

Effect of varying media formulations on microtuberization in the potato cultivars

A Thesis

*Submitted in partial fulfillment of the requirement
for the award of degree of
Masters of Science in Biotechnology*

**Under the guidance of
Dr. N. DAS
Associate professor
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Dedicated to.....

My parents.....

Certificate

This is to certify that the thesis entitled "**Effect of varying media formulations on microtuberization in the potato cultivars**" submitted by Sonal Bansal (Roll no: 300901017) in partial fulfillment of the requirement for the award of Degree of Master of Sciences in biotechnology, to Thapar University (Deemed University), Patiala, is a record of Student's own work carried out by her under our supervision and guidance. The report has not been submitted for the award of any other degree or certificate in this or any other university or institute.


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Candidate's Declaration

I hereby declare that the work which is being presented in this thesis "**Effect of varying media formulations on microtuberization in the potato cultivars**" submitted by the undersigned in partial fulfillment of the requirement for the award of Degree of Master of Sciences in biotechnology, Thapar University, Patiala, is true and original record of my own independent and original research work carried out under the supervision of Dr. N. Das, Associate professor, Department of Biotechnology and Environmental Sciences, Thapar University, Patiala, India. The matter embodied in this thesis has not been submitted in part or full to any other university or institute for the award of any degree.

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Abstract

In vitro microtuberization represents the transitory phase between *in vitro* multiplication of a healthy plant material and field growing stage. Microtubers are small in size, less in weight, having similar morphology and biochemical features with traditional tubers and are produced *in vitro* in a plethora of different growing systems with varying environment and media constituents. Microtubers offer a lot of advantages to storage, transport and mechanization and are used as in basic and strategic research covering the areas of plant metabolism, germplasm selection and molecular farming. Microtuberization is a complex physiological process regulated by many factors like genotype of the cultivar and environmental conditions. So, its response varies from cultivar to cultivar. In the present study, we mainly focussed on some of our Indian potato cultivars viz. Kufri Chipsona 1, Kufri Chipsona 2, Kufri Pukhraj and Kufri Ashoka along with an exotic variety Desiree, which are suitable to our agro-climatic zones. Various media formulations were used to study the effect of sucrose and chloro choline chloride (CCC) on different cultivars in the same environmental conditions. In the media containing 8% sucrose concentration along with benzyl amino purine (BAP) and CCC, the potato cultivar Kufri Pukhraj showed good response, Kufri Ashoka and Kufri Chipsona 1 showed average response whereas, Desiree and Kufri Chipsona 2 showed poor response with respect to average tuber weight, length and tuber number per plantlet. In 6% sucrose, again the cultivar Kufri Pukhraj showed good response, Kufri Chipsona 1 and Kufri Ashoka showed average response whereas, Kufri Chipsona 2 and Desiree showed poor response. In 4% sucrose, the cultivars Kufri Pukhraj and Kufri Ashoka showed maximum response Desiree and Kufri Chipsona 2 showed average response while, Kufri Chipsona 1 showed poor response. Early tuber induction was observed in the cultivars Kufri Pukhraj and Desiree while late tuberization was observed in the cultivar Kufri Chipsona 1. Similarly, the effect of CCC on microtuberization of these cultivars was also studied. In media containing 8% sucrose and 500 mg/L CCC, the best results were shown by the cultivar Kufri Pukhraj, followed by Kufri Ashoka and Kufri Chipsona 2 whereas, Kufri Chipsona 1 and Desiree showed least response. In media containing 8% sucrose and 250 mg/L CCC, the best response was shown by Kufri Ashoka and Kufri Pukhraj followed by Desiree and Kufri Chipsona 1 while, Kufri Chipsona 2 showed least response. Kufri Chipsona 1 and Kufri Pukhraj showed early tuber induction while, Kufri Chipsona 2 showed late tuber induction in all CCC concentrations. When both CCC and BAP were omitted from the tuberization media containing only sucrose, the cultivars Kufri Pukhraj and Kufri Chipsona 1 showed best response in 8%, 6%, 4% sucrose whereas Desiree showed good response in 8% and 6% sucrose, followed by Kufri Chipsona 2 and least response was shown by Kufri Ashoka. Early tuber induction was seen in case of Desiree and Kufri Pukhraj whereas, Kufri Chipsona 1 and Kufri Chipsona 2 showed late response to tuber induction. As a whole, the cultivar Kufri Pukhraj showed best performance in terms of tuber yield and size, as compared to other cultivars when grown at various media formulations.

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Chapter 1

Introduction

1.1 Potato

Potato (*Solanum tuberosum*, Solanaceae) is the major tuber crop that is grown in temperate regions. It is a cool season crop that requires an optimal average temperature between 20–25°C. From its origins in the Andes, the cultivated potato has spread to most parts of the world in the last 400 years. It is now a major source of nutrition for people from a wide variety of cultural and ethnic backgrounds. It is also the most important tuber crop in terms of production, accounting for about 45% of the total world production of all tuber crops. In the developing countries of Asia and Africa, the rate of increase in potato production has been higher than that of the most other crops. Presently, potato ranks fourth in terms of total global food production after maize, rice and wheat. So, research into tuber initiation and development is of great relevance to global food security.

1.1.1 Taxonomy of Potato

The potato is botanically known as *Solanum tuberosum* L., (a tetraploid with 48 chromosomes), and is a member of the Solanaceae or Nightshades family of plants, that consists of about 2000 species. It is estimated that probably 700 out of them are really distinct. It is also interesting to know that only six out of these species grow potato tubers at all, remaining all are known in the world as most plants do for their flower, fruit, and seed. The six tuber bearing species are- *Solanum tuberosum*, *Solanum magila*, *Solanum commersoni*, *Solanum cardiophyllum*, *Solanum jamessi*, *Solanum oxycarpum*.

Solanum tuberosum is a hybrid between the diploid species *S. stentotomum* and the diploid weed *S. sparsipilum* with subsequent chromosome doubling. The potato has a series of ploidy levels, based on a haploid number of 12, ranging from diploid ($2n=2x=24$) to hexaploid ($2n=6x=72$), and including triploids, tetraploids and pentaploids (Dodds, 1962). The cultivated potatoes are autotetraploid ($2n=4x=48$); many wild species are diploid, but may range up to hexaploid. The tetraploid cultivated potatoes are not diploid, so that there are four interchangeable genes at each locus (Ross, 1986).

1.1.2 Nutritional aspects of potato

In a potato tuber, about 80% is water and the rest is dry matter. In terms of nutrition, the potato is best known for its carbohydrate content. The predominant form of this carbohydrate is starch. The average raw material composition of a potato tuber consist of Dry matter (20 %), Starch (13-16 %), Total sugars (0-2 %), Protein (2 %), Fibre (0.5 %), Lipids (0.1 %), Vitamin A (trace/100 g fresh weight), Vitamin C (31 mg/100g fresh weight), Minerals (trace), Ash (1-1.5 %), Glycoalkaloids (<1 mg/100 g fresh weight).

1.1.3 Potato productivity

The United Nations FAO reports that the world production of potatoes in 2008 was 314 million tonnes. The annual diet of an average global citizen in the first decade of the 21st century included about 33 kg (or 73 lb) of potato. However, the local importance of potato is extremely variable and rapidly changing. It remains an essential crop in Europe (especially eastern and central Europe), where per capita production is still the highest in the world, but the most rapid expansion over the past few decades has occurred in Southern and Eastern Asia. China is now the world's largest potato-producing country, and nearly a third of the world's potatoes are harvested in China and India.

1.1.4 Morphology

The potato is a starchy, tuberous crop from perennial *Solanum tuberosum* of the family Solanaceae (also known as the nightshades). The word potato may refer to the plant itself as well as the edible tuber. The potato is a tuber grown underground on a specialized plant part (subterranean stem) known as stolon. Therefore, it is a modified stem in a strict botanical sense. A potato tuber is usually oval to round in shape, although intermediate shapes are also frequently encountered. It consists of an inner flesh and an outer protective cover known to be envelope or the skin which comprises the corky or outer covering of the tuber, corresponding to the bark of an above ground stem. There is a great variation in flesh colour and skin finish. The eye shaped depressions on a potato tuber is known as its eyes, and actually these are the dormant buds, which give rise to new shoots under suitable conditions. Potato plants are herbaceous perennials that grow about 60 cm high, depending on variety.

1.1.5 Development and Anatomy of the tuber

The potato has a complex life cycle. Like most plants, it produces seed and future generations can grow from seed. Unlike most plants, it also produces tubers that gardeners harvest and

then use to produce more plants. These tubers are seed potatoes and they are not the same as potato seed.

Tubers are fundamentally different from seeds in their anatomy and development in that seeds represent the end point in a complex and tightly regulated developmental pathway that is initiated by fertilization and ends in the programmed dehydration of the seed. In contrast, tubers are somatic storage tissues which develop by the modification of somatic structures of the plant (Cutter, 1982). The ability to form tubers is limited to only about 160 wild and 7 cultivated species out of approximately 2000 members of the genus *Solanum*. Most of these are wild diploids with tri, tetra, penta and hexaploid species accounting for nearly a third of the tuber bearing species.

Potato tubers represent underground stems which have undergone a morphological differentiation due to a drastic radial expansion and limited cell division. Biologically, tubers are important as they store carbon and usually also nitrogen in a form that can be mobilized when required. Also most of the plant tubers act as propagules, thus they can give rise to new plants on sprouting. In this case, they need to contain a sufficiently wide range of nutrients to support the requirements of the plant until it is capable of independent growth. Under field conditions, induction of this differentiation process is controlled by a number of environmental conditions such as photo-period, temperature and nitrogen supply.

A potato plant tuberizes only when two specific environmental conditions are met with. These are short-day photoperiod (daylight) and cool night temperature. High temperatures are inhibitory for tuberization in both short and long photoperiods, although the inhibitory effect is much greater in long photoperiods. And that is why the potato growing conditions in India are entirely different from those in temperate countries of Europe and North America. High temperatures affect the partitioning of assimilates by decreasing the amount going to the tubers and increasing the amounts to other parts of the plant. There is also some evidence that the inhibitory effect of high temperatures is mediated through increased GA (Gibberellic acid) levels. High light intensity is known to promote tuberization and the promotive effects of high levels of irradiance can ameliorate the inhibitory effects of high temperatures.

1.1.6 Propagation of potato

A potato plant is propagated through tubers, in contrast to many of the cereal crops which are propagated through seeds (sexual hybridization). The tubers meant for propagation are known

as seed tubers or seed potatoes which are small potatoes. This type of propagation has advantages and disadvantages as well.

The main advantage is that a good potato clone can be maintained with a high degree of genetic purity. The disadvantage is that many deadly viruses and seed-borne pathogens are progressively accumulated in the tubers and carried over repeated multiplications resulting in the gradual degeneration of a clone. For this reason, successful potato cultivation and production depend upon the availability of disease-free high-quality seed tubers. This is mostly important in tropical and sub-tropical warm climates as in India where there is an abundance of various vectors, e.g. aphids, mites, white flies, etc. for virus transmission. As a consequence, the cost of good quality seed potatoes alone accounts for about 40-60 % of the total production cost in many countries.

A potato plant can also be propagated through these botanical seeds, which are known as True Potato Seeds (TPS). In areas where seed potato production is not feasible or economic, TPS is an alternative means of propagation. This type of propagation has advantages and disadvantages as well.

The advantages could be listed as-

- Potato production through TPS can not only reduce the production cost, but also increase the net profit of the farmers.
- This technique is used for disease free seed production. It is the technique for virus free seed production.
- Cost of tubers used in conventional method of planting is very high whereas the production of tubers in nursery for planting in next year is relatively very low.

However, TPS technology is presently not full-proof for a large-scale commercial exploitation due to one or other agro-technical as well as techno-economical problems.

The main disadvantages could be listed as-

- Difficulty in producing potatoes those are uniform in shape, colour, size and performance (as a requirement in food industry).
- Instead of producing a genetically identical clone of the mother plant as is the case when growers use seed tubers, each plant grown from open TPS is genetically different.

In order to overcome such problems related to the production of disease free good quality potato, tools of plant tissue culture are applied with conventional methods.

1.2 Plant Tissue Culture

Plant tissue culture is a practice used to propagate plants under aseptic conditions, often to produce clones of a plant for indefinite period on a nutrient medium. For successful plant tissue culture it is best to start with an explant rich in undetermined cells i.e. those from cortex or meristem, because such cells are capable of rapid proliferation. The usual explants are buds, shoot tips, root tips, nodal segments or germinating seeds.

Different techniques in plant tissue culture may offer certain advantages over traditional methods of propagation, including:

- The production of exact copies of plants that produce particularly good flowers, fruits, or have other desirable traits.
- The production of multiples of plants in the absence of seeds or necessary pollinators to produce seeds.
- The regeneration of whole plants from plant cells that have been genetically modified.
- The production of plants in sterile containers that allows them to be moved with greatly reduced chances of transmitting diseases, pests, and pathogens.
- The production of plants from seeds that otherwise have very low chances of germinating and growing, i.e. orchids and nepenthes.
- To clean particular plant of viral and other infections and to quickly multiply these plants as 'cleaned stock' for horticulture and agriculture.

Plant tissue culture relies on the fact that many plant cells have the ability to regenerate a whole plant (totipotency).

It is possible to use tissue culture technology to propagate, conserve, and transfer potato germplasm. Tissue culture allows the rapid clonal propagation of large numbers of plantlets in a short period and the conservation of potato germplasm under controlled conditions requiring reduced space and labour (Espinoza *et al.*, 1984).

1.2.1 Micropropagation

The term propagation means the generation (reproduction) of plants by asexual means. It is the practice of rapidly multiplying stock plant material to produce a large number of progeny plants, using modern plant tissue culture methods. It is carried out in controlled

and aseptic conditions rather than in greenhouse or nursery. The objective of micropropagation is to obtain large numbers of clonal plants in a short period.

Micropropagation of potato is carried out by two methods-

Nodal cuttings- Single nodes with leaves are excised from small *in vitro* plantlets. The single node is then inoculated onto the surface of agar solidified medium. The axillary bud will quickly grow out and in 3-4 weeks culture will be ready for further propagation. This is commonly used in the production of disease free seed tubers, germplasm exchange and conservation (Roca *et al.*, 1979; Ranalli *et al.*, 1994).

Shaker cultures- *In vitro* plantlets are cut in pieces with three to four nodes and large leaves are removed. These segments are then placed in liquid medium and the flask is shaken. After 2-3 weeks very rapid growth has taken place and each flask will contain 60-70 nodal sections for further propagation.

The final products of potato micropropagation are either plantlets or microtubers (*in vitro* tubers). The use of microtubers in the storage and exchange of germplasm and seed potato production is advantageous (Hussey and Stacy, 1981; Tovar *et al.*, 1985; Seabrook *et al.*, 1993; Ranalli *et al.*, 1994) because microtubers can be stored longer and are easier to handle and to transport than plantlets (Struik and Lommen, 1991).

Micropropagation has a number of advantages over traditional plant propagation techniques:

- The main advantage of micropropagation is the production of many plants that are clones of each other.
- Micropropagation can be used to produce disease-free plants.
- It is the only viable method of regenerating genetically modified cells or cells after protoplast fusion.
- It is useful in multiplying plants which produce seeds in uneconomical amounts, or when plants are sterile and do not produce viable seeds or when seed can't be stored.
- Micropropagation often produces more robust plants, leading to accelerated growth compared to similar plants produced by conventional methods - like seeds or cuttings.
- A greater number of plants can be produced per square meter and the propagules can be stored longer and in a smaller area.
- Potato has always been a challenge to functional genomics.

The cultivated potato, *Solanum tuberosum* L., is a tetraploid ($2n = 4x = 48$) and exhibits complex tetrasomic inheritance. It is highly heterozygous and segregates on sexual

reproduction. Elite parental lines and cultivars of potato are thus maintained through vegetative propagation in order to maintain their genetic integrity. Maintenance of potato germplasm through field clonal propagation is time consuming, requires large amounts of space and is labour intensive. This also exposes the plants to disease, pests, and risks of loss due to abiotic stresses and natural calamities (Withers *et al.*, 1990). Therefore, throughout the world potato gene banks prefer to conserve elite parental lines and clones as *in vitro* propagated micro plants under disease free tissue culture conditions (Westcott *et al.*, 1977; Golmirzaie *et al.*, 1999; Gopal *et al.*, 2002).

1.2.2 *In vitro* Microtuberization

Microtubers are the miniature tubers developed under the tuber inducing conditions *in vitro*. Thus, Microtuberization is the process which allows large scale asexual multiplication of desired cultivars. Because of their small size and weight, (they are very small, average weight 100-150 mg) these tubers are particularly convenient for handling, storage and distribution.

Wang and Hu (1982) first reported *in vitro* mass tuberization in potato and its successful integration in to virus free seed potato production programme at Taiwan. This technique was then adopted at the International potato centre (CIP), Lima, Peru (Estrada *et al.*, 1986), at the University of Wisconsin (Wattimena *et al.*, 1983), at the Central Potato Research Institute, Shimla, India (Chandra and Naik, 1993) in developing complementary methods of clonal propagation for germplasm exchange and commercial potato production.

Microtubers have tremendous advantages in terms of storage, transportation and mechanization. Unlike micro propagated plantlets, they do not require time consuming hardening period in greenhouses. They can be directly sown into the soil and can be produced in lab in bulk in any season. They have the similar morphological and biochemical characteristics to field produced tubers (Amina Kanwal *et al.*, 2006).

Economical use of microtubers is possible if the *in vitro* tuberization rate is satisfactorily high and reliable (atleast one microtuber per explant, or higher) and if the size of developed microtubers is sufficiently large.

Microtubers larger than 2 mm can be further propagated but only microtubers larger than 4 mm are suitable for long term storage. It is necessary to increase the tuber size because the larger the microtuber, the lower the loss during storage (Tabori *et al.*, 1999) and the better is the initial vigour, emergence and performance (Wiersema *et al.*, 1987; Ranalli *et al.*, 1994).

1.2.3 Applications of Microtubers

- The application of microtubers in germplasm conservation is widely accepted. Microtubers are produced *in vitro* in a plethora of different growing systems with varying environment, media constituents, and storage intervals. Many of the interactions between growth parameters *in vitro* and subsequent productivity appear to be genotype-specific.
- The use of microtuber technology in seed tuber production, breeding programmes, germplasm conservation, germplasm storage and exchange (Tovar *et al.*, 1985; Donnelly *et al.*, 2003) and research appears to have enormous potential. Microtubers are utilized for minituber (small tubers produced from *in vitro* produced propagules) production in greenhouses or screen houses and less commonly, are directly field planted.
- Can also be used as research tools in the areas of plant metabolism, germplasm selection and evaluation, transformation, somatic hybridization or molecular farming (reviewed by Coleman *et al.*, 2001) and for *in vitro* selection of agronomically important characters such as maturity, abiotic stress tolerance and others (Lentini and Earle, 1991; Gopal and Minocha, 1997; Gopal and Minocha, 1998; Gopal *et al.*, 1998; Vermendi *et al.*, 2000; Donnelly *et al.*, 2003; Gopal and Iwama, 2007).

1.3 Factors influencing the initiation and growth of microtubers

Microtubers have become an important mode of rapid multiplication for pre-basic stock in seed tuber multiplication as well as germplasm exchange. The number and size of microtubers produced *in vitro* depend on many factors, such as optimum concentration of sugar, growth regulators and anti-gibberellin compounds in the culture medium (Tovar *et al.*, 1985; Dodds *et al.*, 1988) as well as various environmental factors which presumably take effect through the hormonal balance of the plant. Among the substances used to induce microtubers, coumarin, CCC (Chloro choline chloride) and cytokinins have received adequate attention (Wang and Hu, 1985; Chandra *et al.*, 1988). Cytokinins are believed to have strong promotive effects on tuberization and to constitute major part of the tuberization stimulus, either alone or in combination with other substances (Palmer and Smith, 1969; Pelacho and Mingo-Castel, 1991). Growth retardant stimulates tuberization of plants under unfavourable environmental conditions (Menzel, 1980) and CCC is widely used in tissue culture media to promote microtuber formation (Tovar *et al.*, 1985 and Rosell *et al.*, 1987). It induces

microtuber formation by a wide range of *Solanum* genotypes of diverse genetic backgrounds (Estrada *et al.*, 1986). Although CCC stimulates tuber initiation by recalcitrant genotypes, it can inhibit microtuber growth in *Solanum tuberosum* cultivars that form tubers readily in its absence. Vecchio *et al.*, (1994) reported that *in vitro* tuberization is linked with the genotype and the culture substratum. According to them, the potato variety Desiree tuberized readily than other varieties with a low CCC concentration.

1.3.1 Environmental Factors

Light- Day length and phytohormones affect considerably potato tuberization. However, biochemical mechanisms underlying these effects are obscure until now. Potato wild species and subspecies can produce tubers only under conditions of short day (SD) as longer light conditions promotes photosynthesis in the plant that contributes to plant growth. During dark conditions the plant assimilates are directed towards the storage tissue for tuber production. Many cultivars manifest a weaker SD response of tuberization. Since the magnitude of the photoperiodic response affects the time and synchronicity of potato yield production in the regions at various latitudes, the investigation of tuberization photoperiodic control is of importance not only for basic but also for applied aspects of science.

Temperature- *In vitro* temperature can affect microtuber production (Wang and Hu, 1982; Akita and Takayama, 1994). Although the growth of micropropagated plantlets is optimal at 20–25°C, microtuber formation is often best at (slightly) lower temperatures. According to Wang and Hu (1982) the average number of microtubers is lower at higher *in vitro* temperature (28–28°C day/night) than at lower temperature (20–20°C day/night). The optimum temperature for *in vitro* tuberization has been suggested to be 20°C (Wang and Hu, 1982) or between 15 and 18°C (Akita and Takayama, 1994). Moreover, it has been suggested that with equal average temperatures, constant temperatures will give higher levels of microtuber formation than alternating day night temperatures (Wang and Hu, 1982).

1.3.2 Composition of medium

1.3.2.1 Sucrose: One of the most important factors is the carbon source in the medium; both its type and concentration have profound effects on tuber growth. Sucrose is considered to be the optimal carbon source as compared to its constituent hexoses—glucose and fructose (Dodds *et al.*, 1992; Khuri and Moorby, 1995). Using radiolabelled sugars, Khuri and Moorby (1995) demonstrated that more sugar is translocated to microtubers, when sucrose rather than glucose or fructose is the carbon source. The reported optimal concentration of sucrose ranges

from 60 to 80 g (Abbot and Belcher, 1986; Dodds *et al.*, 1992; Garner and Blake, 1989; Hussey and Stacey, 1984; Lawrence and Baker, 1963). A too high or low concentration of sucrose leads to slower tuberization, fewer and smaller microtubers. It plays a key role in coordinating the occurrence or 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 phytohormone 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 at higher concentrations it serves as a signal for microtuber formation. Sucrose may play a dual role in microtuber development. Apart from being a suitable carbon source that is easily assimilated by the microplants and converted to starch in developing microtubers, sucrose at a concentration of 80 g/L, also provides a favourable osmolarity for microtuber development (Khuri and Moorby, 1995).

1.3.2.2 Phytohormone: Many studies have implicated growth regulators i.e. phytohormones in tuberization.

Gibberellic acid abolishes tuberization when applied to induced cuttings. Various auxins stimulate tuber formation *in vitro*. Exogenous GAs can promote tuber sprouting (Van Ittersum, 1992) but usually only after dormancy is complete.

Cytokinins are commonly included in the media for *in vitro* tuberization of potatoes (Wang and Hu, 1982). Cytokinins promote *in vitro* tuberization of potato by altering GA balance in non induced stems (Lentini and Earle, 1991), inhibiting root formation and transferring the upright leafy shoots into horizontal stolons. Cytokinins can enhance microtuberization (Wang and Hu, 1985) as well as modify tuber dormancy duration depending on the cultivar (Wattimena, 1983).

Benzyl Amino Purine (BAP) is a synthetic cytokinin that is predominantly used in the media for microtuberization. It is believed to have strong promotive effects on tuberization, and to constitute major part of the tuberization stimulus, either alone or in combination with other substances (Palmer and Smith, 1969; Pelacho and Mingo-Castel, 1991). It induces microtuber formation by a wide range of *Solanum* genotypes of diverse genetic backgrounds (Estrada *et al.*, 1986). Cytokinins play a major role in cell division and elongation which helps in tuber growth and development. Although cytokinin is not directly responsible for tuberization as

reported by many workers, undoubtedly, it plays a key role in cell division and thus creating sink activity of the developing tuber.

Ethylene seems to stimulate tuberization and is antagonistic to the inhibitory effects of gibberellic acid (Stallknecht and Farnsworth, 1982). Endogenous ethylene may be an important component of early dormancy development in microtubers (Suttle, 1998). Whether endogenous ethylene plays a role in dormancy release of microtubers, it is still to be demonstrated.

Abscisic acid's possible role in tuberization is surrounded by uncertainty. It has a stimulatory effect on tuber formation on intact plants but inhibits *in vitro* formation (Kumar and Wareing, 1974).

1.3.2.3 Chemicals

Chloro Choline Chloride- CCC has strong promoting effects on potato microtuberization. It acts as a growth retardant and stimulates tuberization of plants under unfavorable environmental conditions (Menzel, 1980). CCC transfers plant assimilates towards the tuber for its growth and development. Other than above mentioned factors physiological age of explant, culture density, type of explant also affects the total production of microtubers. Although CCC stimulates tuber initiation by recalcitrant genotypes, it can inhibit microtuber growth in *Solanum tuberosum* cultivars that form tubers readily in its absence. Vecchio *et al.*, (1994) reported that *in vitro* tuberization is linked with the genotype and the culture substratum. According to them, the potato variety Desiree tuberized readily than other varieties with a low CCC concentration.

To promote research in the field of potato production worldwide many organizations are working. Foremost of all is the International Potato Centre (known by its Spanish acronym, CIP) La Molina, Lima (Peru) that seeks to reduce poverty and achieve food security on a sustained basis in developing countries through scientific research and related activities on potato. In India, the Central Potato Research Institute (CPRI) was established in 1949. Since its inception CPRI has grown rapidly. It has the responsibility of producing disease free basic seed to meet the country's requirement.

Chapter 2

Review of Literature

Microtuberization, a technique used to produce microtubers is an alternative means of maintaining the germplasms and has tremendous applications in the area of plant biotechnology. Since its invention to till date, process of microtuberization has been studied extensively with regard to media formulations and environmental factors. The process of microtuberization is influenced by many factors out of them the most important being media components, environmental factors and genotype dependent. In all potato growing regions the availability of high quality clean seed tuber has been the most limiting factor owing to the conventional clonal propagation that favours disease build-up that drastically reduces yield (Gebre and Sathyanarayana, 2001). The potato is one of the important vegetable crop, is a staple food crop in many countries of the world. The following review is mainly focussed on the efforts made by many contributors to improve the microtuberization process by varying different parameters in different potato cultivars.

2.1 *In vitro* Microtuberization

Murashige and Skoog, 1962 firstly given MS media that is used by various multiplication programmes in its original or modified form with variable supplements of vitamins and plant bioregulators (PBRs) (Estrada *et al.*, 1986). Several media for propagation under aseptic conditions were also tested by Miller *et al.*, 1985; Estrada *et al.*, 1986; Dodds, 1988.

Harmey *et al.*, 1966; Hussey and Stacey, 1984; Lillo, 1989 reported *in vitro* potato microtuberization using different types of explants including nodal and *in vitro* cuttings.

The major factors limiting the rates of multiplication in nodal culture are the short height of the plantlets and low number of nodes on plantlets obtained. Improvement has been made possible by addition of growth regulators to the medium. Gas stimulated development of nodal cutting on MS but at high concentration produced narrow and elongated shoots (Novak *et al.*, 1980) depending on the genotype.

In vitro tuber can be produced throughout the year. The potential value of tissue culture in potato production has been widely recognized. This technology has been used for disease free seed production in many countries (Wang and Hu, 1982). Seed production technique of potato

can be designed with *in vitro* multiplication through either plantlet regeneration or microtuber production. But microtuber method has several merits over plantlet regeneration.

The propagation of potato (*Solanum tuberosum* L.) by *in vitro* culture of axillary buds is commonly used in the production of disease-free seed tubers, germplasm exchange, and conservation (Roca *et al.*, 1979; Ranalli *et al.*, 1994). *In vitro* propagated plantlets produce microtubers (approx. 2-10 mm in diameter) when incubated under suitable conditions (Wang and Hu 1982; Estrada *et al.*, 1986). While microtubers generally originate as aerial structures on the stem, occasionally a few microtubers may be formed in the medium (Hussey and Stacey 1981, 1984).

Propagation by tissue culture provides the opportunity for having virus free *in vitro* cultures that could be used for micropropagation of large quantities of plantlets to produce microtubers *in vitro* or minitubers *in vivo* was stated by Estrada *et al.*, 1986.

Microtubers can be used as an additional component to the standard methods of rapid propagation used in seed tuber production was told by Dodds, 1988; Espinoza *et al.*, 1989 and it is utilized for the distribution of germplasm quite conveniently.

Microtubers have become an important mode of rapid multiplication for pre-basic stock in seed tuber multiplication as well as germplasm exchange. The number and size of microtubers produced *in vitro* depend on many factors, such as optimum concentration of sugar, growth regulators and anti-gibberellin compounds in the culture medium (Tovar *et al.*, 1985; Dodds *et al.*, 1988). Among the substances used to induce microtubers, coumarin, CCC and cytokinins have received adequate attention (Wang and Hu, 1985; Chandra *et al.*, 1988).

In vitro production of microtubers by serial culture of individual nodes with axillary buds is routinely used for disease-free seed production in potato (Gopal *et al.*, 1998). Microtubers are also convenient for handling, storage and transport of germplasm (Thieme, 1992).

In the rapid multiplication of clean material *in vitro*, the use of single nodal cutting is the most preferred method of propagation since it ensures higher propagation rates with maximum genetic uniformity in potato (Chandra and Naik, 1993).

Longest main shoot and highest node numbers are reported to be obtained in medium containing NAA and BAP (Yousef *et al.*, 1997). Among these methods, the direct use of microtubers has gained a considerable interest owing to their ease of handling, storage and transport of germplasm and reduced period to produce seed tubers (Jones, 1994).

Media conditions such as Nitrogen concentration, sucrose or osmolarity of the medium have either a direct or indirect effect on induction or development processes of *in vitro* produced microtubers (Garner and Blake, 1989; Khuri and Moorby, 1995).

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 rate of degeneration varying from place to place and from cropping season to cropping season (Tadesse, 2000).

However, there are limitations both in shoot regeneration and microtuber production. The limitations in many ways are ascribed to the components of the culture environment and to the low photosynthetic ability of the explants or plantlet. Most current systems of microtubers production have problems of obtaining sufficient number and size of microtubers produced per cycle. Thus both shoot and microtuber production systems are still less competitive and economical compared with *in vitro* rapid multiplication (Gebre and Sathyanarayana, 2001).

Microtubers are very convenient and easy to transport, it can be easily stored for long time. Hence, it is necessary to establish a protocol for *in vitro* production of micro tuber for rapid multiplication. Extensive physiological research has revealed that tuberization is controlled by several factors, such as hormonal combination, ratio of photo period, nutrient compositions etc. (Vreugdenhil & Struik, 1989, Coleman *et al.*, 2001; Tugrul & Samanci, 2001).

The viruses are transmitted through different ways including through planting infected tubers. If the seed stock is not maintained well or frequently replaced with fresh ones, 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). This is the major problem faced by seed producers. Conventional seed multiplication methods take a long time and are prone to virus problems (Biniam and Tadesse, 2008).

Microtubers displaying enhanced dormancy seem to be advantageous for potato germplasm conservation since these can be stored for longer periods without having recourse to specialized infrastructure and stringent maintenance via regeneration schedule as required for conventional storage of microplants *in vitro* (Gopal *et al.*, 2003).

The use of *in vitro* tubers (microtubers) as the final products of potato micropropagation in addition to or rather than *in vitro* plantlets have several advantages in seed tuber production was suggested by Dobranszki *et al.*, 2008.

In vitro microtuber formation potentiality of potato to establish a rapid disease free seed production system in potato was investigated by Hoque (2010).

2.2 Factors affecting Microtuberization

Gopal *et al.*, (2004) worked with the objective of using microtubers for conservation of potato germplasm, the main effects of genotype, abscisic acid (ABA) and sucrose level and of their interactions on biomass production, microtuberization, microtuber dormancy, and dry matter content were studied. ABA decreased both microtuber production and microtuber dormancy, whereas higher concentrations (60–80 g/L) of sucrose promoted biomass production, microtuber production as well as microtuber dry matter content. Microtubers stored under diffused light had longer dormancy than those kept continuously in the dark. The level of sugar in the induction medium, especially sucrose, as well as the genotype also affects microtuberization (Koda and Okazawa, 1983; Andre *et al.*, 1998; Gopal *et al.*, 1998).

In recent days, research related to the various factors that affect *in vitro* microtuberization are most attractive areas of study for researchers to produce virus free seed potato germplasm. These factors could be elaborated under following sections:

2.2.1 Genotype

Vecchio *et al.*, (1994) reported that *in vitro* tuberization is linked with the genotype and the culture substratum. Although CCC stimulates tuber initiation by recalcitrant genotypes, it can inhibit microtuber growth in *Solanum tuberosum* cultivars that form tubers readily in its absence. According to them, the potato variety Desiree tuberized readily than other varieties with a low CCC concentration.

There was considerable similarity between *in vitro* and *in vivo* systems with regard to pattern of coefficient of variation (both phenotypic and genotypic), heritability (broad sense), genetic advance (as % of mean) and correlation coefficients (both phenotypic and genotypic) between characters. The magnitude of various genetic parameters was higher under *in vitro* than *in vivo* conditions, particularly for tuber yield and its components. Variation in genetic parameters and character associations in different treatments was higher under *in vivo* than *in vitro* conditions.

Various studies have been done in previous years to see the differences among various genotypes. The genotype and cultural conditions interactions were significant indicating the importance of developing genotype specific protocols to maximize microtuberization. This

was concluded by Gopal *et al.*, by studying twenty-two genotypes of potato (*Solanum tuberosum* L.) that were induced to form microtubers under six *in vitro* culture conditions.

Gopal (1996) reported a faster rate of microtuberization and an early senescence of plantlets cultured under continuous darkness. How this and other culture conditions affect the number of eyes, which represent the nodes/buds on the stem, is not known. This character is important as a greater number of eyes per microtuber results in a better performance for most of the characters, including tuber yield in a crop raised from microtubers.

The effect of photoperiod, light intensity, temperature, and BAP on yield, number and size of microtubers, and number of eyes per microtuber, was studied. Effectiveness of *in vitro* selection for agronomic characters was studied by finding correlation coefficients between *in vitro* and *in vivo* performance of 22 potato genotypes (Gopal *et al.*, 1997).

Evaluation was performed under eight *in vitro* and two *in vivo* conditions. Genotypic differences were highly significant for various characters under all *in vitro* and *in vivo* conditions. Error mean squares were much lower in *in vitro* experiments than in *in vivo* experiments. *In vitro* selection was found to be highly effective for tuber colour, stem pigment and number of eyes, and moderately effective for average tuber weight, plant vigour and foliage senescence under specific conditions. The results also indicated the possibility of *in vitro* selection for heat tolerance. For tuber yield and number of tubers, effectiveness of *in vitro* selection was low to very low, because differences in phenotypic expressions of genotypes were much enlarged under *in vitro* conditions compared to under *in vivo* conditions.

Correlation coefficients for certain characters were better when *in vitro* conditions were closer to *in vivo* conditions. To improve the efficiency of *in vitro* selection, there is perhaps a need to simulate and identify the *in vitro* conditions under which genotypic differences are reflected in phenotypes more realistically as they are expressed under *in vivo* conditions.

The effect of explant source in indigenous potato variety “PARS-70” and a Dutch introduction Spunta for microtuber induction at different sucrose levels supplemented in Murashige and Skoog medium was studied by B. Fatima *et al.*, (2005).

In vitro grown shoots and nodal explants proved superior to shoot tips for shoot formation, shoot length, number of roots, root length, number of microtubers and microtuber weight in PARS-70 at 8% sucrose concentration in the medium. Cultivar PARS-70 also showed better response for above mentioned parameters at different sucrose levels than Santa. Such studies

might help to reduce the cost of production of virus free potato seed from indigenous cultivars. These all studies show that various characteristics of plant and tuber vary with genotypes. Genotype affects the nutritional and environmental requirements of a plant to grow and also for optimum tuberization.

2.2.2 Sucrose

Although, researches have been carried out on microtuber production in potato, very little attention has been paid on the *in vitro* tuberization with sucrose concentration and different types of explants to establish suitable regeneration protocol.

Potato microtubers are used as pathogen tested *in vitro* stocks for certified seed potato production by W. C. Yu *et al.*, Microtubers grown in a rotating bioreactor grew at a faster rate when the medium was replaced frequently. Although the total microtuber number was not affected, the number of microtubers over 1 g quadrupled when 75% of the medium was replaced every 2 weeks when compared with no medium refreshment. Significantly slower microtuber growth rates resulted when a lower sugar concentration (40 g instead of 80 g) was used or when a mixture of glucose and fructose replaced sucrose. Although high sucrose levels are necessary for optimal microtuber production, the sucrose supplied was rapidly hydrolyzed into glucose and fructose, making the long-term maintenance of desirable sucrose levels difficult. These results indicate that successful strategies to reduce sucrose hydrolysis without inhibiting microtuber growth will improve the efficiency of sucrose utilization in potato microtuber bioreactors.

2.2.3 Chloro Choline Chloride (CCC)

Tuberization in potato is a highly complex developmental process, which may be modified in various ways. Tuberization can be induced under *in vitro* condition. Many researchers used different growth regulators for *in vitro* induction of microtuber in potato (Hossain and Sultana, 1998). The antigibberellin compound Chloro Choline Chloride (CCC) has strong effect on *in vitro* tuberization (Tovar *et al.*, 1985; Dodds *et al.*, 1988).

Growth retardant stimulates tuberization of plants under unfavourable environmental conditions (Menzel, 1980) and CCC (Chloro choline chloride) is widely used in tissue culture media to promote microtuber formation (Tovar *et al.*, 1985 and Rosell *et al.*, 1987). It induces microtuber formation by a wide range of *Solanum* genotypes of diverse genetic backgrounds (Estrada *et al.*, 1986).

2.2.4 Benzyl Amino Purine (BAP)

Cytokinins (eg. BAP) are believed to have strong promotive effects on tuberization, and to constitute major part of the tuberization stimulus, either alone or in combination with other substances (Palmer and Smith, 1969; Pelacho and Mingo-Castel, 1991).

Anoop Badoni and J.S. Chauhan used three concentration of BAP (8 mg/L, 10 mg/L and 12 mg/L) with MS liquid medium for tuberization. The plantlets were shifted to tuberization media and data were reported for the number of microtuber on per original shoot, average weight of microtuber and number of eyes in each microtuber. Formation and development of microtubers were least with 8 mg/L and higher in 10 mg/L BAP concentration, while with the increasing concentration, BAP inhibit the average number, weight and eyes number of microtubers.

2.2.5 Sucrose, Benzyl Amino Purine and Chloro Choline Chloride

Protocols for *in vitro* tuberization of potato (*Solanum tuberosum* L.) variety Cardinal were studied by Iqbal Hussain, Zubeda Chaudhary. Virus free cultures were multiplied on Murashige & Skoog (1962) medium containing 1.0 mg/L GA₃. When the number of shoots was maximum the cultures were shifted on tuber induction medium. *In vitro* tuberization media consisted of MS along with different concentrations of CCC (Chloro choline chloride), BAP (Benzyl amino purine) and Sucrose. It was observed that 200 mg/L of CCC or 90 g/L sucrose in the mediums resulted in maximum tuber induction followed by BAP at 4.0 mg/L producing 9.0 tubers per flask. It was also observed that complete darkness was essential for tuber induction.

Effect of different levels of 6-benzylaminopurine (BAP) and naphthalene acetic acid (NAA) and BAP and sucrose on *in vitro* shoot (SH) proliferation and microtuberization of 'Spunta' potato was studied using virus-free plantlets on modified Murashige and Skoog (MS) media by A.A.R. Yousef *et al.*, NAA at 2.0 mg/L and BAP at 0.5 mg/L resulted in the longest main SH (22 cm) with highest node numbers (23 nodes). A maximum of 15 axillary SHs per main SH was produced after 10 weeks of incubation on the modified MS proliferation media in presence of 2.0 mg/L BAP and 0.1 mg/L NAA. Sucrose at 40 g/L when associated with 0.1 mg/L BAP was optimal for obtaining the maximum number of microtubers (MTs) *in vitro*. Seven MTs per SH and 1.35 MTs per node were obtained after 20 and 8 weeks, respectively. Largest MT weight and size resulted from media with 80 g/L sucrose supplemented with 0.1 mg/L BAP. On basis of space required and media used, single nodal

cuttings with leaf (SNCs) seem to be more applicable for induction of MTs *in vitro*; contamination hazards are also reduced.

In an experiment, six levels of benzyl adenine (0 mg/L, 2.5mg/L, 5.0 mg/L, 7.5 mg/L, 10 mg/L, 12.5 mg/L and 15.0 mg/L) in combination with three levels of (CCC) chloro choline chloride (0 mg/L, 125 mg/L, 250 mg/L and 500 mg/L) were evaluated against control treatment (0 mg/L) by M. Zakaria *et al.*, in each case to find out their optimum levels for microtuberization in potato variety “Diamant”. Microtuberization was the earliest by 13 days at 10 mg/L BA. The number and average weight of microtuber per flask was increased with increasing rate of BA and reached maximum of 12.9 and 252.1 mg respectively at 10 mg/L BA and decreased onwards with further increase of BA concentration. The increase rate of CCC increased the number of microtuber but decreased average weight. The maximum number and average weight of microtuber were recorded at 500 mg/L CCC and in absence of CCC respectively.

In vitro microtubers in potato (*Solanum tuberosum* L.) cultivar Kuroda, a new variety of potato introduced in Pakistan was obtained by Kanwal *et al.*, (2006). The cotton based liquid MS medium supplemented with 8 % sucrose and 0.75 mg/L BAP. The pH 5.7 gave better results when using node as a source of explant. Since microtubers can be induced economically at low concentration of BAP, it is recommended that this cultivar may be adopted for commercial cultivation in Pakistan.

2.2.6 Other Growth Regulators

Work on microtuberization in potato has mainly focused on the use of growth regulators (Palmer and Smith, 1969; Wang and Hu, 1982; Estrada *et al.*, 1986; Vecchio *et al.*, 1994), and there is considerable variation in the results of these studies, i.e. the response obtained depended upon a range of factors including sucrose concentration, temperature, photoperiod, light intensity, and cultivar (Hussey and Stacey, 1984; Ortiz- Montiel and Lozoya-Saldana, 1987; Garner and Blake, 1989).

Little is known about the effect of sucrose on microtuber dormancy (Choi *et al.*, 1994; Coleman and Coleman, 2000). In addition, it would be interesting to study the effect of possible interactions between the genotype, ABA and sucrose on microtuber production and subsequent dormancy release. How these factors affect total biomass production during microtuberization and subsequent microtuber dry matter content has also not been reported.

The influence of different concentrations of BA (6-benzyladenine) and Kinetin on microtuberization was compared by Serkan Uranbey. *In vitro* single node stem segments of potato were cultured on solid MS media supplemented with different concentrations of BA and Kinetin in dark at $22\pm 1^{\circ}\text{C}$. No tuberization occurred 2.5 and 5.0 μM BA. Plantlets cultured on media containing 15 μM Kinetin exhibited the greatest microtuberization response. Moreover, considerable increases in yield of microtubers per plantlet were achieved on media containing 15 μM Kinetin. It was observed that high Kinetin concentrations promoted massive microtubers compared to BA at the same concentrations.

The effects of jasmonic acid (JA) and methyl jasmonate (MeJA) on potato (*Solanum tuberosum* L.) tuberization were studied by Debabrata Sarkar, Suman Kumar Pandey, Sushruti Sharma in relation to cytokinins using single-node cuttings (SNCs) *in vitro*. In three potato cultivars differing in maturity levels, JA or MeJA (0.0, 2.5, 5.0, 7.5 and 10.0 M) action was examined with N₆-benzyladenine (0.0, 22.0 and 44.0 M) under optimum tuber-inducing treatment with 80 g/L sucrose. Although jasmonates had a stimulatory role in root growth from SNCs, BA inhibited the root growth and antagonized the jasmonates action on root growth promotion. There was a strong inhibitory effect of BA on stoloniferous shoot growth, and in combination with jasmonates it could exert a much pronounced inhibitory effect.

Jasmonates did not have any role in tuber induction in terms of tuber number and source sink coordination (harvest index), rather their effects were counteracted in the presence of 22.0 M BA in early cultivar. However, they had a promoting effect on tuber growth after induction in early cultivar, possibly due to its lower levels of endogenous gibberellins. Cytokinins detrimentally affected the tuber growth, and antagonized the jasmonates action irrespective of the maturity levels of the cultivars. It could, however, increase the tuber dry matter concomitant with a higher accumulation of starch. The promoting effect of jasmonates on tuber dry matter and starch accumulation was apparent only in early cultivar. There were significant interactions between cytokinin and jasmonates for accumulation of reducing and total sugars in tubers. The results show that exogenous cytokinins antagonize the jasmonates action, and the correlated effects of these two growth hormones interact with the maturing time of the cultivar during potato tuber formation *in vitro*.

There appears to be very little information available on the effect of ABA on microtuber dormancy when supplied exogenously in the (microtuber) induction medium. There are conflicting reports on the role of ABA in potato tuberization. It has been identified as

promoting (Abdullah and Ahmad, 1980; Andre *et al.*, 1998) as well as inhibiting (Palmer and Smith, 1969; Hussey and Stacey, 1984) tuberization.

Koda and Okazawa (1983) reported that ABA increased the rate of tuberization when its concentration was increased in combination with 2% sucrose, but the tubers thus formed characteristically ceased their development at the bulking stage.

Performance of the crop raised from microtubers is affected by their dormancy (Gopal *et al.*, 1997). Of all the different growth regulators, abscisic acid (ABA) is considered to be the principal dormancy-inducing agent. ABA levels in potatoes are generally highest in freshly harvested (deeply dormant) tubers, and decline as sprouting initiates (van den Berg *et al.*, 1991; Suttle and Hultstrand, 1994).

The extensive survey of literature review revealed that the process of microtuberization depends upon many parameters like sucrose concentration, phytohormones concentration environmental factors like light, temperature etc. The response of various cultivars in different experimental sets with regard to the microtuberization process as reported in the literature can be briefed as:-

- PARS-70 showed better response in media containing 8% sucrose with regard to shoot formation, shoot length, number of roots, root length, number of microtubers and microtuber weight. PARS-70 also showed better response for these parameters at different sucrose levels than the cultivar Santa. These all studies show that various characteristics of plant and tuber vary with genotypes. Genotype effects the nutritional and environmental requirements of a plant to grow and also for optimum tuberization.
- Spunta was studied under six different levels of BAP and NAA, and BAP and sucrose. NAA at 2.0 mg/L and BAP at 0.5 mg/L resulted in the longest main Shoot (22 cm) with highest node numbers (23 nodes). A maximum of 15 axillary Shoots per main Shoot was produced after 10 weeks of incubation on the modified MS proliferation media in presence of 2.0 mg/L BAP and 0.1 mg/L NAA. Sucrose at 40 g/L when associated with 0.1 mg/L BAP was optimal for obtaining the maximum number of microtubers (MTs) *in vitro*.
- Diamant showed earliest microtuberization by 13 days in 10 mg/L BAP, when it was studied in six levels of BAP (0 mg/L, 2.5mg/L, 5.0 mg/L, 7.5 mg/L, 10 mg/L, 12.5 mg/L and 15.0 mg/L) in combination with three levels of (CCC) chloro choline chloride (0 mg/L, 125 mg/L, 250 mg/L and 500 mg/L).

- Kuroda, a new variety of potato introduced in Pakistan, gave better results when the MS medium was supplemented with 8 % sucrose and 0.75 mg/L BAP. The pH 5.7 gave better results when using node as a source of explant.
- The cultivar Benimaru was studied in the media containing 500 mg/L CCC, in a condition of total darkness at 20°C or 25°C, and 10h photoperiod at 20°C or 25°C. In total darkness, the formation of small microtubers was observed 2 days after the beginning of culture on the medium with CCC and BAP. The number increased rapidly, attaining an almost constant level after 12 days of culture. The increase in the number of microtubers on the medium was more rapid than that on the medium with BAP alone. This indicates that CCC accelerated tuberization at the early stage of culture.
- The cultivar Danshakuimo, and Mau Queen were also studied along with Benimaru, and the experiments were designed so as to study the effect of CCC and BAP on the formation of microtubers. All the three cultivars showed a common tendency of responding the best in media containing CCC (500 mg/L) and BAP (5 mg/L) with regard to number and fresh weight of microtubers produced per 10 shoots. However, the mean of the fresh weight of microtubers of Mau Queen was far lower than the other two cultivars.

Objectives

In vitro microtuberization is a tissue culture technique, where microtubers are grown under tuber inducing conditions. *In vitro* microtuberization is a complex developmental process influenced by various factors, the most important being light, temperature and constituents of the media. Moreover, the response of the potato cultivars with regard to microtuberization process vary under the same experimental conditions indicating the effect of genotype.

In the literature, studies related to the response of various potato cultivars with regard to microtuberization in the presence of varying concentrations of Sucrose, Chloro Choline Chloride and phytohormones have been reported. Cultivar Kufri Himalini, 'Spunta' potato, Diamant were studied by varying phytohormone concentration. Similarly, four potato cultivars Santa, Cardinal, Diamant and Desiree were used to study the effect of sucrose and CCC on microtuberization. Cardinal was studied by varying sucrose and phytohormone concentrations. 'Benimaru', 'May Queen', and 'Danshakuimo' were studied by varying sucrose and CCC concentrations.

As per literature survey, no comprehensive report is available till date with regard to the studies on the effect of sucrose and CCC on microtuberization in the Indian potato cultivars. Keeping in view of this, the following objectives were framed accordingly:

- Establishment of pathogen free potato germplasms through apical meristem culture
- To study the effect of varying concentrations of sucrose and chloro choline chloride on *in vitro* microtuberization
- To check the response of the potato cultivars with respect to number, size and overall microtuber yield per plantlet

Chapter 3

Materials and Methodology

3.1 MATERIALS

3.1.1 Cultivars used for study

Various potato cultivars such as Kufri Chipsona-1, Kufri Chipsona-2, Kufri Ashoka, Kufri Pukhraj and Desiree were routinely maintained in our laboratory on MS medium. These cultivars are abbreviated as, cv. CS1, cv. CS2, cv. AS, cv. PR, and cv. De respectively.

Variety	Year of release	Salient features	Areas of adaptations
Kufri Ashoka	1996	Early maturing; tuber white, large oval-long with fleet eyes	North Indian plains
Kufri Pukhraj	1998	Medium maturing, tubers white, large, oval with fleet eyes, resistant to early blight ; moderately resistant to late blight	North Indian plains and plateau regions
Kufri Chipsona 1	1998	Medium maturing; tuber white, medium to large ,oval with fleet eyes; resistant to late blight, suitable for processing	North Indian plains
Kufri Chipsona 2	1998	Medium maturing; tuber white, medium round-oval with fleet eyes; resistant to late blight, tolerant to frost, suitable for processing	North Indian plains
Desiree(foreign variety)	1962	Late maturing; tuber Medium oval-round red skinned tuber with fleet red eyes, low to medium resistance to late blight	Origin at Netherlands

3.1.2 Glasswares and Chemicals

All glassware and plasticware were procured from Borosil India Ltd. and Tarsons Pvt. Ltd. Chemicals used for the preparation of MS medium were procured from HiMedia. N₆-benzyl adenine was procured from HiMedia and Chloro choline chloride from Sigma.

3.1.3 Media used

MS Basal Medium (Murashige and Skoog Medium)

Major salts	Concentration (mg/L)
KNO ₃	1900
NH ₄ NO ₃	1650
MgSO ₄ ·7H ₂ O	370
CaCl ₂ ·2H ₂ O	440
KH ₂ PO ₄	170
Minor Salts	
MnSO ₄ ·H ₂ O	22.3
ZnSO ₄ ·7H ₂ O	8.6
H ₃ BO ₃	6.2
KI	0.83
Na ₂ MoO ₄ ·2H ₂ O	0.25
CuSO ₄ ·5H ₂ O	0.025
CoCl ₂ ·6H ₂ O	18
Na ₂ Fe-EDTA	30.0
Vitamins & Organic supplements	
Myoinositol	100
Nicotinic Acid	0.5
Pyridoxine HCl	0.5
Thiamine HCl	0.1
Glycine	2.0
Sucrose	25.0 g/L

3.1.4 Preparation of stock solutions of Phytohormones:

Preparation of BAP

Dissolve BA (benzyl adenine) in required amount of 0.1 N HCl and make up the volume with sterile double distilled water, heat gently. Working concentration of BAP was 10 mg/L.

Preparation of CCC

2-chloroethyltrimethylammoniumchloride (CCC) was prepared in sterile double distilled water and its working concentration was 500 mg/L.

3.2 METHODOLOGY

3.2.1 Establishment of virus free culture through apical meristem culture (AMC) :

- The previously fumigated laminar air flow chamber was prepared by disinfecting the internal surfaces with alcohol just before work. The tools (scalpel, glass plate and forceps) were sterilized by washing with detergent, oven drying and subsequent autoclaving and were placed in laminar flow hood.
- The mother culture bottle was opened, plantlet was taken and placed on the plate with the help of forceps.
- The explants were cut at apical meristem and the apical dome was excised with the help of scalpel.
- A bottle containing fresh sterile medium was taken, flame sterilized and 7-8 apical domes were placed inside it, in upright position.
- The cultures were then incubated under a 16 ± 8 hr photoperiod at 25 °C.

3.2.2 Initial multiplication (micropropagation):

- After 21 days of the establishment of the AMC, when shoots had attained sufficient growth, the explants were cut internodes with the help of scalpel and thus intermodal segments were obtained.

3.2.3 Mass micropropagation in liquid medium:

- When large numbers of plants are produced in initial multiplication, liquid cultures are initiated for mass micropropagation.
- 10 stem segments (each having 3-4 nodes) obtained from 21 days old plantlets were inoculated in each bottle, containing 25 ml of liquid MS media.

- The liquid cultures were incubated under the same cultural conditions as in semisolid propagation.

3.2.4 Microtuber production:

- After 21 days of incubation, the liquid micropropagation medium was replaced by 40 ml of tuber induction medium.

[The microtuber induction medium is based on MS basal nutrients supplemented with 10 mg/L N₆- Benzyl adenine (BA), 500 mg/L Chlorocholine chloride and 80 g/L Sucrose as per protocol by CPRI.]

- According to the interest of study experimental variations were applied at this stage while adding microtuber induction medium.
- These cultures were incubated under dark conditions at 20°C in BioMatrix BOD incubators.

3.2.5 Studies on the effects of various parameters on microtuberization:

Our objective is to study the effect of varying concentrations of sucrose and CCC on microtuberization in selected Indian potato cultivars. Thus, in various experiment sets following media compositions were adopted.

Varying concentrations of sucrose in presence of growth regulators:

Sr. no.	Sucrose (g/L)	BAP (mg/L)	CCC (mg/L)
1	80	10	500
2	60	10	500
3	40	10	500

Varying concentrations of CCC with 8% sucrose and in absence of BAP:

Sr. no.	Sucrose (g/L)	BAP (mg/L)	CCC (mg/L)
1	80	-	500
2	80	-	250
3	80	-	0

Varying concentrations of sucrose in absence of growth regulators:

Sr. no.	Sucrose (g/L)	BAP (mg/L)	CCC (mg/L)
1	80	-	-
2	60	-	-
3	40	-	-

3.2.6 Harvesting and storage:

- The microtubers were hand harvested in plastic trays so as to avoid damage during harvesting.
- The harvested microtubers were washed under running tap water to remove adhering constituents of the medium. Treated harvested microtubers with 0.2 % Bavistin (to avoid fungal contamination) for 10 mins and allowed them to dry.
- The dried microtubers were packed in perforated polythene bags or muslin cloth and stored at 4°C in a refrigerator.

3.2.7 Data collection and analysis:

- Microtubers obtained from each cultivar were measured for total weight by using electronic weighing balance.
- Total number of tubers produced by each cultivar in each bottle was counted manually and data was noted properly.
- Average diameter and length of microtubers was measured by using electronic vernier callipers and data was noted and analyzed.

FLWSHEET OF THE PROCESS OF MICROTUBERIZATION

*Apical dome is excised and multiplied on semi solid MS media to establish
Apical Meristem culture (AMC)*



*Initial multiplication of desired cultivar on semi solid MS medium
(obtain sufficient amount of initial cultures)*



Mass multiplication of 21 day old in vitro plantlets in liquid MS medium



*After 21 days of incubation liquid media is replaced by the microtuber
induction media (variable experimental parameters will be adopted at this
stage)*



*Incubation at 20°C till the microtubers are produced
(approx. 60-90 days will be the incubation period)*



Harvesting



Data collection and analysis

Chapter 4

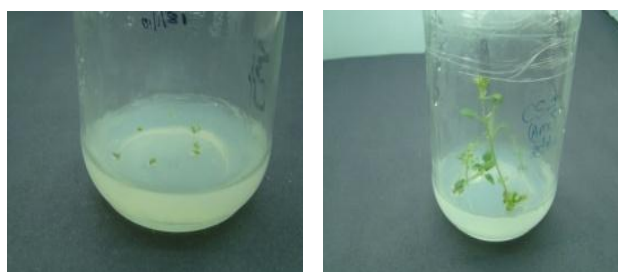
Results and Discussion

In the present study, techniques of plant tissue culture were applied to produce microtubers aseptically under various nutritional conditions to examine the varying response of Indian potato cultivars during the process of microtuberization. In particular, firstly attempts were made to see the effect of varying sucrose concentration and secondly the effect of CCC on microtuberization process.

4.1 Mass propagation of potato cultivars in MS Media

4.1.1 Establishment of virus free potato germplasms through apical meristem culture:

Potato plant nodal explants were continuously subcultured by using previously established mother cultures on semi solid MS media. This process enabled us to maintain the germplasm in laboratory as well as for mass propagation of plantlets to be used as experimental material, as shown in the figures 1 and 2.



Figs 1 & 2. Apical Meristem Culture of CS1 grown on semi solid MS media

4.1.2 Mass micropropagation of the potato cultivars in liquid MS media:



Fig. 3. cv. CS1



Fig. 4. cv. CS2



Fig. 5. cv. AS



Fig. 6. cv. PR



Fig. 7. cv. De

Figs. 3 to 7. Potato cultivars CS1, CS2, AS, PR, De, grown on liquid MS media (21 days old culture) After establishment of cultures as per requirement on semisolid media, these explants were transferred to liquid MS media to obtain a healthy culture in such a state that can be easily induced for tuberization media. For this, almost 21 days old culture was transferred to sterile liquid MS media (composition same as semi solid media except agar was not added). Here, plantlets having 2-3 nodes shown negative geotropism and grown in full plantlets.

4.2 Induction of microtuberization:

As per the standard protocols after 21 days of incubation, liquid cultures were ready for induction of microtuberization. Protocol given by CPRI which was referred in present study states that microtuberization induction medium contains three major components in following concentrations for optimum tuberization consists of Sucrose- 80 g/L, N₆ Benzyl Adenine -10 mg/L, Chloro choline chloride- 500 mg/L. Potato cultivars suitable to different agro-climatic conditions prevailing in different countries vary with regard to genotypes, maturation period, over all yield, size, colour and texture of the tubers, tuber dry matter, disease resistant phenotypes and many other attributes. Therefore, it is very likely that *in vitro* nutritional requirements of different potato cultivars would vary with respect to microtuberization process.

In this study, we focused mainly on Indian potato cultivars that best suits our agro-climatic conditions. Here, attempts were made to study the response of different potato cultivars towards various sucrose concentrations on microtuberization process. Moreover, the effect of CCC was also studied. The purpose is to optimize the media formulations for the production of healthy and disease free microtubers. In fact, a number of media compositions were formulated (as mentioned in previous chapter) and used in this study.

The results obtained are provided in the following sections:

4.2.1 Effect of varying sucrose concentrations in the presence of CCC, BAP

Along with 80 g/L, two other concentrations 60 g/L and 40 g/L of sucrose were used to study the effect of sucrose on microtuberization in the presence of BAP 10 mg/L and CCC 500 mg/L as given in the following table:

Sr. no.	Sucrose (g/L)	BAP (mg/L)	CCC (mg/L)
1	80	10	500
2	60	10	500
3	40	10	500

4.2.1.1 Media having 8% sucrose concentration: In the media having 8% sucrose concentration along with 10 mg/L BAP and 500 mg/L CCC, the cultivar PR showed best response with regard to microtuberization in terms of tuber number, size and yield per plantlet; the cultivars AS and CS1 showed average response, whereas least response was observed in cultivars CS2 and De. The response of some of the potato cultivars in microtuber induction medium and their corresponding harvested microtubers are shown in the Figs. 8 to 13. Usually microtubers were harvested after 60-90 days and studied further for their size, yield, weight etc.



Fig. 8. cv. AS



Fig. 9. cv. PR



Fig. 10. cv. De

Figs. 8 to 10. Microtuberization in the media containing 8% sucrose (45 days old culture)



Fig. 11. cv. AS



Fig. 12. cv. PR



Fig. 13. cv. De

Figs. 11 to 13. Harvested microtubers from different potato cultivars

The microtubers were harvested from each individual cultivar and further sorted out to study the following attributes such as yield per bottle, number per bottle, average weight, length and diameter covering from relatively large microtubers to the smaller ones. Compiled data is shown in the following table.

Cultivar	Total no. of tubers/bottle	Total wt. g/bottle	Weight range (g) \pm SD		Length range (mm) \pm SD		Diameter range (mm) \pm SD	
			Max	Min	Max	Min	Max	Min
CS1	12	2.07	0.30 \pm 0.06	0.11 \pm 0.03	7.73 \pm 0.32	5.70 \pm 0.55	6.86 \pm 0.63	5.11 \pm 0.61
CS2	11	1.60	0.24 \pm 0.02	0.10 \pm 0.05	7.77 \pm 0.30	5.79 \pm 1.40	6.46 \pm 0.54	4.21 \pm 1.13
AS	8	2.53	0.34 \pm 0.07	0.08 \pm 0.02	9.63 \pm 1.05	5.53 \pm 0.57	7.56 \pm 1.07	4.69 \pm 0.37
PR	15	3.38	0.64 \pm 0.17	0.27 \pm 0.06	10.83 \pm 0.81	8.41 \pm 0.96	8.92 \pm 1.29	6.35 \pm 0.17
De	14	1.54	0.17 \pm 0.11	0.08 \pm 0.005	7.45 \pm 1.86	4.98 \pm 0.40	5.66 \pm 0.71	4.62 \pm 0.13

Table 1. Varying response of the potato cultivars with regard to tuber number and size of the microtubers.

The data in Table 1 clearly showed that not only tuber number and weight of microtubers per bottle but average length, weight and diameter of microtubers also differ among the cultivars.

For instance, under the above condition among the all cultivars, the cultivar PR showed maximum number of tubers, maximum total weight, maximum average weight, maximum average length, and maximum diameter. Although, De showed larger number of tubers but the total weight was minimum. Here, based on the data as given in Table 1, we calculated the yield of microtubers per plantlet corresponding to the individual cultivars as summarized in the Table 2.

Cultivar	Total wt. of microtubers/ bottle (g)	No. of plantlets/bottle	Microtuber yield (g/plantlet)
CS1	2.07	10	0.20
CS2	1.60	10	0.16
As	2.53	10	0.25
PR	3.38	10	0.33
De	1.54	10	0.15

Table 2. Comparative response of the potato cultivars with regard to microtuber yield.

Total number of tubers per bottle was highest in PR (15), followed by De (14) and CS1 (12). PR showed maximum yield 0.33 g/plantlet, followed by As 0.25 g/plantlet and CS1 0.20 g/plantlet. De with 0.15 g /plantlet was least responding cultivar in this study.

4.2.1.2 Media having 6% sucrose concentration: In the media having 6% sucrose concentration along with 10 mg/L BAP and 500 mg/L CCC, the cultivar PR showed best response with regard to microtuberization in terms of tuber, size and yield per plantlet; the cultivars CS1 and AS showed average response. Although, CS2 and De showed maximum number of tubers but least response was observed in them overall. The response of some potato cultivars are shown in Figs. 14 to 19. Usually microtubers were harvested after 60-90 days and studied further for their size, yield, weight etc.



Fig. 14. cv. PR



Fig. 15. cv. AS



Fig.16. cv. De

Figs. 14 to 16. Microtuberization in the media containing 6% sucrose (45 days old culture)



Fig. 17. cv. PR



Fig. 18. cv. AS



Fig. 19. cv. De

Figs. 17 to 19. Harvested microtubers from different potato cultivars

The microtubers were harvested from each individual cultivar and further sorted out to study the following attributes such as yield per bottle, number per bottle, average weight, length and diameter covering from relatively large microtubers to the smaller ones. Compiled data is shown in the following table.

Cultivar	Total no. of tubers/bottle	Total wt. g/bottle	Weight range (g) ± SD		Length range (mm) ± SD		Diameter range (mm) ± SD	
			Max	Min	Max	Min	Max	Min
CS1	10	1.79	0.32 ± 0.09	0.22 ± 0.03	8.34 ± 1.01	7.13 ± 0.73	5.14 ± 0.83	6.74±0.25
CS2	16	1.06	0.15 ± 0.01	0.06±0.005	8.44 ± 1.45	6.10 ± 0.41	5.22 ± 0.81	4.15±0.04
AS	13	1.59	0.40 ± 0.06	0.07 ± 0.01	10.57±0.60	5.45 ± 0.57	7.37 ± 0.78	4.78±0.36
PR	11	3.18	0.75 ± 0.23	0.18 ± 0.08	10.68 ± 1.23	7.11 ± 1.60	10.93 ± 1.70	6.55±1.09
De	15	1.05	0.10 ± 0.02	0.05±0.005	6.34 ± 0.34	4.94 ± 0.21	5.05 ± 0.38	3.82±0.35

Table 3. Varying response of the potato cultivars with regard to tuber number and size of the microtubers.

The data in Table 3 clearly showed that not only tuber number and weight of microtubers per bottle but average length, weight and diameter of microtubers also differ among the cultivars. For instance, under the above condition among the all cultivars, although the cultivars CS2 and De showed maximum number of tubers, but their total weight was minimum. While, PR showed maximum total weight, maximum average weight, maximum average length, and maximum diameter. Here, based on the data as given in Table 3, we calculated the yield of microtubers per plantlet corresponding to the individual cultivars as summarized in the Table 4.

Cultivar	Total wt. of microtubers/ bottle (g)	No. of plantlets/bottle	Microtuber yield (g/plantlet)
CS1	1.79	10	0.17
CS2	1.06	10	0.10
As	1.59	10	0.15
PR	3.18	10	0.31
De	1.05	10	0.10

Table 4. Comparative response of the potato cultivars with regard to microtuber yield.

However, same nutritional conditions and incubation time was provided to each cultivar. The cultivar PR showed maximum microtuber yield 0.31 g/plantlet followed by CS1 0.17 g/plantlet and AS 0.15 g/plantlet. CS2 and De showed least response at 0.10 g/plantlet each.

4.2.1.3 Media having 4% sucrose concentration: In the media having 4% sucrose concentration along with 10 mg/L BAP and 500 mg/L CCC, the cultivar PR and AS showed best response with regard to microtuberization in terms of tuber number, size and yield per

plantlet; De and CS2 showed average response. While least response was observed in CS1. The response of some potato cultivars are shown in Figs. 20 to 25. Usually microtubers were harvested after 60-90 days and studied further for their size, yield, weight etc.



Fig. 20. cv. PR



Fig. 21. cv. AS



Fig. 22 cv. CS1

Figs. 20 to 22. Microtuberization in the media containing 4% sucrose (45 days old culture)



Fig. 23. cv. PR



Fig. 24. cv. AS



Fig. 25 cv. CS1

Figs 23 to 25. Harvested microtubers from different potato cultivars

The microtubers were harvested from each individual cultivar and further sorted out to study the following attributes such as yield per bottle, number per bottle, average weight, length and diameter covering from relatively large microtubers to the smaller ones. Compiled data is shown in the following table.

Cultivar	Total no. of tubers/bottle	Total wt. g/bottle	Weight range (g) ± SD		Length range (mm) ± SD		Diameter range (mm) ± SD	
			Max	Min	Max	Min	Max	Min
CS1	7	0.40	0.10 ± 0.007	0.05±0.005	6.18 ± 0.26	6.32 ± 0.50	4.62 ± 0.09	4.0 ± 0.08
CS2	15	1.01	0.22 ± 0.16	0.10±0.007	8.00 ± 0.79	5.46 ± 0.51	6.46 ± 0.86	5.66±0.03
AS	8	2.02	0.81 ± 0.26	0.19 ±0.13	17.08 ± 2.37	7.63 ± 1.72	7.85 ± 0.11	6.06±1.08
PR	12	2.55	0.72 ± 0.21	0.14 ±0.01	13.59 ± 0.01	6.81 ± 0.22	9.73 ± 1.87	6.05±0.14
De	15	1.29	0.21 ± 0.10	0.11 ± 0.02	7.68 ± 0.92	6.45 ± 0.53	6.68 ± 1.38	5.31±0.46

Table 5. Varying response of the potato cultivars with regard to tuber number and size of the microtubers.

The data in Table 5 clearly showed that not only tuber number and weight of microtubers per bottle but average length, weight and diameter of microtubers also differ among the cultivars. For instance, under the above condition among the all cultivars, although the cultivars CS2 and De showed maximum number of tubers, but with comparatively lesser total weight. PR showed maximum total weight, and maximum diameter. AS showed maximum average weight and maximum average length. CS1 showed least response in each regard. Here, based on the data as given in Table 5, we calculated the yield of microtubers per plantlet corresponding to the individual cultivars as summarized in the Table 6.

Cultivar	Total wt. of microtubers/ bottle (g)	No. of plantlets/bottle	Microtuber yield (g/plantlet)
CS1	0.40	10	0.04
CS2	1.01	10	0.10
As	2.02	10	0.20
PR	2.55	10	0.25
De	1.29	10	0.12

Table 6. Comparative response of the potato cultivars with regard to microtuber yield.

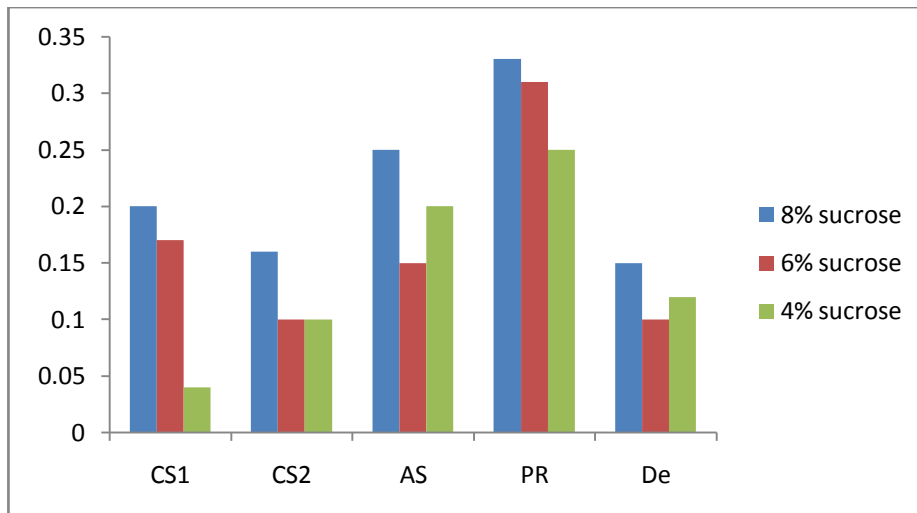
Cultivar CS1 had given negligible production at 4% sucrose concentration. Among 8%, 6% and 4% sucrose concentrations, 4% sucrose containing media was least productive. Among cultivars PR was found to be most promising cultivar with 0.25 g/plantlet microtuber yield followed by AS with 0.20 g/plantlet. Cultivars De (0.12 g/plantlet), and CS2 (0.10 g/plantlet) showed average response. CS1 (0.04 g/plantlet) showed least response.

4.2.1.4 Comparison of microtuber yield from the potato cultivars:

Microtuber yield per plantlet among all the cultivars in case of each 8%, 6% and 4% sucrose concentration was compared and graph was plotted.

Cultivar	8% sucrose (g/plantlet)	6% sucrose (g/plantlet)	4% sucrose (g/plantlet)
CS1	0.20	0.17	0.04
CS2	0.16	0.10	0.10
As	0.25	0.15	0.20
PR	0.33	0.31	0.25
De	0.15	0.10	0.12

Table 7. Comparative response with regard to microtuber yield in 8%, 6% and 4% sucrose



Graph 1. Comparison among the cultivars at 8%, 6% and 4% sucrose concentrations with 500mg/L CCC and 10 mg/L BAP with regard to microtuber yield/plantlet.

With regard to treatment with different concentrations of sucrose, the following inferences were made:

While using 8% sucrose among all the mentioned cultivars, we observed from the data that PR has shown maximum microtuber production per plantlet (0.33 g/plantlet), followed by AS and CS1 (0.25 and 0.20 g/plantlet respectively). Cultivar De showed least response with 0.15 g/plantlet.

In 6% sucrose containing experimental set, again cultivar PR has shown maximum response (0.31 g/plantlet) followed by CS1 (0.17 g/plantlet) and AS (0.15 g/plantlet). Here, CS2 and De showed least response with 0.10 g/plantlet each.

In last set containing 4% sucrose, cultivar PR has shown maximum response (0.25 g/plantlet) with regard to microtuber production followed by AS1 (0.20 g/plantlet). Here, cultivar CS2 showed least response with 0.04 g/plantlet.

In other way, it was also noted that tubers produced in 8% sucrose concentration containing medium responded in better way as compared to 6% sucrose with regard to tuber yield. 4% sucrose containing media produced least microtubers.

4.2.2 Effect of CCC on microtuberization

Along with 80 g/L sucrose, three media formulations were carried out with 500 mg/L CCC, 250 mg/L CCC and 0 mg/L CCC (control) to study the effect of CCC in microtuberization and in the absence of BAP as given in the following table:

Sr. no.	Sucrose (g/L)	BAP (mg/L)	CCC (mg/L)
1	80	-	500
2	80	-	250
3	80	-	0

4.2.2.1 Media having 8% sucrose concentration without BAP and 500

mg/L CCC: Here, our interest was to evaluate the mentioned cultivars for various parameters with regard to microtuberization in the presence of varying concentration of CCC. Thus, the experimental set (sucrose 8%, 10 mg/L BA without CCC) was compared with tubers grown under normal media conditions supplemented with varying CCC concentrations. The cultivar PR showed best response with regard to microtuberization in terms of tuber number and yield per plantlet, followed by the cultivar AS with maximum tuber size, followed by CS2 in terms of microtuber yield. Least response was observed in the cultivar CS1 and De. The response of some potato cultivars are shown in Figs. 26 to 31. Usually microtubers were harvested after 60-90 days and studied further for their size, yield, wt. etc.

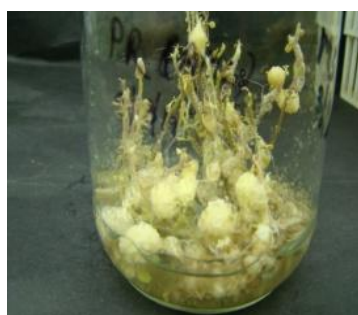


Fig. 26 cv. PR



Fig. 27. cv. AS



Fig. 28. cv. De

Figs. 26 to 28. Microtuberization in the media containing 500 mg/L CCC (45 days old culture)



Fig. 29 cv. PR



Fig. 30. cv. AS



Fig. 31. cv. De

Figs 29 to 31. Harvested microtubers from different potato cultivars

The microtubers were harvested from each individual cultivar and further sorted out to study the following attributes such as yield per bottle, number per bottle, average weight, length and diameter covering from relatively large microtubers to the smaller ones. Compiled data is shown in the following table.

Cultivar	Total no. of tubers/bottle	Total wt. g/bottle	Weight range \pm SD		Length range (mm) \pm SD		Diameter range (mm) \pm SD	
			Max	Min	Max	Min	Max	Min
CS1	15	2.46	0.35 \pm 0.02	0.05 \pm 0.01	8.75 \pm 0.53	4.98 \pm 4.89	8.18 \pm 0.19	4.31 \pm 0.12
CS2	12	3.43	0.97 \pm 0.01	0.06 \pm 0.01	12.69 \pm 0.01	4.64 \pm 0.01	10.40 \pm 0.01	4.40 \pm 0.01
AS	9	3.78	1.42 \pm 0.01	0.05 \pm 0.01	14.72 \pm 0.01	4.83 \pm 0.01	12.16 \pm 0.01	3.67 \pm 0.01
PR	21	4.50	1.02 \pm 0.03	0.07 \pm 0.01	13.38 \pm 0.19	5.22 \pm 0.01	11.18 \pm 0.12	5.04 \pm 0.01
De	16	2.09	0.26 \pm 0.02	0.03 \pm 0.005	8.74 \pm 1.62	4.02 \pm 0.07	6.81 \pm 0.26	3.51 \pm 0.21

Table 8. Varying response of the potato cultivars with regard to tuber number and size of the microtubers.

The data in Table 8 clearly showed that not only tuber number and weight of microtubers per bottle but average length, weight and diameter of microtubers also differ among the cultivars.

For instance, under the above condition among the all cultivars, the cultivar PR showed maximum number of tubers and maximum total weight. While, the cultivar AS showed least number of tubers but with maximum average weight, average length and diameter. De with a greater number of tubers responded poorly in each regard. Here, based on the data as given in Table 8, we calculated the yield of microtubers per plantlet corresponding to the individual cultivars as summarized in the Table 9.

Cultivar	Total wt. of microtubers/ bottle (g)	No. of plantlets/bottle	Microtuber yield (g/plantlet)
CS1	2.46	10	0.24
CS2	3.43	10	0.34
As	3.78	10	0.37
PR	4.50	10	0.45
De	2.09	10	0.20

Table 9. Comparative response of the potato cultivars with regard to microtuber yield.

Total number of tubers per bottle was highest in PR (21) followed by De (16) and CS1 (15). PR showed maximum yield 0.45 g/plantlet, followed by AS 0.37 g/plantlet and CS2 0.34 g/plantlet. The cultivar De showed least response with microtuber yield 0.20 g/plantlet.

4.2.2.2 Media containing 8% sucrose without BAP and 250 mg/L CCC: In the media containing 8% sucrose concentration without BAP and CCC, the cultivars AS and PR showed good response with regard to microtuberization in terms of tuber size and yield per plantlet; the cultivars De and CS1 with maximum number of tubers showed average response in other regards, whereas least response was observed in the cultivar CS2 with regard to all the parameters. The response of some of the potato cultivars are shown in the Figs. 32 to 37. Usually microtubers were harvested after 60-90 days and studied further for their size, yield, weight etc.



Fig. 32 cv. AS



Fig. 33. cv. PR



Fig. 34. cv. CS2

Figs. 32 to 34. Microtuberization in the media containing 250 mg/L CCC (45 days old culture)



Fig. 35 cv. AS



Fig. 36. cv. PR



Fig. 37. cv. CS2

Figs 35 to 37. Harvested microtubers from different potato cultivars

The microtubers were harvested from each individual cultivar and further sorted out to study the following attributes such as yield per bottle, number per bottle, average weight, length and diameter covering from relatively large microtubers to the smaller ones. Compiled data is shown in the following table.

Cultivar	Total no. of tubers/bottle	Total wt. g/bottle	Weight range \pm SD		Length range (mm) \pm SD		Diameter range (mm) \pm SD	
			Max	Min	Max	Min	Max	Min
CS1	15	2.74	0.70 \pm 0.01	0.05 \pm 0.01	11.82 \pm 0.01	7.33 \pm 0.01	10.33 \pm 0.01	3.97 \pm 0.01
CS2	9	2.28	0.45 \pm 0.06	0.04 \pm 0.01	10.21 \pm 0.84	4.83 \pm 0.01	8.56 \pm 0.23	3.72 \pm 0.01
AS	10	3.93	0.73 \pm 0.05	0.04 \pm .007	11.60 \pm 0.33	4.42 \pm 0.02	9.90 \pm 0.08	3.60 \pm 0.05
PR	10	3.63	0.77 \pm 0.12	0.03 \pm 0.01	12.24 \pm 0.40	4.56 \pm 0.01	10.34 \pm 0.44	3.38 \pm 0.01
De	14	2.83	0.36 \pm 0.06	0.02 \pm 0.01	9.03 \pm 0.56	4.60 \pm 0.01	7.63 \pm 0.60	3.11 \pm 0.01

Table 10. Comparative response of the potato cultivars with regard to tuber number and size of the microtubers.

The data in Table 10 clearly showed that not only tuber number and weight of microtubers per bottle but average length, weight and diameter of microtubers also differ among the cultivars.

For instance, under the above condition, cultivar AS showed maximum total weight, and maximum average weight of tubers. While CS1 had maximum number of tubers per bottle and maximum length while PR showed maximum diameter.

Overall, a good response was reported in all the cultivars. Here, based on the data as given in Table 10, we calculated the yield of microtubers per plantlet corresponding to the individual cultivars as summarized in the Table 11.

Cultivar	Total wt. of microtubers/ bottle (g)	No. of plantlets/bottle	Microtuber yield (g/plantlet)
CS1	2.74	10	0.27
CS2	2.28	10	0.22
As	3.93	10	0.39
PR	3.63	10	0.36
De	2.83	10	0.28

Table 11. Comparative response of the potato cultivars with regard to microtuber yield.

From the Table 11 it is clearly observed that the cultivar AS showed best response in terms of microtuber yield i.e. maximum microtubers (0.39 g/plantlet) followed by PR (0.36 g/plantlet) and De (0.28 g/plantlet). The cultivar CS2 showed poor response (0.22 g/plantlet).

4.2.2.3 Media containing 8% sucrose without BAP and 0 mg/L CCC (control):

In the media containing 8% sucrose without BAP and 0 mg/L CCC (control), the tuber number was almost same in all the cultivars except AS which had the minimum tuber number. The cultivars CS1 and PR showed best response with regard to microtuberization in terms of microtuber yield per plantlet, tuber number and tuber size. The cultivar De showed average response followed by CS2. Whereas, least response was observed in the cultivar AS. The response of some of the potato cultivars are shown in the Figs. 38 to 43. Usually microtubers were harvested after 60-90 days and studied further for their size, yield, weight etc.



Fig. 38 cv. PR



Fig. 39. cv. CS2



Fig. 40. cv. AS

Figs. 38 to 40. Microtuberization in the media without CCC (45 days old culture)



Fig. 41 cv. PR



Fig. 42. cv. CS2



Fig. 43. cv. AS

Figs 41 to 43. Harvested microtubers from different potato cultivars

The microtubers were harvested from each individual cultivar and further sorted out to study the following attributes such as yield per bottle, number per bottle, average weight, length and diameter covering from relatively large microtubers to the smaller ones. Compiled data is shown in the following table.

Cultivar	Total no. of tubers/bottle	Total wt. g/bottle	Weight range \pm SD		Length range (mm) \pm SD		Diameter range (mm) \pm SD	
			Max	Min	Max	Min	Max	Min
CS1	16	4.30	0.82 \pm 0.06	0.11 \pm 0.01	14.0 \pm 1.91	6.50 \pm 0.01	9.73 \pm 0.98	5.40 \pm 0.01
CS2	12	1.37	0.18 \pm 0.01	0.02 \pm 0.00	8.4 \pm 1.52	4.68 \pm 0.47	5.87 \pm 0.61	3.20 \pm 0.19
AS	11	0.79	0.18 \pm 0.01	0.03 \pm 0.02	6.9 \pm 0.86	4.71 \pm 0.82	5.68 \pm 0.25	3.28 \pm 0.12
PR	14	4.22	1.00 \pm 0.14	0.06 \pm 0.01	13.03 \pm 0.49	5.30 \pm 0.67	11.80 \pm 0.01	4.22 \pm 0.20
De	15	3.02	0.60 \pm 0.01	0.02 \pm 0.01	10.17 \pm 0.01	4.20 \pm 0.01	9.27 \pm 0.01	3.35 \pm 0.01

Table 12. Comparative response of the potato cultivars with regard to tuber number and size of the microtubers.

The data in Table 12 clearly showed that the tuber number was almost same but other parameters like the weight of microtubers per bottle, average length, total weight and diameter of microtubers differ among the cultivars.

For instance, under the above condition, cultivar CS1 showed maximum number of tubers, maximum total weight, and maximum length, while PR showed maximum average weight of tubers and maximum diameter.

Here, based on the data as given in Table 12, we calculated the yield of microtubers per plantlet corresponding to the individual cultivars as summarized in the Table 13.

Cultivar	Total wt. of microtubers/bottle (g)	No. of plantlets/bottle	Microtuber yield (g/plantlet)
CS1	4.30	10	0.43
CS2	1.37	10	0.13
As	0.79	10	0.07
PR	4.22	10	0.42
De	3.02	10	0.30

Table 13. Comparative response of the potato cultivars with regard to microtuber yield.

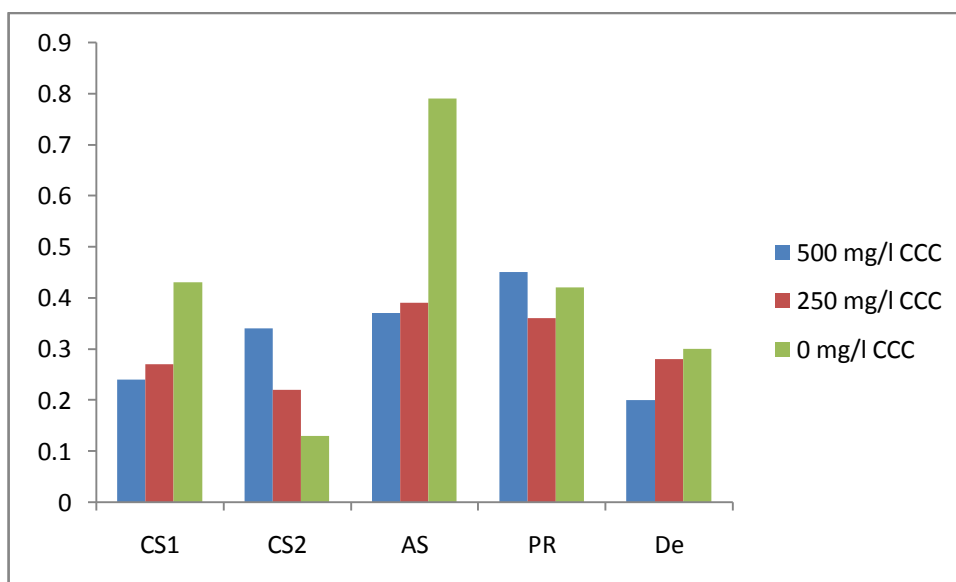
From the Table 13 it is clearly observed that the cultivar CS1 showed best response in terms of microtuber yield i.e. maximum microtubers (0.43 g/plantlet) followed by PR (0.42 g/plantlet) and De (0.30 g/plantlet). Cultivar AS showed least response (0.07 g/plantlet).

4.2.2.4 Comparison of microtuber yield from the potato cultivars:

Microtuber yield per plantlet among all the cultivars in case of each 500 mg/L, 250 mg/L and 0mg/L CCC concentration was compared and graph was plotted.

Cultivar	500 mg/L CCC (g/plantlet)	250 mg/L CCC (g/plantlet)	0 mg/L CCC (g/plantlet)
CS1	0.24	0.27	0.43
CS2	0.34	0.22	0.13
As	0.37	0.39	0.07
PR	0.45	0.36	0.42
De	0.20	0.28	0.30

TABLE 14. Comparative response with regard to microtuber yield in 8%, 6% and 4% sucrose



Graph 2: Comparison among the cultivars at 8% sucrose concentration with varying concentrations of CCC without BAP with regard to microtuber yield/plantlet.

With regard to treatment with different concentrations of sucrose, the following inferences were made:

While using 500 mg/L CCC among all the mentioned cultivars, we observed from the data that PR has shown maximum microtuber production per plantlet (0.45 g/plantlet), followed by AS and CS2 (0.37 and 0.34 g/plantlet respectively). Cultivar De showed least response with 0.20 g/plantlet.

In 250 mg/L CCC containing experimental set, again cultivar AS has shown maximum response (0.39 g/plantlet) followed by PR (0.36 g/plantlet) and De (0.28 g/plantlet). Here, CS2 showed least response with 0.22 g/plantlet each.

In last set containing no CCC (control), the cultivars CS1 (0.43 g/plantlet) and PR (0.42 g/plantlet) showed maximum response followed by De (0.30 g/plantlet) and CS2 (0.13 g/plantlet) with regard to microtuber production. Here, cultivar AS showed least response with 0.07 g/plantlet.

4.2.3 Effect of varying sucrose concentrations in the absence of growth regulators

Along with 80 g/L sucrose, two other concentrations 60 g/L sucrose and 40 g/L sucrose were used to study the effect of sucrose in tuberization in the absence of BAP and CCC as given in the following table:

Sr. no.	Sucrose (g/L)	BAP (mg/L)	CCC (mg/L)
1	80	-	-
2	80	-	-
3	80	-	-

4.2.3.1 Media containing 8% sucrose without CCC and BAP: In the media containing 8% sucrose without CCC and BAP, the cultivars AS and De showed maximum yield per plantlet. The cultivar PR showed average response with regard to yield per plantlet but it showed best response with regard to microtuberization in terms of tuber number, average weight and diameter, whereas least response was observed in the cultivars CS1 and CS2. The response of some of the potato cultivars are shown in the Figs. 44 to 49. Usually microtubers were harvested after 60-90 days and studied further for their size, yield, wt. etc.



Fig. 44 cv. AS



Fig. 45. cv. De



Fig. 46. cv. CS1

Figs. 44 to 46. Microtuberization in the media with 8% sucrose (45 days old culture)



Fig. 47 cv. AS



Fig. 48. cv. De



Fig. 49. cv. CS1

Figs 47 to 49. Harvested microtubers from different potato cultivars

The microtubers were harvested from each individual cultivar and further sorted out to study the following attributes such as yield per bottle, number per bottle, average weight, length and diameter covering from relatively large microtubers to the smaller ones. Compiled data is shown in the following table.

Cultivar	Total no. of tubers/bottle	Total wt. g/bottle	Weight range \pm SD		Length range (mm) \pm SD		Diameter range (mm) \pm SD	
			Max	Min	Max	Min	Max	Min
CS1	12	2.47	0.43 ± 0.03	0.30 ± 0.05	10.08 ± 0.46	11.60 ± 0.89	8.43 ± 0.12	6.33 ± 0.24
CS2	13	2.28	0.45 ± 0.04	0.07 ± 0.01	10.09 ± 0.72	4.96 ± 0.40	8.78 ± 0.37	4.65 ± 0.38
AS	13	3.93	0.63 ± 0.23	0.09 ± 0.01	14.04 ± 3.02	5.32 ± 0.09	8.68 ± 0.47	5.04 ± 0.19
PR	17	3.40	0.64 ± 0.16	0.06 ± 0.007	12.54 ± 1.78	5.59 ± 0.28	9.75 ± 0.79	4.41 ± 0.26
De	22	3.94	0.40 ± 0.01	0.09 ± 0.00	10.37 ± 0.36	5.47 ± 0.12	8.00 ± 0.45	4.91 ± 0.11

Table 15. Comparative response of the potato cultivars with regard to tuber number and size of the microtubers.

The data in Table 15 clearly showed that not only tuber number and weight of microtubers per bottle but average length, weight and diameter of microtubers also differ among the cultivars.

For instance, under the above condition PR showed maximum number of tubers, maximum diameter and maximum average weight. Cultivars De and AS showed maximum total weight. Although AS showed minimum tuber number but it showed maximum tuber length. While CS1 and CS2 showed minimum response in each regard.

Here, based on the data as given in Table 15, we calculated the yield of microtubers per plantlet corresponding to the individual cultivars as summarized in the Table 16.

Cultivar	Total wt. of microtubers/ bottle (g)	No. of plantlets/bottle	Microtuber yield (g/plantlet)
CS1	2.47	10	0.24
CS2	2.28	10	0.22
As	3.93	10	0.39
PR	3.40	10	0.34
De	3.94	10	0.39

Table 16. Comparative response of the potato cultivars with regard to microtuber yield.

From the Table 16 it is clearly observed that the cultivar AS and De showed best response in terms of microtuber yield i.e. maximum microtubers (0.39 g/plantlet each) followed by PR (0.34 g/plantlet). The cultivar CS2 showed poor response (0.22 g/plantlet).

4.2.3.2 Media containing 6% sucrose without CCC and BAP: In media containing 6% sucrose without CCC and BAP, the cultivar De showed best response with regard to tuber number and microtuber yield per plantlet. The cultivar PR showed average response, followed by CS1 and AS. Whereas least response was observed in the cultivar CS2. The response of some of the potato cultivars are shown in the Figs. 50 to 55. Usually microtubers were harvested after 60-90 days and studied further for their size, yield, wt. etc.



Fig. 50 cv. De



Fig. 51. cv. CS1



Fig. 52. cv. CS2

Figs. 50 to 52. Microtuberization in the media with 6% sucrose (45 days old culture)



Fig. 53 cv. De



Fig. 54. cv. CS1



Fig. 55. cv. CS2

Figs 53 to 55. Harvested microtubers from different potato cultivars

The microtubers were harvested from each individual cultivar and further sorted out to study the following attributes such as yield per bottle, number per bottle, average weight, length and diameter covering from relatively large microtubers to the smaller ones. Compiled data is shown in the following table.

Cultivar	Total no. of tubers/bottle	Total wt. g/bottle	Weight range \pm SD		Length range (mm) \pm SD		Diameter range (mm) \pm SD	
			Max	Min	Max	Min	Max	Min
CS1	7	1.92	0.67 \pm 0.12	0.09 \pm 0.01	11.82 \pm 0.36	6.90 \pm 0.55	9.98 \pm 0.85	4.63 \pm 0.94
CS2	20	1.18	0.15 \pm 0.05	0.04 \pm 0.00	7.05 \pm 0.02	4.10 \pm 0.22	5.83 \pm 0.79	3.75 \pm 0.10
AS	11	1.91	0.66 \pm 0.01	0.06 \pm 0.00	14.32 \pm 0.00	4.77 \pm 0.00	8.55 \pm 0.01	3.61 \pm 0.00
PR	13	2.40	0.87 \pm 0.01	0.04 \pm 0.014	11.68 \pm 0.00	4.44 \pm 0.48	11.45 \pm 0.01	4.14 \pm 0.22
De	17	3.02	0.37 \pm 0.03	0.05 \pm 0.01	10.79 \pm 1.41	5.63 \pm 0.60	7.57 \pm 0.94	3.55 \pm 0.45

Table 17. Comparative response of the potato cultivars with regard to tuber number and size of the microtubers.

The data in Table 17 clearly showed that not only tuber number and weight of microtubers per bottle but average length, weight and diameter of microtubers also differ among the cultivars. For instance, De showed maximum number of tubers and maximum total weight. AS showed maximum length while PR showed maximum average weight and diameter. CS2 showed minimum total weight.

Here, based on the data as given in Table 17, we calculated the yield of microtubers per plantlet corresponding to the individual cultivars as summarized in the Table 18.

Cultivar	Total wt. of microtubers/bottle (g)	No. of plantlets/bottle	Microtuber yield (g/plantlet)
CS1	1.92	10	0.19
CS2	1.18	10	0.11
As	1.91	10	0.19
PR	2.40	10	0.24
De	3.02	10	0.30

Table 18. Comparative response of the potato cultivars with regard to microtuber yield.

From the Table 18 it is clearly observed that the cultivar De showed best response in terms of microtuber yield (0.30 g/plantlet each) followed by PR (0.24 g/plantlet). The cultivar CS2 showed least response (0.11 g/plantlet).

4.2.3.3 Media containing 4% sucrose without CCC and BAP:

In the media containing 4% sucrose without CCC and BAP, the cultivar PR showed best response in terms of tuber size, weight and yield per plantlet. The cultivar De with maximum tuber number showed average response along with AS in terms of yield per plantlet. Whereas, least response was observed in the cultivar CS1. The response of some of the potato cultivars are shown in the Figs. 56 to 61. Usually microtubers were harvested after 60-90 days and studied further for their size, yield, weight etc.



Fig. 56 cv. PR



Fig. 57. cv. AS



Fig. 58. cv. CS1

Figs. 56 to 58. Microtuberization in the media with 4% sucrose (45 days old culture)



Fig. 59 cv. PR



Fig. 60. cv. AS



Fig. 61. cv. CS1

Figs 59 to 61. Harvested microtubers from different potato cultivars

The microtubers were harvested from each individual cultivar and further sorted out to study the following attributes such as yield per bottle, number per bottle, average weight, length and diameter covering from relatively large microtubers to the smaller ones. Compiled data is shown in the following table.

Cultivar	Total no. of tubers/bottle	Total wt. g/bottle	Weight range (g) ± SD		Length range (mm) ± SD		Diameter range (mm) ± SD	
			Max	Min	Max	Min	Max	Min
CS1	8	0.67	0.20 ± 0.01	0.05 ± 0.01	7.48 ± 0.01	4.98 ± 0.01	6.55 ± 0.01	4.16±0.00
AS	11	1.07	0.19 ± 0.02	0.04± 0.01	9.59± 0.03	4.22± 0.01	5.79± 0.57	3.09±0.00
PR	11	2.76	0.46± 0.08	0.05±0.007	10.13± 0.26	4.86± 0.12	8.71 ± 0.49	4.41±0.14
De	14	1.07	0.18± 0.04	0.065±0.00	9.67± 0.51	5.37± 1.11	5.34± 0.47	4.29±0.19

Table 19. Varying response of the potato cultivars with regard to tuber number and size of the microtubers.

The data in Table 19 clearly showed that not only tuber number and weight of microtubers per bottle but average length, weight and diameter of microtubers also differ among the cultivars

For instance, De showed the maximum number of tubers. PR showed maximum total weight, maximum average weight, maximum length and diameter.

Here, based on the data as given in Table 19, we calculated the yield of microtubers per plantlet corresponding to the individual cultivars as summarized in the Table 20.

Cultivar	Total wt. of microtubers/bottle (g)	No. of plantlets/bottle	Microtuber yield (g/plantlet)
CS1	0.67	10	0.06
CS2	none	-	-
As	1.07	10	0.10
PR	2.76	10	0.27
De	1.07	10	0.10

Table 20. Comparative response of the potato cultivars with regard to microtuber yield.

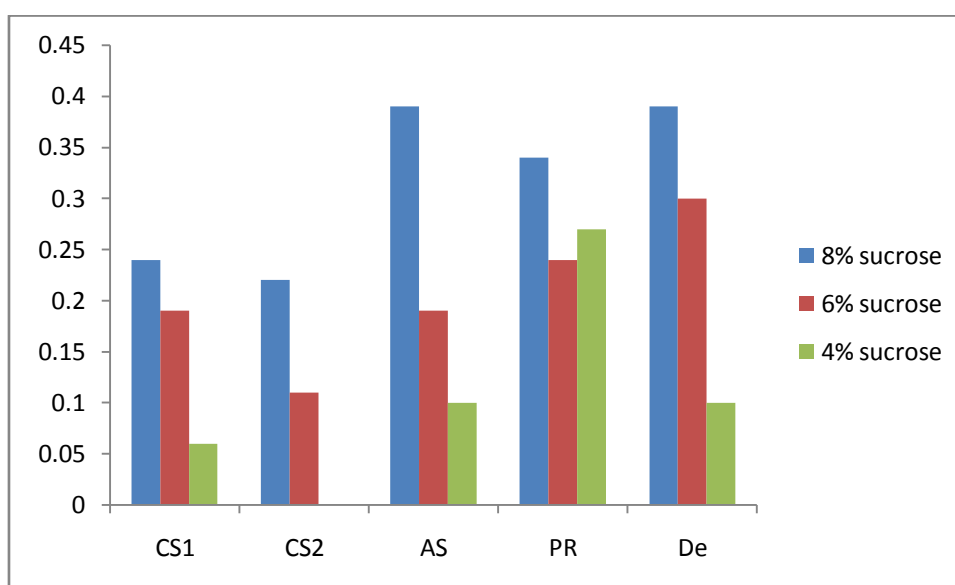
From the Table 20 it is clearly observed that the cultivar PR showed best response in terms of microtuber yield i.e. maximum microtubers (0.27 g/plantlet each) followed by AS and De (0.10 g/plantlet). The cultivar CS1 showed poor response (0.06 g/plantlet). While CS2 showed no response.

4.2.3.4 Comparison of microtuber yield from the potato cultivars:

Microtuber yield per plantlet among all the cultivars in case of each 8%, 6%, and 4% sucrose concentration was compared and graph was plotted.

Cultivar	8% sucrose (g/plantlet)	6% sucrose (g/plantlet)	4% sucrose (g/plantlet)
CS1	0.24	0.19	0.06
CS2	0.22	0.11	-
As	0.39	0.19	0.10
PR	0.34	0.24	0.27
De	0.39	0.30	0.10

Table 21. Comparative response with regard to microtuber yield in 8%, 6% and 4% sucrose without CCC and BAP.



Graph 3 - Comparison among the cultivars at 8%, 6% and 4% sucrose concentrations without CCC and BAP with regard to microtuber yield/plantlet.

With regard to treatment with different concentrations of sucrose, the following inferences were made:

While using 8% sucrose among all the mentioned cultivars, we observed from the data that AS and De has shown maximum microtuber production per plantlet (0.39 g/plantlet) followed by PR (0.34 g/plantlet). Cultivar CS2 showed least response with 0.22 g/plantlet.

In 6% sucrose containing experimental set, again cultivar De has shown maximum response (0.30 g/plantlet) followed by PR (0.24 g/plantlet), AS and CS1 (0.19 g/plantlet). Here, CS2 showed least response with 0.11 g/plantlet each.

In last set containing 4% sucrose, cultivar PR has shown maximum response (0.27 g/plantlet) with regard to microtuber production followed by AS and De (0.10 g/plantlet). Here, cultivar CS1 showed least response with 0.06 g/plantlet. While no response was shown by CS2.

Relevance of the present study-

The aim of the present study was to study the effect of various media formulations on microtuberization process in some Indian potato cultivars along with an exotic cultivar Desiree. Microtubers have tremendous applications in the area of potato biotechnology in terms of germplasm storage, transport and development of agronomically important characters.

The technology serves to understand the many aspects of potato biology both at biochemical and molecular level, signal transduction pathways, spatio-temporal nature of gene expression and to unravel the many aspects of basic research.

Tuber development and related processes at biochemical and molecular level are difficult to study in the field and/or in soil-grown plants due to a low level of synchrony of the tuberization process under these conditions and to the obscuring effect of soil.

By Molecular Pharming and Recombinant DNA technology microtubers can be used to study the role and expression of specific gene products. This can be helpful in producing disease resistant varieties, edible vaccines, potato with high nutrition value etc.

To overcome the increasing demands of the growing population, there is a need to develop a pathogen free seed stock to increase the potato productivity which can be done through microtuberization.

Since 1988, when Dodds reported that the microtubers can be used as an additional component to the standard methods of rapid propagation used in seed tuber production and utilized for distribution of germplasm quite conveniently, the researchers are continuously trying to find out optimum media compositions and culture conditions that may contribute to tuberization potential of potato. As potato is now a major food crop all over the world, recent advances are always awaited in the field of potato research.

In the literature, studies related to response of various potato cultivars with regard to tuberization in presence of varying concentrations of sucrose, CCC and phytohormones have been reported.

The major salient findings of our present study were quite interesting from the previous literature studies. Some of them are highlighted below:

Our studies related to the response of the mentioned potato cultivars towards varying concentrations of sucrose were found to be quite interesting. While considering the treatment of different potato cultivars with different concentrations of sucrose, we found that cultivars under study showed best response at 8% sucrose concentration and responded well even without phytohormones.

While standard protocols cited in the literature states that 8% sucrose with 10 mg/L BA and 500 mg/L CCC is optimum for microtuberization. This clearly shows that there is an endogenous level of these phytohormones in the potato plantlet which help tubers grow even in their absence in the media. For example-

The cultivar AS gave good response at 8% sucrose, without CCC and BAP. This indicates that AS do not require any growth stimulator for initiation and development of microtubers. Only high sucrose is sufficient for good microtuber yield. Other cultivars like De and PR also showed good response in this media formulation.

(However, CCC is classified as a carcinogenic agent so it can be conveniently replaced by sources for the same purpose. This study therefore, indicates that sucrose may serve as a cheap, safe and superior agent for microtuberization, as compared to CCC and plant growth regulators.)

The best response was of the cultivar PR at varying concentrations of sucrose with CCC (500 mg/L) and BAP (10 mg/L), and at 8% sucrose with varying concentration of CCC (500 mg/L, 250 mg/L, and 0 mg/L) without BAP. PR at 8%, 6%, and 4% sucrose without CCC and BAP also showed good response.

As in laboratory practices, we know that cost of phytohormones and CCC makes the product more costly. Thus, these cultivars could be used for tuberization in agronomic practices in an economic way. As microtuberization is performed under aseptic conditions, so pathogen free microtubers are produced.

Therefore, our study will be useful in formulating a media composition optimum for microtuberization of each individual genotype. As our first need is to get maximum yield in most cost effective manner to produce cheap, healthy, virus free seed potato microtubers as they are important in germplasm storage, and propagation of high yielding varieties.

Summary

In this study, four Indian potato cultivars which are suitable for our agro-climatic conditions namely Kufri Chipsona 1, Kufri Chipsona 2, Kufri Ashoka and Kufri Pukhraj along with an exotic cultivar Desiree were used.

These cultivars were routinely maintained through Apical Meristem Culture (AMC) in our laboratory on MS medium. The standard microtuberization protocol was performed in which 10 nodal segments were inoculated in liquid MS medium and these were shifted to tuberization media after 21 days. The work done is summarized as follows-

- Different concentrations of sucrose 80 g/L, 60 g/L and 40 g/L along with 10 mg/L BA and 500 mg/L CCC were used in different experimental sets to study its effect on the above mentioned genotypes with regard to microtuberization. Microtubers were obtained and microtuber yield was calculated and cultivar to cultivar variation was notified. In case of variation in sucrose concentration in presence of growth regulators, microtuberization was affected in different cultivars and varied response was observed. The different cultivars showed their best response at a specific concentration of sucrose and thus optimization was done. PR gave maximum microtuber yield at 8% and 6% sucrose, at 4% sucrose followed by AS. De showed good response at 4%.
- In another set, to study the effect of CCC on microtuberization, tuber induction in varying CCC concentrations (500 mg/L, 250 mg/L, 0 mg/L) with 80 g/L sucrose, without BAP was compared among the cultivars. In case of absence of CCC, CS1 and PR showed maximum tuber production. The exotic variety De also showed some good results at various concentrations.
- To study the effect of sucrose on microtuberization in absence of growth regulators, three experimental sets, 80 g/L sucrose, 60 g/L sucrose and 40 g/L sucrose without CCC and BAP were studied and response in terms of microtuber yield was compared among all the cultivars. In case of variation in sucrose concentration in absence of growth regulators, the best results were shown by AS at 8%, PR at 6% and 4%. While De gave maximum yield at both 8% and 6% sucrose concentrations.

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