

Continuing Education Article

Commercial Role of *In Vitro* Production of Micro and Mini Potato Tubers (*Solanum tuberosum* L.)

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ABSTRACT

Potato is ranked highly valuable crop and is consumed as staple food in many parts of the world. The conventional methods of seed production of potato have a number of constraints to develop the healthy and quality plants which effect the yield of the crop. Tissue culture (*in vitro*) technology has enormous advantages in potato seed production. One of the major advantages is the eradication of different kinds of viruses that cause the drastic reduction of yield in terms of quality and quantity of the crop. This paper presents many aspects of the production principles, prospectus of micro and mini tuber production of potato. A detailed discussion is undertaken to elaborate the standard procedures of crop production encompassing the seedling and micro tubers production in the *in vitro*. Parallel to these standards, those methods adopted for how such seedlings are performed in the controlled environment of greenhouse for further multiplication *in vivo* have also been addressed. The results of some important trials conducted in tissue culture laboratory and in the greenhouse on the vitals aspects relating to the improvement of the tubers are added to signify their importance.

Key Words: Explant; Seedlings; Tubers; Potato (*Solanum tuberosum*); Greenhouse; Planting media; Yield

INTRODUCTION

There was a time when potato seeds could be produced only by conventional method in the field. Old practice of seed production is costly along with the lower quality and also infected with various pathogens of bacteria, fungi and viruses respectively. As a result, the yield remains limited under natural climatic exposure and risk of loss of lucrative profit when compared with the total incurred cost of potato production. The use of low quality potato tubers increase the yield losses in the field due to different pests and diseases that threaten the crop considerably, e.g. Colorado beetle, potato root eel worm, late blight and several virus diseases (Butt, 2003). Some viruses, such as potato viruses X and S, multiply in the plant without noticeable symptoms. Gomeç (1977) reported that potato crop infected by X virus reduced more than 10% potato yield without showing any physical symptoms. However, some other viruses cause even greater losses. Potato leaf roll virus and strains of potato viruses Y frequently reduce the tuber yield by 5-80% (Gomeç, 1977). The average loss due to all potato viruses has been estimated 13%, which amounts to about 30 millions tons of valuable food each year (Anonymous, 2001a). The Turkish potato has a long history in Turkey, have been introduced to Anatolia and the Black Sea Coast from 19th century. Today, Turkey is the largest producer of potatoes in the Middle East, almost 2-3 times that of its major regional competitors, Iran and Egypt (Gomeç, 1977). Average regional yields vary from 8-12 t ha⁻¹ in Black Coast to 18-20 t ha⁻¹ along the Aegean and Mediterranean Coast (Haverkort, 1981). Fungal diseases are serious problems in Turkey, especially in the lower warmer

plains as Verticillium wilt, Fusarium dry rot and wilt, early blight and black scurf. Late blight is especially severe in the Black Sea area. Viruses, especially the PVX and PVY are widespread in the plains and affect periodically crops in the highlands. The most serious bacterial disease is *Streptomyces scabies*, especially in areas of alkaline soil (Sahtiyanci, 1990). With the objectives to ascertain various factors of lowering potato yield; some standard procedures were adopted in the system from *in vitro* to *in vivo* for healthy tuber production. The pertinent trials to improve the quality of micro and mini tubers were conducted so as to minimize the risk of contamination of different pathogens, which are the major cause of yield reduction in potato crop.

Production principles of mini and micro tubers around the world. Mini tubers are generally produced in the greenhouse in many countries around the world. The source of material is either the plantlets developed inside the growth chamber of tissue culture laboratory or the already produced mini or micro tubers in the greenhouse and in the laboratory, respectively. Greenhouse should be facilitated with temperature, light, humidity and air circulation for the production of mini tubers. In many potato seed certification organizations *viz.*, Oregon Seed Certification, USA, the very first produced mini tubers derived from pre-nuclear source (e.g. micro tubers) are referred as nuclear seeds, provided it must have met the basic seed quality production schedule (Anonymous, 2000a). By accepting this way, in some cases, 5 consecutive generations of mini tubers can be maintained for stock multiplication. The major rule pertaining to the production of greenhouse tubers in Minnesota, USA is that there is a zero tolerance for virus diseases. A lot grown as and intended to be pre-nuclear must be grown from plants

tested and shown to be free from the pathogens of *Clavibacter michiganensis* ssp. *sepedonicus* (ring rot), *Erwinia carotovora* (blackleg), potato virus X, potato virus S, potato virus A, potato virus M, potato virus Y, potato spindle tuber viroid and potato leaf roll virus (Anonymous, 2000b). Prenuclear class seed potatoes must be produced in a greenhouse or screen house under sanitary conditions, free from insects and weeds that can harbor or transmit potato diseases or other conditions of possible disease contamination. All facilities must be sufficiently insulated from insects by screens and double doors. Sprouts are surface-sterilized and placed on a semi-solid nutrient medium. The resultant plantlets are then pathogen tested for many diseases including potato viruses S, X, Y leaf roll, A, M, bacteria, which cause ring rot (*Clavibacter michiganense*) and soft rot (*Erwinia carotovora*) fungi and potato spindle tuber viroid (PSTV). Sprouts from each individual tuber receive a numeric "clonal" designation. Once pathogen-free plantlets are obtained and then can be multiplied on large scale (Coltman, 1998).

Prospects of micro and mini tubers. The production of commercial crops by the use of tissue culture technology is a new introduction all over the world. The advancement of micro propagation (a fundamental tool of biotechnology) has provided an excellent opportunity for progressive potato growers to produce virus free seed potatoes. One of the primary objectives of micro and mini tubers for potato production is to provide higher quality and disease-free basic seed stock for seed potato industry. By the use of seed potato produced by modern technology, 25 tons ha⁻¹ of potato yield can be easily obtained (Mahmood *et al.*, 2000). In Turkey, the coastal areas of highlands produce well established seed potatoes for markets. Farmers commonly purchase seed for every third of the two crops. To supply seed for an area of approximately 185,000 ha, around 370,000 tons of seed is required. Every year, a bulk of foreign exchange is being spent to import the seed potatoes in Turkey from Europe to fulfill the requirements of potato growers (Gomeç, 1977).

The tubers produced in the lab and/or in the greenhouse and consequently, in the open field would become a source of prosperity amongst the potato growers of Turkey. This would also provide a chance to export good quality seed potatoes in different parts of the world by improving productivity of quantity and quality standards of the seed tubers. This would also protect the integrity of potato industry by ensuring only high quality potato seed to the growers. A recent report has indicated that the aggregate income benefits from true seed potatoes in a small country like Vietnam are estimated at US\$ 1.075 million per year and in the Red River Delta of Northern Vietnam about 100,000 rural households have been planting their potato by using true potato seeds (TPS) over the past seven years (Anonymous, 2001b).

Micro and mini tuber production in tissue culture laboratory of Turkey. Kartarim is a segment of Kar Group

of Companies. It has got a well equipped and wide ranged biotechnology/tissue culture laboratory located in Aegean region, which was established some years back and has a huge potential for the quality production of potato seedlings and micro and mini tubers by means of various techniques of micro propagation. A bulk quantity of potato seedlings is being produced every year to meet the demand of potatoes as a raw material of crispy chips to Kar-Gida Industry. With the passage of time, the stock materials of potato lose their multiplication potential and reduce the capability of healthy seedlings production. To maintain the plant stock to be physiological active used for further multiplication and with the aim of more quality seedlings and to produce the micro and mini tubers of seed potatoes derived from tissue cultured plantlets, under the supervision of qualified team, a research and development section that is affiliated with greenhouses and highland area of Kozak, was set up in this laboratory in the mid 2000 where different experiments on seedlings and tubers production have given highly encouraging results. The establishment of research and development activities would, undoubtedly, enhance the local marketability and approaching the target of export promotion to predict the future prosperity in the area of potato tuber production.

Research work. Research work have been started for the production of micro or mini tubers, the source of explants and the type of media are extremely important for growth and development of the plantlets. The results indicated that modified MS media supplemented with different vitamins enhanced the tissue cultured seedlings (Bhojwani & Razdan, 1996). The growth pattern of some particular plant materials remained prominent than the others. The age of seedlings/plantlets at the time of inoculation in the new media is a useful consideration for good quality seedlings and the subsequent production of tubers.

For microtubularization, the standard procedures of tissue culture were adopted and the inoculants in the jars were placed on the oscillator in the growth chambers for root shoot growth and subsequently the tubers formation, depending on the use of specific MS media (George, 1996; Bhojwani & Razdan, 1996).

Results of the above experiment indicated a great difference of weight and volume of tubers formed (Table I). Such variations may be attributed to genetic diversity found amongst a large number of potato cultivars.

Table I. Number and weight of micro tubers grown in Kartarim tissue culture laboratory

Variety code	Replications	Number of tubers/node	Single tuber weight (g)
KAR-01-1	5	1.8	0.17
KAR-01-9	5	1.7	0.14
KAR-04	5	1.9	0.18

Selection and screening of potato seedlings in the growth chamber are legal and valid criteria for mini tuber production in the greenhouse. This method allows keeping

Table II. Effect of seedlings selection on the yield and quality of mini tubers of different potato cultivars codes

Parameter		*Variety codes									
		1	2	3	4	5	6	7	8	9	10
Weight (g)/minituber	Selected	1.7	1.1	1.3	2.5	3.0	2.2	1.2	1.7	2.0	1.2
	Control	0.9	1.1	1.1	1.3	1.5	1.6	0.6	1.2	1.1	0.75
Size (mm)/minituber	Selected	11	10.5	11.5	15.5	16	16	11.5	14	16	12.5
	Control	10.5	11	10.8	13	13	14	8.5	13	12.5	11

*1= KAR 01-1, 2= KAR 01-14, 3= KAR 01-15, 4= KAR 02, 5= KAR 04, 6= KAR 06, 7= KAR 07, 8= KAR 08, 9= KAR 09, 10= KAR 11

Table III. Effect of different growing media on the yield and quality of mini tubers of the potato

Parameter	Growing media							
	1	2	3	4	5	6	7	8
Single tuber wt. (g)	2.60	1.30	3.25	1.75	1.65	2.20	2.70	2.90
Stem dia (mm)	16.20	12.50	17.50	14.00	14.00	17.00	17.00	16.00
Total tubers wt./plant (g)	52.50	23.40	63.80	38.60	28.10	54.10	39.80	62.70

1: 1/3 perlite+1/3 sand+1/3 loam soil; 2: 1/2 loam soil+1/4 sand+1/2 pine bark; 3: 3/4 sand+1/4 loam soil; 4: 1/2perlite+1/4 sand+1/4 loam soil; 5: 2/3 perlite+1/4 loam soil; 6: 1/4 perlite+1/4 sand+1/2 loam soil; 7: 1/4 sand+3/4 loam soil; 8: 1/2sand+1/2 loam soil

the ultimate crop homogenized. Useful step in this method is the direct regeneration of potato seedlings than protoplast or callus culture. The successful selected seedlings of potato (3-4 weeks older) were grown for mini tuberization through bag culture technique in the greenhouse.

The results pertinent to quality and yield of mini tubers are given in the Table II. The result has indicated that selected seedlings had exhibited superceding performance over the control in terms of weight and size. The cultivar of code KAR 04, both in weight and size excelled at the top compared with the control.

The data on the use of various growing media in the bag had shown significant results (Table III). As a whole, perlite mixed with sand and loam gave encouraging outcome for tuber yield and quality. However, the mixture of $\frac{3}{4}$ sand + $\frac{1}{4}$ loam soil gave the single tuber weight (3.25g) and total tuber weight (63.8 g), which was the highest compared with the rest of the media. These results are in agreement with the findings of Butt and Varis (2000) and Solomon and Mosley (2000).

Protocol for Minutubers in the Field. The vigor of seed potatoes is greatly reduced in the plan with low altitude. At colder and higher lands, little viral infection is experienced which is the major problem in tuber production of potatoes.

In the present study, the field trials were conducted in the area of Izmir city (referred as Kozak which is 639 meter high) (Anonymous, 2001c). The seedlings (plantlets) developed in the *in vitro* are transferred in the greenhouse round the year. From winter to spring period, these seedlings of different cultivars used as source materials of mini tubers being produced in the greenhouse through bag culture technique (Butt & Varis, 2000). During summer season, however, the seedlings developed by tissue culture are kept in the greenhouse for acclimatization by protecting them with permeable cotton sheet at low height and providing frequent water sprays to reduce the temperature and to increase the humidity. The acclimatized potato seedlings then shifted after 15-20 days by covered loader to

Kozak area for plantation. Seedlings planted in the field during early days to reduce the risk of wilting due to high temperature. The mini tubers produced in the greenhouse also sown in the field in summer period to get super-elite and subsequent generations of elite seeds of potato (Haverkort, 1981). The plants to be grown in the field and suspected to various diseases (including bacterial, fungal and viruses of different strains) roughed out when and where require. Irrigation depends on the weather conditions.

CONCLUSION

A series of experiments relating to the quality seed potatoes of different cultivars in response to selected plantlets in the *in vitro* and the selected mini tubers in the greenhouse being conducted in the *in vivo* at Kozak area of Izmir, Turkey. It can be concluded that a real improvement for getting true seed potatoes may be possible by using appropriate proportion of various chemicals and phytohormones and by culturing meristem and apical shoot culture, and also selection, regeneration and short way cloning of *in vitro* stock to be multiplied for the production of tubers. In order to develop a sound seed programme, and ensure its continued growth and strength, a continuing, reliable source of high quality basic seed stocks is essential. In this way ample supply of established cultivars as well as the introduction of disease-tested new cultivars could be manipulated. For this purpose, ELISA test, for the identification and eradication of pathogens particularly the viruses, are enormously important. This is because production practices have been based on sound seed production and pathological principles with emphasis on the elimination as well as the control of tubers-borne diseases and diseased plants were eliminated or following detection of virus diseases using specific serological test.

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