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Full Length Research Paper

Effect of interaction of 6-benzyl aminopurine (BA) and sucrose for efficient microtuberization of two elite potato (*Solanum tuberosum* L.) cultivars, Desiree and Cardinal

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Single node and multinode explants of two potato cvs., Desiree and Cardinal were tested for *in vitro* microtuber production. Explants were taken from tissue culture laboratory of Seed Centre, University of the Punjab, Lahore, Pakistan in 2004. Experiments were designed in completely randomized pattern. 34 media treatments of Murashige and Skoog (1962) with varying concentrations of sucrose (4, 6, 8, 10 and 12%) either alone or in combination (5-8% sucrose) with 6-benzyl amino purine (BA) were studied. In the case of cv. Desiree, medium MB10 (BA 6 mg/l, sucrose 6%) and for cv. Cardinal, medium MK20 (BA 5 mg/l, sucrose 8%) in term of induction, mean number and mean fresh weight per single node explant were optimized. In comparison, cv. Desiree genotypically was observed to be slightly slow in response to *in vitro* microtuber induction and development than cv. Cardinal.

Key words: Potato, microtuberproduction, 6-benzyl aminopurine (BA), sucrose.

INTRODUCTION

Potatoes, with the conventional method of vegetative propagation are often prone to attack by pathogens such as fungi, bacteria and viruses, thereby resulting in poor quality and yields (Aafia et al., 2007). Seed tubers are the most common source of plant material in potato reproduction. Recently, plant tissue culture technology has become very popular and has a visible impact on the production of virus free seed potatoes. Basic and prebasic seeds of potato produced through tissue culture are free of viruses (viruses like PVY, PVX, PVM, PVA, PVA and PLRV). With evidence for strong and consistent analogies between microtubers and field grown tubers for their induction, growth and development, several components such as the rapid and near synchronous induction and growth, which can be modified by a range of exogenous compounds or conditions, make the microtuber a valuable model system (Coleman et al., 2001). Microtuber production is one of the strategies under this perspective. Because of their small size and weight, microtubers have tremendous advantages in terms of disease free, storage, transportation and mechanization (Kanwal et al., 2006). A number of research groups all over the world are trying to show this revolution (Gopal et al., 2004; Zhijun, et al., 2005; Zhang, 2006). Nowadays, exogenous supply of cytokinin and cytokinin-like compounds in microtuber growth media has been getting much attention for future perspective (Shibli et al., 2001). However, cytokinin stimulates transition of axillary buds into stolons, which could be useful in tuberization *in vitro* but not maintenance of shoot cultures (Vinterhalter et al., 1997).

The objective of the present study was to produce virus free *in vitro* microtubers in terms of induction time, mean number and mean fresh weight of micro-tubers per single node and multinode explants, and

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Abbreviations: BA, 6-Benzyl aminopurine; **MS,** Murashige and Skoog.

Table 1a. Effect of sucrose concentrations on *in vitro* induction, mean number and mean fresh weight of microtubers from single and multinode explants of *Solanum tuberosum* L. Var. Desiree.

Media number	Sucrose (%)	Single node explant			Multinode explant		
		Microtuber induction (days)	Mean number of microtubers	Mean FW (g) of microtubers	Microtuber induction (days)	Mean no. of microtuber	Mean FW (g) of microtubers
M1	4	48^{b}	1.2 ± 0.2^{b}	0.03 ± 0.03^{a}	41^{b}	1.9 ± 0.02^{ab}	0.03 ± 0.02^{a}
M2	6	34^{e}	1.6 ± 0.3^{a}	0.03 ± 0.02^{a}	31^{c}	2.1 ± 0.21^{a}	0.04 ± 0.02^{a}
M3	8	38^{d}	1.5 ± 0.3^{a}	0.04 ± 0.30^{a}	33^{c}	1.9 ± 0.32^{ab}	0.04 ± 0.03^{a}
M4	10	43^{c}	1.3 ± 0.3^{ab}	0.03 ± 0.02^{a}	39^{b}	2.0 ± 0.21^{a}	0.03 ± 0.04^{a}
M5	12	52^{a}	1.2 ± 0.2^{b}	0.03 ± 0.52^{a}	48^{a}	1.7 ± 0.09^{b}	0.03 ± 0.18^{a}
LSD		2.678	0.293	0.026	3.608	0.244	0.017

Means followed by different letters in the same column differ significantly at P = 0.05 according to Duncan's new multiple range test.

evaluation of genotypic responses of two potato cultivars. The protocols developed in this study can be used for the production of disease free, high yielding and premium quality microtubers throughout the year without seasonal limitations. These developed microtubers can be grown under controlled conditions for the production of pre-basic potato seed which after a couple of generations can be supplied to farmers for commercial crop production.

MATERIALS AND METHODS

Healthy virus free potato tubers were obtained from Tissue Culture Laboratory of Seed Centre, University of the Punjab, Lahore, Pakistan in 2004. These tubers were washed several times with detergent followed by several times rinses with distilled water, dried and placed in dark room for eight weeks till sprouting started. One week old sprouts were dipped in 15% NaOCl solution for 15 to 20 min, given three washings with autoclaved distilled water and inoculated on prepared MS medium. After 4 weeks of inoculation, the buds were sprouted into full plantlets that contained 7 to 8 nodes. These were excised into singlenode (one node) and multimode (three nodes) explants and used for microtuberizaton experiments. The MS media used was supplemented with sucrose (4, 6, 8, 10 and 12%) either alone or in combination with BA at varying concentrations. The pH of the medium was adjusted at 5.74. In each test tube, 10 ml media was dispensed and capped before autoclaving. The media was autoclaved at 121°C for 15 min under the pressure of 15 lb/ln^2. After inoculation, the vials were transferred to growth room where temperature was kept at 27 ± 1°C and 16 h day light. Data was recorded for time taken for microtuber formation, mean number of microtubers per plant and mean fresh weight of microtubers, both from multinode and single node explant at different concentrations of BA and sucrose. Experiments were designed in completely randomized pattern. When microtubers became matured, they were harvested into sterilized Petri plates aseptically. Following the analysis of variance (ANOVA), means were used to find simple correlation between the performance of genotypes in various *in vitro* treatments and the corresponding performances of these genotypes in *in vitro* conditions. Duncan's new multiple range test was also used where applicable (Steel and Torrie, 1980).

RESULTS AND DISCUSSION

Effect of sucrose on microtuberization

Table 1 summarizes the results of microtuber induction in cultivars Desiree and Cardinal on MS medium supplemented with different concentrations of sucrose (4, 6, 8, 10 and 12%) without any growth regulator. The medium M2 containing 6% sucrose was proved to be optimal in terms of minimum time of induction (34 and 31 days), mean number (1.2 and 1.9) and fresh weight (0.03 and 0.04 g) of microtuber per single and multinode explant, respectively in cv. Desiree. For cv. Cardinal, the medium M8 containing 8% of sucrose, the minimum time of induction (22 and 17 days), mean number (1.9 and 2.3) and fresh weight (0.03 and 0.04 g) of microtuber per single and multinode explant, respectively was optimized. In comparing both cvs. in terms of microtuber induction (Tables 1 and 2), multinode explants were observed to be earlier tuberized *in vitro* than single node explants (Figures 1 and 2). It might be due to the presence of some endogenous level of cytokinin in multinode explant than single node. Among media without any addition of hormone (Table 1a and b), 6 and 8% sucrose level was found to be optimal for both cultivars, respectively. Khuri and Moorby (1995) proposed that the high sucrose level on one hand provides a good carbon source which was easily assimilated and converted to starch for the microtuber growth and on the other it secures an uninterrupted synthesis of starch due to high osmotic potential provided by the excess sucrose. Carlson (2004) and Sushruti et al. (2004) also reported best microtuber supplemented with 10% sucrose contents. Data presented in Table 1a and b showed that by further increasing the concentration of sucrose, not only time taken for microtuberization was increased but mean number of microtubers per culture vial were also decreased both in single node as well as multinode

Figure 1. Microtuber induction of multinode explant var. Cardinal (2x).

Figure 2. Different stages of microtuber formation from multinode explant Var. Desiree (1x).

explants.

Effect of BA and sucrose on microtuberization

As far as the combined action of BA and high concentration of sucrose is concerned, it was observed that low concentration (1.0 to 3.0 mg/l BA) failed to show significant effect on microtuberization response as shown in Table 2. Aksenoa et al. (2000) reported that cytokinin and sucrose at high concentration stimulated induction response in MS medium supplemented with

Table 1b. Effect of sucrose concentrations on *in vitro* induction, mean number and mean fresh weight of microtubers from single and multinode explants of *Solanum tuberosum* L. Var. Cardinal.

Media number	Sucrose (%)	Single node explant			Multinode explant		
		Microtuber induction (days)	Mean number of microtubers	Mean FW (g) of microtubers	Microtuber induction (days)	Mean number of microtuber	Mean FW (g) of microtubers
M1	4	47^{a}	0.8 ± 0.21^{c}	0.02 ± 0.02^{a}	43^{a}	1.1 ± 0.32^{d}	0.03 ± 0.09^{a}
M2	6	44^{b}	0.9 ± 0.30^{c}	0.03 ± 0.09^{a}	38^{b}	1.4 ± 0.44^{cd}	0.03 ± 0.01^{a}
M3	8	22^{e}	1.9 ± 0.04^{a}	0.03 ± 0.07^{a}	17^{e}	2.3 ± 0.32^{a}	0.04 ± 0.02^{a}
M4	10	29^{d}	1.5 ± 0.04^{b}	0.03 ± 0.42^{a}	22^{d}	1.6 ± 0.26^{bc}	0.04 ± 0.21^{a}
M5	12	34^{c}	1.5 ± 0.04^{b}	0.03 ± 0.07^{a}	26^{c}	1.9 ± 0.29^{ab}	0.03 ± 0.21^{a}
LSD		2.018	0.226	0.013	3.220	0.469	0.016

Means followed by different letters in the same column differ significantly at P=0.05 according to Duncan's new multiple range test.

Table 2a. Effect of BA and sucrose concentrations on *in vitro* induction, mean number and mean fresh weight of microtubers from single and multinode explants of *Solanum tuberosum* Var. Desiree.

Media number	BA (mg/l)	Sucrose (%)	Single node explant			Multinode explant		
			Microtuber induction (days)	Mean number of microtubers	Mean FW (g) of microtubers	Microtuber induction (days)	Mean number of microtuber	Mean FW (g) of microtubers
MB1	4.0	5	24^{a}	1.6 ± 0.30^{de}	0.02 ± 0.07^{b}	21^{a}	2.6 ± 0.50^{abc}	0.04 ± 0.05^{b}
MB2	4.0	6	19^{b}	1.7 ± 0.20^{e}	0.04 ± 0.40^{b}	16^{bc}	2.9 ± 0.37^{ab}	0.06 ± 0.04^{ab}
MB3	4.0	7	18^{bc}	1.7 ± 0.30^{cde}	0.04 ± 1.17^{b}	17^{b}	2.8 ± 0.39^{abc}	0.04 ± 0.06^{b}
MB4	4.0	8	18^{bc}	1.9 ± 0.24^{cd}	0.04 ± 0.93^{b}	15^{bcd}	3.0 ± 0.38^{a}	0.04 ± 0.01^{b}
MB5	5.0	5	17^{bcd}	1.7 ± 1.10^{cde}	0.03 ± 0.02^{b}	14^{cde}	2.5 ± 0.72^{bc}	0.08 ± 0.82^{ab}
MB6	5.0	6	16^{bcd}	1.8 ± 0.62^{cd}	0.04 ± 0.04^{b}	13^{de}	2.8 ± 0.83^{abc}	0.08 ± 0.29^{ab}
MB7	5.0	7	16^{bcd}	1.7 ± 0.30^{cde}	0.10 ± 0.03^{ab}	14^{cde}	2.4 ± 0.43^{c}	0.06 ± 1.00^{ab}
MB8	5.0	8	15^{cd}	2.0 ± 1.10^{abc}	0.12 ± 0.03^{ab}	13^{de}	2.5 ± 0.41^{bc}	0.11 ± 0.34^{ab}
MB9	6.0	5	14^{d}	2.3 ± 0.68^{ab}	0.14 ± 0.60^{ab}	12^{e}	2.9 ± 0.31^{ab}	0.08 ± 0.29^{ab}
MB10	6.0	6	14^{d}	2.4 ± 0.13^{a}	0.21 ± 0.91^{a}	12^{e}	3.0 ± 0.40^{a}	0.13 ± 0.48^{a}
MB11	6.0	7	16^{bcd}	1.9 ± 0.59^{cd}	0.19 ± 0.08^{a}	14^{cde}	2.9 ± 0.24^{ab}	0.10 ± 0.09^{ab}
MB12	6.0	8	15^{cd}	1.4 ± 0.54^{e}	0.18 ± 0.04^{a}	13^{de}	2.9 ± 1.10^{ab}	0.11 ± 0.47^{ab}
LSD			3.027	0.345	0.119	2.482	0.409	0.065

Means followed by different letters in the same column differ significantly at P = 0.05 according to Duncan's new multiple range test.

8% sucrose. According to Nawsheen (2001), the optimal production of microtubers was obtained in MS medium tuber initiation. Best response for cv. Desiree was obtained in MB10 medium containing 6.0 mg/l BA with 6% sucrose. At this concentration, microtuber formation started after 14 and 12 days of inoculation for both single node and multinode explants, respectively (Figures 3, 5 and 7). The mean number (2.4 and 3.0 microtubers) and the maximum mean fresh weight (0.21 and 0.13 g) of microtuber per single node and multinode explant, respectively was optimized. Azzopardi (1997) used tuberization medium containing high level of BA (5.0 mg/l) and sucrose (8%) to get optimal production of microtubers (Figures 4, 6 and 8). With the same medium composition but with the addition of CCC (2-chloroethyltrimethy-lammonium chloride) in concentration of 500 mg/l, the maximum mean number of 44.5 microtubers per 100 ml flask were obtained by Haque (1996). The BA at 14 mg/l in MS medium supplemented with 8% sucrose was found to be an optimum medium by Mogollon et al. (2000). In the case of cv. Cardinal, results were found to be optimal in the medium MB20 containing 5.0 mg/l BA and 8% of sucrose in terms of minimum time of induction (11 and 09 days), mean number (2.6 and 4.1) and fresh weight (0.23 and 0.05 g) of microtuber per single and multinode explant, respectively. Size of microtubers was crucial for sprouting *in vivo*. It was suggested that only microtubers larger than 250 mg can be used to produce

Table 2b. Effect of BA and sucrose concentrations on *in vitro* induction, mean number and mean fresh weight of microtubers from single and multinode explants of *Solanum tuberosum* Var. Cardinal.

Media number	BA (mg/l)	Sucrose (%)	Single node explant			Multinode explant		
			Microtuber induction (days)	Mean number of microtubers	Mean FW (g) Of microtubers	Microtuber induction (days)	Mean number of microtuber	Mean FW (g) of microtubers
MB1	4.0	5	14^{bcd}	2.1 ± 0.49^{bc}	0.09 ± 0.02^{cd}	12^{bc}	2.3 ± 0.33^{c}	0.05 ± 0.13^{b}
MB2	4.0	6	12^{fg}	1.7 ± 0.34^{d}	0.13 ± 0.06^{bcd}	12^{bc}	2.8 ± 0.38^{bc}	0.04 ± 0.52^{b}
MB3	4.0	7	13^{cdef}	1.9 ± 0.44^{cd}	0.11 ± 0.56^{cd}	12^{bc}	2.9 ± 0.43^{bc}	0.04 ± 0.49^{b}
MB4	4.0	8	12^{fg}	2.1 ± 0.31^{bc}	0.05 ± 0.03^{d}	11^{cd}	3.1 ± 0.27^{abc}	0.03 ± 0.42^{b}
MB5	5.0	5	15^{abc}	2.3 ± 0.34^{ab}	0.04 ± 0.04^{d}	12^{bc}	3.2 ± 0.06^{abc}	0.03 ± 0.04^{b}
MB6	5.0	6	17^{a}	2.1 ± 0.16^{bcd}	0.05 ± 0.03^{d}	14^{ab}	3.8 ± 0.09^{ab}	0.04 ± 0.71^{b}
MB7	5.0	7	14^{bcde}	2.2 ± 0.16^{bc}	1.20 ± 0.03^{a}	14^{ab}	3.8 ± 0.09^{ab}	0.04 ± 0.04^{b}
MB8	5.0	8	11^{g}	2.6 ± 0.47^{a}	0.23 ± 0.02^{b}	9^{d}	4.1 ± 0.39^{a}	0.05 ± 0.51^{b}
MB9	6.0	5	14^{bcd}	1.9 ± 1.25^{cd}	0.16 ± 0.04^{bc}	13^{abc}	2.8 ± 0.90^{bc}	0.05 ± 0.63^{b}
MB10	6.0	6	16^{ab}	1.7 ± 1.29^{d}	0.13 ± 0.06^{bcd}	15^{a}	2.9 ± 1.4^{bc}	0.06 ± 0.54^{b}
MB11	6.0	7	13^{defg}	1.8 ± 1.01^{cd}	0.11 ± 0.06^{cd}	14^{ab}	3.2 ± 0.62^{abc}	0.65 ± 0.54^{a}
MB12	6.0	8	12^{efg}	2.1 ± 0.29^{bc}	0.048 ± 0.34^{d}	12^{bc}	3.2 ± 0.03^{abc}	0.03 ± 0.32^{b}
LSD			1.850	0.352	0.092	2.017	0.897	0.076

Means followed by different letters in the same column differ significantly at P = 0.05 according to Duncan's new multiple range test.

Figure 3. *In vitro* tuberization of nodal explant on MS medium containing 6% sucrose and 6.0 mg/l BA Var. Cardinal (1x)

minitubers *in vivo* (Al-Safadi et al., 2000).

Conclusion

From the results, it appears that BA in combination with high sucrose promotes *in vitro* microtuber induction and development. To obtain higher number and larger size microtubers, the media supplemented with BA and higher sucrose level were found to be optimal for both cvs. Desiree and Cardinal. Single node explants were observed to be preferred over multinode explant. The BA is stimulatory to starch metabolizing enzymes, thus creating a strong metabolic sink. As a result, subsequent accumulation of starch occurred which is seen as the swelling of the microtuber. This combined ability can be termed as an excessive substrate (high sucrose level) and stimulus (BA) that triggers the enzymatic activity in the tuberization processes. The cv. Desiree genotypically, was found to be slightly slow in growth in *in*

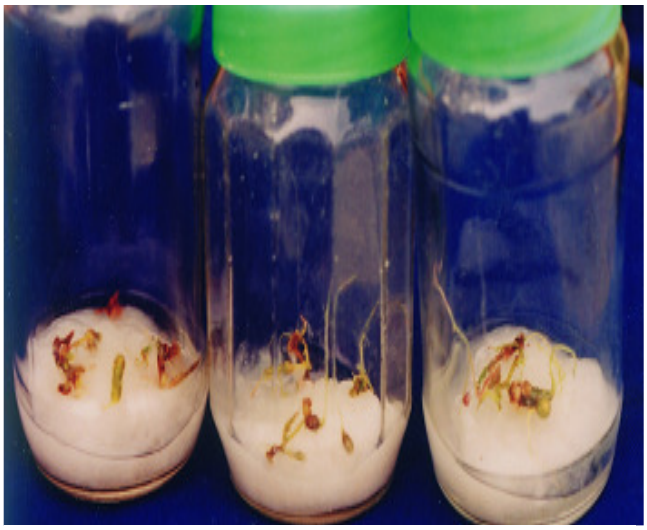

Figure 4. *In vitro* tuberization of nodal explant on MS medium containing 8% sucrose and 5.0 mg/l BA Var. Desiree (1x).

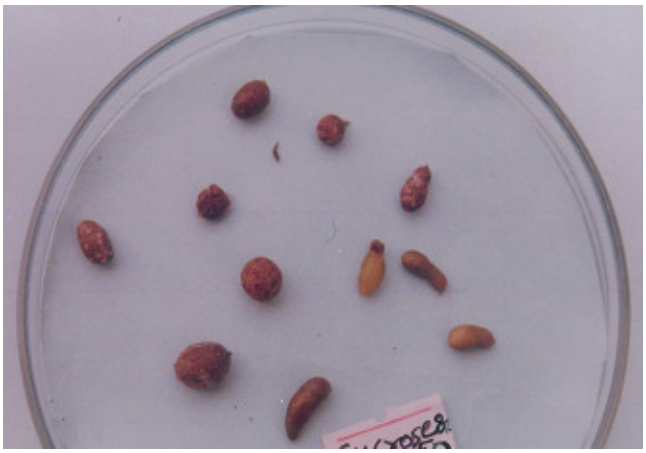

Figure 5. Microtubers harvested from MS medium containing 6% sucrose and 6.0 mg/l BA (1x).

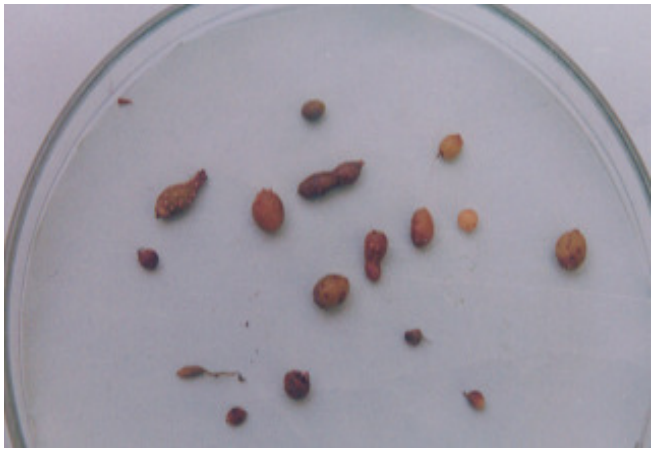

Figure 6. Microtubers harvested from MS medium containing 8% sucrose and 5.0 mg/l BA (1x).

Figure 7. Well developed microtubers of Cardinal obtained from MS medium containing 6% sucrose and 6.0 mg/l BA.

Figure 8. Well developed microtubers of Desiree obtained from MS medium containing 6% sucrose and 6.0 mg/l BA.

vitro microtuberization experiments than cv. Cardinal.

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