **(Plate 2.1, Figure A-B)**. Every phytomer consisted of a node to which a leaf is attached, a subtending internode, and an axillary bud at the base of the leaf. Among the three concentrations of Kin combination with GA_3, 0.4 mg/l Kin + 0.5 mg/l GA_3 was found to be the most effective in resuming new growth and development of the explanted meristems. The highest length of meristem derived shoots was also noticed in this media formulation. Root formation of the cultured tissue was not observed in any of the treatments. Among the six potato cultivars, explanted meristems of cultivar Asterix was the most responsive to morphogenic growth and exhibited the highest shoot length when it was cultured onto media having 0.4 mg/l Kin + 0.5 mg/l GA_3 formulation. The meristems of cultivars Diamant, All Blue and All Red also performed well in this media formulation.

Table 2.2. Effect of different concentrations and combinations of Kin with GA_3 in MS liquid medium on the establishment of primary meristem culture. Ten isolated meristems were incubated for each treatment.

Cultivars	Kin + GA_3 (mg/l)	% explants resumed new growth	Shoot length (cm) of meristem derived plantlets (after 45 days of culture)
All Blue	0.1+0.5	40	3.22 ± 0.45
	0.2+0.5	50	3.41 ± 0.26
	0.4+0.5	60	3.74 ± 0.43
All Red	0.1+0.5	40	3.43 ± 0.33
	0.2+0.5	60	3.56 ± 0.26
	0.4+0.5	70	3.72 ± 0.37
Asterix	0.1+0.5	60	3.52 ± 0.31
	0.2+0.5	70	3.85 ± 0.39
	0.4+0.5	80	4.26 ± 0.18
Diamant	0.1+0.5	60	3.68 ± 0.42
	0.2+0.5	70	3.73 ± 0.27
	0.4+0.5	70	4.11 ± 0.33
JPR	0.1+0.5	30	2.78 ± 0.41
	0.2+0.5	40	3.12 ± 0.25
	0.4+0.5	60	3.33 ± 0.26
Indurkani	0.1+0.5	30	2.98 ± 0.21
	0.2+0.5	40	3.08 ± 0.11
	0.4+0.5	50	3.66 ± 0.19

PLATE 2.1: Different stages of meristem culture and subsequent shoot multiplication

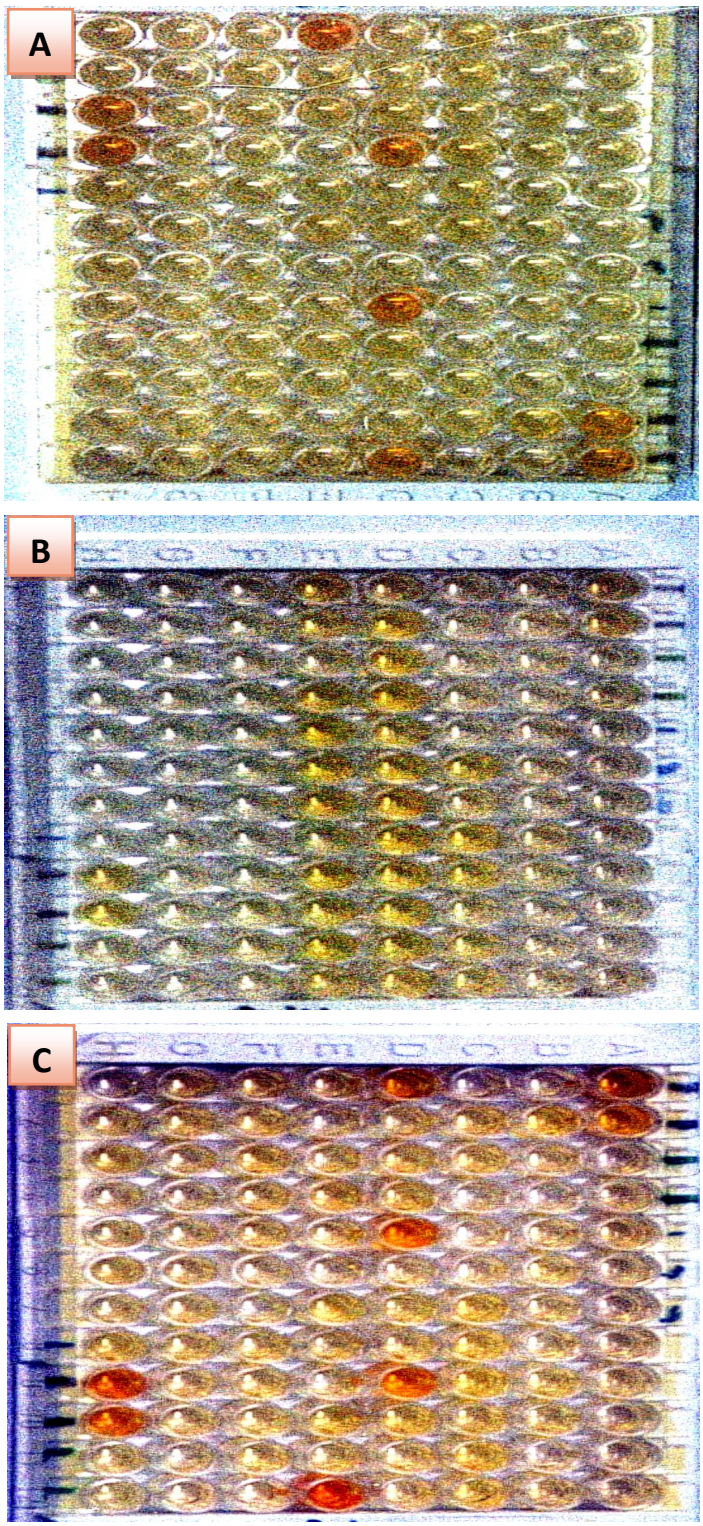


Figures: A: An isolated meristem cultured on filter paper bridge in liquid MS medium containing 0.4 mg/l Kin + 0.5 mg/l GA_3, 7 days after culture; **B:** A shoot developed from explanted meristem, 15 days after inoculation; **C:** 28 days after inoculation; **D:** Meristem grown shoot onto agar gelled MS medium, 7 days after subculture; **E:** A plantlet derived from cultured meristem, 25 days after subculture and **F:** Micropropagation of meristem grown plantlets through node cultures, 21 days after subculture onto agar gelled MS0 medium.

2.3.2. Virus Indexing of Meristem Derived Plantlets through ELISA Test

Among the meristem derived plantlets, a serological DAS-ELISA test was carried out to detect important viral diseases viz. PLRV, PVX and PVY before micropropagation. No positive colour was found in the microtitre plates when the *in vitro* grown meristem derived plantlets were used as samples (**Plate 2.2).** On the other hand colour appeared when field grown plants were used as samples that proved *in vitro* grown meristem derived plantlets were free from the viruses PVY, PVX and PLRV.

PLATE 2.2: ELISA test for the detection of viral diseases among the meristem grown plantlets



Figures: A: Microtitre plate of PLRV detection; **B:** Microtitre plate of PVX detection and **C:** Microtitre plate of PVY detection.

2.3.3. Micropropagation of Virus Free Plantlets

Micropropagation (or *in vitro* shoot multiplication) was established from the nodal cuttings of virus free meristem derived plantlets. Micropropagation was started when the plantlets became 4-5 cm in height. Single nodal cuttings were also used as explants for the different experiments viz. *in vitro* growth response of different nodal positions of explants and effect of different modified MS media formulations on *in vitro* growth of potato.

2.3.3.1. Effect of different nodal positions of the explants on micropropagation of potato

In this experiment, the meristem derived plantlets were carefully rescued from the culture vessel and they were divided to shoot tips and 1st, 2nd, 3rd and 4th nodal cuttings (from the top). Then, they were cultured onto semisolid MS basal medium in order to observe there *in vitro* growth responses. Three parameters on shoot and root induction (viz. mean shoot length, number of usable nodes/shoot and number of roots/plantlet) were considered for evaluating the growth and development of *in vitro* plantlet. The results of nodal position of the explants on the micropropagation of six potato cultivars are shown in **Table 2.3.**

Table 2.3 shows that the *in vitro* growth and development of plantlets of six potato cultivars were significantly influenced both by the nodal positions of the explants and by the cultivars. From the mean results of nodal positions and cultivars it is revealed that 3rd and 2nd nodal explants were better for *in vitro* growth and development of six potato cultivars. Among the cultivars, Asterix was found to be superior to other cultivars.

The results given in **Table 2.3** for cultivar All Blue show that the difference in observed length among the shoots develops from shoot tip and 1st, 2nd, 3rd and 4th nodal explants were no significant. The observed difference in number of usable nodes/shoot develops from shoot tip and 2nd and 3rd nodal explants were also non significant. In respect of root number, no significant variations were observed among the plantlets develop from shoot tip and 1st, 2nd, 3rd and 4th nodal explants of the cultivar All Blue.

In All Red, significant variations were observed among the shoots derived from derived nodal explants. The highest shoot length was noticed in 2nd node derived plantlets and the lowest shoot length was found in 4th nodal explants derived plantlets of the cultivar All

Red. The difference in length among the shoots developed from shoot tip and 1st, 2nd and 3rd nodal explants were not significant. For number of usable nodes/shoot, no significant variations were observed. On the other hand higher number of roots/plantlet was found in 2nd nodal explants derived plantlets and the number of roots at par with 3rd node, shoot tip and 1st nodal explants derived plantlets. The 4th nodal explants derived plantlets exhibited the lower number of roots/plantlet than that of 2nd nodal explants derived plantlets.

In Asterix, the 3rd nodal explants derived plantlets exhibited the longer shoot which was at par with 2nd and 4th nodal explants derived plantlets. The lowest shoot length was recorded from shoot tip explants derived plantlets. The observed difference in length between the shoots develop from 1st and 2nd nodal explants was not significant. On the other hand higher number of usable nodes/shoot was found in shoot develop from 3rd nodal explants which was followed by 4th and 2nd nodal and shoot tip explants. The lowest number of usable nodes was found in 1st nodal explants derived shoot. In respect of root numbers/plantlet, no significant variation was observed in cultivar Asterix.

In Diamant, the longest shoot was produced from 2nd nodal explants and the shortest shoot was produced from 4th nodal explants which were significantly different from each other. The difference in length among the shoots develops from shoot tip and 1st, 2nd and 3rd nodal explants were not significant. For number of usable nodes/shoot and number of roots/plantlet, no significant variations were observed among the plantlets derived from shoot tip and 1st, 2nd, 3rd and 4th nodal explants in cultivar Diamant.

In JPR, the observed difference in both characters of shoot growth among the plantlets develops from shoot tip and 1st, 2nd, 3rd and 4th nodal explants were not significant. On the other hand significantly higher number of roots/plantlet was recorded from shoot tip derived plantlets than that of 4th node derived plantlets. The variation in root number of plantlets develops from 1st, 2nd and 3rd nodal explants were not significant.

For Indurkani, significantly higher shoot length was noticed in 3rd nodal explants derived plantlets than that of shoot tip explants derived plantlets. The difference in length among the shoots develops from 1st, 2nd, 3rd and 4th nodal explants were not significant. On the other hand the observed variations in number of usable nodes/shoot were not significant in Indurkani. The number of roots/plantlet produced from shoot tip explants was higher

and lower was in 1st nodal explants derived plantlets. They were significantly different. The variation in root number of plantlets develops from shoot tip and 2nd, 3rd and 4th nodal explants were not significant.

In conclusion, it is need to be mentioned here that the length of internode in plantlets develop from shoot tip explants was lower than that of 2nd and 3rd nodal explants derived plantlets. As a result the length of plantlets develop from shoot tip explants was lower than that of 2nd and 3rd nodal explants derived plantlets although the observed difference in number of usable nodes in the plantlets develops from shoot tip and 2nd and 3rd nodal explants were not significant.

Table 2.3. Effect of different nodal positions of the explants on micropropagation of potato. Data were collected 21 days after culture.

Characters	Cultivars	Nodal positions of the explants					
		Shoot tip	1st node	2nd node	3rd node	4th node	**Mean**
Shoot length (cm)	All Blue	2.93^{A}	2.53^{A}	4.00^{A}	3.26^{A}	2.46^{A}	3.04^{cd}
	All Red	3.13^{AB}	3.26^{AB}	4.80^{A}	4.33^{AB}	2.73^{B}	3.65^{bc}
	Asterix	4.40^{C}	6.00^{BC}	6.40^{AB}	7.80^{A}	7.76^{A}	6.47^{a}
	Diamant	4.80^{AB}	3.66^{AB}	5.20^{A}	4.73^{AB}	3.20^{B}	4.32^{b}
	JPR	2.73^{A}	2.40^{A}	3.20^{A}	3.60^{A}	2.33^{A}	2.85^{d}
	Indurkani	3.13^{B}	3.66^{AB}	4.20^{AB}	4.93^{A}	4.40^{AB}	4.06^{b}
	Mean	3.52^{b}	3.58^{b}	4.63^{a}	4.77^{a}	3.81^{b}	-
	LSD value (at 5%): 0.68 for cultivars (C); 0.75 for nodal positions (NP) and 1.68 for C× NP						
Number of usable nodes/ shoot	All Blue	3.73^{A}	2.33^{B}	3.51^{AB}	3.13^{AB}	2.14^{B}	2.96^{bc}
	All Red	3.46^{A}	2.53^{A}	3.26^{A}	3.00^{A}	2.20^{A}	2.89^{bc}
	Asterix	2.80^{AB}	2.60^{B}	3.53^{AB}	4.12^{A}	4.00^{A}	3.41^{ab}
	Diamant	3.10^{A}	2.11^{A}	3.06^{A}	3.00^{A}	2.33^{A}	2.72^{cd}
	JPR	2.66^{A}	1.60^{A}	2.26^{A}	2.60^{A}	2.06^{A}	2.24^{d}
	Indurkani	4.13^{A}	3.13^{A}	3.73^{A}	3.80^{A}	3.53^{A}	3.66^{a}
	Mean	3.31^{a}	2.38^{b}	3.22^{a}	3.27^{a}	2.71^{ab}	-
	LSD value (at 5%): 0.57 for cultivars (C); 0.62 for nodal positions (NP) and 1.39 for C × NP						
Number of roots/ plantlet	All Blue	3.65^{A}	3.00^{A}	3.73^{A}	3.82^{A}	3.53^{A}	3.54^{b}
	All Red	4.13^{AB}	4.00^{AB}	4.86^{A}	4.27^{AB}	3.33^{B}	4.11^{a}
	Asterix	4.06^{A}	3.53^{A}	3.00^{A}	3.98^{A}	4.06^{A}	3.72^{ab}
	Diamant	4.66^{A}	4.00^{A}	4.66^{A}	4.13^{A}	3.33^{A}	4.16^{a}
	JPR	3.52^{A}	2.53^{AB}	3.00^{AB}	3.18^{AB}	2.13^{B}	2.87^{c}
	Indurkani	3.00^{A}	1.46^{B}	2.53^{AB}	2.80^{AB}	2.53^{AB}	2.46^{c}
	Mean	3.82^{a}	3.08^{c}	3.63^{abc}	3.69^{ab}	3.15^{bc}	-
	LSD value (at 5%): 0.54 for cultivars (C); 0.59 for nodal positions (NP) and 1.34 for C × NP						

The same letters indicate no significant difference at LSD = 0.05. Small letters used for cultivars (C) or nodal positions (NP) and capital letters used for C × NP.

2.3.3.2. Effect of modified MS media formulations on micropropagation of potato

The experiment was conducted to standardization of suitable media compositions for production of low cost high quality plantlets. In this experiment, the individual nodal cutting was sub cultured onto different modified MS media formulations viz. M_1 (Thiamine HCl free MS medium), M_2 (Nicotinic acid free MS medium), M_3 (Pyridoxine HCl free MS medium), M_4 (Myo-inositol free MS medium), M_5 (Glycine free MS medium) and MS with full supplement of all organic components was used control medium (MS0) in search of *in vitro* growth response of potato plantlets. Shoot length, number of usable nodes/shoot and number of roots/plantlet were considered for evaluating the *in vitro* growth responses. The results of modified MS media formulations on the micropropagation of six potato cultivars are shown in **Table 2.4.**

Table 2.4 shows that the *in vitro* growth and development of plantlets of six potato cultivars were significantly influenced both by the media and by the cultivars. From the mean results it is revealed that M_2 and M_3 media had no significant effect on *in vitro* growth and development of six potato cultivars. Among the cultivars, Asterix was found to be superior to other cultivars followed by Diamant.

The results given in **Table 2.4** for cultivar All Blue show that the observed difference in shoot growth on MS0, M_1, M_2, M_3 and M_4 media was not significant. So the shoot growth in All Blue was not significantly affected by M_1, M_2, M_3 and M_4 media formulations. Shoot length was significantly affected by M_5 medium. On the other hand root growth was not affected by M_2, M_3 and M_4 media.

In All Red the observed variations in shoot growth on MS0, M_1, M_2, and M_3 media was not significant. So the shoot growth was unaffected by M_1, M_2 and M_3 media formulations but affected by M_4 and M_5 media. The number of roots/plantlet was unaffected by all tested media formulations.

In respect of Asterix M_2, M_3 and M_4 media had no significant effect on shoot growth of potato which was affected by M_1 and M_5 media. Because the similar shoot growth was noticed in MS0, M_2, M_3 and M_4 media. The number of roots/plantlet was unaffected by M_1, M_3 and M_4 media.

In Diamant, the observed difference in shoot length and usable node number on MS0, M_1, M_2, M_3 and M_5 media was not significant. So the M_1, M_2, M_3 and M_5 media had no significant effect on *in vitro* shoot growth of potato which was significantly affected by M_4 medium. On the other hand root growth was not affected by M_3 and M_5 media.

In JPR, shoot length was not significantly affected by M_1, M_2, M_3 and M_5 media formulations but affected by M_4 medium. Because the variations in shoot length on MS0, M_1, M_2, M_3 and M_5 media was not significant. The number of usable nodes/shoot was unaffected by M_1, M_2 and M_5 media formulations and the number of roots/plantlet was unaffected by M_5 medium.

For Indurkani the observed difference in shoot growth on MS0, M_1, M_2, M_3 and M_4 media was non significant. Therefore, M_1, M_2, M_3 and M_4 media had no significant effect on *in vitro* shoot growth of potato but the shoot length was significantly affected by M_5 medium. On the other hand root growth was not affected by M_1, M_3, M_4 and M_5 media formulations.

Table 2.4. Effect of modified MS media formulations on micropropagation of six potato cultivars. Data were collected 21 days after culture.

Characters	Cultivars	Modified MS media formulations						
		MS0	M_1	M_2	M_3	M_4	M_5	**Mean**
Shoot length (cm)	All Blue	3.35^{A}	2.80^{AB}	3.86^{A}	3.02^{AB}	3.14^{AB}	2.23^{B}	3.06^{abcd}
	All Red	3.75^{A}	3.12^{AB}	2.92^{AB}	3.62^{A}	2.33^{B}	2.22^{B}	2.99^{bcd}
	Asterix	4.23^{A}	2.73^{BC}	3.60^{AB}	4.00^{A}	4.18^{A}	2.00^{C}	3.45^{a}
	Diamant	3.98^{A}	3.56^{A}	3.30^{AB}	3.39^{A}	2.25^{B}	3.15^{AB}	3.29^{abc}
	JPR	2.92^{A}	3.32^{A}	2.50^{AB}	2.25^{AB}	1.73^{B}	3.25^{A}	2.65^{d}
	Indurkani	3.16^{A}	2.85^{AB}	2.88^{AB}	3.61^{A}	2.85^{AB}	2.02^{B}	2.89^{cd}
	Mean	3.56^{a}	3.05^{bc}	3.17^{abc}	3.31^{ab}	2.74^{cd}	2.47^{d}	-
	LSD value (at 5%):0.45 for cultivar (C); 0.45 for media (M) and 1.10 for C× M							
Number of usable nodes/ shoot	All Blue	3.40^{A}	3.13^{A}	3.98^{A}	3.93^{A}	3.56^{A}	2.72^{A}	3.45^{ab}
	All Red	3.66^{A}	3.01^{AB}	3.17^{AB}	3.88^{A}	2.12^{B}	2.44^{AB}	3.04^{bc}
	Asterix	4.72^{A}	2.66^{B}	4.67^{A}	4.12^{AB}	4.03^{AB}	2.66^{B}	3.81^{a}
	Diamant	4.12^{A}	4.01^{AB}	4.11^{A}	4.09^{A}	2.50^{B}	3.32^{AB}	3.69^{a}
	JPR	2.77^{AB}	3.66^{A}	2.42^{AB}	2.00^{B}	1.33^{B}	2.52^{A}	2.45^{c}
	Indurkani	3.00^{A}	3.50^{A}	2.12^{A}	3.00^{A}	2.53^{A}	2.50^{A}	2.77^{c}
	Mean	3.61^{a}	3.32^{a}	3.41^{a}	3.50^{a}	2.67^{b}	2.69^{b}	-
	LSD value (at 5%): 0.62 for cultivar (C); 0.62 for media (M) and 1.51 for C × M							
Number of roots/ plantlet	All Blue	2.97^{AB}	1.76^{B}	3.33^{A}	2.37^{AB}	2.14^{AB}	1.83^{B}	2.40^{c}
	All Red	3.36^{A}	2.76^{A}	2.63^{A}	3.11^{A}	2.52^{A}	3.44^{A}	2.97^{b}
	Asterix	5.33^{A}	4.32^{AB}	3.66^{B}	3.96^{AB}	4.33^{AB}	1.98^{C}	3.93^{a}
	Diamant	3.33^{AB}	3.00^{BC}	2.66^{BC}	3.47^{AB}	1.88^{C}	4.45^{A}	3.13^{b}
	JPR	3.00^{B}	2.33^{B}	2.50^{B}	2.62^{B}	2.66^{B}	5.50^{A}	3.10^{b}
	Indurkani	2.74^{AB}	2.04^{AB}	1.50^{B}	2.33^{AB}	2.00^{AB}	3.00^{A}	2.26^{c}
	Mean	3.45^{a}	2.70^{b}	2.71^{b}	2.97^{ab}	2.58^{b}	3.36^{a}	-
	LSD value (at 5%): 0.56 for cultivar (C); 0.56 for media (M) and 1.37 for C × M							

The same letters indicate no significant difference at LSD = 0.05. Small letters used for cultivars (C) or media (M) and capital letters used for C × M.

2.3.4. Microtuber Production

Single nodal cuttings from *in vitro* grown plantlets of six potato cultivars were cultured onto agar solidified MS medium supplemented with different concentrations of sucrose (4, 6, 8, 10 and 12%) and different concentrations of BA or Kin supplemented with 8% sucrose. Single nodal cuttings were also cultured onto cotton based and cotton free MS liquid medium. The results of these experiments are described in different subheads.

2.3.4.1. Effect of different concentrations of sucrose on microtuberization of potato

Effect of different concentrations of sucrose (4, 6, 8, 10 and 12%) in MS medium without any growth regulator on microtuber development of six potato cultivars are presented in **Table 2.5 and Plate 2.3.** From the **Table 2.5** it is revealed that significant variations were observed among the sucrose concentrations and the cultivars in respect of mean number of microtubers/shoot and the mean fresh weight of microtubers. For microtuber formation and growth, 8% sucrose concentration was the most effective formulation followed by 10%, 12% and 6% sucrose concentrations. The microtubers were not formed on MS medium supplemented with 3% sucrose, whereas 4% sucrose had little effect on tuberization. Among the six potato cultivars Diamant was the superior for microtuber formation and growth.

The interactions between the potato cultivars and the sucrose concentrations were significant. The results given in **Table 2.5** show that All Blue produced the significantly higher number of microtubers/shoot in MS medium supplemented with 8% sucrose. Similar trends were observed in Diamant, Asterix and All Red. For JPR, the observed difference in number of microtubers/shoot in tuberization medium containing 8% and 10% sucrose was not significant and it was followed by cultivar Indurkani.

Among the six potato cultivars, Diamant exhibited the significantly highest weight of microtubers in 8% sucrose containing medium. In this medium formulation, All Blue, Asterix, All Red and Indurkani produced the highest fresh weight of microtubers. JPR produced the highest fresh weight of microtubers in 10% sucrose containing medium. For Asterix, All Red, JPR and Indurkani, the observed difference in fresh weight of microtubers in tuberization medium containing 8% and 10% sucrose was not significant.

Table 2.5. Effect of different concentrations of sucrose on number of microtubers/shoot and weight of microtubers.

Characters	Cultivars	Different concentrations of sucrose					
		4%	6%	8%	10%	12%	**Mean**
Number of microtuber /shoot	All Blue	0.43^{D}	0.49^{CD}	0.73^{A}	0.61^{B}	0.56^{BC}	0.56^{a}
	All Red	0.25^{C}	0.41^{B}	0.61^{A}	0.48^{B}	0.39^{B}	0.43^{c}
	Asterix	0.33^{D}	0.49^{BC}	0.66^{A}	0.56^{B}	0.43^{C}	0.49^{b}
	Diamant	0.30^{D}	0.54^{BC}	0.72^{A}	0.57^{B}	0.47^{C}	0.52^{ab}
	JPR	0.20^{C}	0.42^{B}	0.60^{A}	0.57^{A}	0.46^{B}	0.45^{c}
	Indurkani	0.21^{C}	0.35^{B}	0.53^{A}	0.54^{A}	0.49^{A}	0.42^{c}
	Mean	0.28^{d}	0.45^{c}	0.64^{a}	0.56^{b}	0.47^{c}	-
	LSD value (at 5%): 0.040 for cultivars (C); 0.044 for sucrose concentrations (SC) and 0.099 for C× SC						
Fresh weight of microtuber (mg)	All Blue	81.00^{C}	121.89^{B}	138.78^{A}	129.78^{B}	125.40^{B}	119.37^{c}
	All Red	39.77^{B}	46.77^{B}	75.33^{A}	69.00^{A}	67.56^{A}	59.69^{d}
	Asterix	79.77^{D}	133.89^{B}	152.11^{A}	146.44^{A}	114.90^{C}	125.40^{b}
	Diamant	89.77^{D}	139.56^{C}	161.67^{A}	151.44^{B}	149.60^{B}	138.40^{a}
	JPR	33.66^{D}	46.11^{C}	73.33^{A}	79.55^{A}	60.44^{B}	58.62^{d}
	Indurkani	22.00^{D}	33.66^{C}	44.22^{A}	38.88^{AC}	34.11^{C}	34.58^{e}
	Mean	57.66^{e}	86.98^{d}	107.57^{a}	102.52^{b}	92.00^{c}	-
	LSD value (at 5%): 3.42 for cultivars (C); 3.72 for sucrose concentrations (SC) and 8.33 for C× SC						

The same letters indicate no significant difference at LSD = 0.05. Small letters used for cultivars (C) or sucrose concentrations (SC) and capital letters used for C × SC.

2.3.4.2. Effect of different concentrations of BA on microtuberization of potato

Table 2.6 shows that the efficiency of microtuberization was significantly dependent on the concentrations of BA and cultivars. Among the five concentrations of BA 10 mg/l was the most effective formulation for microtuber formation and growth followed by 12.5 mg/l formulation. All Blue was the superior among the six potato cultivars.

For the interactions between the potato cultivars and the different concentrations of BA with 8% sucrose, significant variations were observed. From the Table **2.6** it appears that significantly the highest number of microtubers/shoot was observed in MS medium supplemented with 10 mg/l BA and 8% sucrose in All Blue. Similar trends were observed in Diamant, JPR, All Red and Indurkani. Cultivar Asterix showed the significantly higher number of microtubers/shoot in 12.5 mg/l BA with 8% sucrose containing medium.

Among the studied potato cultivars, Diamant exhibited the highest weight of microtubers in MS medium supplemented with 10 mg/l BA and 8% sucrose. In this medium formulation, All Blue, All Red, Asterix and JPR produced the highest fresh weight of microtubers. Indurkani produced the highest fresh weight of microtubers in 12.5 mg/l BA with 8% sucrose containing medium. The observed difference in fresh weight of microtubers in tuberization medium containing 10 mg/l BA + 8% sucrose and 12.5 mg/l BA + 8% sucrose was not significant in Indurkani. For All Blue, All Red, Asterix, Diamant and JPR, the observed difference in fresh weight of microtubers in tuberization medium containing 10 mg/l BA + 8% sucrose and 12.5 mg/l BA + 8% sucrose was significant.

Table 2.6. Effect of different concentrations of BA with 8% sucrose on number of micro-tubers /shoot and weight of microtubers.

Characters	Cultivars	Different concentrations (mg/l) of BA with 8% sucrose					
		5 mg	7.5 mg	10 mg	12.5 mg	15 mg	**Mean**
Number of microtuber/ shoot	All Blue	0.85^{C}	0.94^{C}	1.42^{A}	1.16^{B}	0.87^{C}	1.05^{a}
	All Red	0.58^{D}	0.80^{C}	1.16^{A}	0.92^{B}	0.78^{C}	0.85^{c}
	Asterix	0.77^{D}	0.88^{C}	1.15^{B}	1.30^{A}	0.80^{CD}	0.98^{b}
	Diamant	0.72^{E}	0.94^{C}	1.31^{A}	1.06^{B}	0.83^{D}	0.97^{b}
	JPR	0.60^{D}	0.81^{C}	1.22^{A}	0.93^{B}	0.80^{C}	0.87^{c}
	Indurkani	0.43^{D}	0.62^{C}	0.97^{A}	0.87^{B}	0.58^{C}	0.70^{d}
	Mean	0.66^{e}	0.83^{c}	1.20^{a}	1.04^{b}	0.781^{d}	-
	LSD value (at 5%): 0.038 for cultivars (C); 0.042 for concentrations of BA and 0.094for C× BA						
Fresh weight of microtuber (mg)	All Blue	154.67^{C}	161.67^{C}	192.11^{A}	175.33^{B}	145.30^{D}	165.8^{b}
	All Red	75.11^{E}	115.67^{D}	164.56^{A}	150.44^{B}	125.20^{C}	126.2^{d}
	Asterix	179.89^{C}	173.33^{C}	233.67^{A}	205.11^{B}	164.30^{D}	191.3^{a}
	Diamant	165.78^{D}	177.89^{C}	242.33^{A}	194.89^{B}	166.00^{D}	189.4^{a}
	JPR	84.55^{E}	105.00^{D}	179.00^{A}	167.22^{B}	145.30^{C}	136.2^{c}
	Indurkani	53.22^{C}	72.44^{B}	79.44^{AB}	84.11^{A}	51.00^{C}	68.04^{e}
	Mean	118.87^{d}	134.33^{c}	181.85^{a}	162.85^{b}	132.90^{c}	-
	LSD value (at 5%): 3.40 for cultivars (C); 3.70 for concentrations of BA and 8.29 for C× BA						

The same letters indicate no significant difference at LSD = 0.05. Small letters used for cultivars (C) or concentrations of BA (BA) and capital letters used for C × BA.

2.3.4.3. Effect of different concentrations of Kin on microtuberization of potato

Effect of different concentrations of kinetin ranging from 0.5, 1.5, 2.5, 3.5 and 4.5 mg/l with 8 % sucrose were also studied for microtuberization. From the **Table 2.7** it is revealed that 3.5 mg/l of kinetin with 8 % sucrose gave best response for microtuber induction which was followed by 2.5 mg/l of kinetin with 8 % sucrose. On the contrary 0.5 mg/l kinetin with 8 % sucrose was less effective for microtuberization.

The mean number of microtubers/shoot and the mean fresh weight of microtubers of the cultivars differed significantly. The maximum mean number of microtubers was found in the plantlets of cultivar Diamant and which was at par with All Blue. The lowest mean number of microtubers/shoot was recorded from the plantlets of cultivar Indurkani. The difference in mean number of microtubers/shoot in All Red, Asterix and JPR was no significant. On the other hand the similar higher mean fresh weight of microtubers was found in Diamant and Asterix which was significantly higher than that of other cultivars. The lowest mean fresh weight of microtubers was recorded from the plantlets of cultivar Indurkani.

For the interactions between the potato cultivars and the different concentrations of Kin with 8% sucrose, significant variations were observed. From the **Table 2.7** it is revealed that All Blue produced the significantly higher number of microtuber/shoot in MS medium supplemented with 3.5 mg/l of kinetin + 8 % sucrose followed by media having 2.5 mg/l kinetin + 8 % sucrose. Similar trends were observed in Diamant, Asterix and Indurkani. Cultivars All Red and JPR showed the significantly higher number of microtuber/shoot in media having 2.5 mg/l kinetin + 8 % sucrose followed by media having 3.5 mg/l of kinetin + 8 % sucrose.

Among the six potato cultivars, Diamant exhibited the significantly highest weight of microtubers in tuberization medium supplemented with 3.5 mg/l of kinetin + 8 % sucrose. In this medium formulation, All Red, Asterix and JPR produced the highest fresh weight of microtubers. All Blue produced the highest fresh weight of microtubers on media having 2.5 mg/l kinetin + 8 % sucrose and Indurkani exhibited the highest fresh weight of microtubers on media having 4.5 mg/l kinetin + 8 % sucrose.

Table 2.7. Effect of different concentrations of Kin with 8% sucrose on number of micro-tubers/shoot and weight of microtubers.

Characters	Cultivars	Different concentrations (mg/l) of Kin with 8% sucrose					
		0.5 mg	1.5 mg	2.5 mg	3.5 mg	4.5 mg	**Mean**
Number of microtuber/ shoot	All Blue	0.83^{D}	0.92^{CD}	1.32^{B}	1.43^{A}	0.97^{C}	1.09^{a}
	All Red	0.70^{D}	0.84^{C}	1.20^{A}	1.10^{B}	0.74^{D}	0.92^{b}
	Asterix	0.75^{D}	0.86^{C}	1.05^{B}	1.20^{A}	0.80^{CD}	0.93^{b}
	Diamant	0.80^{E}	0.90^{D}	1.30^{B}	1.42^{A}	1.20^{C}	1.12^{a}
	JPR	0.76^{C}	0.82^{C}	1.16^{A}	1.03^{B}	0.77^{C}	0.90^{b}
	Indurkani	0.63^{B}	0.71^{B}	0.86^{A}	0.87^{A}	0.68^{B}	0.75^{c}
	Mean	0.74^{c}	0.84^{b}	1.15^{a}	1.17^{a}	0.86^{b}	-
	LSD value (at 5%): 0.040 for cultivars (C); 0.043 for concentrations of Kin and 0.097 for C× Kin						
Fresh weight of microtubers (mg)	All Blue	155.11^{C}	174.56^{B}	184.44^{A}	175.67^{B}	146.1^{D}	167.20^{b}
	All Red	84.88^{E}	115.22^{D}	163.56^{B}	171.33^{A}	143.7^{C}	135.70^{d}
	Asterix	183.44^{E}	195.56^{D}	216.44^{C}	235.33^{A}	225.1^{B}	211.20^{a}
	Diamant	173.89^{E}	185.67^{D}	224.11^{B}	256.11^{A}	215.3^{C}	211.00^{a}
	JPR	95.33^{D}	105.22^{C}	167.44^{B}	174.33^{A}	165.8^{B}	141.60^{c}
	Indurkani	55.88^{C}	59.66^{C}	83.11^{B}	84.55^{B}	94.0^{A}	75.44^{e}
	Mean	124.76^{e}	139.31^{d}	173.19^{b}	182.89^{a}	165.0^{c}	-
	LSD value (at 5%): 2.32 for cultivars (C); 2.52 for concentrations of Kin and 5.65 for C× Kin						

The same letters indicate no significant difference at LSD = 0.05. Small letters used for cultivars (C) or sucrose concentrations of Kin (Kin) and capital letters used for C × Kin.

2.3.4.4. Effect of physical nature of media on microtuberization of potato

The experiment was conducted in search of the effect of cotton free liquid medium and cotton based liquid medium with 8% sucrose on microtuber induction of six potato cultivars**.** Agar solidified MS medium supplemented with 8% sucrose was used as a control medium. In this experiment the both liquid media (cotton free and cotton based) gave significantly more number of microtubers/shoot and the higher mean fresh weight of microtubers than that of control medium **(Table 2.8 and Plate 2.3)**. Control medium was ranked the lowest among the media.

Among the six potato cultivars significantly the higher mean number of microtubers/shoot was observed in cultivar Diamant and the lower were in cultivar Indurkani. The similar mean number of microtubers was found in All Blue and Asterix. In respect of mean fresh weight of microtubers Asterix exhibited the best performance which was at par with cultivar Diamant. Cultivar Indurkani was ranked the lowest among the cultivars.

The interactions between the potato cultivars and the media were significant. **Table 2.8** shows that the cultivar Diamant exhibited the significantly higher number of microtubers/shoot in cotton based liquid media. Similar trends were observed in All Blue, Asterix and Indurkani. Cultivars All Red and JPR exhibited the similar higher number of microtubers/shoot in both cotton free and cotton based liquid medium.

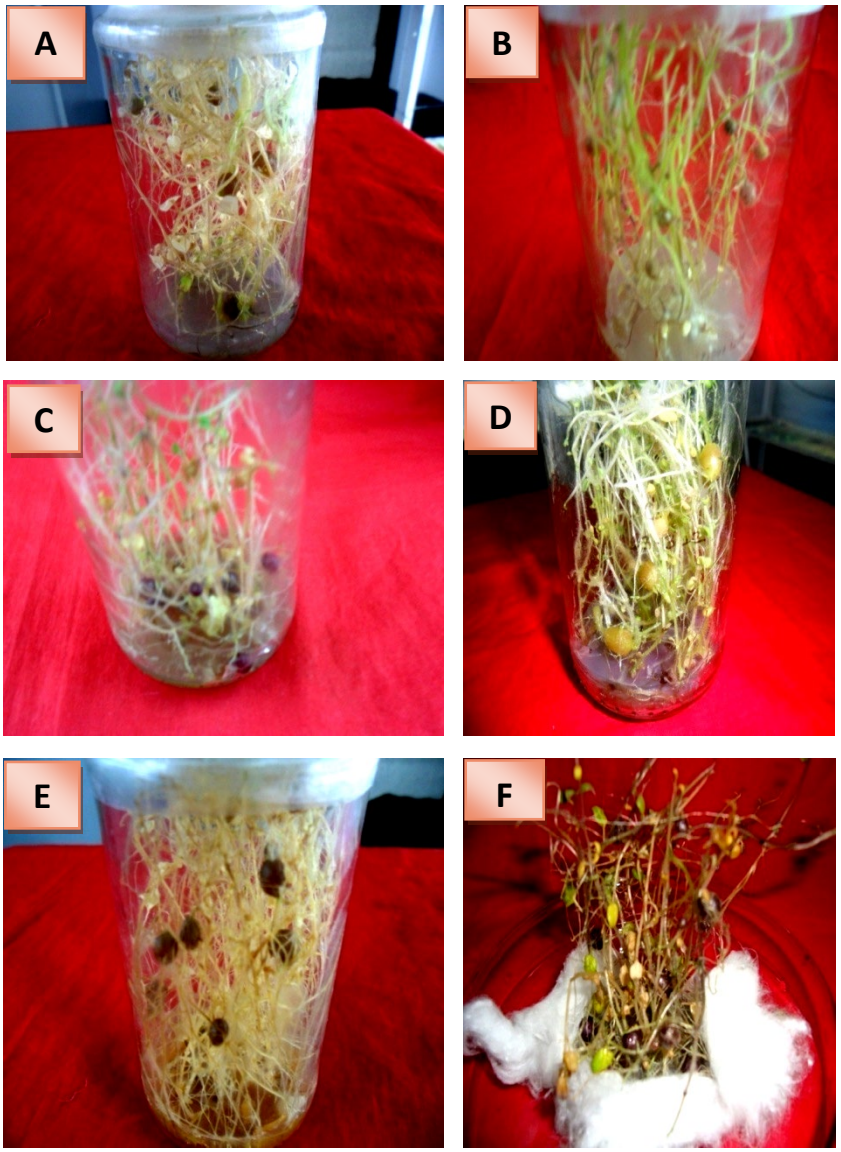
Among the six potato cultivars, Diamant exhibited the highest weight of microtubers in cotton based liquid medium. In this medium cultivars All Red, Asterix and Indurkani also exhibited the highest weight of microtubers. All Blue exhibited the highest weight of microtubers in cotton free liquid medium. The observed difference in fresh weight of microtubers in cotton free and cotton based liquid medium was not significant in All Blue, Asterix and Indurkani.

Table 2.8. Effect of physical nature of media on number of microtubers/shoot and weight of microtubers.

Characters	Cultivars	Physical nature of media			
		Control	Cotton free liquid culture	Cotton based liquid culture	**Mean**
Number of microtubers/ shoot	All Blue	0.72^{C}	0.78^{B}	0.84^{A}	0.78^{b}
	All Red	0.63^{B}	0.73^{A}	0.71^{A}	0.69^{c}
	Asterix	0.64^{C}	0.80^{B}	0.88^{A}	0.78^{b}
	Diamant	0.73^{C}	0.92^{B}	1.00^{A}	0.88^{a}
	JPR	0.61^{B}	0.68^{A}	0.71^{A}	0.66^{d}
	Indurkani	0.47^{B}	0.44^{B}	0.58^{A}	0.50^{e}
	Mean	0.63^{c}	0.73^{b}	0.79^{a}	-
	LSD value (at 5%): 0.024 for cultivars (C); 0.034 for media (M) and 0.059 for C × M				
Mean fresh weight of microtubers (mg)	All Blue	157.44^{B}	200.11^{A}	196.33^{A}	184.62^{b}
	All Red	73.44^{C}	96.55^{B}	122.33^{A}	97.44^{d}
	Asterix	183.11^{B}	218.88^{A}	224.22^{A}	208.73^{a}
	Diamant	184.11^{C}	201.44^{B}	226.11^{A}	203.88^{a}
	JPR	96.22^{B}	105.55^{B}	120.88^{A}	107.55^{c}
	Indurkani	60.88^{B}	71.77^{A}	74.66^{A}	69.11^{e}
	Mean	125.87^{c}	149.03^{b}	160.75^{a}	-
	LSD value (at 5%): 5.62 for cultivars (C); 7.95 for media (M) and 13.77 for C × M				

The same letters indicate no significant difference at LSD = 0.05. Small letters used for cultivars (C) or media (M) and capital letters used for C × M.

PLATE 2.3: Microtuberization in potato



Figures: A: Micrtubers induced to develop in 8% sucrose fortified MS0 medium, 55 days after culture incubation in dark; **B:** Micrtubers develop in MS medium supplemented with 10 mg/l BA + 8% sucrose, 45 days after culture incubation in dark; **C:** Micrtubers develop in MS medium supplemented with 12.5 mg/l BA + 8% sucrose, 45 days after culture incubation in dark; **D:** Micrtubers develop in MS medium supplemented with 3.5 mg/l Kin + 8% sucrose, 45 days after culture incubation in dark; **E:** Micrtubers develop in MS medium supplemented with 2.5 mg/l Kin + 8% sucrose, 65 days after culture incubation in dark; **F:** Micrtubers develop in cotton based MS Liquid medium with 8% sucrose, 55 days after culture incubation in dark.

2.3.5. Field Evaluation of Plantlets and Microtubers of Potato

Plantlets (PL) and microtubers (MT) of the six potato cultivars produced *in vitro* were grown in the field. The plantlets and the sprouted microtubers were gradually acclimatized and successfully established in the field **(Plate 2.4-2.5)**. Visual evaluation of the morphological traits of the plantlet derived plants and the microtuber derived plants indicates that all plants did not show any symptoms of viral diseases (Such as PLRV, PVX and PVY).The morphological and yield characteristics measured on their plants and harvested tubers were compared. The results on the field performances of PL and MT are summarized and presented in **Tables 2.9 – 2.12**.

Plant height: The plant height was significantly influenced both by seed sources (PL and MT) and by potato cultivars **(Table 2.9).** It was found that the height of PL propagated plants was significantly higher than that of MT propagated plants. Among the cultivars Diamant produced the tallest plants which were observed in PL propagated plants followed by Asterix. The observed difference in height of plants in cultivars All Red, JPR and Indurkani was not significant.

Number of stems/hill: Significantly the higher number of stems/hill was noticed in PL propagated plants than that of MT propagated plants **(Table 2.9).** Among the cultivars Indurkani exhibited the maximum number of stems/hill in PL propagated plants followed by Diamant and All Blue. Cultivar Asterix produced the lower number of stems/hill which was recorded from MT propagated plants.

Number of leaves/hill: The number of leaves/hill of PL propagated plants was significantly higher than that of MT propagated plants for all the potato cultivars **(Table 2.9).** On the other hand wide variation was observed among the cultivars in respect of number of leaves/hill. Among the cultivars Indurkani produced the maximum number of leaves/hill followed by Diamant and All Blue. JPR produced the lower number of leaves/hill which was at par with All Red and Asterix.

Table 2.9. Plant height, stem number and leaf number of the plantlets (PL) and the microtubers (MT) propagated crops of six potato cultivars.

Characters	Cultivars	Seed sources		
		Plantlets	Microtubers	**Mean**
Plant height (cm)	All Blue	25.37 ± 0.99^{A}	21.07 ± 0.89^{A}	23.22^{d}
	All Red	28.23 ± 1.41^{A}	24.27 ± 0.62^{A}	26.25^{c}
	Asterix	39.47 ± 1.40^{A}	33.27 ± 0.92^{B}	36.37^{b}
	Diamant	44.53 ± 1.76^{A}	36.53 ± 0.92^{B}	40.53^{a}
	JPR	26.93 ± 1.00^{A}	23.4 ± 0.72^{A}	25.16^{c}
	Indurkani	29.23 ± 0.90^{A}	22.67 ± 0.69^{B}	25.95^{c}
	Mean	32.29^{a}	26.86^{b}	-
	LSD value (at 5%): 1.77 for cultivars (C); 3.07 for seed sources (SS) and 4.34 for C× SS			
Number of stems/hill	All Blue	4.73 ± 0.34^{A}	2.33 ± 0.27^{B}	3.53^{c}
	All Red	2.93 ± 0.26^{A}	1.46 ± 0.13^{B}	2.19^{d}
	Asterix	2.80 ± 0.22^{A}	1.40 ± 0.13^{B}	1.40^{e}
	Diamant	5.53 ± 0.42^{A}	4.06 ± 0.44^{B}	4.79^{b}
	JPR	2.33 ± 0.27^{A}	1.60 ± 0.19^{A}	1.96^{d}
	Indurkani	30.6 ± 1.04^{A}	27.53 ± 1.14^{B}	29.06^{a}
	Mean	9.22^{a}	6.39^{b}	-
	LSD value (at 5%): 0.42 for cultivars (C); 0.74 for seed sources (SS) and 1.05 for C× SS			
Number of leaves/hill	All Blue	53.60 ± 3.34^{A}	32.40 ± 1.48^{B}	43.00^{c}
	All Red	37.07 ± 2.78^{A}	21.93 ± 0.81^{B}	29.50^{d}
	Asterix	36.93 ± 1.60^{A}	22.53 ± 0.70^{B}	29.73^{d}
	Diamant	75.07 ± 5.71^{A}	60.60 ± 1.51^{B}	67.83^{b}
	JPR	$33.0\ 0 \pm 2.29^{A}$	21.67 ± 1.21^{B}	21.67e
	Indurkani	147.30 ± 4.69^{A}	108.90 ± 1.58^{B}	128.10^{a}
	Mean	69.99^{a}	44.67^{b}	-
	LSD value (at 5%): 2.88 for cultivars (C); 5.00 for seed sources (SS) and 7.07 for C× SS			

The same letters indicate no significant difference at LSD = 0.05. Small letters used for cultivars (C) or seed sources (SS) and capital letters used for C × SS.

Third leaf area (cm^2)/hill: The 3rd leaf area of PL propagated plants was higher than that of MT propagated plants but this variation was not significant **(Table 2.10).** On the other hand there was excited cultivar difference in respect of 3rd leaf area. Among the cultivars Diamant produced the highest leaf area which was observed in PL propagated plants followed by Asterix and All Blue and they were significantly different from the rest of cultivars. JPR and All Red produced the similar 3rd leaf area and Indurkani produced the lowest leaf area which was observed in MT propagated plants.

Canopy (cm^2)/hill: Table 2.10 shows that the canopy/hill was significantly higher in PL propagated plants in comparison to MT propagated plants. On the contrary the canopy of the cultivars differed significantly. Maximum canopy was found in the PL propagated plants of cultivar Diamant which was followed by Asterix and All Blue and the lowest canopy was recorded from the MT propagated plants of JPR.

Chlorophyll content (mg/g): The leaf chlorophyll content of PL propagated plant was statistically similar with MT propagated plant. On the other hand different cultivars had significant effect on leaf chlorophyll content as revealed from **Table 2.10**. The PL propagated plant leaves of cultivar Asterix had more chlorophyll content than that of other cultivars. The difference in leaf chlorophyll contents was not significant in JPR and Diamant. The minimum leaf chlorophyll content was found in Indurkani.

Table 2.10. Third leaf area (cm^2)/hill, canopy (cm^2)/hill and leaf chlorophyll content (mg/g) of the plantlets (PL) and the microtubers (MT) propagated crops of six potato cultivars.

Characters	Cultivars	Seed sources		
		Plantlets	Microtubers	**Mean**
3^{rd} leaf area/hill	All Blue	68.22 ± 1.49^{A}	63.13 ± 1.24^{A}	65.67^{c}
	All Red	53.76 ± 0.77^{A}	54.67 ± 1.33^{A}	54.21^{d}
	Asterix	103.2 ± 1.62^{A}	96.13 ± 1.07^{A}	99.66^{b}
	Diamant	109.8 ± 1.25^{A}	99.47 ± 1.41^{B}	104.63^{a}
	JPR	55.53 ± 1.40^{A}	51.33 ± 2.08^{A}	53.43^{d}
	Indurkani	19.94 ± 1.01^{A}	15.0 ± 2.08^{A}	17.47^{e}
	Mean	68.40^{a}	63.28^{a}	-
	LSD value (at 5%): 3.45 for cultivars (C); 6.03 for seed sources (SS) and 8.53 for C× SS			
Canopy/hill	All Blue	975.46 ± 17.29^{A}	663.10 ± 36.35^{B}	819.28^{b}
	All Red	669.40 ± 10.38^{A}	550.70 ± 9.83^{B}	610.05^{d}
	Asterix	1066 ± 17.53^{A}	598.7 ± 15.15^{B}	832.35^{b}
	Diamant	1447.3 ± 23.1^{A}	790.1 ± 14.01^{B}	1118.7^{a}
	JPR	652 ± 19.27^{A}	531.4 ± 10.36^{B}	591.7^{d}
	Indurkani	821.2 ± 25.41^{A}	564.5 ± 8.49^{B}	692.85^{c}
	Mean	938.56^{a}	616.41^{b}	-
	LSD value (at 5%): 39.07 for cultivars (C); 67.67 for seed sources (SS) and 95.71 for C× SS			
Chlorophyll content	All Blue	0.75 ± 0.05^{A}	0.77 ± 0.06^{A}	0.76^{d}
	All Red	0.99 ± 0.09^{A}	0.98 ± 0.03^{A}	0.98^{b}
	Asterix	1.12 ± 0.05^{A}	1.09 ± 0.37^{A}	1.10^{a}
	Diamant	0.95 ± 0.08^{A}	0.93 ± 0.38^{A}	0.94^{c}
	JPR	0.98 ± 0.06^{A}	0.92 ± 0.03^{B}	0.95^{c}
	Indurkani	0.35 ± 0.06^{A}	0.33 ± 0.08^{A}	0.34^{e}
	Mean	0.85^{a}	0.83a	-
	LSD value (at 5%): 0.017 for cultivars (C); 0.03 for seed sources (SS) and 0.042 for C× SS			

The same letters indicate no significant difference at LSD = 0.05. Small letters used for cultivars (C) or seed sources (SS) and capital letters used for C × SS.

Fresh weight (in g) of plants/hill: The fresh weight of plants/hill was significantly influence both by seed sources (PL and MT) and by potato cultivars **(Table 2.11).** It was found that the fresh weight of plants/hill of PL propagated plants was significantly higher than that of MT propagated plants for all the studied potato cultivars. There was an also significant variation in fresh weight of plants/hill among the cultivars. The highest fresh weight of plants/hill was observed in PL propagated plants of cultivar Diamant followed by Indurkani, Asterix and All Blue. The lowest fresh weight of plants/hill was observed in JPR.

Dry weight (g) of plants/hill: The dry weight of plants/hill was significantly influence both by seed sources (PL and MT) and by potato cultivars **(Table 2.11).** It was found that the dry weight of plants/hill was significantly higher than that of MT propagated plants for all the potato cultivars. On the other hand significant variations were observed among the cultivars in dry weight of plants/hill. The highest dry weight of plants/hill was observed in PL propagated plants of cultivar Diamant followed by Indurkani, Asterix and All Blue. The lowest dry weight of plants/hill was observed in JPR.

Table 2.11. Fresh weight of plants/hill and dry weight of plants/hill of the plantlets (PL) and the microtubers (MT) propagated crops of six potato cultivars.

Characters	Cultivars	Seed sources		
		Plantlets	Microtubers	**Mean**
Fresh weight of plants/hill (g)	All Blue	130.53 ± 1.63^{A}	99.93 ± 1.25^{B}	115.23^{b}
	All Red	113.60 ± 2.04^{A}	82.13 ± 4.16^{B}	97.86^{c}
	Asterix	140.40 ± 12.47^{A}	100.9 ± 3.24^{B}	120.65^{b}
	Diamant	259.70 ± 12.13^{A}	130.60 ± 5.14^{B}	195.15^{a}
	JPR	107.13 ± 2.02^{A}	74.20 ± 10.68^{B}	90.66^{c}
	Indurkani	164.26 ± 4.66^{A}	85.33 ± 3.03^{B}	124.79^{b}
	Mean	152.60^{a}	95.51^{b}	-
	LSD value (at 5%): 11.4 for cultivars (C); 19.74 for seed sources (SS) and 27.92 for C× SS			
Dry weight of plants/hill (g)	All Blue	12.21 ± 0.16^{A}	8.58 ± 0.148^{B}	10.39^{c}
	All Red	10.47 ± 0.10^{A}	7.85 ± 0.36^{B}	9.16^{d}
	Asterix	13.58 ± 0.37^{A}	9.18 ± 0.30^{B}	11.38^{b}
	Diamant	22.11 ± 0.59^{A}	11.97 ± 0.49^{B}	17.04^{a}
	JPR	9.95 ± 0.18^{A}	7.32 ± 0.65^{B}	8.63^{d}
	Indurkani	14.64 ± 0.13^{A}	7.9 ± 0.28^{B}	11.27^{b}
	Mean	13.82^{a}	8.80^{b}	-
	LSD value (at 5%): 0.72 for cultivars (C); 1.26 for seed sources (SS) and 1.78 for C× SS			

The same letters indicate no significant difference at LSD = 0.05. Small letters used for cultivars (C) or seed sources (SS) and capital letters used for C × SS.

Number of tubers/hill: The number of tubers/hill of PL propagated plants was significantly higher than that of MT propagated plants for all the potato cultivars **(Table 2.12).** On the other hand wide variation was observed among the cultivars in respect of number of tubers/hill. Among the cultivars Diamant produced the higher number of tubers/hill in PL propagated plants followed by All Blue and Indurkani. All Red produced the lower number of tubers/hill in MT propagated plants. In respect of range of variation, cultivar Diamant exhibited the highest range of variations in PL propagated plants of and All Red showed the lowest range of variations in MT propagated plants.

Weight (g) of tubers/hill: From the **Table 2.12** it is revealed that the weight of tubers/hill of PL propagated plants was significantly higher than that of MT propagated plants for all the potato cultivars. On the other hand significant variations were observed among the cultivars in weight of tubers/hill. In case of tuber weight/hill Diamant was found to be superior to other cultivars. Asterix and All Blue exhibited better performance. Indurkani exhibited the lowest tuber weight/hill which was found in MT propagated plants. The difference between Indurkani and Diamant in total tuber weight/hill was significant. For tuber weight/hill Diamant exhibited the highest range of variation and Asterix showed the minimum range of variation which was noticed in PL propagated plants.

Table 2.12. Number of tubers/hill and tuber weight/hill of the plantlets (PL) and the microtubers (MT) propagated crops of six potato cultivars.

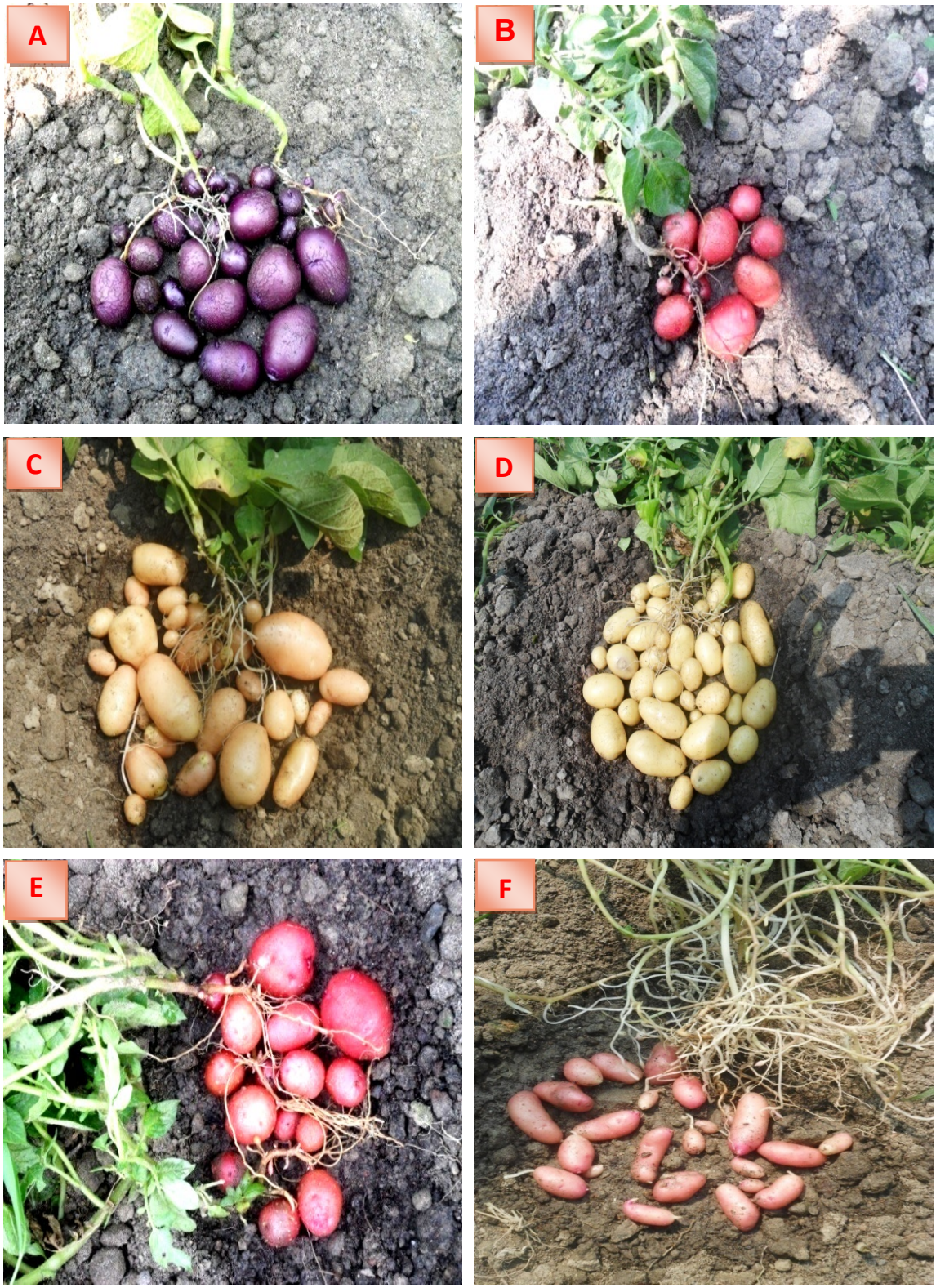
Characters	Cultivars	Seed sources				**Mean**
		Plantlets		Microtubers		
		Range	($\overline{X}$ ± SE)	Range	($\overline{X}$ ± SE)	($\overline{X}$)
Number of tubers/hill	All Blue	16-30	25.53 ± 1.10^{A}	7-19	12.20 ± 0.53^{B}	18.86^{b}
	All Red	9-25	16.13 ± 1.10^{A}	4-11	8.26 ± 0.78^{B}	12.19^{e}
	Asterix	10-30	20.60 ± 1.92^{A}	6-15	10.27 ± 0.57^{B}	15.43^{c}
	Diamant	20-45	33.40 ± 1.63^{A}	8-20	14.33 ± 0.82^{B}	23.86^{a}
	JPR	8-26	18.00 ± 2.02^{A}	5-13	9.13 ± 0.92^{B}	13.56^{d}
	Indurkani	11-35	25.00 ± 4.17^{A}	7-18	11.87 ± 0.65^{B}	18.43^{b}
	Mean	-	23.11^{a}	-	11.01^{b}	-
	LSD value (at 5%): 1.26 for cultivars (C); 2.18 for seed sources (SS) and 3.09 for C× SS					
Weight of Tuber/hill (g)	All Blue	130-210	186.26 ± 5.8^{A}	68-162	108.40 ± 2.3^{B}	147.33^{b}
	All Red	79-182	127.06 ± 7.3^{A}	39-87	63.60 ± 5.9^{B}	95.33^{c}
	Asterix	100-250	198.53 ± 12.0^{A}	88-176	120.10 ± 7.2^{B}	159.31^{b}
	Diamant	210-460	343.2 ± 18.4^{A}	70-212	150.7 ± 3.8^{B}	246.95^{a}
	JPR	30-170	114.86 ± 19.3^{A}	33-69	56.73 ± 12.6^{B}	85.79c
	Indurkani	20-110	76.26 ± 8.92^{A}	28-53	33.52 ± 2.8^{B}	62.23^{d}
	Mean	-	174.36^{a}	-	91.28^{b}	-
	LSD value (at 5%): 17.22 for cultivars (C); 29.84 for seed sources (SS) and 42.2 for C× SS					

The same letters indicate no significant difference at LSD = 0.05. Small letters used for cultivars (C) or seed sources (SS) and capital letters used for C × SS.

PLATE 2.4: Field establishment of microtubers and meristem derived plantlets

Figures: A: Plantlets acclimatized in Thump pots, 21 days after transplantation; **B:** Plants sprouted from microtubers, 25 days after transplantation; **C:** Plantlet derived plants, 30 days after transplantation; **D:** Plantlet and microtuber grown potato plants in a net house, 65 days after transplantation; **E-F:** Minitubers developing from cultivars All Blue and Diamant, 50 days after transplantation.

PLATE 2.5: Minitubers developed from *in vitro* grown planting materials



Figures A - F: Minitubers of six potato cultivars, 90 days after transplantation. **A:** All Blue; **B:** All Red; **C:** Asterix; **D:** Diamant; **E:** JPR and **F:** Indurkani.

2.3.6. Field Performances of Minitubers (G_1 Seed), Breeder (G_2 Seed), Foundation (G_3 Seed) and Source Seed (SS) Potato Tubers

The minitubers (MNT), breeder seed (BS) tubers and foundation seed (FS) tubers originated from the plantlets (PL) and the microtubers (MT) were tested in a field trial. In field trial, the disease incidence and the tuber yield performances (number of tubers/hill and weight of tubers/hill) were studied. The yield performances of minitubers, breeder seed tubers and foundation seed tubers compared with source seed (SS) tubers propagated crop. The results on this experiment are described in the following heads.

2.3.6.1. Viral disease incidence

Incidence of the following viruses PLRV, PVX and PVY was identified based on characteristic symptoms of the viruses appeared on plants. At advanced stage of disease development, severity of PVX, PVY and PLRV infection and their mixed infection varied with generations of potato seed tubers. The incidence of viral diseases was not observed in minituber propagated crop (2^{nd} generation). The viral diseases (such as PLRV, PVX and PVY) were first appeared in BS propagated crop (in both of PL and MT groups) and its incidence increased gradually with the increase of crop generations. Disease incidence ranged from 0.56 to 1.54% in different generations **(Figure 2.1)**. The highest incidence of viruses 1.54% was observed in MT-FS (foundation seed originated from MT) propagated crop (4^{th} generation) followed by PL-FS (foundation seed originated from PL) propagated crop (4^{th} generation). The lowest virus incidence 0.56% was found in PL-BS (breeder seed originated from PL) propagated crop (3^{rd} generation). Visual evaluation of the morphological traits of the MNT, BS tuber and FS tuber propagated crop of the PL and the MT groups indicated that all plants did not show any symptoms of fungal and bacterial diseases.

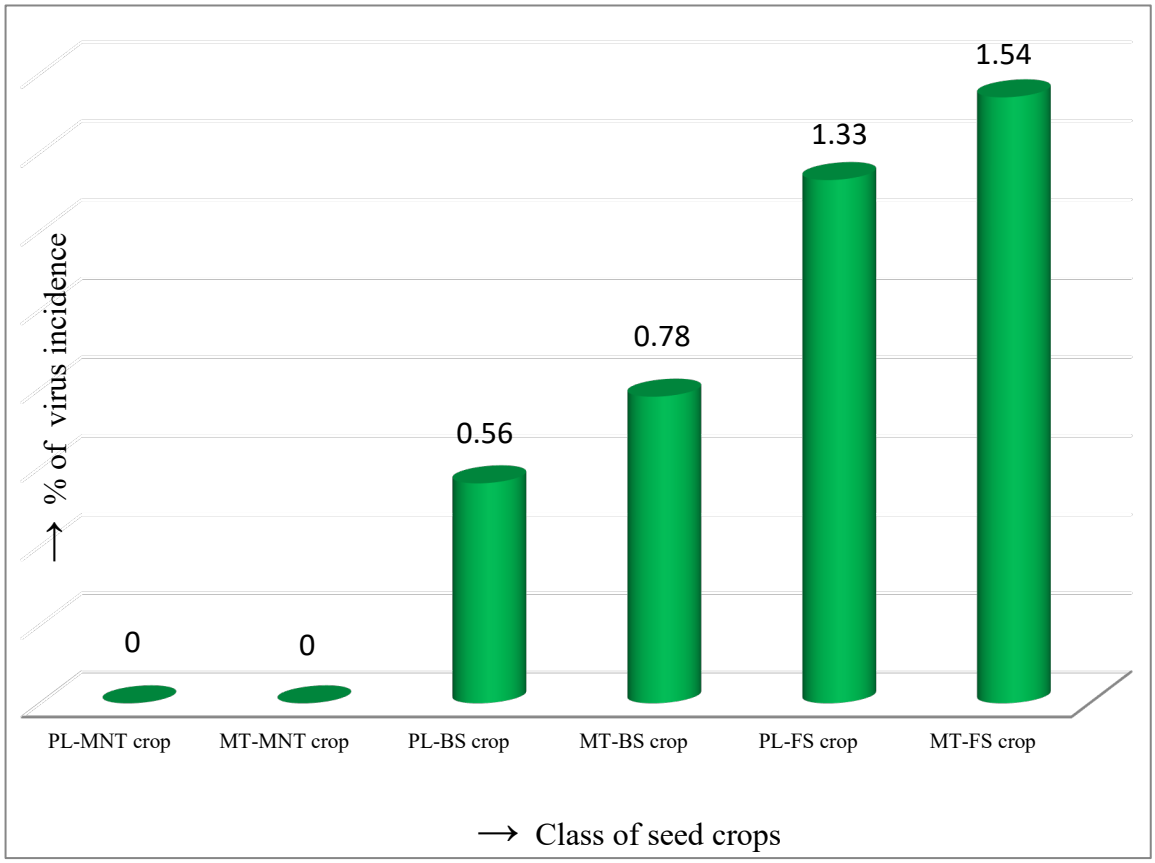


Figure 2.1: Viral disease incidence (%) in minituber, breeder and foundation seed tuber propagated crops (originated from the plantlets and the microtubers groups).

*PL-MNT= Minitubers originated from plantlets

*MT-MNT= Minitubers originated from microtubers

*PL-BS = Breeder seed originated from plantlets via minitubers

*MT-BS = Breeder seed originated from plantlets via microtubers

*PL-FS = Foundation seed originated from plantlets via minitubers and breeder seed

*MT-FS = Foundation seed originated from microtubers via minitubers and breeder seed

2.3.6.2. Number of tubers/hill

Table 2.13 shows that the number of tubers/hill of the PL and the MT groups was significantly higher than that of SS propagated crop and there were no significant differences between the PL and the MT groups for number of tubers/hill in the same generation but number of tubers/hill decreased gradually with the increase of crop generations. On the other hand wide variations were observed among the cultivars in respect of number of tubers/hill. Significantly higher number of tubers/hill was harvested from PL-MNT propagated plants of the cultivar Indurkani followed by All Blue, Asterix and JPR. Diamant was ranked the lowest among the cultivars.

Table 2.13. Number of tubers/hill in minituber, breeder and foundation seed tubers (originated from the plantlets and the microtubers groups) and source seed tubers propagated crops.

Potato cultivars	Class of seed crops							
	2nd generation		3rd generation		4th generation		SS crop	**Mean**
	PL-MNT crop	MT-MNT crop	PL-BS crop	MT-BS crop	PL-FS crop	MT-FS crop		
All Blue	11.45^{A}	10.38^{AB}	10.56^{AB}	10.11^{AB}	9.22^{B}	9.16^{B}	6.59^{C}	9.63^{b}
All Red	9.93^{A}	9.65^{AB}	9.12^{AB}	9.35^{AB}	8.06^{B}	8.11^{B}	6.14^{C}	8.62^{c}
Asterix	10.51^{A}	10.73^{A}	9.89^{A}	9.73^{A}	9.77^{A}	9.54^{A}	6.7^{B}	9.55^{b}
Diamant	9.98^{A}	9.39^{AB}	9.12^{AB}	9.34^{AB}	8.09^{B}	8.12^{B}	5.93^{C}	8.58^{c}
JPR	10.84^{A}	10.44^{A}	9.28^{AB}	9.13^{AB}	8.63^{B}	8.42^{B}	6.38^{C}	9.01^{bc}
Indurkani	16.13^{A}	15.43^{AB}	15.88^{A}	15.2^{ABC}	13.9^{BC}	13.53^{C}	10.66^{D}	14.38^{a}
Mean	11.47^{a}	11.0^{ab}	10.64^{b}	10.47^{b}	9.61^{c}	9.5^{c}	7.06^{d}	
LSD value (at 5%): 0.73 for cultivars (C); 0.68 for class of seed crops (SC) and 1.79 for C × SC								

The same letters indicate no significant difference at LSD = 0.05. Small letters used for cultivars (C) or seed crops (SC) and capital letters used for C × SC.

*PL-MNT= Minitubers originated from plantlets

*MT-MNT= Minitubers originated from microtubers

*PL-BS = Breeder seed originated from plantlets via minitubers

*MT-BS = Breeder seed originated from plantlets via microtubers

*PL-FS = Foundation seed originated from plantlets via minitubers and breeder seed

*MT-FS = Foundation seed originated from microtubers via minitubers and breeder seed

*SS = Source seed

2.3.6.3. Tuber yield/hill

Table 2.14 shows that the tuber yield performance of the PL and the MT groups was significantly higher than that of SS propagated crop but there were no significant differences between the PL and the MT groups for tuber yield in the same generation but tuber yield decreased gradually with the increase of crop generations. On the other hand significant variations were observed among the cultivars for weight of tubers/hill. In case of tuber weight/hill Asterix was found to be superior to other cultivars and Diamant exhibited better performance. They were significantly different from other cultivars. The lowest tuber weight/hill was observed in cultivar Indurkani.

Table 2.14. Tuber weight/hill (g) in minituber, breeder and foundation seed tubers (originated from the plantlets and the microtubers groups) and source seed tubers propagated crops.

Potato cultivars	Class of seed crops							
	2nd generation		3rd generation		4th generation		SS crop	**Mean**
	PL-MNT crop	MT-MNT crop	PL-BS crop	MT-BS crop	PL-FS crop	MT-FS crop		
All Blue	329.44	324.1	312.5	315.1	292.44	288.71	215.3	296.8^{c}
All Red	338.22	321.4	317.64	313.22	308.12	296.34	231.8	303.8^{c}
Asterix	572.11	568.6	554.78	542.44	498.91	486.73	311.9	505.1^{a}
Diamant	424.33	420.8	408.36	411.62	386.13	382.15	243.8	382.4^{b}
JPR	314.66	306.8	302.44	296.33	278.54	266.49	212.4	282.5^{d}
Indurkani	94.44	83.89	88.32	77.14	79.61	72.27	44.22	77.1^{e}
Mean	345.5^{a}	337.6^{a}	330.6^{b}	326.0^{b}	307.3^{c}	298.8^{c}	209.9^{d}	-
LSD value (at 5%): 12.18 for cultivars (C); 11.28 for class of seed crops (SC) and 29.84 for C× SC								

The same letters indicate no significant difference at LSD = 0.05.

*PL-MNT= Minitubers originated from plantlets

*MT-MNT= Minitubers originated from microtubers

*PL-BS = Breeder seed originated from plantlets via minitubers

*MT-BS = Breeder seed originated from plantlets via microtubers

*PL-FS = Foundation seed originated from plantlets via minitubers and breeder seed

*MT-FS = Foundation seed originated from microtubers via minitubers and breeder seed

*SS = Source seed

2.3.6.4. Percentage of yield increase

Table 2.15 shows that the higher tuber yield increasing percentage was recorded from PL-BS propagated crop followed by MT-BS propagated crop and they were significantly different from PL-FS and MT-FS propagated crops in respect of tuber yield increasing percentage. From the **Table 2.15** it also appears that no significant differences between the PL and the MT groups for tuber yield increasing percentage in the same generation and tuber yield increasing percentage decreased with the increase of crop generations.

On the other hand significant variations were observed among the cultivars in respect of yield increasing percentage. The maximum tuber yield increasing percentage was found in cultivar Idurkani followed by Asterix and Diamant and the lowest yield increasing percentage was observed in All Red. The observed difference in yield increasing percentage was not significant in All Red and JPR. Similar trends were observed between Asterix and Diamant.

Table 2.15. Percentage of yield increase in breeder and foundation seed tuber (originated from the plantlets and the microtubers groups) propagated crops over SS propagated crop.

Cultivars	Class of seed crops				
	3rd generation		4th generation		**Mean**
	PL-BS crop	MT-BS crop	PL-FS crop	MT-FS crop	
All Blue	45.17 ± 0.78	46.39 ± 0.48	35.82 ± 0.87	34.09 ± 0.39	40.36[c]
All Red	37.03 ± 0.56	35.12 ± 0.74	32.92 ± 0.36	27.84 ± 092	33.22[d]
Asterix	77.87 ± 0.88	73.91 ± 0.93	59.95 ± 1.02	56.05 ± 0.66	66.94[b]
Diamant	67.49 ± 0.73	68.83 ± 0.27	58.37 ± 0.74	56.74 ± 0.73	62.85[b]
JPR	42.39 ± 1.23	39.51 ± 0.91	31.13 ± 1.06	25.46 ± 082	34.62[d]
Indurkani	99.72 ± 0.98	74.44 ± 0.66	80.03 ± 0.79	63.43 ± 0.29	79.40[a]
Mean	61.61[a]	56.36[a]	49.70[b]	43.93[b]	
LSD value (at 5%): 5.50 for cultivars (C); 6.73 for class of seed crops (SC) and 11.43 for C× SC					

The same letters indicate no significant difference at LSD = 0.05.

*PL-BS = Breeder seed originated from plantlets via minitubers

*MT-BS = Breeder seed originated from plantlets via microtubers

*PL-FS = Foundation seed originated from plantlets via minitubers and breeder seed

*MT-FS = Foundation seed originated from microtubers via minitubers and breeder seed

*SS = Source seed

2.4. DISCUSSION

Potato is usually vegetative propagated crop and very much susceptible to a number of viral diseases, which cause remarkable decrease in yield. Meristem culture is one of the important methods to produce virus free stock plants (Martin *et al.,* 1952). The apical meristem and the first set of primordial leaves are generally not connected to the vascular system of the plant. Therefore, they are not contaminated by viruses that travel through the vascular system (Prakash *et al.,* 1993 and Rahman, 1998). If these explants are carefully excised so as not to contaminate it with sap from more mature leaves or stem tissue and it is placed in culture tube a virus free plant can be established. Many important horticultural crops (e.g. citrus, strawberry, potato, orchids) are routinely freed of viral contamination by using this procedure (Prakash *et al.,* 1993 and Ali, 1998). Murashige and Smith (1970) accomplished the first true meristem culture of an isolated angiosperm meristem into a complete plant. Generally, to establish virus-free plantlets one can culture the apical clone + two or four subjacent primordial leaves. This technique is also valuable for the maintenance of carefully defined stock of specific cultivars in disease free state.

The size of the meristem explants is critical for virus eradication. Often so called meristem tip cultures have failed to eliminate virus infection because the explant contains shoot apices with vascular tissue instead of true meristem (Adams, 1975 and Rahman, 1998). For virus eradication, the double antibody sandwich enzyme linked immuno-sorbent assay (DAS-ELISA) is extensively applied for its various merits like high sensitivity, rapidness and reliability over the order methods as reported by Clark and Bar-Joseph, 1984. DAS-ELISA test has proved to be very effective in virus eradication. Commercial production of virus-free seed potatoes through meristem culture has been a regular practice in many developed countries. Present investigation was therefore designed to conduct experiments using the meristem explants from field grown plants. The results of which are discussed to justify them.

In the present investigation, apical meristems of 25-35 days old field grown plants were successfully cultured on M-shaped filter paper bridge in MS liquid medium supplemented with GA_3 and Kin in order to find out the best culture media formulation for the establishment of primary meristem culture and also find out the best variety

among the six cultivars of potato. Among the various concentrations and combinations of plant growth regulators used for this study, 0.5 mg/l GA_3 + 0.4 mg/l Kin was found to be the best medium formulation for the establishment of primary meristem culture of six potato cultivars. Similarly, 0.5 mg/l GA_3 was also found better medium formulation for primary culture and MS0 was less responsive for the growth response of meristem culture. Ahmmad (1999) used same plant growth regulator formulation and reported satisfactory results for the establishment of primary meristem culture of potato.

Among the six cultivars of potato, Asterix and Diamant were the best responsive cultivars for resuming new growth of cultured meristems for all media compositions. Cultivars All Blue and All Red showed better growth response of cultured meristem. However, cultivar Indurkani and JPR were less responsive than that of all other cultivars in all media formulations. Many researchers also got satisfactory results with MS liquid medium in different crops (White, 1943; Heller, 1949; Stone, 1963; Goodwin, 1966; Vine, 1968; Walkey, 1968; Meller and Smith, 1969; Pennazio and Redolfi, 1973; Rahman, 1998 and Ali, 1998). Use of liquid culture methods of potato tissue culture has been also reported previously (Goodwin, 1966).

From the results it appears that the *in vitro* growth and development of plantlets of six potato cultivars were significantly influenced both by the nodal positions of the explants and by the cultivars. The mean results shows that the 2nd and 3rd nodal cuttings (from the top of individual potato plantlets) with fully expended and matured leaf were exhibited the higher shoot growth than that of shoot tips and 1st and 4th nodal cuttings derived plantlets. Although the similar higher number of roots/plantlet was shown in shoot tips, 2nd nodes and 3rd nodal explants derived plantlets. The results also indicate that very young leaf bearing explants (viz. shoot tip and 1st nodal cuttings) and old or very old leaf bearing explants (4th nodal cuttings) were exhibited the lower shoot growth. The variation in shoot growth among the plantlets develop from different nodal positions of the individual plantlets was probably due to variation of active growing points and quality of explants.

The above results support that the findings of Perennec (1966) and Khan *et al.* (1983). Shambhu *et al.* (2004) reported that the single node cuttings of potato plants with fully expanded and matured leaves were exhibited higher shoot growth than that of very

young and very old leaf bearing node cuttings. Dodds (1989) reported that during excision, the large leaves were discarded to ensure and unvarying growth of the new shoot. This is because when the large leaves senesce, they produce growth regulators, which can hinder the growth of the newly developing shoots.

From the results **Table 2.4** shows when the single nodal explants were cultured onto M_1, M_2, M_3 M_4, M_5 and control media significant variations were observed among the cultivars in respect of shoot length, number of usable nodes/shoot and number of roots/plantlet

From the results it appears that among the five modified MS media formulations, M_2 (Nicotinic acid free MS medium) and M_3 (Pyridoxine HCl free MS medium) media had no significant effect on *in vitro* shoot growth of six potato cultivars. The mean shoot length of six potato cultivars was affected by M_1 (Thiamine HCl free MS medium), M_4 (Myo-inositol free MS medium) and M_5 (Glycine free MS medium) media formulations. The mean number of usable nodes/shoot was not significantly affected by M_1, M_2 and M_3 media. It was significantly affected by M_4 and M_5 media. The results also revealed that M_5 medium formulation had no significant effect on number of roots/plantlet but number of roots/plantlet was affected by M_1, M_2, M_3 and M_4 media formulations.

There are many reports describing in respect of vitamin effects on *in vitro* growth response of plant. Rahman *et al.* (2007) observed statistically similar results on *in vitro* growth response of potato in vitamin free and vitamin containing MS medium. Bhadra (2002) reported that vitamins are internally produced in plants which were used to catalyst in different metabolic functions. When cell or tissue is cultured in nutrient medium a small amount of vitamins are produce there (Bhadra, 2002). He reported that Thiamine is a non essential vitamin on nutrient medium for cell or tissue growth. In case of cell growth of many plant species is no role of Nicotinic acid and Pyridoxine. He also reported that essential amino acid naturally synthesis in cultured cell or tissue for metabolic functions. Syeda (1998) and Paul (1990) reported that a lot of Thiamine HCl is produced in apical zone of plant.

Abrahamian *et al.* (2011) reported that vitamins are necessary compounds synthesized and utilized in plants. In tissue culture media, vitamin addition is not always common; since the amount needed by plants is relatively unknown and varies. George *et al.* (2008)

reported that in tissue culture, some plants can become deficient in vitamin synthesis. Hence, supplementing plant tissue with sub-optimal levels is essential to obtaining vigorous growth. Plant cell requirements for vitamin concentration vary according to the plant species and type of culture. Gamborg *et al*. (1968) cultured soybean root cells onto several media containing different concentrations of Thiamine, and to a complete B_5 culture media. Pyridoxine, Nicotinic acid and Myo-inositol present in the media had no adverse effects on growth individually. Polikarpochkina *et al*. (1979) observed that the removal of Inositol and Pyridoxine from the MS (Murashige *et al*., 1962) medium did not give any significant difference on growth of maize calli.

Welander (1977) observed that omitting vitamins (Thiamine, Pyridoxine, Nicotinic acid, Folic acid, and Biotin) from a Bourgin and Nitsch (1967) media *in vitro* did not affect 16 cultivars, except one, of *Begonia x hiemalis* shoot and root formation. Also, Soczek and Hempel (1988) studied the shoot multiplication of three *Gerbera* cultivars in the presence and absence of Thiamine, Pyridoxine, Nicotinic acid and other compounds. It was concluded that reducing the concentration, to half or quarter of the Murashige *et al*. (1974) medium, or removing the vitamins, did not have any significance on growth over three passages (each 4 weeks), except in the case of one cultivar requiring Nicotinic acid (Soczek and Hempel,1988).

From the results it appears that among the five concentrations of sucrose in media without any addition of hormone, 8% sucrose level was found to be optimal for all the cultivars except JPR and 10% sucrose level was found to be moderate. In summary cultivars Diamant and Asterix showed the best results in 8% sucrose containing medium. According to the obtained results, it is quite obvious that using very low or high sucrose concentration for optimal microtuberzation was unsuitable. The very low sugar concentration is not enough to induce microtubers and very high concentration increased the osmotic properties of medium which disturbed the pH and balance of nutrient. Dodds *et al*. (1992) also reported the same observation that low and high sucrose concentrations influenced microtuberization negatively and a fewer minitubers were produced. Potato shoots growing on MS medium with 2-3% sucrose can be induced for microtuberization by transferring them to MS medium with 8% sucrose (Nistor *et al*., 2010). Carlson (2004) and Sushruti *et al*. (2004) also reported best microtuber induction response in MS medium supplemented with 8% sucrose. In agreement to this, it was a continuous

observation that the higher sucrose concentration 8 and 10% showed the positive results for number of microtubers and mean fresh weight of microtubers. It is demonstrated that the starch synthesis is regulated by the osmolarity of the media which can be increased by mixing high amount of sucrose in the media (Oparka and Wright, 1988). Alternatively, sucrose can work as inducer that stimulates some special genes in the potato plants for tuberization (Johnson and Ryan, 1990). Khuri and Moorby (1995) has proposed that the high sucrose levels in media are easily assimilated and converted to starch without interruption for the microtuberization. Keeping in view the studies of other researchers, it seems that sucrose contributes in microtuberization as an inducer, nutritive and osmoticum. Thus the higher sucrose concentrations trigger rapid starch biosynthesis and induction of microtubers. In addition according to Ranalli (2007) the high sucrose concentrations as 6-8 % (60-80 g/l) are necessary for the increase of biomass and dry matter of microtubers. It is important to report the leaf wilting in plantlets grown on 10 and 12% sucrose during present study; this could be due to the high osmotic potential which suggests the use of 8% sucrose in media.

The higher mean number of microtubers was achieved by the combined effect of cytokinin and sucrose promotes *in vitro* microtuberization than sucrose alone. Maximum mean number of microtubers was observed in MS medium supplemented with 10 mg/l BA and 8% sucrose in cultivar All Blue. The mean number of microtubers/shoot increased with increasing rate of BA upto 10 mg/l and then gradually decreased with further increase of BA concentration. The results are similar to the findings of Wang and Hu (1982) who reported that at a higher concentration of BA above 10 mg/l in the medium decreased the number of microtubers. The concentration of BA at 10 mg/l produced the highest mean fresh weight of microtubers in cultivar Damant as compared to other concentrations. This agreed well with the results of similar works by other researchers (Wang and Hu, 1982; Teixeira and Pinto, 1991). Zakaria *et al*. (2008) obtained earliest microtuberization by 13 days at 10 mg/l BA in cultivar Diamant.

The media supplemented with high concentrations of kinetin (3.5 mg/l) produced the maximum number of microtubers in cultivars All Blue and Diamant. The MS medium containing 3.5 mg/l kinetin was also produced the highest mean fresh weight of microtubers in cultivar Diamant. The promotion of microtuberization on cultured shoots by cytokinins has been demonstrated by many researchers (Palmer and Smith, 1969;

Wang and Hu, 1982; Hussey and Stacey, 1984; Estrada *et al.*, 1986; Ortiz- Montiel and Lozoya-Saldana, 1987). High sucrose concentration, Kinetin and growth retardants are required for tuberization as an inductive medium (Lawrence and Barker, 1963; Hussey and Stacey, 1984: Estrada *et al.*, 1986; Chandra *et al.*, 1988; Peri *et al.*, 1991; Alchanatis *et al.*, 1994). The findings of present study with regard to effect of kinetin on microtuberization agree with previous studies (Palmer and Smith, 1970; Wang and Hu 1982; Chandra *et al.,* 1988; Alchanatis *et al.,* 1994). Aksenova *et al.* (2000) also reported that cytokinin and sucrose at high concentration stimulated tuber initiation.

Commonly, cytokinins are included in the media for *in vitro* tuberization of potatoes (Wang and Hu, 1982). Cytokinins promote *in vitro* tuberization of potato by altering GA balance in non-induced stems (Lentini and Earle, 1991), inhibiting root formation and transferring the upright leafy shoots into horizontal stolons (Shibli *et al.,* 2001). At 2% sucrose, cytokinin fails to exert stimulating effect of tuherization at any concentration (Koda and Okazawa, 1983). They further noted that sucrose concentration at above 4%, cytokinins exhibited a promoting effect on tuberization. Requirement of high concentration of sucrose by cytokinins for *in vitro* tuberization was also reported by Palmer and Smith (1969) and Wang and Hu (1982). For several reasons, cytokinin has often been considered to be an important factor for tuberization process. Firstly, cytokinin is known to stimulate cell division (Skoog and Miller, 1957); secondly, there are indications that it inhibits cell elongation (Vanderhoef and Key, 1968), and promote cell expansion (Scott and Liverman, 1956). These phenomena are required for tuber formation and development. Several researchers have, therefore, suggested that the unknown tuberization stimulus could be a cytokinin like substance (Madec, 1963; Courduroux, 1966). Although cytokinin is not directly responsible for tuberization as reported by many researchers, without doubt, it plays a key role in cell division and thus creating sink activity of the developing tuber.

Maximum mean number of microtubers was obtained in liquid with cotton based MS medium supplemented with 8% sucrose, while their number was low even in prolonged time on the same formulation of agar solidified MS medium. Although many studies recommend solidified media for microtuberization of potato (Myeong *et al.,* 1990; Kiji *et al.,* 1997; Pelacho *et al.,* 1999), reports favouring liquid medium are also available. Khomyak (1998) and Jimenez (1999) reported that use of liquid medium instead of solid

medium increased microtuber yield of potato. Miranda *et al.* (2005) used MS medium without plant growth regulators to induce microtuberization by the addition of liquid MS medium supplemented with 80 g/l sucrose.

Plantlets (PL) and microtubers (MT) of six potato cultivars produced in the laboratory were grown in the field and there morphological, physiological and yield characteristics were measured and compared. The performance of PL group was significantly better than that of MT group for various characters specially tuber yield and its components. The PL group plantlets and the MT group microtubers were transplanted and planted at the same time in the field. But the PL group plantlets were already rooted and ready to development contrary to the microtubers of the MT group. Therefore they had different physiological at planting, so the PL plants transferred to the field, the development of plants started earlier than the microtubers. Thus the PL group had long time for tuber development and bulking so the PL group had high means for various characters specially tuber yield related traits than those of the MT group.

On the other hand significant variations were observed among the cultivars in respect of morphological, physiological and yield traits. In general, cultivar Diamant exhibited the high means for plant height, 3rd leaf area, canopy/hill, fresh weight of plants/hill and dry weight of plants/hill. It was noticed that the vegetation period of cultivar Diamant was faster than other cultivars in the early growing period. Therefore, Diamant could grow fast and had vigorous vegetation as compared to other cultivars, so it has higher means. Moreover, Diamant had also the highest means in yield characteristics such as number of tubers and tuber weight/hill. Cultivar Indurkani produced the higher number of stems and leaves/hill but its stem was very narrow, size of leaves was very small, leaf chlorophyll content was the lowest and growth was late. So, yield was low. Many earlier researchers reported similar and dissimilar results in tuber yield for different cultivars of potato (Bremner and Reedley, 1966; Carlson, 1975; Breukema and vander zagg, 1979; Hussain, 1993 and Karim, 2001). It was observed that the positive effect of early plant development on tuber yields. The results related to the tuber number and the tuber yields are in agreement with earlier reports (Wiersema *et al*., 1987; Haverkort *et al*., 1991; Yildirim *et al.,* 2003). Hussain (1993) demonstrated the maximum tuber number/plant for Diamant in respect of meristem culture derived plants.

Field performance of PL-MNT (minitubers originated from plantlets), MT-MNT (minitubers originated from microtuber), PL-BS (breeder seed originated from PL), MT-BS (breeder seed originated from MT), PL-FS (foundation seed originated from PL), MT-FS (foundation seed originated from MT) and SS (source seed) were observed in order to study the selection efficiency at minituber, BS tuber and FS tuber crop level. In the field trial, the viral diseases (such as PLRV, PVX and PVY) were first appeared in BS tuber propagated crop (in both of PL and MT groups) and it's increased in FS tuber propagated crop (in both of PL and MT groups). This result is in agreement with the findings of Rahman *et al*. (2010) who reported that the viral disease incidence increased gradually with the increase of crop generations. The yield performance of the PL and the MT groups was significantly better than that of SS propagated crop. The maximum number of tubers/hill and the highest tuber weight/hill were harvested from PL-MNT propagated crop followed by MT-MNT propagated crop. The number of tubers was gradually decreased in BS and FS tuber propagated crop and it was significantly different (in both of PL and MT groups). Similar results were observed by Rahman *et al*. (2010) who recorded in 2005-06 crop season significantly different numbers of tubers in 2^{d}, 3^{rd}, 4^{th}, 5^{th} and 6^{th} generations. Tuber weight/hill was also gradually decreased in BS and FS tuber propagated crop and it was significantly different (in both of PL and MT groups). Rahman *et al*. (2010) reported that in case of healthy seed, the difference in tuber yield in various generations was significant. In this experiment, there were no significant differences between the PL and the MT groups for tuber number and tuber weight/hill in the same generation. These results are partly in agreement with Wattimena *et al.* (1983) and Gulsum *et al*. (2010) who reported that PL-MNT propagated crop and MT-MNT propagated crop were not significantly different from each other for their yield performances. Wide variations were observed among the cultivars in respect of number of tubers and weight of tubers/hill. In case of tuber weight/hill Asterix was found to be superior to other cultivars and Diamant exhibited better performance.

From the results it appears that the higher tuber yield increasing percentage was recorded from PL-BS propagated crop followed by MT-BS propagated crop and they were significantly different from PL-FS and MT-FS propagated crops in respect of tuber yield increasing percentage. On the other hand significant variations were observed among the cultivars in respect of tuber yield increasing percentage. The maximum tuber yield

increasing percentage was found in cultivar Idurkani followed by Asterix and Diamant and the lowest yield increasing percentage was observed in All Red. The result is nearer to the findings of Bawden and Kassanis (1965) who reported that the virus-free clone produced more vigorous haulm and about 30% higher yields, attributed to more tubers rather than large ones. Zhang (1995) reported 40% yield increase in potato using virus free tuber seed.

2.5. SUMMARY

The present investigation was conducted with a view to develop an effective meristem culture protocol, to develop micropropagation method, to establish a suitable *in vitro* tuberization protocol and to evaluate the performance of *in vitro* grown planting materials of six potato cultivars in field condition.

In this study, apical meristems of 25-35 days old field grown plants were successfully cultured on filter paper bridge in liquid MS medium supplemented with different concentrations and combinations of GA_3 and Kin. Among the various growth regulator formulations 0.5 mg/l GA_3 + 0.4 mg/l Kin was found to be the best medium formulation for the establishment of meristem culture.

The effect of different nodal positions of the explants on *in vitro* growth and development of potato plantlets was studied. The *in vitro* growth and development of plantlets of six potato cultivars were significantly influenced both by the nodal positions of the explants and by the cultivars. The highest shoot growth was found when 3rd node was used as explants followed by 2nd nodal explants. The observed difference in shoot growth between the shoots develop from 2nd and 3rd node was not significant.

From the results it appears that among the five modified MS media formulations, M_2 (Nicotinic acid free MS medium) and M_3 (Pyridoxine HCl free MS medium) media had no significant effect on *in vitro* shoot growth of six potato cultivars. The mean shoot length of six potato cultivars was affected by M_1 (Thiamine HCl free MS medium), M_4 (Myo-inositol free MS medium) and M_5 (Glycine free MS medium) media formulations. The mean number of usable nodes/shoot was not significantly affected by M_1, M_2 and M_3 media. It was significantly affected by M_4 and M_5 media. The results also revealed that M_5 medium formulation had no significant effect on number of roots/plantlet but number of roots/plantlet was affected by M_1, M_2, M_3 and M_4 media formulations.

In respect of microtuberizaton, single nodal cuttings from *in vitro* grown plantlets of six potato cultivars were cultured onto agar solidified MS medium supplemented with different concentrations of sucrose (4, 6, 8, 10 and 12%) and different concentrations of BA or Kin supplemented with 8% sucrose. Single nodal cuttings were also cultured onto cotton based and cotton free MS liquid medium. Among the different treatments 8%

sucrose with 10 mg/l BA or 3.5 mg/l Kin was the best formulation for microtuberizaton of potato.

The plantlets (PL) and the microtubers (MT) of six potato cultivars produced in the laboratory were grown in the seedbeds. The performance of PL group was significantly better than that of MT group for various characters specially tuber yield. Visual evaluation of morphological trails of the PL and MT group showed all plants were found normal and free from viral diseases.

Field performance of PL-MNT (minitubers originated from plantlet), MT-MNT (minitubers originated from microtuber), PL-BS (breeder seed originated from PL), MT-BS (breeder seed originated from MT), PL-FS (foundation seed originated from PL), MT-FS (foundation seed originated from MT) and SS (source seed) were observed. In the field trial, virus incidence ranged from 0.56 to 1.54% in different generations. The tuber yield performance of the PL and the MT groups was significantly better than that of SS propagated crop but the PL and the MT groups were statistically similar for their tuber yield performances. The maximum number of tubers/hill and the highest tuber weight/hill were harvested from PL-MNT propagated crop followed by MT-MNT propagated crop. The number of tubers/hill was significantly different in MNT, BS and FS propagated crop level. Tuber weight/hill was significantly different in MNT, BS and FS propagated crop level. In case of tuber weight/hill Asterix was found to be superior to other cultivars.

The higher tuber yield increasing percentage was recorded from PL-BS propagated crop followed by MT-BS propagated crop. On the other hand the highest yield increasing percentage was found in cultivar Idurkani followed by Asterix and Diamant and the lowest yield increasing percentage was observed in All Red.

The average yield increasing percentage of six potato cultivars was 61.61 for PL-BS propagated crop, 56.36 for MT-BS propagated crop, 49.70 for PL-FS propagated crop and 43.93 for MT-FS propagated crop.

CHAPTER III

NUTRITIONAL QUALITY DETERMINATION OF SELECTED POTATO CULTIVARS

3.1. INTRODUCTION

The potato is a staple food crop in many countries. Potato tuber is containing different nutritional components such as starch, sugars, protein, vitamins, antioxidants and minerals. But there is a considerable genetic variation in concentrations of nutritional components of tuber both between and within *Solanum* species. Several other factors, including environmental conditions and cultivation practices during growth are also important for the concentrations of nutritional components (Kumar *et al*., 2004).

Starch is a major nutritional component of the potato tuber. Starch percentage in potato tubers varied both with variety and environment (Gall *et al*., 1965). On the other hand quantity or kind of sugars and dry matter is a heritable character, but is also affected by a number of environmental factors (Ezekiel *et al*., 1999). Sugar level in potatoes during tuberization and at harvest is largely dependent on cultivar (Sinha *et al*., 1992).The genetic component; however, has the strongest influence since the reducing sugars content is a heritable trait that can be screened for in tubers (Stephenson *et al*., 1964). Early harvesting of tubers resulted in significantly higher levels of reducing sugars than harvesting at maturity. Tubers from early plantings were lower in glucose and sucrose than those from later planting. Freshly harvested potatoes contain very little sugar. Small tubers are higher in sugar than that of big tubers. Reducing sugars and sucrose increased steadily during sprouting of tubers.

Protein exists in potato tubers in both soluble and insoluble forms. The difference in protein content in potato tuber may be due to genotype (Ereifej *et al*., 1997). The percentage of true protein in total protein is subject to environmental effects. Some authors (Mulder and Bakema, 1956; Labib, 1962) have found that the ratio of protein N: total N is lower with high nitrogen fertilization than with low. In contrast, others (Hoff *et al*., 1978; Li and Sayre, 1975) have shown that increasing nitrogen fertilization increases not only the percentage of total N, but also the percentage of true protein, and that true protein and total N increase at the similar rate (Hoff *et al*., 1978). Greater accumulation

of protein occurred when N, P, K as chloride were applied than when N, P, K as sulfate were applied. Nitrogen fertilizations increased the content of total, ammonium–nitrate non-proteins and, in part, protein N of potato tubers. Tkornholm *et al.* (1975) demonstrated that true protein content was increased by nitrogen fertilization.

A series of experiments in the 1940s (Leichsenring, 1951) showed that the reduced ascorbic acid content of potatoes varies with variety, locality, crop year and maturity at time of harvest (values were highest when plants were at their maximum vigor and declined thereafter as vines began to die off). β-carotene (pro- vitamin A) a common carotenoid in many other plants and also presents in the aerial parts of the potato plant is absent or present in only trace amounts in the tubers (Burton, 1989). There is a direct correlation between yellow flesh colour and total carotenoid content, which is a heritable characteristic. Phenolic compounds are distributed mostly between the cortex and skin (peel) tissues of the potato. There are about ten times as much phenolic compounds in the peel as in the flesh of the potato (Lisinka and Leszczynski, 1989). The amount of phenolic compounds varies, depending on flesh color of potato tuber.

Tuber Ca, Fe, and Zn concentrations have been shown to vary significantly between *Solanum* species grown under identical conditions (Andre *et al*., 2007; Bamberg *et al*., 1993, 1998). Among the *Solanum* species, *S. gourlayi* and *S. microdontum* had the highest tuber Ca concentrations, whereas *S. kurtzianum* and *S. tuberosum* had the lowest tuber Ca concentrations when supplied with ample Ca (Bamberg *et al*., 1993). Although the skin generally has a greater Ca concentration than the flesh (Ereifej *et al*., 1998; McGuire and Kelman, 1984, 1986; Wszelaki *et al*., 2005), differences in tuber Ca concentration between *Solanum* species do not appear to be associated simply with differences in skin to flesh ratios (Bamberg *et al*., 1993). Andre *et al*. (2007) observed a strong relationship between tuber Ca and Fe concentrations and a weak but significant correlation between Zn and Fe concentrations among 74 Andean landraces. They observed that some genotypes from the Ajanhuiri group had exceptionally high tuber Ca and Fe concentrations and that tuber size explained 13% of the variability in tuber Fe concentrations. When grown under identical conditions, *S. tuberosum* genotypes have been shown to differ in tuber N (Augustin, 1975; Fitzpatrick *et al*.,1969; Rexen, 1976), K (Brown *et al*., 2005; Ereifej *et al*., 1998; Tekalign and Hammes, 2005; Van Marle *et al*., 1994; Workman and Holm, 1984), P (Dampney *et al*., 2002; Ereifej *et al*., 1998;

Randhawa *et al*., 1984; Tekalign and Hammes, 2005; Trehan and Sharma, 2003), S (Tekalign and Hammes, 2005), Ca (Ereifej *et al*., 1998; Karlsson *et al*.,2006; McGuire and Kelman, 1986; Randhawa *et al*., 1984; Tekalign and Hammes, 2005; Tzeng *et al*., 1990; Van Marle *et al*., 1994), Mg (Allison *et al*., 2001a; Ereifej *et al*., 1998; Randhawa *et al*., 1984; Tekalign and Hammes, 2005), Fe (Brown *et al*., 2005; Ereifej *et al*., 1998; Randhawa *et al*., 1984), Zn (Brown *et al*., 2005; Ereifej *et al*., 1998; Randhawa *et al*., 1984; Tekalign and Hammes, 2005), Cu (Ereifej *et al*., 1998; Randhawa *et al*., 1984; Tekalign and Hammes, 2005), and Mn concentrations (Brown *et al*., 2005). Systematic differences in tuber K, Mg, Fe, Zn, Cu, and Mn concentrations have also been observed between potato varieties obtained commercially (Casanas *et al*., 2003; Di Giacomo *et al*., 2007). It is likely, therefore, that tuber mineral concentrations can be manipulated genetically through commercial breeding programs.

It has been hypothesized that higher yielding potato genotypes have lower concentrations of mineral elements in their tubers than those of lower yielding genotypes when grown in the same environment because of a ‘‘dilution effect’’ caused by plant growth rates exceeding the ability of plants to acquire these elements (Jarrell and Beverly, 1981) that is impacted by both environmental and genetic factors (Davis, 2005; Davis *et al*., 2004). Moreover, Randhawa *et al*. (1984) found insignificant positive relationships between tuber FM yield and tuber K, Mg, Zn, and Cu concentrations in their comparison of eight potato varieties.

From these points of view, it can be noted that potato genotypes also will differ in concentrations of nutritional components. This information may be useful for designing future breeding efforts to improve potato nutritional quality management. Little information on the magnitude of variation in nutritional components of potato genotypes is currently available in literature and more data would benefit future nutritional studies.

3.1.1. Objectives

This part of the research has following objectives:

1. Nutritional quality determination of selected potato cultivars.
2. Selection of nutritionally enriched potato cultivars.
3. Processing quality determination of selected potato cultivars and selection of the cultivars with better processing quality.

3.2. MATERIALS AND METHODS

3.2.1. Materials

Fresh harvested tubers of 44 potato cultivars were used as plant materials for nutritional and processing quality determination. The 44 potato cultivars were grown at the research field of the Department of Botany, University of Rajshahi, Bangladesh. The field soil condition was a sandy loamy with low organic matter. Nitrogen (ammonium sulphate), phosphorus (triple super phosphate) and potassium (potassium sulphate) fertilizers were applied wring broadcast method at a rate of 342 kg urea/ha, 220 kg TSP/ ha and 259 kg MP/ha, respectively. Tuber plantation was done by hand in November 21, 2009. Pond water was used for irrigation and fungicides were sprayed when needed. The tubers were harvested 90 days after plantation. Harvested tubers were taken in laboratory and after 20-25 days they were used for nutritional quality analysis**.**

3.2.2. Methods

The different methods used for the determination of nutritional quality of 44 potato cultivars are described below.

3.2.2.1. Determination of total sugar content

Total sugar content in the potato tuber was determined by the Anthrone method, as described in laboratory manual in Biochemistry (Jayaraman, 1981).

A. Chemicals: i) Anthrone, ii) Conc. H_2SO_4, iii) Glucose, iv) Water, v) Ethyl alcohol, vi) 80% alcohol.

B. Equipments: i) Volumetric flask, ii) Mortar and pestle, iii) Pipette, iv) Conical flask, v) Double layers of muslin cloths, vi) Steam bath, vii) Glass marbles, viii) Test tubes, ix) Whatman # 41 filter paper and x) Spectrophotometer (Model CECIL CE2021 SERIES 2000, CECIL Instruments Ltd., Cambridge, England).

C. Reagent preparation: i) Anthrone reagent: The anthrone reagent was prepared by dissolving 2 g of anthrone in 1 litre of concentrated H_2SO_4.

ii) Standard glucose solution: A standard solution of glucose was prepared by dissolving 10 mg glucose in 100 ml of distilled water.

D. Extraction of sugar from potato tubers: Extraction of sugar from potato tuber was done following the method described by Loomis and Shull (1937). Approximately 3 g of potato tuber were plunged into boiling ethyl alcohol and allowed to boil for 5-10 minutes (5 to 10 ml of alcohol was used/g of tuber). The extract was cooled and pasted thoroughly in a mortar with a pestle. Then the extract was filtered through double layers of muslin cloth and re-extracted the pasted tissue for three minutes in hot 80% alcohol, using 2 to 3 ml of alcohol/g of sample. Then, it treated as before. This second extraction ensured complete removal of alcohol soluble substances. The extract was cooled and passed through muslin cloth. Then both the extracts were mixed and filtered through Whatmann No-41 filter paper. The volume of the extract was evaporated to about $1/4^{th}$ the volume over a steam bath and cooled. This reduced volume of the extract was then transferred to a 100 ml volumetric flask and made up to the mark with distilled water. Then 1 ml of the diluted solution was taken into another 100 ml volumetric flask and made up to the mark with distilled water (working standard).

E. Procedure: Aliquot of 1 ml of the extract was pipette into test tubes and 4 ml of the anthrone reagent was added to each of this solution and mixed well. Glass marbles were placed on the top of each tube to prevent loss of water by evaporation. The test tubes were heated for 10 minutes in a boiling water bath and then cooled. A reagent blank was prepared by taking 1 ml of water and 4 ml of anthrone reagent in a tube and treated similarly. The absorbance of the blue-green solution was measured at 680 nm in a spectrophotometer. A standard curve of glucose was prepared by taking 0.0, 0.1, 0.2, 0.4, 0.6, 0.8 and 1 ml of standard glucose solution in different test tubes containing 0.0, 10, 20, 40, 60, 80 and 100 µg of glucose respectively and made the volume up to 1 ml with distilled water. Then 4 ml of anthrone reagent was added to each test tube and mixed well. Then it treated as before. The absorbance was measured at 680 nm using the blank containing 1 ml of water and 4 ml of anthrone reagent.

F. Calculation: The amount of total sugar content was calculated from the standard curve of glucose. Finally, the percentage of total sugar present in the potato tuber was determined using the formula given below:

Percentage of total sugar (mg/100 g of potato tuber)

$$= \frac{\text{Weight of total sugar obtained}}{\text{Weight of potato tuber}} \times 100$$

3.2.2.2. Determination of reducing sugar content

Reducing sugar content in the potato tuber was determined by the dinitrosalicylic acid method (Miller, 1959).

A. Chemicals: i) Dinitrosalicylic acid, ii) Crystalline phenol, iii) Sodium sulphite, iv) 1% NaOH, v) K-Na Tartarate (Rochelle salt), vi) Distilled water and vii) Standard glucose (0.1mg/ml).

B. Equipments: i) Beaker, ii) Test tubes, iii) Pipette and iv) Spectrophotometer.

C. Reagent Preparation: i) Dinitrosalicylic acid (DNS) reagent: Simultaneously 1g of DNS, 200 mg of crystalline phenol and 50 mg of sodium sulfite were placed in a beaker and mixed with 100 ml of 1% NaOH solution by stirring (If it is need to store then sodium sulphite must be added just before use).

ii) 40% solution of Rochelle salt: Approximately 40 g of Rochelle salt dissolved in 100 ml distilled water.

D. Extraction of reducing sugar from potato tuber: Reducing sugar extract from tubers was done by the procedure as described determination of total soluble sugar.

E. Procedure: Aliquot of 3 ml of the extract was pipette into test tubes and 3 ml of DNS reagent was added to each of this solution and mixed well. The test tubes were heated for 5 minutes in a boiling water bath. After developing the colour, 1 ml of 40% Rochelle salt was added when the solutions of the tubes were still warm. The test tubes were then cooled under a running tap water. A reagent blank was prepared by taking 3 ml of water and 3 ml of DNS reagent in a tube and treated similarly. The absorbance of the solutions was measured at 575 nm in a spectrophotometer.

A standard curve of glucose was prepared by taking 0.0, 0.3, 0.6, 1.2, 1.8, 2.4 and 3.0 ml of standard glucose solution in different test tubes containing 0.0, 30, 60, 120, 180, 240

and 300 µg of glucose respectively and made the volume up to 3 ml with distilled water. Then 3 ml of DNS reagent was added to each test tube and mixed well. Then it treated similarly. The absorbance was measured at 575 nm using the blank containing 3 ml of water, 3 ml of DNS reagent and 1 ml of 40% Rochelle salt.

F. Calculation: Percentage of reducing sugar (g/100 g of potato tuber).

$$= \frac{\text{Weight of reducing sugar obtained}}{\text{Weight of the sample}} \times 100$$

3.2.2.3. Estimation of non reducing sugar content

Non reducing sugar or sucrose content was determined by the following formula (Golder, 2000).

Percentage of non-reducing sugar = (% Total sugar - % Reducing sugar) × 0.95.

3.2.2.4. Determination of starch content

The starch content in the potato tuber was determined by the Anthrone method as described in laboratory manual in Biochemistry (Jayaraman, 1981).

A. Chemicals: i) Anthrone reagent (0.2% anthrone in conc. H_2SO_4), ii) Standard starch solution (0.1% in1M HCl), iii) 1M HCl, iv) Distilled water, v) Ethanol

B. Equipments: i) Mortar and Pestle, ii) Double layer of muslin cloth iii) Centrifuge machine, iv) Water bath, v) Volumetric flasks, vi) Test tubes, vii) Glass marbles and viii) Spectrophotometer.

C. Procedure: About 3 g of the potato tuber were cut into small pieces and homogenized well with 20 ml of water. It was then filtered through double layer of muslin cloth. To the filtrate, twice the volume of ethanol was added to precipitate the polysaccharide, mainly starch. After kept it overnight in cold, the precipitate was collected by centrifugation at 3,000 rpm for 15 minutes. The precipitate was then dried over a steam bath. Then 40 ml of 1M HCl was added to the dried precipitate and heated to about 70°C. Then it was transferred to a volumetric flask and diluted up to 100 ml with 1M HCl. Then 1 ml of diluted solution was taken in another 100 ml volumetric flask and made up to the mark with 1M HCl (working standard). Aliquot of 1 ml of the

extract of each treatment was pipette into test tubes and 4 ml of anthrone reagent was added to the solution of each tube and mixed well. Glass marbles were placed on top of each tube to prevent loss of water by evaporation. The tubes were placed in a boiling water bath for 10 minutes, then removed and cooled. A reagent blank was prepared by taking 1 ml of water and 4 ml of anthrone reagent in a test tube and treated as before. The absorbance of the blue-green solution was measured at 680 nm in a spectrophotometer.

A standard curve of glucose was prepared by taking 0.0, 0.1, 0.2, 0.4, 0.6, 0.8 and 1 ml of standard starch solution in different test tubes containing 0.0, 10, 20, 40, 60, 80 and 100 μg of starch respectively and made the volume up to 1 ml with 1M HCl. Then 4 ml of anthrone reagent was added to each test tube and mixed well. Then it treated similarly. The absorbance was measured at 680 nm using the blank containing 1 ml of 1M HCl and 4 ml of anthrone reagent. The amount of starch content in the potato tuber was calculated from standard curve of starch.

D. Calculation: Percentage of starch (g/100 g of potato tuber)

$$= \frac{\text{Weight of starch obtained}}{\text{Weight of the sample}} \times 100$$

3.2.2.5. Determination of soluble protein content

Soluble protein content in the potato tuber was determined following the method of Lowry *et al.* (1951).

A. Equipments: i) Whatman # 1 filter paper, ii) Spectrophotometer, iii) Microfuge tubes, iv) Pipette, v) Mortar and pestle, vi) Centrifuge machine and vii) Muslin cloth.

B. Reagents: i) 2% Na_2CO_3 solution in 0.1N NaOH (Approximately 0.4 g of NaOH dissolved in 100 ml of distilled water and then 2 g of Na_2CO_3 dissolved it),

ii) 0.5% CuSO4. $5H_2O$ in 1% Na-Ka tartarate (Approximately 1 g of Na-Ka tartarate dissolved in 100 ml of distilled water and then 0.5 g copper sulphate dissolved it)

iii) Folin-Ciocaltea's Reagent-FCR (diluted with equal volume of distilled water, just before used) and

iv) Protein standard (Approximately 10 mg of bovine serum albumin diluted in100 ml distilled water).

C. Extraction of potato tubers: About 5 g of the potato tuber were cut into small pieces and homogenized well with 5 ml of distilled water. It was the filtered through double layer of muslin cloth. The filtered was centrifuged at 3000 rpm for 15 minutes and the clear supernatant was transferred in a 50 ml volumetric flask and made up to mark with distilled water. Then 10 ml of diluted solution was taken in another 100 ml volumetric flask and made up to the mark with distilled water (working standard).Water was carefully added avoiding formation of foam.

D. Procedure: Reagents (i) and (ii) were mixed in the ratio of 50:1 and the reagent (iii) was diluted just before use. In 9 glass test tubes, 0.0, 0.1, 0.2, 0.3, 0.4, 0.5, 0.6 and 0.8 ml of the standard protein solution were taken and the volume was made up to 1 ml with distilled water. One (1) ml of the sample was taken in a test tube and a duplicate was made. To each of the tubes 5.0 ml of (i: ii) mixture was added and after 10 minutes 0.5 ml of FCR solution was added. Absorbance of the solutions was recorded after 30 minutes at 650 nm. A graph was drawn with the data obtained from the standards and the amount of protein in the sample was calculated from the graph.

E. Calculation: Percentage of soluble protein content (g/100 g of the potato tuber)

$$= \frac{\text{Amount of protein obtained}}{\text{Weight of potato tuber}} \times 100$$

3.2.2.6. Determination of vitamin c content

Vitamin C content in the potato tuber was determined by the titrimetric method (Bessey and king, 1933).

A. Equipments: i) Burette, ii) Centrifuge machine iii) Conical flask, iv) Volumetric flask, v) Muslin cloth, vi) Mortar and pestle and vii) Scalpel.

B. Chemicals: i) Ascorbic acid, ii) Metaphosphoric acid, iii) Acetic acid, iv) 2, 6-dichlorophenol indophenols, v) Sodium bicarbonate and vi) Distilled water.

C. Reagent preparation: i) Dye solution: Approximately 200 mg of 2, 6-dichlorophenol indophenol dissolved in water and then 210 mg of sodium bicarbonate dissolved it and made up to 1000 ml with water. The solution was then filtered.

ii) 3% metaphosphoric acid reagent: Approximately 3 g of metaphosphoric acid was dissolved in 20 ml of distilled water and then made up to 100 ml with 80 ml of acetic acid.

iii) Standard solution of vitamin C (0.1mg/ml): Approximately 10 mg of ascorbic acid was dissolved in 3% metaphosphoric acid and made up to 100 ml with 3% metaphosphoric acid.

D. Procedure: Approximately 10 ml of standard ascorbic acid solution was taken in a conical flask and titrate it with prepared dye solution from a burette. The titration was terminated by the appearance of a permanent pink color in the titration medium. The operation was repeated for two times and burette readings were recorded each time.

About 02 to 04 g sample of the potato tubers were cut into small pieces and homogenized well with 3% metaphosphoric (approximately 20 ml) and filtered it through double layer of muslin cloth. The filtered was centrifuged at 6000 rpm for 10 minutes and the clear supernatant was transferred in a 100 ml volumetric flask and made up to mark by 3% metaphosphoric acid which called mother stock solution. 10 ml of this solution was taken in to a conical flask and titrated with the dye solution. The operation was repeated for two times and reading was taken for each time. The amount of vitamin C present in the extract was determined by comparing with the titration result of standard vitamin C solution.

E. Calculation: Calculate the vitamin C content of the extract by using the following formula:

$$\frac{I \times S \times D \times 100}{A \times W} = \text{mg of ascorbic acid/100 g of potato tuber}$$

Where, I = ml of indophenol reagent used in the titration; S = mg of ascorbic acid reacting with 1 ml of the reagent; D = total volume of the extract in ml; A = the aliquot titrated in ml, (in titration used extract) and W = the weight of the sample in g.

3.2.2.7. Determination of β-carotene content

β-carotene content in the potato tuber was determined according to the procedure reported in the methods of vitamin assay (Anonymous, 1960) and methods of biochemical analysis (Glick, 1957)

A. Chemicals: i) β-carotene, ii) Ammonium sulphate, iii) Acetone, iv) Petroleum ether (40-60°C), v) n-hexane, vi) Activated alumina or aluminium oxide anhydrous (Al_2O_3) and vii) KOH.

B. Equipments: i) Mortar and pestle, ii) Pipette, iii) Conical flask, iv) Separating funnel, v) Column and vi) Spectrophotometer.

C. Reagent preparation: i) 5.6% potassium hydroxide solution: Approximately 5.6 g of KOH dissolve in 100 ml distilled water.

ii) Standard solution of β -carotene: Standard solution of β -carotene was prepared by dissolving 2 mg of β -carotene in 100 ml of petroleum ether.

iii) 10% acetone in petroleum ether: Aliquot of 10 ml acetone mixed with 90 ml of petroleum ether.

iv) Column preparation: A column (40 × 2.5 cm) was prepared by using activated alumina as a packing material. 10% acetone in petroleum ether was used as eluant buffer.

D. Procedure: About 10 g of potato tuber and 8 g of ammonium sulphate were taken in a mortar, and rubbed to an even paste with pestle. The extraction was carried out with acetone (12 ml) and small amount of hexane (8 ml). Extraction was continued until the acetone extract became colorless. Then the extraction was filtered by double layer of muslin cloth. Aliquot of 10 ml of potassium hydroxide solution (5.6%) was added to the filtrate extract and mixed well. Then it was kept in a dark place for half an hour. The mixture was then transferred to a separating funnel. 20 ml of petroleum ether, a few (3-5) ml of hexane and 10 ml of water were added to the separating funnel and shacked gently. The ether layer (upper layer) was collected and the process was repeated until the petroleum ether layer became colorless. After the ether layer collection, its volume was measured. The extract was equal volume before applied onto the top of the column with

ether.The concentrated extract (2 ml) was applied onto the top of the alumina column and eluted with 10% acetone in petroleum ether. The absorbance of the eluant was taken at 440 nm in a spectrophotometer.

E. Construction of standard curve of β-carotene: A standard curve was prepared by taking 0.0, 0.1, 0.2, 0.4, 0.6, 0.8 and 1.0 ml standard solution of β-carotene and the volume was made up to 2 ml with petroleum ether and mixed well. The absorbance of the solutions was taken at 440 nm in a spectrophotometer and a standard curve of β-carotene was prepared by plotting the data. The amount of β-carotene content in each variety of potato was calculated by using the standard curve of β-carotene.

F. Calculation: Amount of β -carotene in the potato tuber (μg/100 g of potato tuber)

$$= \frac{\text{Amount of } \beta\text{ - carotene obtained}}{\text{Weight of potato tuber}} \times 100$$

3.2.2.8. Determination of phenolic compounds content

Phenolic compounds or total phenol content in the potato tuber was determined spectrophotometrically by Folin-Ciocaltea's method (Bray and Thorpe, 1954).

A. Reagents: i) Folin- Ciocalteu's Reagent; ii) Na_2CO_3, 2% and iii) Catechol (0.1 mg/ml distilled water)

B. Extraction of phenol from potato tubers: Extraction of phenol from potato tuber was done following the methods described by Loomis and Shull (1937). About 6 g of potato tuber were plunged into boiling ethyl alcohol and allowed to boil for 5-10 minutes (5-10 ml of alcohol was used/g of tuber). The extract was cooled and crushed thoroughly in a mortar with a pestle. Then the extract was filtered through double layers of muslin cloth and re-extracted the pasted tissue for three minutes in hot 80% alcohol, using 2-3 ml of alcohol/g of tissue. Then, it treated as before. This second extraction ensured complete removal of alcohol soluble substances. The extract was cooled and passed through muslin cloth. Then both the extracts were mixed and filtered through Whatmann No-41 filter paper. This alcohol extract was used for the estimation of total phenol.

C. Procedure: Aliquot of 1 ml of the extract and 1 ml of Folin-Ciocalteu's reagent (FCR) was pipette into test tubes followed by addition of 2 ml of sodium carbonate solution (2%) and mixed well. The test tubes were heated for 2 minutes in a boiling water bath and then cooled. The blue solution was transferred to a 25 ml volumetric flask and made up to the mark with distilled water. Then the solution was filtered. A reagent blank was prepared by taking 1 ml of water and 1ml of FCR in a tube and treated similarly. The absorbance of the blue solution was measured at 650 nm in a spectrophotometer.

A standard curve of catechol was prepared by taking 0.0, 0.1, 0.2, 0.3, 0.4, 0.5 and 0.6 ml of standard catechol solution in different test tubes containing 0.0, 10, 20, 30, 40, 50 and 60 μg of catechol respectively and made the volume up to 1 ml with distilled water. Then 1 ml of FCR was added to each test tube followed by addition of 2 ml of sodium carbonate solution (2%) and mixed well. Then it treated as before. The absorbance was measured at 650 nm using the blank containing 1 ml of water and 1 ml of FCR and treated similarly.

D. Calculation: The amount of total phenol content was calculated from the standard curve of catechol. Finally, the percentage of total phenol present in the potato tuber was determined using the formula given below:

Percentage of total phenol (mg/100 g of potato tuber)

$$= \frac{\text{Weight of total phenol obtained}}{\text{Weight of potato tbuber}} \times 100$$

3.2.2.9. Determination of minerals and heavy metals content

The minerals content in the potato tuber was determined by the atomic absorption spectrophotometric method (described by Anonymous, 2009 and Robert *et al*., 1987).

A. Equipments: i) Electric balance, ii) Scalpel, iii) Mortar and pestle, iv) Pipette, v) Test tubes, vi) Water bath, vii) Volumetric flasks, viii) Whatman No 41 filter paper, ix) Round test tube holders and x) Atomic absorption spectrophotometer.

B. Chemicals: i) HNO_3, ii) Perchloric acid ($HClO_4$), iii) Minerals viz. K, Ca, Fe, Zn, Cu, Mn, Cd, As, Pb, Cr and Co and iv) Deionized distilled water.

C. Standards: The standards were prepared from the individual 1000 mg/l standards (Merck) supplied in de-ionized distilled water. A series of working standards were prepared from these standard stock solutions.

D. Digestion of the samples: About 50 g of potato tuber was taken from each cultivar and all samples were washed with tape water followed with de-ionized distilled water. Samples were cut into small pieces and sun dried on a concrete floor for about 12 hours. After drying the samples were ground into powder form. Approximately 0.5 g of each sample in duplicate taken into digestion tubes and to each of the tubes 5 ml of nitric acid and 2.5 ml of perchloric acid (followed by 2:1 ratio) was added and mixed well. The test tubes were heated to about 100°C for 10 -12 hours in boiling water bath and cooled. Then 2.5 ml of nitric acid was further added to each of the tubes and heated to about 90°C for 3-4 hours in a boiling water bath till the solutions becomes transparent (water colour). The solutions were cooled and filtered through Whatmann No 41 filter paper and made up to 100 ml with de-ionized distilled water (working standard). All glasswares including digestion tubes were soaked with 30% HNO_3 for 8 hours and finally washed with de-ionized distilled water.

E. Procedure: Aliquot of 14 ml of the solution of each treatment was pipette into test tubes. A reagent blank was prepared by taking 14 ml of de-ionized distilled water in a test tube. The absorbance and concentrations (ppm) of the solutions were measured at 769.9 (K), 422.67 (Ca), 327.40 (Cu), 346.58 (Co), 288.80 (Cd), 425.44 (Cr), 371.99 (Fe), 403.08 (Mn), 307.59 (Zn), 197.20 (As) and 283.30 (Pb) nm in an atomic absorption spectrophotometer.

The amount of minerals content in the potato tuber was calculated from concentrations (ppm) of the solutions.

F. Calculation: The percentage of minerals (mg/100 g of fresh potato tuber)

$$= \frac{C \times V \times P \times TPW}{W \times Mg \times P} \times DF$$

Where, C = Concentrations (ppm) of the solutions; V = Volume of stock solutions; P = Percentage; W = Weight (g) of used powder; Mg = Miligram factor; DF = Dilution factor and TPW= Total powder weight (in g) obtained from 100 g fresh tuber.

3.2.2.10. Determination of moisture content

Moisture content was determined by the conventional procedure (Karmas, 1980).

A. Materials: i) Porcelain crucible, ii) Electric balance, iii) Oven and iv) Desiccators.

B. Procedure: About 50 g of potato tuber was weighed in a porcelain crucible which was previously cleaned, heated to 70ºC, cooled and weighed. The crucible with the sample was heated in an electric oven for about 48 hours at 70ºC. It was then cooled in desiccators and weighed again.

C. Calculation: Percentage of moisture content = $\frac{\text{Amount of moisture}}{\text{Weight of potato tuber}} \times 100$

3.2.2.11. Determination of dry matter content

Dry matter content was determined from the data obtained for percentage of moisture content.

3.2.2.12. Determination of ash content

Ash content was determined following the method of A. O. A. C. (1980).

A. Materials: i) Porcelain crucible, ii) Balance, iii) Muffle furnace and iv) Desiccators.

B. Procedure: About 50 g of potato tuber was weighed in a porcelain crucible which was previously cleaned, heated to 100ºC, cooled and weighed. The crucible was placed in a muffle furnace for about 4 hours at 600ºC. It was then cooled in desiccators and weighed. To ensure completion of ashing, the crucible was again heated in the muffle furnace for half an hour, cooled and weighed again. This was repeated till two consecutive weights were the same and the ash was almost white in colour.

C. Calculation: Percentage of ash content

$$= \frac{\text{Amount of ash obtained}}{\text{Weight of potato tuber}} \times 100$$

3.2.2.13. Measurement of specific gravity

Specific gravity was determined by using the following equation (Nissen, 1955).

$$Y = 214\ (V - 0.988)$$

Where, Y = Dry matter content in 100 g of potato tuber and

V = Specific gravity.

3.3. RESULTS

Biochemical analysis in order to determine the nutritional status of the freshly harvested potato tubers of 44 cultivars was carried. The biochemical analysis included starch, sugars, soluble protein, vitamins, phenolics, minerals and heavy metals. Dry matter content, moisture content and specific gravity of 44 potato cultivars were also determined for the evaluation of processing quality of the tubers. Details of the results so far obtained from each of the experiments are described under the different heads.

3.3.1. Starch Content (%)

Potato cultivars under investigation were significantly different in total content of starch **(Table 3.1).** The starch contents of the cultivars ranged from 65% to 75.53% (mean of 71.35%) on the basis of dry weight and 10.60% to 20.23 % (mean of 15.72%) on the basis of fresh weight. On the basis of dry weight, higher amount of starch was recorded from JPR (75.53%) followed by Jam Alu (75.5%) while the lowest amount of starch was noted from Lara (65%). In contrast, on the basis of fresh weight, higher starch content was estimated in cultivar Hagri (20.23%) followed by JPR (20.12%). The lowest amount of starch was noticed in Ultra (10.6%).

3.3.2. Total Sugars Content (%)

Significant difference was found in terms of total sugar in potato cultivars **(Table 3.1).** The genotype Banana had the maximum total sugar (2.04 %) followed by Lal Pakri (1.98 %) both being at par. The genotype Lady Rosetta had the lowest value of total sugar (0.77%). The mean of total sugar in the cultivars was 1.36 %. Total sugar ranged from 1.14 to 1.67% for most of the cultivars.

3.3.3. Reducing Sugars Content (%)

Significant difference was recorded among the genotypes with respect to the reducing sugar percentage. Maximum reducing sugar (0.89%) was recorded from Chipita followed by Shaita Red having 0.88% reducing sugars and the variation was insignificant**. Table 3.1** also revealed that reducing sugars ranged from 0.89% in Chipita to 0.32% in Courage, while the other genotypes were between these two limits.

3.3.4. Non- reducing Sugars Content (%)

It is evident from **Table 3.1** that all the genotypes were significantly different in terms of non reducing sugar. The highest percentage (1.26%) of non reducing sugar was noticed in cultivar Banana followed by Cardinal having 1.25% non-reducing sugar, while statistically similar lowest value (0.35%) was recorded in genotypes All Blue and Lady Rosetta.

Table 3.1. Starch, total sugars (TS), reducing sugar (RS) and non-reducing sugar (NRS) content in 100 g tuber of 44 potato cultivars.

Cultivars	% starch in DM	Fresh weight basis			
		% starch	% TS	% RS	% NRS
Kenne	71.86 ± 0.49	15.24 ± 0.14	1.19 ± 0.051	0.58 ± 0.061	0.57 ± 0.034
All Blue	72.40 ± 0.23	15.97 ± 0.14	0.83 ± 0.034	0.45 ± 0.064	0.35 ± 0.024
Granola	70.02 ± 0.57	13.73 ± 0.30	1.16 ± 0.057	0.41 ± 0.048	0.70 ± 0.023
Shepody	72.03 ± 0.57	15.46 ± 0.36	1.46 ± 0.069	0.79 ± 0.059	0.63 ± 0.029
L. Rosetta	74.63 ± 0.36	18.21 ± 0.50	0.77 ± 0.063	0.40 ± 0.049	0.35 ± 0.044
Patrones	73.39 ± 0.22	16.78 ± 0.75	1.59 ± 0.025	0.52 ± 0.043	1.01 ± 0.043
Courage	73.58 ± 0.33	17.12 ± 0.50	0.99 ± 0.051	0.32 ± 0.046	0.63 ± 0.051
Call White	71.57 ± 0.32	14.98 ± 0.57	1.62 ± 0.092	0.49 ± 0.057	1.07 ± 0.038
Banana	74.43 ± 0.24	17.98 ± 0.46	2.04 ± 0.089	0.70 ± 0.061	1.26 ± 0.131
Quiency	68.84 ± 0.48	12.79 ± 0.42	1.30 ± 0.046	0.64 ± 0.085	0.62 ± 0.067
Baraka	72.63 ± 0.36	16.09 ± 0.51	1.31 ± 0.063	0.45 ± 0.050	0.82 ± 0.059
Blondy	74.00 ± 0.57	16.50 ± 0.75	1.61 ± 0.132	0.53 ± 0.058	1.02 ± 0.100
JPR	75.53 ± 0.30	20.12 ± 0.27	1.43 ± 0.112	0.55 ± 0.086	0.83 ± 0.072
S. White	69.21 ± 0.12	13.09 ± 0.23	1.28 ± 0.080	0.64 ± 0.055	0.61 ± 0.036
Ultra	65.31 ± 0.17	10.60 ± 0.46	1.30 ± 0.127	0.74 ± 0.058	0.53 ± 0.051
Febula	73.43 ± 0.24	16.85 ± 0.20	1.25 ± 0.141	0.62 ± 0.049	0.59 ± 0.060
Chieftain	69.61 ± 0.35	13.35 ± 0.42	0.95 ± 0.069	0.46 ± 0.046	0.46 ± 0.060
Chipita	75.47 ± 0.06	19.24 ± 0.52	1.37 ± 0.138	0.89 ± 0.086	0.45 ± 0.040
Innovator	71.46 ± 0.26	15.50 ± 0.75	1.51 ± 0.012	0.40 ± 0.046	1.05 ± 0.119
Jam Alu	75.50 ± 0.57	19.36 ± 0.36	1.14 ± 0.095	0.54 ± 0.041	0.57 ± 0.073
Akhira	69.91 ± 0.57	13.59 ± 0.34	0.80 ± 0.011	0.39 ± 0.034	0.38 ± 0.045
Dumini	72.15 ± 0.37	15.53 ± 0.48	1.38 ± 0.025	0.55 ± 0.058	0.79 ± 0.055
Shilbilati	70.30 ± 0.17	14.69 ± 0.36	1.30 ± 0.115	0.62 ± 0.062	0.64 ± 0.069
Mondial	71.24 ± 0.14	14.69 ± 0.40	0.92 ± 0.109	0.40 ± 0.042	0.49 ± 0.049
Bellini	70.00 ± 0.5	14.64 ± 0.13	1.38 ± 0.121	0.42 ± 0.020	0.91 ± 0.037
Atlanta	75.14 ± 0.57	18.89 ± 0.57	1.14 ± 0.043	0.54 ± 0.045	0.57 ± 0.053
Hagri	73.13 ± 0.57	20.23 ± 0.43	1.77 ± 0.005	0.63 ± 0.043	1.08 ± 0.081
Challisa	74.00 ± 0.40	17.48 ± 0.56	1.23 ± 0.05	0.59 ± 0.056	0.60 ± 0.040

Contd...

Cultivars	% starch in DM	Fresh weight basis			
		% starch	% TS	% RS	% NRS
All Red	73.06 ± 0.31	16.52 ± 0.56	0.85 ± 0.069	0.42 ± 0.040	0.40 ± 0.027
Deshi-5	68.92 ± 0.28	12.85 ± 0.23	1.78 ± 0.127	0.54 ± 0.044	1.17 ± 0.084
Lara	65.00 ± 0.57	12.06 ± 0.27	1.55 ± 0.069	0.54 ± 0.051	0.96 ± 0.049
Asterix	69.47 ± 0.27	16.22 ± 0.33	1.52 ± 0.109	0.40 ± 0.056	1.06 ± 0.108
Atlas	66.63 ± 0.38	11.65 ± 0.11	1.61 ± 0.066	0.42 ± 0.061	1.13 ± 0.056
Lal Pakri	69.30 ± 0.17	15.86 ± 0.42	1.98 ± 0.029	0.7 ± 0.061	1.21 ± 0.032
Voyager	73.06 ± 0.43	16.47 ± 0.85	1.27 ± 0.086	0.60 ± 0.042	0.63 ± 0.029
Shaita Red	74.14 ± 0.57	17.64 ± 0.20	1.73 ± 0.115	0.88 ± 0.030	0.81 ± 0.065
Marabel	72.02 ± 0.40	15.47 ± 0.47	1.57 ± 0.083	0.45 ± 0.043	1.06 ± 0.131
Diamant	69.37 ± 0.21	16.18 ± 0.47	1.04 ± 0.098	0.37 ± 0.037	0.63 ± 0.056
Indurkani	70.02 ± 0.34	16.98 ± 0.13	1.03 ± 0.031	0.45 ± 0.068	0.55 ± 0.061
Gaforgaon	70.21 ± 0.57	16.93 ± 0.15	1.21 ± 0.017	0.60 ± 0.080	0.57 ± 0.045
B.Mountain	66.51 ± 0.29	11.28 ± 0.16	1.33 ± 0.109	0.62 ± 0.060	0.67 ± 0.044
Rodeo	70.56 ± 0.32	15.68 ± 0.11	1.67 ± 0.080	0.62 ± 0.041	0.99 ± 0.046
Martin	69.46 ± 0.26	15.01 ± 0.34	1.77 ± 0.063	0.50 ± 0.046	1.20 ± 0.096
Cardinal	71.07 ± 0.32	16.12 ± 0.35	1.81 ± 0.043	0.59 ± 0.043	1.25 ± 0.024
Mean	71.35	15.72	1.36	0.54	0.77
LSD at 5%	1.107	1.213	0.231	0.157	0.251
Value of coefficient of correlation (r) between starch and specific gravity : 0.987					

*L. Rosetta = Lady Rosetta; *S. White = Shaita White; *B. Mountain = Blue Mountain

3.3.5. Soluble Protein Content (%)

The statistical analysis for soluble protein content of different potato genotypes showed significant variation. The highest amount of soluble protein was found in Indurkani (2.58%) followed by Diamant (2.56%). The lowest amount of soluble protein was observed in cultivar Call White (0.67%). The average soluble protein percentage in potato tuber was 1.54 % and range was 0.67% to 2.58% **(Table 3.2).**

3.3.6. Vitamin C Content (mg/100 g)

Potato is considered to be a good source of vitamin C or ascorbic acid. Wide range of variation was observed among the cultivars in respect of vitamin C content **(Table 3.2).** The highest amount of vitamin C was obtained from Jam Alu (21.47) followed by Chieftain (20.67) and All Red (20.61) and the lowest amount of vitamin C was recorded from Atlas (12.44). The average amount of vitamin C was 15.64 mg and the range was 12.44 – 21.47 mg/100 g fresh tubers.

3.3.7. β-carotene Content (µg/100 g)

Significant variation was also existed in β-carotene content among the different potato cultivars. The highest amount of β- carotene was found in All Red (61.58 µg) which was followed by JPR (57.66 µg) and Akhira (29.48 µg). Rest of the potato cultivars showed either no or very negligible (trace) amount of β-carotene **(Table 3.2).**

3.3.8. Phenolics Content (mg/100 g)

Significant variation was noticed for phenolics content among the different potato cultivars. The highest amount of phenolics content was found in Indurkani (79.4 mg) followed by Gaforgaon (78.41 mg). The lowest amount of phenolics content was observed in cultivar Kenne (21.5 mg). The average phenolics content in potato tuber was 31.68 mg and range was 21.5 mg to 79.8 mg **(Table 3.2)**. Generally, purple and red fleshed cultivars contained higher amounts of phenolics than cultivars with a cream or white flesh.

Table 3.2. Vitamin C, β-carotene and phenolics contents in 100 g fresh tuber of 44 potato cultivars.

Cultivars	% protein	Vitamin-C (mg)	β-carotene (µg)	Phenolics (mg)
Kenne	1.26 ± 0.058	14.10 ± 0.12	trace	21.50 ± 0.288
All Blue	1.03 ± 0.052	13.23 ± 0.17	-	68.26 ± 0.819
Granola	1.62 ± 0.083	15.16 ± 0.35	trace	30.35 ± 0.350
Shepody	1.38 ± 0.070	16.22 ± 0.43	trace	23.45 ± 0.296
Lady Rosetta	1.08 ± 0.059	14.54 ± 0.26	trace	22.34 ± 0.406
Patrones	1.00 ± 0.053	15.73 ± 0.15	trace	29.59 ± 0.352
Courage	1.39 ± 0.073	13.47 ± 0.29	trace	32.47 ± 0.318
Call White	0.67 ± 0.035	15.02 ± 0.32	trace	22.18 ± 0.621
Banana	0.94 ± 0.048	16.61 ± 0.21	trace	24.47 ± 0.318
Quiency	1.26 ± 0.064	16.30 ± 0.17	trace	27.73 ± 0.215
Baraka	1.35 ± 0.068	14.09 ± 0.31	trace	29.44 ± 0.294
Blondy	1.26 ± 0.062	15.61 ± 0.30	trace	26.98 ± 0.187
JPR	1.77 ± 0.090	18.47 ± 0.27	57.66 ± 0.24	44.54 ± 0.655
Shaita White	1.48 ± 0.075	13.23 ± 0.39	trace	32.38 ± 0.310
Ultra	1.12 ± 0.057	14.53 ± 0.46	trace	25.30 ± 0.358
Febula	1.22 ± 0.061	15.20 ± 0.25	trace	29.40 ± 0.221
Chieftain	1.58 ± 0.079	20.67 ± 0.53	trace	22.46 ± 0.413
Chipita	1.10 ± 0.057	14.39 ± 0.22	trace	26.76 ± 0.144
Innovator	1.90 ± 0.097	19.66 ± 0.39	trace	41.56 ± 0.554
Jam Alu	1.27 ± 0.069	21.47 ± 0.45	trace	29.2 ± 0.435
Akhira	1.62 ± 0.082	14.42 ± 0.31	29.48 ± 0.36	32.38 ± 0.658
Dumini	1.19 ± 0.059	15.29 ± 0.41	trace	27.77 ± 0.467
Shilbilati	1.65 ± 0.083	17.82 ± 0.38	trace	23.78 ± 0.148
Mondial	1.40 ± 0.071	16.32 ± 0.36	trace	27.61 ± 0.310
Bellini	1.59 ± 0.084	14.02 ± 0.29	trace	28.12 ± 0.591
Atlanta	1.70 ± 0.084	15.38 ± 0.35	trace	22.44 ± 0.294
Hagri	2.24 ± 0.104	14.43 ± 0.17	trace	27.02 ± 0.577
Challisa	1.48 ± 0.073	16.08 ± 0.12	trace	27.06 ± 0.238
All Red	1.79 ± 0.092	20.61 ± 0.12	61.58 ± 0.38	34.40 ± 0.377

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Cultivars	% protein	Vitamin-C (mg)	β-carotene (μg)	Phenolics (mg)
Deshi-5	1.31 ± 0.065	15.25 ± 0.12	trace	28.42 ± 0.479
Lara	1.77 ± 0.091	19.58 ± 0.12	21.43 ± 0.29	33.78 ± 0.717
Asterix	2.07 ± 0.108	13.5 ± 0.12	-	27.25 ± 0.387
Atlas	1.39 ± 0.071	12.44 ± 0.12	trace	26.32 ± 0.355
Lal Pakri	1.91 ± 0.096	17.98 ± 0.12	trace	34.53 ± 0.370
Voyager	1.61 ± 0.080	16.24 ± 0.12	trace	28.54 ± 0.291
Shaita Red	1.70 ± 0.088	15.98 ± 0.12	trace	31.38 ± 0.312
Marabel	1.37 ± 0.065	14.39 ± 0.12	trace	29.42 ± 0.395
Diamant	2.56 ± 0.180	18.48 ± 0.12	trace	26.09 ± 0.584
Indurkani	2.58 ± 0.182	13.24 ± 0.12	-	79.4 ± 0.305
Gaforgaon	2.11 ± 0.107	12.54 ± 0.12	-	78.41 ± 0.838
Blue Mountain	1.42 ± 0.073	13.58 ± 0.12	trace	23.13 ± 0.589
Rodeo	1.84 ± 0.098	14.33 ± 0.12	trace	28.61 ± 0.310
Martin	2.06 ± 0.112	13.25 ± 0.12	trace	27.60 ± 0.302
Cardinal	1.54 ± 0.080	15.56 ± 0.12	trace	30.06 ± 0.233
Mean	1.54	15.64	-	31.68
LSD at 5%	0.23	0.911	-	0.221

3.3.9. Ash Content (%)

Mineral substances, called ashes, average 1.054 % in potato tubers. The results regarding ash content of different potato cultivars are given in **Table 3.3** which showed that the ash content of genotypes differed significantly. The range of ash content in potato tuber was 0.788 to 1.386 %. The ash content was found the highest (1.386%) in cultivar Jam Alu which closely related to Chipita (1.365%). The potato genotype Ultra showed the lowest ash content (0.788%).

3.3.10. Potassium (K) Content (mg/100 g)

The content of K in potatoes is high. Different cultivars showed significant variation in respect of K contents. The range of K contents in the cultivars was 173.89 - 908.19 mg/100 g and average K content of the cultivars was 417.88 mg **(Table 3.3).** Ultra (173.89 mg) was the lowest K containing cultivar. JPR was the highest K containing cultivar (908.19 mg) followed by Banana (903.39 mg) and Quiency (901.64 mg) and the mean differences of these cultivars were insignificant.

3.3.11. Calcium (Ca) Content (mg/100 g)

Wide variation was observed among the cultivars in respect of Ca contents. Maximum amount of Ca was found in Baraka (44.41 mg) followed by Rodeo (42.44) and Call White (39.58 mg) while the lowest amount of Ca was recorded from Mondial (8.42 mg). The Ca contents of the potato cultivars ranged from 8.42 to 44.41 mg and average Ca content of the cultivars was 18.57 mg (**Table 3.3).**

3.3.12. Iron (Fe) Content (mg/100 g)

Potato is the modest source of Fe. Of all the micronutrients, Fe is required by plants in the largest amount. Fe contents of the cultivars investigated significantly differed. The Fe content of the potato cultivars ranged from 0.62 to 1.97 mg. Diamant (0.62 mg) was the lowest Fe containing cultivar while Indurkani (1.97 mg) and All Red (1.96 mg) were the similar higher Fe containing cultivars. The mean of Fe content of the cultivars was 1.40 mg **(Table 3.3).**

Table 3.3. Ash, K, Ca and Fe content in 100 g fresh tuber of 44 potato cultivars.

Cultivars	% ash	K(mg)	Ca(mg)	Fe(mg)
Kenne	0.960 ± 0.014	463.02 ± 0.13	8.59 ± 0.30	1.62 ± 0.063
All Blue	1.026 ± 0.032	229.06 ± 0.28	9.52 ± 0.28	1.92 ± 0.060
Granola	0.930 ± 0.038	701.27 ± 0.64	8.50 ± 0.28	1.51 ± 0.075
Shepody	0.981 ± 0.020	639.24 ± 0.38	9.49 ± 0.28	1.96 ± 0.063
Lady Rosetta	1.007 ± 0.039	629.19 ± 0.39	8.99 ± 0.57	1.90 ± 0.057
Patrones	1.040 ± 0.018	314.2 ± 0.34	12.46 ± 0.29	1.43 ± 0.063
Courage	1.078 ± 0.030	736.07 ± 0.29	17.52 ± 0.28	1.46 ± 0.066
Call White	0.962 ± 0.022	299.29 ± 0.40	39.58 ± 0.30	0.97 ± 0.054
Banana	1.060 ± 0.063	903.39 ± 0.45	11.37 ± 0.31	1.36 ± 0.066
Quiency	0.924 ± 0.060	901.64 ± 0.32	12.52 ± 0.29	1.62 ± 0.066
Baraka	1.089 ± 0.066	632.28 ± 0.31	44.41 ± 0.30	1.87 ± 0.054
Blondy	1.202 ± 0.092	359.02 ± 0.19	27.55 ± 0.29	1.50 ± 0.054
JPR	1.140 ± 0.019	908.19 ± 0.43	12.17 ± 0.16	1.47 ± 0.054
Shaita White	0.859 ± 0.015	242.64 ± 0.32	11.46 ± 0.29	0.81 ± 0.023
Ultra	0.788 ± 0.042	173.89 ± 0.58	8.55 ± 0.47	1.86 ± 0.028
Febula	1.057 ± 0.026	410.59 ± 0.30	26.52 ± 0.28	1.36 ± 0.023
Chieftain	0.921 ± 0.043	834.50 ± 0.28	15.56 ± 0.29	0.97 ± 0.034
Chipita	1.365 ± 0.138	691.29 ± 0.20	15.46 ± 0.29	1.18 ± 0.031
Innovator	1.292 ± 0.193	462.47 ± 0.28	13.36 ± 0.31	0.93 ± 0.017
Jam Alu	1.386 ± 0.147	222.44 ± 0.29	22.53 ± 0.29	1.66 ± 0.034
Akhira	1.036 ± 0.103	260.61 ± 0.31	10.39 ± 0.30	1.26 ± 0.017
Dumini	1.308 ± 0.045	210.49 ± 0.28	12.50 ± 0.28	1.28 ± 0.023
Shilbilati	1.192 ± 0.156	224.61 ± 0.31	22.39 ± 0.30	1.90 ± 0.034
Mondial	1.006 ± 0.056	193.39 ± 0.30	8.42 ± 0.29	0.86 ± 0.037
Bellini	1.033 ± 0.063	260.44 ± 0.29	31.35 ± 0.32	1.18 ± 0.023
Atlanta	1.174 ± 0.038	437.37 ± 0.31	18.52 ± 0.26	1.72 ± 0.028
Hagri	1.217 ± 0.063	316.60 ± 0.30	34.11 ± 0.48	1.92 ± 0.043
Challisa	1.094 ± 0.030	279.56 ± 0.29	10.40 ± 0.30	1.42 ± 0.014
All Red	1.072 ± 0.044	199.63 ± 0.31	25.56 ± 0.29	1.96 ± 0.020
Deshi-5	0.909 ± 0.050	190.18 ± 0.15	29.43 ± 0.29	1.13 ± 0.023

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Cultivars	% ash	K(mg)	Ca(mg)	Fe(mg)
Lara	0.924 ± 0.062	401.48 ± 0.28	14.23 ± 0.14	0.92 ± 0.028
Asterix	1.025 ± 0.014	191.69 ± 0.55	21.55 ± 0.24	0.91 ± 0.026
Atlas	0.954 ± 0.099	610.24 ± 0.14	14.54 ± 0.24	1.91 ± 0.047
Lal Pakri	1.116 ± 0.062	366.65 ± 0.32	23.50 ± 0.28	1.31 ± 0.049
Voyager	1.066 ± 0.041	174.59 ± 0.30	17.44 ± 0.29	1.04 ± 0.014
Shaita Red	1.045 ± 0.025	696.58 ± 0.29	13.51 ± 0.28	1.22 ± 0.043
Marabel	0.970 ± 0.014	775.61 ± 0.31	15.56 ± 0.29	1.15 ± 0.028
Diamant	1.024 ± 0.014	209.63 ± 0.31	14.40 ± 0.30	0.62 ± 0.060
Indurkani	1.080 ± 0.046	344.62 ± 0.31	20.42 ± 0.29	1.97 ± 0.011
Gaforgaon	1.064 ± 0.037	314.28 ± 0.14	23.43 ± 0.29	1.92 ± 0.023
Blue Mountain	0.985 ± 0.077	238.37 ± 0.31	15.65 ± 0.32	1.39 ± 0.025
Rodeo	0.989 ± 0.023	213.45 ± 0.29	42.44 ± 0.29	1.13 ± 0.017
Martin	1.007 ± 0.031	279.60 ± 0.30	19.56 ± 0.29	1.24 ± 0.031
Cardinal	1.013 ± 0.013	243.66 ± 0.33	21.49 ± 0.28	0.98 ± 0.020
Mean	1.054	417.88	18.57	1.40
LSD at 5%	0.187	0.963	0.867	0.118

3.3.13. Zink (Zn) Content (mg/100 g)

Analysis of variance revealed that, the cultivars studied significantly differed in terms of Zn content. The highest Zn content (1.21 mg) was determined in the cultivar Lady Rosetta followed by Patrones (0.99 mg) and JPR (0.92 mg). Among the cultivars, Cardinal had the lowest Zn content (0.23 mg). The range and mean of Zn contents of the cultivars were 0.23-1.21 mg and 0.54 mg respectively **(Table 3.4).**

3.3.14. Manganese (Mn) Content (mg/100 g)

In this study there were differences with regard to Mn contents among the potato cultivars **(Table 3.4).** The range and mean of Mn contents of the cultivars were 0.084-0.681 mg and 0.229 mg respectively. The cultivars Kenne and Courage (0.681 and 0.449 mg respectively), were the cultivars having the highest Mn contents. In contrast, lowest amount of Mn was detected in the cultivars Chieftain (0.084 mg).

3.3.15. Copper (Cu) Content (mg/100 g)

As can be seen in the **Table 3.4** the average Cu content significantly differed among the potato cultivars. Blondy had the highest Cu content (0.416 mg) followed by Banana (0.333 mg). The lowest Cu value was obtained from Akhira (0.09 mg). The range and mean of Cu contents of the cultivars were 0.09-0.416 mg and 0.167 mg respectively.

3.3.16. Cobalt (Co) Content (mg/100 g)

There was a significant variation in Co content among the cultivars. Co contents ranged from 0.014 to 0.151 mg and average content was 0.074 mg **(Table 3.4)**. Statisticaly similar higher Co content was calculated from Lady Rosetta (0.151 mg), Lal Pakri (0.151 mg), Febula (0.150 mg) and Hagri (0.150 mg) while the lowest Co content was recorded from Shilbilati and Lara (0.014 mg).

Table 3.4. Zn, Mn, Cu and Co content in 100 g fresh tuber of 44 potato cultivars.

Cultivars	Zn(mg)	Mn(mg)	Cu(mg)	Co(mg)
Kenne	0.7 ± 0.028	0.681 ± 0.063	0.253 ± 0.014	0.090 ± 0.005
All Blue	0.51 ± 0.011	0.385 ± 0.060	0.17 ± 0.011	0.081 ± 0.005
Granola	0.51 ± 0.011	0.1864 ± 0.020	0.176 ± 0.014	0.087 ± 0.006
Shepody	0.693 ± 0.063	0.163 ± 0.026	0.174 ± 0.011	0.141 ± 0.023
Lady Rosetta	1.213 ± 0.020	0.146 ± 0.026	0.233 ± 0.017	0.151 ± 0.017
Patrones	0.993 ± 0.020	0.366 ± 0.037	0.273 ± 0.014	0.033 ± 0.003
Courage	0.686 ± 0.029	0.449 ± 0.028	0.22 ± 0.023	0.040 ± 0.002
Call White	0.486 ± 0.023	0.191 ± 0.011	0.203 ± 0.031	0.048 ± 0.002
Banana	0.546 ± 0.024	0.099 ± 0.017	0.333 ± 0.026	0.066 ± 0.009
Quiency	0.46 ± 0.034	0.090 ± 0.010	0.256 ± 0.029	0.055 ± 0.002
Baraka	0.573 ± 0.043	0.355 ± 0.025	0.19 ± 0.017	0.120 ± 0.023
Blondy	0.646 ± 0.029	0.104 ± 0.020	0.416 ± 0.023	0.097 ± 0.001
JPR	0.923 ± 0.023	0.155 ± 0.029	0.256 ± 0.018	0.057 ± 0.004
Shaita White	0.463 ± 0.037	0.144 ± 0.020	0.14 ± 0.020	0.062 ± 0.006
Ultra	0.38 ± 0.046	0.094 ± 0.014	0.123 ± 0.014	0.045 ± 0.002
Febula	0.62 ± 0.011	0.257 ± 0.028	0.16 ± 0.011	0.150 ± 0.011
Chieftain	0.426 ± 0.013	0.084 ± 0.003	0.13 ± 0.017	0.092 ± 0.006
Chipita	0.69 ± 0.017	0.158 ± 0.028	0.163 ± 0.020	0.081 ± 0.005
Innovator	0.46 ± 0.034	0.186 ± 0.026	0.166 ± 0.012	0.099 ± 0.005
Jam Alu	0.543 ± 0.026	0.215 ± 0.031	0.186 ± 0.017	0.111 ± 0.011
Akhira	0.59 ± 0.051	0.113 ± 0.003	0.09 ± 0.017	0.025 ± 0 .008
Dumini	0.556 ± 0.029	0.264 ± 0.037	0.156 ± 0.017	0.022 ± 0.001
Shilbilati	0.35 ± 0.040	0.174 ± 0.014	0.126 ± 0.012	0.014 ± 0.001
Mondial	0.413 ± 0.037	0.199 ± 0.028	0.143 ± 0.020	0.087 ± 0.002
Bellini	0.63 ± 0.017	0.135 ± 0.020	0.16 ± 0.023	0.070 ± 0.005
Atlanta	0.7 ± 0.017	0.162 ± 0.028	0.123 ± 0.020	0.132 ± 0.011
Hagri	0.536 ± 0.012	0.184 ± 0.025	0.155 ± 0.025	0.150 ± 0.017
Challisa	0.55 ± 0.028	0.311 ± 0.034	0.126 ± 0.029	0.067 ± 0.004
All Red	0.53 ± 0.017	0.163 ± 0.026	0.173 ± 0.026	0.090 ± 0.005
Deshi-5	0.33 ± 0.017	0.216 ± 0.037	0.116 ± 0.012	0.092 ± 0.002

Contd...

Cultivars	Zn(mg)	Mn(mg)	Cu(mg)	Co(mg)
Lara	0.586 ± 0.052	0.249 ± 0.028	0.16 ± 0.023	0.014 ± 0.001
Asterix	0.506 ± 0.034	0.223 ± 0.043	0.12 ± 0.028	0.029 ± 0.002
Atlas	0.41 ± 0.034	0.104 ± 0.025	0.12 ± 0.023	0.024 ± 0.001
Lal Pakri	0.583 ± 0.049	0.089 ± 0.017	0.12 ± 0.015	0.151 ± 0.002
Voyager	0.563 ± 0.037	0.294 ± 0.025	0.133 ± 0.020	0.021 ± 0.002
Shaita Red	0.556 ± 0.034	0.434 ± 0.020	0.166 ± 0.029	0.021 ± 0.002
Marabel	0.696 ± 0.057	0.434 ± 0.049	0.14 ± 0.023	0.086 ± 0.004
Diamant	0.323 ± 0.014	0.273 ± 0.031	0.103 ± 0.020	0.071 ± 0.003
Indurkani	0.36 ± 0.034	0.336 ± 0.020	0.113 ± 0.014	0.05 ± 0.011
Gaforgaon	0.386 ± 0.052	0.274 ± 0.020	0.116 ± 0.012	0.076 ± 0.003
Blue Mountain	0.34 ± 0.023	0.220 ± 0.011	0.14 ± 0.011	0.025 ± 0.02
Rodeo	0.43 ± 0.017	0.223 ± 0.026	0.101 ± 0.014	0.076 ± 0.02
Martin	0.5 ± 0.023	0.276 ± 0.034	0.13 ± 0.011	0.035 ± 0.01
Cardinal	0.23 ± 0.017	0.230 ± 0.017	0.113 ± 0.014	0.111 ± 0.011
Mean	0.549	0.229	0.167	0.074
LSD at 5%	0.091	0.080	0.055	0.024

3.3.17. Chromium (Cr) Content (mg/100 g)

Cr content of the cultivars differed significantly. Cr content ranged from 0.059 to 0.204 mg and average content was 0.133 mg/100 g tuber **(Table 3.5).** Maximum Cr content was estimated from Martin (0.204 mg) followed by Cardinal (0.197 mg) and Shaita Red (0.184 mg) while the lowest Cr content was recorded from Ultra (0.059 mg).

3.3.18. Cadmium (Cd) Content (mg/100 g)

Different cultivars showed significant variation in respect of Cd content. The range of Cd contents in the cultivars was 0.074-0.196 mg/100 g and average Cd content of the cultivars was 0.138 mg **(Table 3.5).** Ultra (0.074 mg) was the lowest Cd containing cultivar while JPR (0.196 mg) was the highest Cd containing cultivar followed by Hagri (0.184 mg) and Shaita Red (0.169 mg).

3.3.19. Lead (Pb) Content (mg/100 g)

Significant differences were observed among the potato cultivars for Pb concentrations. The mean of Pb contents varied between 0.878 mg obtained for the cultivar Febula to 0.259 mg found for the cultivar Atlas. The average Pb content in the cultivars was 0.474 mg/100 g tuber **(Table 3.5).**

3.3.20. Arsenic (As) Content (µg/100 g)

As contents of the cultivars was differed significantly. As content ranged from 3.16 µg to 9.34 µg and average content was 5.33 µg/100 g tubers **(Table 3.5).** Maximum As content was estimated from Call White (9.34 µg) followed by Lal Pakri (8.30 µg) and JPR (7.22 µg) while the lowest As content was recorded from Atlas (3.16 µg).

From the above mentioned results it can be noted that the potato cultivars showed a considerable variation in concentrations of different biochemical components.

Table 3.5. Cr, Cd, Pb and As content in 100 g fresh tuber of 44 potato cultivars.

Cultivars	Cr (mg)	Cd (mg)	Pb (mg)	As (µg)
Kenne	0.085 ± 0.006	0.126 ± 0.014	0.662 ± 0.057	4.12 ± 0.30
All Blue	0.099 ± 0.005	0.145 ± 0.025	0.678 ± 0.051	6.46 ± 0.29
Granola	0.095 ± 0.004	0.113 ± 0.017	0.670 ± 0.051	4.94 ± 0.31
Shepody	0.097 ± 0.001	0.135 ± 0.020	0.662 ± 0.040	4.86 ± 0.37
Lady Rosetta	0.105 ± 0.002	0.134 ± 0.014	0.805 ± 0.031	5.15 ± 0.20
Patrones	0.110 ± 0.005	0.153 ± 0.026	0.764 ± 0.043	6.82 ± 0.46
Courage	0.108 ± 0.010	0.135 ± 0.020	0.704 ± 0.060	6.19 ± 0.34
Call White	0.066 ± 0.001	0.126 ± 0.014	0.604 ± 0.029	9.34 ± 0.20
Banana	0.131 ± 0.011	0.154 ± 0.031	0.679 ± 0.040	5.00 ± 0.57
Quiency	0.110 ± 0.005	0.114 ± 0.020	0.469 ± 0.032	3.74 ± 0.43
Baraka	0.121 ± 0.006	0.144 ± 0.020	0.532 ± 0.046	4.97 ± 0.57
Blondy	0.136 ± 0.008	0.153 ± 0.020	0.651 ± 0.028	4.42 ± 0.29
JPR	0.161 ± 0.007	0.196 ± 0.026	0.743 ± 0.026	7.22 ± 0.40
Shaita White	0.077 ± 0.002	0.095 ± 0.014	0.401 ± 0.058	4.06 ± 0.34
Ultra	0.059 ± 0.001	0.074 ± 0.019	0.273 ± 0.014	3.29 ± 0.17
Febula	0.114 ± 0.002	0.143 ± 0.026	0.878 ± 0.043	5.71 ± 0.40
Chieftain	0.114 ± 0.003	0.124 ± 0.014	0.455 ± 0.031	4.47 ± 0.28
Chipita	0.115 ± 0.002	0.136 ± 0.020	0.508 ± 0.037	6.79 ± 0.28
Innovator	0.129 ± 0.005	0.143 ± 0.026	0.466 ± 0.026	5.49 ± 0.34
Jam Alu	0.155 ± 0.006	0.155 ± 0.025	0.611 ± 0.051	6.06 ± 0.14
Akhira	0.105 ± 0.002	0.128 ± 0.017	0.354 ± 0.031	3.84 ± 0.20
Dumini	0.131 ± 0.006	0.155 ± 0.014	0.446 ± 0.026	6.23 ± 0.26
Shilbilati	0.143 ± 0.008	0.138 ± 0.017	0.436 ± 0.020	5.53 ± 0.72
Mondial	0.108 ± 0.004	0.126 ± 0.020	0.350 ± 0.017	4.52 ± 0.28
Bellini	0.146 ± 0.008	0.166 ± 0.020	0.444 ± 0.025	4.73 ± 0.43
Atlanta	0.163 ± 0.009	0.146 ± 0.020	0.442 ± 0.028	6.01 ± 0.57
Hagri	0.162 ± 0.006	0.184 ± 0.020	0.485 ± 0.037	4.40 ± 0.23
Challisa	0.134 ± 0.002	0.138 ± 0.028	0.415 ± 0.037	4.89 ± 0.57
All Red	0.163 ± 0.007	0.155 ± 0.014	0.431 ± 0.028	3.98 ± 0.57
Deshi-5	0.128 ± 0.005	0.136 ± 0.014	0.335 ± 0.020	5.29 ± 0.40

Contd...

Cultivars	Cr (mg)	Cd (mg)	Pb (mg)	As (µg)
Lara	0.170 ± 0.005	0.155 ± 0.020	0.371 ± 0.023	5.50 ± 0.28
Asterix	0.143 ± 0.001	0.134 ± 0.025	0.327 ± 0.029	3.73 ± 0.40
Atlas	0.14 ± 0.008	0.119 ± 0.028	0.259 ± 0.023	3.16 ± 0.26
Lal Pakri	0.146 ± 0.003	0.167 ± 0.034	0.275 ± 0.014	8.30 ± 0.40
Voyager	0.157 ± 0.004	0.149 ± 0.017	0.375 ± 0.043	3.60 ± 0.34
Shaita Red	0.184 ± 0.005	0.169 ± 0.028	0.390 ± 0.023	5.39 ± 0.32
Marabel	0.170 ± 0.005	0.159 ± 0.023	0.355 ± 0.025	6.38 ± 0.23
Diamant	0.162 ± 0.005	0.135 ± 0.014	0.278 ± 0.017	4.93 ± 0.26
Indurkani	0.159 ± 0.005	0.146 ± 0.020	0.310 ± 0.034	6.98 ± 0.57
Gaforgaon	0.157 ± 0.004	0.139 ± 0.017	0.270 ± 0.011	6.52 ± 0.28
Blue Mountain	0.150 ± 0.005	0.114 ± 0.014	0.286 ± 0.014	4.33 ± 0.37
Rodeo	0.147 ± 0.004	0.116 ± 0.023	0.273 ± 0.020	5.01 ± 0.57
Martin	0.204 ± 0.007	0.108 ± 0.023	0.373 ± 0.020	6.35 ± 0.20
Cardinal	0.197 ± 0.007	0.115 ± 0.020	0.362 ± 0.017	6.00 ± 0.57
Mean	0.133	0.138	0.474	5.339
LSD at 5%	0.016	0.060	0.095	1.099

3.3.21. Dry Matter (DM) Percentage

In the present experiment, significant variations were observed among the cultivars in respect of DM percentage of potato tubers. DM ranged from 16.23% (Ultra) to 27.64 % (Hagri) and average DM was 21.99 %. **Table 3.6** reveals that most of the genotypes had higher dry matter over 20%. Potato cultivars Hagri (27.64%), JPR (26.63%), Jam Alu (25.64%), Chipita (25.51%), Atlanta (25.14%), Lady Rosetta (24.41%) and Indurkani (24.22%) were found the most suitable quality for processing.

3.3.22. Specific Gravity

Genotypes varied with respect to specific gravity, which ranged from 1.063 to 1.117 and mean was 1.090 **(Table 3.6).** It was observed that Hagri had the highest specific gravity (1.117) followed by JPR (1.112) with non significant difference, while the minimum value for specific gravity was noted for Ultra (1.063). **Table 3.6** and **3.1** showed that DM content is positively correlated with specific gravity (r = 1) and starch content (r = 0.987). From the **Table 3.6** revealed that most of the potato genotypes had higher specific gravity over 1.080 and hence is suitable for processing.

3.3.23. Moisture Percentage

There was a significant variation in moisture percntage of potato tubers among the cultivars **(Table 3.6).** The range of moisure percentage in cultivars was 72.35-83.76% and average percentage was 78%. The maximum moisture was observed in Ultra (83.76%) and Blue Mountain (83.03%). The lowest moisture content was noticed in cultivar Hagri (72.35%).

Table 3.6. Dry matter, moisture and specific gravity content in fresh tuber of 44 potato cultivars.

Cultivars	% dry matter	% moisture	Specific gravity
Kenne	21.21 ± 0.39	78.78 ± 0.23	1.087 ± 0.019
All Blue	22.06 ± 0.34	77.93 ± 0.23	1.091 ± 0.012
Granola	19.59 ± 0.57	80.40 ± 0.23	1.079 ± 0.045
Shepody	21.45 ± 0.37	78.54 ± 0.23	1.088 ± 0.016
Lady Rosetta	24.41 ± 0.64	75.58 ± 0.23	1.102 ± 0.058
Patrones	22.87 ± 0.79	77.12 ± 0.23	1.094 ± 0.048
Courage	23.27 ± 0.34	76.72 ± 0.23	1.096 ± 0.038
Call White	20.93 ± 0.92	79.06 ± 0.23	1.085 ± 0.048
Banana	24.16 ± 0.26	75.83 ± 0.23	1.100 ± 0.058
Quiency	18.58 ± 0.53	81.42 ± 0.23	1.074 ± 0.043
Baraka	22.134 ± 0.59	77.86 ± 0.23	1.091 ± 0.052
Blondy	23.65 ± 0.37	76.35 ± 0.23	1.098 ± 0.056
JPR	26.63 ± 0.68	73.36 ± 0.20	1.112 ± 0.064
Shaita White	18.91 ± 0.64	81.09 ± 0.28	1.076 ± 0.022
Ultra	16.23 ± 0.33	83.76 ± 0.43	1.063 ± 0.036
Febula	22.94 ± 0.54	77.05 ± 0.26	1.095 ± 0.054
Chieftain	19.19 ± 0.34	80.81 ± 0.46	1.077 ± 0.044
Chipita	25.51 ± 0.36	74.48 ± 0.27	1.107 ± 0.061
Innovator	21.7 ± 0.69	78.3 ± 0.17	1.089 ± 0.022
Jam Alu	25.64 ± 0.44	74.35 ± 0.20	1.107 ± 0.062
Akhira	19.44 ± 0.25	80.56 ± 0.32	1.078 ± 0.016
Dumini	21.52 ± 0.10	78.47 ± 0.27	1.088 ± 0.029
Shilbilati	20.90 ± 0.52	79.09 ± 0.29	1.085 ± 0.029
Mondial	20.62 ± 0.36	79.37 ± 0.21	1.084 ± 0.019
Bellini	20.92 ± 0.53	79.07 ± 0.30	1.085 ± 0.023
Atlanta	25.14 ± 0.47	74.85 ± 0.20	1.105 ± 0.060
Hagri	27.64 ± 0.37	72.35 ± 0.20	1.117 ± 0.067
Challisa	23.62 ± 0.36	76.37 ± 0.21	1.098 ± 0.029
All Red	22.58 ± 0.34	77.41 ± 0.23	1.093 ± 0.030

Contd...

Cultivars	% dry matter	% moisture	Specific gravity
Deshi-5	18.65 ± 0.32	81.34 ± 0.19	1.075 ± 0.043
Lara	18.5 ± 0.57	81.5 ± 0.28	1.074 ± 0.042
Asterix	23.36 ± 0.49	76.64 ± 0.29	1.097 ± 0.027
Atlas	17.76 ± 0.37	82.24 ± 0.13	1.070 ± 0.040
Lal Pakri	22.88 ± 0.51	77.11 ± 0.57	1.094 ± 0.028
Voyager	22.54 ± 0.31	77.46 ± 0.26	1.093 ± 0.034
Shaita Red	23.8 ± 0.46	76.2 ± 0.11	1.099 ± 0.028
Marabel	21.46 ± 0.65	78.53 ± 0.27	1.088 ± 0.029
Diamant	23.33 ± 0.26	76.66 ± 0.29	1.097 ± 0.030
Indurkani	24.22 ± 0.39	75.77 ± 0.28	1.101 ± 0.058
Gaforgaon	24.11 ± 0.22	75.88 ± 0.28	1.100 ± 0.011
Blue Mountain	16.96 ± 0.50	83.03 ± 0.28	1.067 ± 0.021
Rodeo	22.22 ± 0.45	77.77 ± 0.28	1.091 ± 0.029
Martin	21.60 ± 0.75	78.39 ± 0.23	1.088 ± 0.025
Cardinal	22.66 ± 0.38	77.33 ± 0.19	1.093 ± 0.035
Mean	21.99	78.00	1.090
LSD at 5%	1.367	0.860	0.113
Value of coefficient of correlation (r) between DM and specific gravity : 1			

3.4. DISCUSSION

In the present investigation was carried out for biochemical analysis of potato tubers of selected cultivars and selection of nutritionally enriched cultivars. Dry matter content, moisture content and specific gravity of selected potato cultivars were also determined for evaluation of processing quality. The potato cultivars show a considerable variation in concentrations of different biochemical components. In particular, the uptake of minerals and heavy metals and their concentrations are mostly affected by genotypic differences. Moreover, minerals and heavy metal content could also be influenced by location, soil condition, fungicide and climate properties, and other factors. Excessive fertilization and irrigation water pollution may cause higher heavy metal content in potato cultivars.

Starch is a major component of the potato tuber. Starch normally constitutes the greater part of the DM and its content is influenced by different factors reported by many earlier researchers. Starch contents are modified by factors affecting the DM content of the tuber. Maturity type is far more important than remaining genetic variation for tuber yield and starch content (Van Eck, 2007). The late-maturing cultivars tend to produce much greater tuber and starch yield compared with the early maturing cultivars. Starch was higher in potato tubers of 25-30 g than that of 50-80 g (Usoltsev, 1974). Intensity of starch synthesis is higher in potato plants grown from the basal parts of tubers, which contain about 2% more starch than the apical portions, than in plants grown from apical portion of tubers. Potato tuber transferred from 32^0 to 69^0 F, starch is re-synthesized with a simultaneous decrease in the concentration of monosaccharide and sucrose. Chlorocholine chloride solution applied to potato tubers before planting or sprayed on plants at bud formation or flowering increased tuber yield and the starch and protein content of the tuber (Fisher and Pyshtaleva, 1974). Distribution of starch follows that of the DM, increasing from the skin inwards as far as the vascular ring and then decreasing inwards to the central medullary region, while the 'heel' end contains more starch than the 'rose' end. Starch content was proportional to the dry matter (Uppal, 1999). Since the DM content of potato tuber is mostly dependent on starch (Dean and Thornton, 1992).

In the present investigation, the starch contents of the cultivars ranged from 65% to 75.53% (mean of 71.35%) on the basis of dry weight and 10.60% to 20.23 % (mean of

15.72%) on the basis of fresh weight while maximum starch content was shown in cultivar Hagri. Similar phenomenon was reported by many earlier researchers.

Starch comprises 65-80% of the dry weight of tubers (Kadam *et al*., 1991). Starch is a major component of the potato tuber at approximately 17.5 g (range of 8.0-29.4 g)/100 g FM or 75.30% of its dry weight (Lisinkaa and Leszczynski, 1989). Jansen *et al*. (2001) reported that cultivated potatoes contain 11.0–30.4% starch on a fresh weight basis (mean of 18.8%), wild species ranged from 3.8 to 39.6% with a mean of 18.1%. However, these data were not grouped based on maturity type. Potato tuber contains 15 g starch/100 g FM reported by CIP (1982). Starch content of 15 varieties of potatoes was 13. 7 – 22.2% and amylose in starch was 32.0- 78.5% (Janicki*,* 1967). With increase in future growth from 1 cm to 2.5 cm in diameter, the starch gradually increases from 11.4 to 16.0% and from 6.6 to 11.5% with white skin and red skin varieties, respectively (Khuda, 1964).

Sucrose, glucose and fructose comprise the major sugars of the potato tuber and its content is influenced by different factors reported by many earlier researchers. The content of sugars in the potato tuber is influenced by genotype, location, degree of maturity of the tubers, growing conditions, storage temperature and physiological development of the tuber (Gray and Hughes, 1978). In this study, the mean of total sugar content in the cultivars was 1.36 % and range was 0.77 - 2.04% although most of the cultivars ranged from 1.14 to 1.67%. From the results **Table 3.1** revealed that reducing sugars ranged from 0.89% in Chipita to 0.32% in Courage, while the other genotypes were between these two limits. **Table 3.1** also revealed that non reducing sugars ranged from 0.35 to 1.26%. The results of the present trial completely or partly support the findings of many earlier researchers. Leo Mustonen (2004) found that the range of total sugar, reducing sugar and sucrose content in different cultivars fluctuated from 1.2 to 1.8%, 0.3 to 0.7% and 0.7 to 1.2% respectively. The range of total sugar and reducing sugar in different potato cultivars was respectively 1.17-1.57% and 0.86-1.11% observed by Lucia *et al.* (1981). Total sugar and reducing sugar content of the potato tubers ranges from about 0.05-8% and 0-5% respectively on the basis of dry weight (Lisinkaa and Leszczynski, 1989). Potato tubers contain 0.01-0.6% reducing sugars on fresh weight basis (Storey, 2007). Regarding reducing sugars, Hasbun *et al.* (2009) indicates a 0.076 and 1.384 g/100 g FM range. Ghulam *et al.* (2011) found that reducing sugars in

32 cultivars ranged from 0.67% to 0.01%. In the present work suitable genotypes Courage (0.32%) had far low content of reducing sugar compared to other tested cultivars which is fit for processing.

Protein exists in potato tubers in both soluble and insoluble forms. The soluble protein (true protein) constitutes over 50% and insoluble protein about 10% of the total nitrogen content (Rahman, 1990). The proportion of soluble protein N to total nitrogen, although shown as 50%, it can vary widely. A range of 29.5% to 51.2% was noted among 11 different *S. tuberosum* varieties (Neuberger and Sanger, 1942) and of 40% to 74% in 50 clones of *S.tuberosum* group andigena (Li and sayre, 1975). In the present experiment, the average soluble protein percentage was 1.54 and range was 0.67 to 2.58 in the tested cultivars although most of the cultivars fluctuated between 1.0% and 1.65%. This finding is in conformity with the earlier findings. Soluble protein ranged from 0.37 to 1.24 g/100 g fresh tuber reported by Van Gelder and Vonk, 1980. Potato tubers contain 0.72- 3.4% protein on fresh weight basis (Ghulam *et al*., 2011). The average protein percentage in potato is 2% and range is 0.7 to 4.6% (Singh and Kur, 2009). In a previous report, protein content of Kufri Jyoti and Kufri Sinduri was 1.82 and 2.12%, respectively (Sandhu and Parhawk, 2002). The results also showed more protein in genotype NARC 1- 2006/1 (3.38%) than variety Chipsona-111 (3.04%).

Potato is considered to be a good source of vitamin C or ascorbic acid. In the tested cultivars, the range of vitamin C content was 12.44-21.47 mg/100 g FM. There are many reports describing in respect of vitamin C content in potato tubers. The total amount of the vitamin C (ascorbic acid and dehydroascorbic acid) in potato tubers ranges from 1 to 54 mg/100 g FM, although most frequently it is between 10 and 25 mg/100 g. Vitamin C ranges in content between 84 to 145 mg/100 g dry weight depending on cultivar, planting site, and storage conditions (Augustin, 1975). As for the vitamins, potato contains on average 20 mg/100 g FM of vitamin C (Brown, 2005). Woolfe (1987) reported that vitamin C ranged from 13.1 to 26.6 mg/100 g FM of potato tubers. Daniele *et al*. (2012) reported that the amount of vitamin C in potato cultivars ranged from 8.6 to 26.4 mg/100 g FM of potato tubers.

The most potent dietary source of vitamin A is β-carotene (pro-vitamin A). Potato contains low amounts of carotenoids, such as β-carotene (Brown, 2005), indicating that

potato is not a good sources of pro-vitamin A (carotenes). In the present experiment the highest amount of β-carotene/ 100 g FM was obtained from cultivar All Red (61.58 µg) followed by JPR (57.66 µg), Akhira (29.48 µg), Lara (21.43 µg) and most of the cultivars contains either no or very negligible (trace) amount of β-carotene. There are many reports describing on the amounts of pro-vitamin A or carotene or β-carotene in potato tubers. Potato pro-vitamin A ranged from 11 to 56 µg/100 g FM (Lisinkaa and Leszczynski, 1989). Carotene content in potato tubers is 24 µg/100 g FM (Ahmed, 1977). The amount of vitamin A in potato is 96.5 µg/100 g DM (AST/CIDA, 1983). Daniele *et al*., (2012) reported that the amount of β-carotene in potato cultivars ranged from 2 to 10 µg/100 g FM of tubers. The amount of β-carotene varies, depending on flesh color. Cream flesh and yellow flesh coloured potato tuber contains respectively 16 and 8 µg β-carotene/100 g of fresh-weight potato (CIP, 2000). β-carotene content is directly correlated with yellow flesh colour and total carotenoid content, which is a heritable characteristic. Typical "white" flesh potatoes contain 0.01-0.05 mg of carotenoids/100 g FM while varieties with "yellow" flesh contain 0.11-0.34 mg of carotenoids/100 g FM (Gross, 1991). It is thought that the tendency for a high carotenoid content is determined by a single dominant gene, although there are modifying genes (Brown *et al.,* 1993). Different methods of vitamins analysis can lead to varying results (Finglas and Faulks, 1984). K increases resistance to disease and pro-vitamin A content.

Potato tubers contain a number of phenolic compounds, but their percentage is rather low. The average phenolics content in the tested cultivars was 31.68 mg and range was 21.5 mg to 79.8 mg/100 g fresh potato tubers. Similar phenomenon was reported by many earlier researchers. In a US study total phenol content of potato (peeled) was 28 mg/100 g FM and it was ranked twentieth out of 23 commonly consumed vegetables. However, it was ranked ninth in terms of antioxidant activity (Vinson *et al*., 1998). Phenolic content of the potato tubers ranges from 5 to 30 mg/100 g FM reported by Lisinka and Leszczynski (1989). Woolfe (1987) reported that phenolics content ranged from 17 to 59 mg/100 g FM of potato tubers. The amount of phenolic compounds varies, depending on flesh color. Cream flesh, yellow flesh and purple flesh coloured potato tuber contains respectively 30, 95 and 550 mg phenolic compounds/100 g of fresh-weight potato (CIP, 1982). Polyphenolic compounds in potatoes show antioxidative activity in several systems.

The average ash content in potato tuber was 1.054% and ranged from 0.788% (Ultra) to 1.386% (Jam Alu). The results supported the findings of Singh and Kur (2009) who reported that average ash content in potato is 1% and range for ash percent is 0.44 to 1.9. Ash constitutes about 1% of the tuber fresh weight (Woolfe, 1987).Variation in ash may be a varietal character as mentioned by earlier researchers (Ereifej *et al*., 1997; Sandhu and Parhawk, 2002).

Potato contains some important minerals and trace elements essential to various human body structures and functions. Determinations of the major minerals and trace elements in raw potatoes have been carried out by various researchers. It has been reported that genotypes, soil type and fertilizer content of soil can cause variations in the minerals content of potato tubers. The higher-yielding potato genotypes have lower concentrations of some mineral elements in their tubers than lower-yielding genotypes when grown in the same environment, but this is not universally observed.

Potassium (K) is found as the major cation in potato tubers. Higher concentrations of K are present in the skin and directly beneath it than in the interior of the potato tuber. In this experiment, the range of K contents in the cultivars was 173.89 - 908.19 mg and average K content of the cultivars was 417.88 mg/100 g FM. This finding is in conformity with the earlier findings. Woolfe (1987) reported that K content ranged from 204.9 to 900.5 mg and mean was 564 mg/100 g FM of potato tubers. Potato tuber contains 1.6% K of FM reported by Vandder (1981). K varies from 3550-8234 µg/g FM (Casanas *et al*., 2002; Rivero *et al*., 2003; Sanchez-Castillo. 1998). One report listed K as low as 5.6 µg/g of FM (True *et al*., 1978). Potato tuber contains 421 mg K/ 100 g FM reported by CIP. The K content of 100 g fresh weight of potatoes is 425 mg reported by Philip *et al*. (2009). Variation in tuber K concentrations among *Solanum* genotypes is 13.8 g (Ereifej *et al*., 1998), 24.9 g (Tekalign and Hammes, 2005) and 21.3 g (SCRI, unpublished data)/kg dry matter. The minerals present in greatest concentrations in raw potato include (mg/g FM): K (564), phosphorus (30-60), Ca (6-18) (Burton, 1989; Buckenhuskes, 2005). Variations in the K contents of 100 g potato DM are 1400-2500 mg (adapted from Lisinka and Leszczynski, 1989) and 1394-2825 mg (Lampit and Goldenberg, 1940). Applying K fertilizers increases tuber K concentrations (Addiscott, 1976; Allison *et al*., 2001b; Harris, 1992; Maier, 1986). The application of Ca fertilizers generally reduces tuber Mg concentrations but can increase tuber P, S, and K

concentrations (Clough, 1994; Simmons and Kelling, 1987). K content increases during the entire growing season (Lisinka and Leszczynski, 1989). Excessive quantity of K can poison plants. K stimulates leaf growth, tuber growth and tuber enlargement. It plays a part in producing proteins and counteracts the effects of excessive application of P.

Potatoes are a significant source of Calcium (Ca), with a wide range reported. Mostly it is present in the skin and the vascular system in the potato. The Ca contents of the tested cultivars ranged from 8.42 to 44.41 mg and an average Ca content was 18.57 mg/100 g FM. These results are in agreement with earlier findings. Two studies reported Ca content in the potato tubers up to 130 mg/100 g DM and 455 mg/kg FM (Lisinka and Leszczynski, 1989; Randhawa, 1984). Variation in tuber Ca concentrations among different potato cultivars is 10-130 mg/100 g DM (Lampit and Goldenberg, 1940). Potato tuber contains 0.05% (50 mg/100 g) Ca of FM reported by Vandder (1981). Among 74 Andean landraces, Ca ranged from 271-1093 µg/g of DM (Andre *et al*., 2007). Wild *Solanum* species vary in the ability to accumulate tuber Ca (Bamberg, 1998). High levels of tuber Ca are associated with resistance to pathogens (McGuire, 1986) and abiotic stress (Tawfik, 1996).

Iron (Fe) is also present in fairly low amounts but may make a contribution to dietary intake. Fe, in association with chlorogenic acid, causes after-cooking darkening of potatoes. Of all the micronutrients, Fe is required by plants in the largest amount. The Fe contents of the tested cultivars ranged from 0.62 to 1.97 mg while mean was 1.40 mg/100 g FM. These results are nearer to the findings of Ozturk *et al*., (2011); Lisinka and Leszcynki, (1989) and Lampit and Goldenberg, (1940) who found that the Fe content of the potato cultivars ranged from 48.87 to 72.64 mg/kg and 2.5 to 72 mg and 2.61 to 71.5 mg/100 g DM respectively. A study of cultivated varieties showed 0.3-2.3 mg of Fe in a 100 g tuber (True *et al*., 1978). Ranges of Fe content from 6 to 158 µg/g of DM have been reported Andre *et al*., (2007) and Wills *et al*. (1984). Fe contains in potato tuber is 1.8 mg/100 g FM reported by CIP. In a study of 74 Andean landraces, the iron content ranged from 29.87 to 157.96 µg/g DM (Andre *et al*., 2007).

Zinc is an essential component of various enzyme systems for energy production, protein synthesis, and growth regulation. Significant differences in zinc content occur in potatoes. In this study, average Zn contents (0.549 mg/100 g tuber) is nearer to the

findings of Ereifej *et al.*(1998) and True *et al*.(1978) who found that 20.4 mg/kg DM and 0.41 mg/100 g FM respectively. The highest Zn content (1.213 mg/100 g FM) was determined in the cultivar Lady Rosetta whereas Lampit and Goldenberg (1940) and Lisinka and Leszcynki (1989) reported that the highest Zn contents in potato tuber are 2.17 mg and 2.2 mg/100 g DM respectively. The zinc content ranges from 1.8 to10.2 µg/g FM (Andre *et al*., 2007; Randhawa *et al*., 1984 and Rivero *et al*., 2003). Yellow-fleshed potatoes from different cultivars contain zinc in 0.5-4.6 µg/g FM (Dugo *et al*., 2004). In a study of 74 Andean landraces, the zinc content from 12.6 to 28.83 µg/g DM (Andre *et al*., 2007).

Manganese (Mn) is an essential element for plants, intervening in several metabolic processes, mainly in photosynthesis and as an enzyme antioxidant-cofactor. Nevertheless, an excess of this micronutrient is toxic for plants. The range and mean of Mn contents in the tested cultivars were 0.084-0.681 mg and 0.229 mg/100g tuber respectively. This finding is in conformity with the earlier findings. Lisinka and Leszcynki, (1989) reported that the range of Mn distribution in potato tubers is 0.5-8 mg/100 g DM. The range of potato Mn content has been reported from 0.73-3.62 µg/g FM (Rivero *et al*., 2003) to 9-13 µg/g DM (Orphanos, 1980). Sharma, *et al*. (2005) found that the range of Mn concentrations in potato tubers is 8.25-18.25 mg/kg DM. Mn content could also be influenced by location, soil and climate properties, and other factors (Caussy *et al*., 2003; Tok, 1997; White and Zasoski, 1999). The application of P fertilizers may reduce tuber Mn concentrations (Hammond and White, 2005),

Copper (Cu) is one of the essential micronutrients and its adequate supply for growing plants should be ensured through artificial or organic fertilizers (Itanna, 2002). Cu occurs in the compounds with no known functions as well as enzymes having vital function in plant metabolism (Kabata and Pendias, 2001). In this study, significant variations in Cu content occur in potatoes. Cultivar Blondy had the highest Cu content (0.416 mg) while the lowest Cu value was obtained from Akhira (0.09 mg). The results are nearer to earlier findings. Cu in potatoes varies from 0.23 to 11.9 mg/kg FM (Randhawa *et al*., 1984; Rivero *et al.,* 2003). Tekalign and Hammes, (2005) observed that Cu concentrations in potato tuber is 20.0 mg/Kg DM. Like zinc, Cu is also high in yellow-fleshed potatoes (Dugo *et al*., 2004). Lisinka and Leszcynki (1989) reported that Cu concentrations in potato tuber are 0.06-2.8 mg 100/g DM.

Chromium (Cr) is an element occurring in food products of both plant and animal origins. It is regarded as an essential trace element in humans and animals, taking part in various metabolic processes. The availability of Cr to the body may depend on the form in which it is present in food. Cr concentration of the cultivars differed significantly. In this study, the maximum Cr content was estimated from Martin (0.204 mg) while the lowest Cr content was recorded from Ultra (0.059 mg) and average Cr concentration was 0.133 mg/100 g FM. There are many reports describing on the content of Cr in potato tubers. Zbigniew *et al*., (2007) observed that the mean concentrations of Cr in potato cultivars are 47.72-129.61 µg/kg DM. Heavy metal concentrations of plants is directly associated with their concentrations in soils, but their levels significantly differ with plant species, and even can also affected by genotypes within the same species (Kabata and Pendias, 2001). In particular, the uptake of Cr and their concentrations are mostly affected by genotypic differences (Prosba and Mydlarski, 2000) and it was also be influenced by location and climate properties, and other factors (Caussy *et al*., 2003; Tok, 1997; White and Zasoski, 1999).

Cobalt (Co) is a trace element that forms part of the structure of vitamin B_{12}, one of the vitamins. There was a significant variation in Cobalt contents among the cultivars. Co contents ranged from 0.014 to 0.151 mg and average content was 0.074 mg/100 g tuber. Statistically similar higher Co content was calculated from Lady Rosetta (0.151 mg), Lalpakri (0.151 mg), Febula (0.150 mg) and Hagri (0.150 mg) while the lowest Co content was recorded from Shilbilati and Lara (0.014 mg). Woolfe (1987) reported that the average Co concentration in potato tubers was 0.065 mg/100 g FM. Sharma *et al*. (2005) found that the range of Co concentrations in potato tubers is 1.5-2.5 mg/kg DM.

Cadmium (Cd) is a nonessential element in foods and natural waters. Potato tubers can accumulate high concentrations of Cd in edible portions, so that techniques to determine high risk Cd environments are required by growers. Cd in foods is mostly derived from various sources of environmental contamination (Adriano, 1984). In this study, among the tested cultivars, Ultra (0.074 mg) was the lowest Cd containing cultivar while JPR (0.196 mg) was the highest Cd containing cultivar. There are many reports describing on the content of Cd in potato tubers. Bokyoung (1994) mentioned that in contaminated soils the average content of Cd in tubers can reach level of 1.3-2.2 mg/kg. The Cd ranged from 0.08 to 0.32 mg/kg tuber reported by Ozturk *et al.* (2011). Potato cultivars have

been shown to differ in their ability to accumulate Cd, although the differences between cultivars are not consistent. Harris *et al.* (1981) found no differences in tuber Cd concentration of cultivars. Mclaughlin *et al.* (1997) found that cultivars grown commercially exhibited significant differences in tuber Cd concentration. In particular, the uptake of Cd, Cu, Pb and Mb and their concentrations are mostly affected by genotypic differences (Prosba and Mydlarski, 2000) and it was also be influenced by location, soil and climate properties, and other factors (Caussy *et al.*, 2003; Tok, 1997; White and Zasoski, 1999). Though Cd and As are not necessary for the growth of plants they are absorbed by roots and leaves, they get into plant tissue where they are accumulated, making worse the transport of nutrients, thus growth and quality of produced biomass.

Significant differences were observed among the tested potato cultivars for lead (Pb) contents. The mean Pb contents varied between 0.878 mg obtained for the cultivar Febula to 0.259 mg found for the cultivar Atlas. The average Pb content in the cultivars was 0.474 mg/100 g tuber which was very much higher than the maximum permissible level of Pb content (0.1 mg/kg; Anonymous, 2011) in potato tuber. Ozturk *et al.* (2011) observed that the range of Pb contents in potato tuber is 0.51 - 0.77 mg/kg. Heavy metal concentrations of plants is directly associated with their concentrations in soils, but their levels significantly differ with plant species, and even can also affected by genotypes within the same species (Kabata and Pendias, 2001). It was also be influenced by location and climate properties, and other factors such as irrigation with Pb-contaminated water, and application of wastes, and Pb-containing pesticides and herbicides (Caussy *et al*., 2003; Tok, 1997; White and Zasoski, 1999). So in this experiment, the higher content of Pb may be due to the absorption of Pb directly from soil or above mentioned sources.

Arsenic (As) is one of the major global environmental pollutants because of its highly toxic and carcinogenic properties. The maximum permissible level of As content in food grade is 0.5 mg/kg (Anonymous, 2011). Soil As is the major source for the As uptake of crops (Huang *et al.,* 2006). Besides its natural origin, various anthropogenic activities like mining, smelting, coal burning, irrigation with As contaminated water, and application of wastes, animal manures, and As containing pesticides and herbicides may also contribute As to soil (Dutre *et al.,* 1998; Flynn *et al.,* 2002; Mandal and Suzuki, 2002; Alam *et al.,* 2003; Warren *et al.,* 2003; Baroni *et al.,* 2004; Camm *et al*., 2004).

Some previous reports clearly showed that the As contents in the agricultural plants were correlated to the degree of As contamination in irrigation water and soil (Roychowdhury *et al.,* 2005; Dahal *et al.,* 2008). Dahal *et al.* (2008) further concluded that the uptake of As by agricultural plants was far better correlated with the As concentrations in irrigation water than soil As contents.

The present results showed that the accumulation of As in potato tubers was between 3.16 and 9.34 µg and average content was 5.33 µg/100 g FM. Bhattacharya *et al.* (2010) found that the average As concentrations in potato tubers are 0.654 mg and the range is 0.19-1.02 mg/kg DM. Although cultivation of potato, an underground vegetable, requires less amount of water for irrigation, the higher content of As (0.19 - 1.02 mg/kg DM) may be due to the absorption of As directly from soil. Das *et al.* (2004) found that the range of As concentrations in vegetable crops is 0.02-3.99 mg/kg. As is also absorbed by roots and leaves of plants and they get into plant tissue.

The dry mater (DM) or 'solids content' of tubers is one of the prime characters used by potato processors to evaluate a crop. Potatoes with high DM are most suitable for the manufacture of dehydrated food products and stock feed and is especially good for the production of fried foods. For chips, French fries and dehydrated products tuber dry matter needs to be more than 20% (Ezekiel *et al*., 1999). DM content is extremely variable in potato tuber. The interactive factors (viz. variety, climate and soil conditions, agricultural practices, length of growing season, incidence of pests and diseases) which influence tuber DM have been reviewed at length by Burton (1966) and more briefly by Grison (1973). Tuber dry matter content differs considerably between cultivars and is a strongly genetic based character (Toolangi, 1995). DM content increases during the growing season and is highest in the vascular system, intermediate in the cortex and lowest in the pith.

In this study, the DM content varied from 16.23% (Ultra) to 27.64% (Hagri) and its mean was 21.99%. These results are in good agreement with many earlier researchers. The potato tuber DM ranged from 13.1% to 36.8% (mean of 22.5%) reported by Schwimmer *et al.* (1967). Ghulam *et al.* (2011) found that dry matter of 32 cultivars ranged from 14.86% to 25.65%. Another study showed the highest values for dry matter content (24–26%) in La Molina (Amoros, 2000). A sample of 100 g of potato contains,

on average, about 22 g of DM and 78 g of moisture (Wu Leung and Flores, 1961; Wu Leung *et al*., 1968 and Wu Leung *et al.* 1978). Dry matter content of potato tubers ranges from about 13.1 to 36.8% with an average of 23.7% and the other 75% of the potato consists of water (Lisinka and Leszczynski, 1989). Anonymous (1987) found that the DM % of the maximum potato cultivars ranged from 15 to 24%. The tuber DM range of 13.7% to 34.8% was found amongst accessions to the germplasm collection at the International Potato Centre (unpublished data). A significant relationship between dry matter and specific gravity was found in earlier reports (Rastovski *et al*., 1981). High dry matter content has been reported to be positively correlated with a lower sugar concentration during storage (Watada and Kunkel, 1955; Iritani and Weller, 1976).

Specific gravity of tubers is another important characters used by potato processors. In general, tubers with high specific gravity are preferred for processing (Adams, 2004). Good quality potatoes should have a specific gravity value of more than 1.080. Potato tubers with specific gravity values less than 1.070 are generally unacceptable for processing. In the present investigation specific gravity of the tested cultivars varied from 1.063 (Ultra) to 1.117 (Hagri) and mean was 1.090 and it was influenced by genotypes. This result is also nearer to that of many earlier researchers. Ghulam *et al.* (2011) observed that 32 potato genotypes varied with respect to specific gravity, which ranged from 1.034 to 1.144. Samih *et al.* (2011) observed that specific gravity of potato tubers ranged from 1.05 to 1.07. Amoros *et al.* (2000) studied six clones of potato and found that specific gravity ranged between 1.121 and 1.141. Specific gravity illustrated a positive relationship with starch content and dry matter (Feltran *et al*., 2004). A decrease in starch would be expected to decrease the specific gravity of the tuber (Rowe and Powelson, 2002). Scheele *el al.* (1937) demonstrated a high correlation between DM and specific gravity when a large number of samples (560) were employed. However, the reliability of the relationship between specific gravity and total solids may be reduced when individual tubers containing intercellular air tissue spaces or the phenomenon known as 'hollow heart' are included in the measurements (Porter *et al*., 1964; Burton, 1966). Also, the regression lines calculated for the relationship can vary with factors such as soil type, growing conditions, location (Porter *et al*., 1964; Schippers, 1976), and even cultivars (Schippers, 1976). It has therefore been recommended that situation-specific regression lines be used.

3.5. SUMMARY

The present investigation was carried out for nutritional quality determination of selected potato cultivars and selection of nutritionally enriched cultivars. For this investigation, biochemical analysis of field grown fresh potato tubers of 44 potato cultivars was studied by different biochemical analysis methods. Among the 44 potato cultivars, Hagri was exhibited the highest content of starch on fresh weight basis of tubers. It was also showed the highest specific gravity and dry matter content. Cultivar Banana was exhibited the maximum contents of total sugar and non reducing sugar. On the other hand cultivars Chipita and Indurkani showed the highest amount of reducing sugar and soluble protein respectively. The highest amount of vitamin C, β-carotene and phenolic compounds was recorded from cultivars Jam Alu, All Red and Indurkani respectively. Different cultivars showed significant variations in respect of minerals contents. The highest contents of K,Ca, Fe, Zn, Mn, Cu, Cr, Co, Cd, Pd and As was noticed in JPR, Baraka, Indurkani, Lady Roseta, Kenne, Blondy, Martin, Lal Pakri/Lady Rosetta, JPR, Febula and Call White respectively. Averaged over the 44 potato genotypes, the minerals accumulation pattern for the potato tubers was in following order; K>Ca>Fe>Zn>Pb>Mn>Cu>Cd>Cr>Co>As.

The present study concluded that the potato cultivars showed considerable variation in concentrations of different biochemical components. In particular, the uptake of minerals and heavy metals and their concentrations are mostly affected by genotypic differences. Moreover, minerals and heavy metal content could also be influenced by location, soil condition, fungicide and climate properties, and other factors. Excessive fertilization and irrigation water pollution may cause higher heavy metal content in potato cultivars. This information may be useful for designing future breeding efforts to improve potato nutritional quality management. It can believe that such information can be beneficial for understanding the importance of cultivar selection in healthy nutrition and improving consumer awareness.

CHAPTER IV
INDUCTION AND EVALUATION OF SOMACLONAL VARIATION IN POTATO

4.1. INTRODUCTION

Potato is one of the major dietary components (after rice and wheat) in Bangladesh. It is an excellent low-fat source of carbohydrate but potato generally contains small amount of iron (Fe) and most of the potato cultivars contain either no or very negligible amount of pro-vitamin A (β-carotene). Iron is necessary for many functions in the body including formation of haemoglobin, brain development and function, regular of body temperature, muscle activity and catecholamine metabolism. Iron deficiency is one of the leading risk factors for disability and mortality worldwide, affecting both developing and developed countries with major consequences for human health as well as social and economic improvement. It is estimated that over 60% of the world's six billion people are iron (Fe) deficient (Welch and Graham, 2002; White and Broadley, 2005). According to UNICEF, nearly two billion people are estimated to be anemic and about double that number, or 3.7 billion are iron deficient the vast majority of them women (Potrykus, 2000)**.** In Africa and Asia UNICEF estimates that IDA contributes to approximately 20% of all maternal deaths (Potrykus, 2000). In Bangladesh the rates of anaemia among children 0-5 years of age were 66.5% for boys and 71.3% for girls (hemoglobin concentration<11.0 g/dl). The rates of anaemia among children 6-14 years of age were highest; 90.7% for boys and 90.6% for girls. The survey reported anaemia rates of 89.1% among adult males (haemoglobin concentration <13 g/dl) and 86.8% among adult females (haemoglobin concentration<12 g/dl) (FAO, 1999). This situation has been attributed to sourcing produce from land with low mineral phytoavailability, eating crops with inherently low tissue mineral concentrations, and/or consuming the refined foods of civilization. In particular, it appears that the occurrences of Fe deficiency anaemia have increased dramatically in populations changing from traditional diets dominated by pulses, vegetables, and fruits to diets dominated by cereals (Graham *et al*., 2001; Welch and Graham, 2002), whose tissue concentrations of these elements appear to be constrained by an ancient evolutionary heritage (Broadley *et al.,* 2004, 2007; White and Broadley, 2003).

On the other hand β- carotene is the most potent dietary source of vitamin A. Vitamin A deficiency (VAD) is widespread and it is one of the major vitamin deficiencies in developing countries. Each year more than one million VAD associated childhood deaths occur. And, according to the World Health Organization, as many as 230 million children are at risk of clinical or subclinical VAD, a condition which is largely preventable. VAD makes children especially vulnerable to infections and worsens the course of many infections. Supplementation with vitamin A is estimated by UNICEF to lower a child's risk of dying by approximately 23 percent (Potrykus, 2000)**.** VAD can lead to eye damage and even blindness and also weaken the protective barriers to infection put up by the skin, the mucous membranes and the immune system (Somer and West, 1966). VAD is the single most important cause of blindness among children in developing countries, about 500,000 per year (Potrykus, 2000)**.**

So, there is an urgent need to take up programs of Fe and pro-vitamin A enrichment in most important and cheapest food crops to ensure the nutritional security of the world. In this case potato will be the future crop and there is a vertically scope of Fe and pro-vitamin A enrichment.

Plant biotechnology has been developed as a new technology to put forward as a potential way of propagation as well as increasing genetic variability for plant improvement. It has now important practical application in potato breeding and production of potato cultivars. During the last 30 years enormous efforts and researches have been going on for the refinement of plant biotechnology especially on tissue culture techniques for the improvement of plants. These techniques have been applied to a large number of important potato cultivars in agriculture (Hashem *et. al.,* 1990). One important way to improve potato cultivars is the callus tissue culture. It is realized that callus tissue culture derived plants display somaclonal variation which is akin to mutations in tissues and cultured cells. Somaclonal variation thus appears to be an important source of genetic variability. Larkin and scowcroft (1981) first reviewed the occurrence of somaclonal variation in plant species. Since then several reviews have been published (Semal, 1986; Morrison and Evans, 1987; Brar and Khush, 1994; Jain *et al.*, 1998). At present somaclonal variation has been reported in a large number of plant species; Potato, Sugarcane, Tobacco, Tomato, wheat, Rice, Brassica and others for various agronomic traits such as disease resistance, plant height, tiller number, maturity

and for various physiological and biochemical traits. Several useful somaclonal variants have been obtained and some of them have been released as cultivars.

4.1.1. Objectives

This part of the research has following objectives:

1. Enhancing the range and magnitude of somaclonal variation through callus tissue culture technique.
2. Selecting desirable lines having good agronomic characters including yield and high levels of iron and β-carotene.

4.2. MATERIALS AND METHODS

4.2.1. Materials

Explants are an important factor for callus induction. The internodal and leaf explants were collected from previously established *in vitro* plantlets through sprouts culture of pro-vitamin A (β-carotene) and iron enriched potato cultivar All Red.

4.2.2. Methods

The methods involved in the present investigation are described in the different heads.

4.2.2.1. Callus induction

To induced callus internodal and leaf segments were taken and cultured in MS medium supplemented with different callus inducing substances and incubated in dark at 22 ± 1°C for 6-7 weeks. MS medium was supplemented with different concentrations of 2, 4-D, NAA, NAA with BA, 2, 4-D with Kin and sucrose (30 g/l) for massive callus induction. Humidity was not controlled for any of the experiment. In each treatment 10 explants were inoculated.

4.2.2.2. Subculture of calli

When the calli attained a size of about 10-15 mm in diameter; these were rescued and subcultured aseptically on same or different PGRs supplemented media for maintenance.

4.2.2.3. Shoot regeneration from calli

The selected calli were placed on shoot regeneration medium supplemented with different concentrations and combinations of BA with GA_3, Kin with GA_3, and BA with NAA and Kin with NAA for shoot regeneration. The cultures were incubated in light at 22 ± 1°C. Well developed shoots from ten selected treatments were excised and cultured separately and individually.

4.2.2.4. Multiplication of the regenerated shoots

The regenerated plantlets from each of individual callus were further multiplied. Accessions of internode and leaf derived plantlets of ten selected treatments were named and maintained as AR-01 to AR-10 line.

4.2.2.5. Acclimatization and transplantation of the regenerated plantlets

After the acclimatization of somaclones they were finally transplanted to the field.The somaclones were gradually acclimatized and successfully established in the field.

4.2.2.6. Data recording on in vitro parameters

After 6 weeks of culture data were recorded on different parameters from different treatments as follows:

i) Percentage of explants induced to callus: Percentage of explants induced in callus was recorded and calculated using following formula:

$$\% \text{ of explants induced callus} = \frac{\text{Number of explants induced callus}}{\text{Total number of cultured explants}} \times 100$$

ii) Callus colour: The colour and the texture of callus varied in respect of plant growth regulators supplements. For this reason, callus colour was recorded.

iii) Degree of callus formation: Induction and growth of callus were varied on the basis of media formulation, so degree of callus growth was denoted

iv) Percentage of calli induced in shoots: The number of calli that produced shoots was expressed as % and data were recorded.

$$\% \text{ of callus formed shoots} = \frac{\text{Number of explants formed shoot}}{\text{Total number of explants cultured}} \times 100$$

v) Mean number of shoots: Number of shoots/callus was calculated.

vi) Shoot length/callus: The length of shoots in cm was recorded.

4.2.2.7. Data recording on field crops

To evaluate field performances and somaclonal variations among the calli derived plants, data on various morphological and agronomical characters were recorded. Chlorophyll content was estimated as earlier described in Methods in Physiological Plant Pathology (Mahadevan and Sridhar, 1982).

4.2.2.8. Determination of DM, iron and β-carotene in potato tubers

DM, iron and β-carotene content in the hervested tubers (obtained from somaclonal accessions) were determined according to the earlier describing (in chapter 3.2) methods.

4.2.2.9. Data analysis

The data were analysed using the standard procedures (Steel and Torrie, 1980).

4.3. RESULTS

This investigation was carried out for enhancing the range and magnitude of somaclonal variation which can be used for selecting desirable lines having good agronomic characters including yield and high levels of iron and pro-vitamin A (β- carotene). The various parameters that were analyzed in this regards are as follows:

4.3.1. Callus Induction

Callus induction and their maintenance for further growth were done from internodal and leaf explants and the explants were collected from pro-vitamin A (β-carotene) and iron enriched potato cultivar (All Red). Explants were cultured onto MS medium solidified with agar and supplemented with different concentrations of 2,4-D , NAA alone and in combinations of 2,4-D with Kin and NAA with BA in order to find out the most suitable culture media formulation to induce maximum callus formation. The rates of callus induction were shown a great variation with different concentrations and combinations of plant growth regulators. Usually callus proliferation started from the cut surface of the explants and gradually covered the whole explant.The growth of a cultured callus over a period of time is characterized by an increase in cell number, an increase in volume or mass and changes in biochemistry and cellular complexity.Three parameters namly percentage of explants induced to callus, callus colour and degree of callus development were used to evaluate the experiments. The results of these experiments are presented below.

4.3.1.1. Effect of 2, 4-D on callus induction from internodal and leaf explants

Among all the treatments of 2, 4-D, maximum callusing rate was found from internodal explants when the explants were cultured onto media having 3.0 mg/1 2,4-D (**Table 4.1**). The second highest callusing rate was found from leaf explants when the explants were cultured onto the same media formulation. The degree of callus formation from intemodal explants was satisfactory. In this case the callus colour was brown. The rate and degree of callus formation from leaf explants was lower than that of internodal explants.

4.3.1.2. Effects of NAA on callus induction from internodal and leaf explants

In this case, the higher callusing rate was recorded from internodal explants when they were cultured onto MS medium supplemented with 2.0 mg/l NAA (**Table 4.1**). The second higher callusing rate was noticed in leaf explants when the explants were cultured on media having 2.0 mg/l NAA. Callus developed in this media formulation was brown colour (for internodal explants) or white brown colour (for leaf explants) and the degree of callus formation was massive in internodal explants and moderate in leaf explants.

4.*3.1.3. Effects of 2, 4-D with Kin on callus induction from internodal and leaf explants*

Among all the treatments of 2, 4-D with Kin, 1 mg/l 2, 4-D + 0.25 mg/l Kin was the best treatment for callus formation. In this media formulation the highest callusing rate was recorded from internodal explants and the second highest callusing rate was observed in leaf explants (**Table 4.1**). The degree of callus formation from intemodal explants was satisfactory and the degree of callus formation from leaf explants was lower than that of internodal explants. Callus developed in this media formulation was greenish or greenish brown colour.

4.3.1.4. Effects of NAA with BA on callus induction from internodal and leaf explants

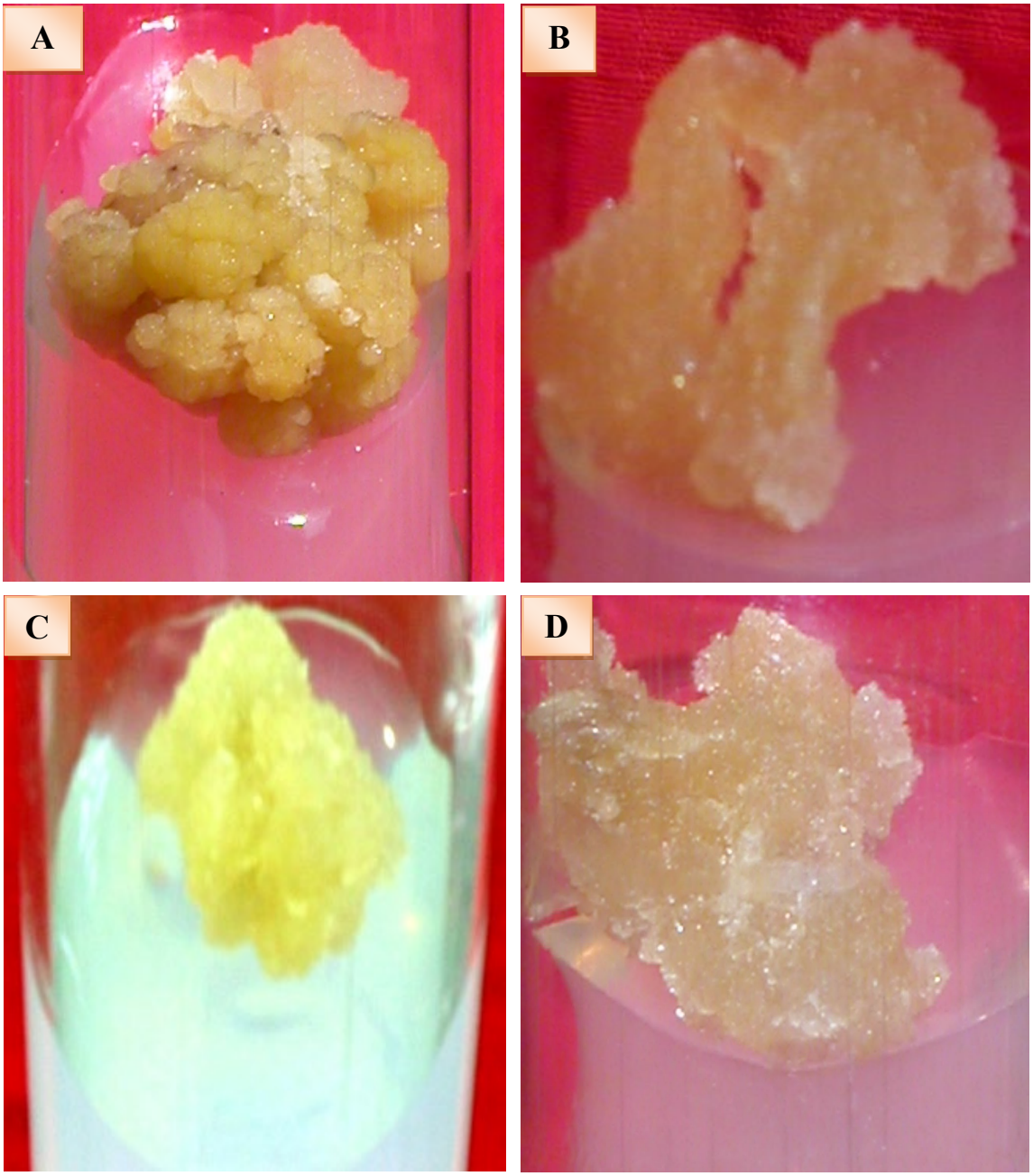
In case of different concentrations and combinations of NAA + BA, maximum callusing rate was found both in internodal and leaf explants when the explants were cultured on media having 3.0 mg/l NAA + 2.5 mg/l BA (**Table 4.1**). Callus developed in this media formulation was white brown and the degree of callus formation was massive.

Table 4.1. Effect of different concentrations of 2, 4-D and NAA singly or combinations of 2, 4-D with Kin and NAA with BA in MS medium on callus induction from internodal and leaf explants. In each treatment 10 explants were inoculated.

Concentration of PGRs (mg/l)	Types of explants	% explants induced to callus	colour of callus	degree of callus formation
2,4-D				
1.0	Internode	10	Br	+
	Leaf	-	-	-
2.0	Internode	40	Br	+
	Leaf	50	Br	+
3.0	Internode	90	Br	+++
	Leaf	70	Br	++
NAA				
1.0	Internode	30	Br	+
	Leaf	20	Br	+
2.0	Internode	60	Br	+++
	Leaf	50	WBr	++
3.0	Internode	40	Br	+
	Leaf	40	Br	
2,4-D + Kin				
1.0 + 0.25	Internode	70	G	+++
	Leaf	50	GBr	++
2.0 + 0.25	Internode	50	GBr	++
	Leaf	40	GBr	+
3.0 + 0.25	Internode	40	GBr	+
	Leaf	30	GBr	+
NAA + BA				
1.0 + 2.5	Internode	10	Br	+
	Leaf	20	Br	+
2.0 + 2.5	Internode	50	Br	++
	Leaf	40	WBr	+
3.0 + 2.5	Internode	90	WBr	+++
	Leaf	90	WBr	+++

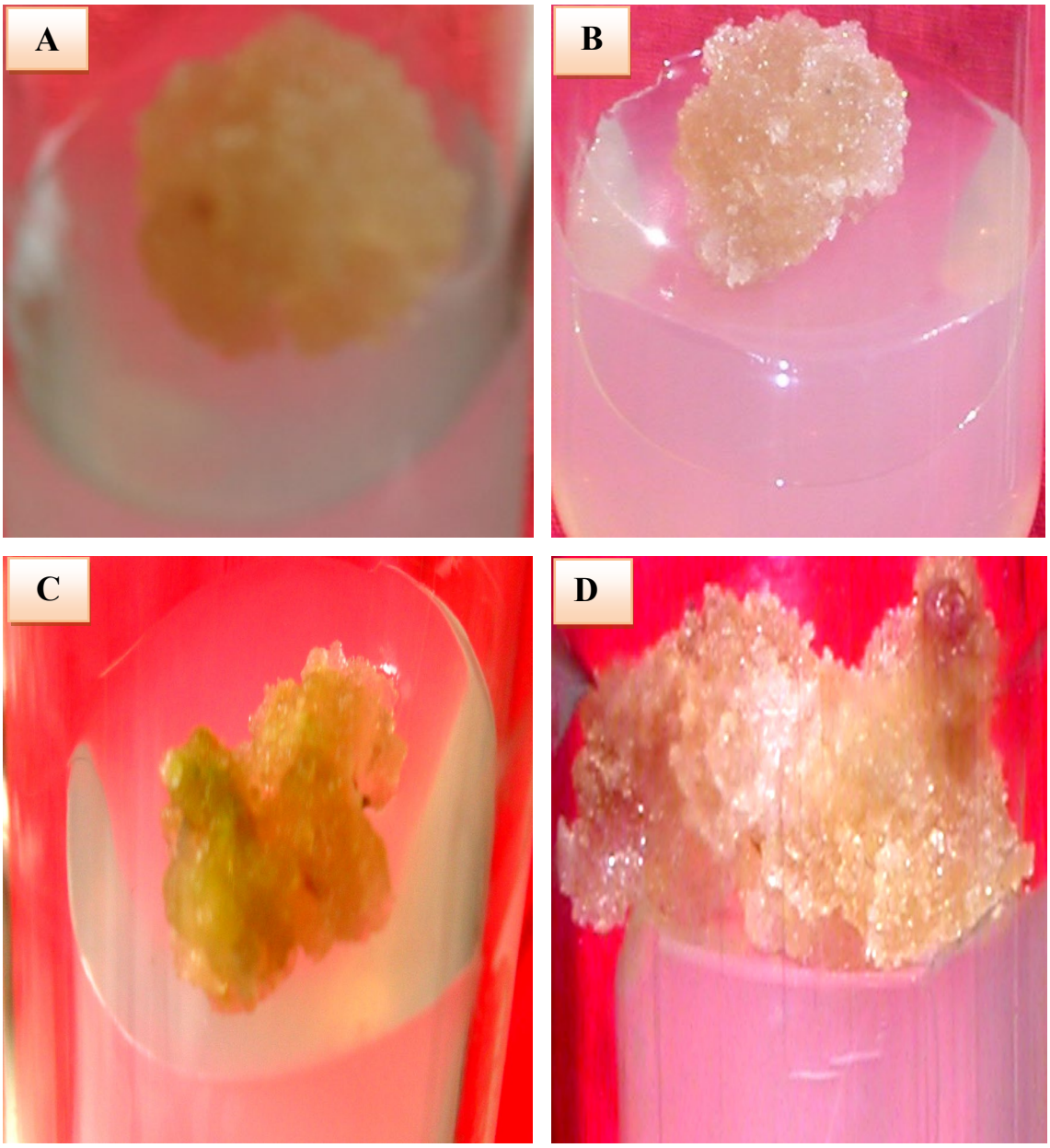
− = No callusing; + = little callusing; + + = Moderate callusing; + + + = massive callusing; Br = Brown, G = Grenish; GBr = Grenish brown; WBr = White brown.

PLATE 4.1: Different stages and types of calli developed from internodal explants



Figures: A: Callus developed in MS medium supplemented with 3.0 mg/l 2,4-D, 28 days after culture incubation; **B:** Callus developed in MS medium supplemented with 2.0 mg/l NAA, 28 days after culture incubation; **C:** Callus developed in MS medium supplemented with 1.0 mg/l 2, 4-D + 0.25 mg/l Kin, 28 days after culture incubation; **D:** Callus developed in MS medium supplemented with 3 mg/l NAA + 2.5 mg/l BA, 28 days after culture incubation.

PLATE 4.2: Different stages and types of calli developed from leaf explants



Figures: A: Callus developed in MS medium supplemented with 3.0 mg/l 2,4-D, 28 days after culture incubation; **B:** Callus developed in MS medium supplemented with 2.0 mg/l NAA, 28 days after culture incubation; **C:** Callus developed in MS medium supplemented with 1.0 mg/l 2, 4-D + 0.25 mg/l Kin, 28 days after culture incubation; **D:** Callus developed in MS medium supplemented with 3 mg/l NAA + 2.5 mg/l BA, 28 days after culture incubation.

4.3.2. Shoot Regeneration from Calli

Different experiments were conducted to investigate plant regeneration ability through callus culture of All Red potato cultivar. The calli derived from internodal and leaf explants, were sub cultured in MS medium supplemented with different concentrations and combinations of BA with GA_3, Kin with GA_3, BA with NAA and Kin with NAA for shoot regeneration. The effects of these growth regulators on percentage of calli induced in shoot regeneration, number of shoots/callus and shoot length/callus was recorded after six weeks of culture and the results obtained are described under the different heads.

4.3.2.1. Effects of BA with GA_3 on shoot regeneration from calli

Calli derived from internodal and leaf explants were subcultured onto MS semisolid medium supplemented with six concentrations and combinations of BA with GA_3 and the results obtained from this experiment have been presented in **Table 4.2.**

For calli derived from internodal explants, among all the formulations of BA with GA_3, maximum number of calli induced to develop shoot regeneration in media having 1.0 mg/l BA + 0.1 mg/l GA_3. The lower number of calli induced to shoot regeneration in media having 3.0 mg/l BA + 0.1 mg/l GA_3. The higher number of shoots/callus was noticed in media having 1.0 mg/l BA + 0.1 mg/l GA_3. The lower number of shoots/callus was in media having 2.0 mg/l BA + 0.5 mg/l GA_3. The highest length of shoot was recorded when the medium was supplemented with 1.0 mg/l BA + 0.1 mg/l GA_3. The lowest length of shoot was found in media having 2.0 mg/l BA + 0.5 mg/l GA_3. When the calli were cultured onto media having 3.0 mg/l BA + 0.5 mg/l GA_3 no shoot was formed.

In case of calli derived from leaf explants, among the formulations of BA with GA_3, maximum number of calli induced to develop shoot regeneration in media having 2.0 mg/l BA + 0.1 mg/l GA_3. The lower number of calli was enabled to shoot regeneration in media having 2.0 mg/l BA + 0.5 mg/l GA_3. The higher number of shoots/callus was observed in media having 1.0 mg/l BA + 0.1 mg/l GA_3. The lower number of shoots/ callus was in media having 2.0 mg/l BA + 0.5 mg/l GA_3. The highest length of shoot/ callus was recorded when the medium was supplemented with 1.0 mg/l BA + 0.1 mg/l GA_3. The lowest length of shoot was noticed in media having 3.0 mg/l BA + 0.1 mg/l

GA_3. When the calli were cultured onto media having 3.0 mg/l BA + 0.5 mg/l GA_3 no shoot was formed.

4.3.2.2. Effects of Kin with GA_3 on shoot regeneration from calli

Among the calli derived plantlets, a great variation was observed due to different concentrations and combinations of Kin with GA_3 and the results obtained from this experiment have been presented in **Table 4.2.**

For calli derived from internodal explants, among the formulations of Kin with GA_3, maximum number of calli induced to develop shoot in media having 2.0 mg/l Kin + 0.5 mg/l GA_3. The lower number of calli was enabled to show shoot regeneration in media having 3.0 mg/l Kin + 1.5 mg/l. The higher number of shoots/callus was recorded in media having 2.0 mg/l Kin + 0.5 mg/l GA_3. The lower number of shoots/callus was in media having 3.0 mg/l Kin + 1.5 mg/l GA_3. The highest length of shoot was observed when the medium was supplemented with 2.0 mg/l Kin + 0.1 mg/l GA_3. The lowest length of shoot was observed in media having 3.0 mg/l Kin + 1.5 mg/l GA_3. When the calli were cultured onto media having 1.0 mg/l Kin + 0.1 mg/l GA_3 no shoot was formed.

For calli derived from leaf explants, among the formulations of Kin with GA_3, maximum number of calli induced to develop shoot regeneration in media having 2.0 mg/l Kin + 0.5 mg/l GA_3. The lower number of calli was enabled to show shoot regeneration in media having 3.0 mg/l Kin + 1.5 mg/l GA_3. The higher number of shoots/callus was noticed in media having 2.0 mg/l Kin + 0.5 mg/l GA_3. The lower number of shoots/ callus was in media having 3.0 mg/l Kin + 1.5 mg/l GA_3. The highest length of shoot was recorded when the medium was supplemented with 2.0 mg/l Kin + 0.1 mg/l GA_3. The lowest length of shoot was found in media having 1.0 mg/l Kin + 0.5 mg/l GA_3. When the calli were cultured onto media having 1.0 mg/l Kin + 0.1 mg/l GA_3 no shoot was formed.

Table 4.2. Effect of different concentrations and combinations of BA with GA_3 and Kin with GA_3 in MS medium on shoot regeneration from calli.

Source of calli	PGRs (mg/l)	% calli induced in shoot regeneration	No. of shoots/ callus	Shoot length/ callus
	BA + GA_3			
	1.0 + 0.1	70	7.6	5.8
	1.0 + 0.5	40	2.4	2.5
	2.0 + 0.1	50	3.5	3.7
	2.0 + 0.5	20	1.7	2.1
	3.0 + 0.1	10	2.1	2.5
	3.0 + 0.5	-	-	-
Internodal	Kin + GA_3			
segments	1.0 + 0.1	-	-	-
	1.0 + 0.5	20	2.6	2.4
	2.0 + 0.1	30	2.5	3.7
	2.0 + 0.5	50	4.2	2.8
	3.0 + 1.0	20	3.8	2.5
	3.0 + 1.5	10	1.0	2.2
	BA + GA_3			
	1.0 + 0.1	50	6.3	4.6
	1.0 + 0.5	40	2.5	2.2
	2.0 + 0.1	60	3.6	3.9
	2.0 + 0.5	20	1.2	2.0
	3.0 + 0.1	30	1.8	1.4
Leaf	3.0 + 0.5	-	-	-
segments	Kin + GA_3			
	1.0 + 0.1	-	-	-
	1.0 + 0.5	20	1.4	2.4
	2.0 + 0.1	30	2.0	3.8
	2.0 + 0.5	40	3.2	3.5
	3.0 + 1.0	20	1.5	3.1
	3.0 + 1.5	10	1.2	2.6

4.3.2.3. Effects of BA with NAA on shoot regeneration from calli

Calli derived from internodal and leaf explants were subcultured onto MS semisolid medium supplemented with different concentrations and combinations of BA with NAA. The results obtained are presented in **Table 4.3.**

For calli derived from internodal explants, among the formulations of BA with NAA, maximum number of calli induced to develop shoot regeneration in media having 3.0 mg/l BA + 0.1 mg/l NAA. The lower number of calli were enabled to show shoot regeneration in media having 2.0 mg/l BA + 1.0 mg/l NAA and 3.0 mg/l BA + 1.5 mg/l NAA. The higher number of shoots/callus was recorded in media having 3.0 mg/l BA + 0.1 mg/l NAA. The lower number of shoots/callus was in media having 2.0 mg/l BA +1.0 mg/l NAA. The highest length of shoot was observed when the medium was supplemented with 3.0 mg/l BA + 1.0 mg/l NAA. The lowest length of shoot was recorded after 45 days of culture in media having 2.0 mg/l BA +1.0 mg/l NAA.

For calli derived from leaf explants, among all the formulations, the highest number of calli induced to develop shoot regeneration in media having 3.0 mg/l BA + 0.1 mg/l NAA and 3.0 mg/l BA + 0.5 mg/l NAA. The lower number of calli was enabled to show shoot regeneration in media having 2.0 mg/l BA + 1.5 mg/l NAA. The higher number of shoots/callus was found in media having 3.0 mg/l BA + 0.1 mg/l NAA. The lower number of shoots/callus was in media having 2.0 mg/l BA + 1.5 mg/l NAA. The highest length of shoot was recorded when the medium was supplemented with 2.0 mg/l BA + 1.5 mg/l NAA. The lowest length of shoot was noticed in media having 3.0 mg/l BA + 1.0 mg/l NAA. When the calli were cultured onto media having 2.0 mg/l BA + 1.0 mg/l NAA and 3.0 mg/l BA + 1.5 mg/l NAA no shoot was formed.

4.3.2.4. Effects of Kin with NAA on shoot regeneration from calli

Calli derived from internodal and leaf explants were subcultured onto MS semisolid medium supplemented with different concentrations and combinations of Kin with NAA. The results obtained are presented in **Table 4.3.** In case of calli derived from internodal explants, the higher number of calli induced to develop shoot regeneration in media having 4.0 mg/l Kin + 1.0 mg/l NAA. The lower number of calli was enabled to develop shoot regeneration in media having 3.0 mg/l Kin + 0.1 mg/l NAA. The higher number of

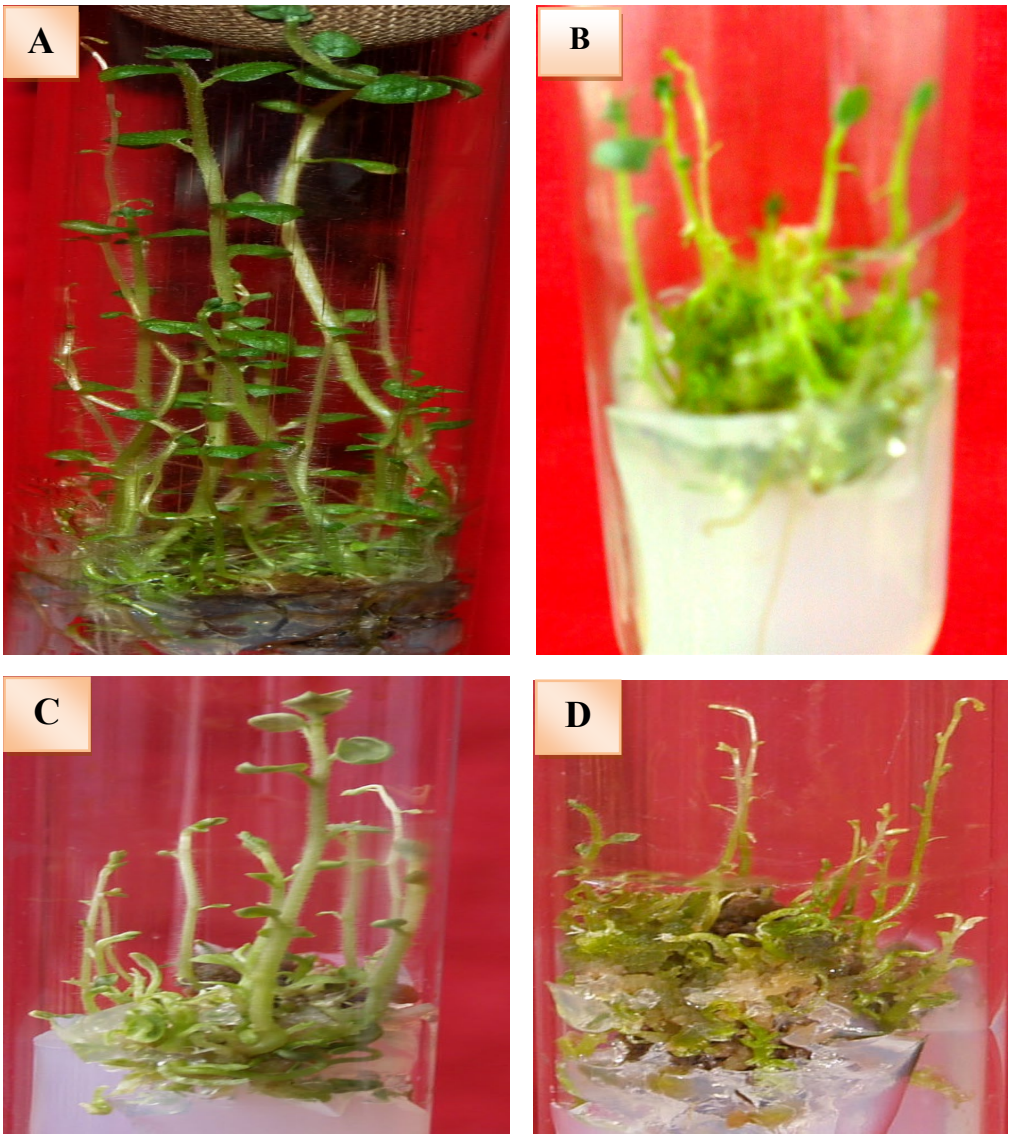
shoots/callus was observed when the media were supplemented with 4.0 mg/l Kin + 1.0 mg/l NAA. The lower number of shoots/ callus was when the media were supplemented with 3.0 mg/l Kin + 0.1 mg/l NAA. The highest length of shoot was recorded in media having 4.0 mg/l Kin + 0.5 mg/l NAA. The lowest length of shoot was when the media were supplemented with 3.0 mg/l Kin + 0.1 mg/l NAA.

In respect of calli derived from leaf explants, maximum number of calli were induced to develop shoot regeneration in media having 3.0 mg/l Kin + 0.5 mg/l NAA. The lower number of calli was enabled to develop shoot regeneration in media having 2.0 mg/l Kin +1.5 mg/l NAA and 3.0 mg/l Kin + 1.5 mg/l NAA. The higher number of shoots/callus was recorded when the media were supplemented with 3.0 mg/l Kin + 0.5 mg/l NAA. The lower number of shoots/callus was when the media were supplemented with 2.0 mg/l Kin + 1.5 mg/l NAA. The highest length of shoot was found in media having 3.0 mg/l Kin + 0.5 mg/l NAA. The lowest length of shoot was oberved when the media were supplemented with 2.0 mg/l Kin + 1.5 mg/l NAA. When the calli were cultured onto media having 2.0 mg/l Kin + 1 mg/l NAA no shoot was formed.

Table 4.3. Effect of different concentrations and combinations of BA with NAA and Kin with NAA in MS medium on shoot regeneration from calli.

Source of calli	PGRs (mg/l)	% calli induced in shoot regeneration	No. of shoots/ callus	Shoot length/ callus
	BA + NAA			
	2.0 + 1.0	10	1.0	2.1
	2.0 + 1.5	20	2.0	2.3
	3.0 + 0.1	30	4.5	2.2
	3.0 + 0.5	20	1.2	2.5
	3.0 + 1.0	20	1.4	3.1
Internodal	3.0 + 1.5	10	1.8	2.7
segments	Kin + NAA			
	3.0 + 0.1	10	1.8	2.1
	3.0 + 0.5	20	2.6	2.5
	3.0 + 1.0	30	3.7	2.3
	4.0 + 0.5	40	3.2	3.8
	4.0 + 1.0	50	4.8	3.4
	4.0 + 1.5	30	3.6	2.2
	BA + NAA			
	2.0 + 1.0	-	-	-
	2.0 + 1.5	10	1.4	3.3
	3.0 + 0.1	40	4.8	2.4
	3.0 + 0.5	40	4.2	2.8
	3.0 + 1.0	20	2.7	1.3
Leaf	3.0 + 1.5	-	-	-
segments	Kin + NAA			
	2.0 + 1.0	-	-	-
	2.0 + 1.5	10	1.2	2.4
	3.0 + 0.1	20	2.0	2.7
	3.0 + 0.5	30	3.0	3.1
	3.0 + 1.0	20	1.6	2.8
	3.0 + 1.5	10	1.4	2.6

PLATE 4.3: Potato shoots regeneration from calli in different culture media formulations



Figures: A: Multiple shoots regenereted from the internodal explant derived callus in MS medium fortified with 1.0 mg/l BA + 0.1mg/l GA_3, 55 days after subculture; **B:** Multiple shoots regenereted from the leaf explant derived callus in MS medium fortified with 2.0 mg/l BA + 0.1 mg/l GA_3, 55 days after subculture; **C:** Multiple shoots regenereted from the internodal explant derived callus in MS medium fortified with 2.0 mg/l Kin + 0.5 mg/l GA_3, 45 days after subculture; **D:** Multiple shoots regenereted from the leaf explant derived callus in MS medium fortified with 2.0 mg/l Kin + 0.5 mg/l GA_3, 45 days after subculture.

4.3.3. Field Evaluation of Somaclones on Agronomic and Morphological Characters

The multiplyed and rooted somaclones were successfully established to the field and the data were recorded on different agronomic and morphological characters and it was collected from 10 randomly selected plants in each replication for individual cell lines. The results are described in the following heads.

Plant height: Significant variation was observed among the somaclones in respect of plant height. Maximum plant height was noticed in AR-05 cell line and it was not significantly different from the height of control plants. On the other hand, the lowest plant height was recorded for the plants of AR-08 cell line **(Figure 4. 1).**

Number of stems/hill: The higher number of stems/hill was recorded for the plants of AR-06 cell line followed by AR-05, AR-10 lines and control plants.They were not significantly different in respect of stem number/hill. The lower number of stems was noticed in the plants of AR-08 cell line **(Figure 4. 2).**

Number of leaves/hill: The higher number of leaves was found in the plants of AR-06 line followed by AR-05 line and control plants. These variations were insignificant. On the other hand, the lower number of leaves was recorded for the plants of AR-08 cell line **(Figure 4.3).**

Canopy (cm^2)/hill: The maximum canopy was found in AR-05 cell line which was followed by AR-06, AR-07 and AR-10 line and control plants. The observed variations in canopy were insignificant in AR-05, AR-06, AR-07 and AR-10 line and control plants. The lowest canopy was recorded in the plants of AR-02 line **(Figure 4.4).**

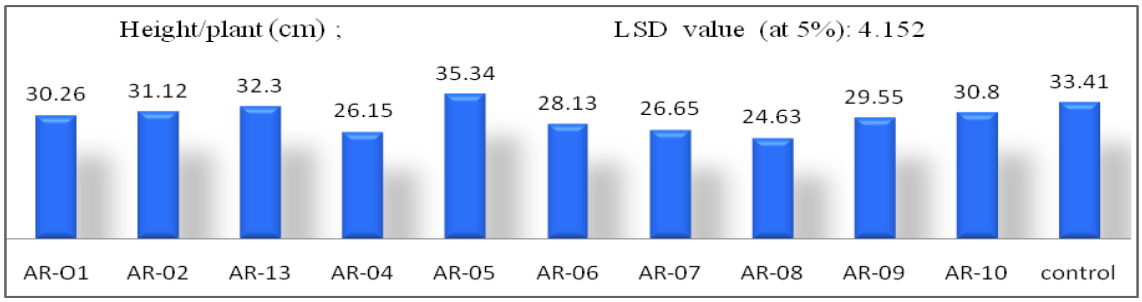


Figure: 4.1

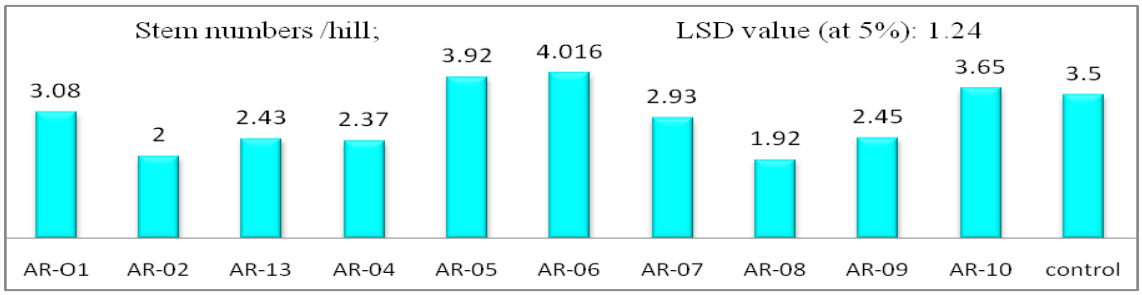


Figure: 4.2

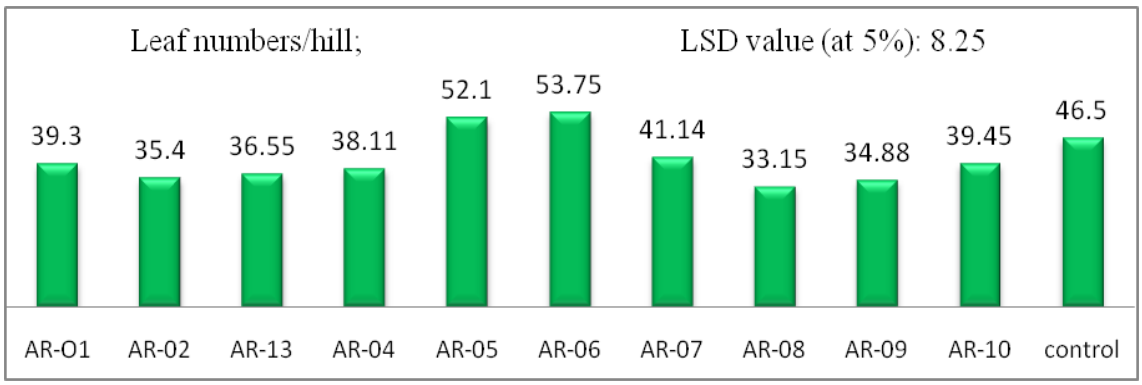


Figure: 4.3

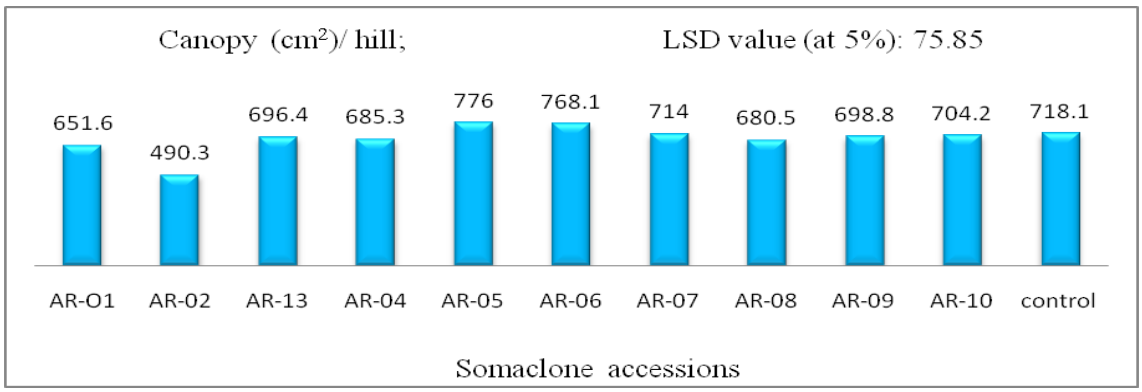


Figure: 4.4

Figures 4.1-4.4: Plant height, number of stems, and number of leaves and canopy size/hill of somaclones (accessions AR-01 to AR-10) of cultivar All Red. Data were recorded after 65 days of plantation.

Third leaf area (cm^2): Among the somaclones AR-06 line produced the highest area of 3rd leaf followed by control plants and AR-03 line. The observed difference in 3rd leaf area was insignificant in AR-06 and AR-03 line and control plants. AR-08 line produced the lowest area of 3rd leaf **(Figure 4.5).**

Chlorophyll content (mg/g): The maximum leaf chlorophyll content was noticed in AR-06 cell line which was followed by control plants. On the other hand, the lowest amount of chlorophyll was recorded for the plants of AR-04 cell line **(Figure 4. 6).**

Number of tubers/hill: The maximum number of tubers was recorded from AR-05 cell line and it was significantly higher as compared to the mother plants. The second highest number of tubers was recorded for plants belong to cell line AR-06. For number of tubers/hill, insignificant variations were noticed in AR-09 line and control plants. On the other hand, the lower number of tubers/hill was recorded from AR-08 line **(Figure 4.7).**

Weight (g) of tubers/hill: The maximum tuber weight/hill was observed in AR-05 cell line followed by AR-06 line and control plants. The observed difference in tuber weight/hill was insignificant in AR-05 and AR-06 line and control plants. The lowest amount of tuber was recorded from AR-02 line**.** The tuber weight/plant increased 7 g in the AR-05 somaclone line as compared to the mother plants **(Figure 4. 8).**

In addition of these, changes or modifications in some other characters were also observed. Plant with more green leaves was found. Plants with deformed leaves such as thick and deep green leaflets; dwarfism and modification in canopy structure were also recorded in some of the plants.

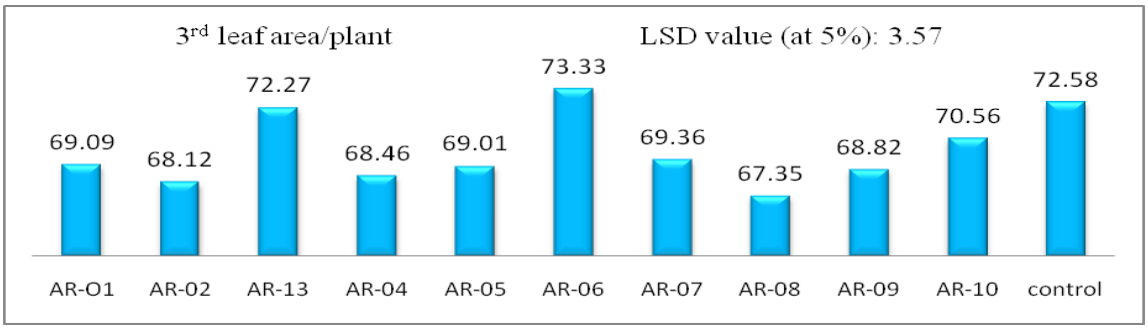


Figure: 4.5

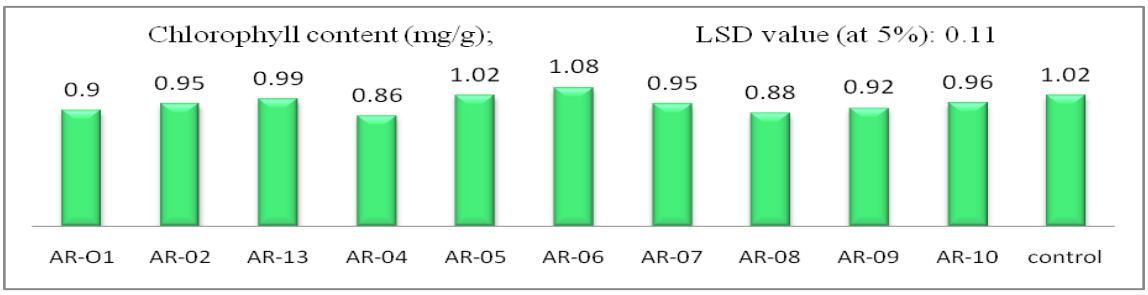


Figure: 4.6

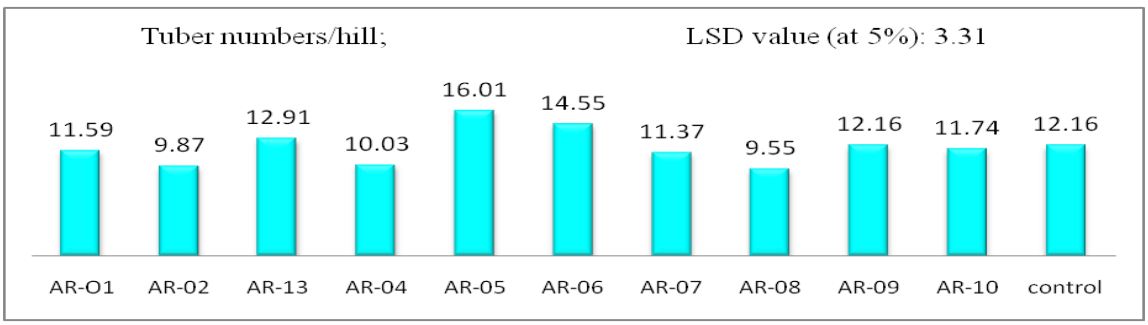


Figure: 4.7

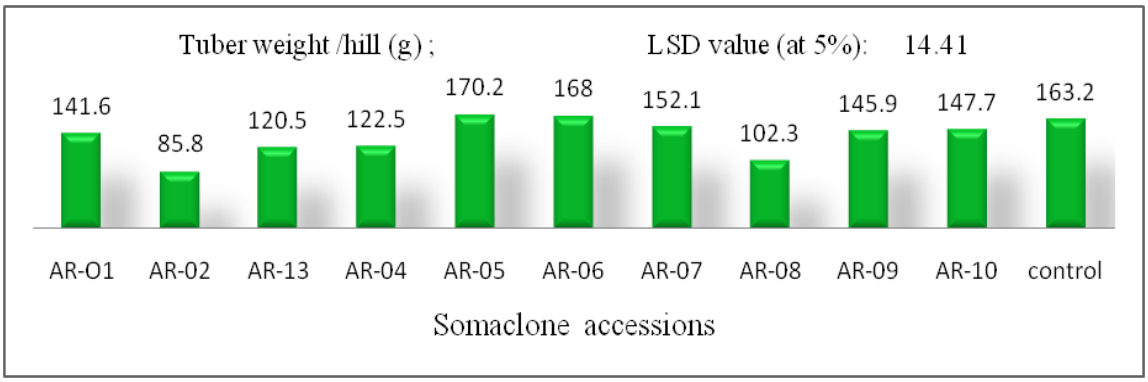


Figure: 4.8

Figures 4.5-4.8: Third leaf area, leaf chlorophyll content, tuber number/plant and tuber weight/plant of somaclones (accessions AR-01 to AR-10) of cultivar All Red. Data were recorded after 65 days for 3rd leaf area and chlorophyll content and 90 days for tuber number and tuber weight.

4.3.4. Evaluation of Somaclones on Processing and Nutritional Quality

Dry matter, iron (Fe) and β-carotene content and somaclonal variations in the potato tubers were evaluated after the harvesting of tubers produced from somaclonal accessions.

Dry matter in tuber (%): The dry mater content of tubers is one of the prime characters used by potato processors to evaluate a crop. In the present study, the higher percentage of dry matter in potato tuber was estimated from AR-05 which was followed by AR-08 cell lines and control plants and these variations were insignificant. Minimum percentage of tuber dry matter was recorded from AR-04 line **(Figure 4.9).**

Iron (Fe) content (mg/100 g tuber): The highest amount of Fe was recorded from the fresh tuber of AR-01 line and it was significantly higher as compared to the control (mother plants). For Fe content, insignificant variations were noticed in AR-02 and AR-08 cell lines and control plants. On the other hand the lowest amount of Fe was recorded from AR-05 line. The Fe content increased 0.46 mg in the AR-01 somaclone line as compared to the control (mother) plants **(Figure 4.10).**

β-carotene content (µg/100 g tuber): The highest amount of β-carotene was obtained from the fresh tuber of AR-09 line followed by AR-02, AR-06 and AR-03 lines but the observed difference in β-carotene content was not significant in AR-09, AR-02, AR-06 and AR-03 lines and control plants. On the other hand the lowest amount of β-carotene was recorded from AR-10 line**.** The β-carotene content increased 5.67 µg in the AR-09 somaclone line as compared to the control (mother) plants but it was not significant **(Figure 4.11).**

From the field evaluation of somaclones, it is clearly focused that many somaclones appeared to display significant or insignificant superiority as compared to the parent various.

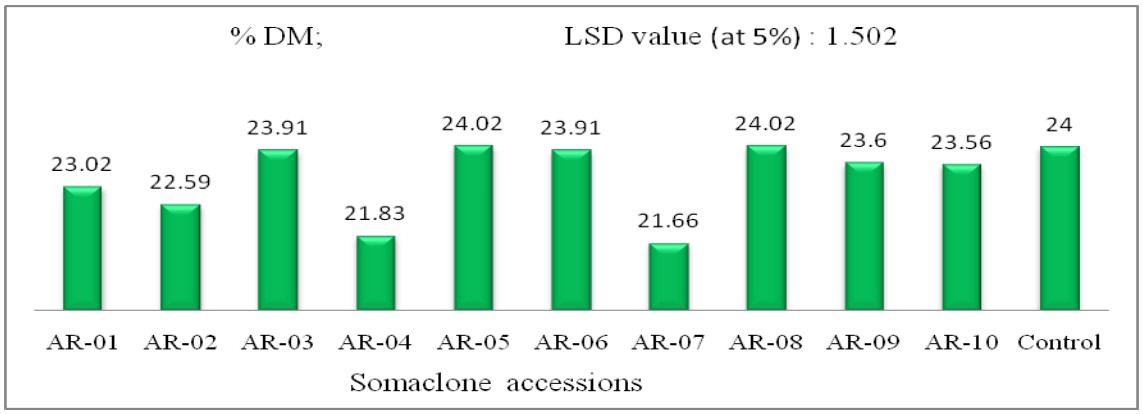


Figure: 4.9

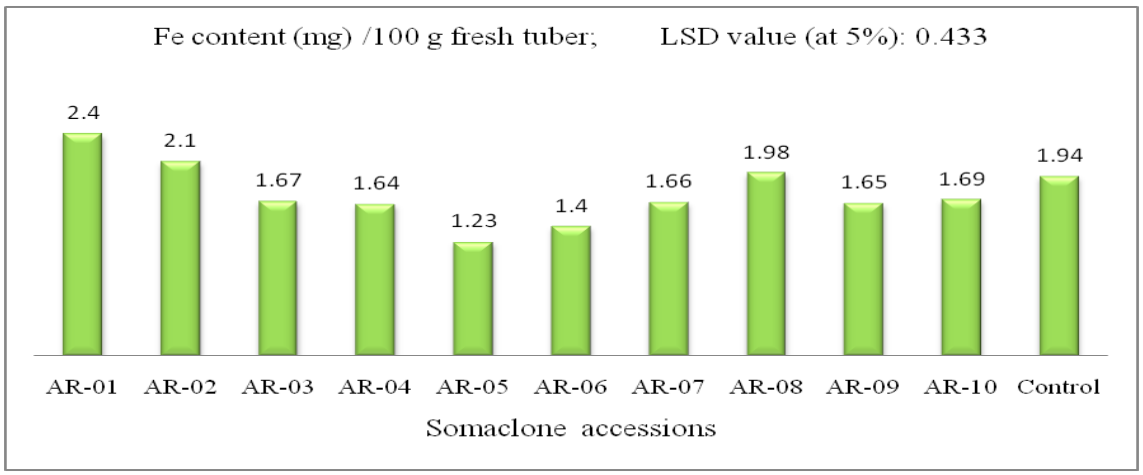


Figure: 4.10

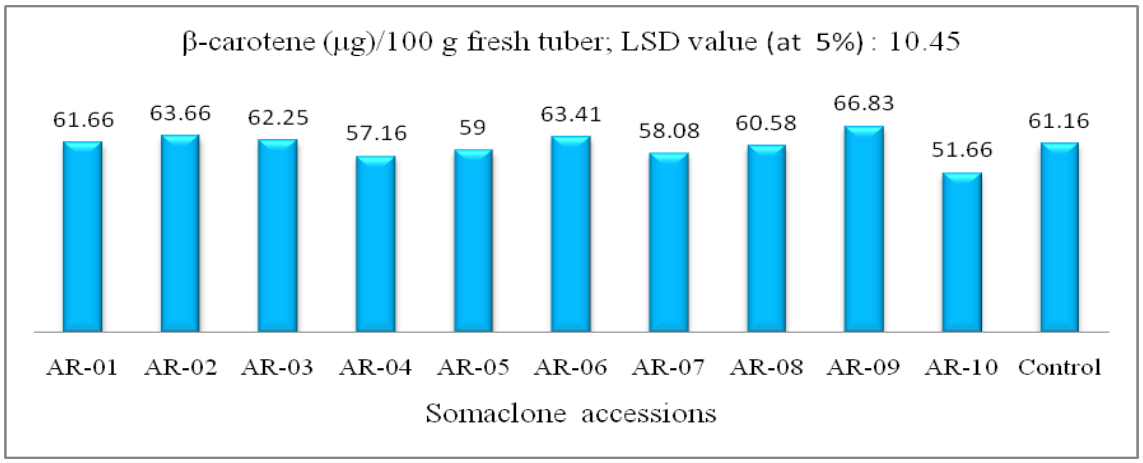
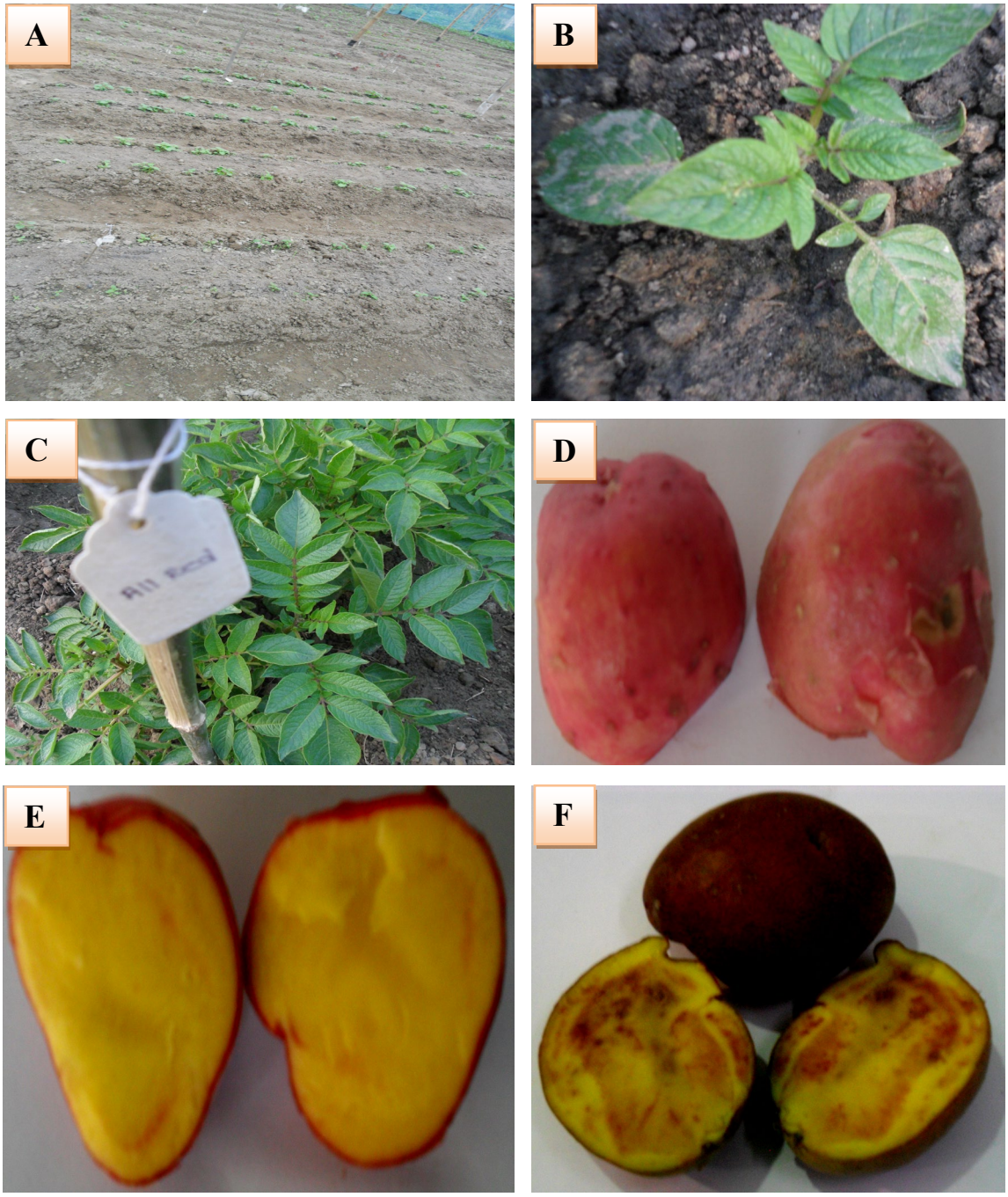


Figure: 4.11

Figures 4.9 - 4.11: Variations among the 10 somaclones (accessions AR-01 to AR-10) of All Red potato cultivar in respect of DM, iron and *β*-carotene content. Data were recorded after 15th days of harvesting.

PLATE 4.4: Field evaluation of somaclones



Photographs showing field evaluation of somaclones. **A-C:** Field establishment of 10 somaclonal accessions of cultivar All Red; **D-E:** Minitubers and its sections of AR-09 somaclonal line. The photographs were recorded after 80 days of planting; **F:** Sections of minituber of AR-01 somaclonal line. The photograph was recorded after 35-40 days of harvesting.

4.4. DISCUSSION

Somaclonal variation provides a valuable source of genetic variations for the improvement of crops through the selection of novel variants. It has been successful in identification of new cultivars in sugarcane, sorghum, tomato, wheat, flax, and *Pelargonium* (Skirvin and Janick, 1976 and Sears *et al*., 1992). Callus culture is the most promising plant tissue culture technique that can introduce somaclonal variation in short time. Variations in morphological characters among callus regenerated plants were observed in rice for grain size, leaf number (Sun and Zheng, 1990) and in potato for maturation time, size, leaf shape and size and yield (Karp, 1990 and Sood, 2006). In the present investigation was carried out for enhancing the range and magnitude of somaclonal variation which can be used for selecting desirable lines having good agronomic characters including yield and high levels of iron and pro-vitamin A (β-carotene). The results obtained in the present investigation have been discussed in the following paragraphs with an endeavor to justify them.

The term callus refers to tissue arising from the disorganized proliferation of cells from segments (explants) of plant organs. Callus formed during *in vitro* culture has some similarities to tissue arising *in vivo* injury to plants (so called wound callus). However, there often are differences in morphology, cellular structure, growth and metabolism between callus derived through tissue culture and natural wound callus.

To initiate callus from different explants, an exogenous supply of growth regulators are often required. Exogenous supplies of auxin and often in combination with cytokinin to medium are essential for callus induction. Rao and Lee (1986) reported that intermediate levels of auxin and cytokinin usually promote callusing. But many other factors like genotypes, compositions of the nutrient medium, physical growth factors such as light, temperature, humidity etc. are important for callus induction (Pierik, 1987). Explants condition is another factor for callus induction.

For callus culture of dicot plants there are many reports with many species. On the contrary, for monocot plants, it has been believed that callus induction is very difficult, because they have no secondary growth which occurs through the activity of vascular cambium (Maeda, 1980). Auxins are usually required for the induction of callus from a variety of tissue explants, except cambium tissues that can proliferate without an

exogenous supply of auxin (Minocha, 1987). It has now been well established that any tissue can be changed into callus if it is cultured on a suitable defined medium under controlled conditions. The history of callus growth from potato tissue culture goes back as early as 1937 when Nobecoyrt obtained callus proliferation from tuber slices. Skoog and Miller (1957) demonstrated hormonal control of differentiation and laid the foundation of clonal propagation of plants through tissue culture techniques. Since then, efforts have been made throughout the world to differentiate potato callus. At first Lam (1975) and Behnke (1995) were able to induce callus in potato. At CIP, callus culture was started in 1975. In the present investigation MS medium was found to be effective for callus induction and plant regeneration. This is an agreement with that of the findings of Reddy and Reddy (1993).

In the present experiments internodal and leaf explants were collected from pro-vitamin A (β-carotene) and iron enriched potato cultivar (All Red). Explants were cultured on MS medium supplemented with different concentrations of 2,4-D , NAA alone and in combinations of 2,4-D with Kin and NAA with BA in order to find out the most suitable culture media formulation to induce the explants to develop maximum callus. The rates of callus induction showed a great variation with different concentrations and combinations of plant growth regulators. Among all the treatments of 2, 4-D, maximum callusing rate was found in media having 3.0 mg/1 2, 4-D. The degree of callus formation was satisfactory. The rate and degree of callus formation from leaf explants was lower than internodal explants. Mutasim *et al. (*2010), Khadiga *et al.* (2009) and Shirin *et al.* (2007) also found the best results when they were used 3.0 mg /l 2, 4-D in potato callus induction. Many researchers also observed 2, 4-D as the best auxin for callus induction as common as in monocot and even in dicot (Evans *et al.,* 1981; Lu *et al.,* 1982; Ho and Vasil, 1983; Jaiswal and Narayan, 1985 and Chee, 1990). Okazawa *et al*. (1976) also obtained good amount of callus in media with 2, 4-D than other auxins in case of a dicot plant mulberry. However, Mamun *et al.* (1996) reported that 2, 4-D proved less effective when used alone for other plant species. Sultana (2001) used 2, 4-D alone for callus induction from internode and leaf explants of potato. She also obtained similar results in three potato cultivars viz. Diamant, Cardinal and Multa. Malamung *et al.* (1991) found better results when using 2, 4-D as a callus inducing growth regulator in potato callus induction. Khatun *et al.* (2003) used 2, 4-D alone for callus induction and also found the best results in potato cultivar Diamant for callus induction. Similar results

were observed by Nasrin *et al.* (2003). Shirin *et al.* (2007) reported that the rate of callus formation from internodal explants was higher than that of leaf explants.

Among all the treatments of NAA, higher number of explants induced to callus formation in media having 2.0 mg/I NAA. The degree of callus formation was satisfactory. Between the two auxins 2, 4-D was found to be better than NAA for callus induction in potato when they were singly used. This finding is in agreement with the finding of Shirin *et al.* (2007).

Among all the treatment concentrations of 2, 4-D with Kin, maximum internodal explants induced to callus formation in media containing 1.0 mg/l 2,4-D + 0.25 mg/l Kin and the degree of callus formation was massive. Haque *et al. (*2009) also found better result when they were used 1.0 mg/I 2, 4-D + 0.25 mg/l Kin in potato callus induction.

In case of different concentrations and combinations of NAA + BA, higher number of internodal and leaf explants induced to callus formation in media having 3.0 mg/l NAA + 2.5 mg/l BA and the degree of callus formation was massive. This finding is nearer to the findings of Biswas *et al.* (2010) who reported that 94.44 % leaf explants of cultivar Granola induced to callus formation in media containing 3.0 mg/l NAA + 2.5 mg/l BA.

Therefore in the present study, it is clearly focused that MS medium containing 3.0 mg/l 2, 4-D or 3.0 mg/l NAA + 2.5 mg/l BA was the best formulation for callus induction from both internodal and leaf explants in All Red cultivar.

The calli derived from internode and leaf explants, were sub cultured onto MS medium supplemented with different concentrations and combinations of BA with GA_3, Kin with GA_3, BA with NAA and Kin with NAA for shoot regeneration. After subculture of callus, shoot regeneration started within 30-35 days of culture along with more callus proliferation.

The necessity of cytokinin for shoot initiation is well established (Torrey, 1968; Engelke *et al.,* 1973; Narayanaswamy, 1977; Beck and Coponetti, 1983 and Evans *et al.,* 1981). But shoot regeneration from nodal, intemodal and leaf explants derived callus do not seem to be an easy process. Regeneration rate of plantlet from callus in potato is generally poor. Although many researchers reported a good amount of callus formation out of tissue culture, regeneration of complete plants from callus still presented a big

problem (Ghislain *et al*., 1999; Gosal and Bajaj, 1979; Mroginski and Kartha, 1984; Singh *et al.,* 1986). Wareh-HA *et al.* (1989) found that primary callus from leaf explants of the North American cultivar Viking and Norgold-M formed stolon like structures while those of Red LA Soda formed both roots and stolon like structures. After three transfers 30% of Red lapsed calli eventually regenerated 1-3 shoots/callus. In the present investigation, different combinations of cytokinin with auxin were used to see the response of multiple shoots from internodal and leaf explants derived callus of All Red cultivar of potato.

The media with different concentrations and combinations of BA with GA_3, Kin with GA_3, BA with NAA and Kin with NAA were found effective for shoot regeneration of All Red potato cultivar from leaf and intemodal explants derived callus. Two successive culture phases are required for inducing multiple shoots as reported by many earlier researchers (Conover and Litz, 1978; Litz and Conover, 1981).

Among all the four combinations of PGRs BA with GA_3 was proved to be more effective than other combinations for maximum shoot induction from intemodal and leaf explants derived calli. In this case, the regeneration rate of internodal explants derived calli was higher than that of leaf explants derived calli. Among all the treatment concentrations and combinations of BA with GA_3, maximum internodal explants (calli) induced to develop shoot regeneration in media having 1.0 mg/l BA + 0.1 mg/I GA_3. The maximum number of shoots and the highest shoot length callus was also observed in this media formulation. Haque *et al. (*2009) also found the best results when they were used 1.0 mg/I BA + 0.1 mg/l GA_3 in potato callus induction.

For different formulations of Kin with GA_3, the regeneration rate of internodal explants derived calli was higher than that of leaf explants derived calli. Maximum calli (derived from internode) induced to develop shoot regeneration in media having 2.0 mg/l Kin + 0.5 mg/l GA_3. Maximum number of shoots/culture was noticed in same media formulation. The highest length of shoot was recorded in media having 2.0 mg/l Kin + 0.1 mg/l GA_3 when the leaf derived calli were cultured.

In case of different concentrations and combinations of BA with NAA, the regeneration rate of leaf explants derived calli was higher than that of internodal explants derived calli. The maximum number of calli induced to develop shoot regeneration in media

having 3.0 mg/l BA + 0.1 mg/l NAA and 3.0 mg/l BA + 0.5 mg/l NAA from leaf derived calli. The maximum number of shoots/callus was also found in media having 3.0 mg/l BA + 0.1 mg/l NAA. Shirin *et al.* (2007) also found the best results when they were used 3.0 mg/l BA + 0.1 mg/l NAA in potato shoot regeneration. Hoque (1995) used BA with NAA for multiple shoots induction and obtained maximum shoots when cytokinin and auxin were used in MS medium in *Chrysanthemum morifolium.* Sultana (2001) also found the same results in potato cultivars. The effect of BA with NAA fortified with MS medium on multiple shoots production has also been demonstrated by Malaure *et al.* (1991) in *Chrysanthemum morifolium.*

MS medium was supplemented with different concentrations and combinations of Kin with NAA to induce shoot regeneration from the internodal and leaf explants derived calli. In this case the regeneration rate of leaf explants derived calli was lower than that of intemodal explants derived calli. It was also noted that Kin with NAA showed better performance than BA with NAA for shoot regeneration percentage on internode derived calli. The highest number of calli (derived from internode) induced to develop shoot regeneration in media having 4.0 mg/l Kin + 1.0 mg/l NAA. The maximum number of shoots/callus was also found in this media composition. The highest shoot length was recorded in media having 4.0 mg/l Kin + 0.5 mg/l NAA. Shirin *et al.* (2007) also found the best results when they were used 4.0 mg/l Kin + 1.0 mg/l NAA in potato shoot regeneration. Sultana (2001) used Kin with NAA in MS medium for shoot regeneration from callus in *Solanum tuberosum.* Suh and Park (1986) recorded that Kin with NAA in MS medium also stimulated proliferation and elongation of shoots in garlic. Ratio of cytokinin and auxin seems to play an important role in the morphogenic differentiation of cultured explants, as suggested by Murashige and Skoog (1962), Steward *et al.* (1969), Thomas and Street (1970), Pareek and Chandra (1981), Beck and Coponetti (1983) and Haider (1992). This differential response with regard to morphogenic response of *Solanum* explants may be due to the genotype differences of the plant material used in the investigation.

In the present study MS medium supplemented with 1.0 mg/l BA + 0.1 mg/I GA_3 was recorded as optimum concentration and combination for maximum shoot regeneration from both types of explants in All Red cultivar.

The multiplied and rooted somaclones were successfully established to the field and different agronomic, morphological and nutritional characters were evaluated. From the results it is revealed that AR-01 and AR-05 somaclone lines appeared to display significant superiority compared to the parent various in respect of iron content and tuber number respectively. However, many somaclones appeared to display insignificant superiority compared to the parent various. Further studies are needed for a complete evaluation of these variations for use in breeding programs. For proper evaluation of variation stability the plants should be grown in different climatic conditions and the studied characters monitored for a long time.

4.5. SUMMARY

The present investigation was conducted for enhancing the range and magnitude of somaclonal variation which can be used for selecting desirable lines having good agronomic characters including yield and high levels of iron and β-carotene. To achieve the objectives internodal and leaf explants were collected from pro-vitamin A (β-carotene) and iron enriched potato cultivar (All Red). Then the explants were cultured on MS medium supplemented with different concentrations of 2,4-D , NAA alone and in combinations of 2,4-D with Kin and NAA with BA in order to find out the most suitable culture media formulation to induce the explants to develop maximum callus. The rates of callus induction showed a great variation with different concentrations and combinations of plant growth regulators. In this case it is clearly focused that MS medium containing 3.0 mg/1 2, 4-D or 3.0 mg/l NAA + 2.5 mg/l BA were the best formulation for callus induction from both internodal and leaf explants.

The calli derived from internode and leaf explants, were sub cultured in MS medium supplemented with different concentrations and combinations of BA with GA_3, Kin with GA_3, BA with NAA and Kin with NAA for shoot regeneration. Shoot regeneration was started within 30-35 days of subculture along with more callus proliferation. In the present study MS medium supplemented with 1.0 mg/1 BA + 0.1 mg/I GA_3 was recorded as optimum concentration and combination for maximum shoot regeneration from both types of explants in All Red cultivar.

The multiplied and rooted somaclones were successfully established to the field and different agronomic and morphological characters were evaluated. Significant variation was observed among the somaclones in respect of plant height, number of stems, number of leaves, canopy size, chlorophyll content tuber number and tuber weight/plant but these variations were not significantly different from the control (mother) plants although significant variation was found between AR-05 somaclone line (16.01 ± 1.62) and mother plant (12.16 ± 1.94) in respect of tuber number/plant.

In the present study, significant variation was also found between AR-01 somaclone line and mother tuber in respect of iron content. The iron content in 100 g mother tuber was found 1.94 mg which is increased in somaclonal variant (line AR 01) and the value was

2.4 mg. On the other hand the amount of β-carotene in 100 g mother tuber was found 61.16 μg which is increased in somaclonal variant (line AR 09) and the value was 66.83 µg but this variation is not significantly different.

So it is noted that AR-01 and AR-05 somaclone line appeared to display significant superiority compared to the parent various in respect of iron content and tuber number respectively. However, many somaclones appeared to display insignificant superiority compared to the parent various.

CHAPTER V

IN VITRO GERMPLASM CONSERVATION OF POTATO

5.1. INTRODUCTION

The cultivated potato, *Solanum tuberosum* L., is a tetraploid (2n = 4x = 48) and exhibits complex tetrasomic inheritance. It is highly heterozygous and segregates on sexual reproduction. Therefore, true (botanical) seeds cannot be used for conserving the selected individual genotypes, though it can be an effective way of storing the total gene pool. Elite parental lines and cultivars of potato are thus maintained through vegetative propagation in order to maintain their genetic integrity. Maintenance of potato germplasm through field clonal propagation is time-consuming, requires large amounts of space and is labour intensive. This also exposes the plants to disease, pests, and risks of loss due to abiotic stresses and natural calamities (Withers *et al*. 1990).Therefore, throughout the world potato gene banks prefer to conserve elite parental lines/clones as *in vitro* propagated plantlets under disease-free tissue culture conditions (Westcott *et al*., 1977; Roca *et al*., 1989). When grown under optimum propagation conditions (MS medium with 3% sucrose, 16 h photoperiod, 22 ± 1ºC temperature they require sub culturing after every 4-8 weeks. In order to reduce the frequency of sub culturing, growth of the plantlets is restricted by employing growth retardants or osmotic stress in combination with a reduced energy source, low temperatures, low light intensity and varied photoperiod (Westcott, 1981a, b; Estrada *et al*., 1983; Ishige, 1995; Siddiqui *et al*., 1996; Lopez-Delgado *et al*., 1998). Use of low temperatures (6-8ºC) and 16 h photoperiod (15-30 µmol m^{-2} s^{-1} light intensity from cool white fluorescent lamps) is almost universal in potato gene banks for conservation. However, in the tropics and sub-tropics, ambient temperatures in summer can be as high as 45-50ºC. Therefore, maintenance of walk-in-chambers at 6-8ºC in tropical and sub-tropical conditions is very energy demanding and costly. In addition this low temperature-osmotic stress method of storage has been shown to result in excessive phenotypic abnormalities (such as vitrification, flaccidity, senescence, etc.) in potato plantlets under protracted *in vitro* storage (Lopez-Delgado *et al*., 1998). Hence, there is need to develop protocols for conserving potato plantlets at temperatures of 22 ± 1ºC that are normally available in

tissue culture rooms used for micropropagation. Non-metabolisable sugar-alcohols (osmoticums) reduce the water availability to the growing cultures by imposing a water-deficit stress (Thorpe, 1979; Thompson *et al*., 1986). This stress may perhaps be responsible for slow growth of potato plantlets. In the present study, the possibility of conserving potato germplasm at 22 ± 1ºC was investigated by employing different concentrations and combinations of metabolically inactive sugar-alcohols (osmoticums) to produce osmotic stress and sucrose as the energy source.

Mechanism of osmoticums in slow-growth *in vitro* conservation: Osmotic agents (osmoticums) are materials that reduced the water potential of cells and reduce mineral uptake by cells through differences in osmotic pressures thereby retarding plant growth (Dodds and Roberts, 1985; Thompson *et al*., 1986). The addition of osmotica to the culture medium has been proved to be efficient reducing growth and increasing the storage life of many *in vitro* grown tissues of different plant species (Shibli, 1991; Wlison *et al*., 2000). According to the hypothesis for turgor driven growth and cell expansion (Zimmermann, 1978), high level of osmotic agents in the medium would act against the creation of a critical turgor pressure, which must be established before cell expansion can occur. This stress condition will inhibit both callus growth and shoots formation (Brown, 1979). *In vitro* plants growing in closed culture vessels have low concentrations of CO_2; C absorption in maintained by supplementing the medium with sugar.

Types of osmoticums or growth retardants: Mannitol, sucrose, sorbitol (Shibli *et al*., 1992), tributyl-2, 4 dichlorbenzylphosphonium chloride (phosphon D), malic hydrazide, succinic acid-2, 2-dimethyl hydrazide (B-995), CCC and ancymidole (Wlison *et al*., 2000) were reported to be good materials to lengthen the storage life of *in vitro* grown tissue.

Commonly used some osmoticums in slow-growth *in vitro* conservation:

Sucrose: Sucrose is a major component of most tissue culture media. It functions as both a carbon/energy source and osmotic agent (Shibli *et al*., 2000; Shibli, 1991). Sucrose can be used to reduce plant growth *in vitro* (Moges *et al.,* 2003; Orlikwska, 1992). However, the growth of explants is dependent on sucrose concentration (Sarkar and Naik, 1998). Micro plant elongation was decreased in potato (Sarkar and Naik, 1998) and wild pear

(Tahtamouni and Shibli, 1999) with increasing concentration of sucrose. For tobacco callus cultures, 30 g/l reduced the capacity of the culture to from shoots which was completely inhibited when sucrose level reached 150 g/l (Brown, 1979).

Mannitol: Mannitol can also be used as an osmotic agent (Lipavska and Vreugdenhil, 1996). It is a sugar alcohol which is produced as a primary photosynthetic product by some plants and can be metabolized by them (Moges *et al.,* 2003; Tahtamouni and Shibli, 1999). The use mannitol did not support tissue growth in tobacco (Shibli *et al*., 1999), but reduced shoot growth of chrysanthemum (Moges *et al.,* 2003) and bitter almond (Ballester *et al*., 1997).

Sorbitol: Sorbitol is another sugar alcohol that inhibited shoot growth of *in vitro* grown chrysanthemum when it was added to the proliferation media (Ballester *et al*., 1997). Shibli *et al.* (1992) reported that elevated sucrose, sorbitol or mannitol reduced growth of better almond microshoots significantly and extended the subculture interval to four months when cultures were kept at room temperature.

Importance of slow-growth *in vitro* conservation: *In vitro* conservation is the most useful and efficient way to distribute clonal materials. It facilitates the availability of planting materials at any time, avoids the transfer of major pests and pathogens and makes possible virus eradication through meristem culture (Roca *et al*., 1979; George, 1993). *In vitro* material can be kept in culture indefinitely, provided sufficient care is taken to avoid contamination and transfers to fresh media are made at appropriate intervals. In this way a pathogen-tested stock can be kept as a reserve for future use. In addition, *in vitro* conservation is less expensive than cryopreservation of field-grown clonal materials (Florkowski and Jarret, 1990).

Limitation of slow-growth *in vitro* conservation: The most serious limitations are a lack of a common method suitable for all species and genotypes, the high costs and the possibility of somaclonal variation and non-intentional cell-type selection in the stored material (e.g. aneuploidy due to cell division at low temperatures or non-optimal conditions giving one cell type a selective growth advantage). Growth retardants can produce some physiological changes or generate mutations, which can threaten the genetic stability of the materials, conserved *in vitro* (Hughes, 1981; Lizarraga *et al*., 1989; Wescott, 1981b).

5.1.1. Objectives

The main objective of this investigation is to find out the most suitable culture media formulation to reduce the frequency of subculturing of conserving potato germplasm by employing different concentrations and combinations of osmoticums.

5.2. MATERIALS AND METHODS

5.2.1. Materials

In vitro single nodal segments of five potato cultivars viz. All Blue, All Red, Asterix, Diamant and JPR were used for conducting different experiments in the present investigation.

5.2.2. Methods

The methods involved in the present investigation are described under the separate heads.

5.2.2.1. In vitro conservation techniques of potato plantlets

Single nodal cuttings (SNCs) were dissected from 4-5 weeks old axenic plantlets that were routinely propagated and maintained on semisolid MS (Murashige and Skoog,1962) medium supplemented with 30 g/l sucrose (adjusted to pH 5.8 before autoclaving), under standard culture conditions (16 h photoperiod; 40 µmol m^{-2} s^{-1} light intensity and 22 ± 1°C). These were sub cultured onto different modified MS media formulations which are presented in **Appendix 3**. Seven SNCs were cultured in each culture vessel (110 × 50 mm) containing 25 ml of medium solidified with 7 g/1 agar (Merck, India). Culture vessels were closed with polypropylene caps and sealed with parafilm "M" (Laboratory Film) (Chicago, IL. 60631, USA), and incubated under a 16 h photoperiod (from cool white fluorescent lamps, approx. 20 µmol m^{-2} s^{-1} light intensity) at 22 ± 1°C in a plant growth chamber.

5.2.2.2. Data recording and statistical analysis

Cultures were checked at 6 and 12 months for the percentage of plantlets survival, plantlet condition (on a visual 0 - 5 preference scale: 0 = dead, 1 = very poor, to 5 = very good) for suitability for sub culturing, and root growth (on visual 0-5 scale: 0 = nil, 1 = very poor, to 5 = very good). Observations were also recorded on the presence or absence of aerial roots, microtubers or phenotypic abnormality. The data were analyzed using the standard procedures (Steel and Torrie, 1980).

5.3. RESULTS

In this investigation the single nodal explants of 5 potato cultivars were sub cultured on MS medium solidified with agar and supplemented with different concentrations of sorbitol and mannitol alone and in combinations of sorbitol with mannitol, sucrose with sorbitol, sucrose with mannitol and sucrose with sorbitol and mannitol in order to find out the most suitable culture media formulation for increasing intervals between subculture of conserving potato plantlets. Four parameters such as growth response of cultured explants, percentage of plantlets survival, plantlets condition and root growth were noted. The results of present piece of work are described under the separate heads.

5.3.1. Effect of Different Concentrations of Sorbitol and Mannitol Alone and in Combinations of Sorbitol with Mannitol on *In Vitro* Conservation of Potato

The explants were sub cultured onto different modified MS media formulations namely MS_1 (MS medium with 30 g/l sorbitol), MS_2 (MS medium with 40 g/l sorbitol), MS_3 (MS medium with 50 g/l sorbitol), MM_1 (MS medium with 30 g/l mannitol), MM_2 (MS medium with 40 g/l mannitol), MM_3 (MS medium with 50 g/l mannitol), $MSTMT_1$ (MS medium with 15 g/l sorbitol + 15 g/l mannitol), $MSTMT_2$ (MS medium with 20 g/l sorbitol + 20 g/l mannitol) and $MSTMT_3$ (MS medium with 25 g/l sorbitol + 25 g/l mannitol) media to observe the response of cultured explants and plantlets survival. Here, sucrose was no added to any treatments. MS medium without sucrose was used as a control medium.

In this experiment, for the absence of sucrose in conservation medium no explants of five potato cultivars were responded or survived although the media were supplemented with different concentrations of sorbitol or mannitol or in combinations of sorbitol with mannitol. On the contrary, the explants were partially responded in control medium but the plantlets produced in control medium were affected by maturity group within 1-2 months of culture. JPR was not responded in control medium due to absence of sucrose.

5.3.2. Effect of Sorbitol with Sucrose on *In Vitro* Conservation of Potato

In this experiment the single nodal explants of five potato cultivars were sub cultured onto different modified MS media formulations viz. MSS_1(MS medium with 20 g/l sucrose + 20 g/l sorbitol), MSS_2 (MS medium with 20/l sucrose + 40 g/l sorbitol), MSS_3 (MS medium with 40 g/l sucrose + 20 g/l sorbitol), MSS_4 (MS medium with 30 g/l sucrose + 40 g/l sorbitol) and MSS_5 (MS medium with 40 g/l sucrose + 30 g/l sorbitol) media to observe the survibility of plantlets with plantlet condition and root growth. The plantlets survibility with plantlet condition and root growth was significantly varied with treatment concentrations and combinations. The detailed results on this experiment are presented in **Table 5.1** and **Plate 5.1.**

Table 5.1 shows that after 6 and 12 months of storage significantly the higher number of plantlets was survived in MSS_2 medium (MS medium with 20 g/l sucrose + 40 g/l sorbitol) with better phenotypic condition and poorer root growth. In respect of cultivars, significantly the best performances were observed in Asterix followed by All Blue and Diamant.

At 6 months conservation of cultivar All Blue, the higher number of plantlets was survived in MSS_2 medium followed by MSS_4 and MSS_3 media. At 12 months of conservation significantly the highest plantlet survival was recorded in MSS_2 medium with better plantlet condition and poorer root growth. Minimum plantlets were survived in MSS_1 medium with poorer plantlet condition and better root growth.

In case of 6 months conservation of cultivar All Red, the highest plantlet survival was recorded when the explants were cultured onto MSS_4 medium followed by MSS_2 medium and the difference between plantlet survival in MSS_4 and MSS_2 media was not significant whereas the lowest survival was recorded in MSS_1 medium. At 12 months of storage, the highest plantlet survival was recorded in MSS_4 medium which was at par with MSS_2 and MSS_3 media whereas the lowest plantlet survival was recorded in MSS_1 medium. Better plantlet condition was found in MSS_4 and MSS_2 media whereas the lowest plantlet condition was found in MSS_5 medium. Root growth was maximum in MSS_1 medium followed by MSS_3 medium and root growth was minimum in MSS_4 medium in All Red cultivars after all storage periods.

In Asterix, the higher number of plantlets at 6 months storage was survived in MSS_2 medium. The difference between plantlet survival in MSS_4 and MSS_2 media was not significant. At 12 months of storage significantly the highest plantlets survival was noticed in MSS_2 medium with better plantlet condition and poorer root growth. Minimum plantlets were survived in MSS_1 medium with better root growth. The lowest plantlet condition was found in MSS_5 medium.

In Diamant, higher number of plantlets at 6 months storage was survived in MSS_2 medium followed by MSS_3 and MSS_4 media. At 12 months of storage significantly the highest plantlets survival was recorded in MSS_2 medium with better plantlet condition and poorer root growth. Minimum plantlets were survived in MSS_1 medium with poorer plantlet condition and better root growth.

At 6 months conservation of cultivar JPR, the highest plantlet survival was recorded when the explants were cultured onto MSS_4 medium which was at par with MSS_2 medium whereas the lowest survival was recorded in MSS_1 medium. At 12 months of storage, the highest plantlets survival was noticed in MSS_4 medium followed by MSS_2 medium whereas the lowest survival was recorded in MSS_5 medium. Better plantlet condition was found in MSS_4 medium followed by MSS_2 medium whereas the lowest plantlet condition was in MSS_1 medium. Root growth was higher in MSS_3 medium and root growth was lower in MSS_2 medium in JPR cultivar after all storage periods.

From the experiment it was also observed that more or less all treatments were exhibited aerial roots but microtuber formation was not observed in any treatment. At 12 months of storage, nearly 30% of the plantlets had turned yellow even on the best treatment in all cultivars.

Table 5.1. Effect of different concentrations and combinations of sucrose with sorbitol in MS medium on plantlet survival, shoot conditions and root growth. In each treatment at least 21 explants were inoculated.

Characters	Cultivars	Different MS media formulations					
		MSS_1	MSS_2	MSS_3	MSS_4	MSS_5	**Mean**
% plantlet survival after 6 months storage	All Blue	73.19^{B}	96.22^{A}	88.42^{A}	93.03^{A}	71.83^{B}	84.54^{a}
	All Red	63.11^{D}	88.89^{A}	78.22^{BC}	89.88^{A}	69.67^{CD}	77.96^{b}
	Asterix	69.89^{C}	100.0^{A}	88.78^{B}	95.66^{AB}	71.33^{C}	85.13^{a}
	Diamant	63.89^{C}	93.11^{A}	86.56^{A}	89.00^{A}	76.00^{B}	81.71^{ab}
	JPR	39.44^{C}	61.56^{AB}	53.89^{B}	64.66^{A}	47.00^{C}	53.31^{c}
	Mean	61.9^{d}	87.96^{a}	79.17^{b}	86.45^{a}	67.17^{c}	
	LSD value (at 5%): 4.27 for cultivars (C); 4.27 for media (M) and 9.56 for C× M						
% plantlet survival after 12 months storage	All Blue	50.56^{D}	85.44^{A}	70.22^{B}	72.00^{B}	61.56^{C}	67.96^{ab}
	All Red	43.22^{C}	71.33^{A}	68.22^{A}	73.88^{A}	57.00^{B}	62.73^{c}
	Asterix	51.03^{D}	89.00^{A}	71.11^{BC}	77.00^{B}	64.33^{C}	70.49^{a}
	Diamant	47.56^{E}	81.33^{A}	67.22^{C}	71.88^{BC}	59.11^{D}	65.42^{bc}
	JPR	26.33^{C}	35.78^{AB}	31.22^{B}	39.33^{A}	21.33^{C}	30.8^{d}
	Mean	43.73^{e}	72.58^{a}	61.6^{c}	66.82^{b}	52.67^{d}	
	LSD value (at 5%): 3.21 for cultivars (C); 3.21 for media (M) and 7.17 for C× M						
Plantlet condition score after 12 months storage	All Blue	2.10^{E}	3.67^{A}	2.50^{CD}	3.16^{B}	2.26^{DE}	2.74^{a}
	All Red	2.01^{C}	2.44^{AB}	2.16^{BC}	2.51^{A}	1.96^{C}	2.23^{b}
	Asterix	2.18^{C}	3.70^{A}	2.42^{C}	3.06^{B}	2.12^{D}	2.69^{a}
	Diamant	2.08^{CD}	3.42^{A}	2.53^{C}	3.12^{B}	2.30^{CD}	2.69^{a}
	JPR	1.57^{E}	2.17^{AB}	1.88^{BD}	2.18^{A}	1.62^{DE}	1.89^{c}
	Mean	1.99^{d}	3.09^{a}	2.30^{c}	2.81^{b}	2.05^{d}	
	LSD value (at 5%): 0.12 for cultivars (C); 0.12 for media (M) and 0.29 for C× M						
Root growth score after 12 months storage	All Blue	3.88^{A}	3.20^{C}	3.74^{A}	3.48^{B}	3.72^{A}	3.60^{ab}
	All Red	3.28^{A}	2.88^{B}	3.22^{A}	2.85^{B}	2.96^{B}	3.04^{d}
	Asterix	3.93^{A}	2.86^{D}	3.79^{A}	3.14^{C}	3.43^{B}	3.43^{c}
	Diamant	3.83^{A}	2.90^{C}	3.73^{A}	3.43^{B}	3.66^{AB}	3.51^{bc}
	JPR	3.95^{B}	3.08^{D}	4.32^{A}	3.26^{D}	3.70^{C}	3.66^{a}
	Mean	3.78^{a}	2.98^{d}	3.76^{a}	3.23^{c}	3.49^{b}	
	LSD value (at 5%): 0.10 for cultivars (C); 0.10 for media (M) and 0.23 for C× M						

The same letters indicate no significant difference at LSD = 0.05. Small letters used for cultivars (C) or media (M) and capital letters used for C × M. [**A.** Plantlet condition (on 0-5 scale, 0 = dead, I = very poor, to 5 = very good); **B.** Root growth (on 0-5 scale, 0 = nil, 1 = very poor, to 5 = very good)].

PLATE 5.1. *In vitro* conservation of potato plantlets in MSS_4 and MSS_2 media

Photographs showing *in vitro* conservation of potato plantlets in MSS_4 and MSS_2 media. **A:** MSS_4 medium and **B:** MSS_2 medium (after 12 months of storage).

5.3.3. Effect of Mannitol with Sucrose on *In Vitro* Conservation of Potato

In this experiment the nodal explants of five potato cultivars were sub cultured onto different modified MS media formulations viz. MSM_1 (MS medium with 20 g/l sucrose + 20 g/l mannitol), MSM_2 (MS medium with 20 g/l sucrose + 40 g/l mannitol), MSM_3 (MS medium with 40 g/l sucrose + 20 g/l mannitol), MSM_4 (MS medium with 30 g/l sucrose + 40 g/l mannitol) and MSM_5 (MS medium with 40 g/l sucrose + 30 g/l mannitol) media to observe the survibility of plantlets with plantlet condition and root growth. The results on this experiment are presented in **Table 5.2** and **Plate 5.2.**

Table 5.2 shows that the plantlets survibility with plantlet condition and root growth of five potato cultivars were significantly influenced both by the different modified MS media formulations and by the cultivars. From the experiment it was observed that after 12 months of storage maximum number of plantlets was survived in MSM_3 medium with the best plantlet condition and poorer root growth. Among the cultivars All Blue and Asterix were found to be superior to other cultivars for plantlet survival with better plantlet condition.

At 6 months storage of cultivar All Blue, maximum plantlet survival was noticed in MSM_3 medium while minimum plantlet survival was observed in MSM_4 medium. At 12 months of conservation the highest plantlet survival was recorded in MSM_3 medium followed by MSM_5 medium while minimum plantlet survival was found in MSM_4 medium. Better plantlet condition was found in MSM_3 medium followed by MSM_5 medium whereas the lowest plantlet condition was in MSM_2 medium. Root growth was higher in MSM_4 medium followed by MSM_2 medium and root growth was lower in MSM_3 medium.

In case of 6 months storage of cultivar All Red, maximum plantlet survival was noticed in MSM_3 medium followed by MSM_5 medium while minimum plantlet survival was observed in MSM_2 medium. At 12 months of conservation significantly the highest plantlet survival was noticed in MSM_3 medium while minimum plantlet survival was found in MSM_4 medium. Better plantlet condition was found in MSM_3 medium followed by MSM_5 medium whereas the lowest plantlet condition was in MSM_4 medium. Root growth was higher in MSM_4 medium followed by MSM_2 medium and root growth was lower in MSM_3 medium.

In Asterix, significantly the higher number of plantlets at 6 months storage was survived in MSM_3 medium while minimum plantlet survival was noticed in MSM_1 medium. At 12 months of conservation the highest plantlet survival was recorded in MSM_3 medium followed by MSM_5 medium while minimum plantlet survival was found in MSM_4 medium. Better plantlet condition was found in MSM_3 medium followed by MSM_5 medium whereas the lowest plantlet condition was in MSM_4 medium. Root growth was higher in MSM_4 medium followed by MSM_1 medium and root growth was lower in MSM_3 medium.

In Diamant, significantly the higher number of plantlets at 6 months storage was survived in MSM_3 medium while minimum plantlet survival was observed in MSM_4 medium. At 12 months of conservation the highest plantlet survival was recorded in MSM_3 medium followed by MSM_5 medium while minimum plantlet survival was found in MSM_1 medium. Better plantlet condition was found in MSM_3 medium followed by MSM_5 medium whereas the lowest plantlet condition was in MSM_1 medium. Root growth was higher in MSM_1 medium followed by MSM_2 medium and root growth was lower in MSM_3 medium.

At 6 months storage of cultivar JPR, maximum plantlet survival was noticed in MSM_5 medium while minimum plantlet survival was observed in MSM_2 medium. At 12 months of conservation the highest plantlet survival was recorded in MSM_5 medium followed by MSM_3 medium while minimum plantlet survival was found in MSM_2 medium. Better plantlet condition was found in MSM_5 medium followed by MSM_3 medium whereas the lowest plantlet condition was in MSM_4 medium. Root growth was higher in MSM_1 medium followed by MSM_2 medium and root growth was lower in MSM_3 medium.

From the experiment it was also observed that more or less all treatments were exhibited aerial roots. Nodal swellings were observed in many treatments. Microtuber formation was not observed in any treatment. At 12 months of storage, nearly 50% of the plantlets had turned yellow even on the best treatment in all cultivars. Plantlet condition deteriorated sharply after 12 months of storage.

Table 5.2. Effect of different concentrations and combinations of sucrose with mannitol in MS medium on plantlet survival, shoot conditions and root growth.

Characters	Cultivars	Different MS media formulations					
		MSM_1	MSM_2	MSM_3	MSM_4	MSM_5	**Mean**
% plantlet survival after 6 months storage	All Blue	71.78^{B}	69.33^{B}	89.89^{A}	62.33^{C}	84.33^{A}	75.53^{b}
	All Red	67.22^{C}	65.78^{C}	85.78^{A}	69.88^{BC}	75.22^{B}	72.78^{b}
	Asterix	68.33^{D}	70.11^{D}	96.89^{A}	77.00^{C}	87.22^{B}	79.91^{a}
	Diamant	70.22^{BC}	66.89^{CD}	97.78^{A}	62.55^{D}	75.33^{B}	74.56^{b}
	JPR	56.11^{BC}	50.33^{C}	59.78^{AB}	51.77^{C}	65.78^{A}	56.76^{c}
	Mean	66.73^{c}	64.49^{c}	86.02^{a}	64.71^{c}	77.58^{b}	
	LSD value (at 5%): 2.82 for cultivars (C); 2.82 for media (M) and 6.31 for C× M						
% plantlet survival after 12 months storage	All Blue	56.89^{BC}	60.56^{B}	71.44^{A}	53.11^{C}	67.78^{A}	61.96^{a}
	All Red	53.67^{BC}	54.67^{B}	67.89^{A}	48.55^{C}	59.22^{B}	56.8^{b}
	Asterix	56.22^{B}	55.67^{B}	72.44^{A}	52.11^{B}	70.78^{A}	61.44^{a}
	Diamant	52.89^{C}	56.44^{BC}	75.67^{A}	55.77^{C}	62.22^{B}	60.6^{a}
	JPR	35.00^{BC}	30.56^{C}	39.43^{AB}	34.44^{BC}	44.56^{A}	36.8^{c}
	Mean	50.93^{cd}	51.58^{c}	65.38^{a}	48.8^{d}	60.91^{b}	
	LSD value (at 5%): 2.62 for cultivars (C); 2.62 for media (M) and 5.86 for C× M						
Plantlet condition score after 12 months storage	All Blue	2.42^{BC}	2.25^{C}	3.64^{A}	2.62^{B}	3.42^{A}	2.87^{a}
	All Red	2.53^{B}	2.76^{AB}	3.03^{A}	2.16^{C}	2.88^{A}	2.67^{b}
	Asterix	2.36^{B}	2.38^{B}	3.39^{A}	2.25^{B}	3.16^{A}	2.71^{b}
	Diamant	2.37^{B}	2.38^{B}	3.36^{A}	2.49^{B}	3.11^{A}	2.74^{ab}
	JPR	1.82^{B}	1.76^{B}	2.31^{A}	1.52^{B}	2.34^{A}	1.95^{c}
	Mean	2.30^{c}	2.31^{c}	3.14^{a}	2.21^{c}	2.98^{b}	
	LSD value (at 5%): 0.15 for cultivars (C); 0.15 for media (M) and 0.34 for C× M						
Root growth score after 12 months storage	All Blue	3.47^{AB}	3.55^{AB}	3.11^{C}	3.63^{A}	3.31^{BC}	3.41^{a}
	All Red	3.33^{BC}	3.42^{AB}	3.16^{C}	3.58^{A}	3.22^{BC}	3.34^{a}
	Asterix	3.43^{AB}	3.38^{B}	3.26^{B}	3.64^{A}	3.32^{B}	3.41^{a}
	Diamant	3.46^{A}	3.33^{AB}	2.95^{C}	3.13^{BC}	3.00^{C}	3.17^{c}
	JPR	3.66^{A}	3.53^{A}	2.44^{C}	2.85^{B}	2.55^{C}	3.00^{d}
	Mean	3.47^{a}	3.44^{a}	2.98^{b}	3.37^{a}	3.08^{b}	
	LSD value (at 5%): 0.11 for cultivars (C); 0.11 for media (M) and 0.24 for C× M						

The same letters indicate no significant difference at LSD = 0.05. Small letters used for cultivars (C) or nodal positions (M) and capital letters used for C × M.

PLATE 5.2. *In vitro* conservation of potato plantlets in MSM_3 and MSM_5 media

Photographs showing *in vitro* conservation of potato plantlets in MSM_3 and MSM_5 Media. **A:** MSM_3 medium and **B:** MSM_5 medium (after 12 months of storage).

5.3.4. Effect of Sorbitol, Mannitol and Sucrose on *In Vitro* Conservation of Potato

In the experiment the nodal explants were sub cultured onto different modified MS media formulations viz. $MSSM_1$ (MS medium with 20 g/l sucrose + 20 g/l sorbitol + 20 g/l mannitol), $MSSM_2$ (MS medium with 20 g/l sucrose + 40 g/l sorbitol + 20 g/l mannitol), $MSSM_3$ (MS medium with 20 g/l sucrose + 20 g/l sorbitol + 40 g/l mannitol) and $MSSM_4$ (MS medium with 40 g/l sucrose + 20 g/l sorbitol + 20 g/l mannitol) media to observe the survibility of plantlets with plantlet condition and root growth. The overall results on this experiment are presented in **Table 5.3** and **Plate 5.3.**

From the **Table 5.3** it appears that the plantlets survibility with plantlet condition and root growth of five potato cultivars were significantly influenced both by the different modified MS media formulations and by the cultivars.

From the experiment it was observed that after 12 months of storage maximum number of plantlets was survived in $MSSM_1$ medium with better plantlet condition and poorer root growth for all the potato cultivars except JPR. In respect of cultivars, the best performances were noticed in All Blue and it showed 72.88 % survibility in $MSSM_1$ medium formulation.

The interactions between the potato cultivars and the media formulations were significant. **Table 5.3** shows that at 6 months storage of cultivar All Blue, maximum plantlet survival was noticed in $MSSM_1$ medium while minimum plantlet survival was observed in $MSSM_4$ medium. At 12 months of conservation the highest plantlet survival was recorded in $MSSM_1$ medium followed by $MSSM_4$ medium while minimum plantlet survival was found in $MSSM_3$ medium. Better plantlet condition was found in $MSSM_1$ medium followed by $MSSM_4$ medium whereas the lowest plantlet condition was in $MSSM_2$ medium. Root growth was higher in $MSSM_3$ medium followed by $MSSM_4$ medium and root growth was lower in $MSSM_1$ medium.

In case of 6 months storage of cultivar All Red, maximum plantlet survival was noticed in $MSSM_1$ medium followed by $MSSM_4$ medium while minimum plantlet survival was observed in $MSSM_2$ medium. At 12 months of conservation the highest plantlet survival was recorded in $MSSM_1$ medium followed by $MSSM_2$ medium while minimum plantlet survival was found in $MSSM_4$ medium. Better plantlet condition was found in $MSSM_1$ medium followed by $MSSM_2$ medium whereas the lowest plantlet condition was in

$MSSM_4$ medium. Root growth was higher in $MSSM_4$ medium followed by $MSSM_3$ medium and root growth was lower in $MSSM_1$ medium.

In Asterix, the higher number of plantlets at 6 months storage was survived in $MSSM_1$ medium while minimum plantlet survival was noticed in $MSSM_3$ medium. At 12 months of conservation significantly the highest plantlet survival was recorded in $MSSM_1$ medium while minimum plantlet survival was found in $MSSM_2$ medium. The observed difference in plantlet survival was not significant in $MSSM_1$, $MSSM_3$ and $MSSM_4$. Better plantlet condition was found in $MSSM_1$ medium whereas the lowest plantlet condition was in $MSSM_2$ medium. Root growth was higher in $MSSM_4$ medium followed by $MSSM_2$ medium and root growth was lower in $MSSM_1$ medium.

In Diamant, the higher number of plantlets at 6 months storage was survived in $MSSM_1$ medium while minimum plantlet survival was observed in $MSSM_2$ medium. At 12 months of conservation the highest plantlet survival was recorded in $MSSM_1$ medium while minimum plantlet survival was found in $MSSM_3$ medium. The variation in plantlet survival was not significant in $MSSM_2$, $MSSM_3$ and $MSSM_4$ media. Better plantlet condition was found in $MSSM_1$ medium followed by $MSSM_2$ medium whereas the lowest plantlet condition was in $MSSM_3$ medium. Root growth was higher in $MSSM_3$ medium followed by $MSSM_2$ medium and root growth was lower in $MSSM_1$ medium.

At 6 months storage of cultivar JPR, maximum plantlet survival was noticed in $MSSM_4$ medium while minimum plantlet survival was observed in $MSSM_3$ medium. At 12 months of conservation the highest plantlet survival was recorded in $MSSM_4$ medium while minimum plantlet survival was found in $MSSM_3$ medium. The observed difference in plantlet survival was not significant in $MSSM_1$, $MSSM_2$ and $MSSM_4$ media. Better plantlet condition was found in $MSSM_4$ medium followed by $MSSM_1$ medium whereas the lowest plantlet condition was in $MSSM_3$ medium. Root growth was higher in $MSSM_3$ medium followed by $MSSM_2$ medium and root growth was lower in $MSSM_4$ medium.

From the experiment it was also observed that aerial roots were observed more or less in all treatments. Nodal swellings were observed in many treatments. Microtuber formation was not observed in any treatment. At 12 months of storage, nearly 45% of the plantlets had turned yellow even on the best treatment in all cultivars. Plantlet condition deteriorated sharply after 12 months of storage.

Table 5.3. Effect of different concentrations and combinations of sucrose with sorbitol and mannitol in MS medium on plantlet survival, shoot conditions and root growth. In each treatment at least 21 explants were inoculated.

Characters	Cultivars	Different MS media formulations				
		$MSSM_1$	$MSSM_2$	$MSSM_3$	$MSSM_4$	**Mean**
% plantlet survival after 6 months storage	All Blue	99.00^{A}	92.11^{B}	86.00^{C}	80.00^{D}	89.28^{a}
	All Red	95.22^{A}	75.66^{C}	79.33^{BC}	81.56^{B}	82.94^{b}
	Asterix	97.66^{A}	89.44^{B}	84.11^{C}	90.11^{B}	90.33^{a}
	Diamant	98.77^{A}	74.88^{C}	81.33^{B}	82.89^{B}	84.47^{b}
	JPR	60.22^{B}	60.00^{B}	50.77^{C}	66.00^{A}	59.25^{c}
	Mean	90.17^{a}	78.42^{bc}	76.31^{c}	80.11^{b}	
	LSD value (at 5%): 2.15 for cultivars (C); 2.41 for media (M) and 4.82 for C× M					
% plantlet survival after 12 months storage	All Blue	72.88^{A}	67.55^{AB}	66.44^{B}	69.22^{AB}	69.03^{a}
	All Red	67.33^{A}	65.66^{AB}	60.55^{BC}	57.33^{C}	62.72^{b}
	Asterix	68.88^{A}	62.11^{B}	65.00^{AB}	63.33^{AB}	64.83^{b}
	Diamant	70.33^{A}	63.44^{B}	60.22^{B}	62.89^{B}	64.22^{b}
	JPR	35.44^{AB}	34.66^{AB}	31.33^{B}	38.89^{A}	35.08^{c}
	Mean	62.97^{a}	58.68^{b}	56.71^{b}	58.33^{b}	
	LSD value (at 5%): 2.59 for cultivars (C); 2.90 for media (M) and 5.81 for C× M					
Plantlet condition score after 12 months storage	All Blue	3.54^{A}	2.78^{C}	2.84^{BC}	3.09^{B}	3.06^{a}
	All Red	3.16^{A}	3.12^{A}	2.81^{B}	2.13^{C}	2.80^{c}
	Asterix	3.17^{A}	2.68^{B}	2.90^{B}	2.82^{B}	2.89^{bc}
	Diamant	3.24^{A}	3.16^{A}	2.54^{C}	2.84^{B}	2.94^{b}
	JPR	2.36^{A}	2.16^{AB}	2.05^{B}	2.38^{A}	2.23^{d}
	Mean	3.09^{a}	2.78^{b}	2.63^{c}	2.65^{bc}	
	LSD value (at 5%): 0.11 for cultivars (C); 0.13 for media (M) and 0.26 for C× M					
Root growth score after 12 months storage	All Blue	3.06^{B}	3.54^{A}	3.75^{A}	3.66^{A}	3.50^{c}
	All Red	3.23^{C}	3.55^{B}	3.76^{AB}	3.89^{A}	3.61^{b}
	Asterix	3.34^{B}	3.54^{AB}	3.40^{AB}	3.62^{A}	3.47^{c}
	Diamant	3.13^{C}	3.51^{AB}	3.69^{A}	3.28^{BC}	3.40^{c}
	JPR	3.68^{AB}	3.86^{A}	3.91^{A}	3.45^{B}	3.72^{a}
	Mean	3.29^{b}	3.60^{a}	3.70^{a}	3.58^{a}	
	LSD value (at 5%): 0.10 for cultivars (C); 0.12 for media (M) and 0.24 for C× M					

The same letters indicate no significant difference at LSD = 0.05. Small letters used for cultivars (C) or nodal positions (M) and capital letters used for C × M.

PLATE 5.3. *In vitro* conservation of potato plantlets in $MSSM_1$ and $MSSM_4$ media

Photographs showing *in vitro* conservation of potato plantlets in $MSSM_1$ and $MSSM_4$ Media. **A:** $MSSM_1$ medium and **B:** $MSSM_4$ medium (after 12 months of storage).

5.4. DISCUSSION

Potato is a tuber growing plant. Tuber is very difficult for long time conservation. The cold storage and *in vitro* conservation are the alternative methods for germplasm conservation. *In vitro* systems for the storage and preservation of potato germplasm have received considerable attention and practical alternatives to conventional means of storage of vegetative material are now available. The last decade has seen growing awareness of the value of *in vitro* germplasm conservation. *In vitro* maintenance of germplasm can offer many advantages as a complement to field maintenance. The International Potato Center (CIP) has actively researched a wide range of methods for effective *in vitro* maintenance in its large potato germplasm collection.

In the present investigation single nodal explants of 5 potato cultivars were sub cultured onto different modified MS media formulations supplemented with different concentrations of sorbitol and mannitol alone and in combinations of sorbitol with mannitol, sucrose with sorbitol, sucrose with mannitol and sucrose with sorbitol and mannitol in order to find out the most suitable culture media formulation for increasing intervals between subculture of conserving potato plantlets. The results obtained in the present investigation have been discussed in the following paragraphs with an endeavor to justify them.

In this investigation the plantlets survibility with plantlet condition and root growth was greatly varied with treatment concentrations and combinations of the tested plant growth regulators (osmoticums). The results also showed that it is not mannitol or sorbitol that is important for conservation, but the combination of specific concentrations of sucrose and mannitol or sorbitol.

For the absence of sucrose in conservation medium no explants of five potato cultivars were responded or survived although the media were supplemented with different concentrations of sorbitol or mannitol or in combinations of sorbitol with mannitol.

Among the five concentrations and combinations of sucrose with sorbitol, significantly higher number of plantlets was survived in MSS_2 medium (MS medium with 20 g/l sucrose + 40 g/l sorbitol) with the best phenotypic condition and poorer root growth. In

respect of cultivars, better performances were observed in Asterix and it was shown the highest survibility in this media formulation.

In case of five concentrations and combinations of sucrose with mannitol higher number of plantlets was survived in MSM_3 medium (MS medium supplemented with 40 g/l sucrose + 20 g/l mannitol) with better plantlet condition and poorer root. Among the cultivars All Blue and Asterix were found to be superior to other cultivars for plantlet survival with better plantlet condition.

For different formulations of sucrose with sorbitol and mannitol maximum number of plantlets was survived in $MSSM_1$ medium (MS medium supplemented with 20 g/l sucrose + 20 g/l sorbitol + 20 g/l mannitol) with better phynotypic condition and poorer root growth. Among the cultivars, the best performances were noticed in All Blue and it was shown the highest survibility in this media formulation.

However, among the different modified MS media formulations, MSS_2 medium (MS medium containing 20 g/l sucrose + 40 g/l sorbitol) was the best formulation for *in vitro* germplasm conservation of potato and cultivar Asterix was found to be superior to other cultivars for maximum plantlet survival with better plantlet condition.

After 12 months without sub culturing, maximum survival coupled with a plantlet condition good enough to provide suitable nodes for sub culturing was observed with the use of conservation medium containing 20 g/l sucrose + 40 g/l sorbitol.

Though this medium could be used for all genotypes, the presence of genotype × medium interaction for plantlet condition suggests that genotypes respond differentially to the different media. This indicates the need of developing genotype-specific protocols to improve the efficiency of tissue culture systems (Gopal *et al*., 1998a, b). However, where thousands of genotypes are conserved, it may be difficult to develop genotype-specific protocols for germplasm conservation. The conservation response was not affected by the maturity class as genotypic differences for plantlet survival were not significant and plantlet condition, and root growth were also not related to maturity class of a genotype. Plantlet survival and plantlet condition were closely associated with each other.

At normal propagation temperature (22 ± 1°C), no observations were made beyond 12 months of conservation as the media became exhausted and were desiccated in most of culture tubes by this time. The survival in the best treatment (MS medium with 20 g/l sucrose + 40 g/l sorbitol) was 89.0% after 12 months of culturing, and in the control treatment, it was as low as 12.46% only after 5 months of culturing. Routinely, at the CPRI gene bank (Simla, India), an accession is sub cultured before 50% of the plantlets have senesced. Hence, 12 months seems to be the upper limit for subculture if the best medium identified in the present study is used. However, cultures can be stored perhaps for a longer period if more medium and bigger culture tubes or other suitable vessels are used (Westcott, 1981a, b).

Interestingly, the conservation medium with 20 g/l sucrose + 40 g/l sorbitol that gave maximum plantlet survival and favorable condition resulted in significantly less root growth than other media in which plantlet condition and survival was lower. The conservation medium with 40 g/l sucrose + 20 g/l mannitol that was the second best for plantlet survival at 12 months of storage was second worst for root growth. The other media which had lower plantlet survival were better for root growth. This negative association between plantlet survival and root growth suggests that the *in vitro* storage period can be prolonged by reducing the root growth. However, such plantlets also had reduced leaf and shoot growth. Westcott (1981b) reported that reduced root growth increases longevity of cultures by delaying the time when medium components become limiting for growth. Contrary to what was found in a previous study (Gopal *et al*., 2002), higher root growth in medium without mannitol/sorbitol than in some treatments with mannitol/sorbitol in the present study suggests that water stress imposed by polyols does not necessarily stimulate root development.

It is generally assumed that plants micropropagted by multiplication of organized meristems or by serial subculture of stem nodes are stable. Potter and Jones (1991) confirmed this and found no variation in DNA fingerprint patterns in any of the 21 plants stored by osmotic reduction of growth on the MS medium supplemented with 30 g/l sucrose and 40 g/l mannitol. However, Harding (1994) reported that use of mannitol in conservation medium resulted in methylation of DNA sequences, which might lead to heritable epigenetic phenotypic changes. There is no study on the genetic stability of the material conserved on medium supplemented with sorbitol.

In most of the previous studies, *in vitro* conservation of potato plantlets has been tried at low temperatures. When MS medium was supplemented with growth inhibitors such as abscisic acid, maleic hydrazide, N-dimethylaminosuccinamic acid, phosphon D (Radatz and Standke, 1978; Westcott 1981b) and acetylsalicylic acid (Lopez-Delgado *et al*., 1998), the interval between subculture s ranged from 6 to 16 months depending on genotype and the type and concentration of the retardant used. Westcott (1981a) reported that interval between subculture could be increased from 4 to 12 months by storing the plantlets at 6^0C instead of 22^0C when normal MS medium with 30 g/1 sucrose was used. The present study shows that a similar subculture period can be achieved even at 22 ± 1^0C by using MS medium with 20 g/l sucrose and 40 g/1 sorbitol. The plantlets conserved in this way had normal phenotype with thick stems and broad leaves. In contrast, plantlets conserved at low temperatures generally look abnormal with stunted growth, thin stems and leaves that are reduced or absent. Such plantlets have also been reported to have a high incidence of abnormalities such as cholorosis, vitrification and flaccidity, particularly when low temperature is combined with media with osmotic stress or growth retardants (Lizarraga *et al*., 1989; Lopez-Delgado *et al*., 1998). Such morphological abnormalities make the recovery of normal plants difficult on subculture. This raises concern about the genetic stability of plants conserved in this way for prolonged periods (Roca *et al*., 1989; Dodds *et al*., 1991; Harding, 1994, 1999). Conservation at normal propagation temperatures is thus desirable not only to save on the cost of energy and maintenance etc., but also for better genetic stability of the germplasm.

5.5. SUMMARY

In the present investigation single nodal explants of five potato cultivars were sub cultured onto different modified MS media formulations with different concentrations of sorbitol and mannitol alone and in combinations of sorbitol with mannitol, sucrose with sorbitol, sucrose with mannitol and sucrose with sorbitol and mannitol in order to find out the most suitable culture media formulation to reduce the frequency of sub culturing of conserving potato germplasm.

From the results it appears that for the absence of sucrose in conservation medium no explants of five potato cultivars were responded or survived although the media were supplemented with different concentrations of sorbitol or mannitol or in combinations of sorbitol with mannitol.

Among the five concentrations and combinations of sucrose with sorbitol, significantly higher number of plantlets was survived in MSS_2 medium formulation (MS medium with 20 g/l sucrose + 40 g/l sorbitol) with better plantlet condition and poorer root growth for the tested potato cultivars.

In case of different concentrations and combinations of sucrose with mannitol maximum number of plantlets was survived in MSM_3 medium formulation (MS medium supplemented with 40 g/l sucrose + 20 g/l mannitol) with the good plantlet condition and poorer root growth for all the potato cultivars except JPR.

For different formulations of sucrose with sorbitol and mannitol maximum number of plantlets was survived in $MSSM_1$ medium (MS medium supplemented with 20 g/l sucrose + 20 g/l sorbitol + 20 g/l mannitol) with better plantlet condition and poorer root growth for all the potato cultivars except JPR.

In conclusion, MS medium with 20 g/l sucrose + 40 g/l sorbitol was the best formulation as it resulted in maximum plant survival, and the lowest root growth, although plantlet condition in this medium was comparable to the one with 40 g/l sucrose + 20 g/l mannitol. The results also showed that it is not mannitol or sorbitol that is important for conservation, but the combination of specific concentrations of sucrose and mannitol or sorbitol. Among the cultivars Asterix was found to be superior to other cultivars for maximum plantlet survival with better plantlet condition.

CHAPTER VI
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ABBREVIATIONS AND SYMBOLS

µ	: Micron
µM	: Micro mole
1M	: 1 Mole
nm	: Nanometre
mm	: Millimetre
cm	: Centimetre
cm^2	: Centimetre square
m	: Metre
mg	: Milligram
g.	: Gram
kg.	: Kilogram
lb.	: Libra, pl. librae (pounds in weight)
PSI	: Pounds per square inch, pressure unit
MT.	: Metric tonne
t	: Ton
ml	: Millilitre (S)
l	: Litre
sq.	: Squire
Sec.	: Second
min.	: Minute
h	: Hour
ha	: Hectare
^0C	: Degree Celsius
rpm	: Rotation per minute
SE	: Standard Error
0.1N	: 0.1 Normal
etc.	: Et cetera (= and the others)
e.g.	: Exempli gratia (= for example)
ex.	: Example; examined

et al.	: Et alia (= and others)
i.e.	: Id est. (= that is)
Viz.	: Videlicet (= namely)
vs.	: Versus
%	: Percentage
/	: Per
Fig.	: Figure
No.	: Number
pH	: Negative logarithm of hydrogen ion (H^+) concentration
w/v	: Weight per volume
v/v	: Volume by volume
Kin	: Kinetin (6-furfurylaminopurine)
IAA	: Indole-3-acetic acid
IBA	: Indole-3-butyric acid
BA	: 6-Benzyle Adenine
BAP	: 6-Benzyl Aminopurine
NAA	: α-Naphthalene Acetic Acid
2,4-D	: 2,4-Dichlorophenoxyacetic acid
GA_3	: Gibberellic Acid - 3
HCl	: Hydrochloric acid
$HgCl_2$	: Mercuric chloride
NaOH	: Sodium hydroxide
KOH	: Potassium hydroxide
Na_2-EDTA	: Sodium salt of ferric ethylene diamine tetra acetate
CCC	: Chlorocholine Chloride
MS	: Murashige and Skoog (1962)
MS0	: MS medium without any plant growth regulators
ELISA	: Enzyme Linked Immuno-Sorbent Assay
IP	: *In vitro* plantlets
MT	: Microtuber
MNT	: Minituber
BS	: Breeder Seed

FS	: Foundation Seed
Cv.	: Cultivar
sp.	: Species
JPR	: Japanese Red
AR	: All Red
L.	: Linneaus (Carolus Linneaus)
DM	: Dry Matter
DW	: Dry Weight
FM	: Fresh Matter
TSP	: Triple Super Phosphate
MP	: Muriate of Potash
NPK	: Nitrogen, Phosphorus and Potassium
BCP	: British Potato Council
BBS	: Bangladesh Bureau of Statistics
BADC	: Bangladesh Agricultural Development Corporation
BES	: Bangladesh Economical Survey
BARI	: Bangladesh Agricultural Research Institute
HBAS	: Handbook of Agricultural Statistics, Bangladesh.
RDA	: Rural Development Academy
NGO	: Non Government Organization
GO	: Government Organization
Govt.	: Government
UK	: United Kingdom
USA	: United State of America
LDC	: Least Developing Countries
UNICEF	: United Nation Children Fund
NY	: New York
CIP	: Centro International de la Papa. Lima, Peru.
CIDA	: Canadian International Development Agency
CPRI	: Central Potato Research Institute, Simla, India
SCRI	: Scottish Crop Research Institute, Dundee, UK

APPENDICES

Appendix 1: MS medium (Murashige and Skoog, 1962)

Components	Concentrations
Macro- nutrients	**(mg/1)**
KNO_3	1900.00
NH_4NO_3	1650.00
KH_2PO_4	170.00
$CaCl_2.2H_2O$	440.00
$MgSO_4.7H_2O$	370.00
Micro-nutrients	
$FeSO_4.7H_2O$	27.80
$Na_2EDTA.2H_2O$	37.30
$MnSO_4.4H_2O$	22.30
H_3BO_3	6.20
$ZnSO_4.7H_2O$	8.60
KI	0.83
$Na_2MoO4.2H_2O$	0.25
$CuSo_4.5H_2O$	0.025
$CoCl_2.6H_2O$	0.025
Organic nutrients	
Glycine	2.00
Nicotinic acid	0.50
Pyridoxine- HCl	0.50
Thiamine- HCl	0.10
Myo-inositol	100.00
Sucrose	30,000.00

P^H adjusted to 5.8 before autoclaving.

Appendix 2: Preparation of stock solutions of different growth regulators.

PGRs	Amount (mg) taken	Appropriate solvents	Final volume with distilled water (ml)	Strength (mg/ml) of the stock solution
BA	100	1N NaOH	100	1
Kin	100	0.1N KOH	100	1
GA_3	100	70% Ethanol	100	1
NAA	100	1N NaOH	100	1
2,4-D	100	Ethanol/1N NaOH	100	1

Appendix 3: Symbolic name and their components of different modified MS media formulations.

Symbolic name of different modified MS media formulations	Components of different modified MS media formulations			
	Basal nutrients	Sucrose (g/l)	Sorbitol (g/l)	Mannitol (g/l)
MS_1	MSBN	-	30	-
MS_2	MSBN	-	40	-
MS_3	MSBN	-	50	-
MM_1	MSBN	-	-	30
MM_2	MSBN	-	-	40
MM_3	MSBN	-	-	50
$MSTMT_1$	MSBN	-	15	15
$MSTMT_2$	MSBN	-	20	20
$MSTMT_3$	MSBN	-	25	25
MSS_1	MSBN	20	20	-
MSS_2	MSBN	20	40	-
MSS_3	MSBN	40	20	-
MSS_4	MSBN	30	40	-
MSS_5	MSBN	40	30	-
MSM_1	MSBN	20	-	20
MSM_2	MSBN	20	-	40
MSM_3	MSBN	40	-	20
MSM_4	MSBN	30	-	40
MSM_5	MSBN	40	-	30
$MSSM_1$	MSBN	20	20	20
$MSSM_2$	MSBN	20	40	20
$MSSM_3$	MSBN	20	20	40
$MSSM_4$	MSBN	40	20	20

*MSBN = MS basal nutrients