

BIOTECHNOLOGICAL APPROACHES FOR
IMPROVEMENT OF POTATO
(*SOLANUM TUBEROSUM* L.)



By

MISS MUSSARAT JABEEN

DEPARTMENT OF BOTANY
UNIVERSITY OF PESHAWAR
PESHAWAR, PAKISTAN
2013

DEDICATED

TO MY

Parents and beloved nephew Hassan Khan

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BIOTECHNOLOGICAL APPROACHES FOR IMPROVEMENT OF
POTATO (*SOLANUM TUBEROSUM* L.)

A dissertation submitted in partial satisfaction

of the requirement for the degree of

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In

BOTANY

By

Mussarat Jabeen

Prof. Dr. Farrukh Hussain, Supervisor

Graduate Studies Committee

1. Prof. Dr. Syed Zahir Shah, Member
2. Prof. Dr. Muhammad Saeed, Member
3. Prof. Dr. Siraj-ud-Din, Member
4. Miss Mussarat Jabeen, Member
5. Prof. Dr. Muhammad Ibrar, Convener

APPROVAL

This thesis, entitled "BIOTECHNOLOGICAL APPROACHES FOR IMPROVEMENT
OF POTATO (*SOLANUM TUBEROSUM* L.)

submitted by Miss. Mussarat Jabeen is hereby approved and recommended as partial
fulfillment for the award of Degree of

Doctor of Philosophy in

BOTANY,

Prof. Dr. Farrukh Hussain,
Research supervisor
Department of Botany,
University of Peshawar.

Farrukh Hussain
3/12/2014

External examiner: _____

[Signature]

DEPARTMENT OF BOTANY,
UNIVERSITY OF PESHAWAR
PESHAWAR, PAKISTAN
2013

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Mussarat Jabeen

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I wish researchers round the globe who are involved in seed potato production could benefit from this work.

Mussarat Jabeen

1977 - M.Sc. Department of Botany, University of Peshawar
1980 - Diploma in Advanced Botany from University of Peshawar
1982 - M. Phil. Department of Botany, University of Peshawar
Professor
Research Officer - (1983-1989) National Science Foundation - Pakistan Science
Foundation
Lecturer (1989-1991) Department of Botany, University of Peshawar
Lecturer (1991-1994) Department of Botany, University of Peshawar
Assistant Professor (1994-2000) Department of Botany, University of Peshawar

Major Field	Botany (Theme Culture)
Chairman of	Teacher
1. Botany & Conservation	Prof. Dr. Farooq Hussain
2. Botany & Ecology	Prof. Dr. Farooq Hussain
3. Botany & Agriculture	Prof. Dr. Farooq Hussain
4. Botany & Horticulture	Prof. Dr. Farooq Hussain
5. Botany & Environmental	Prof. Dr. Farooq Hussain

VITAE

Academic

April 13, 1954, Born, Peshawar.

1970 – Matric Presentation Convent High School, Peshawar.

1972 – Government Frontier College for Women, Peshawar.

1975 – B.Sc. University College for Women Peshawar.

1977 – M.Sc. Department of Botany, University of Peshawar.

1986 – Diploma in Advanced Biotechnology Colorado State University, USA.

1989 – M. Phil, Department of Botany, University of Peshawar.

Professional

Research Officer – (1983-1989) National Science Foundation – Pakistan Science Foundation.

Lecturer (Adhoc) 1989-1994), Department of Botany, University of Peshawar.

Lecturer (Permanent) (1994-1998) Department of Botany, University of Peshawar.

Assistant Professor, (1998- till date) Department of Botany, University of Peshawar.

Major field

Botany (Tissue Culture)

Courses studied

Teacher

1. Biodiversity & its conservation
2. Intensive studies in ecology
3. Pharmacognosy
4. Virology
5. Chemistry & Environmental

Prof. Dr. Farrukh Hussain
Prof. Dr. Farrukh Hussain
Prof. Imtiaz ul Haq
Prof. Sher Hassan
Prof. Dr. Ikhtiar Khan

ABSTRACT

Shoot cultures were obtained by culturing the buds from disease free healthy potatoes of the cultivars Desiree and Ultimus, on both $\frac{1}{2}$ and full strength Murashige and Skoog (MS) (1962) salt solution. Sprouting of the buds in both the cultivars was observed on the 3rd day of culture on $\frac{1}{2}$ MS medium that grew into single leafy shoots, within 3 weeks, whereas on full MS medium sprouting of the buds in both the cultivars was delayed and occurred after the second week of culture. Moreover growth of the plantlets was also very slow on this medium. Nodal segments from these plantlets were then cultured on micropropagation media.

The micropropagation medium consisted of $\frac{1}{2}$ MS with 4% sucrose and supplemented with varying concentrations of NAA (0.1, 0.5mg/l), BAP (0.1, 0.5mg/l) GA₃ (0.2, 0.4, 0.6, 0.8mg/l) either alone or in combination. The experiments were carried out in two different day lengths (8 and 16hrs photoperiod). Best multiple shoot formation was observed on medium supplemented with 0.1mg/l NAA in combination with 0.5mg/l BAP and 0.4mg/l GA₃ in 16hrs photoperiod. The multiple shoots thus obtained were further subjected to different tuberization media. The tuberization media consisted of varying concentrations of different carbon sources (sucrose and glucose); different growth regulators such as BAP, Kinetin, Adenine sulphate (AS), IAA, Coumarin, ABA, GA₃, Chlorocholine Chloride.

Varying sucrose and glucose levels (4, 6, 8 and 10%) were used under different day lengths (8 & 16hrs photoperiod) for *in vitro* tuberization of potato cvs Desiree and Ultimus. Of the two sugars used 8% sucrose under eight hours photoperiod proved to be

the best for *in vitro* tuberization. Though sucrose was the best carbon source but the yield was not promising, therefore in the further studies different growth regulators (cytokinins, auxins, gibberellins, coumarin ABA etc.) were used to improve tuber yield.

For tuberization different cytokinins (BAP, Kinetin, Adenine sulphate) were used. The *in vitro* raised plantlets obtained at stage II were subjected to tuber induction media (MS full strength). This was supplemented with varying concentrations (2.0, 4.0, 6.0, 8.0 and 10mg/l) of BAP and varying sucrose levels (*viz* 4, 6, 8 and 10%). The cultures were kept under varying light regimes *i.e.* 8 and 16hrs photoperiod. Highest number of tubers were obtained on medium supplemented with 10mg/l in combination with 8% sucrose in 8hrs photoperiod, whereas tubers with maximum weight were obtained on medium supplemented with 6.0mg/l BAP in combination with 8% sucrose under short (8hrs) photoperiod. Tuberization response in all the treatments was poor in 16 hrs photoperiod.

In another set of experiments varying levels (2.0, 3.0, 4.0, 6.0 and 8mg/l) of kinetin were used in the basal medium (MS full strength) in combination with sucrose (4, 6 and 8%). Best microtuber yield (both number and weight) was obtained on medium supplemented with 4mg/l kinetin in combination with 8% sucrose in both the cultivars. Still further combined effect of BAP and Adenine sulphate (AS) was also tested in the MS basal medium. The addition of AS to the medium was not of any advantage. Tuber yield was much decreased.

IAA at varying levels (0.5 and 1.0mg/l) in combination with 8% sucrose was supplemented in the MS medium. Tuber number could not be increased in IAA supplemented media, rather tuber weight was greatly increased in both the cultivars. In

further experiments GA₃, Coumarin, ABA and Chlorocholine chloride were also tested. GA₃ strongly hampered tuberization. Stoloniferous shoot formation occurred in the media. The addition of coumarin at (20mg/l) to the basal medium increased both tuber number and weight, whereas the addition of ABA or Chlorocholine chloride was of no added advantage with regard to tuber yield.

Still further in another experiment B-5 medium was compared to MS medium. Both the media were supplemented with BAP (6, 8 and 10mg/l) and 8% sucrose. Tuberization occurred earlier in B-5 medium, but higher tuber yield (both number and weight) was obtained on MS medium.

The *in vitro* plants and the microtubers thus obtained were further tested in the field. Performance of microtubers was better in the field than *in vitro* plants. About 30% of the plantlets died during acclimatization whereas microtubers exhibited 100% sprouting.

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INTRODUCTION

CHAPTER - 1

INTRODUCTION

INTRODUCTION

A major problem facing the world and in particular the developing nations, is the explosive rate of population growth. At present there are over six billion mouths to be fed and with the present rate of population growth this number will almost reach to 8.0 billion by the year 2020 with an annual increase of 100 million. Over 93% of this growth will take place in the developing countries with Asia alone comprising 4.689 billion of the total population by 2020 (Nygaard, 1994).

Conventional agriculture may well not be able to supply sufficient food and in particular protein to satisfy such demands. The UN Food and Agriculture organization (FAO) already predicts a widening of the protein gap between developed and developing countries. At least 25% of the world's population currently suffers from hunger and malnutrition and a disproportionate number of these occur in developing nations where arid climates and infertile lands hamper productive agriculture (FAO, 2008).

The cultivated potato (*Solanum tuberosum* L.) is the third most important food crop in the world after rice and wheat in terms of human consumption. More than a billion people eat potato and global total crop production exceeds 320 million tons. It is a critical crop in terms of food security in the face of population growth and increased hunger rates. It is thus the most important dicotyledonous crop and is one of the world's most efficient crops in converting land, water, labor and capital into a highly nutritious food. It has the capacity to produce more energy and protein per unit land than any other single food crop (FAO, 2008). Among the plant food sources potato contains a better balance of essential amino acids, particularly lysine. The ratio of protein to carbohydrates is higher in potato than in many cereals and other roots and tubers. In addition the value of potato proteins is higher than that of other major food crops. So potatoes are extremely well balanced food source. The favorable protein / carbohydrate balance and the extremely high quality of potato proteins are potential assets for improving the nutritional status of malnourished populations whose diets are based on cereals

and other roots and tubers. Potato ranks first in protein production per hectare per day. Thus, potato can substitute both for vegetable and food grains in the food basket of the consumers in variable proportions. It can be consumed as a vegetable singly or in combination with other vegetables and also in place of rice, wheat and other food grain preparation (Badoni & Chauhan, 2009).

Potato is grown in temperate, sub-tropical and tropical regions. The per capita production of crop in countries of the temperate regions is much higher than those of the tropics and sub-tropics.

In 2008, several international organizations highlighted the potatoes role in world food production, in the face of developing economic problems. They cited its potential derived from its status as a cheap and plentiful crop that grows in a wide variety of climates and localities (Reuters, 2008). The United Nations officially declared 2008 as the International year of the potato, to raise its profile in developing nations; calling the crop a "hidden treasure": (CIP, 2008).

History

Potatoes originated in the Andean mountain region of South America. Researchers estimate that potatoes have been cultivated by the Indians living in these areas from between 4000 and 7000 years (Theisen, 2007). Potatoes were brought to Europe by Spanish explorers who "discovered" them in South America in the early 16th century. Since potatoes are good sources of vitamin C, they were subsequently used on Spanish ships to prevent scurvy. They were introduced into Europe via Spain, but were not widely consumed throughout Europe, even though many governments actively promoted this nutritious food stuff that was relatively inexpensive to produce. The reason for this is that many felt that it was poisonous like some other members of this family. Moreover many judged potatoes with suspicion since they were not mentioned in the Bible. By the early 19th century potatoes rapidly spread as a food crop throughout Europe, the United States, India, China and elsewhere and is now grown in over 100% countries worldwide (Hijmans, 2001 and Nathan & Nancy, 2011). The spread of potato crop presents a fascinating study, as documented by Salaman (1949 & 1989) in the History and

Social Influence of the Potato. Perhaps the best known aspect was the Irish Potato Famine of 1845-1847, when the entire Irish potato crop was decimated by the late blight fungus (*Phytophthora infestans*).

Composition of Potato

In the raw form potatoes consist of 70-80% moisture and 20% solids mainly in the form of easily digestible carbohydrates, which form 65-80% of the dry weight. Potatoes contain up to 10% sugar measured on a dry basis. The sugar content is dependent on variety, maturity, culture conditions and storage. A low sugar content is considered a desirable, varietal character because potatoes required for processing undergo less browning. They also contain a small amount of crude fiber, cellulose, hemicelluloses, pectic substances and other polysaccharides. The small quantity of protein ranging from 2-3% is of good quality, but is deficient in certain essential amino acids. The mineral and vitamin content of potatoes is favorable for human nutrition in terms of balance and availability.

Minerals and Vitamins

Potatoes contain 4.1g ash per 100g dry matter; this is quantitatively a large amount in comparison to 1.7g for wheat and 0.7g for rice. The notable point about potato ash from a therapeutic point of view is that it is rich in potash and is alkaline in contrast to wheat, rice, eggs and animal proteins which are acidic. Among the vitamins, ascorbic acid is the most important; some varieties contain as much as 29mg per 100g which according to the internationally recommended allowances meets the entire requirement of the adult. Storage and preservation decrease the vitamin C content. Potatoes are a good source of thiamine (0.98 – 0.13 mg/100 g) riboflavin (0.4 mg/100 g) and niacin (1.2–3.3 mg/100 g). Storage has been demonstrated to improve the niacin content during the first month followed by subsequent decrease (Page & Hanning, 1963).

Proteins and Amino Acids

In potatoes 40–60% of the total nitrogen is in the non-protein nitrogen (NPN) fraction, free amino acids account for 75%, thus making this fraction nutritionally important. In terms of 100g, an edible portion of potato contains only 2g protein (based nitrogen analysis) while wheat and rice contain 10 and 7g per 100g material. However, if rice and wheat are hydrated to their cooked weight, potatoes compare favorably with rice. The essential amino acids which limit the utilization of potato proteins are methionine – cysteine – cysteine, while cereals are limited by lysine. The amino acid score of potato is similar to that of cereals.

On the basis of solubility the major protein from potatoes was shown to be a water insoluble protein called “tuberin” accounting for 71% of the total nitrogen. Patatin is the name given to the major soluble glycoprotein's which account for 40% soluble protein of potato tubers. Patatin is generally reported to be localized in tubers, but on removal of axillary buds and tubers it accumulates in stems (Paiva *et al.*, 1983).

Nutrition and Health Benefits

The first modern “convenience food” potato is an important food staple and the number one vegetable crop in the world. Potatoes are available around the year as they are harvested some where every month of the year (CIP, 2008).

Potato can supplement the food needs of people in a substantial way. Potato produces dry matter food, well balanced protein and more calories per unit area of land than other major food crop. It contains practically all the essential dietary constituents like cereals, carbohydrates which are the major constituents of potato. Besides it contains essential nutrients as proteins, minerals and vitamins. (Thamburaj & Singh 2001).

Humans can actually survive healthily on a diet of potatoes supplemented only with milk or butter, which contain the two vitamins (A and D) not provided by potatoes. The potatoes besides being a good source of vitamins and minerals are also as an assortment of phytochemicals that have antioxidant activity. Among these important health promoting compounds are carotenoids, flavonoids, and

natural phenols, as well as unique storage proteins, such as patatin, which exhibit activity against free radicals (Liu *et al.*, 2003). Chorogenic acid constitutes up to 90% of the potato tuber natural phenols (Breithaupt & Bamedi, 2002).

A medium size (150g) potato with skin provides 27mg of vitamin C (45% of the daily value (DV)), 620mg potassium (18% DV), 0.54mg vitamin B6 (27% of DV) and trace amounts of thiamin, riboflavin, folate, niacin, magnesium, phosphorus, iron and zinc. The fiber content of a potato with skin (2g) is equivalent to that of many whole grain breads (Ferretti, 2011).

Potato is best known for its carbohydrate content (approximately 26g in a medium potato). The predominant form of this carbohydrate is starch, a very small portion of which is resistant to digestion by enzymes in the stomach and small intestine, and so reaches the large intestine essentially intact. This resistant starch is considered to have similar physiological effects and health benefits as fiber. It provides bulk, offers protection against colon cancer, improves glucose tolerance and insulin sensitivity, lowers plasma cholesterol and triglyceride concentrations increases satiety and possibly even reduces fat storage (Raben *et al.* 1994; Cumming *et al.* 1996; Hylla *et al.* 1998). The amount of resistant starch in potatoes depends much on preparation methods. Cooking and then cooling potatoes significantly increases resistant starch. For example cooked potato starch contains about 7% resistant starch, which increases to about 13% upon cooling (Englyst *et al.* 1992).

Potato Phytochemicals

Potatoes known as a high carbohydrate white starch has removed them from the meals of many weight conscious people. A new analytical method developed by Agricultural Research Service US plant geneticist Navarre (2007) has identified 60 different kinds of phytochemicals and vitamins in the skins and flesh of 100 wild and commercially grown potatoes. Analysis of potatoes revealed that their phenolic content rivals that of Broccoli, Spinach and Brussels sprouts and includes flavonoids with protective activity against cardiovascular disease, respiratory problems and certain cancers. High levels of vitamin C, folic acid,

quercetin and kukoamines were also identified during these analyses. Kukoamines are known to lower blood pressure (Parr *et al.* 2005).

Much concern has recently been generated towards the over consumption of sodium. Restriction of sodium and increase in magnesium intake is frequently recommended for persons prone to high blood pressure and kidney as well as gall bladders stones. The potassium is increased to overcome the excessive renal loss of potassium induced by taking diuretics. In such cases potatoes being low sodium, high potassium food can be effectively utilized.

Vitamin B6

If only for its high concentration of vitamin B6 the potato earns high marks as a health promoting food. Vitamin B6 is involved in more than 100 enzymatic reactions and is virtually active everywhere in the body. Many of the binding blocks of protein, amino acids require B6 for their synthesis. Because amino acids are critical parts of new cell formation, vitamin B6 is essential for the formation of virtually all new cells in the body. *Heme* (the protein center of our blood cells) and phospholipids (cell membrane components that enable messaging between cells) also depend on vitamin B6 for their creation.

Vitamin B6 plays numerous roles in our nervous system, many of which involve neurological activity. B6 is necessary for the creation of amines, a type of messaging molecular or neurotransmitter that the nervous system relies on to transmit messages from one nerve to the next. Neurotransmitters, that require vitamin B6 are serotonin and dopamine, a lack of which is linked to depression; melatonin; the hormone needed for a good night's sleep; adrenaline; epinephrine and nor epinephrine; hormones that help us respond to stress; and GABA which is needed for normal brain function.

Vitamin B6 plays another critically important role in methylation, a chemical process in which methyl groups are transferred from one molecule to another. Many essential chemical events in the body are made possible by methylation, for example, genes can be switched on and off in this way. This is particularly in cancer prevention since one of these genes that can be switched on

and off is the tumor suppressor gene. Another way that methylation helps prevent cancer is by attaching methyl groups to toxic substances to make them less toxic and encourage their elimination from the body. Methylation is also important to cardiovascular health, by changing the potentially dangerous molecule "homocystein" into other benign substances. Since homocystein can directly damage blood vessel walls greatly increasing the progression of atherosclerosis, high homocystein levels are associated with a significantly increased risk for heart attack and stroke. Eating foods rich in vitamin B6 can help keep homocystein levels low. In addition, diets high in vitamin B6 rich foods are associated with lower rates of heart disease, even when homocystein levels are normal, most likely because of all the other beneficial activities of this energetic B vitamin.

Vitamin B6 is also necessary for the breakdown of glycogen, the form in which sugar is stored in our muscle cells, and liver, so this vitamin is a key player in athletic performance and endurance (Navarre, 2007).

Traditional Methods of Potato breeding

Potato is about 30-100 cm tall annual. It is vegetatively propagated through tubers. The tubers bear the buds, commonly known as "eyes which sprout on germination and grow into plants. The tubers, the size of which differs with age and cultivars are grown in fields in ridges to maintain developing tubers under soil, because on exposure to light they become green and unpalatable. The tubers start developing when the plant flowers and their formation ceases when fruit formation begins.

Potato breeding is a difficult task, mainly due to tetrasomic inheritance of characters, the high heterozygosity, self-incompatibility and male sterility in many cultivars. The conventional methods of potato breeding involve selection, crossing for recombination and mutations (Ross, 1986). Selection is limited to existing variation, which not only takes a long time, but the efficiency of selection is also limited. Thus starting with 100,000 seedlings it would take 6-8 years to select desired variety (Wenzel, 1980). The efficiency of obtaining required clones possessing valuable gene combinations is dependent on homo or heterozygosity.

From the tetraploid level the breeding is now carried out at the dihaploid level as well. Superior pollinators are available which help in the extraction of dihaploids in large numbers. The advantages of dihaploids are that the $2x-2x$ crosses lead to the production of high yielding $4x$ progenies, which show heterosis. However, the percentage of seedlings selected from such crosses is higher than $4x-4x$ crosses. At present the variability of dihaploid to tetraploid via colchicine treatment produces chimeras and thus decreases heterozygosity. Meiotic doubling is frequently used (Mendiburu *et al.* 1974).

To preserve the parental properties, potato is propagated by tubers, but sometimes variants called "bud sports" appear, however, their frequency is rather low. Mutations have been induced in potato by X-rays, UV and by chemical mutagens (Broertjes & Van Harten, 1978). Practically all mutation work has been done using tetraploid cultivars performed on tubers. Mutants have been obtained with success with regard to size, shape and color of the tubers. Mutations for earliness, increased resistance to different diseases and increased starch content of tubers have been reported (Solomko, 1965). In many cases, however, mutations reveal themselves as periodical chimeras and hence show pleiotropic effects. Breeding with *S. demissum* which is resistant to many races of potato blight has been attempted. First, resistant gene R_1 and later R_2 was transferred to potato, but it is still not possible to impart resistance to all races of pathogens (Nelson, 1984). One of the obstacles against efficient resistance breeding in potato is its tetraploid nature. The reduction of this ploidy level to $2x$ through in vitro culture and their utilization would facilitate the incorporation of resistance. This has been demonstrated by the use of anther derived plant (Wenzel & Uhrig, 1981).

There is a lack of genetic variability to synthesize new clones. Some of the varieties of potato such as Russet Burbank, Bintje and King Edward are over 50 years old and still popular. The International Potato Center, Lima has done a remarkable job in the collection, storage, improvement and distribution of potato germplasm to bring about a breakthrough at the desired speed. The conventional practices combined with biotechnological approaches would enable the induction of genetic diversity and quicken the pace of release of new and improved

varieties. Potato is thus a prime candidate for *in vitro* manipulation and in general it is amongst the most amenable crop plants in its response to *in vitro* culture (CIP, 2008).

Conventional propagation

Conventional propagation of seed potato production involves the use of potatoes that are propagated by harvesting and replanting the tubers in the field. The tubers used for planting are known as “seed potatoes” as opposed to potato seeds. Seed potato growers select better quality tubers for seed and discard those of poor quality. The diseased and healthy plants are identified and separated and the healthy tubers are used for the next season production. However, this method of seed production has proved to be laborious, prone to pest and disease infestation and time consuming.

Conventional propagation of potato is done vegetatively using seed tubers and ensures uniformity of the crop in terms of growth and yield, but results in degeneration of the crop due to virus infection, the role of degeneration varying from place to place and from cropping season to cropping season (Biniam & Tadesse, 2008). The viruses are transmitted through different ways, even through planting infected tubers. If the seed stock is not maintained well or frequently replaced with fresh one, the virus infiltration can reach up to 100% in 3–4 successive crop seasons resulting in almost – half or one third yields (Khurana *et al.* 2001; Badoni & Chauhan, 2009, 2010; El-Komy *et al.* 2010).

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. During the growing season, growers check fields visually for signs of disease and remove infected plants through the process of roughing. However, visual inspection, particularly for primary infection, is unreliable, time consuming and requires a well experienced eye (Lapierre & Signoret, 2004; Phytocultures Ltd, 2008). The quality of seed potato produced in subsequent generation's declines substantially.

Biotechnological Approaches for Potato Improvement

From the 16th century Andean Highlands to the 21st century Europe Biotechnology has transformed potato to "Pomato" and "Topato" (Melchers *et al.* 1978). Potato is the first major food crop where biotechnology has been successfully applied. It has been subjected to a wide range of *in vitro* manipulation (Fig. 1). The *in vitro* produced disease free plants, somaclones, haploid and somatic hybrids, plants resistant to blight, viruses, nematodes, herbicides and the microtubers produced in test tubes have been moved from the laboratory to the field and propagated on a large scale in various countries (Bajaj & Sopory, 1986).

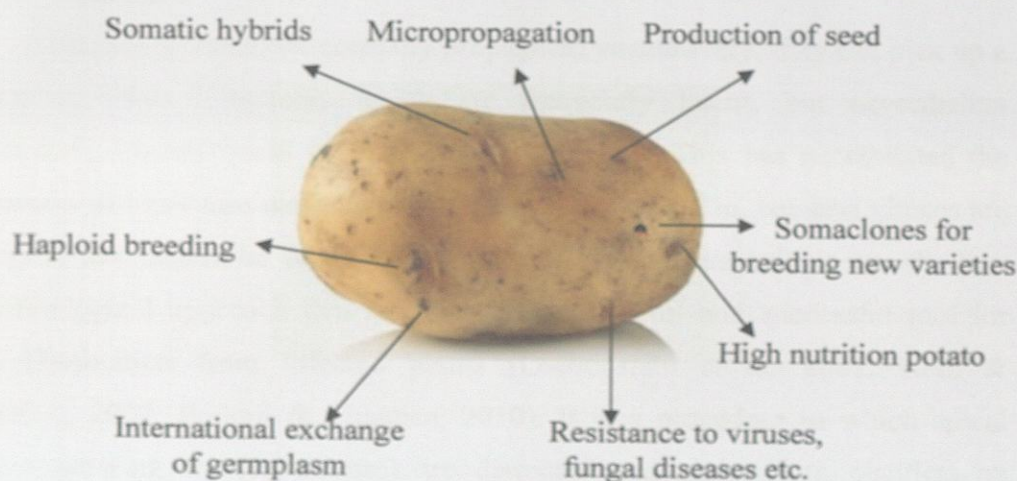


Fig. 1. Some biotechnological approaches for *in vitro* improvement of potatoes.

The *in vitro* manipulation to which potato has been subjected can be divided into two types.

- I. Those in which genetic stability should be maintained, and
- II. Those in which the aim is to achieve some kind of genetic modification.

***In Vitro* Approaches Requiring Genetic Stability**

The following *in vitro* approaches require genetic stability.

- i. Virus elimination.
- ii. Micropropagation.
- iii. Germplasm storage / transport.
- iv. Production of micro and mini tubers.

Virus Elimination

Because potatoes are normally propagated vegetatively, they can pick up a burden of virus infections, which are frequently latent, but nevertheless significantly depress yield (Loebenstein *et al.* 2001). This has necessitated the production of virus free material for healthy seed production, because viruses are pathogens that cannot be destroyed by chemicals. Meristem culture was the first biotechnological approach that has become a powerful and successful tool for virus elimination from infected plants (Loebenstein *et al.* 2001; Naik & Karihaloo, 2007; Badoni & Chauhan, 2010). It is a procedure in which apical axillary growing tip (0.1-0.3mm) are dissected and grown into plantlets on nutrient media under controlled conditions. This technique for virus elimination is based on the principle that, many viruses are unable to infect the apical/axillary meristems of a growing plant and that a virus free plant can be produced if a meristematic tissue is propagated (Kassanis, 2008). This is based on the observation that the extreme tip of a shoot or root is free of viruses.

Meristem is the dome of actively dividing cells, that remain in an active state of division throughout the vegetative phase of the plant forming new tissues and organs; they have therefore the potential of producing whole plants both *in vivo* and *in vitro* (Danci, 2007). Virus particles, which may be present in the

vascular system, can reach the meristematic region of the apex only through cell to cell movement, which is a slow process that might take a few days (Hull, 2002). Therefore, in a virus infected plant, virus concentration decreases acropetally toward the meristem of both apical and axillary buds, on assumption that viruses were unable to reach the meristematic tissues of the bud. Moreover meristem tissues are virus free because of the unique characteristics they possess. Some of the features include:

1. Vascular system through which viruses are spread is not developed in the meristematic region.
2. Chromosome multiplication during mitosis and high auxin content in the meristem may inhibit virus multiplication through interference with viral nucleic acid metabolism.
3. Existence of virus inactivating system with greater activity in the apical region than elsewhere (Kantha-rajah, 2001).

Potato plants can be regenerated from a variety of tissues including leaf tissue, leaf discs, petioles with leaflets (Wang & Song 2000; Yee *et al.* 2001). However, meristem buds develop into plantlets more readily than other tissues and after appropriate treatment of the source plant, are usually virus free. Moreover plantlets derived from meristem tissues are more likely to be identified to the source plant than are the plantlets derived from other tissues, although there are no reports of abnormal potato plants arising from callus cultures.

Meristem culture has been successfully adopted and applied in potato for development of virus free plants and appeared a new venture in producing virus free potato tuber seeds (Wang *et al.* 2011); an increase of 40% in potato yield was obtained when virus free tubers were used. Virus free potato stocks were also obtained by Lobenstein *et al.* (2001), Nagib *et al.* (2003), Ebadi *et al.* (2007), Badoni and Chauhan (2009), Al-Taleb *et al.* (2011) and Danci *et al.* (2012) through meristem culture.

The *in vitro* technology combined with traditional methods can thus increase the commercial production of virus free seeds which is an important pre-requisite to maximize potato yield.

Micropropagation

In vitro Micropropagation are now being employed fairly widely in potato growing areas. Besides the advantage it gives of being able to disseminate virus free clones of old varieties, micropropagation is also of considerable value to potato breeders and is used to bulk up new varieties or breeding lines for germplasm storage. The rate of propagation is much faster than from conventional methods of increase. It would normally take two years to obtain about 3000 plants of a new potato variety; this number can easily be produced in six months by a tissue culture laboratory, with much less manual labor.

Micropropagation is also used for the production of micro and minitubers. It is therefore an alternative to conventional propagation of potatoes (Danci *et al.* 2011). The advantages over annual field growth are savings in man power, space, freedom from pests and diseases, the vagaries of weather and so on.

Microtuberization

Microtubers are produced in *in vitro* cultures when potato shoot cultures are grown in the presence of high levels of sucrose, where minitubers are produced on stolons of micropropagated plants transferred to soil and grown at high density in green houses. The use of microtubers and minitubers should reduce the time taken to produce seed tubers, reduce the number of field generations required and so result in higher quality seed tubers.

Potato microtubers offer several advantages over *in vitro* propagated plants, since they can be stored and transplanted directly into the field without an acclimatization stage. Also handling and shipping are easier thus facilitating commercialization and international exchange of germplasm (Rosu *et al.* 2004).

In vitro microtuberization of potato constitutes the transitory phase, between "*in vitro*" multiplication and establishment of cultures in the field. Microtuber production represents an efficient method for obtaining a healthy material, by which the process of production is reduced with 3–4 years.

The Future Potato

There is no such thing as an ideal "potato" for the world population. This is especially true when one considers the range of material available and the different uses to which the potato is amenable, that can be further enhanced through biotechnology. What one should look for should be potatoes which would meet the requirements of different countries, regions and specific population in relation to their food habits. The diets of the people differ thus there is a varying need to focus on different, specific nutrients and flavors. In the developing countries the emphasis is nutritional. The potato being a short term crop, can quickly yield energy and protein, thus potato would require an improvement in the protein quantity and quality. In the developed countries potato takes the form of a low fat and low sodium food. The multiple uses of potato also indicate the variation in the traits considered desirable for each purpose, for instance, potato to be used as feed for infants and those used for French fries or for fuel energy need different treatment built in qualities. Thus, the potato of the 21st century is not one ideal potato, but different kinds of potatoes which are to be developed to suit the dietary habits, meet the nutritional deficiencies of specific populations and be appealing enough to the concerned consumers.

Some of the qualities of potatoes which appeal to the consumer are their skin color and texture, shape, size, taste, cooking quality and the price. The plant breeder is primarily concerned about disease resistance and yield. A nutritionist would like it to be energy packed and proteinacious. For a post-harvest technologist it should be easy and economical to store and transport.

The future potato is thus a variety of materials that challenge the creative ability of the scientist and the desires of the consumers. Biotechnology, through cell culture and *in vitro* genetic manipulations is quite competent to meet the challenge of specific demands in order to "tailor" the potato to match the need of the people (Bajaj, 1987).

LITERATURE REVIEW

Management

The current literature on the impact of organizational culture on organizational performance is mixed. Some studies have found a positive relationship, while others have found no significant relationship. For example, a meta-analysis by Cameron and Quinn (1999) found that organizations with a strong culture have higher performance than those without a strong culture. However, a study by Schein (1995) found that the relationship between culture and performance is more complex than previously thought. Schein argued that culture can be both a strength and a weakness, depending on the nature of the culture and the nature of the organization.

CHAPTER – 2

LITERATURE REVIEW

The literature on the impact of organizational culture on organizational performance is mixed. Some studies have found a positive relationship, while others have found no significant relationship. For example, a meta-analysis by Cameron and Quinn (1999) found that organizations with a strong culture have higher performance than those without a strong culture. However, a study by Schein (1995) found that the relationship between culture and performance is more complex than previously thought. Schein argued that culture can be both a strength and a weakness, depending on the nature of the culture and the nature of the organization. The current literature on the impact of organizational culture on organizational performance is mixed. Some studies have found a positive relationship, while others have found no significant relationship. For example, a meta-analysis by Cameron and Quinn (1999) found that organizations with a strong culture have higher performance than those without a strong culture. However, a study by Schein (1995) found that the relationship between culture and performance is more complex than previously thought. Schein argued that culture can be both a strength and a weakness, depending on the nature of the culture and the nature of the organization.

LITERATURE REVIEW

Micropropagation

To ensure a faster rate of potato shoot tip multiplication different growth regulators and culture conditions have been used by different workers. Optimum shoot tip growth was fostered on MS medium containing 0.8mM phloroglucinol and 0.2 M sucrose (Sarkar & Naik, 2000). High frequency multiple shoot formation in this medium ensures a faster rate of potato shoot tip multiplication within a limited time and space.

Jasmonic acid is a growth regulator for which initially inhibitory roles were suggested, but more recently an increasing number of other effects have been reported. In contrast to its tuber-inducing effect it has also been reported that Jasmonic acid may stimulate potato plantlet development and therefore can be used in enhancing potato micropropagation by producing more vigorous plantlets of higher quality (Martin *et al.* 2000).

An *in vitro* test with eight potato varieties showed that the addition of 4 mg/l AgNO_3 to plant differentiation medium increased the regeneration frequency from 40% (without AgNO_3)–81% suggesting that AgNO_3 plays an important role in improving plant regeneration *in vitro* (Wang & Song, 2000). Another factor affecting regeneration was abscisic acid (ABA). Regeneration frequency was increased by adding 0.2mg/l ABA to the medium but high concentration of ABA (>1.0mg/l) inhibited plant regeneration.

In vitro morphogenesis of potato cv. Spunta explants showed effects of different culture media and explant types (Al-Momani *et al.* 2000). Shoot tip segments gave shorter shoot lengths than first, second and third nodal segments on Murashige and Skoog (MS) medium supplemented with a combination of 0.3 mg/l gibberellic acid (GA_3) and 1.0 mg/l of BA. Shoot tips gave the highest number of shoots associated with proliferation. On medium supplemented with 0.3 mg/l GA_3 alone, the second nodal segment gave the highest shoot length, aerial shoot number and nodal number of any explants and media combination. The longest induced internodal length of all the explants was on MS

supplemented with 0.3 mg/l GA₃ while the addition of BA to this combination decreased the internodal length.

Micropropagation studies were performed on four potato cultivars (Kaur *et al.*, 2000). Shoot nodal segments were cultured on solid ½ and full strength medium. The shoots obtained were further multiplied in both solid and liquid media. After hardening, the plants were transferred to a soil and sand mixture for minitubers production. From their studies it is revealed that shoot establishment was better in ½ rather than in full strength MS medium and shoot multiplication was faster in liquid than in solid medium.

Manhyun (2001) studied the effect of culture vessel ecosystem on cultured plantlets of the cv. Dejima of *Solanum tuberosum*. The highest plantlet (4777mg/1 plantlet mean fresh wt.) was obtained from explants cultured in a 10 ml bottle typed bioreactor when compared with a 100ml Erlenmeyer flask. Conventional suspension culture, showed dramatic growth promotion of the plantlets. Plantlets grew vigorously in scale up bioreactors than conventional suspension culture. Somewhat similar studies were performed by Paek *et al.* (2001). They suggested that the application of bioreactor culture techniques for plant micropropagation is regarded as one of the ways to reduce production cost by scaling up and automation. About 20,000 cuttings of virus free potato could be obtained from 120 single node explants in a 20 litre balloon-type bubble bioreactor after eight weeks of culture. The percentage of *ex vitro* survival and root induction of the cuttings was more than 95%.

Ullah *et al.* (2007) studied the effect of varying concentrations of Gibberellic acid (0, 0.1, 0.248 and 0.5 mg/l) on morphogenesis of potato (*Solanum tuberosum* L.) cv. Desiree. They obtained best shoot and root growth on MS medium supplemented with 0.248 mg/l GA₃.

Role of different nitrate levels have been tested on the morphogenetic responses of shoot length. Shoot fresh weight and multiplication rate of five potato cultivars. (Rahman *et al.* 2011). The growth significantly varied in media with or without nitrate. The effect of different levels of nitrate on shoot growth

was non-significant but their effect on shoot fresh weight was significant. Shoot multiplication was even not much affected in the different levels of nitrates.

Cost effective measures have been taken by using economically cheaper alternatives to MS salts, agar and sucrose. MS and low cost (LC) media with different hormonal combinations of Kn and IAA were used for shoot and root proliferation (Badoni & Chauhan, 2011). In LC media agar was replaced by tapioca, sucrose by cane sugar. Calcium, ammonium nitrate, single super phosphate and muriate of potash were the salts used in LC media. Their results indicated that among the two media used, LC medium was better for shoot proliferation.

Yasmin *et al.* (2011) studied the effect of four different growth regulators viz. (BAP, NAA, Pantothenic acid and GA_3) on meristem tips for micropropagation of two potato cultivars (Desiree and Patrones). Their results show that best regeneration was obtained on MS medium supplemented with 1.0mg/l pantothenic acid in combination with 0.5 mg/l GA_3 . This combination produced the highest number of plants in the cultivar Desiree in a very short time.

The efficiency of different nodal segments (top, middle and lower portion) of four potato cultivars were tested for micropropagation (Rai *et al.* 2012). Their studies showed that no clear distinction could be made between the explants taken from different parts of the plant in any of the variety. Therefore nodal cuttings taken from any part of the stem can be used for micropropagation.

Mbiyu *et al.* (2012) compared the effects of liquid and solid MS media on three Kenyan potato cultivars i.e. Dutchh, Kenya sifa and Tigoni. Their results showed that liquid media have a significant effect on the plant growth and were found to be cheaper than solid media.

All developed multiplication methods are suitable for practical seed potato production in the field and ensure a reasonable potato crop.

Microtuberization

Tuberization of potato is a survival mechanism and a complex developmental process. The formation of tubers, as a vegetative storage organ in potato, is under hormonal regulation. The developmental process is greatly influenced by environmental factors, which presumably take effect through the hormonal balance of the plant. Potato plants are able to sense changes in environmental conditions that are required to trigger tuberization, after the plant attains adequate physiological development. Leaves play an important role in the perception of environmental stimuli and in the synthesis of endogenous hormones (Seabrook *et al.* 2004; Peres *et al.* 2005), therefore their adequate developmental state is of great importance in the subsequent tuberization process. There are two possible ways of inducing tuberization in *in vitro*.

1. Adding plant growth regulators to the medium.
2. Modification of environmental factors (such as photoperiod, light intensity temperature in order to modify the hormonal balance of *in vitro* plantlets towards tuberization, or the two ways can be combined.

Plant growth regulators

The majority of the published works on *in vitro* tuberization of potato have focused on using plant growth regulators for tuber induction. Role of different growth regulators such as cytokinins, auxins, antigibberellins, coumarin, jasmonic acid have been investigated.

Role of Cytokinins

Four developmental steps are necessary for tuberization in potato plants.

1. Stolon initiation from axillary buds.
2. Its growth and proliferation
3. Cessation of stolon elongation
4. Tuber induction.

The major regulators during the whole process of tuberization are the gibberellins. Their high level promotes stolon initiation and its further growth, after which it should decrease to allow tuber initiation. The addition of other hormones is necessary for the control of the developmental process. For stolon initiation and elongation, low cytokinins level is necessary but tuberization could occur if the gibberellins to ethylene ratio is low, and also if their levels remain low but the level of cytokinins increases as well as the export of jasmonic acid from the leaves. Jasmonic acid is the key controller of tuber initiation in addition to the main controller, the gibberellins (Pruski *et al.* 2003 a, b; Cenzano *et al.* 2007).

The effect of varying concentrations of BAP (0.2, 1.0 & 5.0 μM) and sucrose (3, 6 and 9%) was studied on *in vitro* microtuberization of potato (Rafique *et al.* 2004). It is obvious from their results that 6% sucrose level in combination with 1.0 μM BAP gave the best results regarding both microtuber number and weight.

The influence of different concentration viz. (2.5, 5.0, 10.0, 15.0, 20.0 & 25.0 μM) of BAP (6-benzylaminopurine) and kinetin used individually on microtuberization was compared (Uranbey, 2004). Single node stem segments of potato were cultured on solid MS medium supplemented with varying concentrations of either BAP or Kinetin. The cultures were incubated in dark at $22 \pm 1^\circ\text{C}$. No tuberization occurred at 2.5 & 5.0 μM BAP. Best microtuber yield was obtained on media containing 15 μM kinetin compared to BAP at the same concentration.

Kanwal *et al.* (2006) obtained microtubers in potato cultivar Kuroda, a newly introduced variety in Pakistan. They achieved good results on medium supplemented with 8% sucrose and 0.75mg/l BAP. It is further revealed from their studies that microtubers can also be obtained on cotton based liquid medium.

Hussain *et al.* (2006) studied the effect of chlorocholine chloride, sucrose and BAP on *in vitro* tuberization of potato (*Solanum tuberosum* cv. Cardinal). Maximum tuber induction was obtained on MS medium supplemented with either 200mg/l CCC or 9% sucrose (i.e. 16.5 and 15.6 tubers/flask respectively)

followed by BAP at 4.0mg/l producing 9.0 tubers /flask. They also observed that complete darkness was essential for tuber induction.

Aslam & Iqbal (2010) studied the combined effect of cytokinins (Kinetin & BAP) and sucrose on *in vitro* tuberization of two cultivars (Diamant & Red Norland) of potato (*Solanum tuberosum*). They observed that best results were obtained at 8% sucrose level in terms of tuber number and tuber weight. The number of microtubers was further increased by the addition of cytokinins (Kn or BAP) in the medium. It is further gained from their studies that microtuberization is greatly influenced by genotype also.

Badoni & Chauhan (2010) performed similar studies for the microtuberization of potato cultivar Himalini. Plantlets obtained from nodal segments grown on media supplemented with varying concentration & combination of GA₃ and NAA were subjected to varying concentrations of BAP (8, 10, & 12mg/l) for tuberization. Formation & development of microtubers were least with 8mg/l BAP & maximum in 10mg/l BAP.

The effect of Kinetin and Benzylaminopurine was investigated on *in vitro* microtuberization of two potato cultivars "Arinda and Diamant" (Aryakia & Hamidoghli, 2010). Their results showed that the highest number of tubers / node were obtained in cv Diamant in Kinetin supplemented media, whereas tuber size and weight was more in both the cultivars in media containing BAP.

Imani *et al.* (2010) studied the effects of various concentrations of BAP (0, 12, 15 and 18 g/l and sucrose (0, 60, 80 g/l) on *in vitro* tuberization. They concluded that sucrose and BAP had significant effect on microtubers. Sucrose at 60g/l and BAP 15 g/l showed maximum number of microtubers. Where as the maximum size of tubers were obtained at 60 g/l sucrose in combination with 12g/l BAP.

The effect of IAA and kinetin were tested on tuberization parameters of different cultivars and transgenic lines of potato *in vitro* (Romanov *et al.* 2000). Most of the non-transformed potato cultivars and transgenic lines responded to hormone application by an increase in tuber yield. Auxin and cytokinins acted differently. IAA increased predominantly the tuber size while kinetin increased

the number of tubers. The degree of phytohormone effect on tuberization parameters depended on sucrose content of the medium and potato genotype.

Role of Jasmonic Acid

A broad spectrum of plant processes is regulated by jasmonates (JAs), among them seed germination and root growth, flower and fruit development, senescence and tuber formation (Wasternack & Hause, 2002).

Castro *et al.* (2000) observed a reverse of the delaying effect of gibberellic acid (GA_3) in *in vitro* tuberization of potato plantlets when the medium contained $0.8 \times 10^{-5} M$ jasmonic acid (JA). The promoting effect of JA seemed to be correlated with the initial absence of growing roots, probably through direct activity of JA in microtuber producing buds in the stoloniferous shoots. This is based on the fact that JA did not inhibit root growth *a posteriori*. Endogenous gibberellins (GA) synthesized by roots did not interfere with the previous activity of JA in tuberization. The absence of a promoting effect on tuberization of previously rooted plantlets could be related to the capacity of potato roots to synthesize endogenous GA which might antagonize the JA effect on buds of stoloniferous shoots.

Koda & Kikuta (2001) studied the effects of jasmonates on *in vitro* tuberization in several potato cultivars that differed greatly in maturity. Late maturing cultivars of potato form tubers much later than early maturing cultivars in a field. Jasmonic acid (JA) induced *in vitro* tuberization in various cultivars that differed greatly in maturity time. Airbone methyl jasmonate was also able to induce tuberization in all the cultivars tested.

Abdala *et al.* (2002) reported that levels of endogenous JA increased in stolons of *Solanum tuberosum* L. "Spunta" during the transition from stolons to tubers. Moreover, Cenzano *et al.* (2003) reported exogenously applied jasmonic acid induces changes in apical meristem morphology of potato stolons. JA application increased the meristem thickness and reduced the length of the leaf primordia, whereas in initial swelling stolons narrowing of the apical region, absence of leaf primordial and swelling of the subapical meristem were evident.

Jasmonic acid conditioning of stock plants prior to taking explants for tuberization was proposed as a treatment enhancing the quality of microtubers (Pruski *et al.* 2002b). They tested JA effect on *in vitro* tuberization of potato nodal explant cvs. Sangre and Russet Burbank both in liquid and solid media and under 0, 8 and 16h photoperiod. Explants taken from stock plants grown on 2.5 μ M JA supplemented medium tuberized first. The most pronounced benefits of the JA pretreatment were recorded under 16h photoperiod, which is known to inhibit tuberization. Cultivars Sangre benefited from the JA preconditioning of stock plants more than Russet Burbank. Russet Burbank required the JA supplement in tuberization media to reach the same degree of stimulation. Microtubers produced either from JA preconditioning stock plants or on the JA conditioning tuberization media were more uniform and larger than from other treatments. Eight hours photoperiod was best treatment for the production of high quality uniform microtubers.

Pruski *et al.* (2003) worked on phototropic micropropagation; production of microtubers. They tested the effects of jasmonic acid and photoperiod on *in vitro* tuberization, of six commercial cultivars. Potato plantlets produced photoautotrophically were of same quality or better than the conventional cultivars. Conventional cultivars benefited from CO₂ enrichment, over a 4 week period, by a 20% increase in number of nodes and 50% increase in stem dry weight. Better microtuber, yield was observed on solid agar media than on liquid media under 8 hours (SD) photoperiod compared to no light. JA conditioned plantlets produced more uniform and larger microtubers under short day condition.

Culture Density

Effects of culture density were studied on growth and *in vitro* tuberization capacity of potato plantlets (Tabori *et al.* 2000a). *In vitro* plantlets of four potato (*Solanum tuberosum*) cultivars were grown in Kilner jars at different plant densities (20, 30, 40 plantlets/400 ml Kilner jar) to study the effects on growth and microtuber yield. The responses of the cultivars to plant density varied, as

expressed by changes in developmental characteristics, especially stem length and leaf area. Tuber initiation was earlier at higher plant densities for all cultivars. The microtuber number per plantlet was not affected by the plantlet density. The highest number of well sized ($>4\text{mm}$) microtubers per jar could be harvested from the higher plantlet densities (30–40 plantlets per 400 ml jar).

Role of Gelling agents

The effect of different gelling agents viz. phytagel T.M and Difco Bacto-agar was compared for *in vitro* tuberization of single node segments of six potato cultivars (Jaerla, Blanka, Clauster, Kennebec, Desiree and Baraka) (Arregui *et al.* 2003). It was found that tuberization was higher when phytagel TM (0.2% w/v) was used than Difco Bactoagar (0.8% w/v), regardless of the photoperiodic regimes used (dark or an 8-h photoperiod). Chemical analysis of the two gelling agents revealed a higher mineral content and organic impurities in Bactoagar than in phytagel T.M.

Role of Sucrose

Besides plant growth regulators there are certain other constituents, sugars such as sucrose, play a role in coordinating the occurrence and timing of developmental processes in plants. Much evidence has been published concerning the existence of sugar-specific signaling pathways and sugar response pathways interact with other pathways, such as those for phytohormones and for light responses. High level of soluble sugars can cause the formation of extra organs in different plant species, such as tubers in potato (Gibson, 2005).

Sucrose is essential *in vitro* as an energy source or as an osmotic potent agent and at higher concentration it serves as a signal for microtuber formation (Donelly *et al.* 2003), resulting in an increase in the number of tubers in potato culture (Gibson 2005). The importance of sucrose concentration for *in vitro* tuberization has been described in tuberization systems that used plant growth regulators (Gopal *et al.* 2004, Hussain *et al.* 2006) and in medium free of plant growth regulators (Alix *et al.* 2001). In contrast with the 3–4% sucrose

concentration of the micropropagation medium a high sucrose concentration (6 – 8%) is used in microtuberization.

Banfalvi *et al.* (1997) studied the effect of sucrose in the presence or absence of BAP on tuberization. They observed that tuberization and stolon elongation depended on the level of sucrose in the medium. BAP enhanced the efficiency of tuber formation only at sucrose concentration above 4%.

The effect of different carbon sources of (sucrose and maltose) and varying concentrations (2, 4, 6, 8, 10 and 12%) were studied on *in vitro* microtuberization of potato cultivars (Justine and Agria). It was found that 6% sucrose in Agria and 4% maltose in Justine were optimum for microtuberization. (Altindal & Karadogan, 2010).

Explants used for tuberization

The productivity of *in vitro* tuberization system is greatly affected by explants used for tuberization. The physiological age, type of explants and explant density greatly influence tuberization.

While studying tuberization in the cv Desiree, Tabori & Dobranszki (2002) and Dobranszki & Tabori (2004) observed that microtuber size was affected with the size of the explant. Explants with varying number of nodes were layered on a medium with high sucrose (8%) content. The highest multiplication (1.98) was recorded for explants with 5 nodes. The majority of the microtubers (49.4%) were 6–8mm in the case of the smallest explants (with 1 node). Explants with 2–5 nodes produced microtubers 8–10mm; however, the increase in explant size resulted in the increase in the number of microtubers with large diameter (up to 16mm) and in the average fresh weight of tubers.

Environmental Factors

Role of photoperiod

The most important environmental stimulus that affects the growth, morphogenesis and tuberization of potato is light, including photoperiod, light intensity, wavelength, etc. (Seabrook, 2005). Other environmental factors, such as temperature, nitrogen nutrition also affect the developmental process.

In vitro tuberization responses of different clones of potato were evaluated under different photoperiods and light intensities (Dobranszki, 1996, 2000; Dobranszki *et al.* 2000). No growth regulators were added to the culture media to avoid the potential effect of growth regulators on the response of environmental stimuli. An 8% sucrose treatment was used for tuber initiation. Light (Short-day treatment) applied after induction phase delayed or inhibited tuber initiation. By contrast, darkness applied after the tuber induction stage accelerated and synchronized tuber initiation after high light intensity.

Dobranszki (2001) studied the effects of different combination of short days and dark treatment and different light intensities during the short days on *in vitro* tuberization of three potato cultivars. Tuberization in Desiree, Cleopatra and Garcia was induced by short day treatment after 4 weeks culture under long days. The shorter the duration of the short days the higher the light intensity was necessary for earlier tuber initiation.

Chang-Wen *et al.* (2004) studied the effect of photoperiod on *in vitro* microtuberization. Their findings indicated that different periods of darkness did not have an effect on tuberization number but have significantly affected the weight of microtuber by influencing photosynthesis and bio substance distribution. The two treatments, 8h darkness and 12 h darkness had higher yield. There was a negative correlation of the length of darkness with chlorophyll content and microtuber starch content, while a positive correlation of the amylase activity with darkness length was noticed and a significantly positive correlation of economic yield with chlorophyll content and microtuber starch content. With an increase in dark period the microtuber shape and skin colour changed varying from long oval to round and from green to light green to white respectively.

With an approach to establish a rapid disease free seed production system in potato, Hoque (2010) adopted *in vitro* techniques. The results obtained from his experiments, showed that dark conditions gave a better response to tuberization than light condition. Among the various explants used nodal cuttings gave the best results regarding the initiation of microtubers and average weight of microtuber on MS medium supplemented with 6% sucrose + 4mg/l kinetin.

Role of Temperature

In vitro microtuberization of potato is affected considerably by temperature and gelling agent (Uranbey, *et al.* 2004). Plants were subjected to varying temperature regimes such as 18, 20, 22 & 24°C & two different gelling agents, i.e. agar and gelrite. Best results were obtained in cultures maintained at 20–22°C in dark. Considerable increases in number, average weight & yield of microtubers were obtained on media solidified with gelrite compared to agar, when used as a gelling agent.

Role of other Factors

Al Safadi *et al.* (2000) studied the effects of low doses (2.5, 5.0, 10 and 15Gy) of gamma irradiation on potato microtuber production. Irradiation of the explants with 2.5Gy of gamma radiation led to a significant increase in the number of microtubers (38% increase over the control), without any change in the shape and color of the tubers.

Tuberization and stolon growth are significantly affected by salinity (Silva *et al.* 2001). Three species of potato, (*Solanum tuberosum*, *S. curtilobum* and *S. juzepczuckii*) differing in frost resistance were subjected to 0, 25, 50, 75 and 100m mol/litre NaCl. The highest salinity level (100 m mol/litre NaCl) completely inhibited tuber development in all species but not stolon growth.

Hossain & Siddique (2011) tested the efficacy of nitrogen and coumarin (auxin antagonistic) on microtuberization of *Solanum tuberosum* cv Diamant. Five levels of Nitrogen (30, 45, 60, 75 and 90mM) and three levels of coumarin (30, 50 & 70 mg/l) were used in these experiments. Best results were noted on

medium containing 45 mM of Nitrogen in combination with 30 mg/l coumarin within a culture period of 105 days.

Dhital & Lim (2012) studied the role of supplementary nutrients, plant growth regulators and *in vitro* culture conditions for microtuberization of four potato cultivars. Both $\frac{1}{2}$ and full strength MS medium in solid & in liquid form was used. Promising results regarding number & yield of microtubers (relatively of large size) were obtained by using supplemental liquid MS medium at $\frac{1}{2}$ strength, 8% sucrose, moderate concentration of supplementary nutrient, plant growth regulators, followed by a 50% replacement of the same medium.

El-Sharabasy *et al.* (2012) investigated the effect of KNO_3 and sucrose concentrations on the production of *in vitro* microtubers in potato. Shoot proliferation was observed on MS medium supplemented with 3% sucrose and 1.0mg/l of BA. Multinodal stem segments from the *in vitro* raised plantlets were sub-cultured on MS media supplemented with varying concentrations of potassium nitrate and potassium dihydrogen phosphate, for microtuberization. The cultures were subjected to both long day and dark conditions. Highest tuber number was produced on $\frac{1}{4}$ strength MS medium supplemented with 170 mg/l KH_2PO_4 under long days.

Role of Genotype

Hossain (2005) revealed that microtuberization is a variety-dependent character, largely influenced by environmental factors. He evaluated seventeen potato cultivars of six sources for their response *in vitro* microtuberization in the presence or absence of light. Tuber initiation appeared first in the light and gave higher number of microtubers with higher total as well as mean weight than the dark. Among the cultivars on the average, patrones performed best for most of the parameters such as days to tuber initiation, number of tubers, their weight per plant and mean tuber weight. In terms of microtuber production potential, Chamak was the poorest. Among the different sources, the Dutch & Canadian cultivars showed comparatively better performance, in respect of microtuber production potential than the other varieties. The local high yielding varieties

were the least productive. The weight of microtubers per tube and mean tuber weight were also significantly higher in the light compared to the dark.

Nistor *et al.* (2010) studied the influence of potato genotypes on *in vitro* production of microtubers. Experiments were carried out on semi-early, Romanian varieties (Christian and Roclas) and of early Dutch variety (Ostara). Significant differences were noted between the varieties, regarding the size, number and weight of the microtubers.

Minituberization

Potato mini tubers can be produced by hydroponic culture technique (Rollot & Seutin, 1999). The use of this technique can increase the number of minitubers produced per unit area. The quality of the minitubers produced in such a way was excellent; no infections were observed and the physiological behaviour of these tubers was not different from the ones produced in the open field.

An efficient and low cost modular system for the production of minitubers as a source for conventional seed tubers was reported by Ahloowalia (1999). In this system the microplants were soil grown and produced minitubers of 5 – 25mm. diameter. The system was based on the use of pre-sterilized disposable plastic containers which allow micropropagation, weaning, hardening and soil growing of micropropagated plants in the same container. Depending upon the cultivar the modular system allows production of 2900 to 6600 mini tubers /m² in a glass house.

Micropropagation of 4 potato cultivar through the production of minitubers in polyethylene bags filled with normal field soil was tested (Kaur *et al.* 2000). Plantlets were raised, from *in vitro* grown plants by culturing nodal segments on both ½ and full strength MS medium. Shoots thus obtained were further multiplied through periodic sub culturing in solid or liquid MS medium. For mini tuber production, the hardened plants were transferred to a soil and sand mixture in polyethylene bags kept in a glass house (18–20°C) and harvested after two and a half months. The maximum number of minitubers thus produced ranged from 6.2–7.9 depending upon the cultivar.

Differences between varieties have been reported by various workers. The effect of plant growth regulator cultivars and substrate combination on production of minitubers from *in vitro* raised virus free plants was studied by Shojaei *et al.* (2009). They showed that Marfona produced the greatest number of minitubers while Agria the lowest. No significant differences were in the size of the minitubers. Similar results were reported by Sanavy & Moeinil (2003) and Moeinil *et al.*, 2011. They also studied the effect of different plant growth regulators on micropropagation and different potting mixes on mini tuberization of two potato cultivars (Marfona and Agria).

Tsoka, *et al.* (2012) adopted a new technique for the production of minitubers of potato from *in vitro* plantlets and apical stem cuttings. *In vitro* raised plantlets and apical stem cuttings of three potato cultivars were used as source material for the aeroponic study in the greenhouse. Their results indicated that the *in vitro* plant material yielded significantly better seed potato tuber numbers per plant than apical stem cuttings. This shows that *in vitro* plantlets have potential to give a viable material for seed potato tuber production under aeroponics. Similar studies were performed by Chiipanthenga *et al.* 2012 for the production of quality seed tubers of potato. Plants obtained from *in vitro* meristem culture were subjected to aeroponics in the green house. From their studies it is revealed that under aeroponics high quality potato seed tubers are produced in a short time and reduces the numerous labour steps associated with direct use of plantlets in the field.

Kawakami and Iwama (2012) studied the effect of size ranging (from 0.3-5g) on field performance of potato minitubers. Plants from microtubers lighter than 0.5g showed a slower initial leaf and tuber growth than heavy weight microtubers but no significant difference was noticed in the tuber growth or leaf size at flowering stage. No differences in growing period, number of tubers and tuber fresh and dry yield were observed with the microtuber size. Microtubers of all sizes can therefore be used for direct field planting.

Profile of potato in Pakistan

Pakistan is an agricultural country with 70% of its population being dependent on farming. Potato enjoys great popularity as a food commodity in Pakistan. Most families consume potato at least once everyday. It can substitute the deficiencies in the foods of the people of an underdeveloped country because potato along with milk can provide all the nutrients which the human body needs as it contains plentiful carbohydrates, some proteins, calcium and niacin plus a considerable amount of vitamin C, therefore, it is a complete diet at low cost.

Like most countries in the world, Pakistan depends for the greater part on cereals as a staple food in the national diet. They are grown on a combined area of approximately eleven million hectares, producing annually approximately 21 million tons of grain. The most important is wheat which accounts alone for 75 percent of the area of production. Cereals are easy to produce, do not need high investments from the growers and have a guaranteed market. They are also easy to store, handle and to be transformed into ready to eat products. Potatoes do not have an equal status with other foods in Pakistan as yet. Although the area under production has increased from around 3000 ha at the time of independence to around 131000 ha during 2006–2007. During the same period the average yields rose from around 9 in 1947 to 18 MT per hectare. (MINFAL, 2007) they are still considered a vegetable by the Pakistani consumers. In other countries such as Europe and South America they are equally or more important than cereals as a staple food. They achieved that position due to their unmatched capacity in terms of energy production per unit area over 200 Mj (Mega joules) per hectare per day against around 130 Mj per ha per day for wheat and their high nutritional value equal to wheat and rice in proteins and considerably higher in vitamins. In Pakistan with its growing population and diminishing area of productive land, due to erosion, salt problems and water scarcity, wheat and rice may not be able to cope with the necessity for more intensive production and the potato may have a greater potential to fill the widening gap between the need and availability of food.

Due to favorable agro climatic conditions, three crops of potatoes are grown commercially in Pakistan i.e. Autumn, Spring and Summer. Autumn and spring crops are grown in plains whereas summer crop is in hills. Potato growing regions in Pakistan are shown in Fig. 2. In spite of a dramatic increase in the area and production of potato, the national average yield of 18 tons/hectare is very low compared to 40–60 tons obtained in other potato growing countries of the world (FAO, 2010). The reason being the attack of a variety of diseases which reduce potato yield by 10–16%.

All those who are actively involved in potato production and have to make their living out of it, know that it is a difficult crop from many points of view. Even when the farmer has solved agronomical problems like fertilizer and irrigation application, pests and diseases may create havoc for his crop and when he has reached a good yield, he may find the prices collapsing because all others had the same good results. High quality costly seed forms another constraint. The seed contributes to about 35–40% of the total cost of the production is limited and faces technical, economical and managerial problems. Lack of availability of sufficient quantities of good seed and low purchasing power of the farmers, forces them to rely on seed sources of doubtful quality or own production.

Another problem faced by the farmers is the high cost of seed in general and poor quality seed in particular leaving only profit margins for those who obtain high yields. There is a limited number of commercial varieties available in Pakistan. Desiree, Cardinal and Ultimus are the main red skinned while Diamant, Patronex, Ajax and Multa are the white skinned varieties. In remote areas different local varieties have evolved which are still under cultivation (Zanoni, 1991). The yield and quality of these potatoes is poor. This results in poor economic returns for big and small growers alike. Another draw back is that only 2% of the seed required for planting all the crops is certified. It also includes the imported seed as well. Moreover, these seeds even do not match our environmental conditions and are susceptible to various diseases. Even the seed supply by itself is very irregular. The availability of high quality seed of the right quantity at the right time therefore remains the most consequential bottle neck to efficient potato production. For those, however, who master all the difficulties, it is a crop that gives them high economic returns.

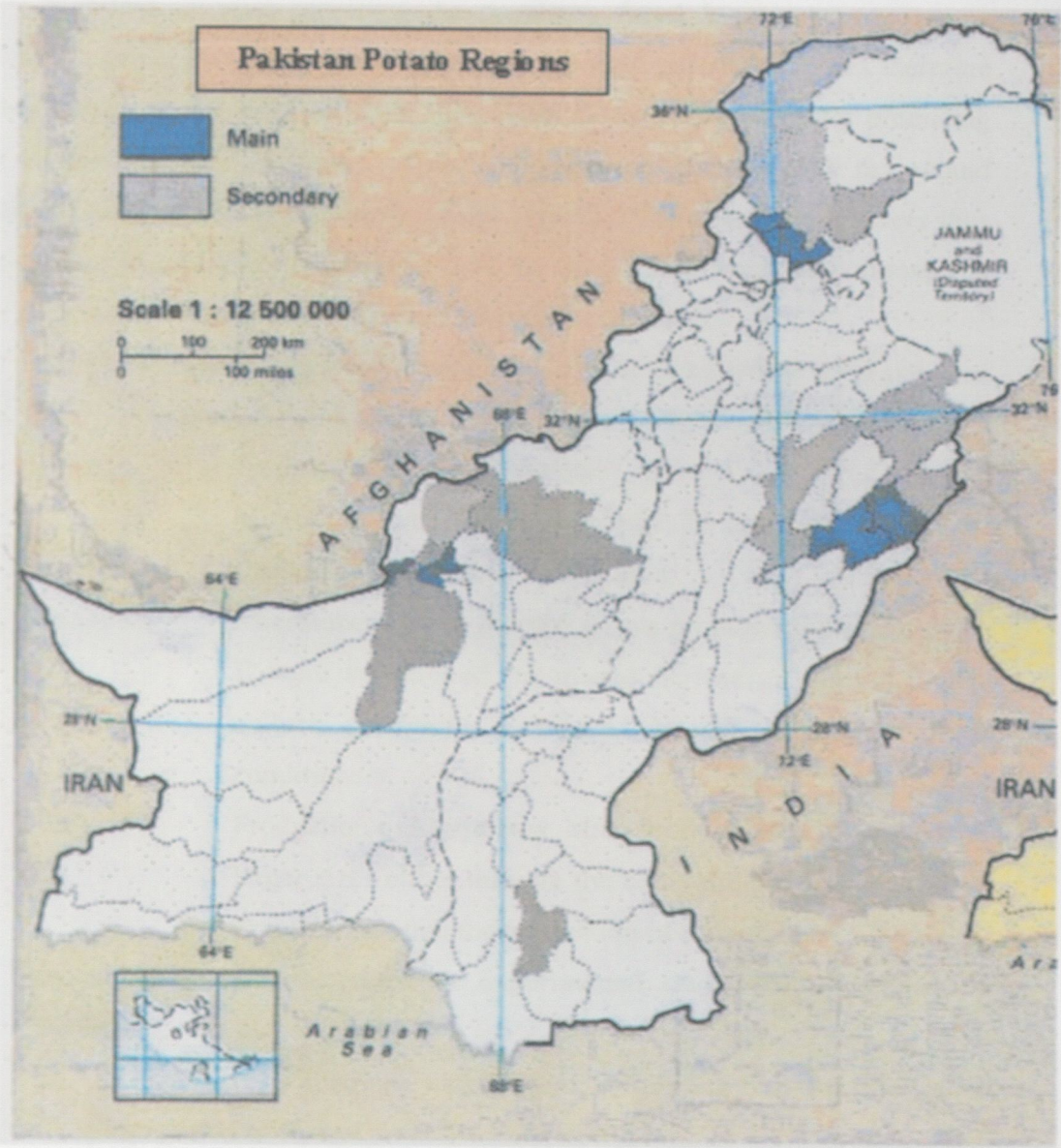


Fig. 2. Potato regions of Pakistan

OBJECTIVES

Keeping in view some of the problems faced by potato growers in Pakistan in general and in the Khyber Pukhtunkhwa in particular, it was therefore desired to micro propagate potatoes using tissue culture technology for producing high yielding, disease free seed tubers in the shortest possible time that would match the environmental conditions of our country. The micro / minitubers thus obtained are to be further experimented in the field and disseminated among the local farmers, so as to revolutionize our seed potato industry.

The core technologies for mass production of disease free seed potatoes were divided into the following stages.

- | | |
|-----------|---|
| Stage I | <i>In vitro</i> bud culture of potato. |
| Stage II | Micropropagation of potato plants. To obtain <i>in vitro</i> plants that are healthy and free of viruses. |
| Stage III | Microtuberization: <i>In vitro</i> shoots propagated through stage II were subcultured for microtuberization under different culture conditions, compared to the stage II culture conditions. |
| Stage IV | Production of Minitubers. Mass production of minitubers of larger sizes either through the cultivation of <i>in vitro</i> raised shoots or through the cultivation of microtubers. |

To achieve these objectives the experimental studies were outlined as follows.

- a). To optimize a medium and culture conditions for mass *in vitro* propagation of shoots.
- b). To optimize a medium and culture condition for microtuberization.
- c). Field performance of micropropagated plants.
- d). Field performance of microtubers.

MATERIAL AND METHODS

Explant source

With the objective of mass production of potato seed tubers, experiments were conducted to find the micropropagation potential and *in vitro* adaptation of potato (*Solanum tuberosum* L.) using tissue culture techniques. Two varieties of potato, i.e. Desiree and Ulmota which are commonly grown in our province (Khyber Pakhtunkhwa) and studies and both these varieties were obtained from Potato Research Institute, Abbottabad.

CHAPTER – 3

Desiree

It is red skinned variety with yellow flesh. It has large, long oval tubers, with 2-3 eyes each and 9-10 eyes per tuber. The good quality of Desiree are that it is better cooking and contains comparatively low sugar content after storage under low temperature. This variety is suitable for growing in all three growing seasons in Khyber Pakhtunkhwa.

MATERIAL AND METHODS

Ulmota

This variety is also red skinned with a yellow flesh. The tubers are smaller in size and long with deep eyes. It is a high yielding variety and produces good quality tubers. Like Desiree, it is also firm on cooking and there is less conversion of starch into sugars after cold storage. This variety has been proved as best tolerant. The crop for early marketing is planted three weeks earlier than the normal planting season, i.e. from 1st of September when the minimum atmospheric temperature is generally 36 °C which is very harmful for potato cultivation in case of most of the presently cultivated varieties in Pakistan.

The culture techniques adopted during the present investigations were divided into 4 stages.

MATERIAL AND METHODS

Explant source

With the objective of mass production of potato seed tubers, experiments were conducted to find the micropropagation potential and *in vitro* tuberization of potato (*Solanum tuberosum* L.) using tissue culture techniques. Two varieties of potato i.e. Desiree and Ultimus which are commonly sown in our province (Khyber Pukhtunkhwa) were used during the present studies and both these varieties were obtained from Potato Research Institute, Abbotabad.

Desiree

A red skinned variety with yellow flesh. It has large, long oval tubers, with shallow eyes and 9–10 eyes per tuber. The good qualities of Desiree are that it is firm on cooking and contains comparatively less sugar content after storage under low temperature. This variety is suitable for growing in all three growing seasons i.e. spring, autumn and summer in Pakistan and is very popular for marketing in the Punjab and Khyber Pukhtunkhwa.

Ultimus

This variety is also red skinned with a yellow flesh. The tubers are medium to large and long with deep eyes. It is a high yielding variety and produces good quality tubers. Like Desiree, it is also firm on cooking and there is less conversion of starch into sugars after cold storage. This variety has been proved as heat tolerant. The crop for early marketing is planted three weeks earlier than the normal planting season i.e. from 1st of September when the maximum atmospheric temperature is generally 36 °C which is very harmful for potato cultivation in case of most of the presently cultivated varieties in Pakistan.

The culture techniques adopted during the present investigations were divided into 4 stages.

- Stage – I Initial propagation through the use of potato buds.
- Stage – II Propagation through the use of single nodes or stem cuttings (with 3–4 nodes) from the above stock (stage-I) for micro/mini tuberization.
- Stage – III Tuber induction of the plantlets obtained in stage-II.
- Stage – IV Field testing of the micro plants of stage II and the microtubers.

Basic requirements

- i. Suitable working area where media could be easily prepared.
- ii. Transfer area for aseptic manipulations equipped with laminar air flow cabinets.
- iii. Culture room: The room for incubating cultures was maintained at a controlled temperature. Air conditioners were used to maintain the temperature at $25 \pm 1^{\circ}\text{C}$. For controlled photoperiodic regimes special incubators with built in fluorescent lights were used.
- iv. Culture Vessels: Pyrex conical flasks of varying sizes (50, 100, 250 and 300ml capacity) and test tubes (50 ml) were used during the present investigations.
- v. All required chemicals (high quality)
- vi. Other requirements include.
 - a. An autoclave for sterilizing media.
 - b. A supply of distilled water.
 - c. A pH meter etc.

Techniques

New glassware was used only after thorough washing. Traditionally glassware was washed with chromic mixture (a mixture of potassium dichromate + H_2SO_4) for about 4 hours followed by thorough washing in a jet of tap water to completely remove all traces of the acid. After soaking in detergent solution for a suitable period the apparatus was thoroughly rinsed first with tap water and then with distilled water. To recycle the glassware that had contaminated tissues or

media, it was autoclaved so that all the microbial contaminants were destroyed. The washed apparatus after drying was stored in dust proof closets.

Nutrient medium

The basal medium used throughout these studies was that of Murashige and Skoog's (1962) inorganic salt solution unless otherwise mentioned. MS medium has the following composition.

Macro-Nutrients

Nutrients	Formula	Mg/l
Ammonium nitrate	NH_4NO_3	1650
Potassium nitrate	KNO_3	1900
Calcium chloride	$\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$	440
Magnesium sulfate	$\text{MgSO}_4 \cdot 6\text{H}_2\text{O}$	370
Monopotassium dihydrogen phosphate	KH_2PO_4	170

Micro - Nutrients

Potassium iodide	KI	0.83
Boric acid	H_3BO_3	6.2
Manganese sulfate	$\text{MnSO}_4 \cdot 4\text{H}_2\text{O}$	22.3
Zinc sulfate	$\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$	8.6
Molybdic acid	$\text{NaMoO}_4 \cdot 2\text{H}_2\text{O}$	0.25
Copper sulphate	$\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$	0.025
Cobalt chloride	$\text{CoCl}_2 \cdot 6\text{H}_2\text{O}$	0.025

Irons as

Sodium ethylene diamine tetra acetate	NaEDTA	37.3
Ferrous sulfate	$\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$	27.8
Vitamins		
Myo-inositol		100
Nicotinic acid		0.5
Pyridoxine HCl		0.5
Thiamine HCl		0.5

Sucrose/glucose was added at varying (4–10%) levels and the medium was solidified with 1% agar.

Growth Regulators

The following growth regulators were used in the experimental studies.

Compound	Formula	Abbreviation	MW
Cytokinins			
Benzyl Amino purine	$C_{12}H_{11}N_5$	BAP	225.2
Kinetin or 6-furfurylamino purine	$C_{10}H_9N_5O$	K	215
Adenine sulfate	$(C_5H_5N_5)_2H_2SO_5 \cdot H_2O$	AS	-
Auxins			
Naphthalene acetic acid	$C_{10}H_7CH_2COOH$	NAA	186.2
Indole acetic acid	$C_{10}H_9NO_2$	IAA	175.2
Gibberellins			
Gibberellic acid	$C_{19}H_{22}O_6$	GA ₃	346.4
Other Growth regulators			
Absciscic acid		ABA	
Coumarin			
Chlorocholine chloride		CCC	

Stock solutions of micronutrients, vitamins and FeEDTA all prepared to 100 times concentration were stored in the refrigerator. Stock solutions of all the growth regulators were prepared at a 100mg/l strength and were refrigerated.

Media preparation

The sequence of steps involved in preparing a medium are as follows.

1. Required quantities of agar and sucrose were weighed.
2. Appropriate quantities of the various stock solutions, including growth regulators and other special supplements were added.
3. The final volume was made up with distilled water and the sucrose was dissolved thoroughly well in it.

4. After mixing well the pH of the medium was adjusted to 5.6 using 0.1 N NaOH and 0.1N HCl.
5. Agar was added after pHing the medium and was dissolved by heating and constant stirring. The medium was then poured into desired culture vessels.

Sterilization of Medium

The microbial contaminants are normally present in the medium right from the start. To destroy them the mouth of the culture vessel containing the medium was properly plugged with non-absorbent cotton. The medium was sterilized in an autoclave (steam heating under pressure) at 15 lbs pressure psi at 121°C for 15 minutes from the time the required temperature was reached.

Sterilization of Plant Material

Surfaces of plants carry a wide range of microbial contaminants. To avoid this source of infection potato buds were thoroughly washed with running tap water. They were then immersed in 70% ethanol for a few seconds followed by immersion in 0.5% mercuric chloride solution for 3 minutes and finally rinsed several times with sterile distilled water.

Sterilization of Transfer Area

Finally, it is also very essential that all precautions are taken to prevent the entry into the culture vessel of any contaminant when its mouth is opened either for subculture or for inoculation of fresh tissue. To achieve this all transfer operations were carried out under strictly aseptic conditions. For this laminar air flow cabinets were used. Before use the entire chamber was sprayed with 95% alcohol with the help of an atomizer and all the required materials (media, sterilized distilled water, sterilized petridishes, spirit lamp, match box, sterilized instruments etc.) were transferred to the chamber.

After spraying with alcohol the blowers in the chamber were switched on. For achieving a satisfactory degree of aseptic environment inside the laminar flow

cabinets the fans were switched on 15 minutes before the aseptic manipulations. The hands were washed thoroughly with soap before starting to work in the chamber. Hands were sprayed with 70% alcohol and let to dry. To prevent the hazards of fire the spirit lamps were lit only after ensuring that the hands and the chamber were dry, and the aseptic operations were then started.

3. Shoot systems having 3-4 nodes

Culture Conditions

The cultures were incubated at a temperature of $25 \pm 1^\circ\text{C}$ and under varying photoperiodic (0, 8 & 16 hrs light) regimes. Observations were done on a weekly basis and all harvest of the microtubers was done after 10 weeks of culture period.

The yield of the microtubers for a specific medium was based on the number of tubers/flask, the mean weight/tuber and mean weight of tubers /flask.

The plantlets obtained were either transferred to the field after 10 weeks or were used for *in vitro* microtuberization.

Experimental

The *in vitro* techniques adopted for microtuber induction were divided into following four stages.

(Schematically represented in Fig. 3)

The micropropagated plants obtained from stage II were subjected to

Stage I

In vitro bud culture

Shoot cultures were obtained by culturing the buds from disease free healthy potatoes of the cultivars Desiree and Ultimus. The buds after surface sterilization were inoculated on Murashige & Skoog (1962) medium supplemented with 4% sucrose and adjusted at pH 5.5. The cultures were incubated at $25 \pm 1^\circ\text{C}$ both in long and short day lengths (16 and 8 hours photo period).

Stage II

Micropropagation

The *in vitro* plantlets obtained in stage I were further used for micropropagation either through the use of:

1. Single node cuttings.
2. Shoot tips.
3. Stem segments having 3 – 4 nodes.

The explants were excised from the *in vitro* plantlets obtained from stage I and inoculated in the propagation medium. The propagation medium consisted of MS basal medium at half strength with 4% sucrose and supplemented with:

- i. NAA
- ii. NAA + BAP
- iii. NAA + BAP + GA₃

The plantlets obtained were either transferred to the field after acclimatization or were used for *in vitro* microtuberization.

Stage III

Microtuberization

The micropropagated plants obtained from stage II were subjected to tuberization media under varying photoperiodic regimes. The tuberization media consisted of MS basal medium with varying sucrose levels and varying concentrations of different growth regulators. The tuber yield was recorded by their number and weight.

Stage IV

Field experiments

The *in vitro* raised plantlets obtained at stage II and the microtubers and minitubers from stage III were tested in the field for their performance.

Stage I
In vitro bud culture

Stage II
Micropropagation through
nodal segments

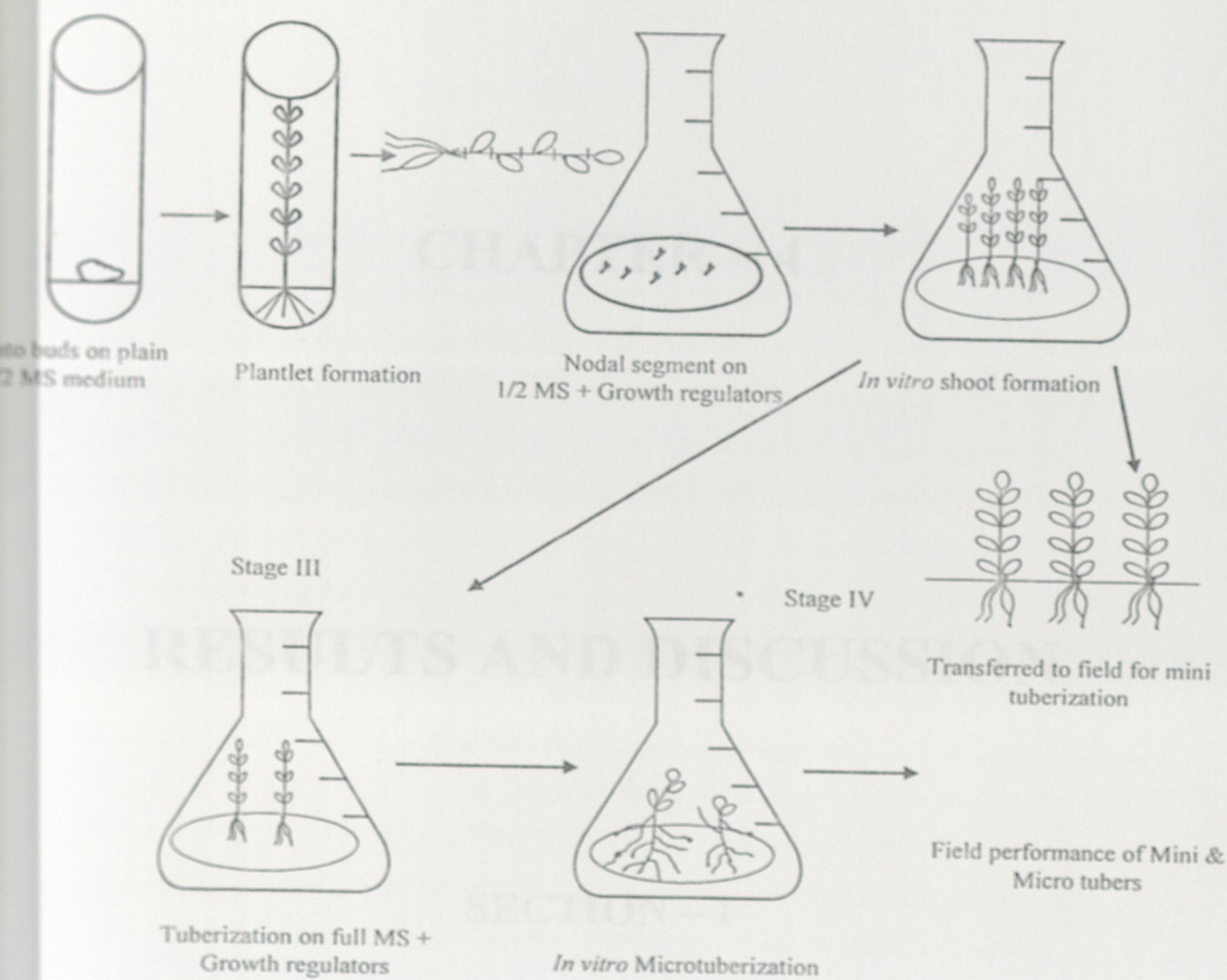


Fig. 3. Schematic representation of the experimental work.

Stage - I *IN VITRO* BUD CULTURE

In view of the problems faced by the potato growers in Pakistan, the present studies were initiated to develop a system for *in vitro* tuber induction, with characteristics such as:

1. Short induction period for tuberization and

2. Production of tubers in minimum number and weight of explants and within the shortest possible time.

CHAPTER - 4

RESULTS AND DISCUSSION

SECTION - I

IN VITRO BUD CULTURE

Stage – I *IN VITRO* BUD CULTURE

In view of the problems faced by the potato growers in Pakistan, the present studies were initiated to develop a system for *in vitro* tuber induction, with characteristics such as:

1. Short induction period for tuberization and
2. Production of high yield (both in terms of number and weight) of micro and mini tubers within the shortest possible time.

Shoot cultures were obtained by culturing the buds from disease free healthy potatoes of the cultivars Desiree and Ultimus. The buds after surface sterilization were inoculated on both full and half strength Murashige & Skoog (1962) hormone free medium supplemented with 4% sucrose and adjusted at pH 5.5. The cultures were incubated at $25 \pm 1^{\circ}\text{C}$ both in long and short day lengths (16 and 8 hour days). Results obtained are outlined in (Tables 1 and 2; Figs. 4 and 5).

The buds from both the cultivars inoculated on 1/2 strength MS basal medium grown under long day (16 hours light) conditions started sprouting on the 3rd day of culture. They grew into single several cms long leafy shoots having 8-10 nodes within a culture period of 3 weeks. Each plant had a healthy root system and with a single large dark green leaf at each node (Figs. 6a and 6b). With increasing duration of culture period there was a considerable increase in the length of the plants together with some branching (Figs. 7a and 7b).

The buds from both the cultivars when grown in full strength MS basal medium did not exhibit good results (Table 1 and 2). Buds sprouted after the second week of culture. Moreover further growth of these plants was very slow and plantlets with one or at the most 2 nodes were obtained within a culture period of eight weeks. Half strength basal medium was therefore used for bud culture during these studies. The plantlets thus obtained were used as the stock cultures for further experiments.

Still further, in another set of experiments the buds when cultured in half strength MS medium and grown under short days (8 hours light per day) did not exhibit a good response (Table 1 and 2). There was comparatively less sprouting. Moreover the plantlets thus produced were weak and etiolated with few nodes, very long internodes and very small leaves in both the cultivars.

The ANOVA (Table 3 and 4) showed that the genotypes have non significant effect while photoperiod and exposure time had highly significant effect on the number of sprouting buds in Desiree and Ultimus. The interactive effect of potato genotypes and photoperiod was non-significant. Similar ANOVA result was also observed for interaction of potato genotypes and exposure period. On the other hand, the interactive effect of photoperiod and exposure time was significant on the number of sprouting buds. A non significant effect for the interaction of potato genotypes, photoperiod and exposure period was observed.

Reported results about the sprouting of potato buds on 1/2 strength plain MS medium (with no added growth hormones) under long photoperiods exactly coincide to those reported by Kaur *et al.* (2000). They compared 1/2 and full strength MS medium and found that shoot establishment was better in half rather than full strength basal medium, which confirm the present results. There are several reports for obtaining potato plantlets from buds in a hormone free medium (Ahsan *et al.* 2003; Mohammad, 2005; Ain, 2005; Nazar, 2005; Rafiq, 2005; Bibi, 2008; Aslam & Iqbal, 2010; Yasmin *et al.*, 2011b). This medium was good to support the growth of the plants, but was not capable of its further multiplication. Consequently, the mere presence of nutrients was insufficient for propagation in potato explants. However, the growth rate of the explant can be improved by supplementing the medium with growth hormones (Yousaf *et al.* 2001; Hoque, 2010). The present work confirmed the validity of previous ones. The need for the presence of growth hormones was considered essential under these circumstances. The majority of the excised plant tissues however require the addition of one or more growth factor in the medium to stimulate its further growth and development.

Conclusion

It could therefore, be gained from the above results that 1/2 strength MS basal medium without any growth regulators was enough for sprouting of the buds and supporting further growth of the plants, but it could not aid in shoot proliferation. The use of various growth regulators was therefore essential for shoot multiplication and its further growth. Furthermore long days (16 hr day) gave a better response towards sprouting of the buds and its further growth, than short days.

	1/2	4	16	2	70	
no buds	1/2	4	16	2	70	single leafy shoot up to 4cm long
no buds	1/2	4	16	2	95	The shoots increased in length with some branching & well developed root system
no buds	1/2	4	16	2	95	The shoots increased considerably in size having several nodes together with an increase in leaf size roots formed a dense mat.
no buds	1/2	4	2	3	45	Sprouting observed after the 6 th day. Each sprout represented by a tiny tiny stem.
no buds	1/2	4	2	2	50	Shoots up to 1cm long having no leaves.
no buds	1/2	4	2	3	65	Slight increase in sprouting noted. Shoot length increased having long internodes.
no buds	1/2	4	2	4	65	Shoots long and branched with long inter nodes and small leaves and a well developed root system.
no buds	Full strength	4	16	2	70	No sprouting observed.
no buds	Full strength	4	15	2	30	Slight rupture of the buds observed.
no buds	Full strength	4	16	2	33	Slight increase in the size of the buds noticed.
no buds	Full strength	4	16	4	25	No further increase in the length of the plants noticed.

Table 1. Effect of media concentration and photoperiod on sprouting of potato buds of the cultivar Desiree.

Explant source	MS medium strength	Sucrose %age	Photo period No. of light hrs/day	Culture period in weeks	Sprouting %age	Remarks
Potato buds	1/2	4	16	1	50	Sprouting observed within 3 days of culture. Each sprout measured up to 1cm in length and had a single root.
Potato buds	1/2	4	16	2	70	The sprouts developed into single leafy shoots up to 4cm long.
Potato buds	1/2	4	16	3	95	The shoots increased in length with some branching & well developed root system.
Potato buds	1/2	4	16	4	95	The shoots increased considerably in size having several nodes together with an increase in leaf size roots formed a dense mat.
Potato buds	1/2	4	8	1	45	Sprouting observed after the 6 th day. Each sprout represented by a thin tiny stem.
Potato buds	1/2	4	8	2	60	Shoots up to 1cm long having no leaves.
Potato buds	1/2	4	8	3	65	Slight increase in sprouting noted. Shoot length increased having long internodes.
Potato buds	1/2	4	8	4	65	Shoots long and etiolated with long inter nodes and small leaves and a well developed root system.
Potato buds	Full strength	4	16	1	-	No sprouting observed.
Potato buds	Full strength	4	16	2	30	Slight rupture of the buds observed.
Potato buds	Full strength	4	16	3	35	Slight increase in the size of the buds noticed.
Potato buds	Full strength	4	16	4	38	No further increase in the length of the plants noticed.

Table 2. Effect of media concentration and photoperiod on sprouting of potato buds of the cultivar Ultimea.

Ex plant source	MS medium strength	Sucrose %age	Photo period No. of light hrs/day	Culture period in weeks	Sprouting %age	Remarks
Potato buds	1/2	4	16	1	49	Each sprout very small having a tiny root.
Potato buds	1/2	4	16	2	65	Single leafy shoots observed measuring up to 4cm.
Potato buds	1/2	4	16	3	90	Shoots increased greatly in length having some branches and a well developed root system.
Potato buds	1/2	4	16	4	92	Considerable increase in shoot length was noticed with an increase in leaf size.
Potato buds	1/2	4	8	1	48	Each sprout small and thin.
Potato buds	1/2	4	8	2	59	Shoots with thin and elongated stems having no leaves have been observed.
Potato buds	1/2	4	8	3	62	Shoots with etiolated stems having very long internodes.
Potato buds	1/2	4	8	4	63	Slight increase in sprouting noticed. The shoots had etiolated stems with very long internodes and scale like leaves.
Potato buds	Full strength	4	16	1	-	No sprouting observed.
Potato buds	Full strength	4	16	2	25	Sprouts very small measuring up to 5mm in length.
Potato buds	Full strength	4	16	3	30	Plants measuring up to 1cm in length with some rooting observed.
Potato buds	Full strength	4	16	4	31	Plants did not increase much in size. Each plant had 2 – 3 nodes and small leaves.

Table 3. ANOVA of factors, potato cultivar, day length, duration in weeks and their interactive effect on the number of sprouting buds.

Source	Degree of freedom	Mean square values	Status
Potato cultivar (A)	1	1.81	NS
Day length (B)	1	120.76	**
A x B	1	0.76	NS
Duration in weeks (C)	3	84.31	**
A x C	3	0.47	NS
B x C	3	19.22	**
A x B x C	3	0.56	NS
Error	144	0.68	

* = Significant at 5% level of significance.

** = Significant up to 1% level of significance.

NS = Non significant.

Table 4. Mean values of potato cultivar, day length, duration in weeks and their interactive effect on the number of sprouting buds.

Cultivar (A)	Day length (B)	Duration in weeks (C)				
		1	2	3	4	
Desiree	Long day	A x B x C				A x B
		4.50	6.00	6.50	6.50	5.88
	Short day	5.00	7.00	9.50	9.50	7.75
Ultimus	Long day	4.80	5.90	6.20	6.30	5.80
	Short day	4.90	6.50	9.00	9.20	7.40
		A x C				A
Desiree		4.75	6.50	8.00	8.00	6.81
Ultimus		4.85	6.20	7.60	7.75	6.60
		B x C				B
	Long day	4.65 ^D	5.90 ^C	6.35 ^{BC}	6.40 ^{BC}	5.83 ^B
	Short day	4.95 ^D	6.75 ^B	9.25 ^A	9.35 ^A	7.58 ^A
C		4.80 ^C	6.35 ^B	7.80 ^A	7.88 ^A	

LSD values for

Duration in weeks (C) = 0.364

B x C = 0.515

At 5% level of significance

112448

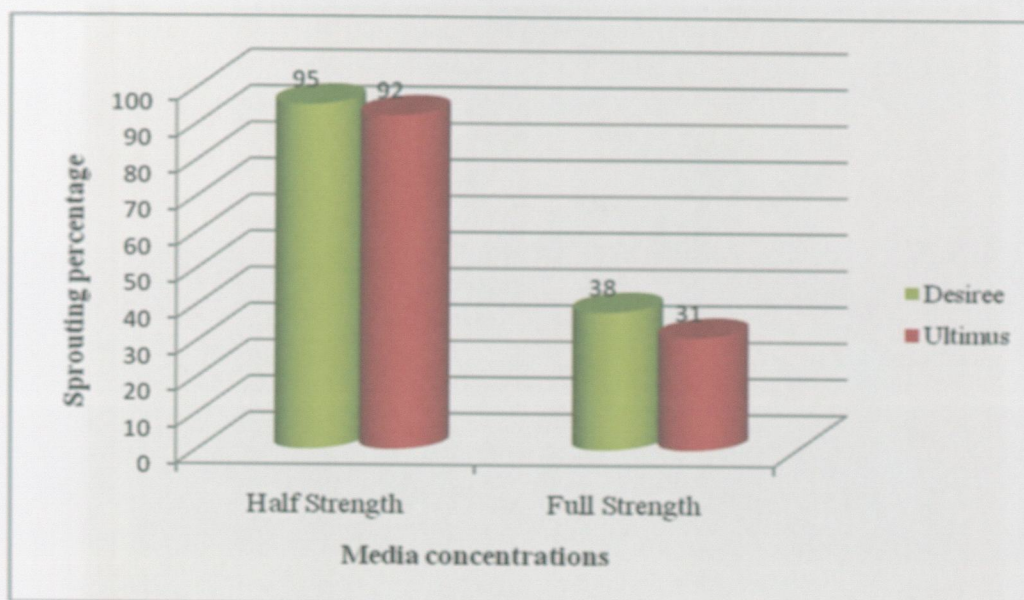


Fig. 4. Effect of media (MS) concentration on sprouting of potato buds of cvs. Desiree and Ultimus.



Fig. 5. Effect of photoperiod on sprouting of potato buds of cvs. Desiree and Ultimus.



a. Desiree

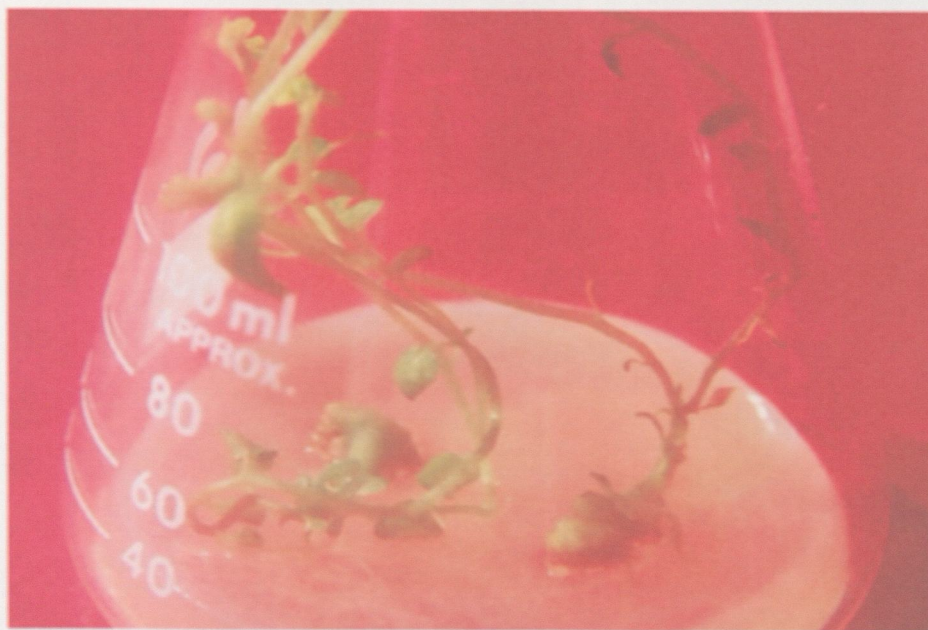


b. Ultimus

Figs. 6a, b. Sprouting of potato buds 3 weeks after inoculation on plain 1/2 strength MS medium.



a. Desiree



b. Ultimus

Figs. 7a, b. The plants at a later stage on plain $\frac{1}{2}$ MS medium.

Stage - II

Micropropagation

The *in vitro* plantlets obtained from potato buds in the above experiments were further used for micropropagation either through the use of:

1. Single node
2. Shoot tips
3. Stem segments having 3-4 nodes.

The effect of different growth hormones was tested in the propagation medium in the following experiments.

Growth of single node

Single node explants were obtained from stage-I. The large leaves were carefully removed. The removal of the leaves ensure uniform growth of the newly developing shoots. If large leaves are left on the single node pieces, it is believed that hormones from the remaining leaf can inhibit growth of the newly developing shoots. The axillary explants were then inoculated in the propagation medium.

Propagation media

The propagation medium consisted of MS basal medium at half strength with 4% sucrose and supplemented with:

- i. NAA
- ii. NAA + BAP
- iii. NAA + BAP + GA₃

1. Effect of NAA

Node explants from both the cultivars were inoculated in basal medium (MS) supplemented with 0.1 and 0.5 mg/l NAA and incubated at 25 °C under 16 hour light photoperiod. (Results are shown in Table 5 and 6, Fig. 8)

Stage – II Formation was observed within 3 days of culture in both the

Micropropagation

The *in vitro* plantlets obtained from potato buds in the above experiments were further used for micropropagation either through the use of:

1. Single node cuttings.
2. Shoot tips.
3. Stem segments having 3–4 nodes.

The effect of different growth hormones was tested in the propagation medium in the following experiments.

Growth of single node cuttings

Single nodes were excised from small *in vitro* plantlets obtained from stage-I. The large leaves were carefully removed. The removal of the leaves ensure uniform growth of the newly developing shoots. If large leaves are left on the single node pieces, it is believed that hormones from the senescing leaf can inhibit growth of the newly developing shoots. The nodal explants were then inoculated in the propagation medium.

Propagation medium

The propagation medium consisted of MS basal medium at half strength with 4% sucrose and supplemented with:

- i. NAA
- ii. NAA + BAP
- iii. NAA + BAP + GA₃

i. Effect of NAA

Nodal explants from both the cultivars were inoculated on-basal medium (1/2MS) supplemented with 0.1 and 0.5 mg/l NAA and incubated at 25 °C under 16 hour light photoperiod-(Results are shown in Table 5 and 6; Fig. 8)

Root formation was observed within 3 days of culture in both the experimental treatments in all the explants of both the cultivars. Roots proliferated very soon forming dense mats at both the concentrations within 2 weeks. However, shoot growth was very slow in these treatments as only a single shoot with a few nodes was obtained within a culture period of 4 weeks. NAA, when used alone in the medium was not enough for shoot proliferation. Therefore, BAP was added in combination with NAA in the medium. The ANOVA (Table 7 and 8) showed that the potato cultivar, NAA concentration and their interactive effect was non-significant.

These results are in line with those obtained by Muqarrab (1994) and Hussain (1995); whereas the findings of Sanavy & Moeinil (2003) and Moeinil *et al.* (2011) strongly negate our results. They reported a decrease in both shoot and root number and length.

ii. Effect of BAP and NAA

With the objective of multiple shoot formation, in another set of experiments, the excised nodal segments were sub cultured in the basal medium (1/2 MS) incorporated with varying concentrations of BAP in addition with varying levels of NAA (Table 5 and 6; Fig. 8). The results indicated that excellent multiple shoot together with root formation was achieved on medium supplemented with 0.5mg/l each of BAP and NAA within a culture period of 4 weeks (Figs. 11a and 11b). Moreover 100% sprouting of the nodes was noted in Desiree; whereas, 98% of the nodes sprouted in Ultimus. Somewhat similar results were also obtained on medium supplemented with 0.1mg/l NAA in combination with 0.5mg/l BAP (Figs. 12a and 12b). The sprouting rate of this set of experiments was 100% in both the cultivars. Some plantlets formation was also observed on BM (1/2 MS) supplemented with 0.1 mg/l each of NAA and BAP (Fig. 13a and 13b) and 0.5 mg/l NAA in combination with 0.1mg/l BAP but to a much lesser degree. Higher concentration of BAP, i.e., 0.5mg/l seems to be responsible for higher number of plantlets formed within these cultures. The ANOVA (Table 7 and 8) showed that BAP concentration had highly significant

effect on the sprouting percentage of nodal cuttings while interaction between cultivar and NAA concentration and BAP concentration was non-significant.

The plantlets thus obtained in these experiments for both the cultivars were healthy, with a number of lateral branches and a well developed root system. Each shoot had 10–12 nodes. When the culture period was prolonged up to eight weeks there was no subsequent increase in the number of plantlets, but further increase in the length of the plantlets together with some tuber formation (1–2 tubers / flask) was observed. The tubers thus produced were very small.

The plantlets produced in the above experiments were either transplanted after acclimatization into the field or were subcultured into tuber induction media. Moreover, these plantlets can further be used for propagation through repeated subcultures of the nodal segments.

Reported results of BAP and NAA being the best set of combination used for shoot proliferation exactly coincide to the findings of Ain (2005), Rafiq (2005) and Nazar (2005) where prolific shoot formation was observed in cvs Desiree and Cardinal on 1/2 MS medium supplemented with BAP and NAA. The results of other researcher on this subject are also similar to those of Hoque (2010) who reported that MS medium with 2mg/l kin and IAA is the best hormones concentration to multiple shoot and root regeneration. Yousaf *et al.* (2007) obtained maximum shoot length and node number on medium supplemented with 2 mg/l NAA and 0.5 mg/l BAP.

However, these results are in striking contrast to those obtained by Hussain (1995) and Moeinil *et al.* (2011), who observed that application of BAP and NAA decreased both shooting and rooting of single nodes. Negative effects of BAP and NAA on plant regeneration were also observed by Yasmin *et al.* (2011a). They obtained callus at the base of the explants. Different response of different potato varieties due to genetic make up towards *in vitro* shoot multiplication and their development were reported by Elaleem (2009) and Ghaffoor *et al.* (2003).

Effect of GA₃ in combination with BAP and NAA on shoot proliferation

The effect of GA₃ in promoting growth and development of *in vitro* derived shoot tips and axillary buds was studied in the cvs. Desiree and Ultimus. Different concentrations of GA₃ (0.2, 0.4, 0.6, 0.8 mg/l) were tested in combination with 0.1 mg/l NAA + 0.5 mg/l BAP and 4% sucrose. Single shoots developed from each shoot tip in the control medium without GA₃. The number however, increased to an average of 2.1 in Desiree and 1.9 in Ultimus (Table 9) within 4 weeks of culture.

The inclusion of GA₃ in the medium readily induced multiple shoot formation. The number of multiple shoots per shoot tip increased at 0.4 mg/l level of GA₃ in both the cultivars. Maximum number of shoots per shoot tip was obtained with this concentration within a culture period of 4 weeks (Table 9). With a further increase in the GA₃ levels a decline in the number of shoots occurred in both the cultivars (Fig. 9).

GA₃ also promoted axillary bud proliferation (Fig. 10). The Axillary buds sprouted within a week of culture, on medium supplemented with 0.4 mg/l GA₃ in both the cultivars (Figs. 14a and 14b).

The ANOVA (Table 10 and 11) showed that potato cultivar had non significant effect; while the interactions (cultivar x explants source, cultivar x GA₃ concentration) had significant effect on the number of shoots/explant while interaction effect of cultivar x explant source x GA₃ concentration was non significant.

These studies are in line with those obtained at CIP (2008). Similar results were also obtained by Al-Momani *et al.* (2000), Mohammad (2005), Ain (2005) and Rafiq (2005), who cultured shoot tips and nodal segments on medium supplemented with BAP and GA₃. Badoni & Chauhan (2009) obtained multiple plantlets from meristem tips on medium supplemented with NAA in combination with GA₃. Abbas & Abbas (2007) studied *in vitro* effects of GA₃ on morphogenesis of potato explants and obtained multiple plantlets. In agreement to the present studies, Hussain *et al.* (2006) also reported that higher concentration of GA₃ (2.0 mg/l) had no effect on the number of shoots.

Liquid shaken cultures

With the objective of obtaining a high frequency of plantlet formation, the *in vitro* raised plantlets from stage-I were cut into segments each with 4–5 nodes. The large leaves were removed and 2–3 segments were inoculated into 250 ml flasks containing 50 ml liquid 1/2 MS medium enriched with BAP and NAA at 0.5 and 0.1 mg/l, respectively. The cultures were kept under 16hrs photoperiod with a temperature of 25°C. The culture vessels were placed on an orbital shaker and shaken at 80 rpm to give the plants a better distribution of nutrients inside the flasks and to disturb the apical dominance of the main shoots.

After 3 weeks of culturing, the culture vessels were filled with vigorous plantlets having small leaves. The number of plantlets was higher in Desiree than in Ultimus. The plantlets thus obtained were further used to tuberize *in vitro* or transferred to non sterile conditions, i.e. transplanted into beds or pots for tuber production.

The results of Kaur *et al.* (2000) are in agreement with the present findings. Vigorous shoot multiplication was obtained in liquid rather than in solid medium. Similar results were also obtained by Mbiyu *et al.* (2012), who compared liquid and solid MS media. They found that liquid media yielded much healthier plants. Maximum number of shoots was observed on MS liquid medium containing GA₃ at 1.0 mg/l producing 15 shoots/flask in contrast 12 shoots/flask on solid medium (Hussain *et al.* 2006). Though promising results were obtained from these experiments but due to lack of facilities could not be performed on a large scale.

Table 5. Effect of different growth regulators on multiple shoot formation in potato cv. Desiree.

Explant source	MS 1/2 strength + growth regulator mg/l			Culture period in weeks	Sprouting percentage	Response noted
	NAA	BAP	GA ₃			
Nodal cuttings from <i>in vitro</i> plants	0.1	-	-	4	70	Growth of shoots was very slow with prolific root growth
	0.5	-	-	4	70	Single leafy shoots with a few nodes and dense mats of roots noted.
	0.1	0.1	-	4	80	Some multiple shoot formation observed
	0.5	0.1	-	4	80	Root growth much prolific than shoot.
	0.1	0.5	-	4	100	Excellent multiple shoot together with root formation observed.
	0.5	0.5	-	4	100	Multiple shoots with an extensive root system observed
	0.1	0.5	0.4	4	100	High rates of growth and shoot proliferation observed.

Table 6. Effect of different growth regulators on multiple shoot formation in potato cv. Ultimus.

Explant source	MS 1/2 strength + growth regulator mg/l			Culture period in weeks	Sprouting percentage	Response noted
	NAA	BAP	GA ₃			
Nodal cuttings from <i>in vitro</i> plants	0.1	-	-	4	68	Sprouting of the axillary buds observed, but shoot growth very slow. Root growth prolific.
	0.5	-	-	4	67	Single shoots together with root formation observed.
	0.1	0.1	-	4	75	Some multiple shoots observed
	0.5	0.1	-	4	78	Multiple shoot formation with prolific root growth observed.
	0.1	0.5	-	4	100	Prolific multiple shoot formation together with root formation.
	0.5	0.5	-	4	98	Multiple shoot formation with prolific root growth.
	0.1	0.5	0.4	4	100	Multiple shoot together with root formation observed.

Table 7. ANOVA of factors, potato cultivar, NAA concentration (mg/l), BAP concentration (mg/l) and their interactive effect on the sprouting percentage of nodal cuttings.

Source	Degree of freedom	Mean square values	Status
Potato cultivar (A)	1	1.63	NS
NAA concentration (B)	1	0.03	NS
A x B	1	0.03	NS
BAP concentration (C)	2	20.21	**
A x C	2	0.06	NS
B x C	2	79.31	NS
A x B x C	2	0.16	**
Error	108	0.61	

* = Significant at 5% level of significance

** = Significant up to 1% level of significance.

NS = Non significant.

Table 8. Mean values of potato cultivar, NAA concentration, BAP concentration and their interactive effect on the number of sprouting nodes.

Cultivar (A)	NAA conc. (mg/l) (B)	BAP Conc. (mg/l) (C)			
		0.1	0.5		
		A x B x C			A x B
Desiree	0.1	7.00	10.00	7.90	8.30
	0.5	8.10	7.00	10.00	8.37
Ultimus	0.1	6.80	10.00	7.50	8.10
	0.5	7.80	6.70	9.80	8.10
		A x C			A
Desiree		7.55	8.50	8.90	8.33
Ultimus		7.30	8.35	8.65	8.10
		B x C			B
	0.1	6.90 ^C	10.00 ^A	7.70 ^B	8.20
	0.5	7.95 ^B	6.85 ^C	9.90 ^A	8.23
C		7.43 ^C	8.43 ^B	8.80 ^A	

LSD values for

BAP conc. (mg/l). (C) = 0.345 B x C = 0.604

At 5% level of significance

A x B	1	3.15	*
CA, concentration (C)	2	4.56	**
A x C	2	3.59	**
B x C	4	6.07	**
A x B x C	4	0.41	NS
Error	180	0.66	

* = Significant at 5% level of significance

** = Significant up to 1% level of significance

NS = Non significant

Table 9. Effect of varying levels of GA₃ in combination with 0.1 mg/l NAA and 0.5 mg/l BAP on multiple shoot formation in explants of potato. Mean number of shoots / explant within 4 weeks culture period.

Cultivars	Explant source	GA ₃ mg/l				
		0	0.2	0.4	0.6	0.8
Desiree	Shoot tips	2.1	3.4	5.3	3.1	1.2
	Axillary	5.9	6.1	8.2	5.2	3.1
Ultimus	Shoot tips	1.9	3.2	5.2	4.1	2.3
	Axillary	4.8	5.8	7.9	5.7	3.2

Table 10. ANOVA of factors, potato cultivar, explants source (shoot tips & axillary buds), GA₃ concentration (mg/l and their interactive effect on the number of shoots /explant.

Source	Degree of freedom	Mean square values	Status
Potato cultivar (A)	1	0.13	NS
Explant source (B)	1	290.41	**
A x B	1	3.65	*
GA ₃ concentration (C)	4	94.56	**
A x C	4	3.59	**
B x C	4	6.07	**
A x B x C	4	0.41	NS
Error	180	0.66	

* = Significant at 5% level of significance

** = Significant up to 1% level of significance.

NS = Non significant.

Table 11. Mean values of potato cultivar, explant source (shoot tips & axillary buds), GA₃ concentration (mg/l) and their interactive effect on the number of sprouting buds.

Cultivar (A)	Explant source (B)	GA ₃ Conc. (mg/l) (C)					A x B
		0	0.2	0.4	0.6	0.8	
Desiree	Shoot tips	A x B x C					3.02B
		2.10H	3.40FG	5.30CD	3.10G	1.20I	
	Axillary buds	5.90BC	6.10B	8.20A	5.20CD	3.10G	5.70A
Ultimus	Shoot tips	1.90HI	3.20G	5.20CD	4.10EF	2.30H	3.34B
	Axillary buds	4.80DE	5.80BC	7.90A	5.70BC	3.20G	5.48A
		A x C					A
Desiree		4.00C	4.75B	6.75A	4.15C	2.15C	4.36
Ultimus		3.35D	4.50C	6.55A	4.90B	2.75E	4.41
		B x C					B
	Shoot tips	2.00E	3.30D	5.25E	3.60D	1.75E	3.18B
	Axillary buds	5.35C	5.95B	8.05A	5.45BC	3.15D	5.59A
C		3.68C	4.63B	6.65A	4.53B	2.45D	

LSD values for

Explants source (B) = 0.226

GA₃ concentration (C) = 0.358

A x B = 0.324

A x C = 0.507

B x C = 0.506

A x B x C = 0.716

At 5% level of significance

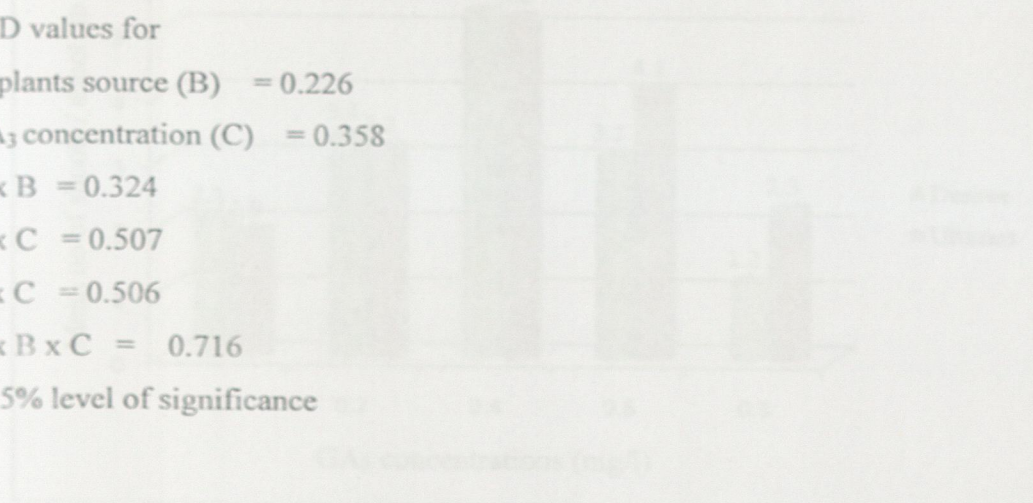


Fig. 9. Effect of GA₃ in combination with 0.1mg/l NAA + 0.5mg/l BAP on mean number of sprouting per shoot tip in potato cvs. Desiree and Ultimus.

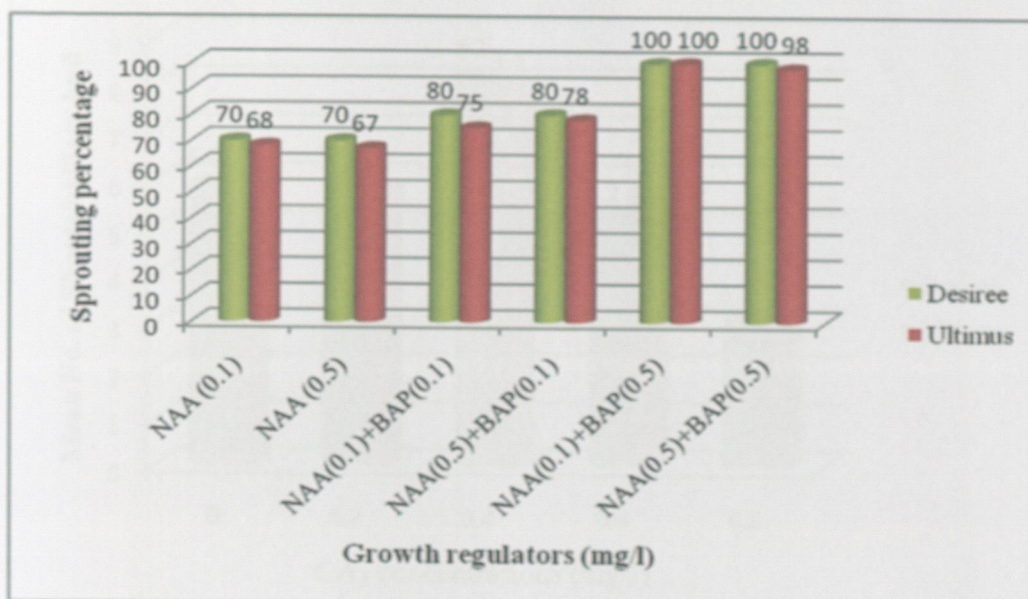


Fig. 8. Effect of NAA and NAA+ BAP at varying concentrations on the sprouting of nodal segments of potato cvs. Desiree and Ultimus.

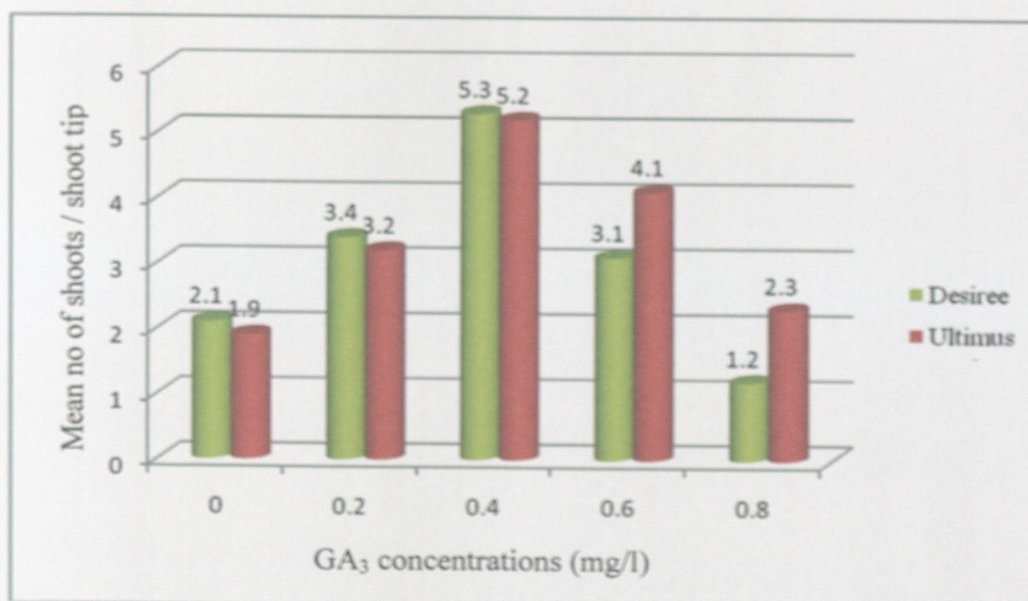


Fig. 9. Effect of GA₃ in combination with 0.1mg/l NAA + 0.5mg/l BAP on mean number of shoots per shoot tip in potato cvs. Desiree and Ultimus.

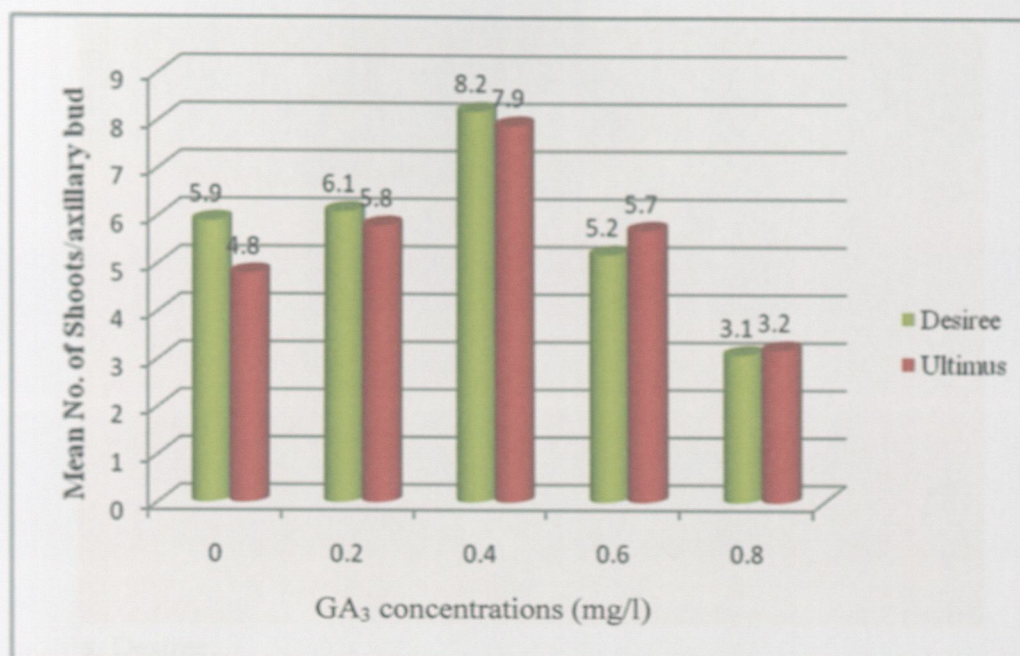
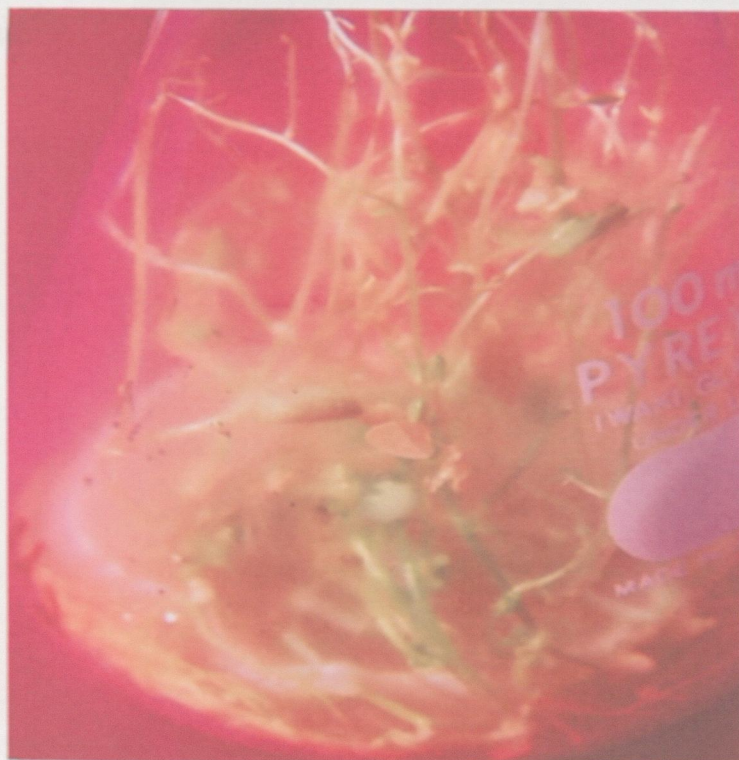


Fig. 10. Effect of GA₃ in combination with 0.1mg/l NAA + 0.5mg/l BAP mean number of shoots per axillary bud in potato cvs. Desiree and Ultimus.



a. Desiree



b. Ultimus

Figs. 11a, b. Multiple shoot formation on 1/2 strength MS supplemented with 0.5mg/l BAP

Figs. 11a, b. Multiple shoot formation on 1/2 strength MS basal medium supplemented with 0.5mg/l each of BAP and NAA.



a. Desiree



b. Ultimus

Figs. 12a, b. Multiple shoot formation on $\frac{1}{2}$ MS supplemented with 0.5mg/l BAP and 0.1mg/l NAA.



a. Desiree

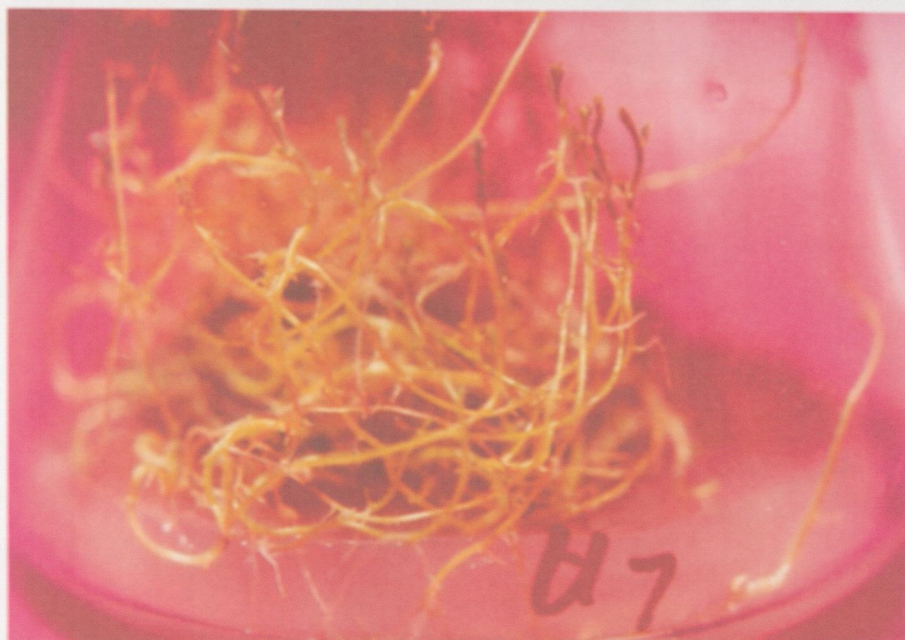


b. Ultimus

Figs. 13 a, b. Some shoot together with root formation on medium (1/2 MS) supplemented with 0.1mg/l each of BAP and NAA.



a. Desiree



b. Ultimus

Figs. 14a, b. Shoots arising from stem explants in the medium supplemented with 0.5mg/l BAP, 0.1mg/l NAA and 0.4mg/l GA₃.

Step – III

In Vitro Tuberization

In vitro tuberization is a highly complex morphogenic process consisting of sequential stages. An important feature of in vitro tuberization is the number of tubers per plant and their size.

The number and size of tubers are regulated by factors such as the genetic level (cultivar characteristics) and exogenous factors (growth regulators).

SECTION – III

The objective of this section is to study the effect of growth regulators on in vitro tuberization.

Several approaches have been made by different workers in order to improve tuber quality and the number of tubers per plant by changing the growth medium composition and light conditions (Kumar et al. 1998; Yu et al. 2000; Ganesan, 2004) and the addition of plant growth regulators (Ramesh et al. 2000; Ashish & Jyoti, 2001; Chandra Sekhara et al. 2004).

MICROTUBERIZATION

In the present studies several experiments were performed for production of large scale tuber crop in vitro. The in vitro plantlets were grown in MS medium supplemented with different concentrations of growth regulators. The effect of growth regulators on in vitro tubers was studied. The results are given in the following paragraphs.

Effect of different concentrations of growth regulators on in vitro tubers

Prior to study the effect of growth regulators on in vitro tubers, the effect of different concentrations of growth regulators on in vitro tubers was studied. The results are given in the following paragraphs.

In the first experiment, the effect of different concentrations of growth regulators on in vitro tubers was studied. The results are given in the following paragraphs.

Stage – III

In Vitro Tuberization

Potato tuberization is a highly complex morphogenetic process consisting of sequential stages. An important characteristic of the ultimate result of tuberization is the number of tubers per plant and their sizes.

The number and size of tubers are regulated at different levels the genetic level (cultivar characteristics) and organism level (donor acceptor relationships) etc. The system of hormonal regulation is assumed to be one of the mechanisms that determines the number and size of tubers.

Several approaches have been made by different workers in order to improve tuber quality and the number of tubers per plant by changing the culture medium components. e.g. high sucrose content (Xu *et al.* 1998; Yu *et al.* 2000, Carlson, 2004) and the addition of plant growth regulators, (Romanov *et al.* 2000; Aslam & Iqbal, 2010) manipulation of culture conditions e.g. temperature, light and photoperiod (Dobranszki 2001; Chang-Wen *et al.* 2004).

In the present studies several experiments were performed for production of large scale and good quality microtubers. The *in vitro* raised plants were subjected to tuberization media with varying concentrations of different growth regulators and also to different cultural conditions. Results are given in the following paragraphs.

Effect of different carbon sources on *in vitro* tuberization

Prior to study the effect of different growth regulators on *in vitro* tuberization, the effect of different sugars was tested on tuber induction. Different carbon sources, viz: sucrose and glucose at concentration ranging from 4–10% were tested in relation to tuberization process. The basal medium used was full strength MS medium and the cultures were kept under 8 and 16 hrs light (short and long day) conditions at a temperature of 25°C.

In the first set of experiments the *in vitro* plantlets from stage-II of both the cultivars were transferred to BM supplemented with either 4% sucrose or 4% glucose, without any growth regulators in the medium. The results obtained are

given in Table 12 and 13; Figs. 15-20. It is evident from the table that some tuber formation was observed at this concentration in both the cultivars (Figs. 21a and 21b). An average of 3.1 and 2.2 tubers/flask were obtained for Desiree and Ultimus, respectively under short days when sucrose was supplemented in the medium, within a culture period of 8 weeks (Figs. 15 and 16). Further increase in the culture period up to 10 weeks could not increase the tuber number. However a slight increase in tuber size was noticed in both the cultivars.

Slight tuberization could also be observed at 4% sucrose level under long day conditions after the 8th week of culture period in both the cultivars. In contrast to sucrose, glucose showed a very poor response towards tuberization when used at 4% level (Figs. 22a and 22b). Very little tuberization was observed only in the short days, with an average of 1.3 and 1.1 tubers/flask being produced in Desiree and Ultimus, respectively within a culture period of 8 weeks. Moreover the tuber size was also very small. No tuberization occurred at all in the long day conditions.

There was a gradual increase in the number of tubers as well as the size of the tubers with an increase in the concentration of both sucrose and glucose (Figs. 23a, b and 24a, b). Maximum number of tubers was obtained for both the cultivars under short days in 8% sucrose levels (Fig. 25a and 25b). An average of 6.3 tubers/flask was obtained for Desiree and 5.8 tubers/flask were produced in Ultimus within a culture period of 8 weeks. Maximum number of tubers obtained for glucose was also at the 8% concentration in the medium (Fig. 26). An average of 3.8 and 3.2 tubers/flask were obtained for Desiree and Ultimus respectively in short days. Long days did not exhibit a better response towards tuberization in both the cultivars and in both sucrose and glucose at all concentrations. Only one or some times 2 tubers were produced in some of the flasks. Further increase in the concentration of both sucrose and glucose was inhibitory for tuber induction (Table 12 and 13, Figs. 27a and 27b).

The ANOVA (Table 14-18) shows that both sucrose and glucose and the concentrations has highly significant effect on the number and weight of tubers in both the cultivars (Desiree and Ultimus), while interaction between varieties and

treatments were non-significant for number and weight of tubers in Desiree and highly significant in Ultimus.

Sucrose has been the carbohydrate source traditionally used for potato microtuber production. It has been added to the culture media at a variety of concentrations, depending on the method and media used to induce microtuber imitiation. As early as 1954 it was established that a high sucrose concentration (5% as opposed to 1%) favored tuberization (Mes & Menge, 1954). There have been several reports comparing the effects on microtuberization of a range of sucrose concentration.

Khuri & Moorby (1995) compared the effect of sucrose, glucose, maltose and fructose. They found that a medium supplemented with sucrose was more suitable for microtuberization than the ones with maltose, glucose or fructose. When glucose was supplied at concentration which had the same number of carbon atoms as 8% sucrose, the high osmolarity inhibited microtuberization. Sugar movement in the tubering plantlets was followed using radio-labeled sucrose, glucose and fructose. The sucrose was translocated and used at a faster rate than the other sugars, which tended to remain in the roots of the plantlets. It was therefore concluded that sucrose acts primarily as a suitable carbon source for uptake and utilization by the plantlets. Nazar (2005) also compared the effect of sucrose, glucose and fructose at varying levels (4, 6, 8 and 10%) and found sucrose to be the best carbon source for *in vitro* tuberization. In the present studies, it was also observed that sucrose served as a better carbon source compared to glucose. *In vitro* tuberization is highly dependent on sucrose concentration (Xu *et al.* 1998) and sucrose is known to induce several genes that are also induced in the potato tuber e.g. patatin, proteinase inhibitor II, and ADP-GLC pyrophosphorylase. Xu *et al.* (1998) reported much higher levels of GA₃ in the tips of stolons growing in the media with 1% sucrose, as opposed to 8% sucrose and suggested that sucrose can modulate endogenous GA₃ levels in the stolon tip. GA₃ can affect carbohydrate metabolism. GA₃ reduces the activity ADP-GLC pyrophosphorylase and thus starch synthesis but increase the activity

of UDPG pyrophosphorylase, an enzyme involved in the production of nucleotide sugars that can be used in cell wall synthesis.

In line with the present findings various other studies (Yu *et al.* 2000; Shibli *et al.* 2001; Rida *et al.* 2001) reported that high level of sucrose was beneficial in producing larger microtubers. Nawsheen (2001), Carlson (2004) and Sushrati *et al.* (2004) also reported best microtuber production at 10% sucrose level. El-Sawy *et al.* 2007 reported that sucrose is an important factor for microtuber formation. They achieved highest tuber formation when 12% sucrose was added to the culture medium, whereas from the studies of Kanwal *et al.* (2006) and Aslam *et al.* (2011) revealed that maximum tuber yield was obtained at 8% sucrose in the medium, which exactly correspond to the present results. Concentrations above 8% level proved detrimental for tuber induction.

Controversial results were however reported by Altindal & Karadogan (2010). They worked on 2 potato cultivars Agria and Justine using 2 different carbon sources (sucrose and maltose). Their results were cultivar based obtaining maximum tuber yield in Agria with 6% sucrose and in Justine with 4% maltose.

Table 13. Effect of different carbon sources on in vitro tuberization in cv Agria. Harvesting done after 10 weeks.

Conclusion

It could be gained from the present studies and from most of the literature reviewed that sucrose is a better carbon source in comparison to glucose as far as tuber yield is concerned both in terms of number and weight. Moreover it is much cheaper than glucose, maltose or fructose. Furthermore it could also be concluded from the above results that sugars when used alone in the medium did not gave a better tuber yield. The addition of growth regulators was therefore essential to obtain a higher yield.

Glucose	10	12	0.533	0.19
4	1.1	0.253	0.23	
6	1.8	0.432	0.24	
8	3.2	0.796	0.28	
10	1.4	0.352	0.18	

Table 12. Effect of different carbon sources on *in vitro* tuberization in cv Desiree. Harvesting done after 10 weeks.

Explants source	Carbon source percentage	Mean No. of tubers / flask	Mean wt. of tubers / flask (g)	Mean wt / tuber (g)
<i>In vitro</i> plants	Sucrose			
	4	3.1	0.775	0.25
	6	4.8	1.536	0.32
	8	6.3	2.268	0.36
	10	3.2	0.608	0.19
	Glucose			
	4	1.3	0.273	0.21
	6	2.1	0.462	0.22
	8	3.8	0.95	0.25
	10	2.0	0.34	0.17

Table 13. Effect of different carbon sources on *in vitro* tuberization in cv Ultimus. Harvesting done after 10 weeks.

Explants source	Carbon source percentage	Mean No. of tubers / flask	Mean wt. of tubers / flask (g)	Mean wt / tuber (g)
<i>In vitro</i> plants	Sucrose			
	4	2.2	0.572	0.26
	6	3.4	0.986	0.29
	8	5.8	1.914	0.33
	10	2.8	0.532	0.19
	Glucose			
	4	1.1	0.253	0.23
	6	1.8	0.432	0.24
	8	3.2	0.896	0.28
	10	1.4	0.252	0.18

Table 14. ANOVA of factors, sugar types, sugar concentration and their interactive effect on the number of tubers in Desiree (CI), weight of tubers in Desiree (CI) number of tubers in Ultimus (C2) and weight of tubers in Ultimus (C2).

Source	Degree of freedom	Mean square values			
		Number of tubers CI	Weight of tubers CI	Number of tuber C2	Weight of tubers C2
Sugar types (A)	1	84.05**	25.40**	59.51**	6.02**
Sugar conc. (B)	3	31.88**	10.92**	32.71**	4.48**
A x B	3	2.35NS	1.84NS	2.42**	0.58**
Error	72	0.87	1.80	0.49	0.03

* = Significant at 5% level of significance.

** = Significant up to 1% level of significance.

NS = Non significant.

Table 15. Mean values of sugar types, sugar concentration (%) and their interactive effect on the number of tubers in Desiree (CI)

Sugar types (A)	Sugar concentration (%) (B)				A
	4	6	8	10	
	A x B				
Sucrose	3.10	4.80	6.30	3.20	4.35A
Glucose	1.30	2.10	3.80	2.00	2.30B
SD values					
B	2.20C	3.45B	5.05A	2.60C	

LSD values for

Sugar types = 0.416

Sugar conc. = 0.589

At 5% level of significance

Table 16. Mean values of sugar types, sugar concentration and their interactive effect on the weight of tubers in Desiree (C1)

Sugar types (A)	Sugar conc. (%) (B)				A
	4	6	8	10	
	A x B				
Sucrose	1.54	2.68	3.25	1.17	2.16A
Glucose	0.51	0.94	1.85	0.84	1.03B
B	1.03B	1.81AB	2.55A	1.00B	

LSD values for

Sugar types = 0.598

Sugar conc. = 0.846

At 5% level of significance.

Table 17. Mean values of sugar types, sugar concentration and their interactive effect on the number of tubers in Ultimus (C2)

Sugar types (A)	Sugar conc. (B)				A
	1	2	3	4	
	A x B				
Sucrose	2.20CD	3.40B	5.90A	2.80BC	3.78A
Glucose	1.10E	1.70DE	3.20B	1.40E	1.85B
B	1.65D	2.55B	4.55A	2.10C	

LSD values for

Sugar types = 0.313

Sugar conc. = 0.443

A x B = 0.626

At 5% level of significance.

Table 18. Mean values of sugar types, sugar concentration and their interactive effect on the weight of tubers in Ultimus (C2)

Sugar types (A)	Sugar conc. (B)				A
	1	2	3	4	
	A x B				
Sucrose	0.775C	0.99B	1.94A	0.53CD	1.00A
Glucose	0.25E	0.41D	0.90B	0.25E	0.45B
B	0.41C	0.70B	1.41A	0.39C	

LSD values for

Sugar types = 0.705

Sugar conc. = 0.100

A x B = 0.141

At 5% level of significance.

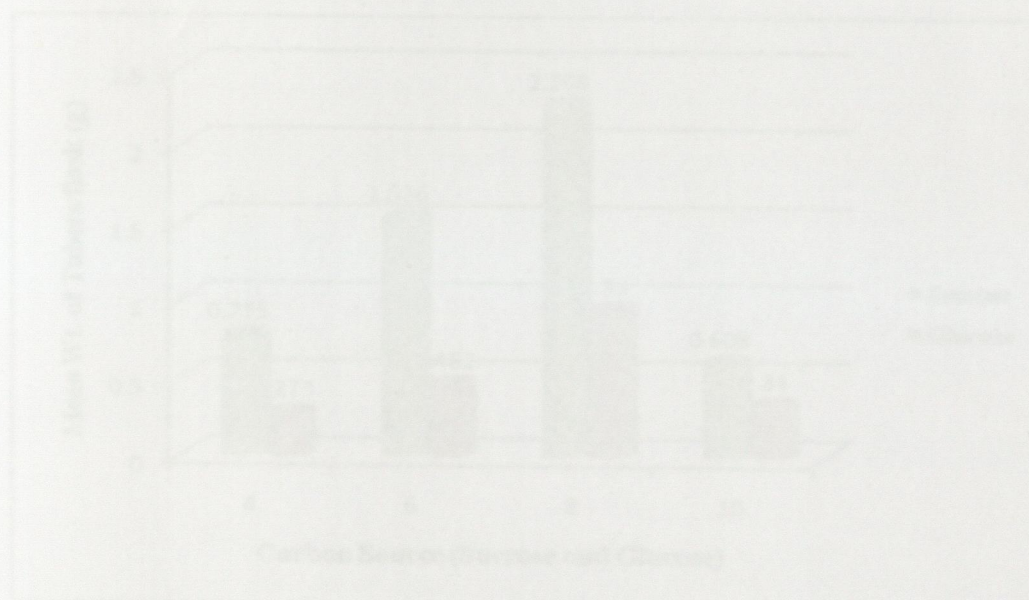


Fig. 18. Effect of different carbon sources on mean weight of tubers per flask in potato cv. Désirée.

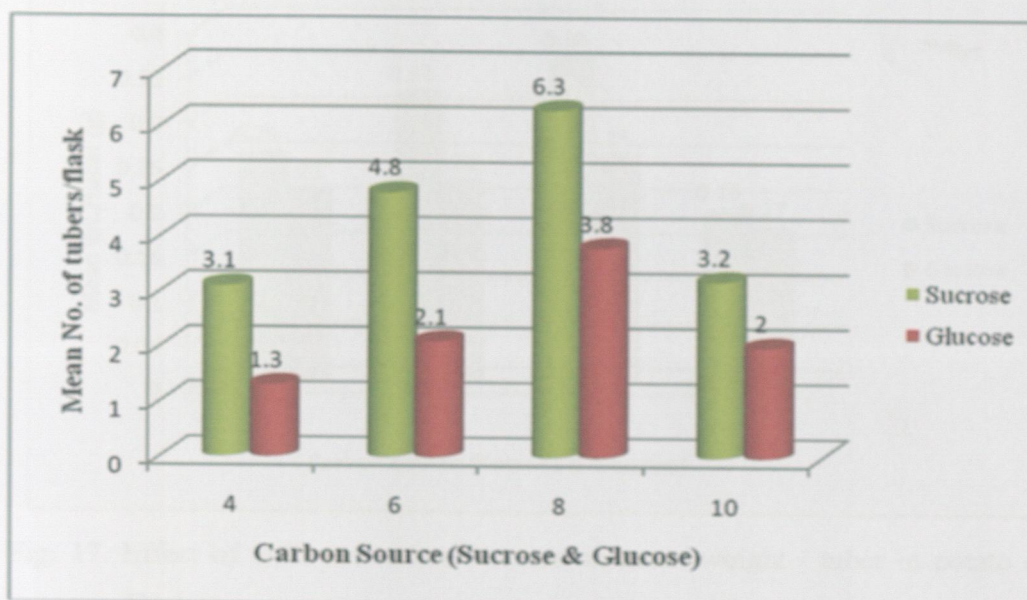


Fig. 15. Effect of different carbon sources on mean number of tubers per flask in potato cv. Desiree.

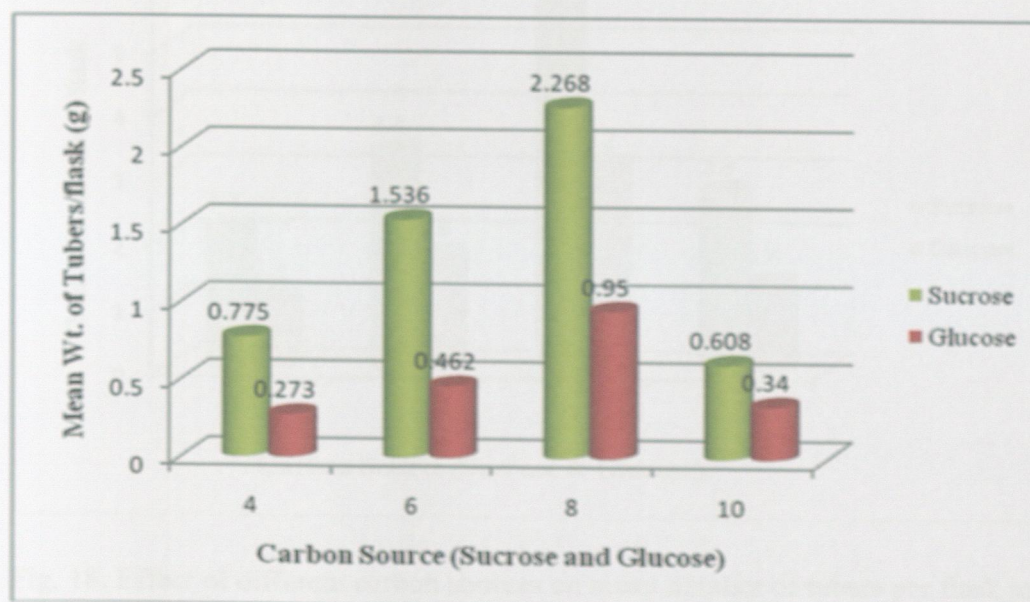


Fig. 16. Effect of different carbon sources on mean weight of tubers per flask in potato cv. Desiree.

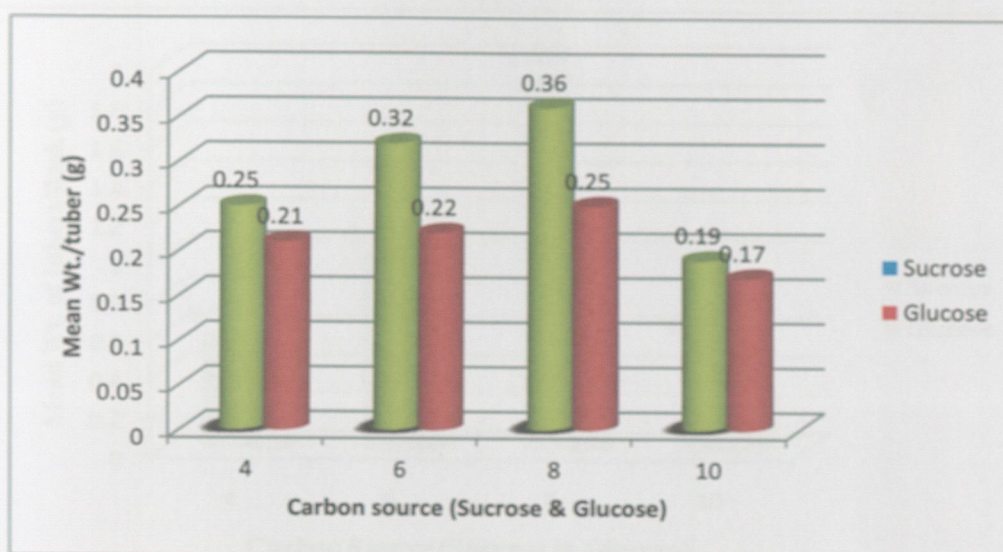


Fig. 17. Effect of different carbon sources on mean weight / tuber in potato cv Desiree.

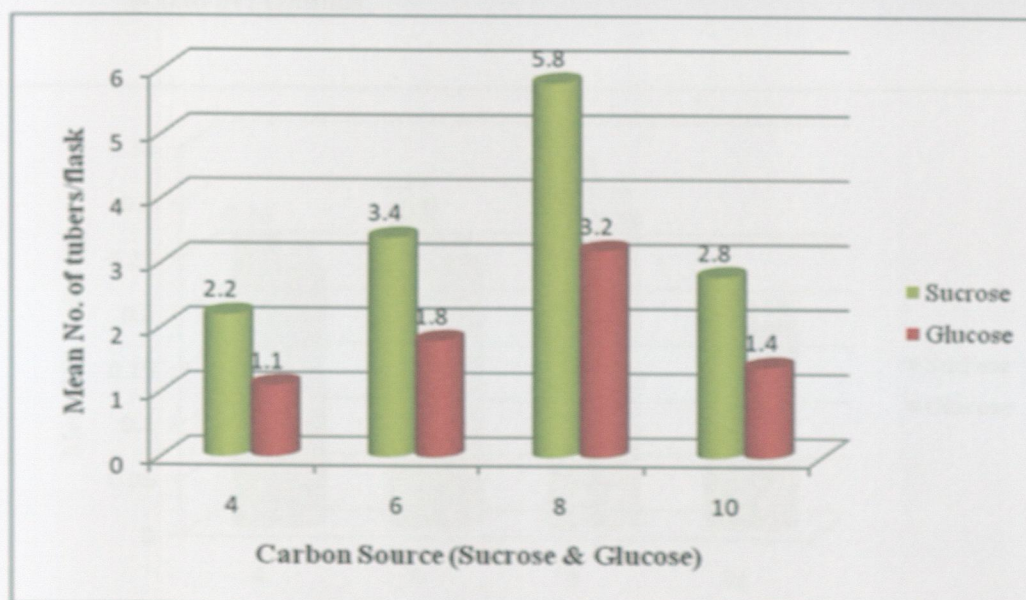


Fig. 18. Effect of different carbon sources on mean number of tubers per flask in potato cv. Ultimus.

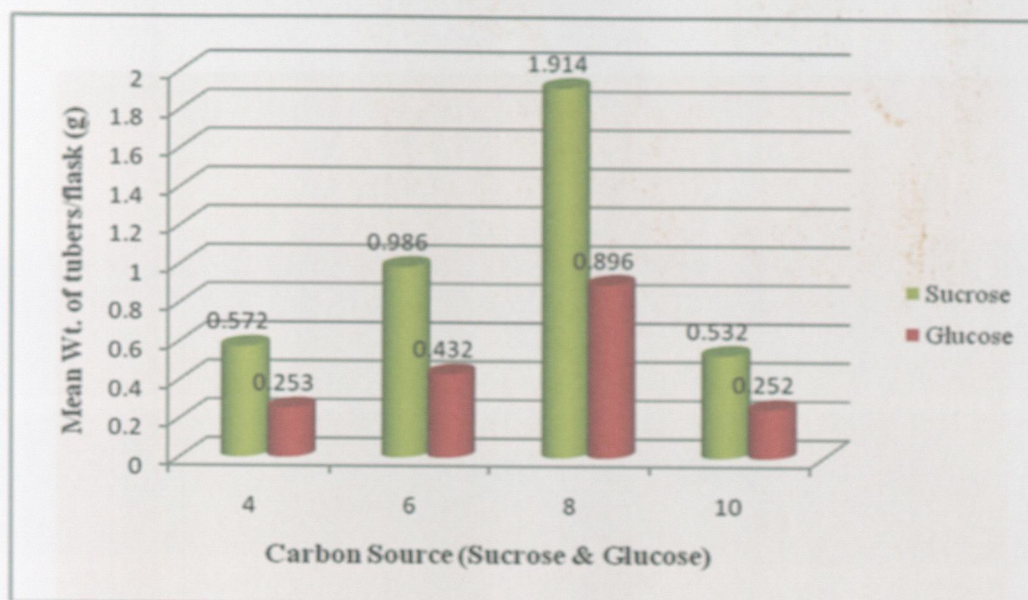


Fig 19. Effect of different carbon sources on mean weight of tubers per flask in potato cv. Ultimus.

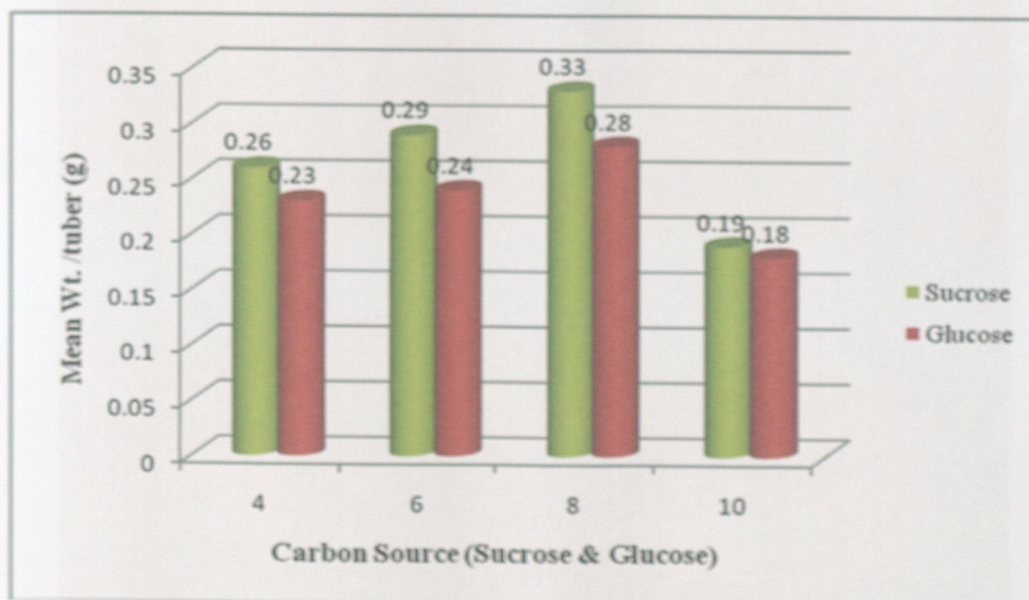
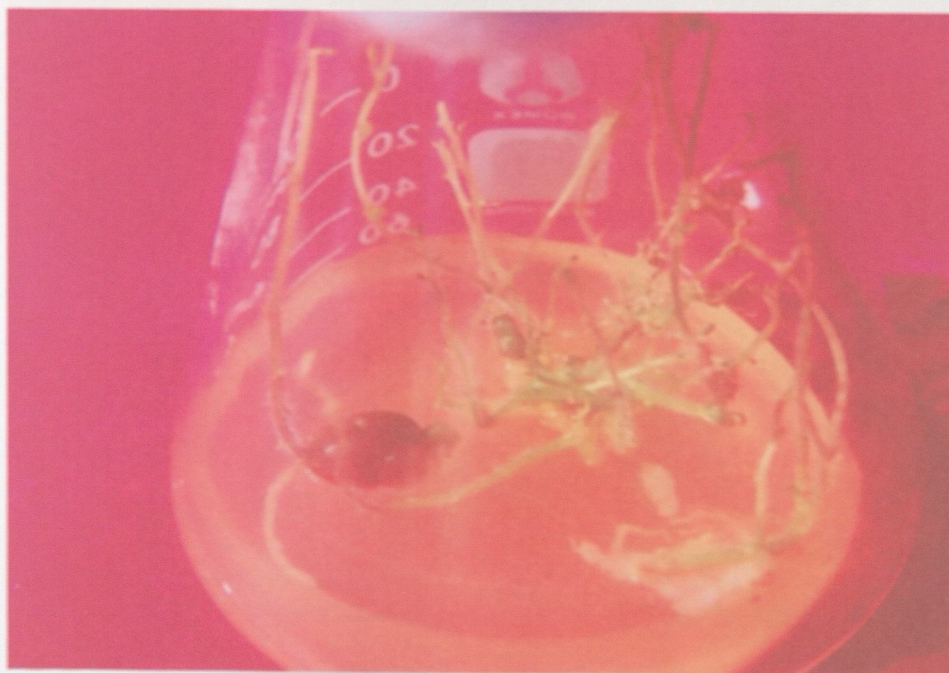


Fig. 20. Effect of different carbon sources on mean weight per tuber in potato cv. Ultimus.



a. Desiree



b. Ultimus

Figs. 21a, b. Tuberization in potato on medium supplemented with 4% sucrose under 8h photoperiod.



a. Desiree

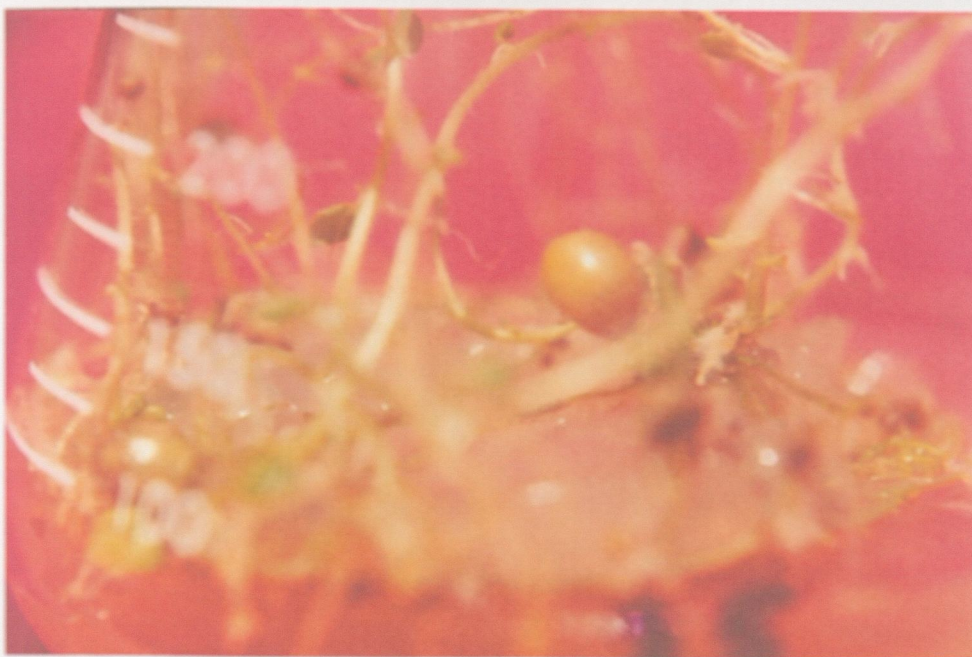


b. Ultimus

Figs. 22a, b. Tuberization in potato at 4% glucose level under short (8h) photoperiods.



a. Desiree

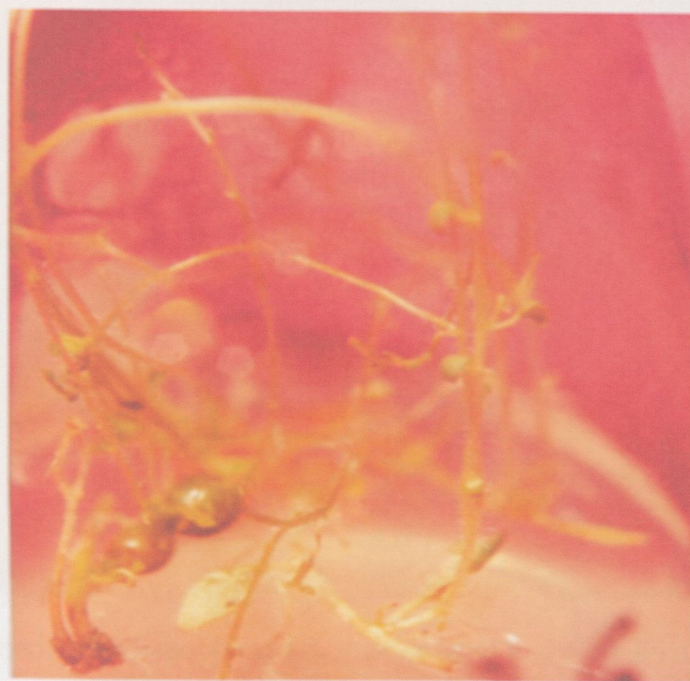


b. Ultimus

Figs. 23a, b. Tuberization in potato on medium supplemented with 6% sucrose under short days.



a. Desiree



b. Ultimus

Figs. 24a, b. Tuberization at 6% glucose level in the medium, under short days.



a. Desiree



Figs. 25a, b. Tuberization in potato at 8% sucrose level in the medium under short photo periods.



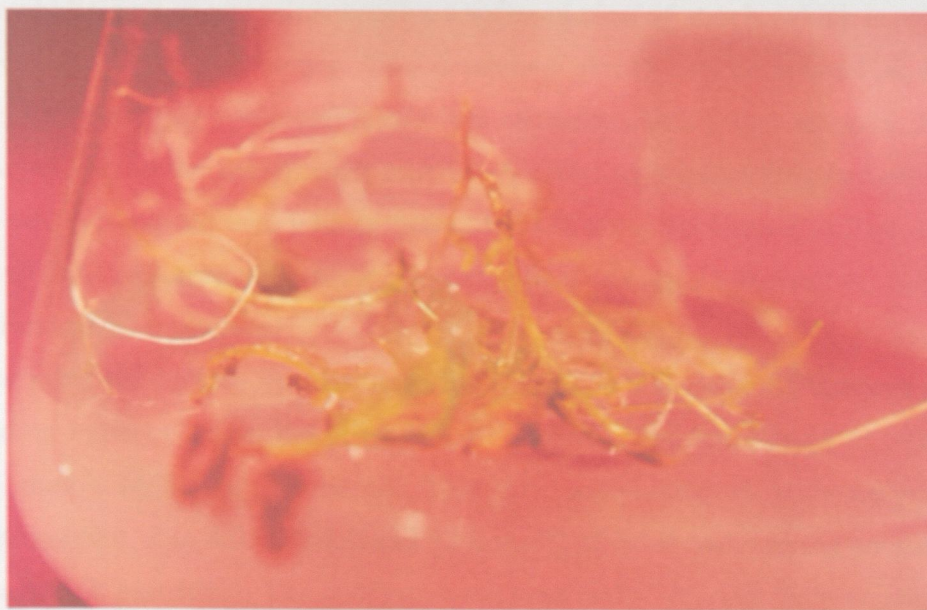
Figs. 26. Tuberization in potato cv Desiree at 8% glucose in the medium under short photo periods.

2. 1. 1970

Figs. 27a, b. Tuberization in potato cv Desiree at 8% glucose in the medium under 24 photo period.



a. Desiree



b. Ultimus

Figs. 27a, b. Tuberization in potato at 10% sucrose level in the medium under 8h photo period.

Effect of BAP, Sucrose and day lengths on *in vitro* tuberization

There are two aspects of tuber formation.

- a. The morphological development of a tuber and.
- b. The biochemical changes resulting in starch synthesis and storage (Xu *et al.* 1998).

The morphogenetic step consists of stolon elongation and tuber initiation. During the early stages of tuber formation the growth habit of stolons is altered, with sub-apical radial growth taking place instead of elongation. The first indication of *in vitro* tuber formation is thickening of an internode (Xu *et al.* 1998). The earliest changes associated with tuber formation are a marked increase in both radial expansion and number of mitotic cells in the sub apical stolon region together with the deposition of starch and protein.

The cytokinins stimulate starch metabolizing enzymes, thus creating a strong metabolic sink as a result subsequent accumulation of starch, which is seen as the swelling of the microtuber. This combined ability can be termed as an excessive substrate (high sucrose level) and stimulus (BAP) that triggers the enzymatic activity in the tuberization process. A series of experiments, (counting to a total of 24 combinations, with or without BAP) were performed to find out the effect of BAP, sucrose and varying day lengths on *in vitro* tuberization. Each combination had 10 replicates.

For tuber production the plantlets of cvs. Desiree and Ultimus, obtained in the preceding experiment on the propagation medium, were transferred to tuber induction media (MS. Full strength). This was supplemented with varying concentrations (0.0, 2.0, 4.0, 6.0, 8.0 and 10.0 mg/l) of BAP and varying sucrose levels (viz 4, 6, 8 and 10%). The cultivars were kept under varying, light regimes i.e. 8 and 16 hour fluorescent light in 24 hours cycle and in complete darkness.

Each plantlet attaining a length of up to 7–8 cm having 6–7 nodes each, was transferred to 250ml capacity flasks containing 100ml of the tuberization medium. Each flask was inoculated with two plantlets.

Some tuber formation was observed in the cv. Desiree in MS medium (with 0 level or without added cytokinins) supplemented with 8% sucrose in 8 hours day length. Tuberization also occurred in the cv. Ultimus within a culture period of 8 weeks (Table 19 and Figs. 25a and 25b).

In long days, the Axillary buds at the nodes grew into leafy shoots. Very few tubers were formed on shoots which grew out of the axils of the lower nodes of the cultured shoots in the both the cultivars. The largest number of tubers was produced after 8 weeks under short day (8 hours light) condition.

The lower sucrose levels, i.e., 4 and 6% without any BAP in the medium however failed to give promising results in both the cultivars as regards tuberization. Tuber formation was observed after the 8th week of culture period and the number of tubers recorded after the 10th week of culture period was very low (Table 19; Figs. 28-33). Moreover, the tuber size was also very small. The response of Desiree was slightly better compared to Ultimus. An average of 3.1 and 4.8 tubers were formed per flask in the 4 and 6% levels respectively in the cv. Desiree and 2.2 and 3.4 in Ultimus.

The plantlets which are produced in long days (16 hours) when cultured in complete darkness did not exhibit good results. Negligible amount of tuberization was observed only at 8% sucrose level in both the cultivars. The stolons arising from the plants were etiolated with scale like leaves and were of a pale color. The tubers thus produced on these shoots were very small, rounded with a reddish skin and hardly had a single eye (Fig. 34).

The ANOVA results (Table 20) showed that the number and weight of tuber significantly varies between potato cultivars (Desiree & Ultimus). Sugar concentration and photoperiod also significantly affected both the number and weight of tubers. The interactive effect of cultivar and sugar concentration were non-significant for both number and weight of tubers. The interactive effect of cultivars & photoperiod and sugar concentration and photoperiod were significant on number and weight of tubers. The total interactive effect of cultivars, sugar concentration and photoperiod was nonsignificant for number of tubers while highly significant for weight of tubers.

Cultures kept under 16 hour's photoperiod were unable to produce microtubers (Hussain *et al.* 2006). During incubation under light GA_3 is synthesized which inhibit tuber induction, while darkness enhances tuberonic acid; a glucoside involved in tuber induction (Alisdair & Willmitzer, 2001). Donnelly *et al.* (2003) have also demonstrated that microtuberization efficiency was increased by short days exposure or continuous darkness during culture condition (Hussain *et al.* 2006). In line with the present findings Pruski (2001) obtained better production of microtubers under 8hr photoperiod in comparison to no light. Moreover these microtubers performed better in the field than the one's produced in darkness.

The above results indicated that sucrose when used alone in the basal medium i.e., without any growth regulators did not have a profound effect on tuberization. BAP was, therefore, incorporated in the basal medium in further experiments. The experiments were carried out under 2 varying photoperiods (8 and 16hr days) because complete darkness was not beneficial for tuber formation.

The effect of BAP at 2.0, 4.0, 6.0, 8.0 and 10.0mg/l in both 8 and 16hr day lengths and at each of the four levels of sucrose, i.e. 4, 6, 8 and 10% was tested on both Desiree and Ultimus. Results given in Tables 23 and 24; Figs. 35-42.

The ANOVA (Tables 25 to 30) showed that the sugar concentration, BAP concentration and day length has highly significant effect on the number and weight of tubers. The interaction of sugar concentration $\times$ photoperiod + BAP $\times$ photoperiod had significant effect on the number and weight of tubers in both Desiree and Ultimus. The interactive effect of (sugar concentration $\times$ BAP concentration + photoperiod on the number and weight of tubers was non significant in both the cultivars.

In the first set of experiments, BAP level was kept constant at 2.0 mg/l while that of sucrose increased from 4-10% level. As evident from the Tables 23 and 24, reasonable amount of shoots were observed in both the cultivars on BM (full strength MS) supplemented with 2 mg/l BAP in combination with either 4 or 6% sucrose in 16hr days within a culture period of 4 weeks. Tuberization

the sucrose level up to 10% in the medium was not of much benefit, but was rather detrimental as the tuber number greatly decreased in most of the cultures (Tables 23 and 24; Figs 35-42).

In another set of experiments BAP level was increased to 4.0 mg/l in combination with varying levels of sucrose (4, 6, 8 & 10%). With this concentration of BAP in combination with 6% sucrose the upright leafy shoots were transformed into horizontal stolons, within 5 weeks of culture and root formation was inhibited. The stolons developed tubers a little earlier i.e., after the 5th week of culture more so in the short than in the long days. The response of both the cultivars was the same towards tuberization (Figs. 48a and 48b). An average of 9.8 tubers/flask were obtained in Desiree and 7.5 tubers/flask in Ultimus within a culture period of 10 weeks after which no further increase in tuber number could be observed. A further increase in tuber number together with an increase in tuber size was noticed when sucrose level was increased to 8% in combination with 4mg/l BAP (Tables 23 and 24).

Moreover with an increase in the BAP level up to 6.0 mg/l in combination with 6% sucrose, a further increase in tuberizing capacity for both the cultivars was observed (Figs. 49a and 49b) when grown under short days. Some very dramatic results were obtained when the sucrose level was raised to 8% in combination with 6 mg/l BAP particularly in Desiree. The tuber number increased in both the cultivars together with an increase in size (Figs. 50a and 50b). Very exotic tubers were obtained in some of the cultures of Desiree at this combination (6.0 mg/l BAP + 8% sucrose) (Figs. 51 and 52). The microtubers were very large measuring up to 2.2cm in length with a weight of 2.5gms. They had several eyes that sprouted readily when transferred to plain MS medium and produced more tubers (Figs. 53 and 54).

BAP (8.0 mg/l) in combination with the varying levels of sucrose (4, 6, 8 & 10%) further increased the tuber number in short day lengths (Figs. 55a, 55b and 56). In contrast to short days, upright shoot growth was observed in long photoperiods, on medium supplemented with 8.0 mg/l BAP and 8% sucrose (Figs.

57a and 57b). Comparatively a much lesser number of tubers were observed under these cultural conditions.

The trend of increase in tuber number with BAP concentration maintained itself up to 10mg/l levels. In these sets of experiments BAP level was kept constant at 10mg/l, while that of sucrose increased from 4–10%. As it is clear from the table at lower sucrose levels i.e., 4 and 6% the number of tubers/flask was 8.3 and 16.2 respectively in Desiree and 4.8 and 13.4 in Ultimus in the short day cultures. In the long day cultures the number of tubers/flask was much smaller.

The maximum number of tuber formation occurred on medium supplemented with 10mg/l BAP in combination with 8% sucrose. An average of 25.3 tubers/flask was produced in Desiree and 21.2 tubers/flask in Ultimus (Figs. 58a and 58b). A further increase in sucrose level, was inhibitory for tuber production, because the average number of tubers produced/flask was greatly decreased in both the cultivars when sucrose level was increased to 10% (Figs. 59a and 59b). Moreover, these results revealed that higher level of BAP increased the number of tubers / flask whereas the weight / tuber were slightly reduced.

In the present studies the addition of BAP in the medium together with sucrose increased both tuber number and weight. Maximum numbers of tubers were obtained at 10mg/l level of BAP, whereas maximum tuber weight was obtained at 6mg/l of BAP in combination with 8% sucrose. Because cytokinins interfere cell division, induction and production of *in vitro* microtubers are greatly increased. For stolon initiation and elongation, low cytokinin level is necessary but tuber initiation could occur if the gibberellin to ethylene ratio is low and also if their levels remain low but the levels of the cytokinins increases (Van den Berg & Ewing, 1991).

BAP as a GA inhibitor has been used in varying concentration from 2mg/l to 10mg/l and due to its GA inhibitory role and the presence of BAP canalize all the resources of plants towards tuberization i.e. anabolic activity rather than elongation of stem (Simko, 1994). Simko, 1994; Banfalvi *et al.* (1997) studied the effect of sucrose in the presence or absence of BAP on tuberization. They

observed that tuberization and stolon elongation depended on the level of sucrose in the medium. BAP enhanced the efficiency of tuber formation only at sucrose concentration above 4%.

Exogenous supplementation of cytokinins and cytokinin like compounds in microtuber growth media are gaining much attention for obtaining higher yields (Shibli *et al.* 2001). Kefi *et al.* (2000); Aksenova *et al.* (2000), and Mogollon *et al.* (2000) also reported that cytokinins and sucrose at high concentration stimulated tuber initiation. Zakaria *et al.* (2008) obtained earliest microtuberization at 10 mg/l of BAP. Aslam & Iqbal (2010) and Aslam *et al.* (2011) also obtained good results on medium supplemented with BAP 6.0mg/l and 6% sucrose for Desiree and 5.0 mg/l BAP and 8% sucrose for cvs Cardinal and Diamant.

The present findings exactly correspond to those of the previous ones. Rafique *et al.* (2004) obtained maximum number of microtubers on MS medium supplemented with 6% sucrose and 1.4 μ M BAP. Similarly, Kanwal *et al.* (2006) obtained maximum number of microtubers with a maximum fresh weight (0.442g) at a very low concentration of BAP in the cv. Kuroda.

Tuber formation started after 3 weeks when medium contained BAP at higher concentration (3.0-5.0 mg/l) under dark conditions. Maximum tuber yield was obtained at 4.0 mg/l BAP in the medium (Hussain *et al.* 2006). Donnelly *et al.* (2003) reported that growth regulators (BAP, 2iP, Kn and NAA) could induce microtubers in potato *in vitro* but in some cases the effect of growth regulators was found to be genotype dependent.

In striking contrast to the above results, Imani *et al.* (2010) reported that 6% sucrose in addition to 15 g/l BAP gave maximum number of microtubers. This infact is a very high concentration of BAP. Cultivars have a different potential in production of microtuber (Al-Safadi *et al.* 2000). Differences between varieties have also been reported by Nistor *et al.* (2010). It seems that under similar conditions, genotypic potential of cultivar has the greatest effect and will result in different yields because of innate capacity of the genotypes in production of endogenous levels of growth regulators.

Conclusions

It can be concluded from these results that BAP together with high sucrose content are best for *in vitro* tuberization. However the concentration of BAP varied greatly among the different cultivars which show that microtuberization of potato is genotype dependent also.

Table 19. Effect of varying levels of sucrose and varying day lengths on *in vitro* tuberization in potato.

CVs	Sucrose %age	Mean number of tubers/flask			Mean weight of tubers / flask g			Mean weight / tuber		
		LD	SD	Dark	LD	SD	Dark	LD	SD	Dark
Desiree	4	1.8	3.1	0.5	0.162	0.775	0.03	0.09	0.25	0.06
	6	2.0	4.8	0.9	0.22	1.536	0.063	0.11	0.32	0.07
	8	2.2	6.3	1.0	0.286	2.268	0.07	0.13	0.36	0.07
	10	1.2	3.2	0.3	0.096	0.608	0.015	0.08	0.19	0.05
Ultimus	4	1.3	2.2	0.4	0.104	0.0572	0.46	0.08	0.26	0.06
	6	1.7	3.4	0.6	0.204	0.986	0.042	0.12	0.29	0.07
	8	1.8	5.8	1.2	0.216	1.914	0.096	0.12	0.33	0.08
	10	0.9	2.8	0.1	0.072	0.532	0.006	0.08	0.19	0.06

Table 20. ANOVA of factors, Potato cultivar, sugar concentration, day length and their interactive effect on the number of tubers and weight of tubers.

Source	Degree of freedom	Mean square values	
		Number of tubers	Weight of tubers
Potato cultivar (A)	1	11.704**	0.93**
Sugar Conc. (B)	3	33.35**	4.14**
A x B	3	0.77NS	0.07NS
Day length (C)	2	230.87**	28.77**
A x C	2	1.87*	0.44**
B x C	6	10.59**	2.87**
A x B x C	6	0.38NS	0.08**
Error	216	0.55	0.03

* = Significant at 5% level of significance.

** = Significant up to 1% level of significance.

NS = Non significant

Table 21. Mean values of potato cultivar, sugar concentration, day length and their interactive effect on the weight of tubers

Cultivar (A)	Sugar (%) B	Day length (C)			
		Long day	Short day	Dark	
A x B x C					A x B
Desiree	4	0.16GHIJ	0.76E	0.15GIJK	0.36
	6	0.22GH	1.54C	0.07IJK	0.61
	8	0.29G	2.27A	0.07IJK	0.88
	10	0.10HIJK	0.61F	0.02JK	0.24
Ultimus	4	0.10HIJK	0.57F	0.02JK	0.23
	6	0.20GHI	0.99D	0.04JK	0.41
	8	0.22GHI	1.91B	0.10HIJK	0.74
	10	0.07IJK	0.53F	0.01K	0.20
A X C					A
Desiree		0.19C	1.30A	0.08DE	0.52A
Ultimus		0.15CD	1.00B	0.04E	0.40B
B x C					B
	4	0.13EF	0.67C	0.09FG	0.30C
	6	0.21DE	1.26B	0.06FG	0.51B
	8	0.25D	2.09A	0.08FG	0.81A
	10	0.08FG	0.57C	0.01G	0.22D
C		0.17B	1.15A	0.06C	

LSD value for

Sugar Conc. (B) = 0.602

Day length (C) = 0.521

A x C = 0.074

B x C level of significance = 0.104

A x B x C = 0.148

At 5% level of significance.

Table 22. Mean values of Potato cultivar, sugar concentration, day length and their interactive effect on the number of tubers.

Day length (C)					
Cultivar (A)	Sugar conc. (%) B	Long day	Short day	Dark	A x B
		A x B x C			
Desiree	4	1.80	3.10	0.80	1.90
	6	2.00	4.80	0.90	2.57
	8	2.20	6.30	1.00	3.17
	10	1.20	3.20	0.30	1.57
Ultimus	4	1.30	2.20	0.40	1.30
	6	1.70	3.40	0.60	1.90
	8	1.80	5.90	1.20	2.97
	10	0.90	2.80	0.10	1.27
		A X C			A
Desiree		1.80C	4.35A	0.75E	2.30A
Ultimus		1.43D	3.58B	0.58E	1.86B
		B x C			B
	4	1.55DE	2.65C	0.60GH	1.60C
	6	1.85D	4.10B	0.75FG	2.23B
	8	2.00D	6.10A	1.10EF	3.07A
	10	1.05FG	3.00C	0.20H	1.42C
C		1.61B	3.96A	0.66C	

LSD value for

Sugar Conc. (B) = 0.266 A x C = 0.326

Day length (C) = 0.231 B x C = 0.461

At 5% level of significance.

Fig. 23. Effect of varying sucrose levels and different photoperiods on mean weight of tubers per flask in potato cv. Desiree.

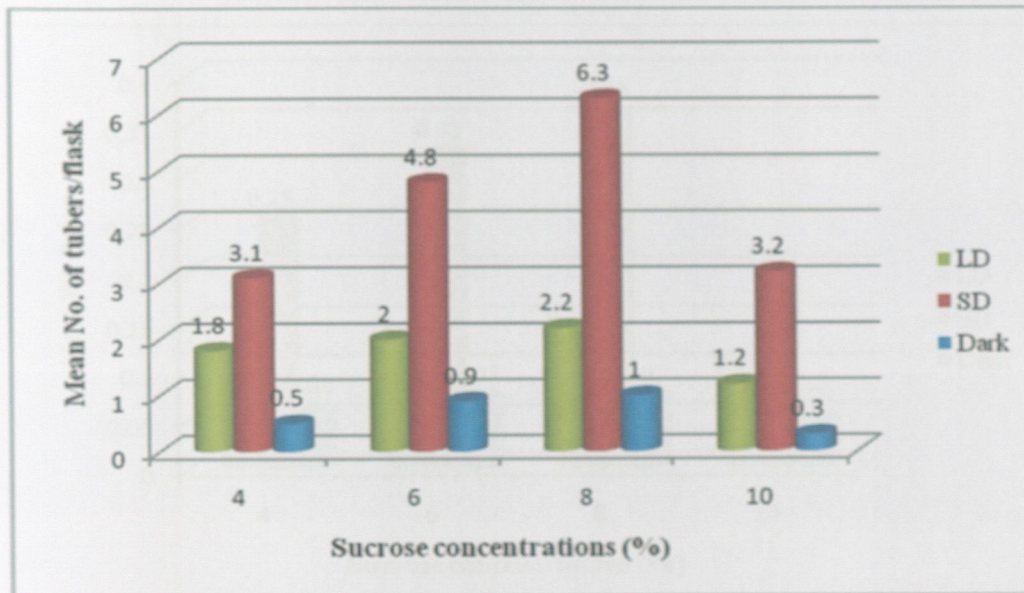


Fig. 28. Effect of varying sucrose levels and different photoperiods on mean number of tubers per flask in potato cv. Desiree.

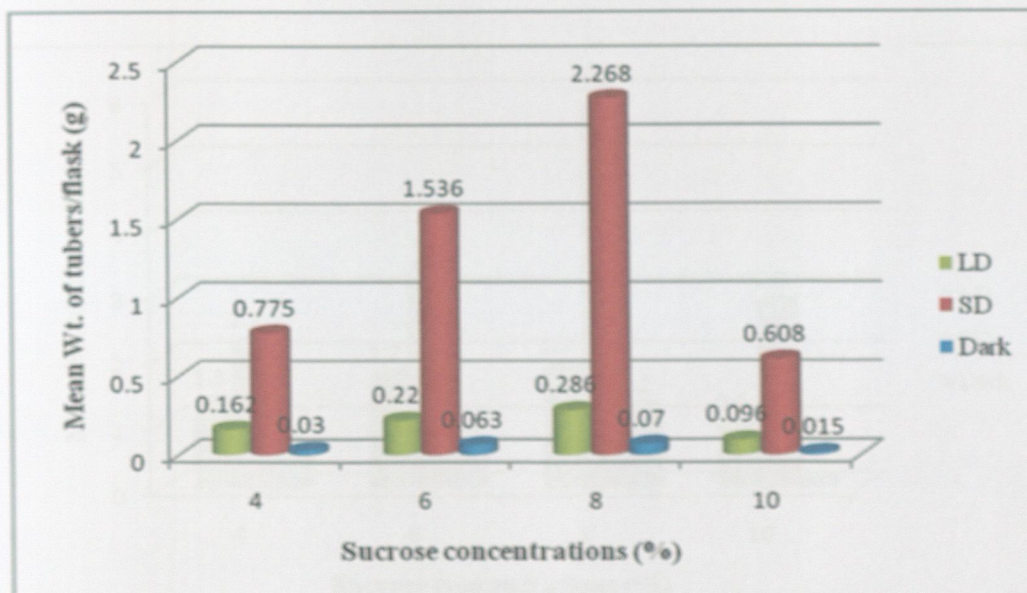


Fig. 29. Effect of varying sucrose levels and different photoperiods on mean weight of tubers per flask in potato cv. Desiree.

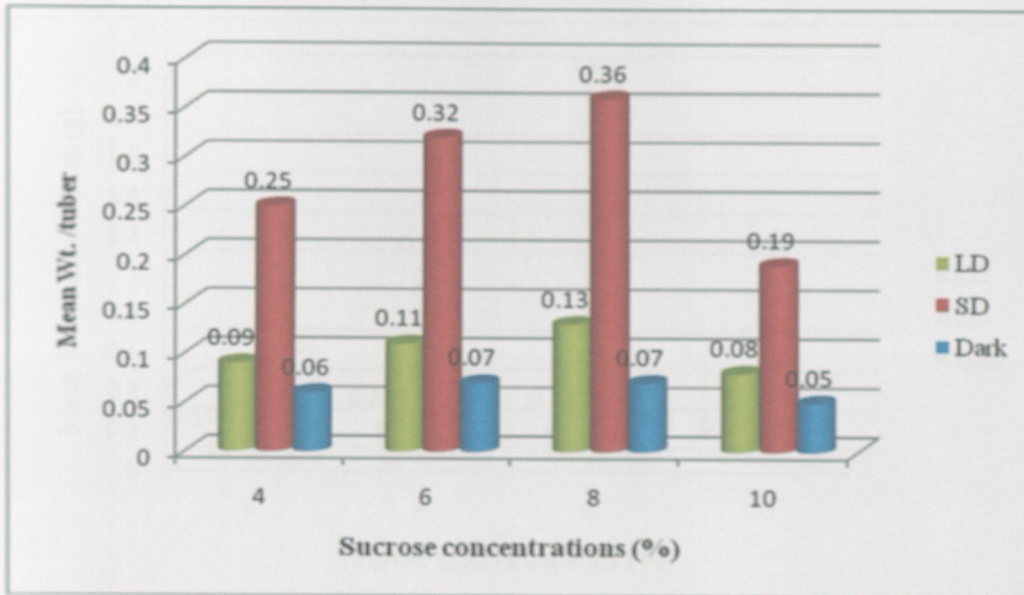


Fig. 30. Effect of varying sucrose levels and different photoperiods on mean weight per tuber in potato cv. Desiree.

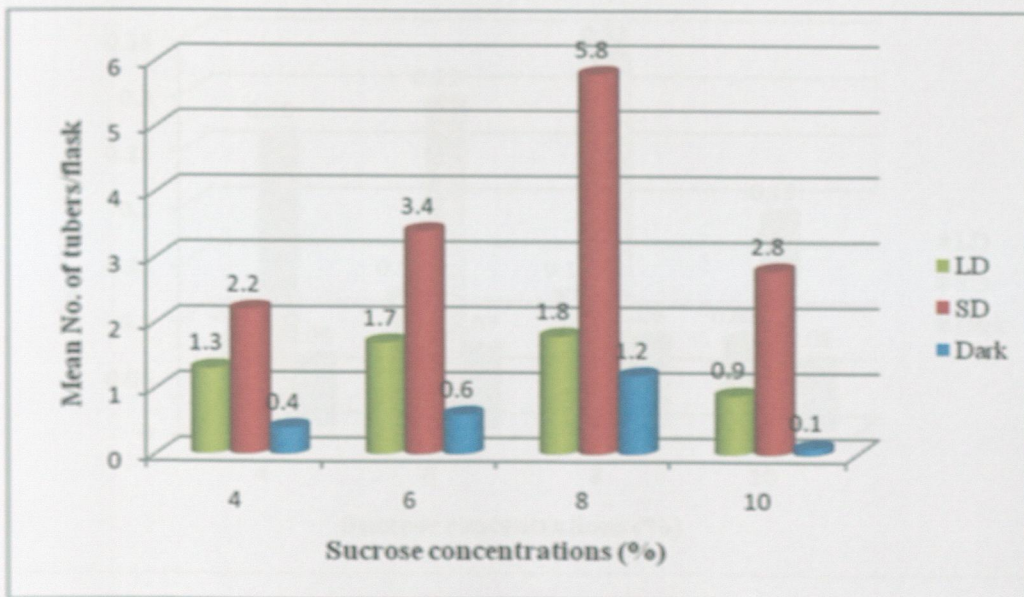


Fig. 31. Effect of varying sucrose levels and different photoperiods on mean number of tubers per flask in potato cv. Ultimus.

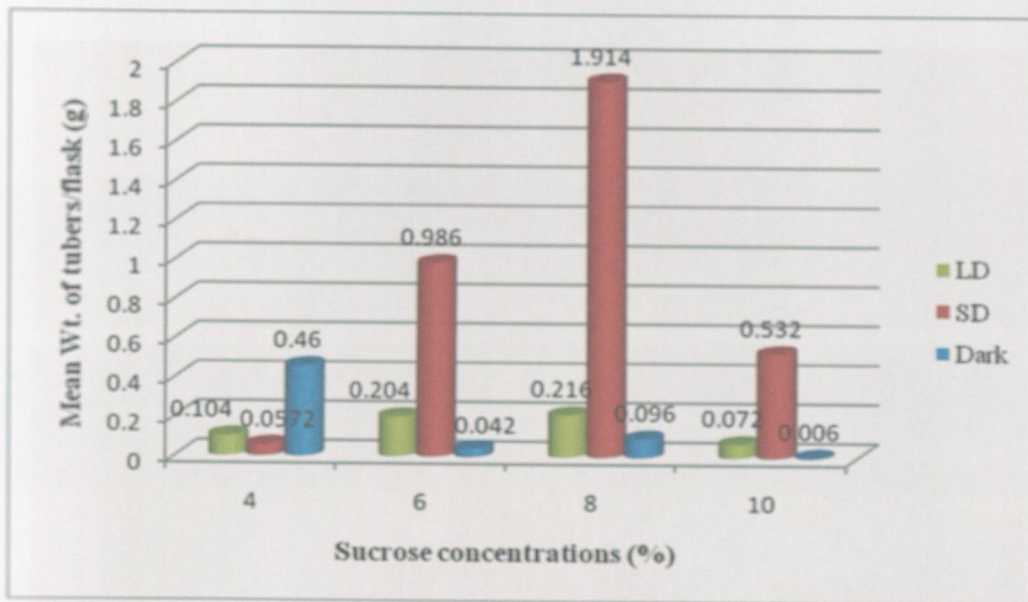


Fig. 32. Effect of varying sucrose levels and different photoperiods on mean weight of tubers per flask in potato cv. Ultimus.

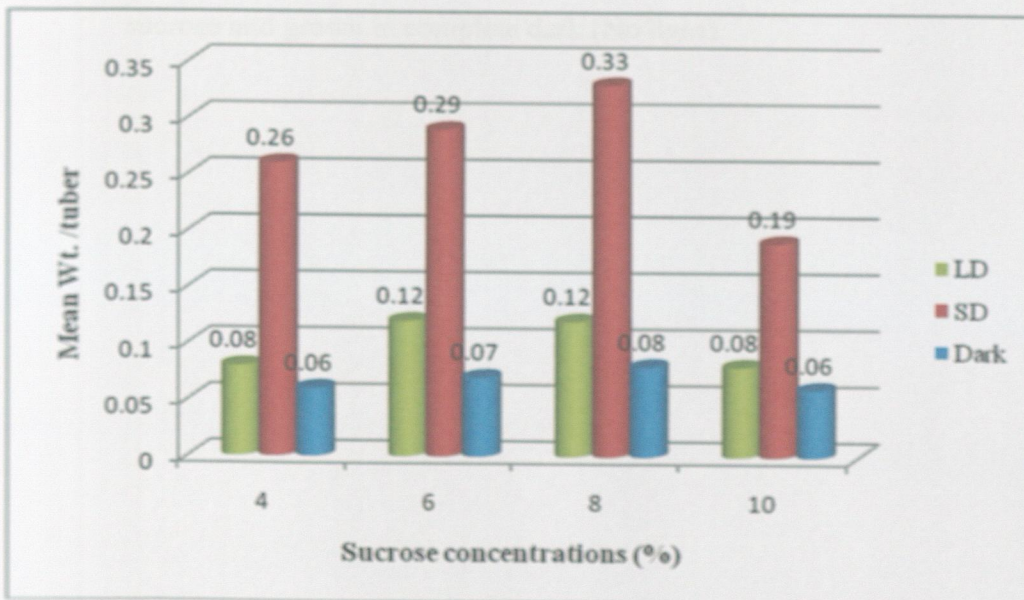


Fig. 33. Effect of varying sucrose levels and different photoperiods on mean weight per tuber in potato cv. Ultimus.

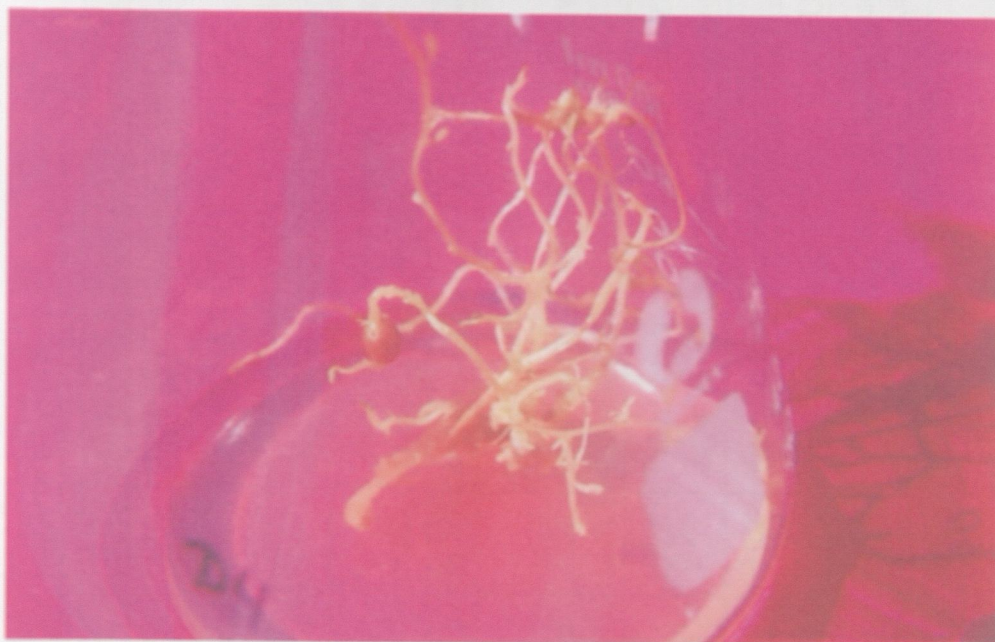


Fig. 34. Tuberization in the cv. Desiree on medium supplemented with 8% sucrose and grown in complete dark (No light).

Explant source	Growth regulator BAP mg/l	Sucrose level (%)	Mean number of tubers/flask		Mean weight of tubers/flask		Mean weight / tuber		General remarks
			Short day (SD)	Long Day (LD)	Short day (SD)	Long day (LD)	Short day (SD)	Long day (LD)	
<i>In vitro</i> plantlets of Desiree	2	4	4.0	1.2	1.12	0.21	0.28	0.21	Multiple shoots observed in LDs in the 1 st 4 weeks. tuberization noticed after 12 th week. Tuberization in SDs earlier after the 6 th week.
	2	6	6.1	2.3	1.769	0.506	0.29	0.22	Multiple shoots with vigorous rooting. Tuberization observed after the 10 th week in LDs.
	2	8	9.4	4.1	3.948	1.148	0.42	0.28	More stoloniferous shoots with early tuberization, occurring after 4 th week in SDs.
	2	10	3.1	1.4	0.589	0.154	0.19	0.11	Tuber yield decreased greatly in both SDs & LDs.
	4	4	4.9	1.5	1.372	0.33	0.28	0.22	Some multiple shoot formation together with tuberization occurring after 8 th weeks in SDs. Tuberization in LDs delayed.
	4	6	9.8	3.2	3.822	0.8	0.39	0.25	Tuberization occurring after 5 th week in SDs. Upright shoots formed in LDs with delayed tuberization.
	4	8	12.5	4.6	5.375	1.334	0.43	0.29	Tuber size and number increased.
	4	10	3.3	1.6	0.528	0.224	0.16	0.14	Tuber yield decreased in both SDs & LDs.
	6	4	5.3	1.5	1.537	0.315	0.29	0.21	Upright shoot growth in SDs depressed. Tuberization occurring after 8 th week in SDs and 10 th week in LDs.

6	6	10.5	3.8	3.99	0.988	0.38	0.26	Tuber number and weight increased in SDs. No pronounced increase in LDs.
6	8	12.9	4.7	5.834	1.41	0.46	0.30	Tuberization occurring after 6 th week in SDs. Some tubers dramatically large measuring up to 2.2cm in length and weighing up to 2.5g.
6	10	3.3	1.8	0.627	0.234	0.19	0.13	Tuberization delayed and decreased.
8	4	8.1	2.0	2.511	0.46	0.31	0.23	Tuberization in SDs occurring after 7 th week in LDs after 9 th week.
8	6	12.6	3.1	4.788	0.837	0.38	0.27	Tuber number increased.
8	8	16.4	5.2	6.396	1.456	0.39	0.28	Tuber number increased further, but weight /tuber decreased compared to 6.0mg/l BAP in combination with 8%.
8	10	3.8	1.9	0.608	0.228	0.16	0.12	Slight increase in tuber number compared to 4 and 6mg/l in combination with 10% sucrose.
10	4	8.3	2.3	0.644	0.529	0.28	0.23	Further increase in tuber number noticed in comparison to 4, 6, 8 mg/l + 4% sucrose.
10	6	16.2	4.5	5.832	1.17	0.36	0.26	An increase in tuber number together with a decrease in tuber size noticed.
10	8	25.3	5.4	8.096	1.35	0.32	0.25	Maximum number of tubers obtained with a slight decrease in weight / tuber occurred.
10	10	4.6	2.0	0.69	0.18	0.15	0.09	Very little tuberization observed.

Explant source	Growth regulator BAP mg/l	Sucrose level (%)	Mean number of tubers/flask		Mean weight of tubers/flask		Mean weight / tuber		General remarks
			Short day (SD)	Long Day (LD)	Short day (SD)	Long day (LD)	Short day (SD)	Long day (LD)	
<i>In vitro</i> plantlets of <i>U. ulmifolius</i>	2	4	2.8	1.0	0.7	0.1	0.25	0.21	Multiple shoots in LD with tuberization observed after 13 th whereas tubering in SDs after 8 th week.
	2	6	3.5	0.9	1.015	0.418	0.29	0.22	Vigorous shoots with roots in LDs with late tuberization after 13 th week. Fewer multiple shoots in SD with early tuberization after 10 th week.
	2	8	6.3	2.5	2.394	0.6	0.38	0.24	Stoloniferous shoots in SDs tuberization occurred after 7 th week.
	2	10	3.6	0.8	0.432	0.096	0.18	0.12	Tuber yield decreased both in terms of number and weight.
	4	4	2.9	1.3	0.667	0.247	0.23	0.19	Stoloniferous shoots together with tuberization after the 8 th week. Tuberization in LD delayed.
	4	6	7.5	2.5	2.325	0.55	0.31	0.22	More stoloniferous shoots, roots inhibited. Tuberization occurring after the 8 th week.
	4	8	9.6	2.9	3.264	0.667	0.34	0.23	Tuberization occurred after 7 th week.
	4	10	3.4	1.1	0.544	0.154	0.16	0.14	Tuber yield decreased in both SDs and LDs.
	6	4	3.0	1.8	0.72	0.396	0.24	0.22	Shoot growth depressed. Tubering observed after 8 th week in SDs and 11 th week in LDs.

6	6	8.2	2.1	2.542	0.483	0.31	0.23	Tuber number and weight increased in SDs.
6	8	11.3	2.9	2.825	0.725	0.38	0.25	Tuberization occurred after the 7 th week in SDs. Whereas in LDs after the 9 th week.
6	10	3.5	0.9	0.525	0.108	0.15	0.12	Very little tuberization after 14 th week.
8	4	3.9	1.6	1.17	0.336	0.30	0.21	Tuberization in SDs observed after 7 th week.
8	6	10.0	2.0	3.3	0.44	0.33	0.22	Shoot growth depressed. Tuber number increased in SDs.
8	8	12.0	3.1	4.56	0.682	0.38	0.22	Slight increase in tuber number observed.
8	10	3.2	0.7	0.48	0.091	0.15	0.13	Negligible amount of tubers formed after the 14 th week.
10	4	4.8	1.8	1.2	0.378	0.25	0.21	Tuberization occurred after 7 th week in SDs.
10	6	13.4	3.1	3.752	0.713	0.28	0.23	Tubers number increased with BAP concentration whereas weight decreased.
10	8	21.2	3.8	5.724	0.836	0.27	0.22	Maximum number of tubers obtained at this combination.
10	10	3.0	1.0	0.36	0.1	0.12	0.10	Very few tubers formed in some of the cultures.

Table 25. ANOVA of factors, sugar concentration (%), BAP concentration (mg/l), day length and their interactive effect on the number of tubers and weight of tubers in Desiree.

Source	Degree of freedom	Mean square values	
		Number of tubers	Weight of tubers
Sugar conc. (%) (A)	3	832.34**	113.29**
BAP conc. (mg/l) (B)	4	1251.88**	72.07**
A x B	12	23.26**	3.59**
Day length (C)	1	2684.75**	321.74**
A x C	3	521.65**	1.83**
B x C	4	317.39**	2.99**
A x B x C	12	0.118NS	0.28NS
Error	720	0.065	0.082

* = Significant at 5% level of significance

** = Significant up to 1% level of significance

NS = Non significant

Table 26. ANOVA of factors, sugar concentration (%), BAP concentration (mg/l), day length and their interactive effect on the number of tubers and weight of tubers in Ultimus.

Source	Degree of freedom	Mean square values	
		Number of tubers	Weight of tubers
Sugar conc. (%) (A)	3	1738.74**	283.04**
BAP conc. (mg/l) (B)	4	383.88**	27.59**
A x B	12	1.16NS	0.43NS
Day length (C)	1	6099.60**	796.94**
A x C	3	611.92**	4.26**
B x C	4	217.29**	3.61**
A x B x C	12	1.31NS	0.119NS
Error	720	0.87	0.164

* = Significant at 5% level of significance

** = Significant up to 1% level of significance

NS = Non significant

Table 27. Mean values representing the effect of sugar concentration (%), BAP concentration (mg/l), day length and their interactive effect on the number of tubers in Desiree.

Day length (C)	Sugar conc. % (A)	BAP Conc. (B)					A x C
		1	2	3	4	5	
		A x B x C					
Short day	4	2.8	2.90	3.0	3.9	4.8	3.48C
	6	3.5	7.5	8.2	10.0	13.4	8.52B
	8	6.3	9.6	11.3	12.0	21.2	12.08A
	10	3.6	3.4	3.5	3.2	3.0	3.34CD
Long day	4	1.0	1.3	1.8	1.6	1.8	1.5F
	6	1.9	2.5	2.1	2.0	3.1	2.32E
	8	2.5	2.9	2.9	3.1	3.8	3.04D
	10	0.8	1.1	0.9	0.7	1.0	0.9G
		B x C					C
Short day		4.04E	5.85D	6.5C	7.27B	10.6A	6.855
Long day		1.55H	1.95G	1.92G	1.85GH	2.425F	1.94
		A x B					A
	4	1.90	2.1	2.4	2.75	3.3	2.49C
	6	2.7	5.0	5.15	6.0	8.25	5.2B
	8	4.4	6.25	7.10	7.55	12.5	7.56A
	10	2.2	2.25	2.20	1.95	2.0	2.12C
B		2.8D	3.9CD	4.21BC	4.56B	6.513A	

Table 28. Mean values representing the effect of sugar concentration (%), BAP concentration (mg/l), day length and their interactive effect on the weight of tubers in Desiree.

Day length (C)	Sugar conc. % (A)	BAP Conc. (B)					A x C
		1	2	3	4	5	
		A x B x C					
Short day	4	4.00	4.90	5.30	8.10	8.30	6.12C
	6	6.10	9.80	10.50	12.60	16.20	11.04B
	8	9.40	12.50	12.90	16.4	25.30	15.30A
	10	3.1	3.3	3.3	3.8	4.60	3.60E
Long day	4	1.0	1.5	1.5	2.0	2.30	1.66F
	6	2.3	3.2	3.8	3.1	4.50	3.38E
	8	4.1	4.6	4.6	5.2	5.40	4.78D
	10	1.4	1.6	1.8	1.9	2.00	1.74F
		B x C					C
Short day		5.65D	7.625C	8.00C	10.225B	13.60A	9.02
Long day		2.20G	2.725F	2.925F	3.05F	3.55E	2.89
		A x B					A
	4	2.50	3.2	3.4	5.05	5.3	3.89C
	6	4.2	6.5	7.15	7.85	10.35	7.21B
	8	6.75	8.55	8.75	10.8	15.35	10.04A
	10	2.25	2.45	2.55	2.85	3.30	2.68D
B		2.925E	5.175D	5.463C	6.638B	8.575A	

Table 29. Mean values representing the effect of sugar concentration (%), BAP concentration (mg/l), day length and their interactive effect on the number of tubers in Ultimus.

Day length (C)	Sugar conc. % (A)	BAP Conc. (B)					A x C
		1	2	3	4	5	
		A x B x C					
Short day	4	1.12	1.37	1.54	2.51	2.32	1.77C
	6	1.77	3.82	4.05	4.79	5.83	4.05B
	8	3.95	5.38	5.93	6.4	8.10	5.95A
	10	0.6	0.53	0.63	0.61	0.69	0.61E
Long day	4	0.21	.33	.32	.46	.53	0.37EF
	6	.51	.8	.99	.84	1.17	0.86E
	8	1.15	1.33	1.41	1.46	1.35	1.34D
	10	.15	.33	.33	.23	.18	0.25F
		B x C					C
Short day		1.86E	2.77D	3.04C	3.58B	4.24A	3.096
Long day		0.51H	0.70G	0.76FG	0.75FG	0.81F	.704
		A x B					A
	4	.66O	.85N	.93N	1.49L	1.43L	1.07
	6	1.14M	2.31H	2.52G	2.81F	3.50D	2.46
	8	2.55G	3.36E	3.67C	3.93B	4.72A	3.64
	10	.37P	.43P	.48P	.42P	.44P	.43
B		1.18D	1.74C	1.90BC	2.16B	2.52A	

Table 30. Mean values representing the effect of sugar concentration (%), BAP concentration (mg/l), day length and their interactive effect on the weight of tubers in Ultimus.

Day length (C)	Sugar conc. % (A)	BAP Conc. (B)					A x C
		1	2	3	4	5	
		A x B x C					
Short day	4	.70	.67	.72	1.17	1.2	0.89C
	6	1.02	2.32	2.55	3.3	3.75	2.59B
	8	2.39	3.26	4.29	4.56	5.76	4.05A
	10	0.65	0.54	0.53	0.49	0.36	0.51E
Long day	4	0.21	0.25	0.40	0.34	0.38	0.31F
	6	0.42	0.55	0.48	0.44	0.71	0.52E
	8	0.60	0.67	0.73	0.68	0.84	0.70D
	10	0.10	0.15	0.11	0.10	0.10	0.11G
		B x C					C
Short day		1.19E	1.70D	2.02C	2.38B	2.77A	2.01
Long day		0.33G	0.40FG	0.43FG	0.39G	0.51F	0.41
		A x B					A
	4	.46G	.46G	.56G	.75F	.79F	0.60
	6	.72F	1.44E	1.52E	1.87D	2.23C	1.55
	8	1.50E	1.97D	2.51B	2.62B	3.30A	2.38
	10	.37GHI	.35HI	.32HI	.29I	.23I	0.31
B		0.76E	1.05D	1.22C	1.38B	1.64A	

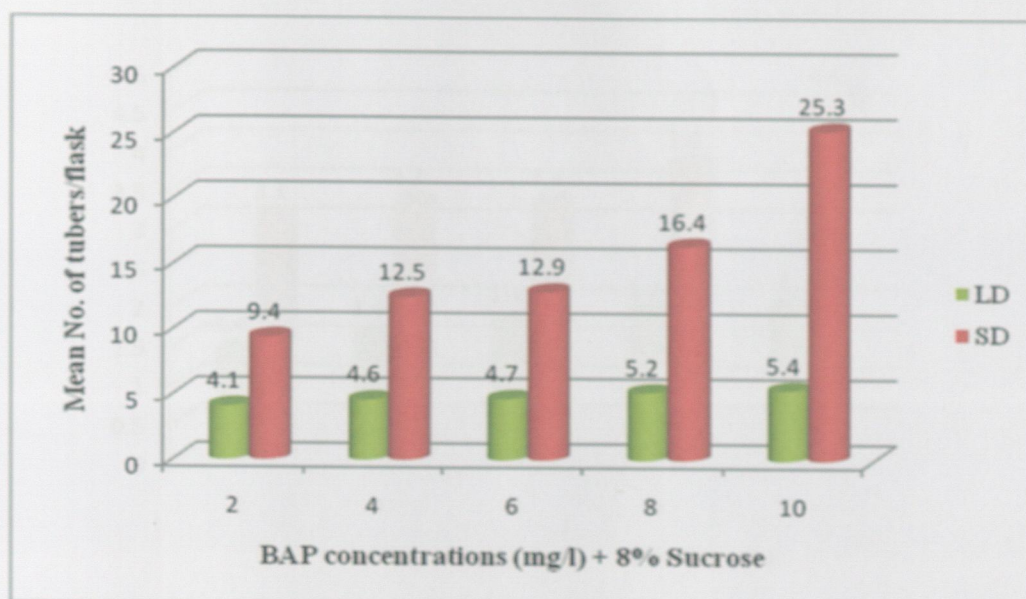


Fig. 35. Effect of varying concentrations of BAP+8% sucrose and different photoperiods on mean number of tubers per flask in potato cv. Desiree.

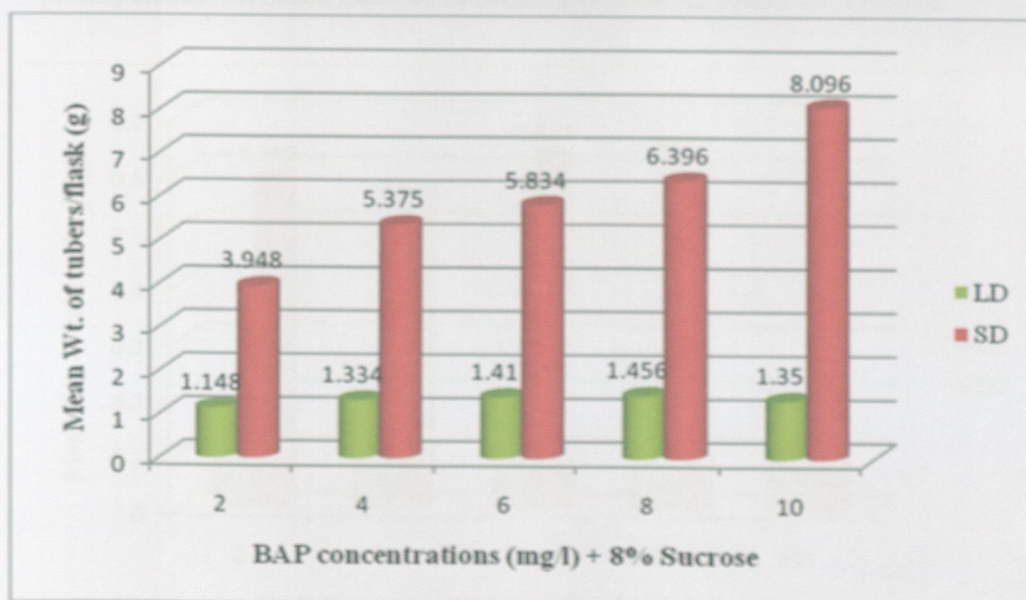


Fig. 36. Effect of varying concentrations of BAP+8% sucrose and different photoperiods on mean weight of tubers per flask in potato cv. Desiree.

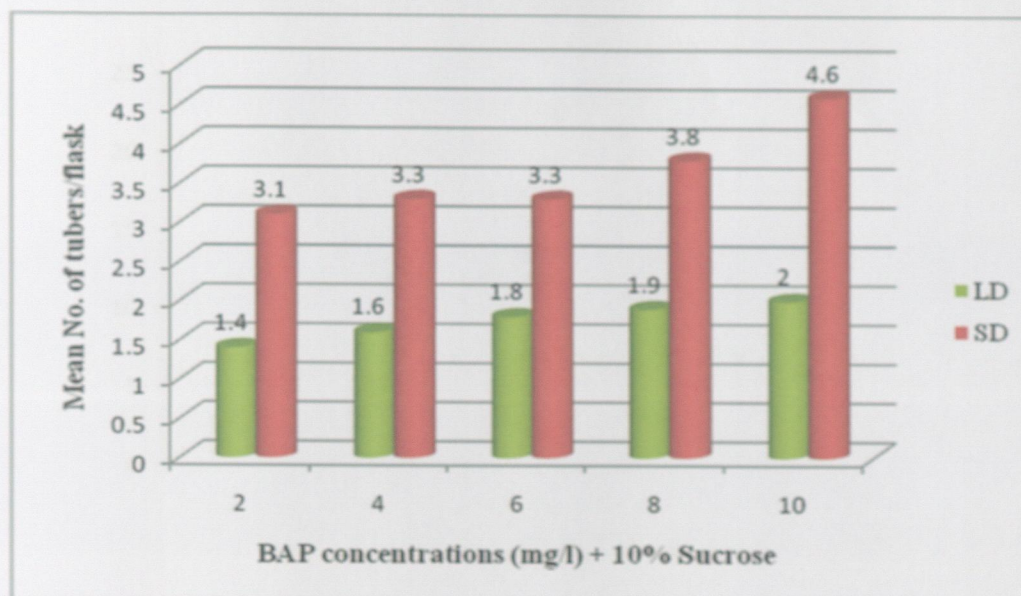


Fig. 37. Effect of varying concentrations of BAP+10% sucrose and different photoperiods on mean number of tubers per flask in potato cv. Desiree.

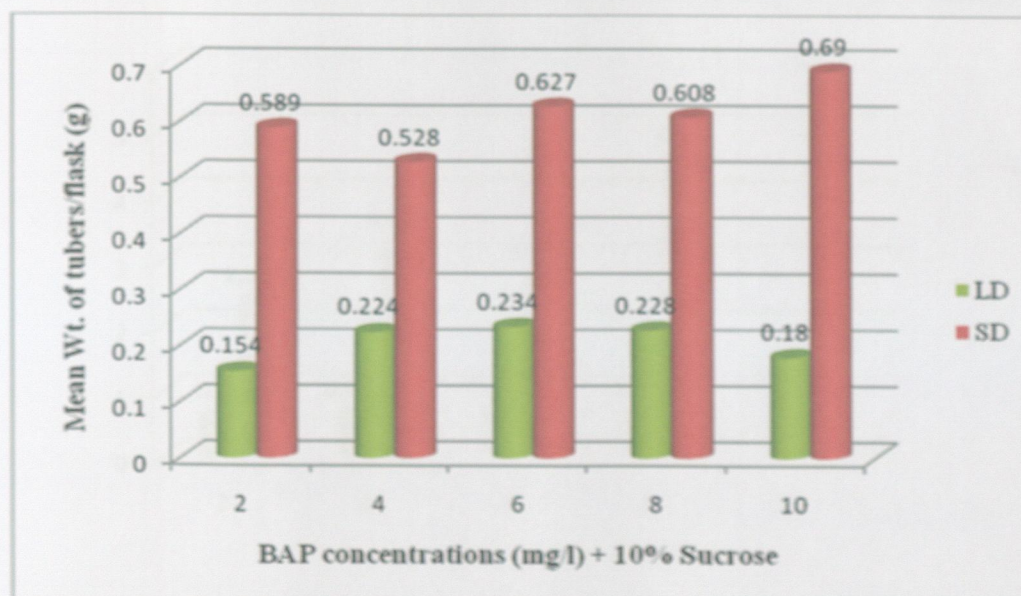


Fig. 38. Effect of varying concentrations of BAP+10% sucrose and different photoperiods on mean weight of tubers per flask in potato cv. Desiree.

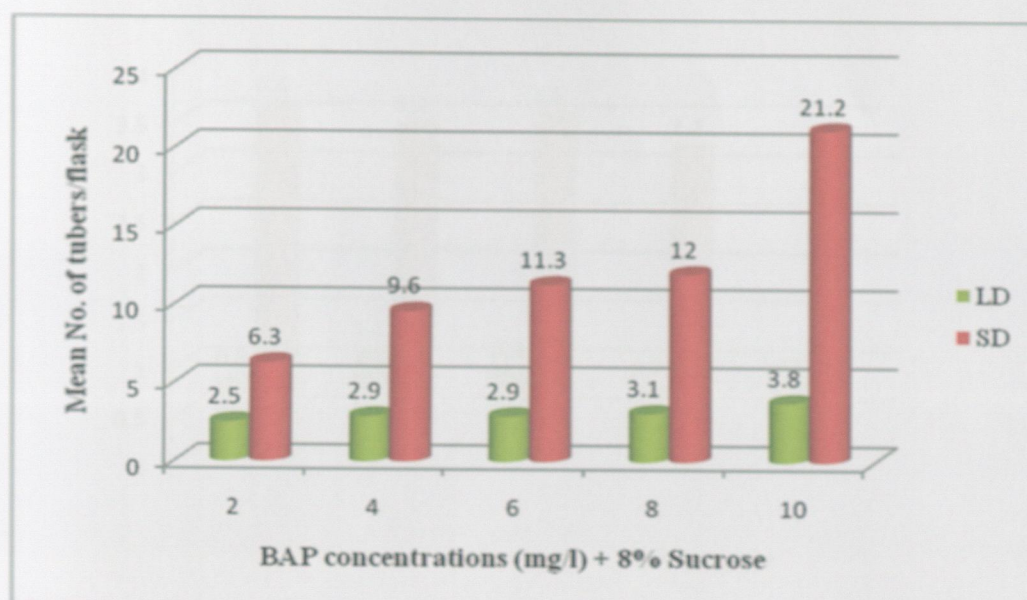


Fig. 39. Effect of varying concentrations of BAP+8% sucrose and different photoperiods on mean number of tubers per flask in potato cv. Ultimus.

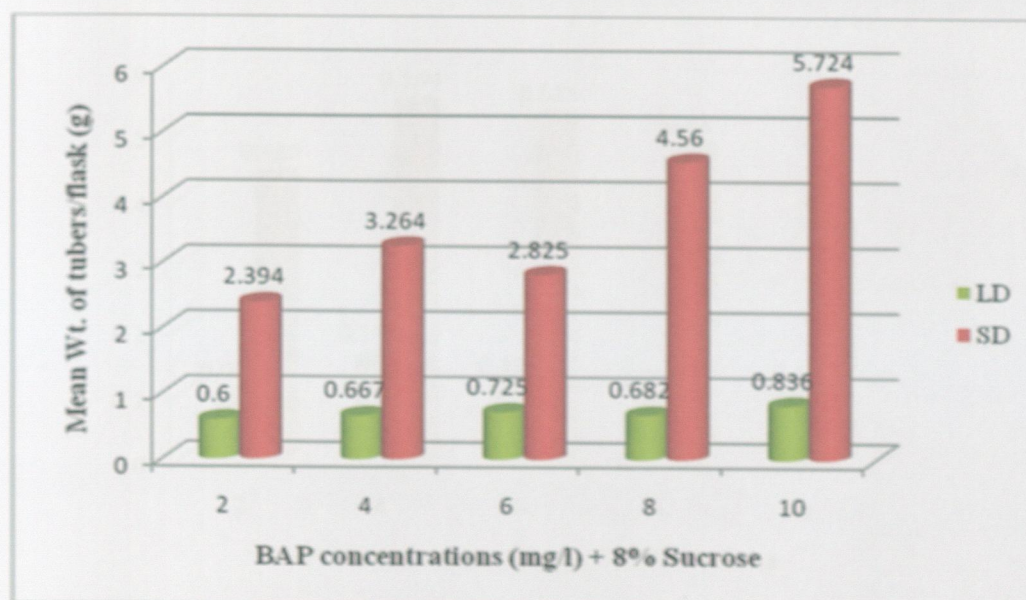


Fig. 40. Effect of varying concentrations of BAP+8% sucrose and different photoperiods on mean weight of tubers per flask in potato cv. Ultimus.

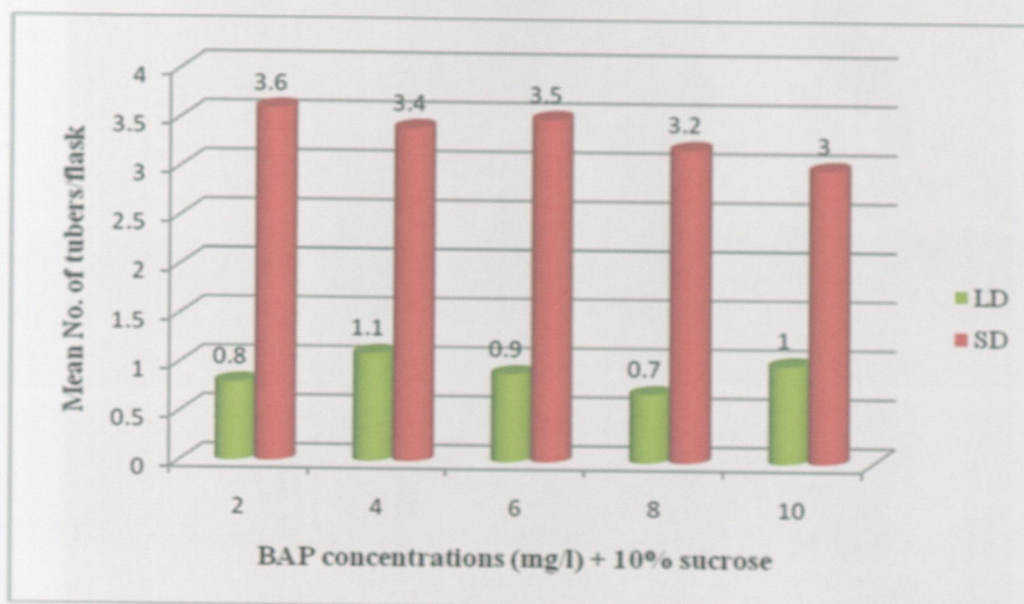


Fig. 41. Effect of varying concentrations of BAP+10% sucrose and different photoperiods on mean number of tubers per flask in potato cv. Ultimus.

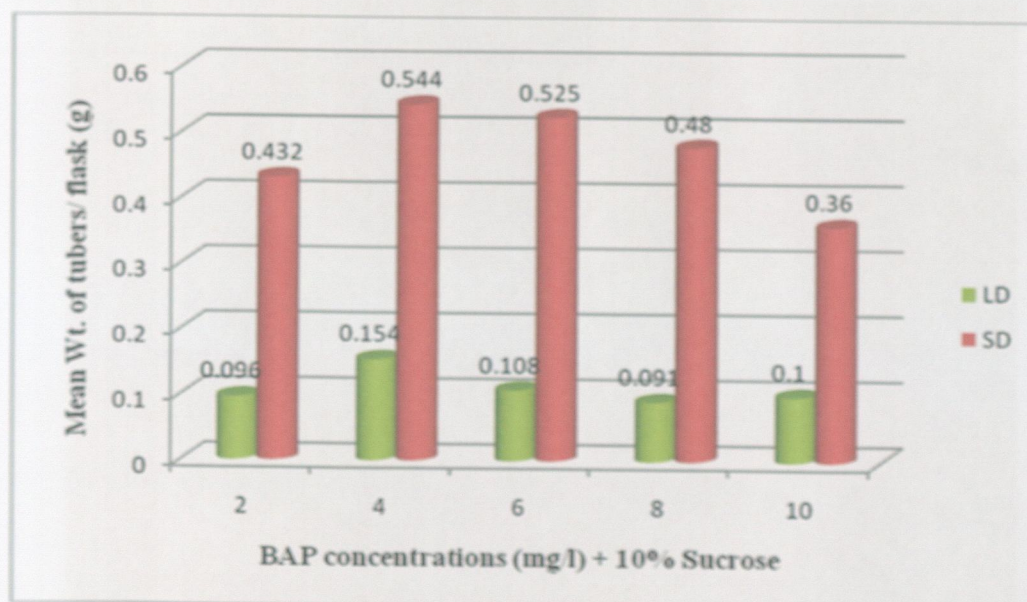


Fig. 42. Effect of varying concentrations of BAP + 10% sucrose and different photoperiods on mean weight of tubers per flask in potato cv. Ultimus.



Fig. 43. Upright shoot formation in potato cv. Desiree on medium supplemented with 2.0mg/l BAP in combination with 4% sucrose under 16h photoperiod.

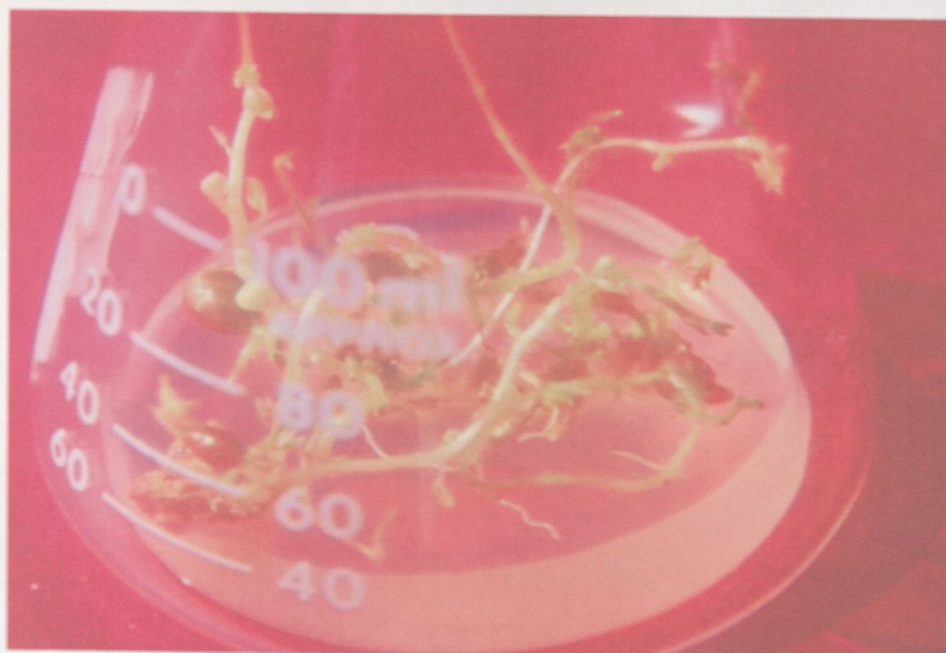
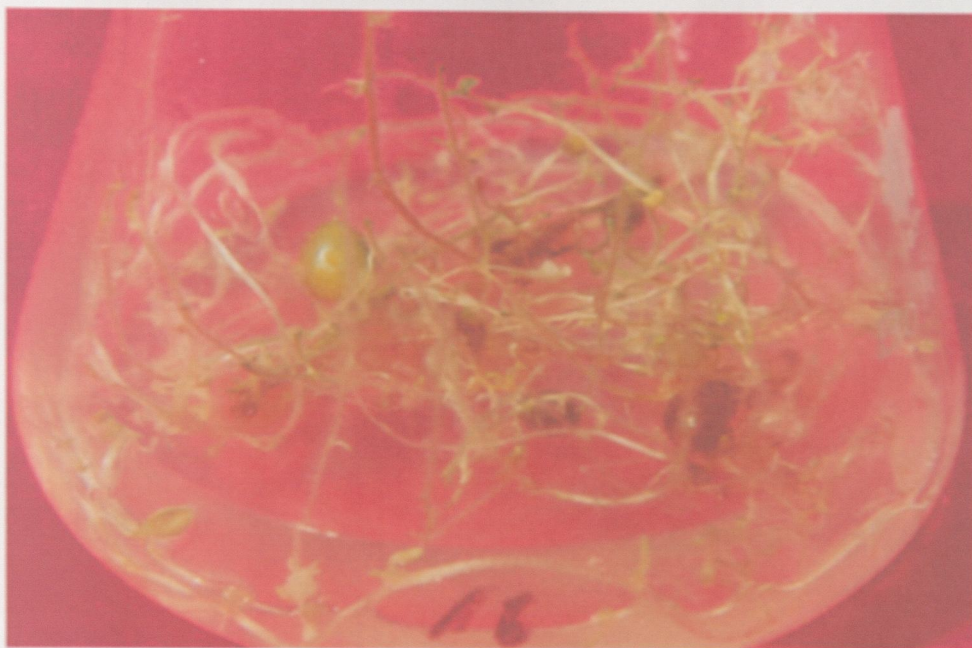
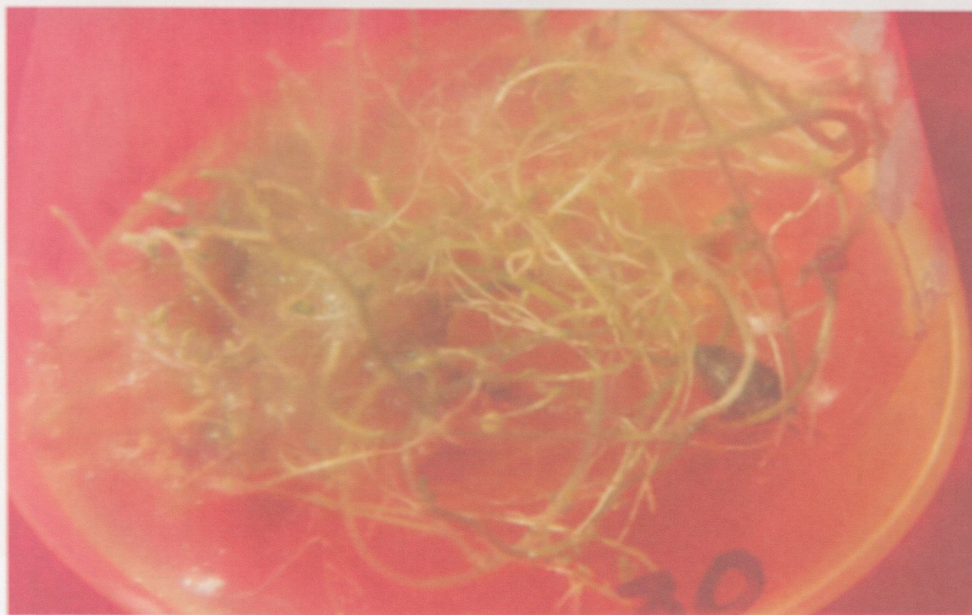


Fig. 44. Tuberization in the cv Desiree as observed on the medium supplemented with 2.0mg/l BAP and 4% sucrose and cultured under short photo period (8h).



a. Desiree



b. Ultimus

Figs. 45a, b. Multiple shoot formation together with some tuberization observed on medium supplemented with 2.0mg/l BAP and 6% sucrose under long day conditions.



a. Desiree



b. Ultimus

Figs. 46a, b. Stoniferous shoots together with tuber formation on BM supplemented with 2.0mg/l BAP and 6% sucrose under short days.

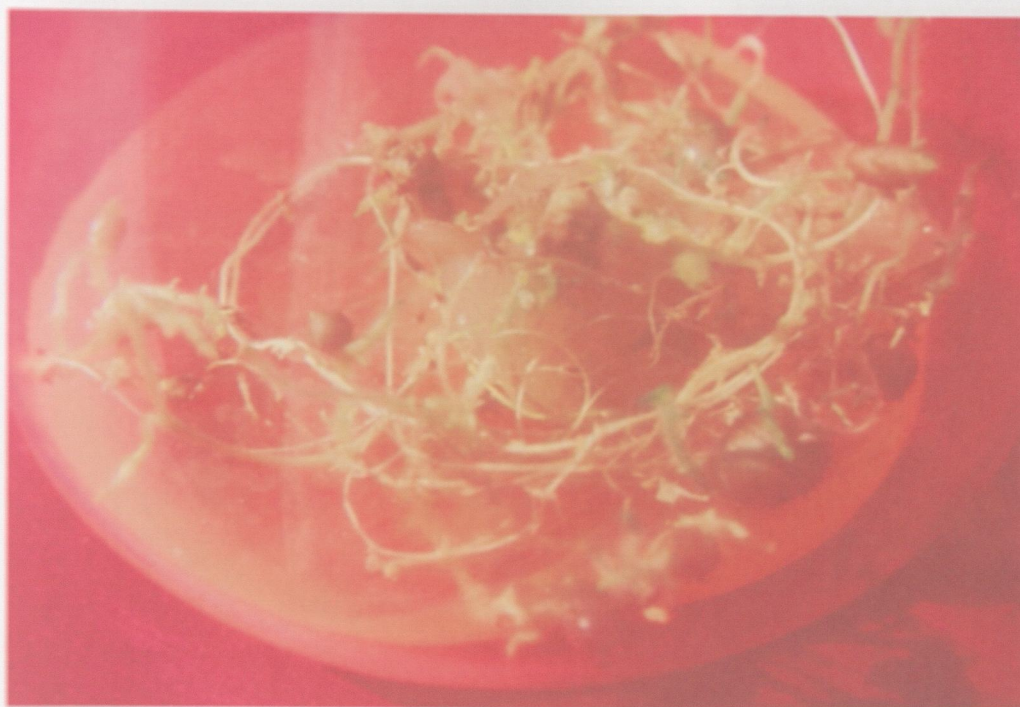


Fig. 47. Stoloniferous shoots and tuber formation on BM supplemented with 2.0mg/l BAP and 8% sucrose under short (8h) photoperiod in Desiree.



a. Desiree



b. Ultimus

Figs. 48a, b. Tuber induction in potato on medium supplemented with 4.0mg/l BAP and 6% sucrose under 8h photoperiod.



a. Desiree



b. Ultimus

Figs. 49 a, b. Tuber induction in potato on medium supplemented with 6.0mg/l BAP and 6% sucrose in 8h photoperiod.



a. Desiree

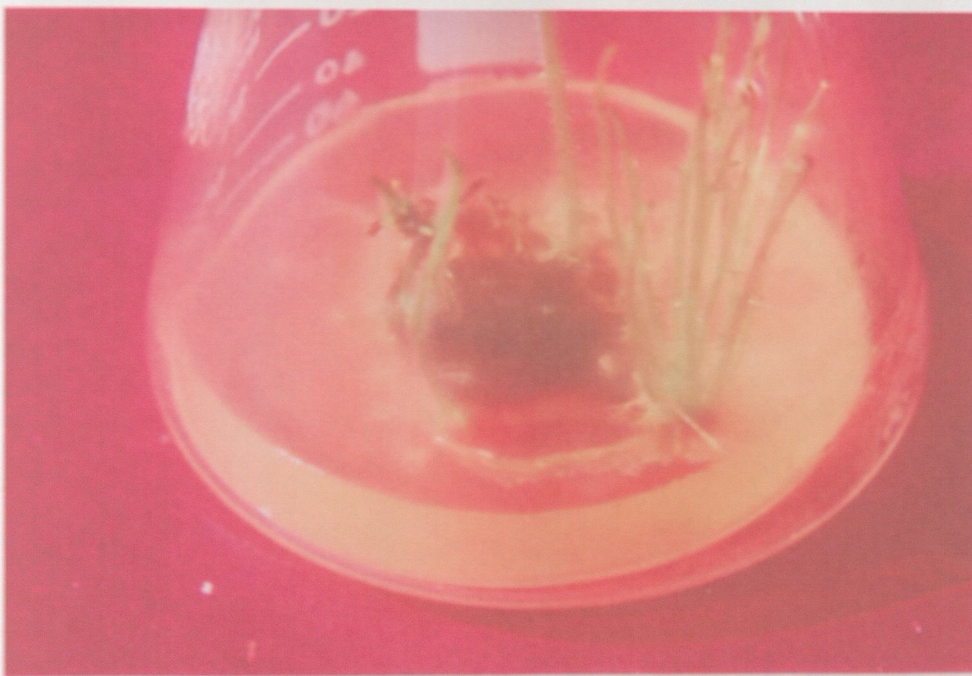


b. Ultimus

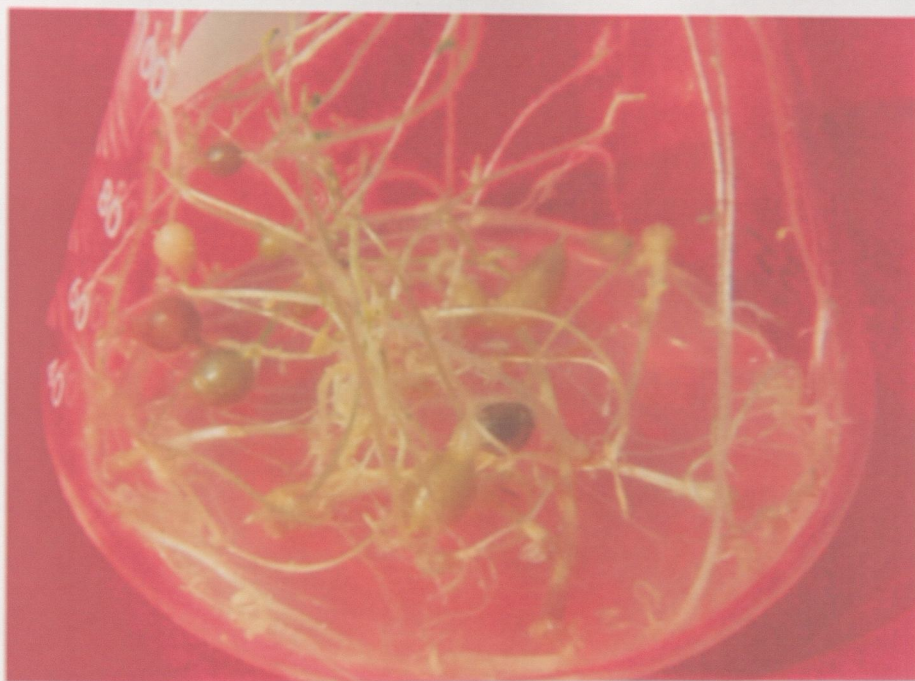
Figs. 50 a, b. Tuber formation on BM supplemented with 6.0mg/l BAP and 8% sucrose under 8h photoperiod. Some large sized tubers measuring up to 1.5cm with several eyes were obtained.



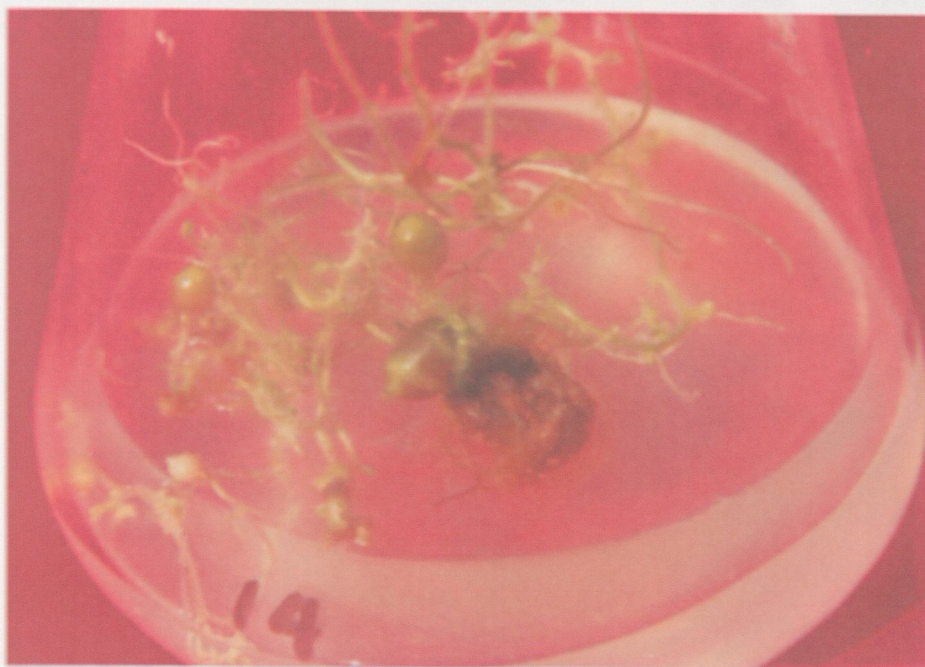
Figs. 51 and 52. Some dramatically large sized tuber measuring up to 2.2cm in length obtained in the cv. Desiree on medium supplemented with 6.0mg/l BAP and 8% sucrose under short days. These on transfer to plain MS medium sprouted readily as seen in Fig. 53 and 54.



Figs. 53 and 54. Sprouting of microtubers on plain MS medium of the cv. Desiree obtained on medium incorporated with 6.0mg/l BAP and 8% sucrose.



a. Desiree

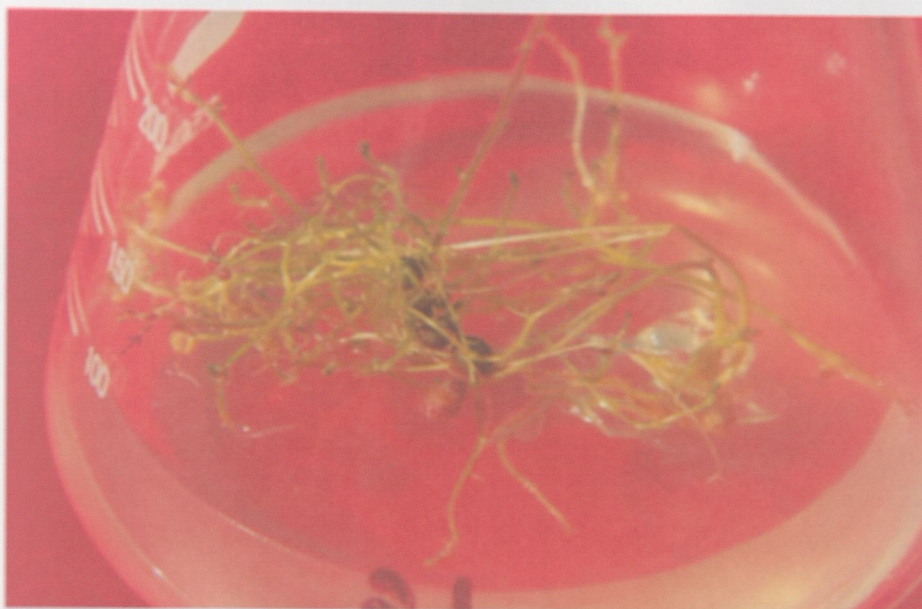


b. Ultimus

Figs. 55 a, b. Tuberization in potato on medium supplemented with 8.0mg/l BAP and 6% sucrose under short (8h) photoperiod.



Fig. 56. Tuber induction on medium supplemented with 8.0mg/l BAP and 8% sucrose under short days (8h) in the cv. Desiree.



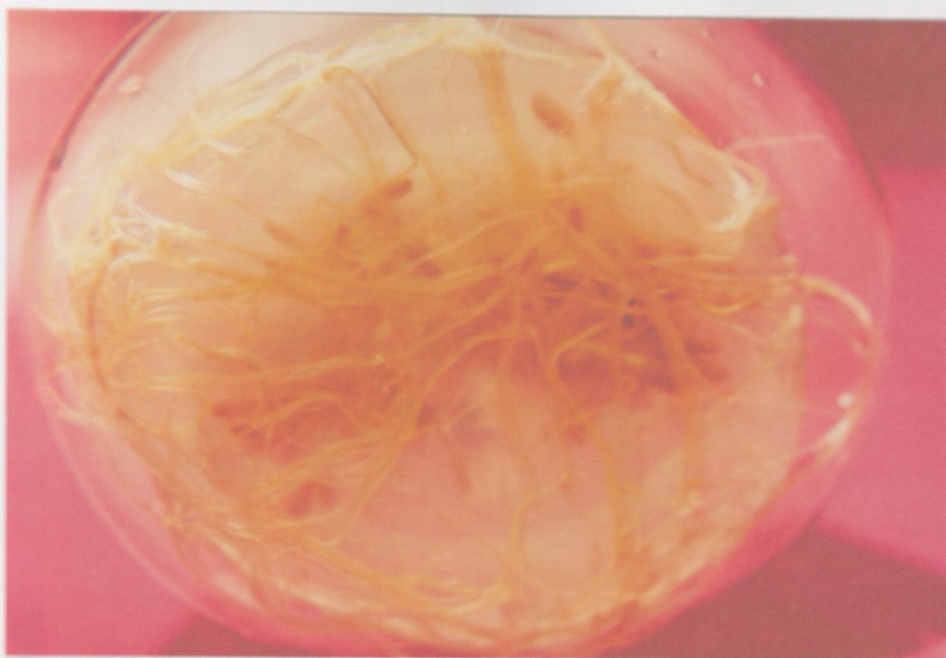
a. Desiree



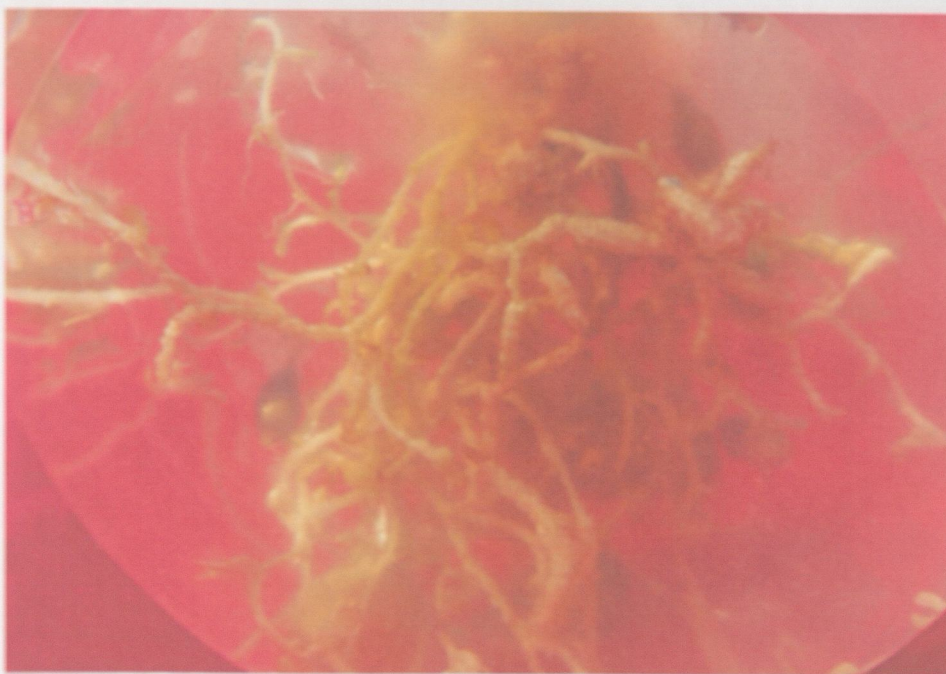
b. Ultimus

Figs. 57 a, b. Some shoots turning upright with tuber formation being observed on a medium supplemented with 8.0mg/l BAP and 8% sucrose, cultured under long (16h) photoperiod.

Note: Some of the tubers on the left in figure extremely small.

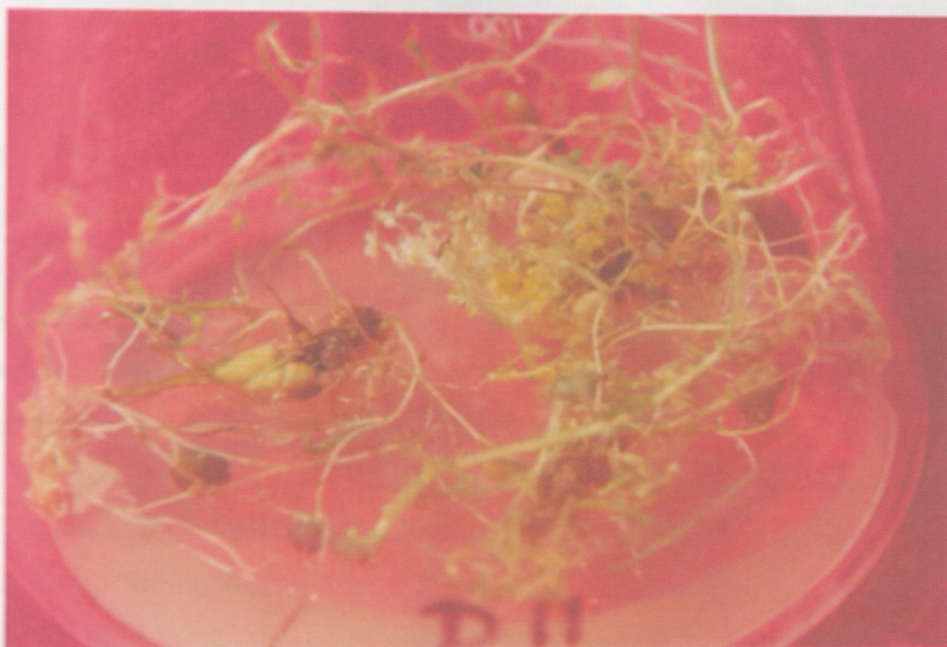


a. Desiree



b. Ultimus

Figs. 58 a, b. Early stages in tuberization of both the cultivars on medium supplemented with 10.0mg/l BAP and 8% sucrose under short days (Large no. of tubers being obtained on this combination).



a. Desiree



b. Ultimus

Figs. 59 a, b. Tuberization being srtrongly hampered in both the cultivars on a medium supplemented with 10mg/l BAP and 10% sucrose under short days. Tuber number and weight both greatly decreased.

Effect of Kinetin and Sucrose on Tuberization

With the objective of *in vitro* tuber formation the plantlets from both the cultivars obtained in Stage-II of the above experiments were isolated and subcultured in tuberization medium. The MS basal medium was used at full strength supplemented with varying levels of Kinetin (2.0, 3.0, 4.0, 6.0 and 8.0 mg/l) and three sucrose levels (4, 6 and 8%). The cultures were incubated under short and long day treatments, i.e. 8 and 16 hours light photoperiod, respectively at a temperature of $25 \pm 1^{\circ}\text{C}$. The results obtained from these experiments are shown in (Tables 31 and 32; Figs. 60-63). The basal medium (MS) when supplemented with 2.0 mg/l K and 4% sucrose could not induce tuberization either in long or in short days within 4 weeks of culture period in both the cultivars. However, some rooting was observed in both the cultivars. Nevertheless, some multiple shoot formation was also observed after the sixth week in Desiree in the long day cultures, whereas no profound effect was observed in Ultimus. Not much increase in shoot growth was noticed in the short day cultures. However, tuber induction was observed during the 6th week of culture period in these cultures. Moreover, when the culture period at both day lengths was extended to 12 weeks, prolific shoot growth together with some tuber formation was observed in the long day cultures (Figs. 64a and 64b), whereas an increase in tuber number was noticed only for short day cultures. Tuber number, however in short day cultures was large as compared to long days. An average of 4.3 tubers per flask was obtained in Desiree in the short day cultures and 1.8 in the long day cultures. Some shoot proliferation together with tuberization also occurred in Ultimus with an average of 3.5 and 1.4 tubers per flask in the short and long day cultures respectively. The size of most of the tubers thus formed in both the cultivars was very small and with one or two eyes in them (Fig. 65).

Since tuberization favors a high concentration of carbon source in the medium sucrose level was therefore, increased in the further experiment to 6 and 8% levels. With an increase in the sucrose level to 6% the number and weight of the tubers increased in both the cultivars particularly in the short days (Table 31 and 32; Figs. 66a and 66b). Still further in another set of experiments, increasing the sucrose level to 8% an increase in tuber number and weight was noticed in both the cultivars. An average of 8.2 and 6.3 tubers per flask were obtained in Desiree and Ultimus respectively in short day

treatments. Tuber formation also occurred in the long day (16hrs days) cultures but to a lesser degree.

When Kinetin level was increased to 3.0 mg/l in the presence of 4, 6 and 8% sucrose in the basal medium, luxuriant shoot growth together with some tuber formation was observed in medium supplemented with 3 mg/l K in the presence of 4% sucrose in the long days (Tables 31 and 32). In the short days (8 hrs day) however, shoot growth was not prolific, whereas tuber number increased in both the cultivars. An average of 8.9 and 7.8 tubers / flask were obtained in Desiree and Ultimus respectively. With a further increase in the sucrose level to 6 and 8% a gradual increase in tuber number and weight was noticed (Tables 31 and 32) in the short days without any increase in the number of shoots, of both the cultivars. Averages of 15.8 tubers / flask were obtained at 8% sucrose level in Desiree and 13.51 in Ultimus. The tubers thus obtained in these experiments were large and had 3–4 eyes (Figs. 67a and 67b) compared to those obtained in 2.0 mg/l K.

In another set of experiments kinetin level was increased to 4.0 mg/l in the basal medium. As 6 and 8% sucrose gave a good tuber yield in combination with 2.0 and 3.0 mg/l kinetin levels, therefore, these concentrations of sucrose were used in the further experiments. The addition of 4.0 mg/l kinetin to the medium in the presence of 6 or 8% sucrose resulted in an increase in tuber number in both the cultivars. More stoloniferous rather than upright shoots were formed that terminated into tubers (Fig. 68). Averages of 19.8 tubers/flask were obtained in the cv. Desiree and 15.1 tubers/flask in Ultimus, within 10 weeks of culture.

As evident from the table further increase in the levels kinetin up to 8.0mg/l did not exhibit any positive effects on tuber formation in any of the 2 cultivars. Even the addition of 8% sucrose to the medium containing 6 or 8.0 mg/l. K was of no added advantage as far as tuberization is concerned. A decrease in tuber number of both the cultivars was observed at 6.0 mg/l level of kinetin whereas as negligible amount of tubers could be obtained at 8.0 mg/l kinetin (Figs. 69 and 70)

The ANOVA (Table 33-38) showed that the sugar concentration and kinetin concentration has significant effect in both Desiree and Ultimus. The effect of kinetin and photoperiod has significant effect on the number and weight of tubers in Ultimus while the sugar concentration has non significant effect on the number and weight of tubers in

Desiree. The interactive effect of sugar concentration x kinetin concentration on the number and weight of tubers was significant in Ultimus while the same interaction was non significant in Desiree. Similarly, sugar concentration x photoperiod interaction was non significant in both Desiree and Ultimus for number and weight of tubers.

The promotion of microtuberization on cultured shoots by cytokinins has been studied by many researchers. There are controversial results regarding *in vitro* tuberization by cytokinins (BAP or K). Effect of kinetin on microtuberization is due to its relationship with the ethylene biosynthesis. Because kinetin influenced the microtuber initiation, therefore microtuber number increased (Kefi *et al.* 2000). Kanwal *et al.* (2006) and Aslam and Iqbal (2010) compared the two cytokinins (BAP and kinetin). They obtained a lesser microtuber yield on kinetin in comparison to BAP. Aryakia and Hamidoghlo (2010) also showed somewhat similar results. They obtained higher microtuber number with kinetin whereas maximum tuber size was obtained on medium supplemented with BAP.

The present results agree to the above studies. Though a considerable number of microtubers were obtained on kinetin supplemented media, but to a lesser degree in comparison to those obtained with BAP in the preceding section. In contrast Uranbey (2004) in a comparative study on BAP and kinetin reported that kinetin at high concentration (15-20 μ M) led to much more tuber formation. Similarly Hoque (2010) obtained a better microtuber yield on kinetin supplemented medium. He obtained maximum number of microtubers on a medium supplemented with 4mg/l kinetin and 6% sucrose. These findings coincide with the present results.

The variations in results shown by different workers in regard to the two cytokinins (BAP and Kinetin) may be attributed to the degree of cell sensitivity towards growth regulators, which depends on the origin of explants and endogenous levels of growth regulators.

Conclusion

It could therefore, be deduced from the above experiments that the addition of kinetin to the medium promoted tuberization at the lower levels (2.0, 3.0 and 4.0 mg/l) in the presence of 6 and 8% sucrose. Higher concentrations supported tuberization but to a lesser degree. At 6.0 mg/l the number of tubers/flask decreased in both the cultivars in comparison to 3 and 4 mg/l levels. However, large size tubers were obtained at this concentration. A further decrease in tuber number was noticed at 8.0mg/l suggesting that this concentration greatly hampered tuberization.

Table 31. Effect of Kinetin and sucrose on *in vitro* tuberization in potato cv. Desiree (All harvests done after 12 weeks).

Explants source	Growth regulator kinetin = mg/l	Sucrose level (%)	Mean number of tubers/flask		Mean weight of tubers/flask		Mean weight /tuber (g)	
			Short day (SD)	Long day (LD)	Short day (SD)	Long day (LD)	Short day (SD)	Long day (LD)
<i>In Vitro</i> Plantlets	2	4	4.3	1.8	0.817	0.108	0.19	0.06
	2	6	7.4	2.2	2.072	0.198	0.28	0.09
	2	8	8.2	2.6	2.624	0.312	0.32	0.12
	3	4	8.9	2.3	1.78	0.089	0.20	0.05
	3	6	13.6	3.0	3.944	0.33	0.29	0.11
	3	8	15.8	3.1	5.372	0.403	0.34	0.13
	4	6	17.1	3.4	5.301	0.51	0.31	0.15
	4	8	19.8	3.2	6.93	0.416	0.35	0.13
	6	8	9.3	2.1	3.255	0.189	0.35	0.09
	8	8	4.8	1.0	1.44	0.08	0.30	0.08

Table 32. Effect of Kinetin and sucrose on *in vitro* tuberization in potato cv. Ultimus (All harvests done after 12 weeks).

Explants source	Growth regulator kinetin = mg/l	Sucrose level (%)	Mean number of tubers/flask		Mean weight of tubers/flask		Mean weight /tuber (g)	
			Short day (SD)	Long day (LD)	Short day (SD)	Long day (LD)	Short day (SD)	Long day (LD)
<i>In Vitro</i> Plantlet	2	4	3.6	1.4	0.432	0.042	0.12	0.03
	2	6	4.2	1.9	0.756	0.095	0.18	0.05
	2	8	6.5	2.4	1.755	0.216	0.27	0.09
	3	4	5.8	2.2	0.87	0.066	0.15	0.03
	3	6	7.3	2.3	1.533	0.184	0.21	0.08
	3	8	13.5	2.6	3.78	0.312	0.28	0.12
	4	6	14.2	3.2	3.266	0.288	0.23	0.09
	4	8	15.1	3.8	4.379	0.608	0.29	0.16
	6	8	6.8	2.2	1.496	0.176	0.22	0.08
	8	8	2.9	1.4	0.58	0.112	0.20	0.08

Table 33. ANOVA of factors, sugar concentration (%), Kinetin concentration. (mg/l), day length and their interactive effect on the number of tubers and weight of tubers in Desiree.

Source	Degree of freedom	Mean square values	
		Number of tubers	Weight of tubers
Sugar conc. (%) (A)	1	0.29NS	1.37NS
Kinetin conc. (mg/l) (B)	2	1.92* *	2.44* *
A x B	2	0.62NS	2.21NS
Day length (C)	1	19.84* *	38.71* *
A x C	1	0.19NS	0.50NS
B x C	2	4.12* *	12.05* *
A x B x C	2	2.33* *	17.93*
Error	216	0.31	1.19

Table 34. Desiree number of tubers

Day length (C)	Sugar concentration (%) (A)	Kinetin conc. (B)			A x C
		1	2	3	
		A x B x C			
Short day	1	7.4E	13.6D	17.1B	12.7
	2	8.2E	15.8C	19.8A	14.6
Long day	1	2.2F	3.0FE	3.4F	2.87
	2	2.6F	3.1F	3.2F	2.97
		B x C			C
Short day		7.8	14.7	18.45	13.65
Long day		2.4	3.05	3.3	2.92
		A x B			A
	1	4.8D	8.3C	10.25B	7.78
	2	5.4D	9.45B	11.5A	8.78
B		6.1C	8.878	10.87A	

Table 35. Desiree weight of Tubers

Day length (C)	Sugar concentration (%) (A)	Kinetin conc. (B)			A x C
		1	2	3	
		A x B x C			
Long day	1	2.07E	3.94C	5.30B	3.77
	2	2.62D	5.37B	6.93A	4.97
Long day	1	0.20G	0.33FG	0.51FG	0.35
	2	0.71F	0.40FG	0.42FG	0.51
		B x C			C
Short day		2.35C	4.66B	6.11A	4.37
Long day		0.46D	0.37D	0.46D	0.43
		A x B			A
		1.14	2.14	2.91	2.06
		1.67	2.84	3.67	2.74
B		1.4C	2.51B	3.29A	

Table 36. ANOVA of factors, sugar concentration (%), Kinetin concentration (mg/l), day length and their interactive effect on the number of tubers and weight of tubers in Ultimus.

Source	Degree of freedom	Mean square values	
		Number of tubers	Weight of tubers
Sugar conc. (%) (A)	1	5.35 *	7.84 **
Kinetin conc. (mg/l) (B)	2	624.87 **	228.64 **
A x B	2	14.02 **	27.37 **
Day length (C)	1	4860.00 **	790.08 **
A x C	1	1.067NS	0.59NS
B x C	2	6.067NS	2.99NS
A x B x C	2	9.82 *	7.43 *
Error	216	2.526	2.883

* = Significant at 5% level of significance.

** = Significant up to 1% level of significance.

NS = Non significant

Table 37. Ultimus number of Tubers

Day length	Sugar concentration (%) (A)	Kinetin conc. (B)			A x C
		1	2	3	
		A x B x C			
Short day	1	4.20D	7.30C	14.20AB	8.57
	2	6.50C	13.50B	15.10A	11.7
Long day	1	1.9E	2.3E	3.1DE	2.47
	2	2.4E	3.60DE	3.8DE	3.27
		B x C			C
Long day		5.35	10.4	14.65	10.16
Short day		2.15	2.95	3.5	2.67
		A x B			A
	1	2.15	2.95	3.5	2.67
	2	4.45B	8.55A	9.45A	7.483
B		3.75C	6.67B	9.07A	

Table 38. Ultimus weight of Tubers

Day length (C)	Sugar conc. (%) (A)	Kinetin conc. (B)			A x C
		1	2	3	
		A x B x C			
Long day	1	0.66E	1.53D	3.27B	1.82
	2	2.06CD	3.78A	3.98A	3.27
Long day	1	0.1F	1.8D	0.29EF	0.19
	2	0.22EF	0.31EF	0.61E	0.38
		B x C			C
Short day		1.35C	2.66B	3.62A	2.54
Long day		0.16E	0.49D	0.49D	0.28
		A x B			A
	1	0.36	0.86	1.78	1
	2	1.14	2.05	2.29	1.83
B		0.75C	1.45B	2.04A	

Mean Wt. of tubers/tuber (g)



Fig. 61. Effect of Kinetin (10, 20, 30 ppm) and different day lengths on mean weight of tubers per tuber in potato cv. Ultimus.

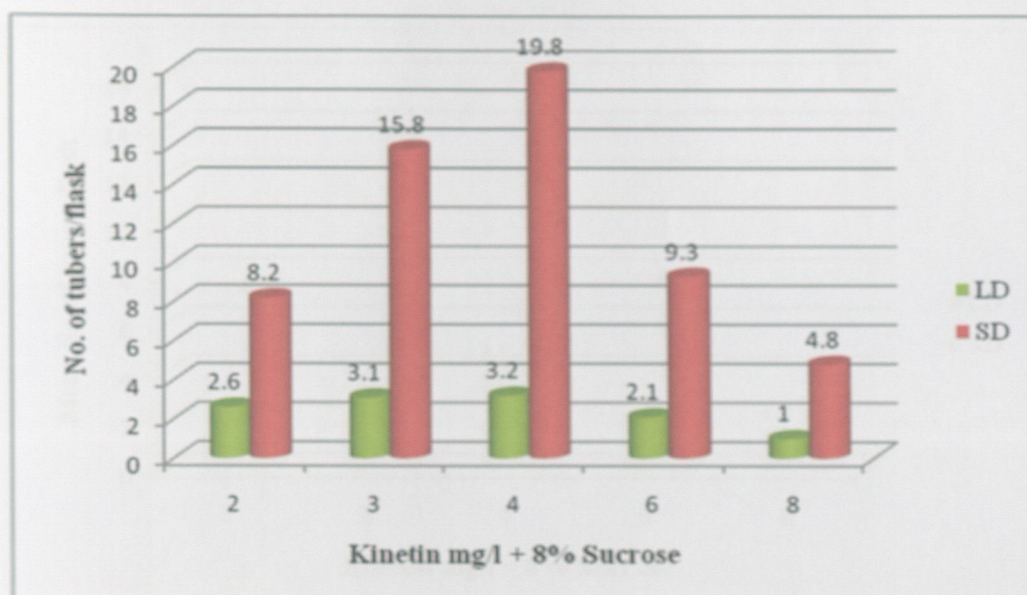


Fig. 60. Effect of Kinetin+8% sucrose and different photoperiod on mean number of tubers per flask in potato cv. Desiree.

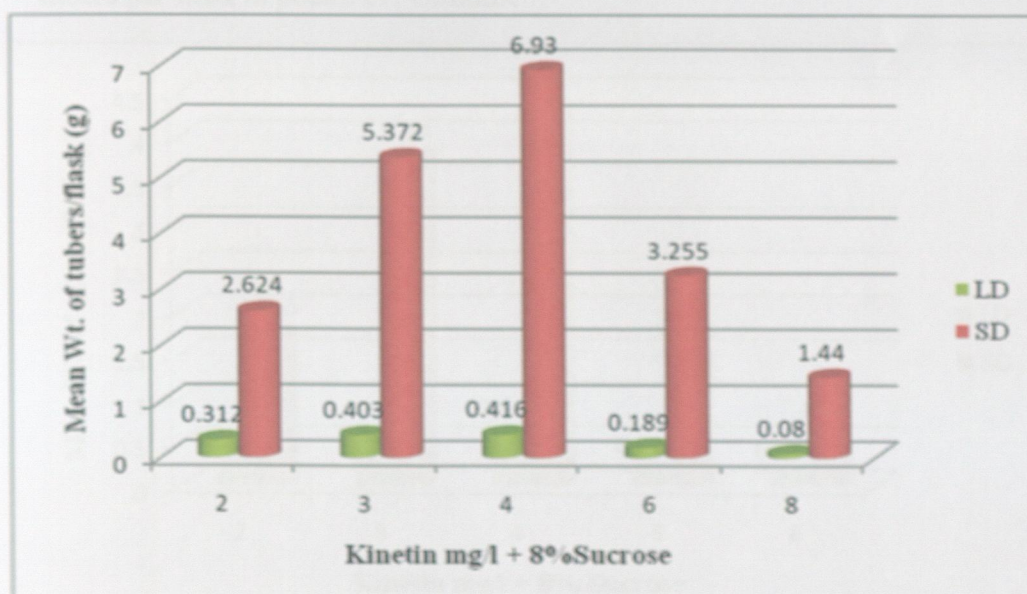


Fig. 61. Effect of Kinetin+8% sucrose and different photoperiod on mean weight of tubers per flask in potato cv. Desiree.

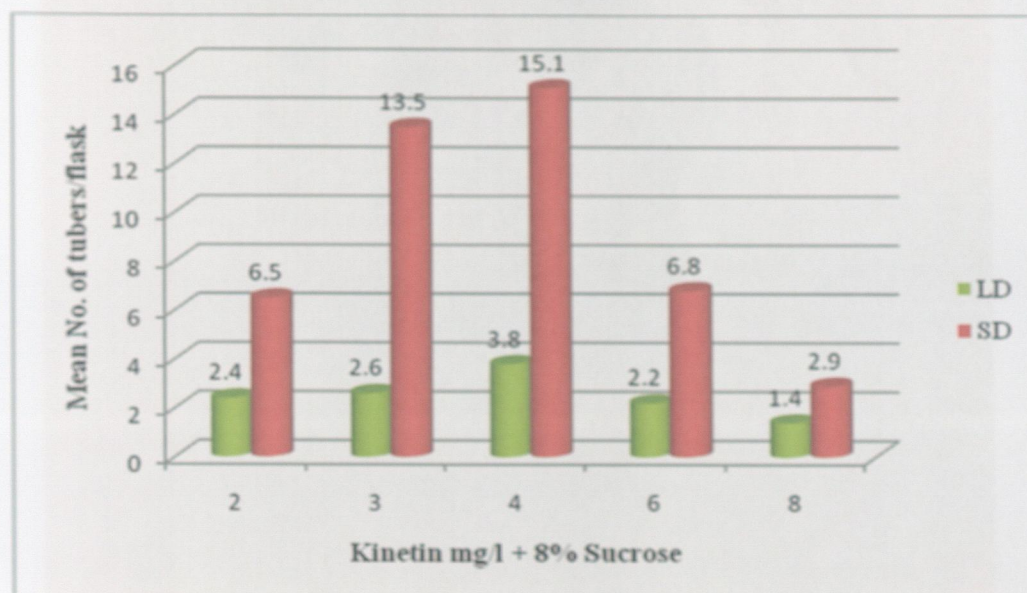


Fig. 62. Effect of Kinetin+8% sucrose and different photoperiod on mean number of tubers per flask in potato cv. Ultimus.

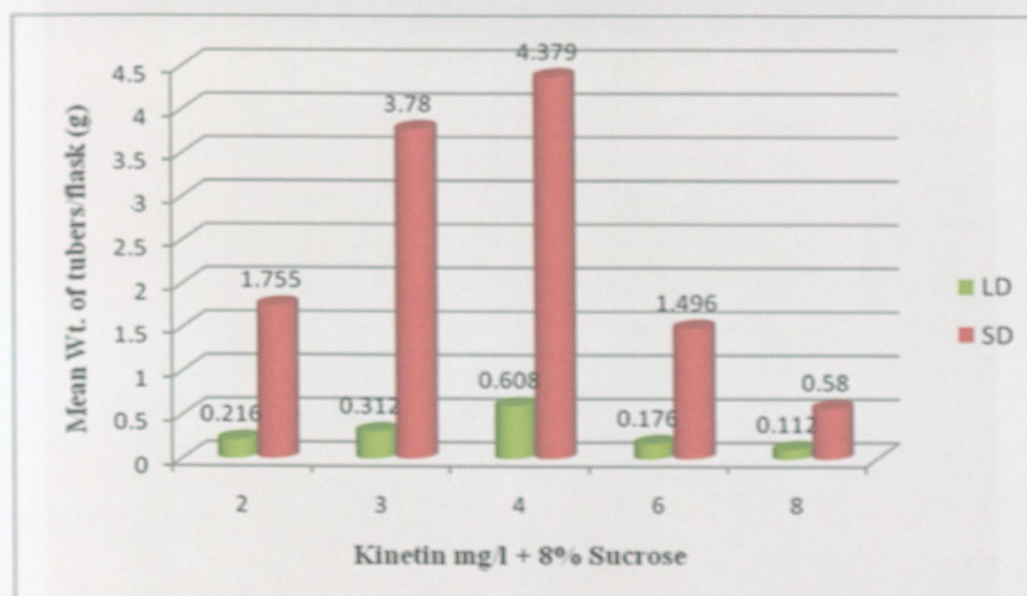


Fig. 63. Effect of Kinetin+8% sucrose and different photoperiod on mean weight of tubers per flask in potato cv. Ultimus.



a. Desiree



b. Ultimus

Figs. 64 a, b. Upright shoot and tuber formation on medium supplemented with 2mg/l K and 4% sucrose under long day conditions.

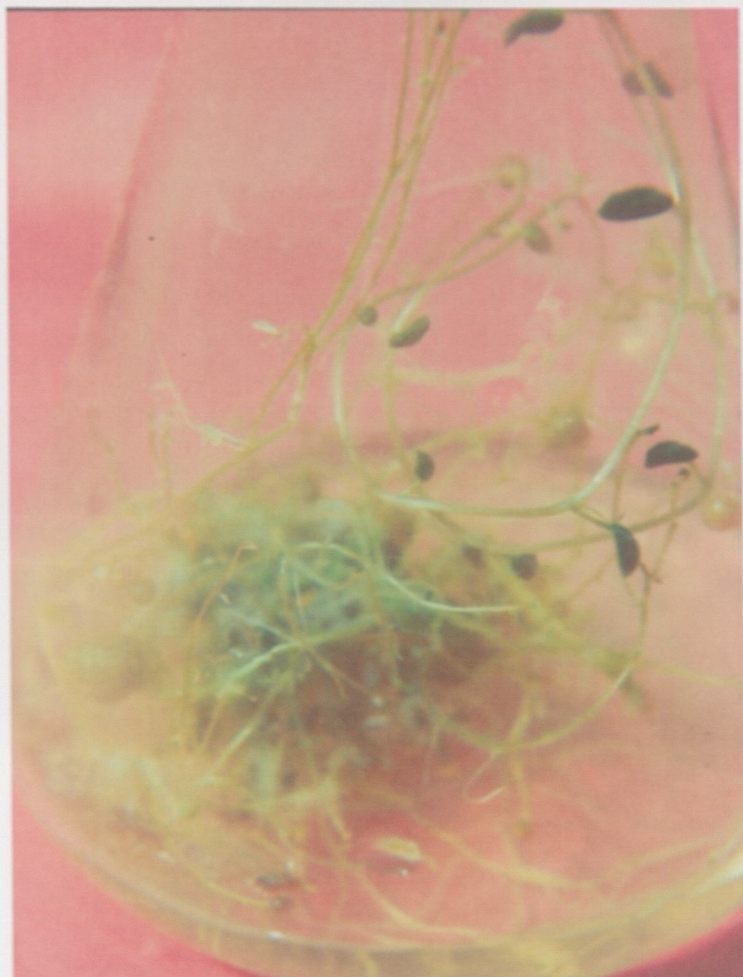
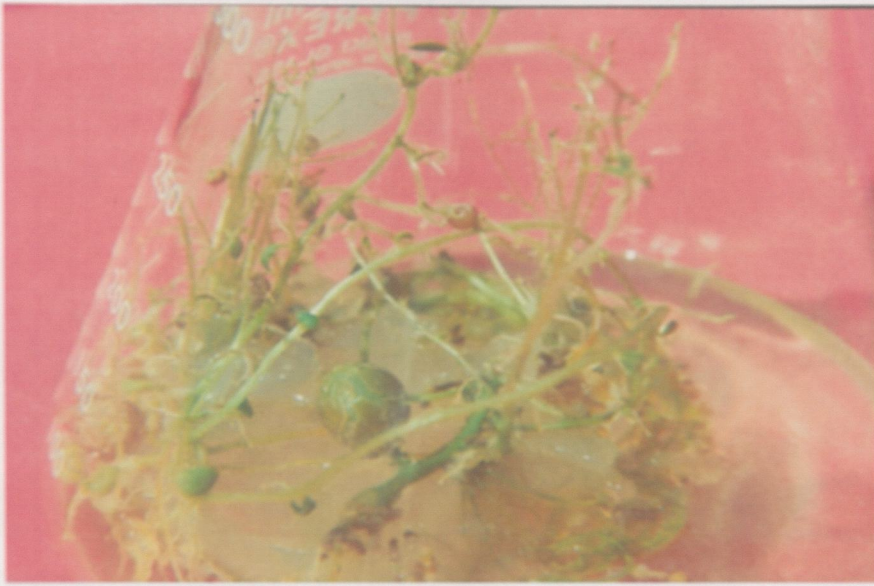
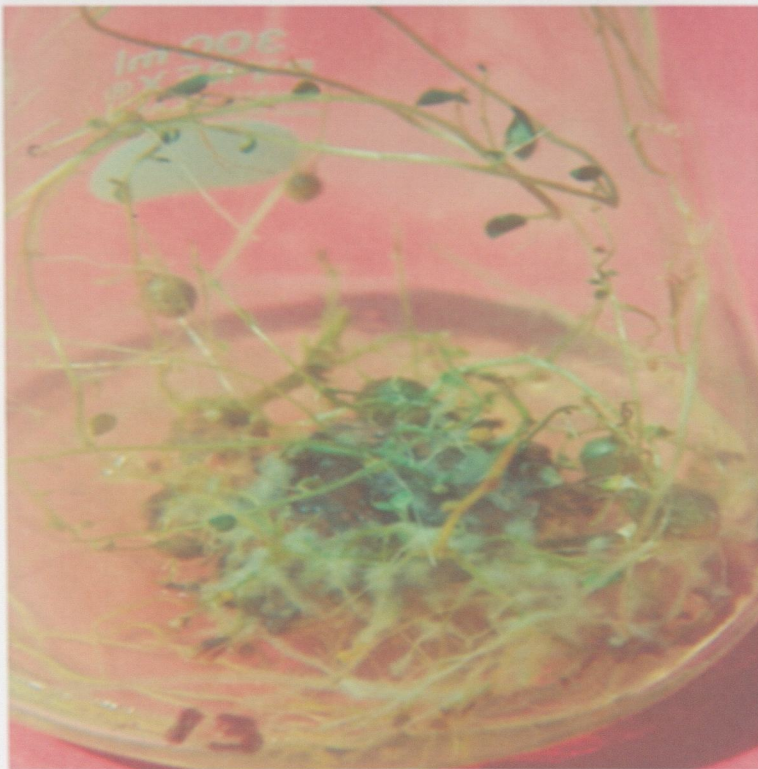


Fig. 65. Multiple shoot and tuber formation on medium supplemented with 2.0mg/l K and 4% sucrose under short day conditions in the cv. Desiree.



a. Desiree



b. Ultimus

Figs. 66 a, b. Tuber induction on medium supplemented with 2.0mg/l K and 6% sucrose under short days.



a. Desiree



b. Ultimus

Fig. 67a, b. Tuberization in potato on medium supplemented with 3mg/l Kand 8% sucrose under short days.

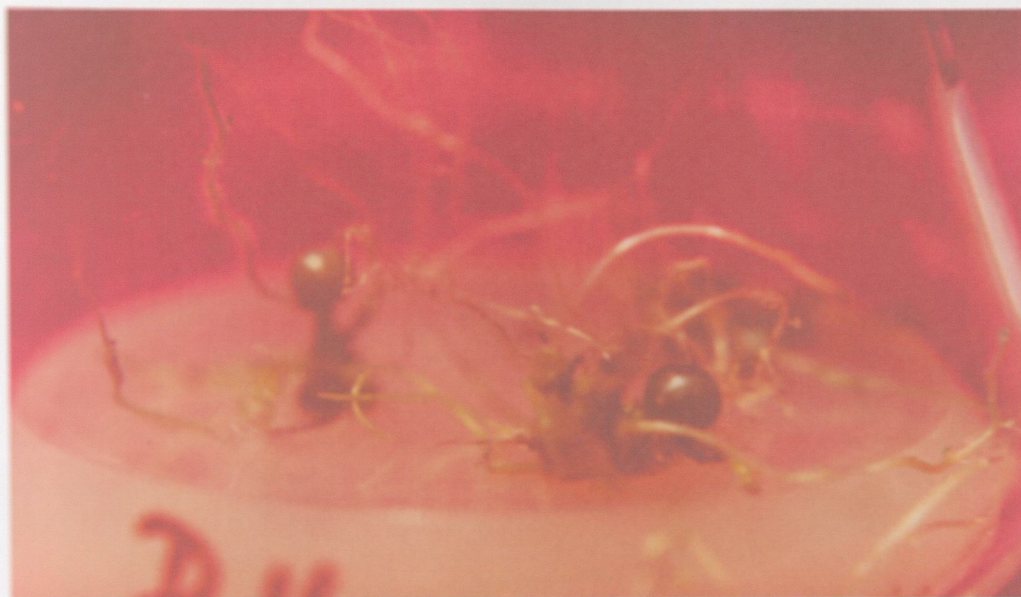


Fig. 68. Tuberization in Desiree on medium supplemented with 4mg/l K and 6% sucrose under short day conditions.



Fig. 69. Tuberization in Desiree on medium supplemented with 6.0mg/ K and 8% sucrose under short day conditions.

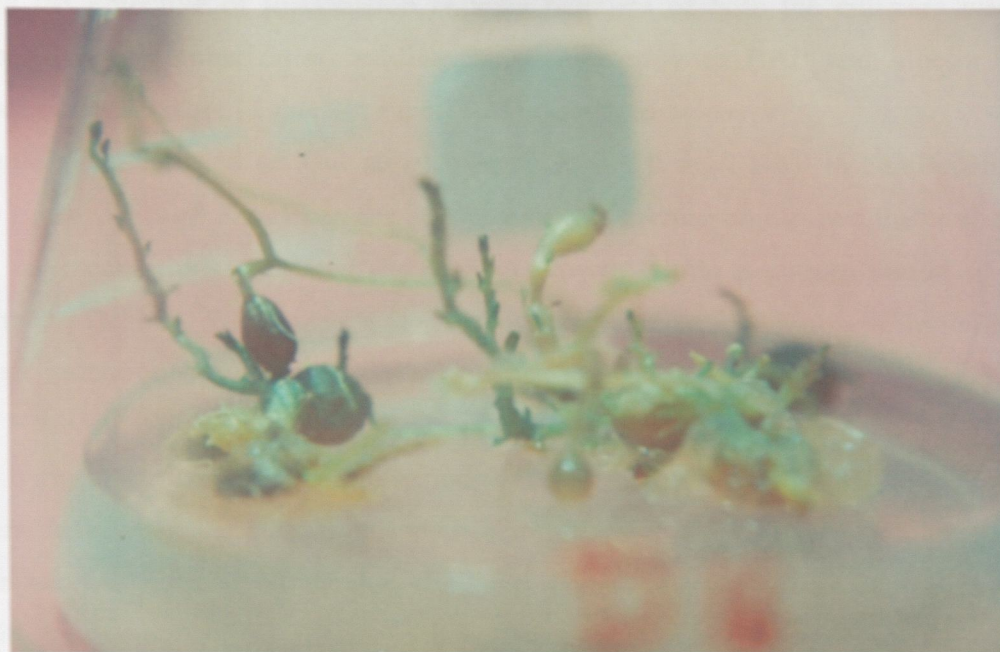


Fig. 70. Another view of figure 69.

Combined effect of BAP and Kinetin on Tuberization

With the objective of tuberization *in vitro* plantlets of both the cultivars were further subjected to a tuberization medium supplemented with BAP and kinetin in combination. The results are (Table 39; Figs 71-74) discussed below.

Plantlets sub-cultured on BM supplemented with 2.0 mg/l BAP in combination with either 1.0 or 2.0 mg/l Kinetin in the presence of 8% sucrose and grown under short day (8 hrs day) conditions exhibited some shoot growth within a culture period of 6 weeks in the cv. Desiree. While only a few shoots were formed in Ultimus within eight weeks. Tuber induction occurred in both the cultivars after the eight week of culture. An average of 3 tubers/flask were noticed in Desiree on basal medium added with 2.0 mg/l BAP and 1.0 mg/l kinetin after a culture period of 9 weeks which increased to an average of 7.8 tubers/flask when the culture period was prolonged to ten weeks. There after no further increase was noticed. Somewhat similar response was also observed in the cv Ultimus with an average of 5.9 tubers/flask produced within 10 weeks of culture. The tubers thus produced were small, somewhat rounded, with 2-3 eyes and of a greenish color (Figs. 75a and 75b).

Increase in the kinetin level to 2.0 mg/l in combination with 2.0 mg/l BAP, caused decline in the tuber number in both the cultivars (Table 39). Resultantly, it could be deduced that BAP and kinetin when used in combination failed to give promising results as far as tuber yield is concerned. However, it promoted shoot growth rather than tuber induction.

Effect of AS in Combination with BAP on Tuberization

To promote a higher tuber yield/flask, the plantlets were further subjected to tuberization media, i.e. MS basal medium supplemented either with 6.0 or 8.0 mg/l BAP in combination with 10.0, 15.0 and 20.0 mg/l AS and 8% sucrose. The cultures were kept under both short (8hrs) and long (16 hrs) photoperiods. No positive response towards tuberization was observed after 4 weeks in any of the photoperiod. However after the 7th week some tuberization was observed in Desiree followed by Ultimus in short day treatment on a medium supplemented with 8.0 mg/l BAP in combination with 10mg/l AS. In both the cultivars, the number of tubers formed on this set of combination was

very low. An average of 4.2 tubers/ flask was achieved in Desiree and 4.0 tubers/flask in Ultimus after 14 weeks in the short (8 hrs) photoperiod. The tubers thus obtained in both the cultivars were elongated and narrow. Each tuber had 5–7 eyes with sprouting buds in them (Figs. 76a and 76b) and Fig. 77a and 77b). The tuberization response in long days was even slower. Tuberization occurred after the 10th week producing only 1.3 tubers/ flask in Desiree and 0.9 tubers/ flask in Ultimus within 12 weeks of culture. There was no further increase in the number of tubers in any of the two cultivars even when the culture period was prolonged to 12 weeks. However, vegetative growth continued in these cultures (Figs. 78-79).

The response of higher concentration of AS (15.0 and 20.0 mg/l) in combination with BAP at 6.0 and 8.0 mg/l level towards tuberization was even poorer in both short and in long days. Negligible amount of tubers were obtained from both the cultivars within 12 weeks in the short day cultures at these combinations (Table 39; Figs. 80 and 81). However, some multiple shoot formation occurred in these cultures.

No tuberization was observed in either of the two cultivars at 15.0 and 20.0 mg/l AS in combination with BAP at 6.0 or 8.0 mg/l and 8% sucrose in the long (16hrs) photoperiods, even after a culture period of 12 weeks.

The ANOVA (Table 40-42) showed that the potato cultivar had non-significant effect while BAP concentration and AS concentration has highly significant effect on number and weight of tubers while their interaction has non-significant effect except for interaction of potato cultivars and AS concentration, that has significant effect on the weight of tubers.

It is concluded that the cytokinin AS did not prove good as a tuber inducer.

Table 39. Combined effect of Cytokinins on *in vitro* tuberization in potato in short day lengths (8 hrs day).

Cultivar	Growth regulator (mg/l)			Sucrose (%)age	Culture period in weeks	Mean number of tuber/flask	Mean wt. of tubers/flask (g)	Mean wt./tuber (g)
	BAP	Kinetin	AS					
Desiree	2.0	1.0	-	8	9	3	0.27	0.09
	2.0	1.0	-	8	12	7.8	1.17	0.15
	2.0	2.0	-	8	12	4.5	0.72	0.16
	6.0	-	10	8	12	3.1	0.372	0.12
	6.0	-	15	8	12	2.8	0.392	0.14
	6.0	-	20	8	12	1.2	0.168	0.14
	8.0	-	10	8	12	4.2	0.798	0.19
	8.0	-	15	8	12	3.4	0.578	0.17
	8.0	-	20	8	12	1.9	0.323	0.17
Ultimus	2.0	1.0	-	8	9	3.1	0.217	0.07
	2.0	1.0	-	8	12	5.9	0.708	0.12
	2.0	2.0	-	8	12	3.5	0.42	0.12
	6	-	10	8	12	3.0	0.33	0.11
	6	-	15	8	12	2.9	0.377	0.13
	6	0	20	8	12	1.5	0.15	0.10
	8	10	10	8	12	4.0	0.76	0.19
	8	15	15	8	12	3.1	0.558	0.18
	8	-	20	8	12	1.4	0.182	0.13

Table 40. ANOVA of factors, potato cultivar, BAP concentration (mg/l), AS concentration (mg/l) and their interactive effect on the number of tubers and weight of tubers.

Source	Degree of freedom	Mean square values	
		Number of tubers	Weight of tubers
Potato cultivar (A)	1	0.41NS	0.03NS
BAPConc.(mg/l) (B)	1	1021**	1.43**
A x B	1	1.41NS	0.00NS
AS Conc. (mg/l) (C)	2	46.56**	1.26**
A x C	2	0.00NS	0.02NS
B x C	2	1.66NS	0.21**
A x B x C	2	0.31NS	0.03NS
Error	108	81.70	0.02

* = Significant at 5% level of significance

** = Significant up to 1% level of significance

NS = Non significant

Table 41. Mean values of Potato cultivar, BAP concentration (mg/l), AS concentration (mg/l) and their interactive effect on the number of tubers.

Cultivar (A)	BAP conc. (mg/l) (B)	AS Conc. (mg/l) (C)			A x B
		10	15	20	
		A x B x C			
Desiree	2* *	3.10	2.80	1.35	2.37
	6	4.20	3.40	1.90	3.17
Ultimus	2	3.00	2.90	1.50	2.47
	6	4.00	3.10	1.40	2.83
		A x C			A
Desiree		3.65	3.10	1.55	2.77
Ultimus		3.50	3.00	1.45	2.65
		B x C			B
	2	3.05	2.85	1.35	2.42B
	6	4.10	3.25	1.65	3.00A
C		3.56A	3.05B	1.50C	

LSD values for

AS concentration = 0.385

At 5% level of significance

Table 42. Mean values of Potato cultivar, BAP concentration (mg/l) AS concentration (mg/l) and their interactive effect on the weight of tubers.

Cultivar (A)	BAP conc. (mg/l) (B)	AS Conc. (mg/l) (C)			A x B
		10	15	20	
		A x B x C			
Desiree	2**	0.37	0.39	0.17	0.31
	6	0.70	0.58	0.32	0.53
Ultimus	2	0.33	0.38	0.15	0.29
	6	0.67	0.56	0.18	0.50
		A x C			A
Desiree		0.54	0.49	0.25	0.42
Ultimus		0.55	0.48	0.17	0.39
		B x C			B
	2	0.35C	0.38C	0.16E	0.30B
	6	0.73A	0.57B	0.25D	0.52A
C		0.54A	0.48B	0.21C	

LSD values for AS concentration = 0.054
 B x C = 0.077 At 5% level of significance.

Fig. 72. Effect of Adenine sulfate + 6 mg/l BAP on mean weight of tubers per disk in potato cvs. Desiree and Ultimus.

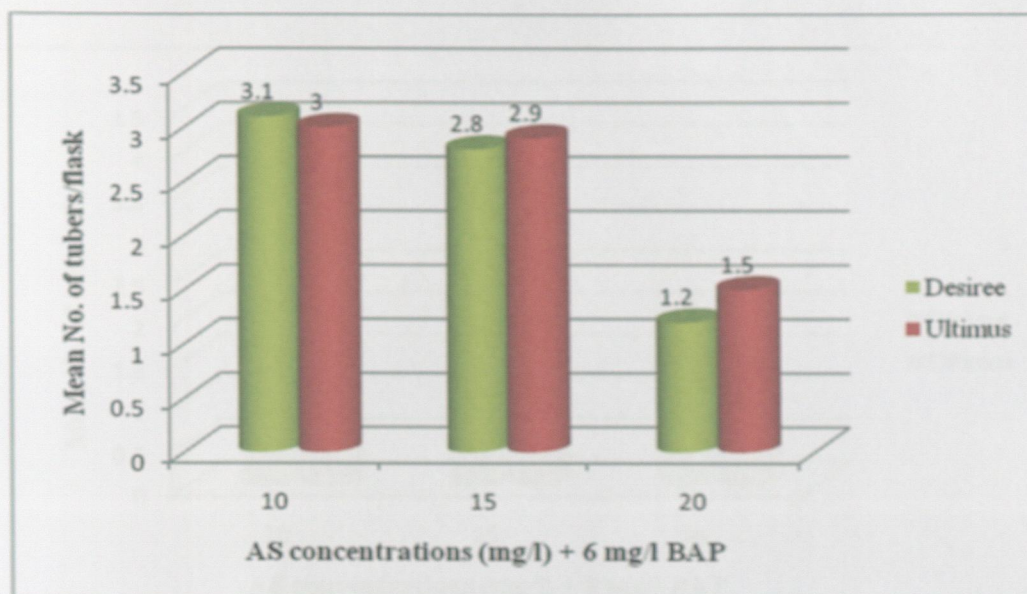


Fig. 71. Effect of Adenine sulfate + 6 mg/l BAP on mean number of tubers per flask in potato cvs. Desiree and Ultimus.

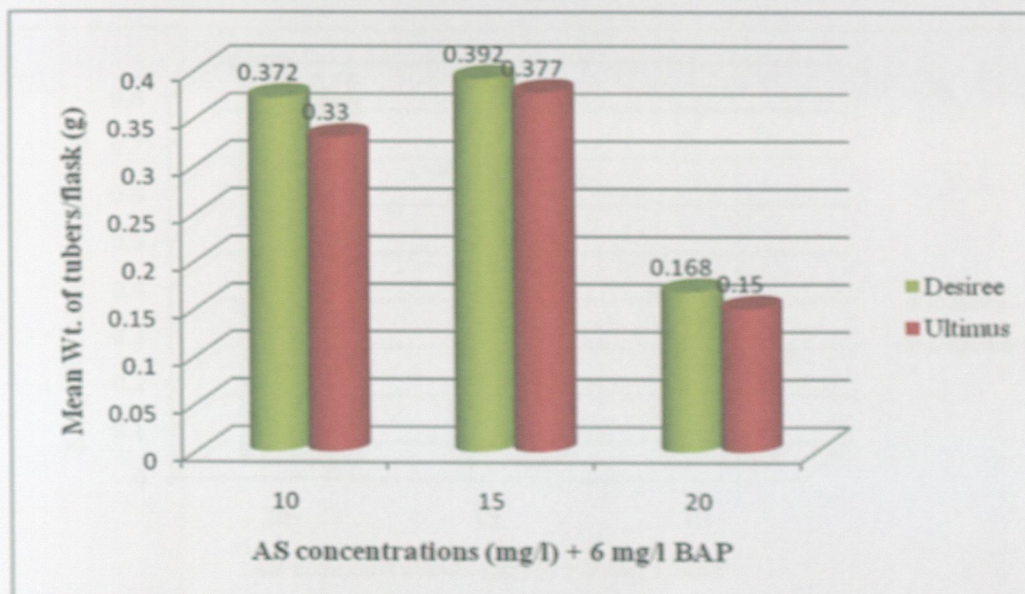


Fig. 72. Effect of Adenine sulfate + 6 mg/l BAP on mean weight of tubers per flask in potato cvs. Desiree and Ultimus.

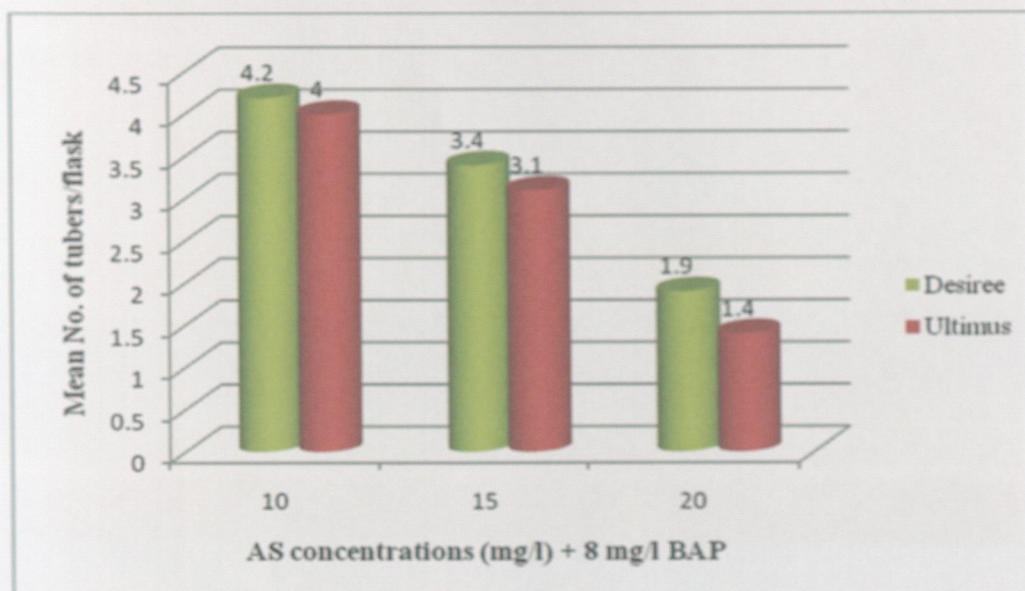


Fig. 73. Effect of Adenine sulfate + 8 mg/l BAP on mean number of tubers per flask in potato cvs. Desiree and Ultimus.

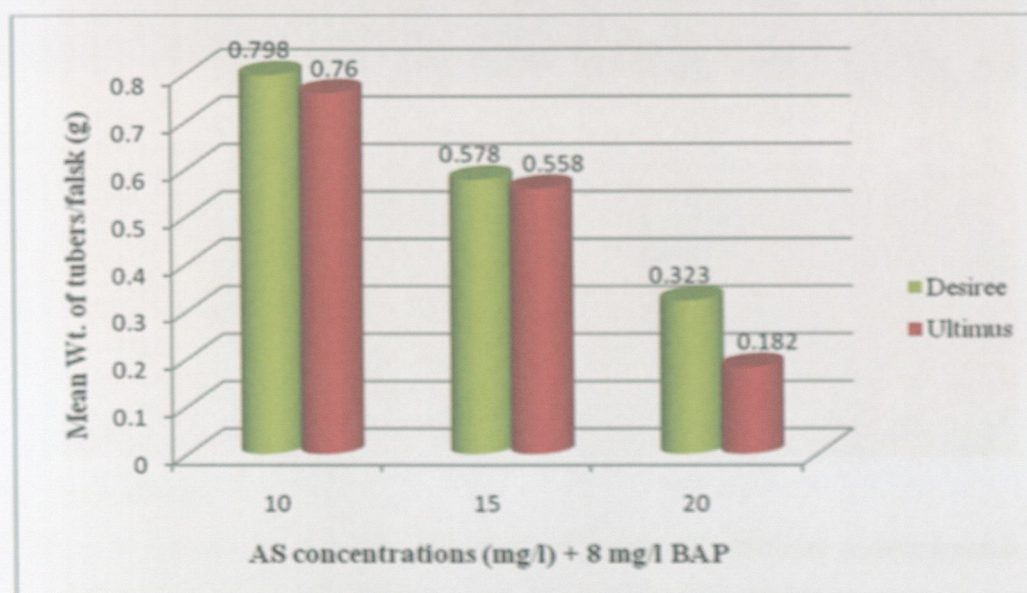
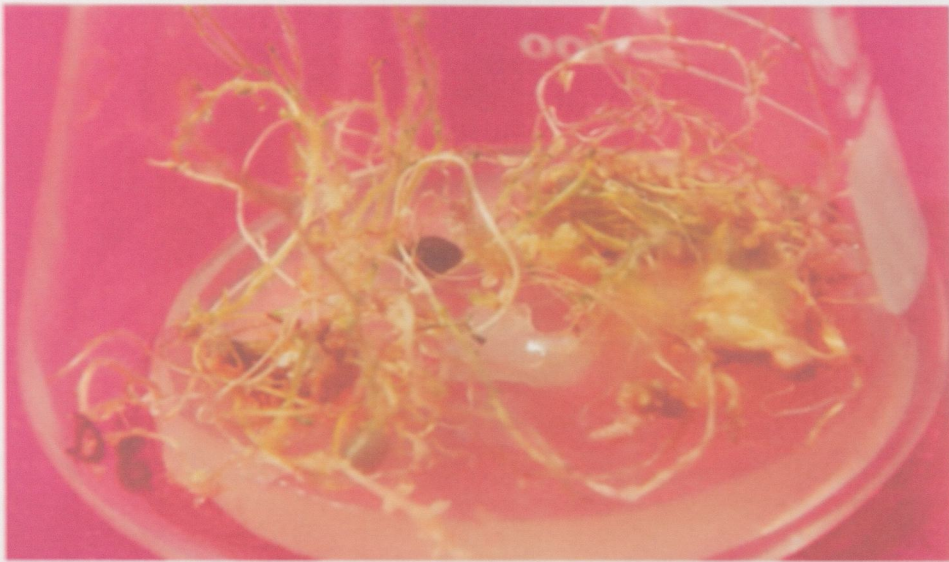
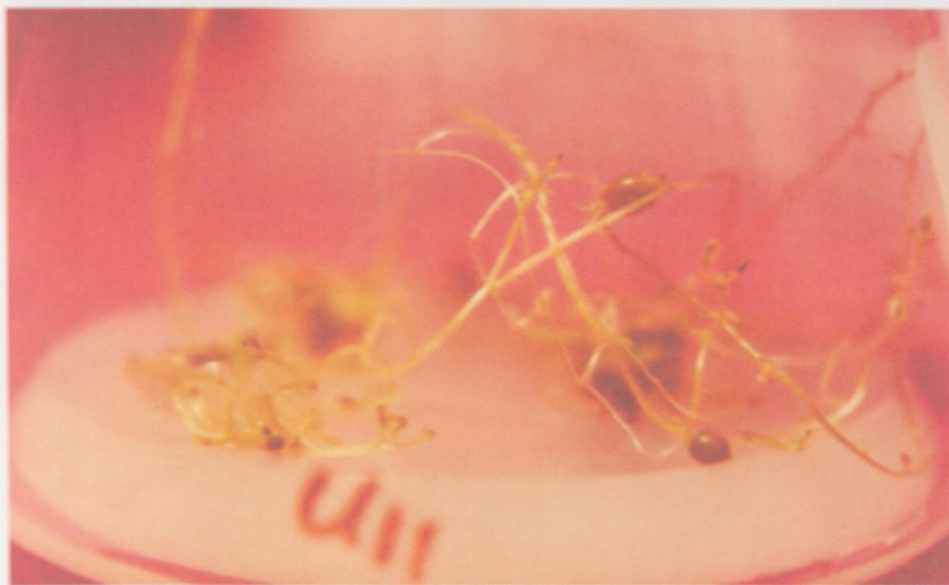


Fig. 74. Effect of Adenine sulfate + 8 mg/l BAP on mean weight of tubers per flask in potato cvs. Desiree and Ultimus.

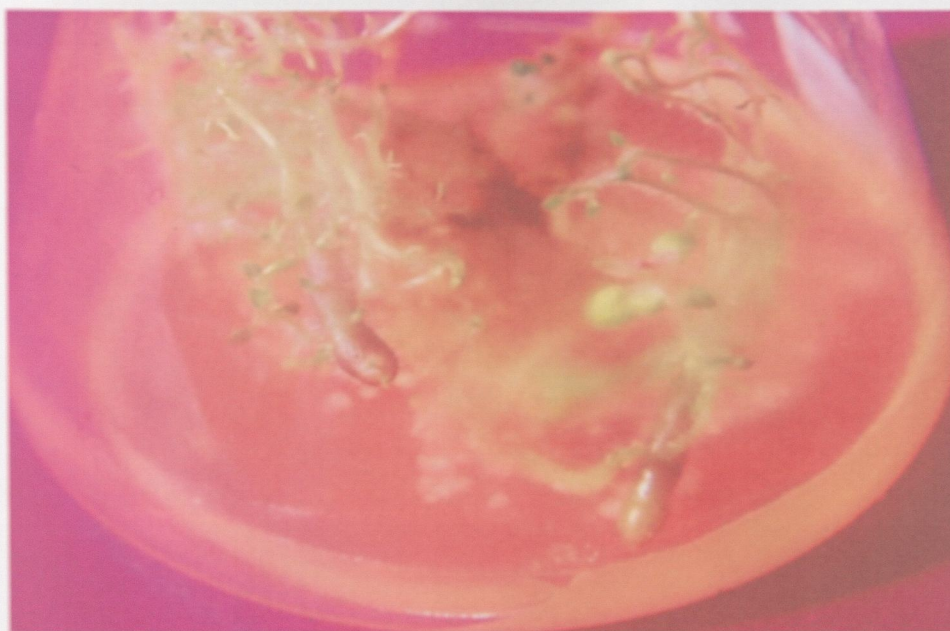


a. Desiree



b. Ultimus

Figs. 75 a, b. Vegetative growth and tuber induction on medium supplemented with 2.0mg/l BAP and 1.0mg/l kinetin in the presence of 8% sucrose and grown under short photoperiod.

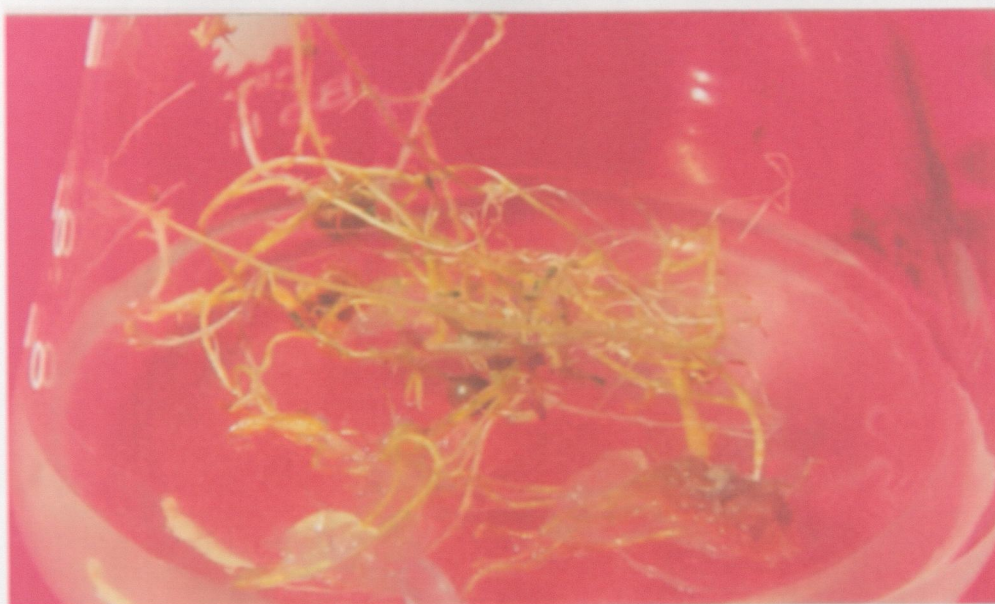


a. Desiree



b. Ultimus

Figs. 76a, b. Long and narrow tubers with sprouting buds could be obtained in both the cultivars on medium supplemented with 6.0mg/l BAP and 10mg/l AS under 8h photoperiod.



a. Desiree



b. Ultimus

Figs. 77a, b. Tuber induction in potato on medium supplemented with 8.0mg/l BAP, 10mg/l AS and 8% sucrose under 8h photoperiod.



Fig. 78. A single tuber could be seen in the long (16h) day cultures on medium supplemented with 6.0mg/l BAP and 10mg/l. AS in potato cv Desiree.



Fig. 79. No tuberization could be seen in the long day cultures on medium supplemented with 6.0mg/l BAP and 10mg/l AS in potato cv Ultimus.



Fig. 80. Tuber induction in Desiree observed on medium supplemented with 8mg/l BAP and 15mg/l AS under short (8h) day lengths.

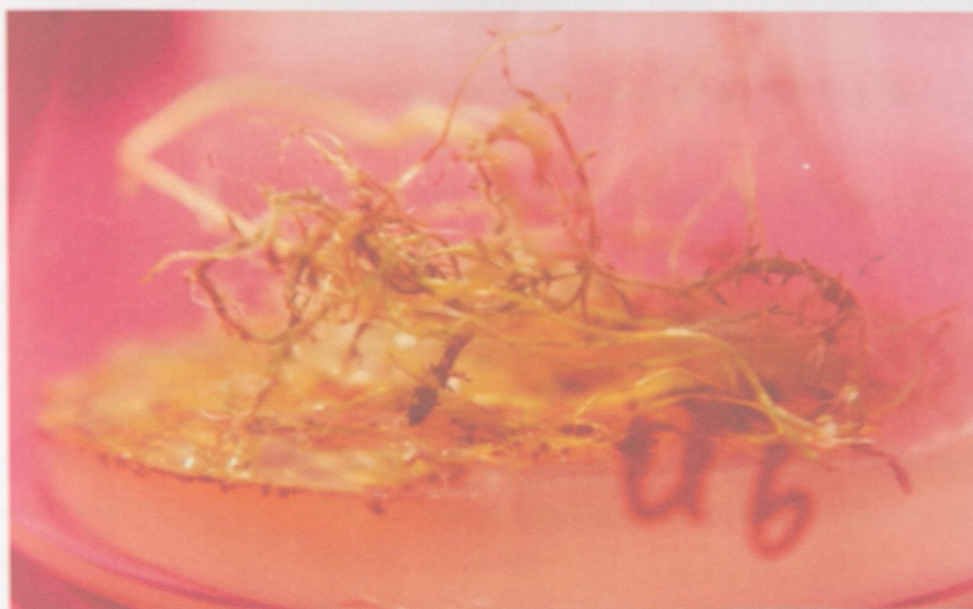


Fig. 81. Tubers very thin and elongated could be obtained in cv Ultimus on medium supplemented with 8.0mg/l BAP and 20mg/l AS under short day lengths.

Effect of IAA on Tuberization

Stem cuttings with 3-4 nodes obtained from the *in vitro* raised plantlets were cultured in tuberization medium, which was composed of MS medium at full strength incorporated with either 0.5 or 1.0 mg/l IAA and 8% sucrose. The cultures were incubated in short day lengths (8 hrs light/day) at a temperature of $25 \pm 1^{\circ}\text{C}$. The results (Table 43) showed that no response towards tuberization could be observed within the first four weeks of culture period. Some rooting together with few stoloniferous shoot formation was observed during this period in both the cultivars. Tuberization was observed after the sixth and seventh week of culture in Desiree and Ultimus respectively on a medium supplemented with 1.0 mg/l IAA. The over all yield of micro tubers was determined either by the weight of the tubers formed or their number.

In this set of experiment, the addition of IAA to the medium did not improve the tuber number per flask as poor numbers of tubers were formed. Although the tuber number could not be increased any further with an increase in the culture period up to 12 weeks, yet the tuber weight was greatly increased in both the cultivars (Figs. 82a and 82b).

The response of tuberization was less pronounced when the IAA level was lowered down to 0.5mg/l in the tuber induction medium (MS + 8% sucrose). Tuber formation was observed after the ninth week of culture period. The number of tubers formed were also less compared to those obtained on 1.0 mg/l of IAA (Table 43); (Figs. 83a and 83b).

The results obtained by Konstantinova *et al.* (1999) and Romanov *et al.* (2000) are in line with the present results. They studied the effect of IAA and kinetin on tuberization parameters of different cultivars of potato. IAA predominantly increased the tuber size while kinetin increased the number of tubers, which exactly coincides to the present studies.

Zhijun *et al.* (2005) in a study on microtuberization reported that IAA at 10 mg/l when added to a medium containing BAP increased both the weight and diameter of microtubers.

Conclusion

It could therefore, be concluded that the addition of IAA to the culture medium increased the tuber weight, whereas its effect on the number of tubers/flask was less pronounced.

		Concentration (mg/l)	1 st week	2 nd week	3 rd week	4 th week	5 th week	Remarks
IAA	0.5	0.5	8	12	68	1.894	0.78	Tubers initiated after the 3 rd week of culture period.
		1.0	8	12	14.2	3.472	0.31	Tuberization occurred after the 4 th week of incubation.
		0.1	6	12	5	-	-	Spore formation occurred after the 4 th week of culture. No signs of tuberization even after 5 weeks.
	GA ₃	0.5	6	12	5	-	-	Upright shoots sprouted within 4 weeks of culture. No tuberization.
		1.0	6	12	-	-	-	Protruding shoot formation observed with no tubers.
		0.5	8	12	-	-	-	Even increased level of hormone could not aid in tuberization.
IAA	0.5	0.5	8	12	44	1.238	0.28	Tuberization observed after the 4 th week of culture.
		1.0	8	12	9	-	-	Shoot elongated growth. No tuberization.
		1.0	8	12	9.1	3.068	0.11	Tuberization observed with the 7 th week of culture.
	GA ₃	0.1	6	12	-	-	-	Shoot formation occurred after the 4 th week of culture. No tuberization observed.
		0.5	6	12	-	-	-	Protruding of the shoot/inflorescence turning into upright shoots. No tuberization.
		1.0	6	12	-	-	-	Protruding of the shoot/inflorescence turning into upright shoot. No tuberization.
GA ₃	0.5	0.5	8	12	-	-	-	No tuberization even at 8th week.

Table 43. Effect of different growth regulator on *in vitro* tuberization in potato.

Cultivars	Growth	Regulator mg/l concentration	Sucrose %age	Culture period in weeks	Mean number of tubers /flask	Mean wt of tubers/ flask	Mean tubers/ flask (g)	General
Desiree	IAA	0.5	8	12	6.8	1.904	0.28	Tubers induced after the 8 th week of culture period.
		1.0	8	12	11.2	3.472	0.31	Tuberization occurred after the 6 th week of inoculation.
	GA ₃	0.1	6	12	-	-	-	Shoot formation occurred after the 4 th week of culture. No signs of tuberization even after 16 weeks.
		0.5	6	12	-	-	-	Upright shoots formed within 4 weeks of culture. No tuberization.
		1.0	6	12	-	-	-	Prolific shoot formation observed with no tubers.
		0.5	8	12	-	-	-	Even increased level of sucrose could not aid in tuberization.
Ultimus	IAA	0.5	8	4	-	-	-	Veg. growth together with some rooting.
		0.5	8	12	4.6	1.288	0.28	Tuberization observed after the eight week of culture.
		1.0	8	9	-	-	-	Some vegetative growth. No tuberization.
		1.0	8	12	9.1	3.003	0.33	Tuberization observed with the 7 th week of culture.
	GA ₃	0.1	6	12	-	-	-	Shoot formation observed after the 4 th week of culture. No tuberization observed.
		0.5	6	12	-	-	-	Prolific of the stoloniferous turning into upright shoots. No tuberization.
		1.0	6	12	-	-	-	Elongation of the stoloniferous turning into upright shoot. No tuberization.
		0.5	8	12	-	-	-	No tuberization even at 8% sucrose.

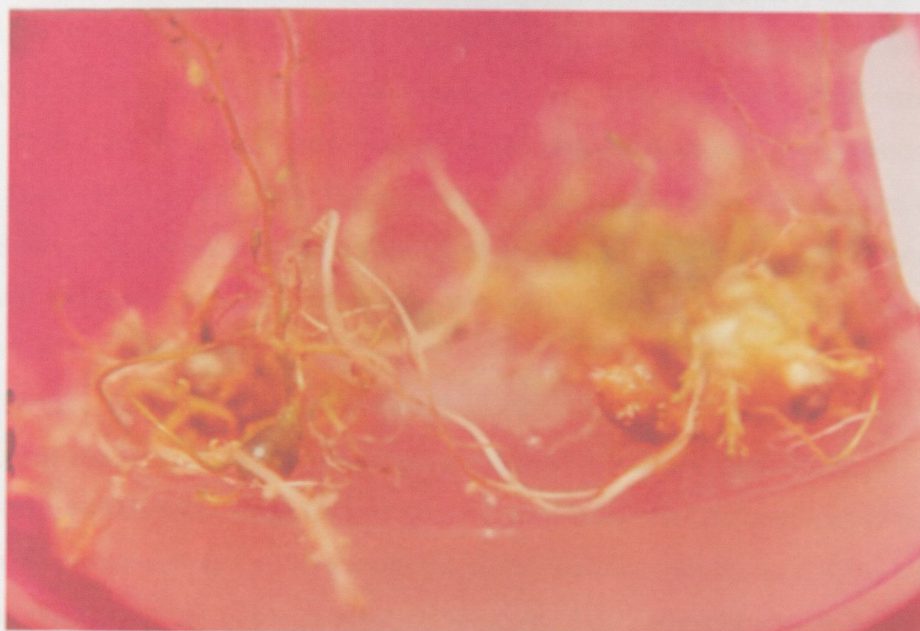


a. Desiree



b. Ultimus

Figs. 82 a, b. Tuberization in potato on BM supplemented with 1.0mg/l IAA and 8% sucrose under short day (8h) conditions.



a. Desiree.



b. Ultimus

Figs. 83a, b. Tuber number decreased with a decrease in IAA level to 0.5mg/l.

Effect of GA₃ on Tuberization

In an attempt to induce early tuberization and obtain a high tuber yield, GA₃ was tested in this set of experiment. Plantlets of both the cultivars were therefore, transferred from the propagation medium and subcultured on MS medium (full strength) incorporated with GA₃ at 0.1, 0.5 and 1.0mg/l levels and 6% sucrose. The cultures were grown in 8 hour days at 25±1°C. Tuberization could not be observed in any of the concentrations used in both the cultivars for up to ten weeks (Table 43). The added GA₃ in the medium however enhanced shoot growth and an elongation of the previously formed stoloniferous shoots.

In a further experiment, single nodes of cv.s Desiree and Ultimus were subcultured on basal medium (full MS) containing 1.0 mg/l BAP for four weeks. All nodes produced stolons which were beginning to grow downwards into the medium (Figs. 84a and 84b). On a further subculture to a medium incorporated with 1.0 mg/l GA₃ led to a reversal of the change in morphology brought about by BAP. Upright shoots grew out of the nodes in both the cultivars (Figs. 85a and 85b). No tubers were formed on these GA₃ treated cultivars, even after a culture period of 10 weeks.

Still further in another effort the plants of cv. Desiree which were already induced to tuberize on a tuberization medium (BAP + high sucrose level) were further subcultured to a medium supplemented with 0.5 mg/l of GA₃. The previously formed tubers (on tuberization medium) could not develop any further. Moreover, sprouting of the tubers could be observed at a very juvenile stage (Figs. 86a and 86b).

GA₃ was withdrawn as it channels all the carbohydrates towards shoot development. Decrease in GA₃ promotes partitioning of biomass to the tubers. Hence tuber induction could be achieved by withdrawal of GA₃ and addition of GA₃ inhibitor.

Zaki (1997) exactly obtained similar results with GA₃ treated plants. Tuber formation was hampered due to prolonged exposure of the plantlets to GA₃.

The results obtained by Zhang *et al.* (2006) are surprisingly different from our findings. They obtained maximum tuber yield in a medium containing BAP+IAA+GA₃. It may be that BAP which is an anti-gibberellins may be masking the effect of GA₃.

Gibberellins are major regulator of potato tuber formation. They inhibit tuberization, cause stolons to elongate rather than to swell, inhibit starch accumulation

and the synthesis of tuber specific proteins. The endogenous levels of GA_3 are low under conditions favoring tuberization, and high under non-inductive conditions.

Gibberellins can affect carbohydrate metabolism. GA_3 reduces the activity of ADP-GLC pyrophosphorylase and thus starch synthesis but increase the activity of UDPG pyrophosphorylase, an enzyme involved in the production of nucleotide sugars that can be used in cell wall synthesis. Hence tuber induction could be achieved by withdrawal of GA_3 and addition of GA_3 inhibitor.

Conclusion

It was therefore concluded that the addition of GA_3 in the medium was of no added advantage either in tuber production or promoting its further growth. However it promoted shoot growth in both the cultivars and moreover caused the stolons to elongate rather than to swell.



Fig. 4. 5. Stoloniferous shoots formed on DM (Pot. M2) supplemented with 1.0 mg/l BAP and 4% sucrose.



a. Desiree

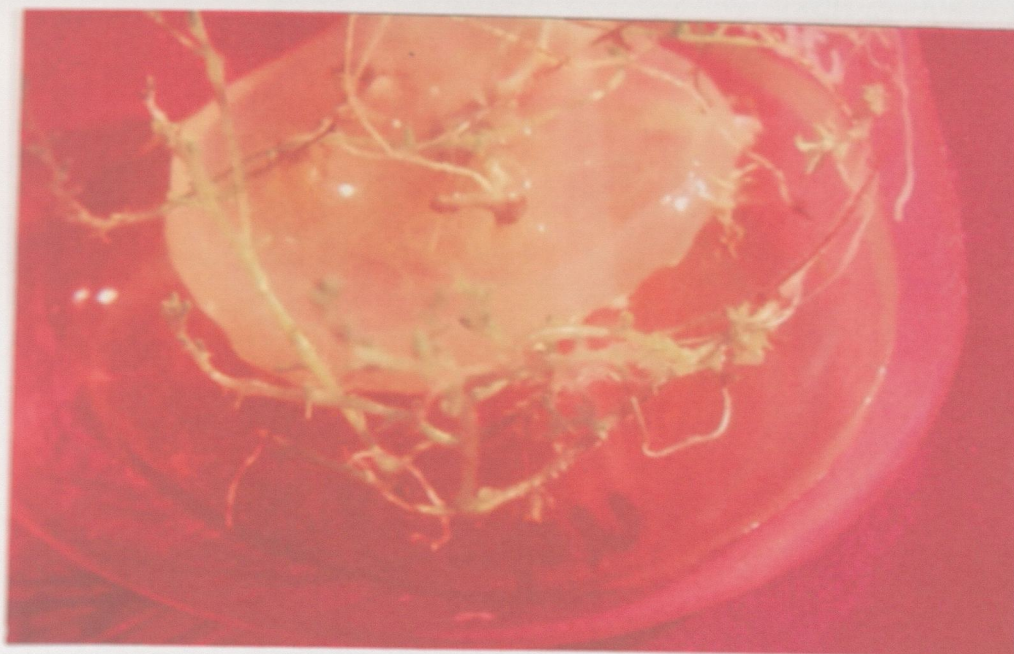


b. Ultimus

Figs. 84 a, b. Stoloniferous shoots formed on BM (full MS supplemented with 1.0mg/l BAP and 4% sucrose).



a. Desiree

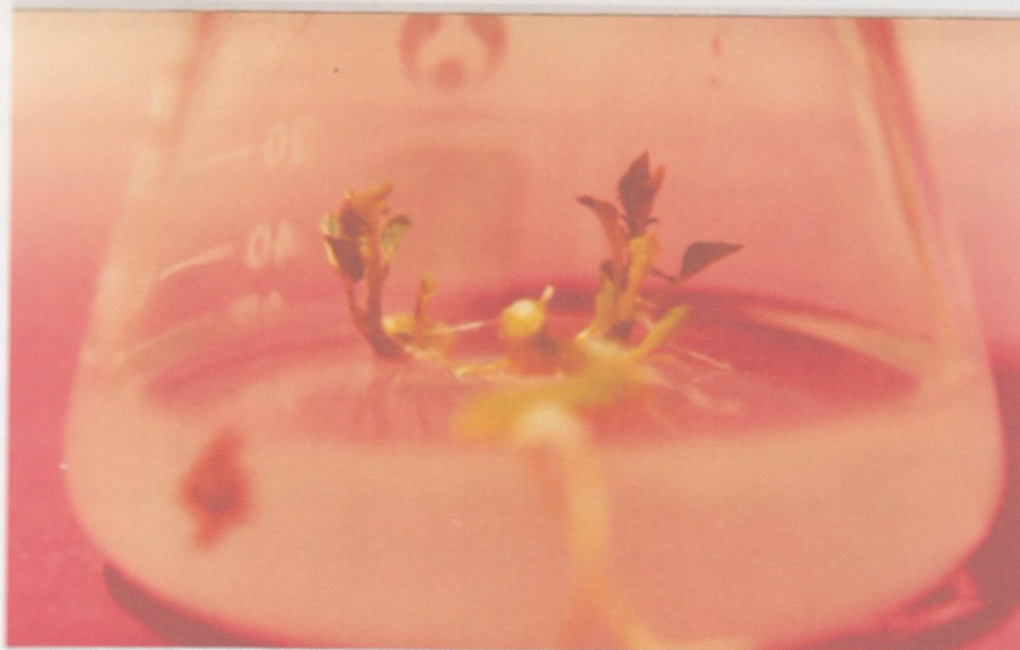


b. Ultimus

Figs. 85a, b. The stoloniferous shoots previously formed on BAP treated medium turned into upright leafy shoots when transferred to medium incorporated with 1.0mg/l GA_3 . No tuberization could be observed.



a. Desiree



b. Ultimus

Figs. 86a, b. The previously induced tubers on a tuberization medium when subcultured to a medium added with 0.5mg/l GA_3 could not develop any further. Sprouting of the young tubers could be seen.

Effect of ABA on Tuberization

While ABA is a growth inhibitor, it is generally considered as a tuberization promoter. Therefore, in another set of experiments ABA was tested in the tuberization medium. The effect of ABA added to the medium was tested at concentrations ranging from 0.01-1.0 mg/l (0.01, 0.05, 0.1, 0.5 and 1.0 mg/l) in the presence of 8% sucrose. The cultures were incubated in 8hrs light /day (short days) and at a temperature of $25 \pm 1^{\circ}\text{C}$.

Results shown in (Table 44; Figs. 87 and 88) indicate that some tuber formation was being observed at 0.01 and 0.05 mg/l levels of ABA in some cultures of Ultimus whereas a negligible amount of tuber formation was observed in the cultivars Desiree. An average of 2.1 and 4.6 tubers/flask were obtained in Ultimus and 1.2 and 1.8 tubers/flask in Desiree at 0.01 and 0.05mg/l, respectively, within a culture period of eight weeks. This number could not be increased any further even when the culture period was prolonged for up to 12 weeks (Figs. 89a and 89b).

With increasing concentrations, ABA progressively inhibited shoot growth whereas a slight increase in tuber number was observed at 0.1 mg/l in both the cultivars (Figs. 90a and 90b). Any further increase in ABA level to 0.5 and 1.0mg/l resulted in stunted and compact shoot growth, together with a decrease in tuber number in both the cultivars. Moreover, the leaves on the shoots at 1.0 mg/l level turned brown within 3 weeks of culture period with no tuberization at all. Death of the plantlets was the net result within 4 weeks of culture.

Cultivars, ABA concentration mg/l as well as their interactive treatment had highly significant effect on both the number and weight of tubers (Tables 45-47). Growth retardants are said to stimulate tuberization of plants under unfavorable conditions. ABA decreases both microtuber number and microtuber dormancy as observed by Nistor *et al.* (2010). These studies exactly coincide the present findings as regards tuber yield both in terms of number and weight.

Table 44. Effect of different tuber inducers on *in vitro* tuberization in *solanum tuberosum* all harvest done after 12 weeks.

Cultivar	MS + 8% sucrose + Tuber inducer	Mg/l	Mean No. of tubers/of flask	Mean wt. tubers/flask	Mean wt/ tuber (g)
Desiree	ABA	0.01	1.2	0.0984	0.082
		0.05	1.8	0.144	0.080
		0.1	2.0	0.16	0.080
		0.5	-	-	-
		1.0	-	-	-
	Coumarin	10	6.9	2.208	0.32
		20	8.2	3.116	0.38
		30	10.9	4.578	0.38
		40	11.1	5.64	0.36
		50	6.3	7.098	0.39
Ultimus		60	4.0	0.95	0.19
	Chloro choline chloride (CCC)	20m	9.4	4.042	0.43
	CCC + BAP	20 + 6.0	15.3	6.273	0.41
	ABA	0.01	2.1	0.357	0.17
		0.05	4.6	0.69	0.15
		0.1	4.9	0.637	0.13
		0.5	-	-	-
		1.0	-	-	-
	Coumarin	10	6.2	1.736	0.28
		20	8.1	2.916	0.36
		30	9.8	3.626	0.37
		40	7.1	4.34	0.35
		50	3.4	5.049	0.33
		60	2.2	1.491	0.21
	CCC	20	6.5	2.535	0.39
	CCC + BAP	20 + 6.0	10.8	4.212	0.39

Table 45. ANOVA factors, potato cultivar, ABA concentration (mg/l) and their interactive effect on the number of tubers and weight of tubers.

Source	Degree of freedom	Mean square values	
Cultivars (A)	1	43.56**	1.90**
ABA conc. (mg/l) (B)	4	55.44**	0.83**
A x B	4	10.44**	0.34**
Error	90	0.37	0.01

* = Significant at 5% level of significance.

** = Significant up to 1% level of significance

NS = Non significant

Table 46. Mean values of potato cultivar, ABA concentration (mg/l) and their interactive effect on the number of tubers.

Potato cultivar (A)	ABA Conc. (mg/l) (B)					A
	***	***	***	***	***	
	A x B					
Desiree	1.20C	1.80B	2.00B	0.00D	0.00D	1.00B
Ultimus	2.10B	4.60A	4.90A	0.00D	0.00D	2.32A
B	1.65B	3.20A	3.45A	0.00C	0.00C	

LSD values for

ABA Concentration (mg/l) = 0.383

At 5% level of significance

Table 47. Mean values of potato cultivar, ABA concentration and their interactive effect on the weight of tubers.

Potato cultivar (A)	ABA Conc. (mg/l) (B)					A
	**	**	**	**	**	
	A x B					
Desiree	1.10C	0.14C	0.16C	0.00D	0.00D	0.08B
Ultimus	0.46B	0.69A	0.64A	0.00D	0.00D	0.36A
B	0.28B	0.42A	0.40A	0.00C	0.00C	

LSD values for ABA concentration (mg/l) = 0.066

A x B = 0.093

At 5% level of significance

Fig. 47. Effect of ABA on mean number of tubers per bush in potato cvs. Desiree and Ultimus.

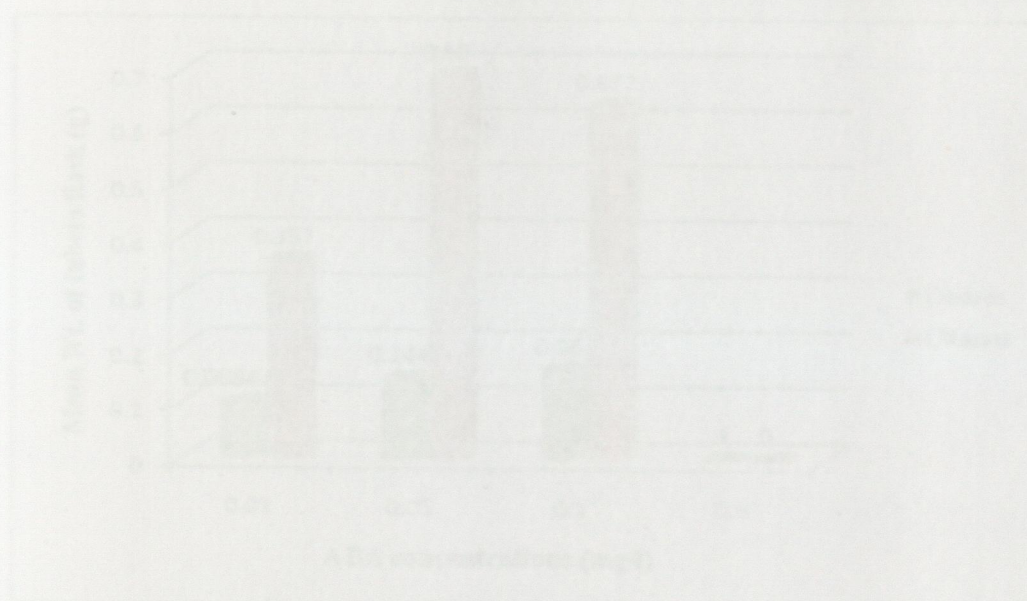


Fig. 48. Effect of ABA on mean weight of tubers per bush in potato cvs. Desiree and Ultimus.

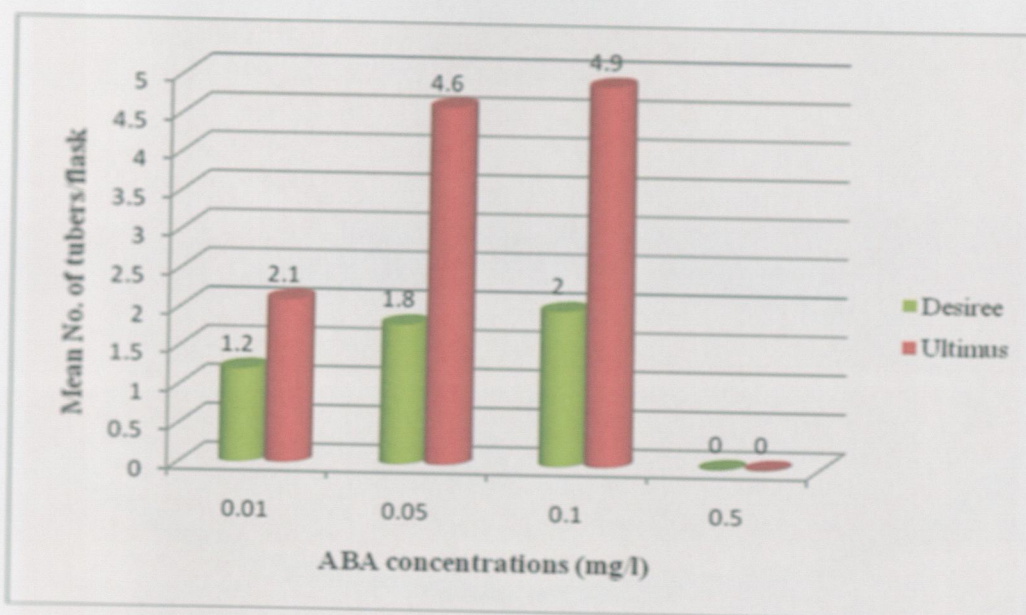


Fig. 87. Effect of ABA on mean number of tubers per flask in potato cvs. Desiree and Ultimus.

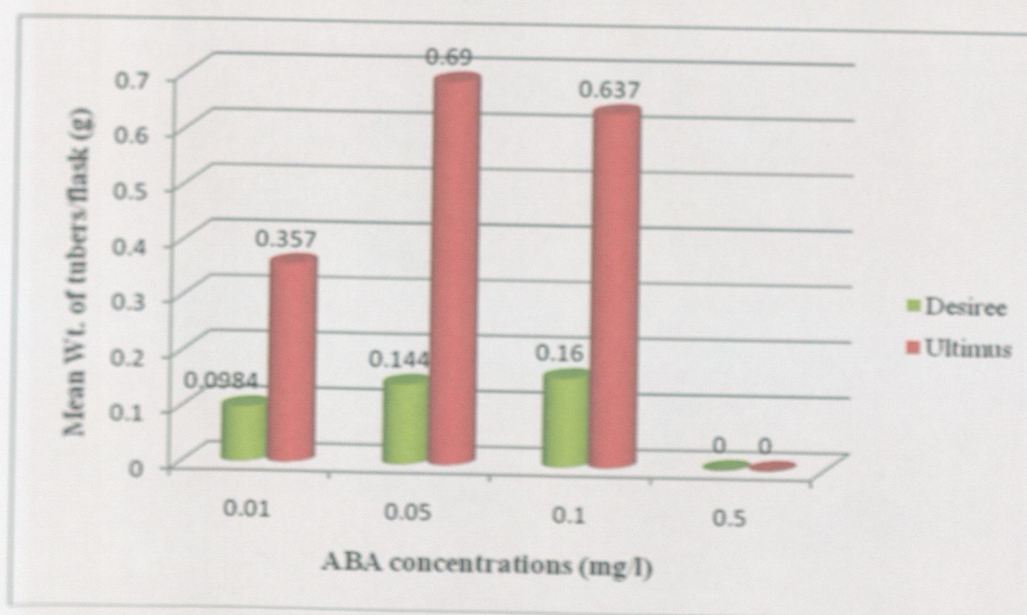


Fig. 88. Effect of ABA on mean weight of tubers per flask in potato cvs. Desiree and Ultimus.



a. Desiree

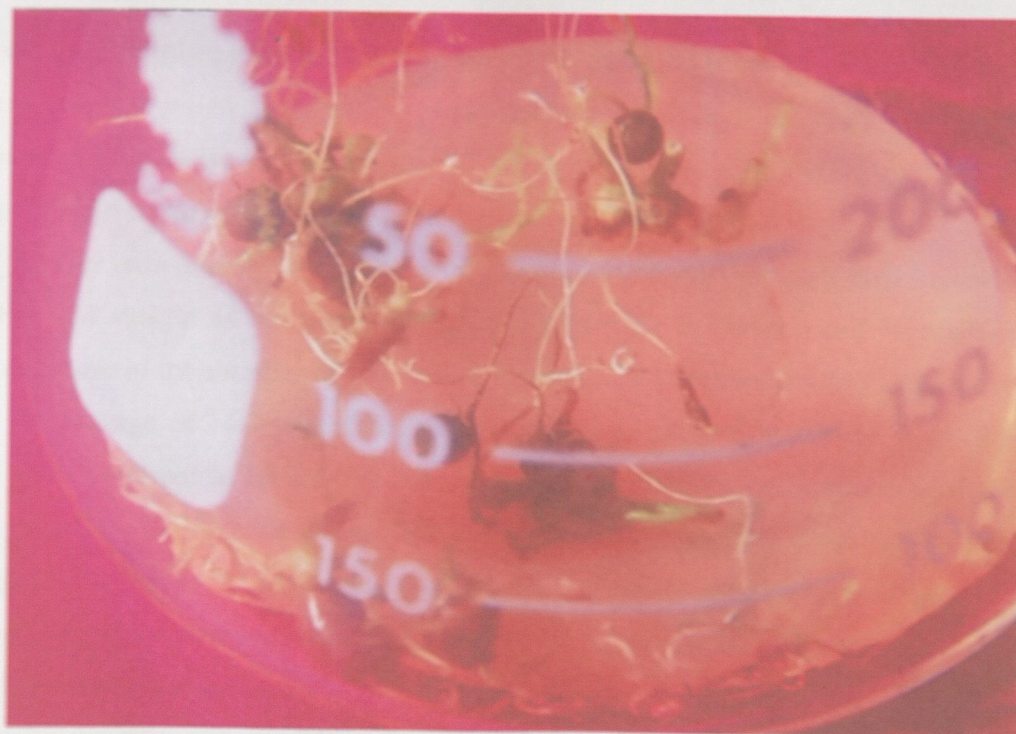


b. Ultimus

Figs. 89a, b. Tuber induction on medium supplemented with 0.05mg/l ABA in the cv Desiree & Ultimus.



a. Desiree



b. Ultimus

Figs. 90a, b. Tuberization on medium supplemented with 0.1mg/l ABA.

Effect of Coumarin on *in vitro* Tuberization

The ABA in the induction medium was replaced by different concentrations of coumarin to study its effect on tuber production. Plantlets obtained from stage II of the cultivars Desiree and Ultimus were further subcultured on BM (MS full strength) supplemented with varying levels of coumarin (10, 20, 30, 40, 50 and 50 mg/l) in addition with 8% sucrose. The cultures were kept under 8 hrs day lengths. The results (Table 44; Figs. 91 and 92) indicate that the addition of coumarin up to a certain level in the medium promoted tuber induction in both the cultivars. It increased both the tuber number and tuber weight compared to the control with no added coumarin.

At 10mg/l levels some tuberization was observed in the cv. Desiree while only a few tubers were produced in Ultimus after the 5th week of culture. The tuber number did not increase much when the culture period was prolonged up to 10 weeks. However, the tuber weight was greatly increased in Desiree and to some extent in Ultimus (Figs. 93a and 93b).

An increase in tuber number together with an increase in tuber weight of both the cultivars was noticed when the medium was supplemented with 20 mg/l coumarin. Tuberization occurred early, i.e., within the 3rd week of culture period compared to the non added coumarin control.

Still further with an increase in the level of coumarin there was a gradual increase in the number and weight of the tubers up to 40 mg/l levels in Desiree and 30 mg/l in Ultimus. A decrease in the number of tubers was noticed at and beyond 40 mg/l coumarin in Ultimus. An average of 11.1 tubers/flask was obtained in Desiree at 40mg/l while 9.8 tubers/flask were obtained in Ultimus at 30mg/l coumarin within a culture period of six weeks. The number of tubers however did not increase any further when the culture period was prolonged up to 10 weeks, but an increase in size of the tubers was noticed.

These were the maximum numbers of tubers, obtained for both the cultivars with coumarin. The tubers thus produced in both the cultivars were large somewhat of rounded shape with 4–5 eyes (Figs. 94 and 95).

Increase in coumarin levels up to 50 or 60 mg/l was of no added advantage. These concentrations exhibited detrimental effects on tuberization in both the cultivars. A drastic decrease in tuber number was noticed. Moreover the tuber size also got decreased.

The results showed that cultivars, coumarin concentrations and their interactive treatments had highly significant effect on both number and weight of tubers (Table 48-50). Coumarin, an auxin antagonistic but auxin-like in activity readily stimulates tuberization *in vitro* micro plants. Axillary shoots cultured on a coumarin-amended medium failed to elongate but produced thickened roots and tuberized readily (Levy *et al.* 1993).

In the present studies maximum tuber yield was obtained at 40 mg/l coumarin in Desiree and 30 mg/l in Ultimus, which are in agreement to the studies of Miklovicova *et al.* (1999). The studies performed by Hossain & Siddique (2011) showed that maximum tuber yield was obtained at 70 mg/l, coumarin, which is in striking contrast to our results, because 50 to 60 mg/l levels had detrimental effect on tuberization in both the cultivars.

Table 48. ANOVA of factors, potato cultivar, coumarin concentration and their interactive effect on the number of tubers and weight of tubers.

Source	Degree of freedom	Mean square values	
		Number of tubers	Weight of tubers
Cultivars (A)	1	93.63**	13.47**
Coumarin conc. (B)	5	147.95**	29.01**
A x B	5	10.63**	1.24**
Error	108	1.27	0.11

* = Significant at 5% level of significance.

** = Significant up to 1% level of significance.

NS = Non significant

Table 49. Mean values of potato cultivar, coumarin concentration and their interactive effect on the weight of tubers.

Potato cultivar (A)	Coumarin Concentration (B)						A
	**	**	**	**	**	**	
	A x B						
Desiree	6.90D	8.20C	10.90A	11.10A	6.30D	4.00E	7.90A
Ultimus	6.20D	8.10C	9.80B	7.10D	3.40E	2.20F	6.13B
B	6.55D	8.15C	10.35A	9.10B	4.85E	3.10F	

LSD values for

Coumarin concentration = 0.707

A x B = 0.999

At 5% level of significance

Table 50. Mean values of potato cultivar, coumarin concentration and their interactive effect on the weight of tubers.

Potato cultivar (A)	Coumarin Concentration (B)						A
	1	2	3	4	5	6	
	A x B						
Desiree	2.21DE	3.12C	4.14A	4.00A	2.14E	0.76H	2.73A
Ultimus	1.74F	2.92C	3.62B	2.49D	1.12G	0.46I	2.06B
B	1.97D	3.02C	3.88A	3.24B	1.63E	0.61F	

LSD values for

Coumarin concentration = 0.208

A x B = 0.294

At 5% level of significance

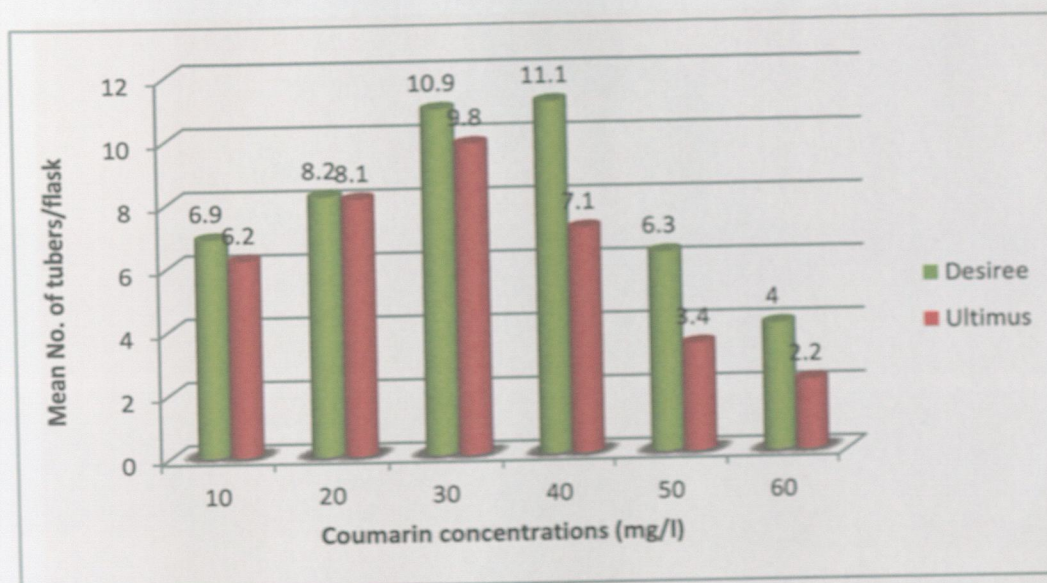


Fig. 91. Effect of coumarin on mean number of tubers / flask in potato cvs. Desiree and Ultimus.

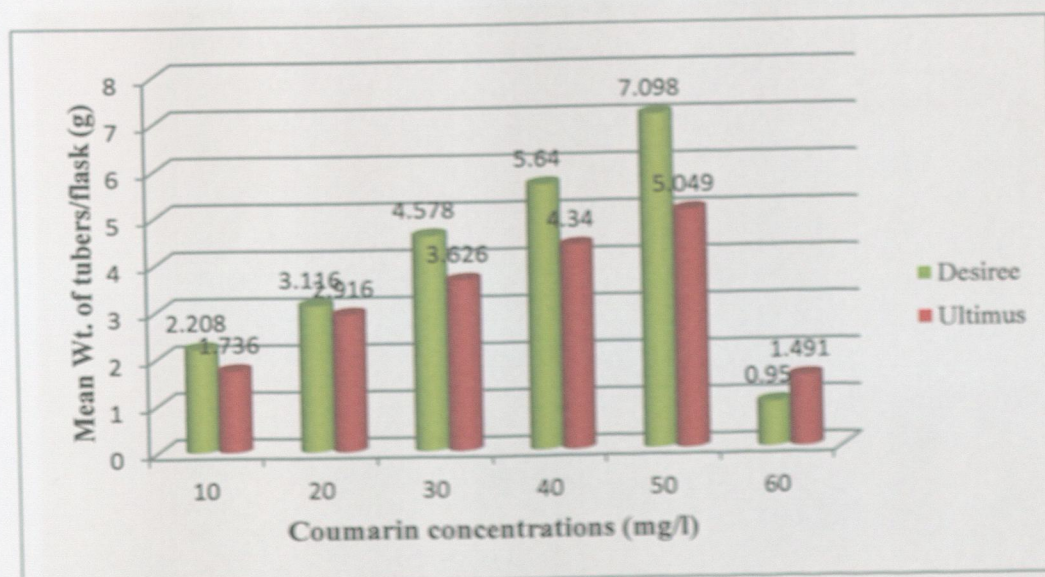
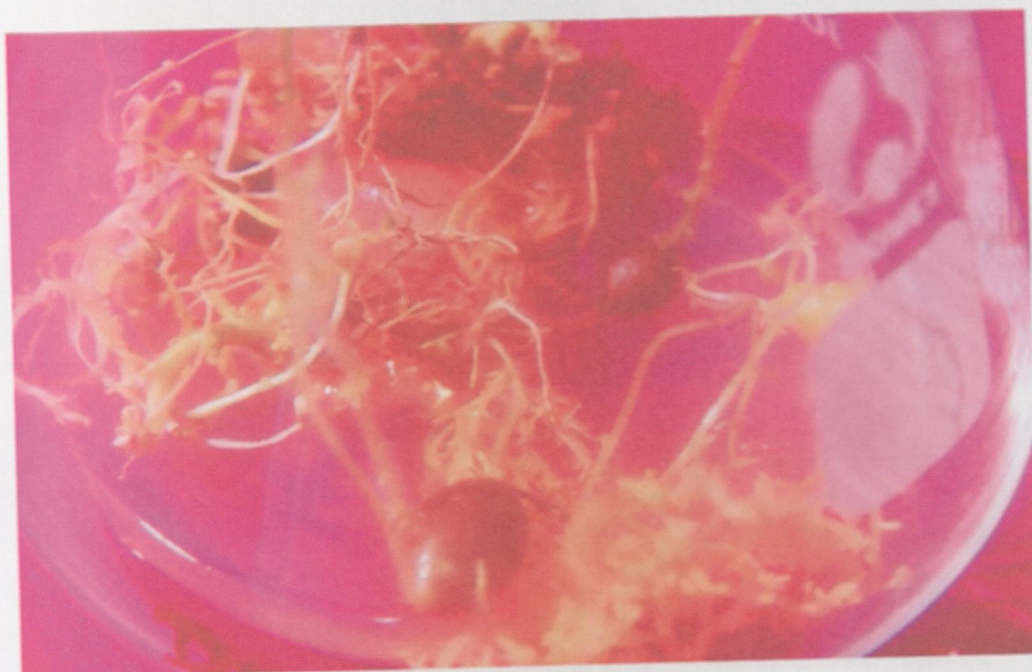


Fig. 92. Effect of coumarin on mean weight of tubers/ flask in potato cvs. Desiree and Ultimus.



a. Desiree



b. Ultimus

Figs. 93a, b. Tuber formation on medium supplemented with 10mg/l coumarin and 8% sucrose.



Fig. 94. Tuberization on medium supplemented with 30mg/l coumarin and 8% sucrose in Ultimus.



Fig. 95. Tuberization on medium supplemented with 40mg/l coumarin and 8% sucrose in Desiree.

Effect of Chlorocholine Chloride on *in vitro* Tuberization

The plantlets that have been growing in long day conditions were further subjected to another tuberization medium. In this set of experiments the basal medium was supplemented with chlorocholine chloride (CCC) either alone or in combination with 6.0 mg/l of BAP and 8% sucrose. A single concentration of chlorocholine chloride i.e. (20 mg/l) was tested on tuberization in both the cultivars and in both day lengths.

The addition of CCC to the medium containing 6.0 mg/l BAP and 8% sucrose turned the color of the leaves to dark green. The effect was more pronounced in Desiree than in Ultimus. Tuberization was promoted by CCC more so in the short than in long days. Tuber formation was observed within five weeks of culture period in both the cultivars (Table 44). The addition of CCC to the medium reinforced the promotive effect of BAP, tubers being formed after less growth of the stolons, induced by BAP.

When used alone, CCC had much less effect on tuber number in both short and in long days. The tuber weight in short days was greatly increased. Moreover, most of the tubers were formed above agar media (Figs. 96a and 96b). In Desiree, short days had a much greatly enhanced tuber number when BAP was also present in the medium in combination with CCC. The effects in Ultimus on tuber number were less pronounced in both day lengths. Tuber weight, however, was greatly increased. The tubers thus formed were rounded to oval in shape having up to six eyes in both the cultivars (Figs. 97a and 97b).

Delgado & Scott (1997) and Zakaria *et al.* (2008) studied the effect of chlorocholine chloride either alone or in combination with BAP. The combined effect of the two was promotive, but these compounds when each tested as sole plant growth regulator, BAP induced up to 70% tuberization, whereas CCC alone was ineffective. The present findings confirmed the validity of the previous ones. The microtubers thus produced in the present study were considerably of a large size.

Contradictory results were reported by Hussain *et al.* (2006), who compared the individual effect of BAP and CCC on *in vitro* tuberization of *Solanum tuberosum* cv. Cardinal and obtained maximum number of tuber on CCC supplemented medium in contrast to medium supplied with BAP.



a. Desiree



b. Ultimus

Figs. 96a, b. Tuber formation on medium supplemented with 20mg/l CCC and 8% sucrose under 8h photoperiod.



a. Desiree



b. Ultimus

Figs. 97a, b. Tuberization on medium supplemented with 20mg/l CCC, 6.0mg/l BAP and 8% sucrose under short (8h) photoperiod.

Effect of B-5 Medium on Tuberization

It has been reported earlier (Dodds *et al.*, 1992) that the number and size of the tubers can be improved by reducing the nitrogen contents in the formulation medium. With the view of obtaining a better tuber yield per flask, B-5 medium was used as the basal medium instead of Murashige and Skoog salt solution because B-5 formula is based on low nitrogen content.

The plantlets obtained from Sage II were further subcultured on a tuberization medium composed of B-5 (Gamborg *et al.*, 1968) as the basal medium and supplemented with varying concentrations of BAP (6, 8 & 10 mg/l) and 8% sucrose. The cultures were kept under short (8 hr) days. Tuber initiation was observed in all the three concentrations of BAP used within a culture period of 6 weeks (Table 51; Figs. 98 and 99) in both the cultivars.

At 6.0 mg/l BAP in the B-5 basal medium stoloniferous shoots together with some upright shoot formation was observed within 4 weeks of culture. The tips of the stoloniferous shoots started swelling after the 5th week of culture that culminated into tubers within the 6th week of culture. Initially the tuber number as well as the sizes was very small, but both the number and sizes increased within 10 weeks of culture. Although there was an increase in the number, yet the tuber size remained small (Figs. 100a and 100b). With an increase in the BAP level to 8.0 mg/l a further increase in tuber number was recorded for both the cultivars. The largest number of tubers were obtained on BM supplemented with 10mg/l BAP in the medium within a culture period of 10 weeks and after that no further increase in tuber number or size was observed. An average of 13.5 tubers/flask was obtained in the cv. Desiree and 11.1 in Ultimus. The tubers thus produced were small and rounded (Figs. 101a and 101b). The tuber number as well as the weight was much less compared to those obtained on MS medium with same set of hormonal and sucrose combination used in our previous experiments in this text.

The ANOVA (Table 52-54) showed that B-5 medium, BAP concentration has highly significant effect on the number and weight of tubers in Desiree and Ultimus. The effect of potato cultivar was found to be significant regarding weight of tubers while non-significant regarding number of tubers. Their interactive effect on the number and weight of tubers was non-significant.

High levels of carbohydrates in the form of sugars and starch favour the formation of storage organs, i.e. tuberization, whereas high nitrogen levels are known to promote shoot and root growth that would utilize much of the available carbohydrate and thereby reduce the amount available for tuberization. The inhibitory effect of increased nitrogen levels on *in vitro* tuberization were observed only at 2% sucrose but not at higher concentrations (Koda & Okazawa, 1983), at which the high carbohydrate levels may be masking the effects by altering the nitrogen levels.

Conclusions

It could therefore be concluded that B-5 medium used in this study initiated earlier tuberization; but it was not beneficial as far as tuber yield (both in terms of number and weight) is concerned. It could be further gained from these studies that the added nitrogen level in MS medium is below the deleterious concentration for tuberization.

Table 22. ANOVA of factors in *in vitro* culture: B-5 medium, BAP and their interactive effect on the number of tubers and weight of tubers.

Source	Degree of freedom	Mean square values	
		Number of tubers	Weight of tubers
Cultivars (A)	3	1.085	0.22*
B-5 med BAP (B)	7	12.32**	1.34**
A x B	21	1.5285	0.0473
Error	54	0.78	0.55

* = Significant at 5% level of significance.

** = Significant up to 1% level of significance.

NS = Non-significant.

Table 51. Effect of B-5 medium on *in vitro* tuberization in potato cvs. Desiree and Ultimus harvesting done after 12 weeks.

Cultivars	B-5 medium + 8% sucrose + BAP mg/l	Mean number of tubers / flask	Mean wt of tubers / flask (g)	Mean weight / tubers (g)	General remarks
Desiree	6	8.3	1.245	0.15	Tuberization observed after the 5 th week of culture.
	8	10.1	1.919	0.19	Early tuberization together with an increase in tuber number and weight.
	10	13.5	2.295	0.17	Increase in tuber number with a slight decrease in tuber weight observed.
Ultimus	6	7.5	1.2	0.16	Tuberization after the 5 th week of culture.
	8	10.3	1.854	0.18	Increase in both tuber number and weight observed.
	10	12.8	2.048	0.16	Slight decrease in weight / tuber observed. Overall yield not affected.

Table 52. ANOVA of factors, potato cultivar, B-5 medium BAP and their interactive effect on the number of tubers and weight of tubers.

Source	Degree of freedom	Mean square values	
		Number of tubers	Weight of tubers
Cultivars (A)	1	2.82NS	0.22*
B-5 med BAP (B)	2	138.52**	4.74**
A x B	2	1.52NS	0.06NS
Error	54	1.70	0.04

* = Significant at 5% level of significance.

** = Significant up to 1% level of significance.

NS = Non significant.

Table 53. Mean values of potato cultivar, B-5medium BAP and their interactive effect on the number of tubers.

Potato cultivar (A)	B – 5med BAP, (B)			A
	6	8	10	
Desiree	8.30	10.10	13.50	10.63
Ultimus	7.50	10.30	12.80	10.20
B	7.90C	10.20B	13.15A	

LSD values for

B – 5med BAP, = 0.826

At 5% level of significance

Table 54. Mean values of potato cultivar, B-5medium BAP, and their interactive effect on the weight of tubers.

Potato cultivar (A)	B – 5med BAP, (B)			A
	6	8	10	
Desiree	1.25	1.92	2.30	1.82A
Ultimus	1.20	1.85	2.05	1.70B
B	1.22C	1.89B	2.17A	

LSD values for

B – 5med BAP, = 0.119

At 5% level of significance

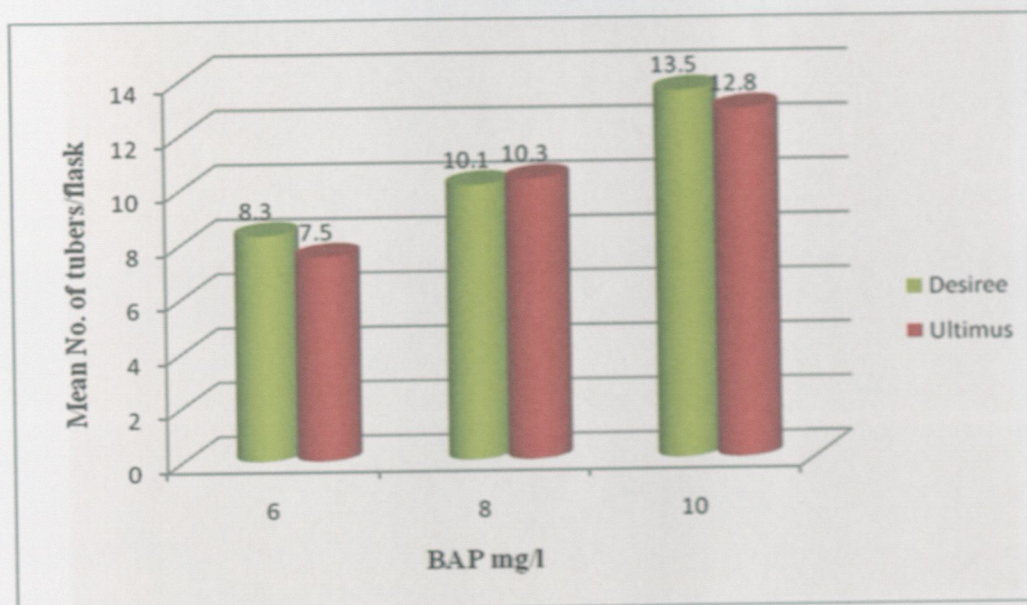


Fig. 98. Effect of B-5 medium supplemented with varying concentrations of BAP on mean number of tubers / flask in potato cvs. Desiree and Ultimus.

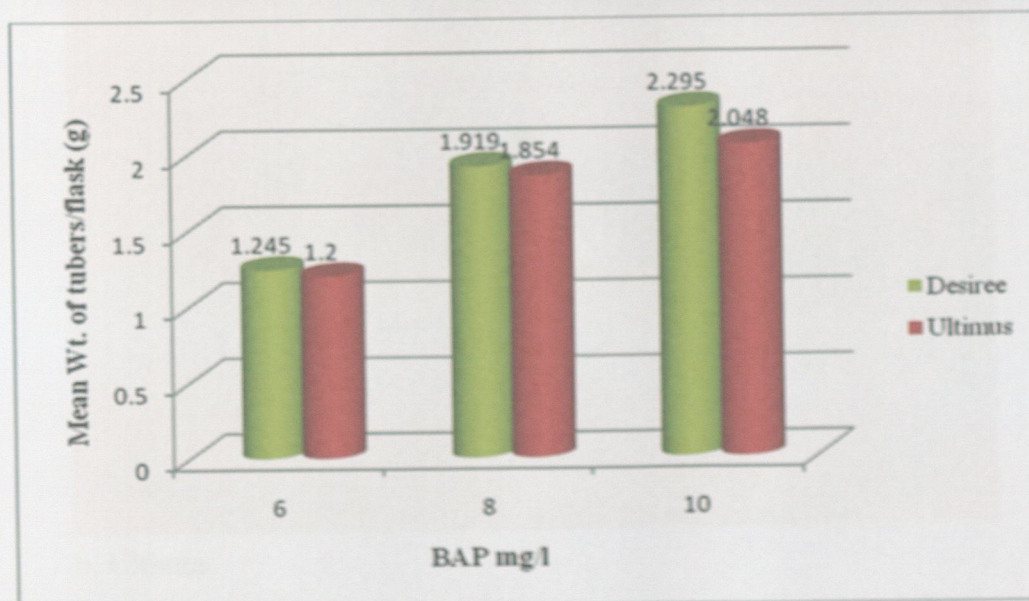


Fig. 99. Effect of B-5 medium on mean weight of tubers / flask in potato cvs. Desiree and Ultimus.

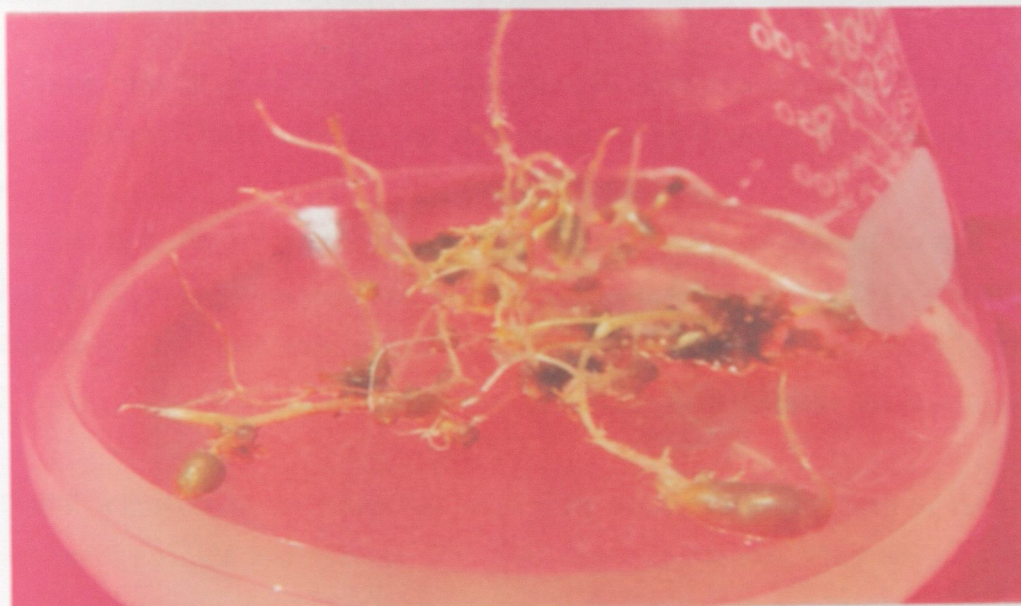


a. Desiree



b. Ultimus

Figs. 100a, b. Tuberization on B-5 medium supplemented with 6.0mg/l BAP and 8% sucrose.



a. Desiree



b. Ultimus

Figs. 101a, b. Tuberization on B-5 medium supplemented with 10mg/l BAP and 8% sucrose under short photoperiod.

Effect of different types and sizes of Culture Vessels on Plantlets and Tuber Formation

Culture vessels of different sizes were used in these experimental studies. These included test tubes of 50ml capacity and wide mouth Pyrex flasks of 50, 100 and 250 ml capacity.

In the first set of experiments nodal cuttings were inoculated in test tubes containing 10 ml propagation medium. The cultures were inoculated at $25 \pm 1^{\circ}\text{C}$ under 16 hrs photoperiod. The tubes were placed in custom made test tube racks. Each rack can hold 60 tubes, standing close to each other. The results obtained in the tubes varied greatly.

Nodal segments in the tubes that were lying towards the periphery of the racks developed healthy single leafy shoots with thick stems and leaves. Each plant had well developed root system, attained a height up to 8cm and had 7-8 nodes/plant within a culture period of 3 weeks. The nodal segments cultured in tubes lying in the 3rd and following rows developed into shoots with rather weaker and etiolated stems, having long internodes with scale like leaves. These plantlets were very delicate. The nodal segments that were cultured in the tubes lying towards the center, either developed shoots with only a single node or failed to develop at all (Table 55).

In another experiment pertaining to propagation, nodal cuttings were also cultured in 50 and 100 ml capacity flasks containing 20 ml and 40 ml propagation medium, respectively. Two nodes/flask were cultured in the small flasks whereas three nodes/flasks were placed in the larger flasks. In striking contrast to the test tubes, the plantlets developed in 100 ml capacity flasks were much healthier, had thicker stems, with large leaves and developed branches. Whereas the nodal segment in the smaller vessels did not exhibit good results. Slight sprouting of the axillary buds on the nodes was noticed in some of the cultures. Some of the buds developed a little further into plantlets with 1-2 nodes, while the others failed to develop (Table 56).

To see the effect of culture vessels on tuberization also, flasks of two different sizes (100 and 250 ml capacity) were used. In the preliminary studies plantlets obtained in the propagation medium were transferred to either 100 or 250 ml capacity flasks

from the developing plant. The interruption can have a variety of consequences, both harmful and beneficial, depending on the type of tissue being cultured, the species and the culture vessel type. The type of vessel used in micropropagation and type of closure employed have been shown to have a variety of effects on culture growth and development.

The vessel closure controls the exchange of gases with the surrounding environment, releasing volatile compounds which build up during plantlet development. Volatile compounds released by a variety of cultures were identified by Thomas & Murashige (1979). They found that carbon dioxide, ethylene, ethane acetaldehyde and ethanol were released from actively growing plant tissue cultures. No detectable levels of the above gases were detected in vessels containing only nutrient medium. With callus production, ethylene levels were found to be the highest, although this varied with the species being cultured. Fully developed shoot cultures had lower levels of carbon dioxide with a further decrease in the presence of light.

Hussey & Stacey (1984) showed that ethylene accumulation in tightly sealed vessels inhibited the formation of potato (*Solanum tuberosum* L.) tubers unless ethylene absorbing agents were used.

Liu *et al.* (1985) reported that the use of parafilm to seal culture vessels of actively growing cultivars of potato resulted in shoot tip necrosis, reduced weight and increased branching. The saturated conditions within the vessel reduced transpiration rates, thereby decreasing translocation of mineral nutrients. These abnormalities were overcome by the addition of calcium to the media and /or increased aeration of the vessel.

Hussey & Stacey (1984) reported a change in morphology in potato culture which was dependent on container closure. Poor aeration resulted in stoloniferous shoots with small leaves. This effect was shown to be a direct result of ethylene accumulation in the vessel. De Proft *et al.* (1985) found that ethylene can have both beneficial and negative physiological effects on the developing culture, depending on the species being cultured.

Vessel size and dimension also affect the culture environment. While culturing grape-vines (*Vitis vinifera*) in liquid culture. Monette (1983) reported that larger vessels yielded a higher number of shoots following 6 weeks in culture. Shoot height was affected by vessel size, with taller shoots occurring in larger vessels. Similar results were

obtained in the present studies also with taller plantlets being obtained in the test tubes, whereas as multiple and branched plantlets were being formed in the 100 ml flasks and still further in the 50 ml flask no plantlets could be obtained at all. Only slight sprouting of the nodal buds could be observed. Vessel volume was also found to affect tuber yield during the present investigations. Tuber number and sizes both were greatly decreased in the smaller (100 ml) flasks in comparison to the 250 and 300 ml flasks. Vessel size also greatly affected the induction and growth of pollen embryos from anthers of *Nicotiana tabacum*. Small volume vessels were found to severely inhibit plantlet formation (Dunwell, 1978).

The culture environment is affected by the type of culture vessel used, its configuration and the type of closure on the vessel. Vessel shape affects the amount of medium exposed to the atmosphere inside the vessel, which can affect the rate of moisture loss and diffusion of gaseous compounds. Vessel closures are important due to the control of the release of gaseous compounds built up during plantlet growth and the control of moisture loss.

Culture	Culture	Spreading Area	Area	Area	Area
	Vessel	Height	Volume	Volume	Volume
250ml	250ml	10.0	10.0	10.0	10.0
100ml	100ml	10.0	10.0	10.0	10.0
50ml	50ml	10.0	10.0	10.0	10.0
100ml	100ml	10.0	10.0	10.0	10.0

Table 55. Effect of culture vessels on *in vitro* shoot growth of potato cultivars Desiree and Ultimus.

Cultivars	Position of test tubes in the rack	Mean No. of shoots/test tube	Mean height/shoot (cm)	Mean No. of nodes/plant
Desiree	1 st row	1.0	5.3	6.5
	2 nd and 3 rd rows	1.0	4.9	2.5
	Middle row	0.3	-	-
Ultimus	1 st row	1.0	5.8	6.7
	2 nd and 3 rd rows	1.0	-	2.3
	Middle row	0.4	-	-

Table 56. Effect of size of culture vessels on *in vitro* tuberization of potato cultivars Desiree and Ultimus on medium supplemented with 8% sucrose and under short days.

Cultivars	Culture vessel flask	Sprouting %age	Mean No. of shoots/flask	Mean height/plant(cm)	Mean No. of nodes /plant
Desiree	50ml	85.3	0.9	1.2	1.0
	100ml	100	5.6	10.3	8.9
Ultimus	50ml	79.5	0.7	1.5	1.0
	100ml	100	6.1	11.1	9.7

Table 57. Effect of size of culture vessels on *in vitro* tuberization of potato cultivars Desiree and Ultimus on medium supplemented with 8% sucrose and under short days.

Cultivars	Culture vessel flasks	No. of tubers/flask	Weight of tubers/flask	Mean weight /tubers
Desiree	100ml	2.1	0.525	0.25
	250/300ml	7.4	3.774	0.51
Ultimus	100ml	2.3	0.483	0.21
	250/300ml	6.8	3.264	0.48

FIELD EXPERIMENTS

SECTION - IV

FIELD EXPERIMENTS

Field Performance of Micropropagated Plants

In vitro plantlets grown in either of the two containers (test tubes or flasks) were transplanted when they were up to 7 cm long. Before transplantation into beds, the plantlets were first hardened (acclimatized) in the laboratory.

Before the plantlets were taken out of the culture vessels, a little water was added into the flasks to soften the agar medium, in order to avoid damage to the roots. Taking the plantlets out of the tubes as compared to the flasks was much easier. The plantlets were then washed gently and thoroughly with water so as to remove the entire agar. To acclimatize the plantlets, after cleaning they were placed in flasks filled with tap water. They were covered with polythene bags in order to prevent dehydration. They were kept in the laboratory for about a week at room temperature. Thereafter the plantlets were either sown in small clay pots or were planted directly into beds in a net house.

The pots were filled with a soil mix composed of organic matter + loam in the ratio of 1:1. The pots were then irrigated with a small amount of tap water. They were prepared to receive the plantlet by making a hole in the center of the soil with a borer. The plantlets were then taken out of the flasks and planted individually in the holes with the roots plus one or two nodes below the surface. The pots with the plantlets were covered with polythene bags to prevent dehydration until the roots were established (for about a week). The plantlets were kept inside the culture room for the first few days in order to protect them from the adverse weather conditions. They were watered lightly with tap water (avoiding over watering) until well rooted. When roots were fully established, they were gradually exposed to the natural atmosphere by removing the polythene bags and taking them out for short periods each day. The plantlets were then taken out and transplanted either to larger clay pots or in beds in the month of February (Fig. 108). The plants were not nurtured with any artificial fertilizer other than organic matter. In the beds the plants were grown in ridges whereas 3 plantlets were grown /pot and in ten pots for each cultivar. The results shown in Table 58. The growth of the plants in the beds / pots was satisfactory. They attained a height of up to 35cm within 6 weeks and also developed a number of lateral branches (Figs. 109 and 110). The plants once established developed rapidly, with the leaves having the same size and morphology as those of the controls. The fully mature vines attained a length of up to 40cm. They were

then harvested in the month of May 14 weeks after planting in beds/pots. An average of 8.5 and 9.3 tubers per plant were produced in Desiree and Ultimus respectively in the pots. (Figs. 102 and 103). There were no differences in morphological characters in the plants or in the tubers. The tubers though smaller in size, were similar in color and shape to the mother tubers, thus ensuring that the plants were genotypically stable. Most of the minitubers measured between 1.0-2.5 cm in size (Fig. 111 and 112). Each tuber had 6-7 eyes having an average weight of up to 3 grams.

After the seventh week the vegetative growth of the *in vitro* plantlets was more prolific in the beds. This has resulted in a higher tuber yield both in terms of number and weight (Table 59; Figs. 104 and 105). The minitubers thus obtained were stored in the refrigerator for further multiplication.

Performance of Microtubers in the field

The microtubers of both the cultivars thus obtained from Stage III of the above experiments shown in (Figs. 113, 114, 115) were further tested in the field. They were tested in the two potato growing seasons (fall and spring) of Peshawar.

Experiments in beds

The microtubers obtained from the different experiments were stored in the refrigerator at 4°C for a period of 12 weeks. They were kept covered in petridishes, so as to prevent them from dehydration. After refrigeration, the tubers were kept at room temperature for about 2 weeks prior to sowing in open. Sprouting of these tubers was observed when kept at room temperature (Figs. 113 and 114). These pre-sprouted microtubers were then taken out in mid September and sown in ridges prepared in beds. The soil composed of loam and farmyard manure mixed in the ratio of 1:1. Only the large size microtubers ranging in size from 1.0–1.5cm were selected for sowing in beds. The smaller ones were sown in pots. Shoots from most of the microtubers emerged in about 3 weeks time. The plants after about eight weeks were almost comparable in size to those of the controls, attaining a height of up to 36 cm having leaves of a similar size and shape (Table 59; Figs. 116 and 117).

The plants were harvested by the end of December (14 weeks after sowing). An average of up to 26 tubers/plant was produced in Desiree and somewhat a similar number of tubers were also produced in Ultimus. (Table 59; Figs. 104 and 105).

The morphology of the tubers, i.e., the shape and colour was the same as the controls. Each tuber had 6–7 eyes and ranging in size from 1.5–3.0 cm in length (Fig. 118). The average tuber yield per plant was 326.12 gms for Desiree and 366.36 for Ultimus. Some of the plants due to their congested nature were transplanted to beds of large sizes, after about five weeks. Swelling of the stolons could be observed at the time of transplantation. The plants got established within a week and reached to maturity.

Harvesting was done 9 weeks after transplantation. It could be observed that none of these plants produced tubers. Transplantation is therefore not suggested after tuber set.

Experiments in Pots

In the pot experiments 3 microtubers with size of 5.0 mm, 9.0mm and 7mm were sown per pot. Hundred percent sprouting of these microtubers was obtained in both the cultivars. The plants though weak in the beginning soon got established. They attained a length of 25 cm within 3 weeks time (Figs. 119a, 119b and 120). The plants were harvested after 14 weeks. Each plant yielded an average of 12.3 tubers, in both the cultivars. Minitubers up to 2.0cm in size were obtained.

Performance of Minitubers in the field

The minitubers thus obtained from these experiments or from the micropropagated plants were further sown in the field the following season. There was 100% sprouting rate in both the cultivars. The plants were then grown to maturity. They produced more tubers in about 12 weeks. The plants were much healthier completely covering the ground in about 6 weeks (Fig. 121) and producing an average of 33.4 tubers/plant in Desiree and average of 32.5 in Ultimus ranging in size from 4–8cm (Table 60; Figs. 106 and 107).

A comparative study of field performance of minitubers and microtubers was made by Ozturk & Yildirim (2010). *In vitro* plantlets (IP) and microtubers (MT) were grown at seed beds and were evaluated for morphological and yield characteristics. The IP and MT plants were not different for plant height, stem number, branch number and leaf number. The IP and MT tubers were not significantly different in terms of yield characteristics except tuber number. The reported results are in agreement to our findings.

Growth and yield of potato microtubers (varying sizes) and conventional tubers were studied by Kawakami *et al.* (2003). At harvest the tuber fresh weight of microtubers was 82% that of conventional tuber plants, suggesting a potential for using microtubers for field planting.

Similarly, Kawakami & Iwama (2012) studied the effect of size (ranging from 0.3–5.0 g, on field performance of potato microtubers. No differences in growing period, number of tubers and tuber fresh and dry yield were observed with the microtuber size. Microtubers of all sizes can therefore be used for direct field planting. In striking contrast to these studies, Bolandi *et al.* (2011) reported that only greater diameter microtubers showed superiority in weight, number and minitubers diameter.

The present study suggests that microtubers performed better than *in vitro* plants in the field. About 30% of the *in vitro* plants died during acclimatization stage, whereas microtubers exhibited 100% sprouting. Even the smallest microtuber sprouted and developed into healthy plants.

The production of microtubers is thus an approach to revolutionize the potato seed industry. The micro/minitubers produced from both the cultivars, were uniform of good quality and disease free. All these had the ability to germinate and could be used for further *in vitro* techniques for potato propagation or can be included in potato seed programs and disseminated to the farmers.

Cultivar		Plant height (cm)	Number of tubers/plant	Tuber fresh weight (g)	Tuber dry weight (g)
Chokila	Conventional tubers	49.5	30.5	1812.91	68.7
	Micropropagated plants	41.5	21.5	713.13	34.1
	Microtubers	45.2	25.3	1422.87	54.1
Shubra	Conventional tubers	50.5	32.8	2941.16	82.2
	Micropropagated plants	42.5	22.8	767.24	30.8
	Microtubers	45.1	26.2	1594.11	50.7

Table 58. Performance of Micropropagated plants and microtubers in pots (under natural conditions) of potato cultivars Desiree and Ultimus.

Potato cultivar	Plant source	Plant height (cm)	Mean No. of tubers	Mean No. of tubers/plant	Mean weight of tubers / plant (g)	Mean weight/tuber (g)
Desiree	Conventional tubers	45.6	40	13.3	932	23.3
	Micropropagated plants	35.7	26	8.5	3952	15.2
	Microtubers	38.2	37	12.3	699.3	18.9
Ultimus	Conventional tubers	46.1	41.5	13.8	1004.3	24.2
	Micropropagated plants	34.8	28	9.3	4396	15.7
	Microtubers	38.6	37	12.3	714.1	19.3

Table 59. Performance of Micropropagated plants and microtubers in beds (under natural conditions) of potato cultivars Desiree and Ultimus.

Potato cultivar	Plant source	Plant height (cm)	Mean No. of tubers/plant	Mean weight of tubers / plant (g)	Mean weight/tuber (g)
Desiree	Conventional tubers	49.4	30.2	1818.04	60.2
	Micropropagated plants	41.5	21.5	733.15	24.1
	Microtubers	46.2	26.3	1422.83	54.1
Ultimus	Conventional tubers	50.3	32.8	2040.16	62.2
	Micropropagated plants	42.5	22.8	702.24	30.8
	Microtubers	45.1	27.3	1384.11	50.7

Table 60. Performance of microtubers and minitubers of potato cultivars Desiree and Ultimus in the field.

Potato cultivar	Plant source	Plant height	Mean No. of tubers/plant	Mean weight of tubers / plant (g)	Mean weight/tuber
Desiree	Conventional tubers	44.2	34.1	2707.54	79.4
	Microtubers	42.5	28.5	1162.8	40.8
	Minitubers	43.6	33.4	2277.8	68.2
Ultimus	Conventional tubers	45.3	35.2	2720.9	77.3
	Microtubers	41.8	30.3	1163.52	38.4
	Minitubers	43.2	32.5	1881.75	57.9

Fig. 192. Mean number of tubers obtained from microtubers, minitubers and conventional tubers of potato cvs. Desiree and Ultimus in the experiments.

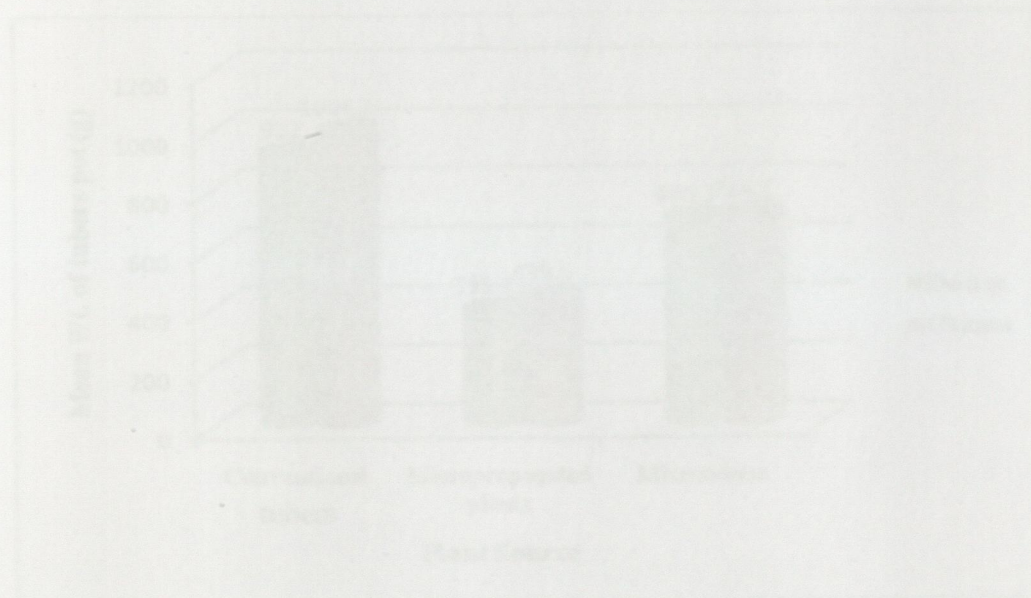


Fig. 193. Mean weight of tubers obtained from microtubers, minitubers and conventional tubers of potato cvs. Desiree and Ultimus in the experiments.

Table 60. Performance of microtubers and minitubers of potato cultivars Desiree and Ultimus in the field.

Potato cultivar	Plant source	Plant height	Mean No. of tubers / plant	Mean weight of tubers / plant (g)	Mean weight / tuber
Desiree	Conventional tubers	44.2	34.1	2707.54	79.4
	Microtubers	42.5	28.5	1162.8	40.8
	Minitubers	43.6	33.4	2277.8	68.2
Ultimus	Conventional tubers	45.3	35.2	2720.9	77.3
	Microtubers	41.8	30.3	1163.52	38.4
	Minitubers	43.2	32.5	1881.75	57.9

Fig. 192. Mean number of tubers obtained from micropropagated plants and conventional tubers of potato cvs. Desiree and Ultimus in pot experiments.



Fig. 193. Mean weight of tubers obtained from micropropagated plants and conventional tubers of potato cvs. Desiree and Ultimus in pot experiments.

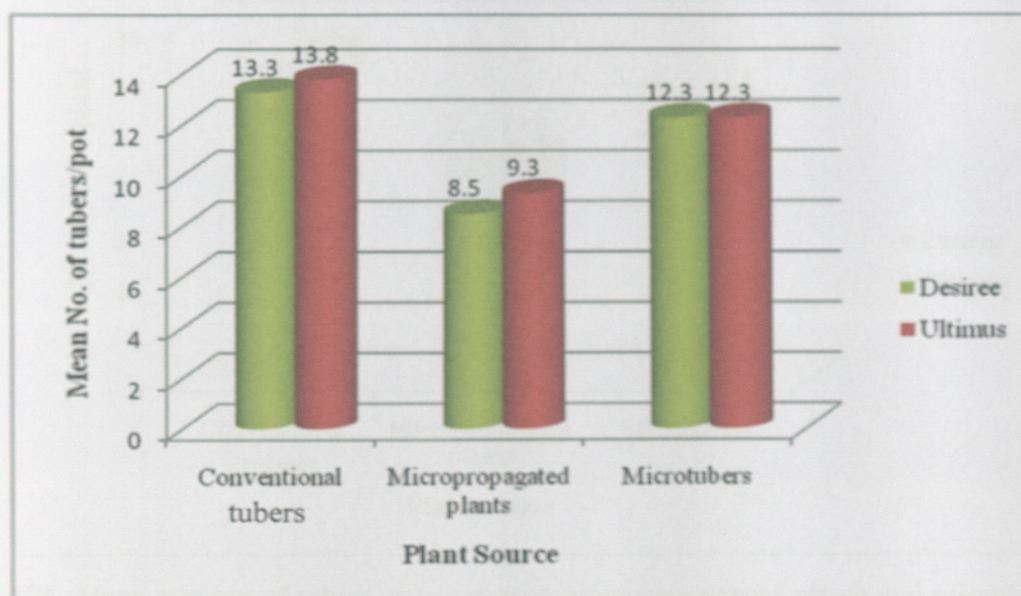


Fig. 102. Mean number of tubers obtained from micropropagated plants and microtubers of potato cvs. Desiree and Ultimus in pot experiments.

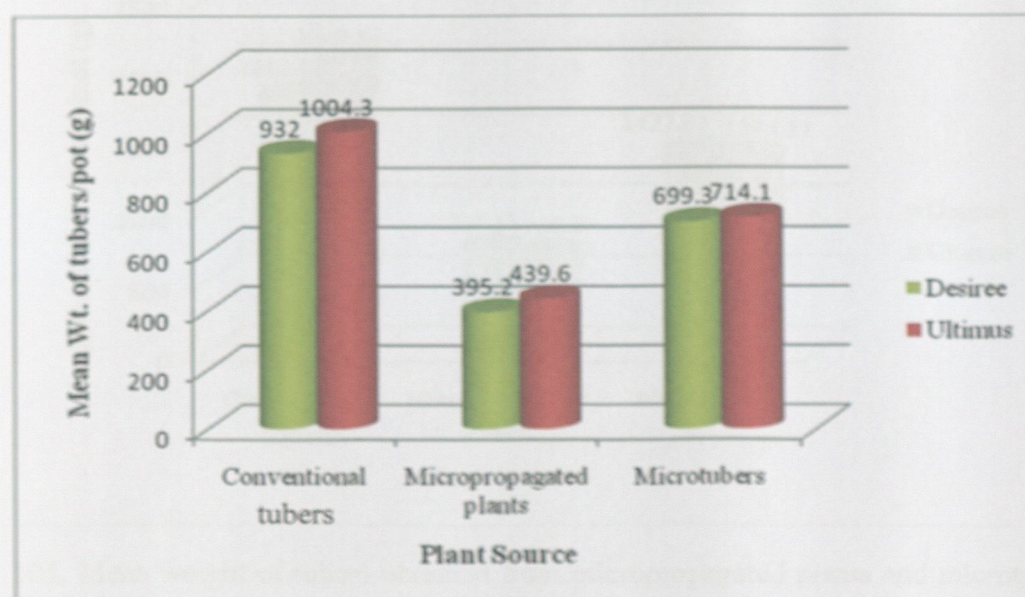


Fig. 103. Mean weight of tubers obtained from micropropagated plants and microtubers of potato cvs Desiree and Ultimus in pot experiments.

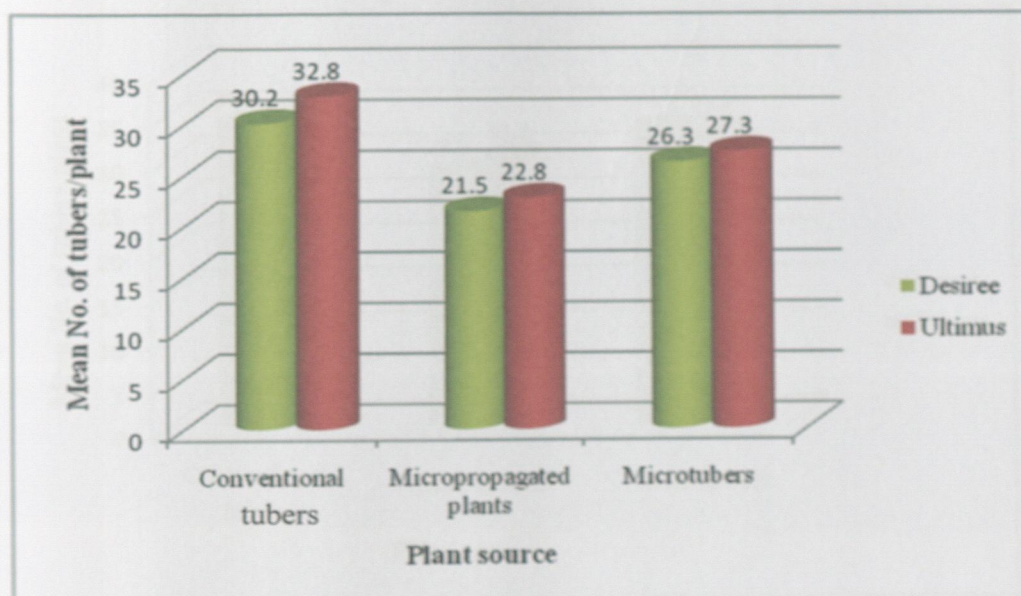


Fig. 104. Mean number of tubers obtained from micropropagated plants and microtubers of potato cvs Desiree and Ultimus in field experiments.

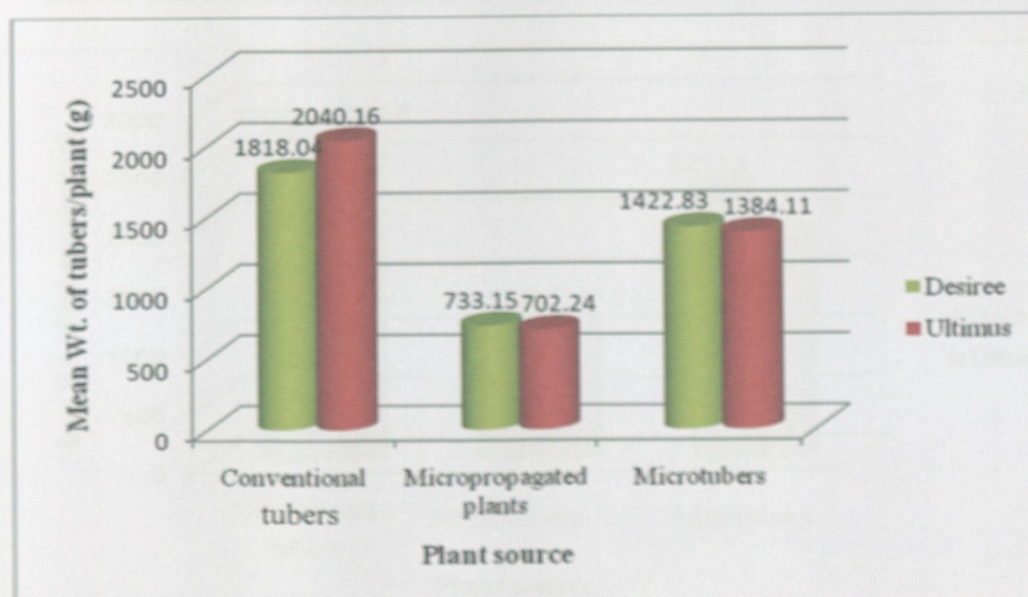


Fig. 105. Mean weight of tubers obtained from micropropagated plants and microtubers of potato cvs. Desiree and Ultimus in field experiments.

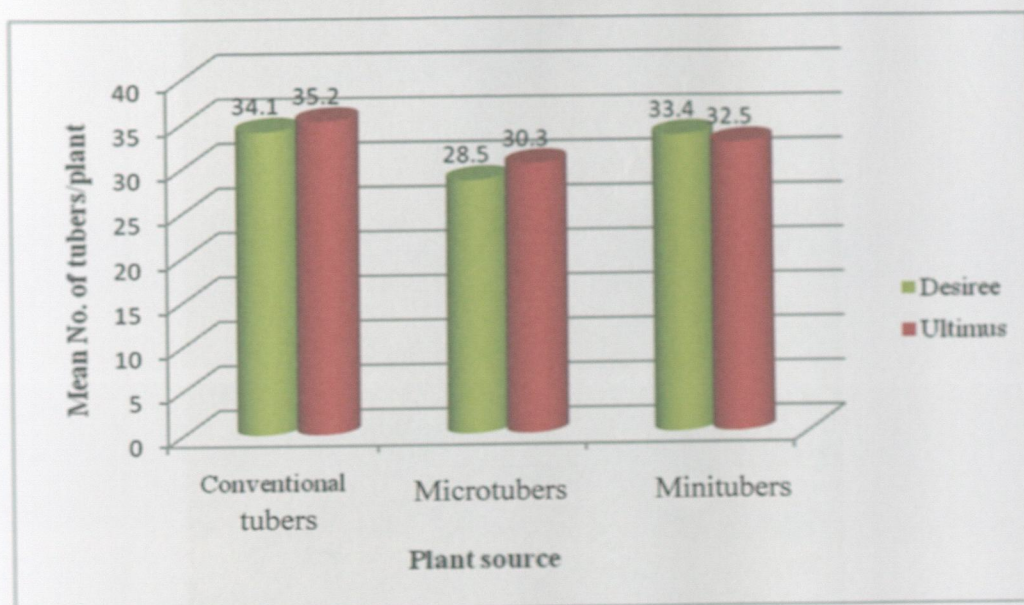


Fig. 106. Mean number of tubers obtained from microtubers and minitubers of potato cvs. Desiree and Ultimus in the field.

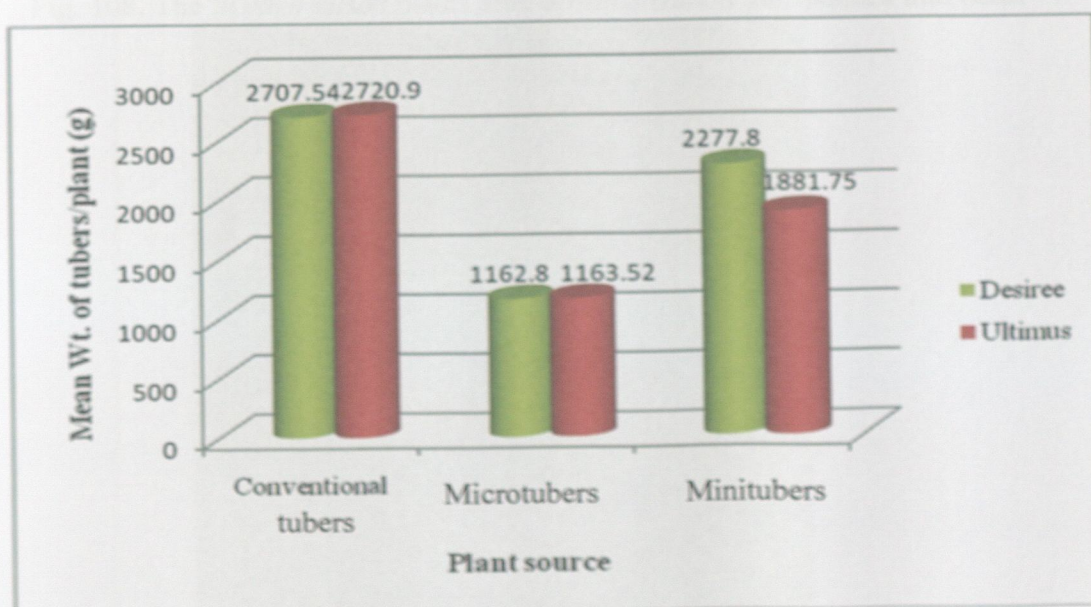


Fig. 107. Mean weight of tubers obtained from microtubers and minitubers of potato cvs. Desiree and Ultimus in the field.



Fig. 108. The *in vitro* raised plants after acclimatization transplanted into beds.



Fig. 109. Micropropagated plants of potato cv. Desiree six weeks after transplantation.



Fig. 110. Micropropagated plants of potato cv. Ultimus six weeks after transplantation.

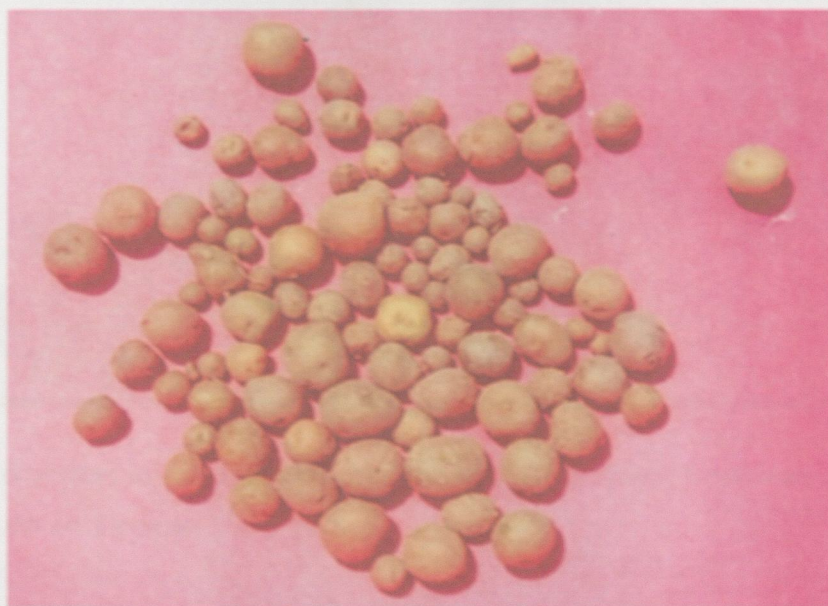


Fig.111. Minitubers obtained from micropropagated plants of potato cv. Ultimus.

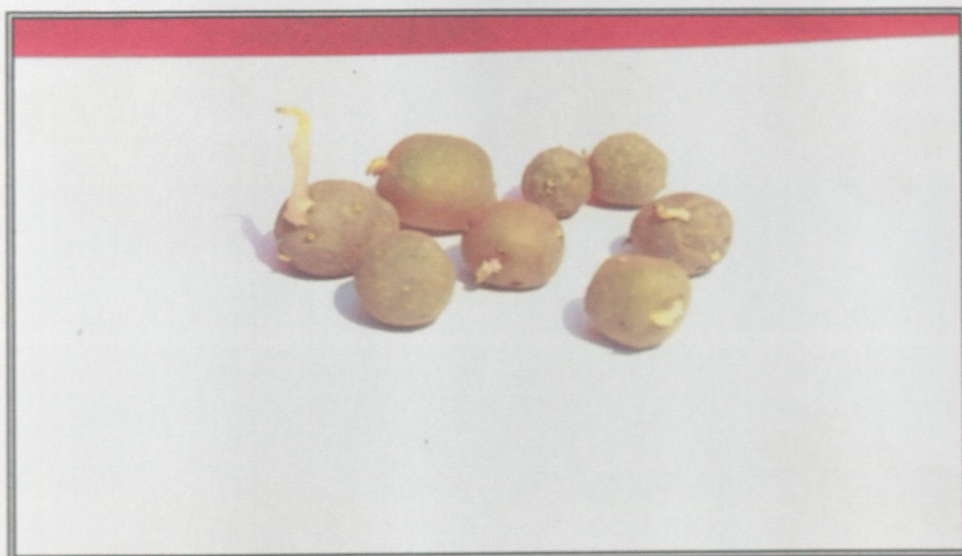


Fig. 112. Minitubers of potato cv. Desiree at sprouting stage.

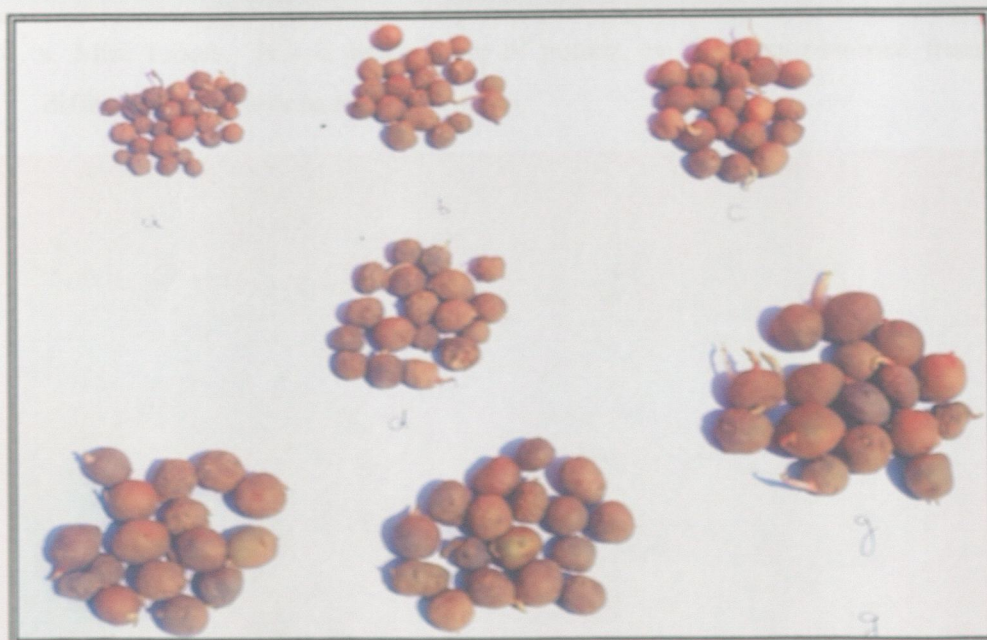


Fig. 113. Microtubers of varying sizes obtained from the different treatments in *in vitro* in potato cv. Desiree.

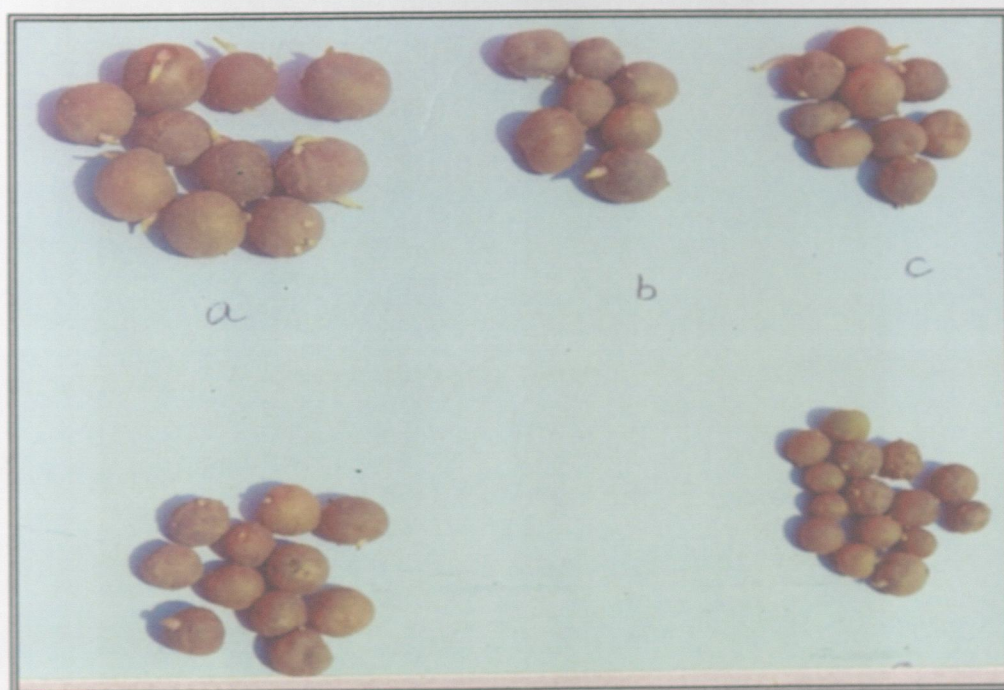


Fig. 114. a. Mini tubers. b. - e. Microtuber of potato. cv. Ultimus obtained from the different treatments in *in vitro*.



Fig. 115. (a). Microtubers and (b). Minitubers from micropropagated plants of potato cv. Desiree.



Fig. 116. Plants raised from microtubers of cv. Ultimus.



Fig. 117. Potato plants raised from microtubers of cv. Desiree.

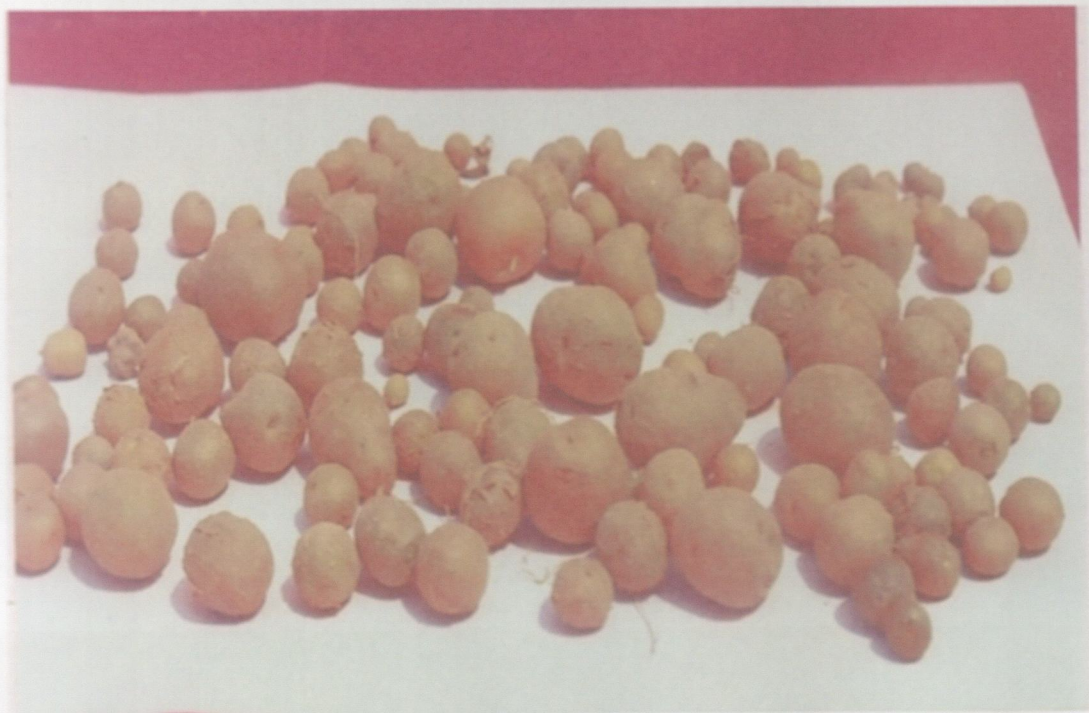


Fig. 118. Minitubers obtained from microtubers.



a. Desiree.



b. Ultimus.

Figs. 119a, b. Potato plants obtained from microtubers ranging in size from 5mm – 7mm, 3 weeks after sowing.



Fig. 120. Potato plants obtained from microtubers.



Fig. 121. Potato plants raised from minitubers.

CONCLUSIONS

It is concluded that the results of the present study suggest that application of the auxin-like growth regulator, IAA, to the culture media of *Chlorella vulgaris* can improve both plant growth and biomass yield.

It would be proved that this study has been a first step in the study of the effect of auxin-like growth regulator on the growth of *Chlorella vulgaris*. Further studies are needed to determine the effect of auxin-like growth regulator on the growth of other algae species.

CHAPTER – 5

CONCLUSIONS

&

RECOMMENDATIONS

Among the different growth regulators (BAP, Kinetin, Auxin, IAA, ABA and Gibberellin) used, BAP gave the best results. Maximum growth was obtained on BAP + 2% sucrose in the presence of 2% sucrose, whereas maximum biomass yield was obtained on BAP + 2% sucrose.

The light induction study showed that BAP gave a better yield both in terms of biomass and weight in culture on BAP + 2% sucrose and no light period.

It is concluded that BAP is a better growth regulator than the other auxin-like growth regulators used in this study. It is recommended that further studies be carried out to determine the effect of BAP on the growth of other algae species.

It is suggested that further studies be carried out to determine the effect of BAP on the growth of other algae species. It is also suggested that further studies be carried out to determine the effect of BAP on the growth of other algae species.

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CONCLUSIONS

In conclusion the results of the present research suggest that standardization of culture media and culture conditions improve both plant growth and microtuber yield.

1. In could be gained from these studies that $\frac{1}{2}$ strength MS basal medium without any growth regulators was enough for sprouting of the buds and supporting further growth of the plants but could not aid in shoot proliferation. The addition of growth regulators was therefore essential for shoot multiplication and its further growth. Furthermore long days (16hrs photoperiod) gave a better response towards sprouting of the buds and its further growth than short days (8hrs photoperiod). Nodal segments (axillary buds) produced higher number of shoots in comparison to apical buds of the stem.
2. The addition of 0.5mg/l BAP + 0.1mg/l NAA + 0.4mg/l GA₃ in the basal medium gave best multiple shoot formation.
3. The results of this study suggest that sucrose is a better carbon source in comparison to glucose as far as tuber yield is concerned both in terms of number and weight. It is further concluded that the mere presence of sugars in medium is not enough for a better tuber yield. The addition of growth regulators is therefore essential to obtain a higher yield.
4. Among the different growth regulators (BAP, Kinetin, Adenine sulphate, IAA, ABA and Coumarin etc.) used, BAP gave the best results. Maximum tuber number was attained on 10mg/l BAP in the presence of 8% sucrose, whereas maximum tuber weight was obtained on 6.0mg/l BAP + 8% sucrose.
5. For tuber induction short photoperiod (8hrs) gave a better yield both in terms of number and weight in contrast to long (16hrs) and no light photoperiods.
6. MS medium in comparison to B-5 medium supplemented with the same set of growth regulators gave a better tuber yield both in terms of number and weight.
7. It is further concluded from the present studies that tuberization response of the cv. Desiree was slightly better than that of Ultimus, which demonstrates that *in vitro* tuberization capacity of potato depends on the genotype.

8. Performance of microtuber was better in the field in comparison to *in vitro* raised plantlets. A great number of the *in vitro* plantlets are wasted during the acclimatization stage whereas the microtuber need not to be acclimatized prior to field transfer and therefore can be produced round the year.

2. Microtubers are the first generation of seed potato from tissue culture. They are used to solve the problems of propagating the plantlets from *in vitro* to *in vivo* conditions. They do not need to be acclimatized and can be directly sown in the soil.

- a. They can withstand the changes in outside temperature better.

- b. They can be easily transported to various destinations, stored, planted and multiplied.

In all potato growing regions the availability of high quality clean seed tuber has been the most limiting factor due to conventional propagation that favours disease build up and degradation of tuber yield. Microtubers may therefore find a solution where the availability of high quality seed tuber forms a constraint due to disease infection.

4. Microtubers are smaller and lighter than conventional seed potatoes. They need a small storage space and are therefore not only convenient for transport but also easy to store and handle.

5. The tissue culture technology of microtubers led to the mass production of seed potatoes, reducing the production cost sharply.

6. The tissue culture technology of microtuber is the year round mass production system of seed potatoes in the laboratory, compared with the conventional production system of seed potatoes which produces a single or double crop a year round.

7. The microtubers could be used for further *in vitro* techniques for potato propagation or can be introduced to potato seed propagating base station or distributed to the farmers. The production of microtubers is thus an approach to revolutionize the potato seed industry.

RECOMMENDATIONS

1. Micropropagation is the alternative to conventional propagation of potatoes. *In vitro* propagation methods using sprouts and nodal cuttings are more reliable for maintaining genetic stability.
2. Microtubers are the first generation of seed potato from tissue culture. They are used to solve the problems of transplanting the plantlets from *in vitro* to *in vivo* conditions. They do not need to be acclimatized and can be directly sown in the soil.
 - a. They can withstand the changes in outside temperature better.
 - b. They can be easily transported to various destinations, stored, planted and multiplied.
3. In all potato growing regions the availability of high quality clean seed tuber has been the most limiting factor due to conventional propagation that favors disease build up and drastically reduces yield. Microtubers may therefore find a solution where the availability of high quality seed tubers forms a constraint due to disease infestation.
4. Microtubers are smaller and lighter than conventional seed potatoes. They need a small storage space and are therefore not only convenient but inexpensive to store and transport.
5. The tissue culture technology of microtubers led to the mass production of seed potatoes, reducing the production cost sharply.
6. The tissue culture technology of microtuber is the year round mass production system of seed potatoes in the laboratory, compared with the conventional production system of seed potatoes which produces a single or double crop a year in soil.
7. The microtubers could be used for further *in vitro* techniques for potato propagation or can be included in potato seed programs and disseminated to the farmers. The production of microtubers is thus an approach to revolutionize the potato seed industry.

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