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Micro-tuber production behaviour of some commercially important potato (*Solanum tuberosum*) cultivars

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ABSTRACT

Micro-tuber production behaviour of six commercially important cultivars of potato (*Solanum tuberosum* L.) was studied under standard medium and culture conditions using ten double node cuttings per flask. Significant differences were observed among the different potato genotypes for most of the characters during *in vitro* tuberization. Per cent micro-tuberization and number of stolons per nodal cutting were found to be maximum in Kufri Badshah and minimum in Kufri Pukhraj. Shoots weight/flask was maximum in Kufri Anand, followed by Kufri Badshah and Kufri Pukhraj, whereas, roots weight was higher in Kufri Badshah and minimum in Kufri Surya. The number of micro-tubers/flask was maximum (14.0) in Kufri Anand, which was almost same and statistically at par with three other varieties, viz Kufri Badshah, Kufri Bahar and Kufri Chipsona 1. Micro-tubers were found to be minimum in Kufri Pukhraj (7.5 tubers/flask). Total yield of micro-tubers/flask and harvest index were also maximum in Kufri Anand (3.7g and 0.29 respectively). Average weight of micro-tubers was maximum (0.27g) in Kufri Anand, whereas, tubers were of lighter weight in Kufri Surya (0.114g). The dry matter content of freshly harvested micro-tubers was maximum in Kufri Chipsona 1(19.65%) and minimum in Kufri Anand (14.75%). The proportions of normal shaped micro-tubers were significantly higher in Kufri Badshah (99%), at par with Kufri Chipsona 1, Kufri Bahar, Kufri Anand and minimum in Kufri Pukhraj (79.2%). The proportion of micro-tubers with burst lenticels was maximum in Kufri Pukhraj (43.5%) and minimum in Kufri Badshah (0.2%).

Key words: Burst lenticels, Cultivar, *in vitro*, Micro-tubers, Stolons, Tuberization

Seed potatoes must be grown in areas where virus transmission is minimal to prevent their spread to the following generations through tubers. In countries without a cold winter, the production of seed potato is difficult because of high disease pressure throughout the year. As a result, these countries (mainly tropical and sub-tropical except India) import much of their seed tubers from regions with better climatic conditions for seed potato production. However, pathogen tested seed tubers are generally expensive and account for up to 50% of the total production cost (van der Zaag and Horton 1983).

In India, traditionally, seed potato is produced by repeated clonal multiplications of initial disease-free tubers, which suffers from low multiplication rate and progressive accumulation of degenerative viral diseases (Naik *et al*. 2000).

In India, one major cause of low potato (*Solanum tuberosum* L.) productivity (19 tonnes/ha) is use of poor quality seed (Singh 2003) as degenerated seed is known to lower productivity up to 40% (Salazar 1996). Availability of quality seed is a major constraint in potato production and thus farmers are often forced to use locally grown seed despite severe yield losses. At present the state and central seed production agencies of the country are able to meet only 20–25% requirement of quality seed (Kumar *et al.* 2007). For bridging this wide gap, large-scale integration of conventional and innovative methods like micro-propagation at commercial level is needed for producing enough quantity of healthy seed tubers in minimum period of time (Pandey 2006). Seed potato production involving *in vitro* asexual multiplication allows quick and round-the-year production of disease-free good quality seed and thus is a way out to supplement the ever high requirement of quality seed. *In vitro* produced disease-free potato clones combined with conventional multiplication methods have become an integral part of seed production in many countries including India (Naik *et al.* 2000). CPRI, the nodal agency in the production of breeder seed of potato in India has been switching over for its 100% production through micro-propagation.

Two alternative propagule types of potatoes can be

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produced from micro-culture; transplants derived from shoot cultures and micro-tubers. The general objective of production and use of micro-tubers and mini-tubers is to improve the health status of conventional seed potatoes by reducing the number of field multiplications (Haverkort *et al.* 1991). Transplants cannot be stored and are sensitive to stress. Micro-tubers have the advantage of transporting over long distances as they do not dry out as readily as plantlet cultures and withstand handling better than plantlets (Seabrook and Coleman 1988). Unlike micro-propagated plantlets, micro-tubers do not require time consuming hardening periods in green houses and can be handled much like normal seed tubers. These have the potential in using for field planting as the fresh tuber yields from micro-tuber plants were 82% that of conventional tuber plants (Kawakami *et al.* 2004).

Prevalence of diseases like brown rot coupled with non-availability of aphid-free period makes the entire region of north-eastern India unfit for seed potato production. Lack of healthy seed potatoes is mainly responsible for the poor potato productivity in the region. Under such circumstances, the use of potato micro-tubers can be a potential alternative to conventional imported seed because they are typically pathogen tested and can be produced in any region under controlled conditions.

Besides rapid multiplication for pre-basic potato seed, micro-tubers also play an important role in germplasm exchange. With the adoption of *in vitro* plantlets for international exchange of potato germplasm, Central Potato Research Institute of India is also receiving *in vitro* forms of potato germplasm from different countries necessitating the production of sufficient quantities of the tubers in short time for field evaluation (Chandra *et al.* 1992a).

Different genotypes have been reported to perform differently during micro-tuberization under same or different culture conditions. Significant interaction of cultivars with culture media, hormones and their concentrations had been reported by many workers (Maroti *et al.* 1980, Miller *et al.* 1985, Mitten *et al.* 1988, Caligari and Powell 1989). Chandra *et al.* (1992a, b), Naik and Chandra (1994a, b) and Singh *et al.* (2001) have also reported the significant differences among the potato cultivars during *in vitro* growth, micro-tuberization and/or during *in vivo* performance of micro-tubers.

Keeping in view the role micro-tuber can play in improving the availability of healthy seed potatoes in areas unsuitable for seed production primarily, the present study was undertaken to know the micro-tuber production behaviour of some commercially important potato cultivars of Indo-Gangetic plains. Such information will be of great help for the advance planning to fulfill the targeted production of potatoes.

MATERIALS AND METHODS

Disease-free plantlets of six potato varieties of Indo-Gangetic plains (Kufri Anand, Kufri Badshah, Kufri Bahar, Kufri Chipsona 1, Kufri Pukhraj and Kufri Surya) maintained and multiplied through shoot cuttings under micro-propagation (16/8 hr photo period) were used as ex-plant source for micro-tuberization (Sarkar *et al*.1997). For micro-tuberization, 10 double node segments were placed in 250ml capacity conical flask containing 25ml of MS liquid media (except for gelling agent) under aseptic conditions. These flasks were closed with cotton plugs and kept in the culture room under 16 hr photo period of 3 000–4 000 lux light using 40watts standard florescent tubes at day and night temperatures of 22$\pm$2^{o}C for 25–28 days until sufficient multi-nodal shoots were developed for mass- tuberization.

The experiment was conducted at Central Potato Research Institute, Shimla, during 2007 and 2008; in completely randomized block design (CRD) with four replications in each treatment (genotype). Each replication consisted of 3-flasks. However, for observations related to per cent micro-tuberization and number of stolons/nodal cutting, extra flasks were prepared for all genotypes. The four replications were placed randomly in four racks/benches of the same tissue culture castor stack representing four locations of height in culture room.

After 28 days, the flasks were taken out from culture room. Under sterile conditions on the bench of laminar flow, the unused medium in the flasks was taken out and 35ml of tuber induction medium was added for tuberization. The induction–medium consisted of MS basal salts and vitamins supplemented with 10mg/l BAP and 8% sugar at *p*H 5.8. The tubers were induced by incubating the cultures under continuous darkness at 16 $\pm$ 1^{0}C for 70 days. Before harvesting, greening of micro-tubers was done by keeping flasks under 16 hr photo period at 22$\pm$1^{o}C for one week. At the time of harvest all the tubers were harvested and the total number and total weight of micro-tubers/flask was recorded. The harvest of different flasks was bulked replication wise before grading of micro-tubers into three grades, viz Large (>300mg), medium (100-300mg) and small (<100mg). Micro-tubers were rinsed with water to remove any excess sugars and salts, and shade dried on soft sterile tissue paper for 24 hr.

Data were collected on the growth parameters like per cent micro-tuberization of nodal cuttings, number of stolons/ cutting, fresh mass of roots, shoots and total fresh bio-mass (including micro-tubers)/flask. Regarding the micro-tuber production behaviour, data were collected on the total number and total yield of micro-tubers, maximum and minimum weight of micro-tubers and average weight of micro-tuber/flask. Also the data was collected on harvest index, percent dry matter and grade-wise number and yield of micro-tubers.

The average of two years data was analyzed statistically in CRD design by applying the technique of analysis of variance (ANOVA) as described by Gomez and Gomez (1984). Mean values were calculated and separated using F-test at 5% level of significance.

RESULTS AND DISCUSSION

Micro-tuberization and number of stolons

Per cent micro-tuberization in nodal cuttings during *in vitro* tuberization reveals significant differences among potato cultivars. It was maximum in Kufri Badshah, closely followed by Kufri Anand and was minimum in Kufri Pukhraj (Table 1). The differences in the number of stolons per nodal cutting among the cultivars were also significant with maximum number of stolons in Kufri Badshah and minimum in Kufri Pukhraj (Table 1). Such differences in the micro-tuberization and number of stolons can be attributed to the differences in the genotypic make up of different potato varieties as well as to the specific response of such genotypes to medium and culture conditions during *in vitro* tuberization. Varietal differences in the rate of tuberization *in vitro* have already been reported to occur (Sharma *et al.* 2005, Levy *et al*. 1994, Ranalli *et al*. 1994).

Fresh weight of shoots and roots

The weight of shoot and root portion (including stolons) developed during *in vitro* tuberization points out significant differences among different cultivars (Table 1). The shoots weight was maximum in Kufri Anand, followed by Kufri Badshah and minimum in Kufri Pukhraj. However, weight of root portion was recorded to be maximum in Kufri Badshah and minimum in Kufri Surya (Table 1). Higher shoot weight in Kufri Anand, followed by Kufri Badshah and more roots weight in Kufri Badshah reflects better utilization of nutrients in liquid media by both the genotypes with respect to shoot and/or root development, whereas, minimum shoot weight in Kufri Pukhraj and roots weight in Kufri Surya are indicative of poor response of these genotypes to the nutrient sources in media. Variable response among potato genotypes for the utilization of medium components has already been reported to exist (Sarkar and Naik 1997, 1998, Sharma *et al.* 2005, Mitten *et al.* 1988).

Total biomass

Total fresh biomass of different potato cultivars during *in vitro* tuberization was significantly more in Kufri Badshah, at par with Kufri Anand and minimum in Kufri Surya (Table 1). The total biomass obtained is a reflection of the variations in the weights of shoots, roots and of micro-tubers of the different genotypes as observed in the present study.

Number and yield of micro-tubers/flask

Total number and yield of micro-tubers (Mts)/flask reveals significant differences among cultivars (Table 2). Number of micro-tubers/flask was almost same and at par with four out of the six varieties evaluated, whereas; it was significantly low in Kufri Pukhraj. The total yield of micro-tubers was significantly more in Kufri Anand (3.7 g) and minimum in Kufri Surya (1.2 g) which was also at par with Kufri Pukhraj (Table 2).

Table 1 Growth behaviour of nodal cuttings during *in vitro* culturing for micro tuberization

Variety	% micro-tuberi-zation	No. of stolons/ NC	Wt. of shoots (g)/flask	Wt. of root portion (g)/flask	Total biomass/ flask (g)
Kufri Anand	61.9	2.5	3.0	6.2	12.9
Kufri Badshah	63.3	2.6	2.8	7.9	13.2
Kufri Bahar	58.7	2.1	1.6	6.1	9.8
Kufri Chipsona-1	53.4	2.1	1.9	6.1	9.8
Kufri Pukhraj	40.2	1.6	1.3	5.8	8.3
Kufri Surya	50.9	2.0	1.5	4.0	6.8
Mean	54.7	2.2	2.0	6.0	10.1
CD (*P*=0.05)	2.6	0.6	0.3	0.6	0.8

Wt. = weight

Almost same number of micro-tubers per flask obtained in four out of six potato cultivars reveals that the micro-tuber (Mt) production potential of these genotypes is almost same than others, viz Kufri Surya and Kufri Pukhraj.

Significant differences among genotypes for the total yield of micro-tubers per flask indicate that every genotype has a different yielding potential under similar medium and culture conditions. Higher yield recorded in Kufri Anand and minimum in Kufri Pukhraj can be attributed to the corresponding micro-tuberization potential of these varieties as observed in the present study.

Maximum, minimum and average weight of micro-tuber/ flask

Significant differences were observed in the weight of the largest and smallest micro-tuber produced by different genotypes. Weight of largest micro-tuber was significantly higher (0.68g) in Kufri Anand, closely followed and at par with Kufri Bahar (Table 2). Among the cultivars, size of the largest micro-tubers was minimum in Kufri Chipsona 1 (0.47g), which was also at par with three other varieties, viz Kufri Badshah, Kufri Pukhraj and Kufri Surya. However, the

Table 2 Production potentiality of different potato cultivars for *in vitro* tuberization

Variety	Total no. of micro-tubers/ flask	Total yield of micro-tubers/ flask (g)	Maxi-mum wt. of micro-tubers/ flask (g)	Mini mum wt. of micro-tubers/ flask (g)	Av. weight of micro-tubers/ flask(g)
Kufri Anand	14.0	3.7	0.68	0.030	0.265
Kufri Badshah	13.9	2.4	0.53	0.045	0.173
Kufri Bahar	12.5	2.2	0.61	0.075	0.173
Kufri Chipsona-1	12.6	1.7	0.47	0.025	0.138
Kufri Pukhraj	7.5	1.3	0.51	0.053	0.170
Kufri Surya	10.7	1.2	0.49	0.022	0.114
Mean	11.9	2.1	0.55	0.042	0.172
CD (*P*=0.05)	2.0	0.3	0.08	0.037	0.022

weight of the smallest micro-tuber was minimum in Kufri Surya and maximum in Kufri Bahar, while, the average weight of micro-tuber/flask was maximum in Kufri Anand and minimum in Kufri Surya (Table 2).

Higher average weights of micro-tubers recorded in Kufri Anand shows the potential of this genotype to produce large micro-tubers and may be attributed to comparatively higher shoot growth and thus synthesis of more food material than other varieties. Ranalli *et al.* (1994) have also reported that the micro-tuber size is affected by the genotype, while, Seabrook *et al.* (1993) have reported that the response of potato varieties to the presence of leaves on nodal cuttings varies for the size of micro-tubers obtained. Getting lighter micro-tubers in Kufri Chipsona 1 and Kufri Surya shows the tendency of these cultivars to produce good number of micro-tubers but of lighter weight and the same can be attributed to the comparatively less shoot development in these genotypes than Kufri Anand. Garner and Blake (1989) have also cited the reason for small size of micro-tubers to the corresponding reduced shoot growth *in vitro*. Such findings indicate the necessity of genotype specific modification of the medium for getting higher shoot growth and thus more number of micro-tubers with better size.

Dry matter in fresh micro-tubers and harvest index

The dry matter content of freshly harvested micro-tubers was found to be significantly different among potato cultivars with maximum in Kufri Chipsona 1 (19.65%) and minimum (14.75%) in Kufri Anand (Table 3). Dry matter content of micro-tubers was generally less than the one found in the conventional tubers, however, it followed a more or less similar trend to the one found for conventionally grown tubers of these genotypes. Reduced proportions of dry matter in micro-tubers than conventional tubers as well as genetic heritability for dry matter of tubers produced *in vitro* have also been reported by Sharma *et al.* (2005).

Harvest index, an indicator of efficiency of a particular genotype to produce more yields with less vegetative growth was significantly different among the potato cultivars. Harvest index was found to be maximum in Kufri Anand (0.29), which was followed by Kufri Bahar and minimum (0.16) in Kufri Pukhraj (Table 3). It indicates that among the varieties evaluated, Kufri Anand is highly efficient for the production of micro-tubers than others while Kufri Pukhraj is least responsive. Variations in the harvest index for micro-tubers during *in vitro* tuberization of different potato varieties have been reported to exist (Sarkar and Naik 1998, Sharma *et al.* 2005).

Proportions of different grades of micro-tubers/flask

Proportions of large, medium and small micro-tubers obtained/flask during *in vitro* tuberization reveal significant differences among cultivars. The proportion of large (>300mg) micro-tubers was significantly higher in Kufri Anand (26.2%) and minimum in Kufri Chipsona 1 and Kufri Surya (Fig 1).

Table 3 Dry matter content of micro-tubers and harvest index for different varieties during *in vitro* tuberization

Variety	Dry matter (%)	Harvest index
Kufri Anand	14.75	0.29
Kufri Badshah	15.45	0.18
Kufri Bahar	17.38	0.22
Kufri Chipsona 1	19.65	0.18
Kufri Pukhraj	15.78	0.16
Kufri Surya	18.15	0.18
Mean	16.86	0.20
CD (*P*=0.05)	0.59	0.03

Proportions of medium (100-300mg) micro-tubers/flask were though higher in Kufri Anand (34.4%) but were closely followed and at par with four other varieties evaluated. Medium micro-tubers were minimum (25.0%) in Kufri Surya (Fig 1). Proportions of small (<100mg) micro-tubers were maximum in Kufri Surya (62.6%) and minimum in Kufri Anand (39.4%).

Significant variations in the proportions of different grades of micro-tubers among different cultivars are indicative of differential response of each and every genotype to the medium and culture conditions for the micro-tuber production and thus necessitate the requirement of genotype-specific growth mediums and culture conditions to harvest more number of tubers of better size.

Proportions of normal and elongated micro-tubers

The data on normal and elongated micro-tubers during *in vitro* tuberization (Table 4) suggest that the potato cultivars differed significantly with respect to shape of the micro-tubers produced. Significantly higher proportions of normal shaped and thus minimum of elongated micro-tubers (99% and 1.0% respectively) were recorded in Kufri Badshah and it were at par with Kufri Chipsona 1, Kufri Bahar and Kufri Anand in a descending order. On the other hand, minimum

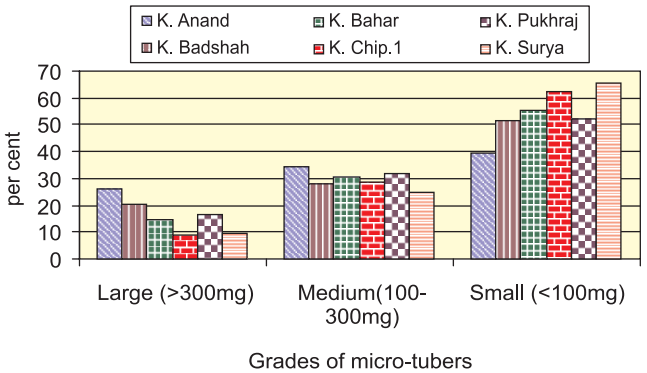

Fig 1 Proportions of different grades of micro-tubers/flask in different potato cultivars

Table 4 Proportions of normal and elongated shaped micro-tubers (Mts) in different varieties

Variety	Normal Mts/ flask (%)	Elongated Mts/flask (%)	Mts with burst lenticels (%)
Kufri Anand	95.1	4.9	27.8
Kufri Badshah	99.0	1.0	0.2
Kufri Bahar	96.9	3.1	11.8
Kufri Chipsona 1	97.5	2.5	26.7
Kufri Pukhraj	79.2	20.8	43.5
Kufri Surya	90.3	9.7	8.9
Mean	93.0	7.0	19.8
CD (*P*=0.05)	5.0	5.0	6.8

of normal shaped (79.2%) and maximum of elongated micro-tubers (20.8%) were found in Kufri Pukhraj (Table 4).

Significantly higher proportions of normal shaped micro-tubers in Kufri Badshah or others and minimum in Kufri Pukhraj again gives a reflection of the better suitability of culture conditions (including medium composition) for Kufri Badshah and others and poor suitability for Kufri Pukhraj.

Proportions of micro-tubers with burst lenticels

Proportions of micro-tubers with burst lenticels also reveal significant differences among different cultivars. Proportions of such micro-tubers were found to be significantly low in Kufri Badshah (0.2%) and maximum (43.5%) in Kufri Pukhraj (Table 4). Higher proportions of micro-tubers with burst lenticels in Kufri Pukhraj necessitate the need for modification of the induction medium for this particular variety to prevent the loss/ damage of already formed micro-tubers and thus to improve the overall quality.

Significant variations in the potentialities of different genotypes for the production of micro-tubers indicate the necessity of genotype specific modification of medium to improve the size of micro-tubers, especially in Kufri Surya and Kufri Chipsona 1. Attention needs to be paid for the improvement of medium (tuber induction) for Kufri Pukhraj to prevent the excessive lenticel bursting of its micro-tubers as well as for improving the rate of micro-tuberization of nodal segments.

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