Plant Tissue Cult.& Biotech. **15**(2): 157-166, 2005 (December)

PTC&B

In vitro Microtuberisation in Potato Obtained from Diverse Sources

M. J. Hossain

Tuber Crops Research Centre, Bangladesh Agricultural Research Institute, Joydebpur, Gazipur-1701, Bangladesh

Key words: Microtuberisation, Potato, Cultivars

Abstract

Seventeen potato cultivars of six diverse sources have been evaluated for their response to *in vitro* microtuberisation in presence or absence of light. Tuber initiation appeared first in the light and gave higher number of microtubers with higher total as well as mean weight than the dark. Among the cultivars, on the average, Patrones performed best for most of the parameters such as, days to tuber initiation, number of tubers, their weight per plant and mean tuber weight. In terms of microtuber production potential, Chamak was the poorest. Among the different sources, the Dutch and Canadian cultivars showed comparatively better performance in respect of microtuber production potential than the others. The local high yielding varieties were the least productive. The weight of microtubers per tube (534.6 mg) and mean tuber weight (104.3 mg) were also significantly higher in the light compared to dark (264 mg/tube and 87.8 mg/tuber, respectively). Microtuberisation potential is a variety-dependent character, which is largely influenced by environmental factor.

Introduction

In vitro microtuberisation is a complex process regulated by different growth regulators like benzyl amino purine (Wang and Hu 1982; Hussey and Stacey 1984; Abbott and Belchel (1986), 2-chloroethyl trimethyl ammonium chloride (CCC) (Tovar et al. (1985), coumarin (Dodds et al. (1988), jasmonoid (Pelacho et al. (1993), etc. Mass microtuberisation of potato is performed in many laboratories (Paet and Zamora 1994; Baoquing et al. 1991). These laboratories have standardized a suitable system of their own by incorporating different factors like culture media, light, temperature, explant, etc. for maximizing microtuber production (Wang et al. 1994; Paet and Zamora 1994).

Researches carried out so far with the standard potato cultivars of the country (Hoque et al. 1996; Hossain and Sultana 1998; Zakaria 2003; Yeasmin 2005) indicated that the production of microtubers is influenced by the presence/absence of hormones in the culture medium, photoperiod and cultivars.

Light is an important factor for plant growth and induction of potato tuber usually occurred in dark. In tissue culture studies, *in vitro* tuberisation has been verified under both light and dark conditions (Sattelmacher and Marschner 1978; Maree 1981; Stallknecht and Farnsworth 1982a,1982b; Thieme and Pett 1982; Wang and Hu 1982; Schilde 1982). As such, there are varied statement on photoperiod requirements for microtuber production. As the tuberisation occurs in darkness in nature, it may be assumed that tuber induction could be better in darkness. But many researches concluded that low light intensity (100 - 500 lux) for a short period (8 h per day) was good for induction of microtuber (Lawrence and Barker 1973). While others observed faster microtuber growth in darkness. However, no report is available on microtuberisation of the materials used in this study.

The production of microtubers has got significant importance in many countries of the world. Many laboratories are producing microtubers commercially for their own national programmes as well as for export. Hyouk et al. (1994) mentioned that, South Korea is producing millions of microtubers annually, and concluded that, mass production system of potato microtuber can revolutionise the world potato agriculture in the near future. Microtubers are produced in China for the elite and progressive seed potato growers (Mou 1996, personal communication). He mentioned that, it is better to plant microtubers rather taking cuttings, which may cause increase of virus infection in mother plants. In Zhongshan city of Guangdong province in China, microtubers are planted in about 1300 ha of land in order to provide a strong support to the national seed potato programme (Jun et al. 1994). In the Philippines, the national breeding project on conservation and multiplication has a strong microtuber production programme in order to produce millions of minitubers every year (Paet and Zamora 1994). Baoquing et al. (1991) harvested at least ten usable size microtubers per flask after 30 days of culture. They tried to give a picture of a large scale and economic production of microtubers for the national programme with a comprehensive research outline. The National Seed Potato Production Programme of Nepal made a success using microtubers. Even they were trying to export microtubers in neighbouring countries (Wattimena personal communication, 1992). In USA, some commercial laboratories are producing millions of microtubers, which are sold @15 cent each, while in Indonesia and China it costs only 5 cent and 1.06 cent, respectively due to cheap labour cost (Baoquing et al. 1991).

Tissue culture laboratory of Tuber Crops Research Centre (TCRC), Bangladesh has been maintaining a large number of potato genotypes including standard cultivars, TPS parental lines and promising germplasm lines aseptically. This laboratory produced *in vitro* microplants and microtubers of the

standard cultivars for the national seed potato production programme. Preliminary studies on microtuberisation showed that the potato cultivars differed widely in this respect i.e. the cultivar Patrones produced about 11 tubers per plant as against one - two for Heera, Dheera or Chamak (Hossain and Sultana 1994). Thus, the present experiment was aimed at investigating microtuberisation over a wide range of potato sources.

Materials and Methods

Seventeen potato cultivars from six diverse sources such as, the Dutch cultivars (Cardinal, Diamant, Multa and Ptarones), Canadian cultivars (Atlantic and Kennebec), local high yielding varieties, namely Heera, Dheera and Chamak, indigenous cultivars (Lalshil and Lalpakri), promising germplasm lines (P-315 and P-430) and true potato parental lines (TPS-25, TPS-2, TPS-67 and TPS-13) were used in this experiment.

The cvs. Atlantic and Kennebec; and TPS parental lines were received from Feredicton Research Station, Canada and International Potato Centre, Lima, Peru, respectively as *in vitro* microplants. The *in vitro* microplants of the other materials have been developed in the TCRC's laboratory following a meristem culture method as described by Hossain (1987). All the materials were subcultured at 20 days interval via nodal cuttings as described by Espinoza et al. (1985). The cultures were incubated at 3 klux florescent light intensity for 16 h daily and at $22 \pm 2°C$.

Organic salts of MS supplemented with 5 mg/l BAP, 500 mg/l CCC and 8% sucrose were used. The pH of the media was adjusted to 5.8 before autoclaving. Tuberisation media (10 ml) were poured into each of 150 mm × 25 mm tube containing three well developed and rooted microplants. The cultures were incubated at $18 \pm 2°C$ for a period of eight weeks. Fifty per cent cultures were incubated at total darkness and the rest 50% at 16 h 3 klux florescence light daily. The experiment was laid in a two factors (cultivar × light/dark) complete randomised design (CRD) with four replications. Mean of sources were taken from cultivars which were again analysed as sources × light/dark factors following a CRD. Five cultures were included in each replication. In total, 680 ($17 \times 2 \times 4 \times 5$) tubes were cultured. The experiment was repeated thrice. After eight weeks, the developed microtubers were harvested inside the clean bench and weighed in a mettler P-163 electric balance. The data on different parameters were collected and statistically analysed. The mean separation was done by LSD.

Results and Discussion

The response of 17 potato genotypes to *in vitro* microtuberisation condition in the absence or presence of light is presented in Tables 1 and 2.Genotypes under

Table 1. Production of microtubers of 17 potato genotypes with MS salts supplemented with 5 mg/l BAP and 500 mg/l CCC in presence of light.

Sucrose	Genotype	Days to tuber initiation		No. of tubers/tube		Wt. of tuber/ tube (mg.)	Mean tuber wt. (mg)	
		Range	Average	Range	Average		Range	Average
Dutch	Cardinal	12 - 17	14.5	5 - 8	6.5	815	61 - 189	125.0
	Diamant	16 - 18	17.0	5 - 9	7.0	657	48 - 138	93.0
	Mu1ta	14 - 17	15.5	4 - 8	6.0	606	55 - 149	102.0
	Patrones	12 - 16	14.0	8 - 11	9.5	939	42 - 192	117.0
Canadian	Atlantic	14 - 17	15.5	4 - 6	5.0	560	55 - 168	111.5
	Kennebec	12 - 18	15.0	5 - 7	6.0	680	49 - 176	112.5
Local	Heera	17 - 25	22.0	2 - 3	2.5	241	59 - 136	97.5
HYV	Dheera	15 - 23	19.0	2 - 3	2.5	287	49 - 182	115.5
	Chamak	20 - 25	22.5	1 - 2	1.5	170	63 - 161	112.0
Indigenous	La1shil	14 - 20	17.0	4 - 7	5.5	598	48 - 169	108.5
	La1pakri	14 - 18	16.0	7 - 8	7.5	591	57 - 142	99.5
Promising	P-315	12 - 18	15.0	3 - 7	5.0	492	63 - 136	99.5
germplasm	P-430	12 - 19	15.5	4 - 7	5.5	516	57 - 129	93.0
TPS	TPS-25	14 - 18	16.0	3 - 6	4.5	427	53 - 136	94.5
	TPS-2	14 - 22	18.0	4 - 7	5.5	516	43 - 144	93.5
	TPS-67	15 - 18	16.5	4 - 7	5.5	492	49 - 132	90.5
	TPS-13	13 - 20	16.5	3 - 8	5.5	573	51 - 158	104.5
Lsd 1%		-	-	-	1.86	21.39	-	4.98

Each figure is the mean of 20 tubes. Each tube containedthree microplants.

Table 2. Production of microtubers of 17 potato genotypes with MS salts supplemented with 5 mg/l BAP and 500 mg/l CCC in absence of light.

Sucrose	Genotype	Days to tuber initiation		No. of tubers/tube		Wt. of tuber/ tube (mg.)	Mean tuber wt. (mg)	
		Range	Average	Range	Average		Range	Average
Dutch	Cardinal	10 - 14	12.0	2 - 4	3.0	295	55 - 132	93.5
	Diamant	12 - 17	14.5	2 - 5	3.5	332	42 - 146	94.0
	Multa	12 - 15	13.5	3 - 5	4.0	379	47 - 139	93.0
	Patrones	8 - 12	10.0	3 - 5	4.0	407	33 - 168	100.5
Canadian	Atlantic	12 - 18	15.0	2 - 4	3.0	265	39 - 136	87.5
	Kennebec	9 - 17	13.0	3 - 5	4.0	392	41 - 155	98.0
Local HYV	Heera	15 - 22	18.5	1 - 2	1.5	116	62 - 101	81.5
	Dheera	14 - 22	18.0	1 - 2	1.5	155	47 - 162	104.5
	Chamak	17 - 24	20.5	0 - 1	0.5	103	69 - 138	103.5
Indige-nous	Lalshil	14 - 16	15.0	2 - 4	3.0	261	35 - 136	85.5
	Lalpakri	11 - 15	13.0	3 - 5	4.0	256	36 - 97	66.5
Promising	P-315	9 - 14	11.5	1 - 4	2.5	207	58 - 105	81.0
germplasm	P-430	12 - 16	14.0	3 - 4	3.5	283	41 - 119	80.0
TPS	TPS-25	12 - 15	13.5	2 - 5	3.5	234	38 - 116	77.0
	TPS-2	15 - 17	16.0	3 - 5	4.0	319	29 - 132	80.5
	TPS-67	10 - 17	13.5	2 - 4	3.0	289	62 - 129	95.5
	TPS-13	10 - 13	11.5	2 - 4	3.0	271	55 - 122	88.5
Lsd 1 %		-	2.09	-	0.98	18.69	-	8.12

Table 3. Response of potato genotypes to microtuberisation in presence and absence of light.

Sucrose	Days to tuber initiation		No. of tubers/tube		Wt. of tuber/tube (mg)	Mean tuber wt. (mg)	
	Range	Average	Range	Average		Range	Average
Dark							
Dutch	10.5 - 14.5	12.5	2.5 - 4.8	3.6	353.3	44.3 - 146.3	95.3
Canadian	10.5 - 17.5	14.0	2.5 - 4.5	3.5	328.5	40.0 - 145.5	92.8
Local	15.3 - 22.7	19.0	0.7 - 1.7	1.2	124.8	59.3 - 133.7	96.5
Indigenous	12.5 - 15.5	14.0	2.5 - 4.5	3.5	258.5	35.5 - 116.5	76.0
Promising germplasm	10.5 - 15.0	12.3	2.0 - 4.0	3.0	245.0	49.5 - 112.0	80.8
TPS	11.8 - 15.5	13.6	2.3 - 4.5	3.4	278.3	41.0 - 124.8	85.4
Mean	11.9 - 16.8	14.2	2.1 - 4.0	3.3	264.7	44.9 - 129.8	87.8
LSD 1 %	-	2.14	-	Ns	11.36	-	5.36
Light							
Dutch	13.5 - 17.0	15.4	5.5 - 9.0	7.3	754.3	51.5 - 167.0	109.3
Canadian	13.0 - 17.5	15.3	4.5 - 6.5	5.5	620.0	52.0 - 172.0	112.0
Local	17.3 - 24.3	21.2	1.7 - 2.7	2.2	232.7	57.0 - 159.7	108.3
Indigenous	14.0 - 19.0	16.5	5.5 - 7.5	6.5	594.5	52.5 - 155.5	104.0
Promising germplasm	12.0 - 18.5	15.3	3.5 - 7.0	5.3	504.0	60.0 - 132.5	96.3
TPS	14.0 - 19.5	16.8	3.5 - 7.0	5.3	502.0	49.0 - 142.5	95.8
Mean	13.9 - 19.3	16.8	3.7 - 6.6	5.1	534.6	53.7 - 154.8	104.3
LSD 1% LSD 1%	-	Ns	-	1.78	22.21	-	9.56

under both dark and light conditions showed wide range of variation in the number of days to tuber initiation, which seemed to be due to varietal differences. This range was wider in the light than that dark (Table 3). Probably light enhanced tuberisation in presence of inducers which conformed to the results of other workers (Wattimena 1983) who applied 0, 8, 16 and 24 h photoperiod for microtuberisation and concluded that the longer the photoperiod, the better the tuberisation. In contrast, Lawrence and Barker (1963) and Schilde (1982) observed faster tuberisation in the dark. In the present study, 8h photoperiod was applied. Most of the genotypes tuberised after 12 - 15 days except the local HYV which took > 20 days. Garner and Blake (1989) cultured two potato cultivars on MS containing 8% sucrose under 16h photoperiod and recorded two weeks for tuberisation while Randhawa and Chandra (1990) and Hossain and Sultana (1998) recorded 10 - 12 and 13 days, respectively, which indicates that different genotypes were the important factor for this variation. The local HYV's were introduced from the International Potato Centre (CIP) as germplasm materials; selected and recommended as local HYV. They are tetraploid in origin under the sub sp. *tuberosum*.

Among the genotypes the number of microtubers per tube varied from 0 - 5 in dark and 1 - 11 in light. However, in the light the maximum number of micro tuber was produced by Patrones (9.5), the minimum by Chamak (1.5) and in the dark these were 4 in a number of genotypes and 0.5 also in Chamak. Drastic reduction in number of microtubers per tube due to dark has been demonstrated by Wattimena (1983). In contrast, Lawrence and Barker (1963) and Schilde (1982) observed better tuberisation in dark. In the present investigation, more than 11 microtubers per tube were produced. However, induction of microtubers in microplant is probably a genetic potential of a genotype which is directly or indirectly influenced by a number of factors (dark/light, chemicals and their interaction). Tovar et al. 1985, Dodds et al. 1988 and Randhawa and Chandra 1990) used 30 nodes in each Erlenmeyer flask and obtained 6 - 26, 8 - 24 and 9 - 20 microtubers per flask, respectively. While Hoque et al. (1996) and Zakaria (2003) harvested 40 and 8 - 12 microtubers per Erlenmeyer flask of the cv. Daimant, respectively. In the present investigation, three microplants in each 150 mm × 25 mm test tube were used in order to obtain more than 11 microtubers. Under the same environmental condition, the local HYV seldom developed microtubers (0 - 1) is possibly due to: (a) the protocol used is not suitable for the local HYV, (b) light or dark is not a important factor, (c) varietal difference and their interaction is mainly responsible for such a varied results. This justifies the use of separate protocol of microtuberisation for Heera, Dheera and Chamak.

Weight of microtuber depends on number and size of microtuber, which is assumed to be environment/chemical-dependent. Light condition produced

much higher microtuber weight per tube than the dark. Light caused greening of microtuber resulted in increasing tuber weight by 10 - 20% (Shah 2002, personal communication). Probably, light enhanced starch granule accumulation in a more compact form than that developed in the dark. In the present investigation, 15% higher microtuber weight were obtained in the dark. The result in respected of weight of microtuber obtained in the present investigation to the findings reported earlier (Simko 1990; Hossain and Sultana 1998; Zakaria 2003; Yeasmin 2005). All of them obtained a mean tuber weight of about 100 mg from the culture in the dark. In contrast, Randhawa and Chandra (1990) obtained much higher microtuber weight, ranging from 650 - 2060 mg in MS + 10 mg/l BAP + 8% sucrose in six Indian potato cultivars which is contradictory to the findings of the present investigation. In the present study, we used 5 mg/l BAP instead of 10 mg/l BAP. Probably, varietal difference and their response are the main reasons for such a good performance.

Table 3 shows that irrespective of light and dark conditions the Dutch and Canadian genotypes were good performers in respect of microtuber number and weight. Moreover, Dutch and Canadian genotypes were also good responder to microtuberisation than the others under the same environmental condition. All the genotypes are of *tuberosum* origin except two indigenous genotypes (*andigena* origin). Local HYVs are the germplasms developed in the CIP were the least responder. This clearly indicates that different genotypes are mainly responsible for such a wide variation (Tovar et al. 1985).

From this study it is evident that microtuberisation of potato is mainly genotype source-dependent, though interaction of other factors such light, dark, temperature, chemicals, etc are there. The present results indicate that genotypes obtained from different sources may require separate protocol of microtuberisation for yield optimization. Moreover, the results obtained in the present study would help us in future planning for microtuber production with these valuable materials.

References

Abbott AJ and **Belcher AR** (1986) Potato tuber formation *in vitro*. *In:* Plant tissue culture and its agricultural applications. LA Withers and PG Alderson (eds.). Butterworths, London, pp. 13122.

Bauqing L, Miaochang Z, Jin LX, Jun W and **Bofu S** (1991) Large scale production of *in vitro* potato tubers: An example. Asian Potato J. **2** : 13-15.

Dodds JH, Tovar P, Chandra R, Estrella D E and **Cabello R** (1988) Improved methods for *in vitro* tuber induction and use of *in vitro* tubers in seed programs. Proc. of the symp. on improved potato planting material. Kunming : China; June 21-24, 1988, pp. 49-64.

Garner N and **Jennet Blake** (1989) The induction and development of potato microtubers *in vitro* on media free of growth regulating substances. Annals of Botany (London), **63**: 663-674.

Hossain MJ (1987) Application of tissue culture techniques for producing virus-free potatoes. M.Sc. thesis submitted to the Dept. of Hort., Bangladesh Agril. University, Mymensingh, pp. 87.

Hossain M J and **Sultana N** (1994) Research report on tissue culture research tuber crops. Tuber Crops Research Centre, BARI, 23-25 July, pp. 1-21.

Hossain MJ and **Sultana N** (1998) Effect of benzylaminopurine (BAP) and choloro-cholinechoride (CCC) on *in vitro* tuberisation of potato. Bangladesh J. Agril. Res. **23**: 685-690.

Hoque MI, Mila NB, Khan S and **Sarker RH** (1996) Shoot regeneration and *in vitro* microtuber formation in potato. Bangladesh J. Bot. **25**: 87-93.

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

Hyouk J, Jeong KS, Jeon HJ and **Jang RL** (1994) Mass production of potato microtubers by novel tissue culture techniques and its agricultural applications. Proc. of the 4th Triennal Conf. and Symp. of the Asian Pot. Assoc., 4-9 July, South Korea. p. 21.

Jun W, Qin LB and **Bofu S** (1994) Commercial *in vitro* tuberlet production: meeting the needs of a tropical climate and beyond. Proc. of the 3rd Triannial Conf. of the Asian Potato Assoc., 5-8 May, Seoul, South Korea. p. 21.

Lawrence CW and **Barker WG** (1963) A study of tuber is at ion in the potato (*Solanum tuberosum* L.). Amer. Pot. J. **40**: 349-356.

Mares DJ, Marschner H and **Kruss A** (1981) Effect of gibberellic acid on growth and carbohydrate metabolism of developing tubers of potato (*Solanum tuberosum* L.). Physiol. Plant. **52**: 267-274.

Paet CN and **Zamora AB** (1994) Prouction of *in vitro* tuberlets and greenhouse planting. Proc. of the 4th Triennal Conf. and Symp. of the Asian Pot. Assoc., 4-9 July, South Korea, pp. 17-18.

Pelacho AM, Viscasillas T and **Mingo-Castel AM** (1993) Photoperiod dependence of *in vitro* jasmonic acid induced tuberisation in (*Solanum tuberosum*) ssp. *andigena*. Proc. of the Ith Triennal Conf. of the European Assoc. for Pot. Res. Paris, France, July 18-23, 1993, pp. 249-250.

Randhawa GJ and **Chandra R** (1990). *In vitro* tuberisation in six potato cultivars. J. Indan Pot. Assoc. **17**: 199-201.

Sattelmacher B and **Marschner H** (1978) Relation between nitrogen, cytokinin activity and tuberisation in *Solanum tuberosum*. Physiol. Plant. **44**: 65-68.

Schilde-Rentschler L (1982) World Potato Facts. International Potato Centre, Lima, Peru, pp. 1-14.

Simko I (1990) Tuberizacia {emiakov v podmiienkach *in vitro* poosetreni Paklobutrazolon. Biologia (Bratislava), **46**: 251-256.

Stallknecht GF and **Farnsworth S** (1982a) General characteristics of coumarin-induced tuberisation of axillary shoots of *Solanum tuberisation in vitro*. Amer. Pot. J. **59**: 17-32.

Stallknecht GF and **Farnsworth S** (1982b) Effect of inhibitors of protein and nucleic acid synthesis on the coumarin-induced tuberisation and growth of excised axillary shoots of potato sprouts *Solanum tuberosum* cultivar Burbank cultured *in vitro*. Amer. Pot. **1**: **59**: 69-76.

Thieme R and **Pett B** (198) Production of tubers in vitro and their use in the establishment of a potato store. Arch. Zuechtunhs forsch. **12**: 257-262.

Tovar P, Estrada R, Schilde-Rentschler L Dodds JH (1985) Induction and use of *in vitro* potato tubers. CIP Circular, International Potato Centre. **13**: 1-4.

Wang PJ and **Hu CY** (1982) *In vitro* mass tuberisation and virus-free seed potato prodcution in Taiwan. Amer. Pot. J. **59**: 33-37.

Wang J, Baoqing Band Bofu S (1994) Commercial *in vitro* tuberlet production: Meeting the needs of a tropical climate and beyond. Proc. of the 4th Triennal Conf. and Symp. of the Asian Pot. Assoc., 4-9 July, South Korea. p. 21.

Wattimena GA (1983) Micropropagation as an alternative technology for potato production in Indonesia. Ph.D. dissertation, University of Wisconsin, Medison, USA.

Yeasmin L (2005) Production of microtubers in potato (*Solanum tuberosum* L.) MS thesis, Department of Biotechnology, Bangladesh Agricultural University, Mymensingh. pp. 1-81.

Zakaria M (2003) Induction and performance of potato microtuber. Ph.D. thesis, Department of Horticulture, BSMRKB, Gazipur, Bangladesh.