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J. Gopal · J. L. Minocha · H. S. Dhaliwal

Microtuberization in potato (*Solanum tuberosum* L.)

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Abstract Twenty-two genotypes of potato (*Solanum tuberosum* L.) were induced to form microtubers under six in vitro culture conditions. Cultures maintained under a short photoperiod (10 h of 6–12 μmol m^{-2} s^{-1}) and low temperatures (day 20°±2°C and night 18°±2°C) had both a higher yield (255 mg/plantlet) and a greater number (2/plantlet) of microtubers than those maintained under long days (16 h of 38–50 μmol m^{-2} s^{-1}) combined with high temperatures (day 28°±2°C and night 25°±2°C) (yield 207 mg/plantlet; microtuber number, 0.9/plantlet), over a wide range of genotypes. After the plantlets had been cultured under long days for an initial period of 60 days, continuous darkness advanced microtuberization by 2–3 months in various genotypes. Under short-day and low-temperature conditions the addition of 6-benzylaminopurine increased microtuber yield from 255 mg/plantlet to 645 mg/plantlet and average microtuber weight from 115 mg to 364 mg. A similar pattern was observed under conditions of long days and high temperature, and continuous darkness and low-temperature. Microtubers produced under light had a greater number of eyes (maximum average: 5.96/microtuber) than those produced in the dark (maximum average: 3.50/plantlet). The genotype × cultural conditions interactions were significant indicating the importance of developing genotype-specific protocols to maximize microtuberization.

Key words Microtuberization · Number of eyes · *Solanum tuberosum* · In vitro culture

Abbreviations *BAP* 6-Benzylaminopurine · *MS* Murashige and Skoog (1962)

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J. Gopal (✉)
Division of Genetics, Central Potato Research Institute,
Shimla-171 001, HP, India

J. L. Minocha · H. S. Dhaliwal
Biotechnology Centre, Punjab Agricultural University,
Ludhiana-141001, Pb, India
Fax no.: 0177-224460
e-mail: cpri@x400.nicgw.nic.in
: jai_gopal@mail.excite.com

Introduction

The propagation of potato (*Solanum tubersoum* 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). 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). Most of these studies were based on a few genotypes only. 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 (Gopal et al. 1997). We report here the effect of photoperiod, light intensity, temperature, and BAP on yield, number and size of microtubers, and number of eyes per microtuber, in 22 genotypes.

Materials and methods

Plant material

Tuber samples of 22 genotypes of *Solanum tuberosum* L. were drawn from the National Potato Breeding Program at the Central Potato Re-

Table 1 In vitro treatments used for microtuberization

Treatment	Media	Incubation conditions
MT1A	40 ml of solid MS media with 8% sucrose, no hormones	16-h day (38–50 μmol m^{-2} s^{-1}; 28°±2°C); 8-h night (25°±2°C)
MT1B	40 ml of solid MS media with 8% sucrose, no hormones and after 60 days of culture, addition of 20 ml of liquid MS media with 8% sucrose and 10 mg/l BAP	——— do ———
MT2A	40 ml of solid MS media with 8% sucrose, no hormones	10-h day (6–12 μmol m^{-2} s^{-1}; 20°±2°C); 14-h night (18°±2°C)
MT2B	40 ml of solid MS media with 8% sucrose, no hormones and after 60 days of culture, addition of 20 ml of liquid MS media with 8% sucrose and 10 mg/l BAP	For initial 60 days: 16-h day (38–50 μmol m^{-2} s^{-1}; 28°±2°C) and 8-h night (25°±2°C) For remaining period: 10-h day (6–12 μmol m^{-2} s^{-1}; 20°±2°C) and 14-h night (18°±2°C)
MT3A	40 ml of solid MS media with 8% sucrose, no hormones	For initial 60 days: 16-h day (38–50 μmol m^{-2} s^{-1}; 28°±2°C) and 8-h night (25°±2°C) For remaining period: continuous dark (19°±2°C)
MT3B	40 ml of solid MS media with 8% sucrose, no hormones and after 60 days of culture, addition of 20 ml of liquid MS media with 8% sucrose and 10 mg/l BAP	——— do ———

search Institute, Shimla. Twenty of these, namely AB455, CP1710 ('Kerr Pondy'), CP2132 ('Tollocan'), E4451, F1277, JE812, JH222 ('Kufri Jawahar'), JN1501, JR465, JTH/C107, MS78-46, MS78-56, MS79-34, MS80-758, MS81-152, MS82-638, MS84-1169, PJ376 ('Kufri Ashoka'), RG1197, and SLB/K23, belong to *Solanum tuberosum* ssp. *tuberosum* and 2, EX/A680-16 and EX/A723, to *S. tuberosum* ssp. *andigena*. These have 38 diverse parents in their immediate pedigree, some with *S. demisum* as a distant ancestor. The material was thus of a wide genetic background.

Establishment of axenic cultures

Single nodal sections (0.5–1.0 cm long) cut from etiolated sprouts were disinfected with a mixture of 0.1% $HgCl_2$ and 0.1% sodium lauryl sulfate for 5 min, rinsed with sterile water thrice, and cultured aseptically in 150×25-mm test tubes, one segment per test tube. Basal and top nodal segments were not used (Le 1991). Each test tube contained 12–15 ml of semisolid (7 g l^{-1} agar) MS basal medium (Murashige and Skoog 1962) with 3% sucrose. The test tubes were closed with cotton plugs and incubated under a 16 h/day photoperiod of 38 μmol m^{-2} s^{-1} and under day and night temperatures of 28°±2°C and 25°±2°C, respectively.

Microtuberization

Six- to eight-week-old axenic plantlets were subcultured aseptically using single nodal segments (0.5–1.0 cm) from the central portion of the plantlets as explants and incubated under six different culture conditions for microtuberization (Table 1).

Experimental design

The experiment was conducted in a randomized complete block design (22 genotypes×six culture conditions) with four replications. Each replication consisted of two culture vessels (jam bottles of 300-ml capacity) with four nodal sections per jam bottle. Thus there were 32 plantlets per genotype per culture condition.

Data recording and statistical analysis

At the onset of foliage senescence (browning of leaves and/or stems), plantlets were taken out of the vessel, media sticking to the roots were rinsed off, and data were recorded for four characters, microtuber yield/plantlet (mg), number of microtubers/plantlet, average microtuber weight (mg), and number of eyes/microtuber. Abnormally slow-growing plantlets, if any, were not included in the data. Analysis of variance and means over genotypes were computed. The fixed-effect model was used as the objective of the study was to compare the effect of various culture conditions on microtuberization over a range of selected genotypes.

Results

All of the treatments employed induced the formation of microtubers (Fig. 1), but none of them in all 22 genotypes (Fig. 2). In treatments MT2A and MT2B, 2 genotypes, RG1197 and MS79-34, did not develop any microtubers; in treatment MT3A 3 genotypes, JN1501, RG1197, and EX/A723, did not produce any microtubers; and in treatment MT3B, 4 genotypes, RG1197, MS79-34, MS82-638, and JN1501 did not form any microtubers. Treatment MT1B was the least effective, i.e., 8 genotypes did not microtuberize, followed by MT1A in which 5 genotypes did not form any microtubers. Genotype MS82-638 microtuberized under all treaments, except in MT3B. RG1197 did

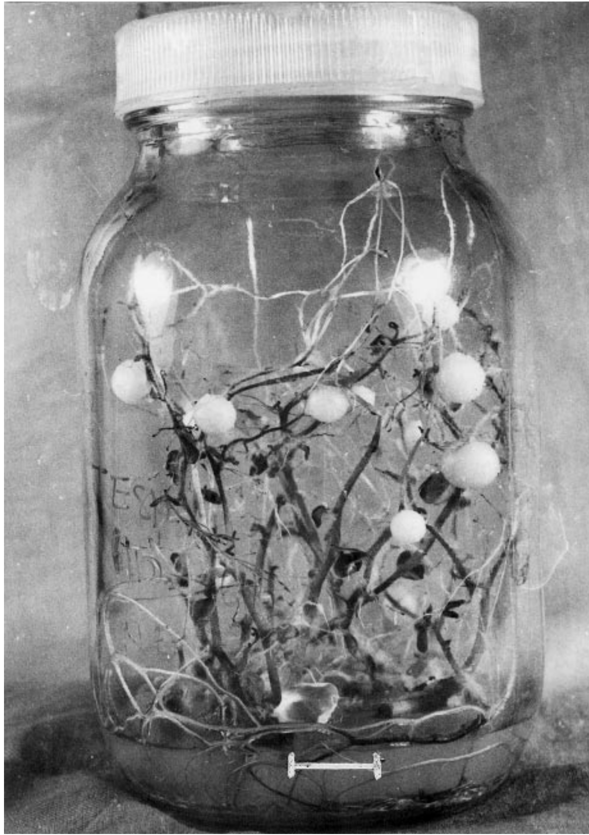

Fig. 1 Microtuberization in CP1710 ('Kerr Pondy') following treatment MT3A. *Bar:* 1.5 cm

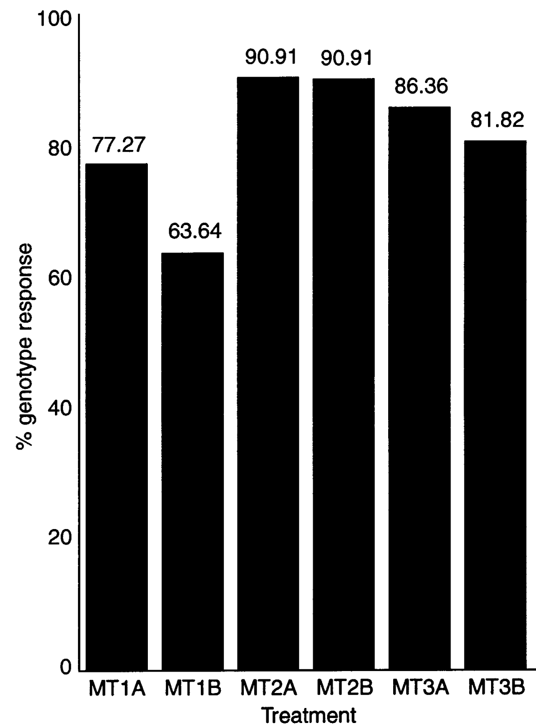

Fig. 2 Genotypes responded (in percentage) to various microtuberization treatments

not microtuberize in any of the treatments. There were only 12 genotypes which microtuberized under all conditions. Microtubers produced under light (treatments MT1A, MT1B, MT2A and MT2B) were green, and those produced in the dark (treatments MT3A and MT3B) were white.

Analysis of variance (Table 2) showed that mean squares due to genotypes, culture conditions, and their interactions were highly significant ($P<0.01$) for various characters. However, mean squares due to interactions were much lower than those of genotypes and culture conditions. Characters' means over 22 genotypes are given in Table 3.

Microtuber yield

A comparison of microtuber yield obtained by culture under three treatments without BAP, i.e., MT1A vs. MT2A vs. MT3A, indicated that MT2A (short days, low-light intensity, low temperature) was the most effective and MT1A (long days, high-light intensity, high temperature) was the least effective (Table 3). A similar comparison among three treatments with BAP, i.e., MT1B vs. MT2B vs. MT3B, indicated that MT2B was the most effective and MT1B was the least. There was considerable increase in microtuber yield in treatments with BAP, i.e., MT2B (average yield 645 mg/plantlet) and MT3B (average yield 414 mg/plantlet) over the corresponding treatments without BAP, i.e., MT2A (average yield 255 mg/plantlet) and MT3A (average yield 225 mg/plantlet). The trend was the same for MT1B (average yield 242 mg/plantlet) vs. MT1A (average yield 207 mg/plantlet), and the differences were significant.

Number of microtubers

The effect of various treatments on this character was similar to that for microtuber yield. Among non-BAP treatments, MT2A resulted in the maximium (2 microtubers/plantlet) and MT1A had the lowest (0.9 microtubers/plantlet) number of microtubers. Among BAP treatments, MT2B was the most effective (1.7 microtubers/plantlet) and MT1B, the least effective (0.9 microtubers/plantlet). However, unlike microtuber yield, the addition of BAP media did not affect the number of microtubers, except in MT2B in which number of microtubers was significantly lower than in MT2A (Table 3).

Average microtuber weight

A comparison among treatments without BAP indicated that MT1A produced the heaviest microtubers (average

Table 2 Analysis of variance of in vitro performance of 22 genotypes in different culture conditions

Character	Mean squares			
	Culture conditions	Genotypes	Interaction	Error
Microtuber yield (mg per plantlet)	2307676**	1124242**	254522**	5332
Microtuber number (per plantlet)	7.76**	8.04**	2.06**	0.07
Average microtuber weight (mg)	694090**	398139**	81835**	2693
Number of eyes (per microtuber)	50.38**	23.54**	5.80**	0.71
df [a]	5	21	105	393

** Significant at P<0.01
[a] *df* of number of eyes were 5, 11, 55 and 213, respectively, as data were available for only 12 genotypes

Table 3 Means over genotypes for various characters in different in vitro culture conditions

Character	Treatment						CD (5%) [a]
	MT1A	MT1B	MT2A	MT2B	MT3A	MT3B	
Microtuber yield (mg per plantlet)	207.49	241.78	254.91	644.84	225.39	414.49	21.85
Microtuber number (per plantlet)	0.91	0.91	2.05	1.69	1.28	1.33	0.08
Average microtuber weight (mg)	226.33	263.42	114.69	363.76	156.58	260.39	15.53
Number of eyes [b] (per microtuber)	5.96	4.75	4.69	5.56	3.50	3.48	0.34

[a] CD, Critical difference=SE×*t* (0.05)
[b] Average over 12 genotypes which tuberized in all the treatments

weight 226 mg) and MT2A the lightest (average weight 115 mg). Among treatments with BAP, MT2B produced the heaviest microtubers (average weight 364 mg), and the difference between the other two treatments, i.e., MT1B and MT3B, which produced smaller microtubers (average weight 263 mg and 260 mg, respectively), was non-significant. Comparison of microtuber weight between MT1A and MT1B, MT2A and MT2B, and MT3A and MT3B indicated that the addition of BAP media increased microtuber weight (Table 3).

Number of eyes

Microtubers produced in the dark (treatments MT3A and MT3B) had a significantly lower number of eyes (3.50 and 3.48 per microtuber, respectively) than those produced in light (treatments MT1A, MT1B, MT2A, and MT2B) (5.96, 4.75, 4.69, and 5.56 per microtuber, respectively). A comparison of long photoperiod (MT1A and MT1B) versus short photoperiod (MT2A and MT2B) did not depict any pattern with regard to their effect on this character. The effect of the addition of BAP media after 60 days varied from treatment to treatment, and there was no consistent trend.

Discussion

Our findings with regard to the effect of photoperiod on microtuberization agree with those of Wang and Hu (1982), Hussey and Stacey (1984), Ortiz-Montiel and Lozoya-Saldana (1987), and Garner and Blake (1989) who also found better microtuber induction when in vitro plantlets were incubated under short-days conditions. Also it is a well-established fact that under in vivo conditions tuberization in whole plants is hastened during short days and at low temperatures (Cutter 1978), particularly at low night temperatures. The desirable effect of low light intensity on microtuberization, as observed in the present study, was also reported by Wang and Hu (1982). Lower microtuber yield under continuous darkness in the present study may be attributed to etiolation. As compared to low light intensity, no further growth occurred under dark conditions (Gopal 1996).

The promotion of microtuberization on cultured shoots by cytokinin has been demonstrated by many workers (Palmer and Smith 1969; Wang and Hu 1982; Hussey and Stacey 1984; Estrada et al. 1986; Ortiz-Montiel and Lozoya-Saldana 1987). The present results show that the increase in microtuber yield upon supplementing the MS me-

dium (1962) with BAP (10 mg/l) following 60 days of culture on a non-BAP medium was mainly due to an increase in average microtuber weight as there was little change in microtuber number under the two conditions (Table 3). Our observation that the promotion of microtuberization by BAP was more pronounced under short days than under long days confirms the findings of Hussey and Stacey (1984). Our results also confirm the findings of Garner and Blake (1989) and Ranalli et al. (1994) who reported that microtubers could be induced even without the use of growth regulators. The use of media without growth regulators, would be desirable where the objective is to judge the innate capacity of genotypes to produce microtubers and to remove the possibility of any undesirable carry-over effect of growth regulators on morphology, dormancy or sprouting.

As the microtuber yield and average microtuber weight increased with the addition of BAP (Table 3), the number of genotypes responding to microtuberization decreased (Fig. 2). This observation indicates that BAP interferred with the innate capacity of the genotypes to initiate microtuberization, perhaps by disturbing the balance of endogenous levels of growth regulators. This effect was evident mainly in treatments with long days and high temperatures or in treatments where plantlets were shifted to total darkness after an initial exposure to long days and high temperatures for 60 days. Thus, it would be interesting to investigate the endogenous levels of various growth regulators in plantlets exposed to varying in vitro conditions.

In the microtuberization protocols used at the International Potato Centre, Lima, Peru (Estrada et al. 1986) and the Central Potato Research Institute, Shimla, India (Dhingra et al. 1992) plantlets are placed in the dark because light delays microtuberization (Dobranski and Mandi 1993). In the present study microtuberization was also faster under continuous darkness and was completed within 4–5 months after culture initiation in comparison to the 6–8 months taken by treatments with light. However, the number of eyes per microtuber was higher in microtubers induced in a light-dark photoperiod. There is no other report on this aspect. The faster rate of microtuberization and early senescence of the plantlets (Gopal 1996) may have resulted in fewer eyes being produced in microtubers cultured in the dark than those cultured in the light. The microtuber yield obtained (Table 3) and the subsequent performance of the crop raised from the microtubers (Gopal et al. 1997) suggest the desirability of inducing microtubers under short days of low light intensity.

In conclusion, it can be said that microtuberization can be induced in potato under a variety of culture conditions. A short photoperiod (10 h) of low light intensity (6–12 μmol m^{-2} s^{-1}) coupled with low temperatures (day 20°±2°C; night 18°±2°C) gives a wide genotypic response, high microtuber yield with more number of tubers; and this combined with media containing BAP results in a higher microtuber yield and larger microtubers. The significance of genotype × culture conditions interaction, however, indicates the need of developing genotype-specific protocols to maximize microtuberization.

References

Cutter EG (1978) Structure and development of the potato plant. In: Harris PM (ed) The potato crop. Chapman and Hall, London, pp 70–152

Dhingra MK, Naik PS, Chandra R, Randhawa GJ (1992) Tissue culture technique for potato health, conservation, micropropagation and improvement. Tech. Bull 39. Central Potato Research Institue, Shimla

Dobranszki J, Mandi M (1993) Induction of in vitro tuberization by short day period and dark treatment of potato shoots grown on hormone-free medium. Acta Biol Hung 44:411–420

Estrada R, Tover P, Dodds JH (1986) Induction of in vitro tubers in a broad range of potato genotypes. Plant Cell Tissue Org Cult 7:3–10

Garner N, Blake J (1989) The induction and development of potato microtubers in vitro on media free of growth regulating substances. Ann Bot 63:663–674

Gopal J (1996) In vitro selection, genetic divergence and cross prediction in potato. PhD thesis, Punjab Agricultural University, Ludhiana (Pb), India

Gopal J, Minocha JL, Sidhu JS (1997) Comparative performance of potato crops raised from microtubers induced in dark versus microtubers induced in light. Potato Res 40: 407–412

Hussey G, Stacey NJ (1981) In vitro propagation of potato (*Solanum tuberosum* L.). Ann Bot 48:787–796

Hussey G, Stacey NJ (1984) Factors affecting the formation of in vitro tubers of potato (*Solanum tuberosum* L.). Ann Bot 53: 565–578

Le CL (1991) Practical aspects of potato (*Solanum tuberosum* L.) micropropagation. Rev Suisse Agric 23:357–358

Murashige T, Skoog F (1962) A revised medium for rapid growth and bioassay with tobacco tissue cultures. Physiol Plant 15: 473–497

Ortiz-Montiel G, Lozoya-Saldana H (1987) Potato minitubers: technology validation in Mexico. Am Potato J 64:535–544

Palmer CE, Smith OE (1969) Cytokinins and tuber initiation in potato *Solanum tuberosum* L. Nature 221:279–280

Ranalli P, Bassi F, Ruaro G, Del Re P, Dicandilo M, Mandolino G (1994) Microtuber and minituber production and field performance compared with normal tubers. Potato Res 37:383–391

Roca WM, Bryan JE, Roca MR (1979) Tissue culture for international transfer of potato genetic resources. Am Potato J 56:1–10

Vecchio V, Andrenelli L, Pagano MT, Benedettelli S (1994) Influence of photoperiod and media culture on potato microtuber production and dormancy (Abstr). Potato Res 37:440

Wang P, Hu C (1982) In vitro mass tuberization and virus free seed potato production in Taiwan. Am Potato J 59:33–37