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Importance of Potato Micro Tuber Seed Material for Farmers of Uttarakhand Hills

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Abstract: In India, still traditional methods are used for potato cultivation. In hills, farmers face many problems in the cultivation and storage of seed material, such as wastage of large food material, low multiplication rate and lake of commercialization. These problems affect the farmers socially as well as economically. Using *In vitro* techniques several problems related to production, cultivation and multiplication can be resolved. A disease free planting material with high yield can be produced, which can solve the problems of hills farmers. The farmers use pieces of potato or whole potato tuber, as a seed so using these conventional methods a large quantity of food material is used for cultivation. Potato is a tetraploid vegetatively propagated crop that poses several problems in seed production. Generally tuber of potato is used as a seed. Due to progressive accommodation of viral diseases in seed stock, availability of good quality seed is a major constraint in potato production, which is approx. 50% of the total production cost. Besides high cost of seed potato, propagation is also characterized by low multiplication rate of only 4-6 times. To large production of clonal material i.e. to produce the uniform, identical seed material of potato, micro propagation is the better alternative over to conventional propagation methods of potato. Saving of food material, development of diseases free and clonal planting material are some of major advantages from *In vitro* propagated micro tuber. This will certainly help in the development of social and economical condition of farmers. Methods used in micro propagation for commercial seed production has moved potato from test tubes to field. The advances in the research are going to prove the being of the second "Green Revolution" in agriculture and expected to make farming more efficient, profitable and environmentally safe.

Key words: Micro tubers % *In vitro* % Clonal and conventional propagation

INTRODUCTION

Agriculture is the mainstay of the economy of Uttarakhand. Around 75-80% of the state's population depends on agriculture for its livelihood. Potato is an important cash crop grown in Uttarakhand. The land holdings are rather small generally less than one hectare in most district, except in two districts, viz. Udham Singh Nagar and Pauri Garhwal [1]. In the hill region, farmers devote a large part of their land holdings for raising cereal crops for their subsistence, although some area is also devoted to horticultural crops and vegetables such as onion, green peas and potato [2]. Vegetables make a valuable contribution to the economy of the state, although these are grown on a limited area. As in the cash of food grains, the productivity of the above mentioned horticultural crops in Uttarakhand is generally lower than the average for the country and substantially below that obtained in the more progressive states.

The main problems to potato cultivation in hills of Uttarakhand, are limited land and water resources, lack of seed agency who provide the quality seed potato and lack of technology intervention. Low yields indicated that the potential which needs to be harnessed. While this offers opportunities for growth in production, income and employment in the rural economy of the state, it also poses an immense challenge that needs to be overcome without losing much time. With the limited land and water resources in the state, it is essential to introduce an improved integrated intensive holistic farming system and adoption of advanced package of practices.

The Potato Crop; Production Area and Productivity: The potato is a dicot plant belonging to the family Solanaceae and the genus *Solanum*. This is a large genus and contains 2000 species. According to the latest classification, the genus has been divided into two sub geneses, *Pachystemonum* and *Leptostemonum.*

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Pachystemonum has been further divided into five sections, of which section potato contains most of the tuber bearing species [3]. Traditionally it was adopted as a commercial food crop in temperate climate, under long day growing condition, mainly in Europe. As a crop of high biological value for its starch, protein and a substantial amount of vitamins, minerals and trace elements, it is undoubtedly a very important crop [4]. The global area under potato cultivation during 1998 was about 18 million ha with a total production of about 295 million tones. Among the major potato growing countries of the world China ranks first, followed by the Russian Federation, Ukraine and Poland. India ranks fifth in the world. India produces a total of about 25-28 millions tones of potato every year. Each hectare of land generally, produces about 16-19 tones of potato. However, in Europe and American countries the productivity is about 30-40 tones per hectare [3].

The country recorded an increase in 1950-51 to 1.3 million hectares in 2004-05 with the corresponding increases in production from 1.66 to 23.6 million tones. The productivity also improved from 69.2 quintals/hectare in 1950-51 to 181.5 quintals/hectare in 2004-05. Thus, there has been a phenomenal increase in the area, as well as the productivity over this period by 44.2, 13.2 and 16.2%, respectively [5]. With the update of modern potato technologies, India now ranks 4th in area and 3rd in production of potato in the world. The productivity of potato in India is better than the world average (166.3 quintals/hectare). By 2020 India will have a population of 1.3 billion bringing about a substantial pressure on land to produce more food. It is also estimated that by 2020 worldwide demand for potato would increase by 40 per cent. Potato is grown in over 130 countries and word's annual crop of potato exceeds 278 million metric tones with an estimated value of around 13 Billion Rupees [6]. By the year 2020 potato production is expected to reach about 49 million tones [7]. As one the principal cash crops, potato contributes to the national economy in many ways. It gives handsome returns to the growers. On the average, the net returns are about Rs. 4,700.00/ha. In other terms; 1 rupee of investment gives a net return of Rs. 1.40 in a short period of 80-100 days. Potato is both a labor and capital intensive crops [8].

Potato as a Food Source and a Raw Material: Potato is an important human food with wider adaptability potential to fill the gap between food needs and cereals. The importance of vegetables in human nutrition is well known. In a country with limited resources, where the nutrition level of the population has to be maintained under inhospitable situations, the potato has a special value as food [9]. As major cereals are fast approaching the practical limits of their production, the yield potential of potato is still underutilized [10].

Potato can supplement the food needs of the country in a substantial way. It produces dry matter food, well-balanced protein and more calories from unit area of land and times then other major food crops. It also contains essential nutrients as proteins and minerals like calcium, phosphorus, iron and vitamins: B1, B2, B6 and C [3]. The rising population is pushing up the demand for food, which needs to be met through enhanced productivity per unit area and time. In this context, importance of potato is noteworthy for several reasons. Firstly, potato crop produces more edible energy and protein per unit area and time than other food crops. Secondly, for the small and marginal farmers, potato fits well into multiple cropping systems prevalent in tropical and subtropical agro-climatic conditions. Thirdly, high profitability of potato as a cash crop has made it a viable commercial enterprise and lastly, rapid technological advances in varietal improvement, agro-techniques, plant protection, storage and processing have led to expansion of potato production even in non-traditional environments [5].

Trends in Potato Production: Conventional propagation of potato is done vegetatively using seed tubers which ensure uniformity of the crop in terms of growth and yield, but results in degeneration of the crop due to virus infection. The rates of degeneration vary from place to place and from one growing season to other growing season [11]. The viruses are transmitted through different ways as well as by 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 [12]. This is the major problem faced by seed producers.

In India, the systematic work on potato tissue and cell culture was initiated in 1972 at the Central Potato Research Institute, Shimla. Varietal improvement program at CPRI over the past more than 50 years has been largely instrumental in nearly thirteen-fold increase in total production and three-fold increase in yield in the country. Further, the development of short duration varieties like Kufri Chandramukhi, K. Ashoka, K. Jawahar, K. Pukhraj and K. Lauvkar has contributed towards higher cropping intensity and higher returns to the farmers. A range of techniques, including tissue culture and *in vitro* rapid

multiplication have been used by 26 national programs in Africa, Asia and Latin America to clean, maintain and reproduce basic stocks of seed potato for later multiplication and used by farmers [13]. This technique aided by biotechnological approaches for virus elimination, micropropagation and effective viral diagnostics has sustained the National Potato Seed Production Program by producing about 2600 tones of breeder's seed annually. This breeder seed is needed to be multiplied to about 4, 32,000 tones of certified seed by the State Department of Agriculture/ Horticulture to meet the seed potato requirements of our farmers [5].

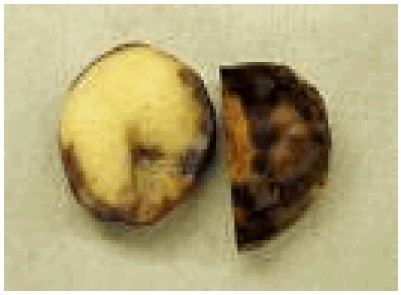
Selection of the Variety: Till to date more than 41 varieties have been released for cultivation for diverse agro-climatic conditions of the country. Some of them are early maturing (70-80 days), some are media maturing (90-100 days) and some are late maturing (110-120 days) [3]. In India till 1956, 16 desi and 38 European varieties were under cultivation. Now almost all of them have been replaced by new potato varieties released by CPRI. A number of improved potato varieties have been breed and released as Kufri series, a name derived from the station where they have been breed and are maintained in diseased free conditions [14].

Potato varieties are distinguished by their habit, pigmentation on the stem, structure of leaf, flower and fruit (berry) color and tuber like shape, size and color, depth of eyes and flesh color etc [5]. Some of the varieties are Kufri Kundan (1958), Kufri Red (1959), Kufri Safed (1958), Kufri Alankar (1968), Kufri Chamatkar (1968), Kufri Chandramukhi (1968), Kufri Badshah (1979), Kufri Jyoti (1968). Kufri Giriraj (SM/85-45), Kufri Pushkar (JE/JC-166), Kufri Chipsona-I, Kufri Chipsona–II etc [15].

The Indian Council of Agriculture Research (ICAR) has identified two new hybrid varieties of potato namely Kufri Chipsona-3 and Kufri Himalini [16]. Nearly 8% of the total area under potato cultivation in the country lies in the hills, where it is an important cash crop. Late blight disease (*Phytophthora infestans*) appears in the hills in epiphytotic form every year and is the most important impediment in potato cultivation. Losses up to 74% in North-Eastern hills and up to 65% in North-Western hills have been recorded in susceptible potato varieties [17].

The main problem of growing potato worldwide is huge economic losses due to late blight. *Phytophthora infestans* can destroy all parts of potato plant within two weeks in wet conditions [18-20] (Plate 1; a-d). *Phytophthora infestans* can survive under adverse conditions and winter over in the form of oospores. The pathogen however, invades and infects potato plants in the field via zoosporangia which disperse via soil water, rain splash and wind [20].

The ICAR has identified Kufri Himalini for commercial cultivation in hilly regions. The new variety, with media maturity of 110-120 days has been recommended for cultivation in the north- western and eastern hills during summer. Kufri Himalini provides a yield advantage of over 10% over Kufri Jyoti and Kufri Giriraj, in the plains and its keeping quality is better than all the cultivars developed so far for hill regions [21].

(a)  (b)  (c) 

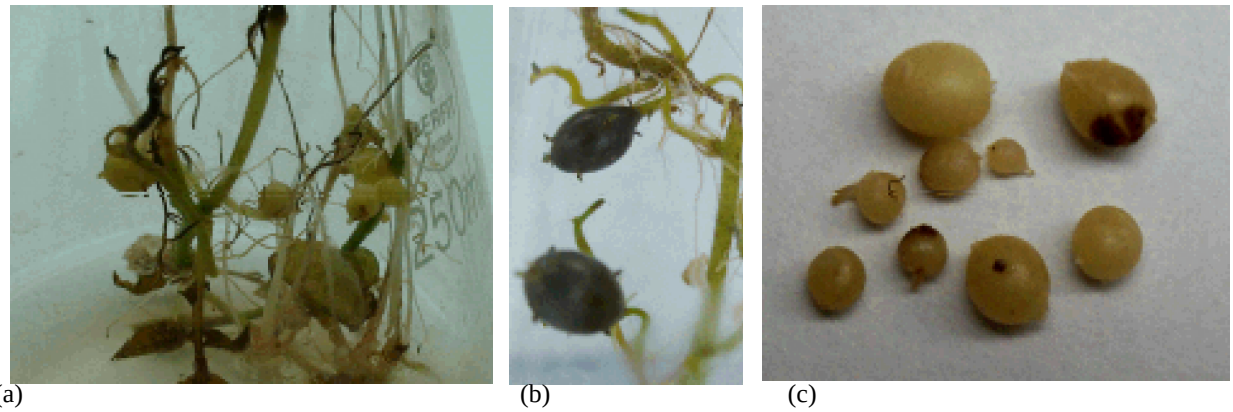
(d) 

Plate 1: (a-c) Late Blight Disease of Potato (d) whole field affected by disease

Problems in Conventional Potato Cultivation: Successful cultivation of seed potato primarily depends upon the availability of disease free seed, plant protection measures, low temperature and short day conditions during tuberization phase. Potato plant is very sensitive to ecological factors such as temperature, rainfall and photoperiod [22]. Seed tuber quality is an extremely important factor for potato yield. Since it is a vegetatively propagated plant, fungal, bacterial and, particularly viral disease, agents are easily transmitted through the tubers [23]. Viral diseases are, for the most part, responsible for degeneration, characterized by a decrease in vigor, productivity and resistance to diseases of potato cultivars after successive cultivation from the same lot of tubers [24, 25]. In India, farmers use the traditional methods for potato cultivation and face many problems. The most severe problem faced by the farmers, regarding is the non-availability of foundation and certified seed. The farmers use pieces of potato or whole potato tuber, as a seed and therefore a large quantity of food material is also lost. Some major problems in conventional potato cultivation are as follows:

- Wastage of a large quantity of food material.
- Disease and Insect problems.
- Absence of uniformity.
- Quantity and quality of the product may be decreased.
- Availability of good quality seed is a major constraint in potato production.
- High cost of potato seed.
- Low multiplication rate.

Microtuber: A Source of Germplasm Conservation: Almost half a century has passed since *In vitro* tubers (microtubers) were first described in potato, but their adoption as a seed propagule has been uneven globally. Consensus is lacking regarding optimal production practices for microtubers and their relative productivity in relation to other propagules for minituber production. There is significant uncertainty regarding the utility of microtubers for evaluation of agronomic characters. However, 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 (Plate 2; a-c). Many of the interactions between growth parameters *In vitro* and subsequent productivity appear to be genotype-specific. Accordingly, microtubers come in different sizes, have different dormancy requirements and differ widely in relative growth potential and productivity. Despite these differences, there is evidence for strong analogies in growth responses between field-grown tubers and microtubers. The use of microtuber technology in seed tuber production, breeding programs, germplasm conservation 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. Wherever microtuber and minituber production technologies have been implemented, they have halved the field time necessary to supply commercial growers (3 or 4 years compared with 7 or more years) and greatly improved seed tuber quality (fewer viral, bacterial, fungal problems) [26]. Production of mini-tubers as a source for seed potato was investigated by growing in soil micropropagated plants and micro-tubers. Micropropagated plants produced mini-tubers in glasshouse after 70–115 days of growth in soil. A large proportion of the mini-tubers produced were between 9 and 15 mm diameter. Several factors, e.g., explants number, duration of *In vitro*culture and genotype influenced mini-tubers production [27, 28].



(a) (b) (c)

Plate 2: (a-c) *In vitro* produced Microtubers

In all potato growing regions the availability of high quality clean seed tuber has been the most limited owing to the conventional clonal propagation that favors disease build-up that drastically reduces yield. Potato seed production programs in many countries have been boosted by using these techniques. In recent years the first multiplication steps in seed production programs are speeded up by using *In vitro*plantlets, Microtubers [29] or mini tubers [30].

Why Rapid Multiplication?: Potato poses various problems to the plant breeders, by being a tetraploid, vegetatively propagated crop. These problems include a high level of heterozygosity, the common occurrence of pollen sterility, difficulties in germplasm storage and transport and the build ups of viruses. The planting material is also quite bulky, as tubers are used for propagation and a seed rate of 2.0 to 2.5 tons/ha is required for it; therefore much amount of potential food is wasted [31].

Generally tuber is used as a seed. Due to progressive accommodation of viral disease in potato seed stock, availability of good quality seed is a major constraint in potato production, which is approximately 50% of the total production cost. Besides high cost of seed potato, the productivity is also influenced by characterized by low multiplication rate of only 4-6 times.

Large scale production of clonal material i.e., to produce uniform, identical seed material of potato, micropropagation can be the better alternative over conventional propagation of potato. The micropropagation method is most suitable alternative to produce microtuber seed material of potato. Micropropagation is a sophisticated technique of regenerating plants using small pieces of plants (so called explants) that is proliferated on an artificial media under sterile conditions. Importance of micropropagation lies in very fast clonal multiplication of vegetable crops. Micropropagation is used mainly for getting disease- free plants of superior vigour and productivity [32].

By using the technique, which involves low cost components, large-scale clonal material can be produced in short time duration. Use of micropropagation for commercial seed production has moved potato from test tubes to field [33]. The current advances are seen as the beginning of the second “Green Revolution” in agriculture and are expected to make farming more efficient, profitable and environmentally safe [34].

Microtubers are small, *In vitro*produced tubers varying in size and weight from 3-12 mm and 0.02 to 1.0 gm. respectively [35]. These are produced under sterile pathogen-free conditions [35, 36]. Such small dormant tubers are particularly convenient in handling, storage and distribution [10]. Potato microtubers are the propagules for quick multiplication of uniform progeny. However, *In vitro* produced microtubers would demand the cost of production of microtubers to be competitive with *in vivo* multiplication techniques with regards to cost and simplicity as well as easy availability of local ingredients [35].

In Vietnam, a simple low - cost rapid multiplication system has been developed for farmers using *In vitro* plantlets. These are multiplied *In vitro*, producing single node cuttings that are transferred and rooted in sand beds at high density. Apical and axillary cuttings are taken and rooted in beds with subsoil-manure mixture. Then cuttings are taken for rooting in the small banana leaf pots. Three *In vitro* plantlets can provide sufficient material to plant 1 ha in 7 months [37]. Many tropical countries are not able to produce high quality seed tubers due to the lack of the vector-free production areas and the import of certified seed which involves very high cost [38]. *In vitro* tubers have been used to overcome the problem of transplanting of tender vegetative plantlets from *In vitro* conditions to external environment which is rather laborious and also has a high mortality rate. Microtubers could be harvested *In vitro* conditions, stored, shipped and planted conveniently. Microtuber production can be easily adopted in seed potato production program in many developing countries where the relative cost of certified seed tubers is high and labor cost is low [39].

Advantages of Non-Conventional Technique over Conventional Methods of Potato Production: Using the tissue culture technique of micropropagation, it is possible not only to reduce the number of field exposures but also to increase the rate of multiplication several times. Plant tissue culture comprises a set of *In vitro* techniques, methods and strategies that are part of the group of technologies called plant biotechnology. Tissue culture has been exploited to create genetic variability from which crop plants can be improved, to improve the state of health of the planted material and to increase the number of desirable germplasm available to the plant breeder. The culture of single cell and meristems can be effectively used to eradicate pathogens

from planting material and thereby dramatically improve the yield of established cultivars. Large-scale micropropagation laboratories are providing millions of plants for the commercial ornamental market and the clonally propagated crop market [40].

One of the most viable methods for obtaining virus-free stocks from propagative material that comes from infected plants is viral eradication by using tissue culture techniques, aided or not by thermo-and/or chemotherapies [41, 42]. The rapid *In vitro* propagation of clonal material is an important area where tissue culture techniques are being routinely used world over for quick multiplication of potato germplasm and raising commercial potato crop [31]. During last two decades several *In vitro* propagation methods using adventitious shoots developed from sprout meristem tips [43], nodal cuttings from *In vitro* plantlets [44, 30, 45, 46], layering of *In vitro* shoots [33], multiplication developed from axillary buds [47], shoot tips [48] and *In vitro* tuberization [49, 50] have been developed.

Hence to produce the disease free planting material and for decreasing the production cost new methods of propagation are to be derived and adopted. To large production of clonal material i.e. to produce the uniform, identical seed material of potato, micropropagation is the better alternative over to conventional propagation of potato. The *In vitro* propagation method is most suitable alternative to produce micro tuber seed material of potato. By using the technique, which involves low cost components the large scale clonal material can be achieved in short time duration. Use of micropropagation for commercial seed production has moved potato from test tubes to field [51].

Need of *In vitro* method for Production of Potato Micro Tuber Seed Material: The conventional planting material for potato seed is the vegetative tuber. Conventional potato seed production involves production of basic seed (also called breeders' seed) on special seed farms, which is further multiplied by seed agencies and registered

Diagrammatic Protocol for Quality Potato Seed Production [55]:

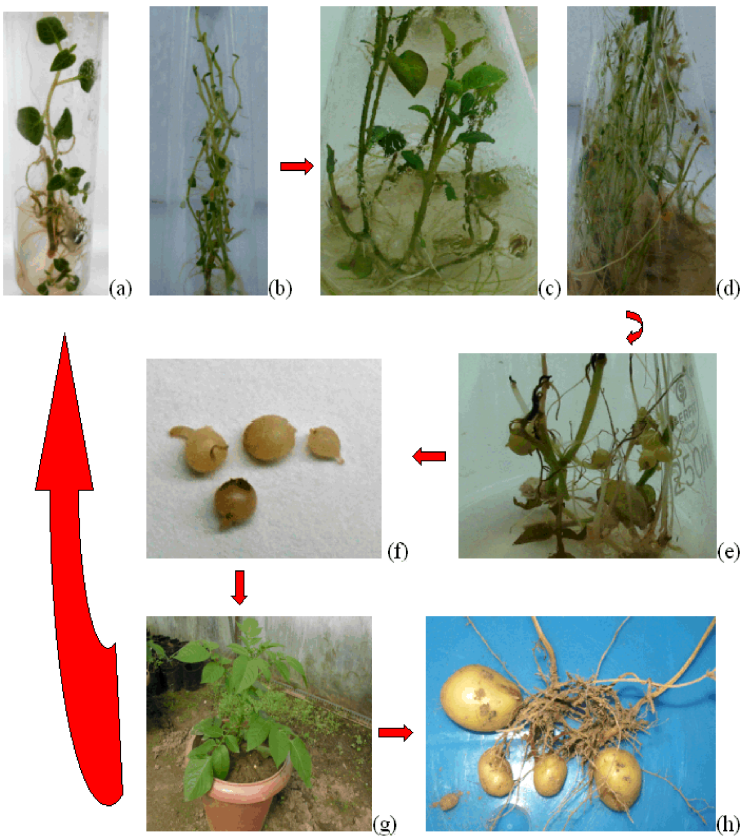


Plate 3 (a-h): Micro and Mini tuber seed production of potato; (a-b) shoot establishment, (c-d) pretuberization stage, (e) tuberization stage (f) harvested microtubers (g) hardening of microtubers and (h) harvested mini tubers

seed growers to produce certified seed. Most seed production programs operate a "flush through" system starting each year with fresh true to type and healthy tubers which have been indexed for freedom from viruses. All conventional potato seed production systems are characterized by low multiplication rate and progressive accumulation of degenerative viral diseases during clonal propagations. About 30 viruses and virus like agents infect potato. These being systemic pathogens, are perpetuated through seed tubers and pose a major threat to potato seed production. In contrast to seed propagated crops, the multiplication rate in potato is low varying from 1:4 to 1:15 (one tuber yields 4 to 15 tubers) depending upon variety, agro-climatic conditions and crop management practices. Therefore, to build up sizeable seed stocks, the initial disease-free tuber material needs to be field multiplied for a number of years. With each such multiplication cycle, viral diseases accumulate progressively causing degeneration or "running out" of seed stocks. Consequently, non-availability of quality planting material in adequate quantities and at affordable prices is the major bottleneck in potato cultivation in many countries. The problem is further aggravated by high seed rate (3 to 4 t/ha) due to which the cost of seed potato alone accounts for about 40 to 60% of the total production costs in many parts of the world [52].

Obtaining quantities of clean planting material has been a major barrior to increases potato production in many developing tropical countries [37]. In India, Professor P. Maheshwari realized the significance of *In vitro* culture technique quite early. Who initiated investigation with complex tissue such as ovary, ovule and control of fertilizers. The technique has developed around the concept that a cell is totipotent that is has the capacity and ability to develop into whole organism [53]. A Systematic effort to promote Biotechnological Research in India began with the establishment of 'National Biotechnological Board' under the Department of Science and Technology in early eighties. Biotechnology has rapidly emerged as an area of activity having a marked realized as well as potential impact on virtually on human welfare, ranging from food processing, protecting the environment, to human health. As a result it now plays a very important role in employment, production and productivity, trades, economics and economy, human health and the quality of human life through out the world [54].

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