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Research Paper

Effect of physical and chemical factors on efficient *in vitro* production of microtuber using three potato genotypes in Bangladesh

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ABSTRACT

Potato microtuberization through *in vitro* culture is a complex development process that is influenced by several chemical and physical factors. For mass production of potato microtubers these factors need to be optimized along with an efficient protocol involving suitable chemicals dosages. Three commercial potato cultivars *viz.* Diamant, Cardinal and Asterix with various doses of sucrose (4, 6, 8, 10 and 12%), plant growth regulators *viz.* cytokinins (BAP or kinetin- 0.5, 0.75, 1.0, 1.5 and 7.0 mg/l), incubation period (4, 6, 8, 10 and 12 weeks) and photoperiodic regimes (0/24, 8/16 and 16/8 light/dark cycles) were applied for enhancing potato microtuberization in conventional semi-solid culture system. It was observed that 8% sucrose + 1 mg/l BAP showed the best performance for all cultivars. For photoperiodic regimes (0/24 h) 10 week incubation period and for microtuber production Diamant was identified as the best cultivar.

Key words: BAP, incubation period, kinetin, photoperiod, potato microtuber, sucrose

Potato microtubers are produced in many laboratories around the world either for research or commercial purposes using *in vitro* procedures. This complex process is influenced by several factors such as chemical (plant growth regulators, vitamins, amino acids, sucrose, etc.) and physical (genotypes, explants, inoculation, effects of pH, incubation period, photoperiodic regimes etc) (Hussey and Stacey, 1984; Ewing and Struik, 1992; Seabrook *et al*., 1993; Simko, 1994). These factors therefore, should be standardized according the potato genotypes to develop suitable protocol for *in vitro* production of microtubers (Paet and Zamora, 1994; Hossain, 2005).

Concentration of sucrose is an influential factor for *in vitro* production of crop plants (Yu *et al*., 2000; Hossain *et al*., 2009; Siddique and Islam, 2015; Morshed *et al*., 2016). Previously, Dodds *et al*. (1992) reported that optimal sucrose concentration is required for *in vitro* microtuber initiation since it determines the osmolarity of culture medium, so higher or lower sucrose content in the medium may result in reduced microtuberization (Khuri and Moorby 1995; Yu *et al*. 2000).

Similarly without any PGR in the culture medium, the quality and quantity of microtubers decreases and it also takes long time for microtuber production (Peng *et al*. 2012; Rahman *et al.* 2015, 2017). Several authors have reported that addition of exogenous PGRs in culture medium have reduced the induction time and enhanced the quality and yield of microtubers (Wang and Hu, 1985; Chandra *et al*., 1988; Zakaria *et al*., 2008). Among different cytokinins, BAP and kinetin were reported to stimulate *in vitro* microtuberization, but responses varied with genotype and concentration (Forti *et al*., 1991; Saddar and Suwwan, 2004; Aslam *et al*., 2011; Ghavidel *et al*., 2012).

A previous study by Leclerc *et al*. (1994) demonstrated that different incubation periods are required for microtuberization of different potato genotypes. Later, Hossain *et al*. (2017) reported that increasing the incubation period significantly increased the number and fresh weight of microtubers but optimization according to genotypes is required. There are several reports regarding potato microtuberization under different photoperiodic conditions, but some researchers observed that short photoperiod was good for microtuber induction (Lawrence and Barker, 1963; Wang and Hu, 1982; Garner and Blake, 1989); while others observed better microtuber growth in darkness (Lawrence and Barker, 1963; Schilde-Rentschler *et al*., 1982; Abdelaleem *et al*., 2015).

In view of above facts an investigation was undertaken to optimize the microtuber production of three different commercial potato genotypes namely Diamant, Cardinal and Asterix in conventional semi-solid

medium with variable sources of sucrose as well as cytokinin concentrations, incubation periods and photoperiodic regimes to improve mass production of potato microtuber through *in vitro* culture.

MATERIALS AND METHODS

The experiment was conducted in the Plant Tissue Culture and Biotechnology Laboratory, Bangladesh Council of Scientific and Industrial Research (BCSIR), Rajshahi. Diseases free *in vitro* raised plantlets of three commercially popular potato cultivars *viz*. Diamant, Cardinal and Asterix were used as stock plants. The plantlets were cultured in test tubes containing semi-solid MS medium with 3% sucrose and kept in growth chamber at 25 ± 1°C with 16/8 h photoperiod. The medium was supplemented with various concentrations of sucrose (4, 6, 8, 10 and 12%) without any PGR. To determine the effect of PGRs, MS medium were supplemented with 8% sucrose and different concentrations of BAP (0.5, 0.75, 1.0, 1.5 and 7.0 mg/l). The pH of the medium was adjusted at 5.8 and solidified with 7.0 g/l agar that was autoclaved at 121°C and 103 kPa for 15 minutes. Cultures were incubated at 15 ± 1°C in darkness. To study the effect of incubation period cultures were incubated in darkness for different periods (4, 6, 8, 10 and 12 weeks) at 25 ± 1°C. To observe the effect of photoperiodic regimes cultures were incubated in different photoperiods (0/25, 8/16 and 16/8 light/dark cycles) for 10 weeks.

The data was collected on the basis of number of microtubers harvested per plantlet, fresh weight (g) at harvest and days to microtuberization when swelling of microtubers was visible. In case of sucrose, cytokinin concentration and photoperiodic regime experimental data was recorded after 10 weeks; whereas data on incubation period was taken at the end of each period. Data was analyzed using ANOVA and the mean value was compared based on Duncan's Multiple Range Test at 5% level of probability.

RESULTS AND DISCUSSION

Microtuberization of potato with different chemical and physical factors using three genotypes of potato are shown in Fig. 1. According to Yu *et al*. (2000), sucrose concentration determines the osmotic potential of culture medium and affects the pH as well as nutrient uptake during microtuber development. Chandra *et al*. (1988) suggested that increasing the osmotic potential would enhance the starch accumulation process and help to trigger rapid starch biosynthesis during microtuberization. In the present study, the number of induced microtubers was highest (0.61) in Diamant with 8% sucrose and lowest (0.13) in Asterix with 10% sucrose (Table 1).

Table 1: Effect sucrose on the number, fresh weight and days to induction of microtubers during *in vitro* microtuberization of three potato cultivars (Mean ± SE)

Characteristics	Sucrose (%)	Cultivars		
		Diamant	Cardinal	Asterix
Number of microtubers	4	$0.37^{c} \pm 0.15$	$0.24^{e} \pm 0.20$	$0.17^{e} \pm 0.19$
	6	$0.43^{c} \pm 0.21$	$0.29^{d} \pm 0.19$	$0.23^{e} \pm 0.13$
	8	$0.61^{a} \pm 0.11$	$0.31^{d} \pm 0.18$	$0.27^{d} \pm 0.17$
	10	$0.55^{b} \pm 0.17$	$0.27^{d} \pm 0.13$	$0.13^{f} \pm 0.14$
	12	$0.57^{b} \pm 0.09$	$0.23^{e} \pm 0.16$	$0.15^{e} \pm 0.16$
Fresh weight of microtuber (g)	4	$0.31^{f} \pm 0.06$	$0.35^{f} \pm 0.05$	$0.44^{e} \pm 0.04$
	6	$0.43^{e} \pm 0.09$	$0.48^{de} \pm 0.07$	$0.45^{e} \pm 0.06$
	8	$0.56^{bc} \pm 0.08$	$0.60^{ab} \pm 0.08$	$0.63^{a} \pm 0.08$
	10	$0.55^{bc} \pm 0.05$	$0.57^{b} \pm 0.05$	$0.59^{b} \pm 0.06$
	12	$0.54^{c} \pm 0.06$	$0.55^{bc} \pm 0.07$	$0.61^{ab} \pm 0.05$
Microtuber induction (days)	4	15.27^{b}	33.80^{d}	38.80^{e}
	6	14.53^{b}	30.13^{d}	30.20^{d}
	8	13.80^{a}	23.73^{c}	29.60^{d}
	10	14.67^{b}	28.27^{c}	33.13^{d}
	12	14.80^{b}	28.40^{c}	33.67^{d}

Mean value indicated with the same letter in a row are not significant and are separated by DMRT.

It was also observed that 8% sucrose produced highest fresh weight of microtubers in Asterix (0.63 g) followed by Cardinal (0.60 g) and Diamant (0.56 g). Similar concentration also demonstrated early induction of microtubers i.e (13.80, 23.73 and 29.60 days, respectively. Our findings are in agreement with Islam *et al.* (2017), Hossain *et al.* (2017) and Aslam *et al.* (2011) who also observed increased growth of microtubers with 8% sucrose in culture medium.

A possible reason is that the microtuberization processes has an accepted level of osmotic potential and sucrose concentration, and above that level osmolarity of culture medium increases, which disturbs the pH and affects the uptake of macro- and micro-nutrient from culture medium resulting in poor growth of the plants. Formation and development of microtuber in potato generally involves stimulation of cell division, inhibition of cell elongation and promotion of cell expen-

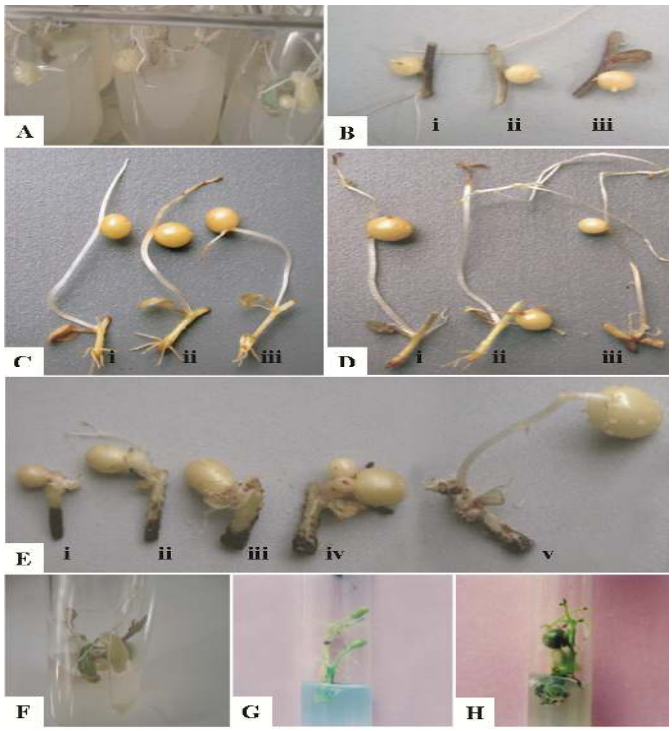

Fig. 1. Microtuberization of potato using different chemical and physical factors using three genotypes of potato: (A) *In vitro* culture of potato microtuber, (B) microtubers obtained from 8% sucrose-i) Diamant ii) Cardinal and iii) Asterix, (C) similarly from 1.0 mg/l BAP, (D) from 2.0 mg/l kinetin, (E) microtubers produced in different incubation periods-i) 4 w, ii) 6 w, iii) 8 w, iv) 12 w and v) 10 w, (F) microtubers produced in 0/24 h, (G) 8/16 h and (H)16/8 h light/dark cycle.

sion and cytokinin is an ideal candidate for this process as it plays a key role in cell division and creates sink activity for microtuber development (Hossain and Sultana, 1998; Zakaria *et al*., 2008). Optimum concentration in the present study for highest number of microtubers in Diamant, Cardinal and Asterix was 1.11, 0.71 and 0.57 for 1.0 mg/l BPA, respectively; also with this concentration highest fresh weight (0.86 g) and lowest days to microtuber induction (12.27 days) was observed in Diamant (Table 2). Whereas, in Cardinal and Asterix, highest fresh weight was observed with 1.0 mg/l kinetin i.e. (0.81 g and 0.82 g, respectively and lowest days to microtuber induction (15.47 days and 15.27 days) was observed with 0.5 mg/l and 1.5 mg/l kinetin, respectively. Aryakia and Hamidoghli (2010) also reported enhanced microtuber production with 0.75 and 1.0 mg/l BAP. We observed a fluctuation in potato microtuberization process according to BAP and kinetin concentration in the medium which is consistent with other reports (Palmer and Smith, 1969; Forti *et al*., 1991). The effect of incubation period on *in vitro* microtuberization of three potato cultivars demonstrated positive correlation of incubation period with increased number and fresh weight of microtubers (Fig. 2).

Table 2: Effect of plant growth regulators on the number, fresh weight and days to microtuber formation during *in vitro* growth of three potato cultivars (Mean ± SE).

PGRs		Number of microtubers			Fresh weight of microtubers			Days to microtuber induction		
		Diamant	Cardinal	Asterix	Diamant	Cardinal	Asterix	Diamant	Cardinal	Asterix
BAP (mg/l)	0.50	$0.74^{c} \pm 0.15$	$0.61^{b} \pm 0.14$	$0.45^{c} \pm 0.11$	$0.74^{cd} \pm 0.06$	$0.65^{d} \pm 0.04$	$0.53^{e} \pm 0.06$	14.67^{ab}	27.53^{d}	29.20^{cd}
	0.75	$0.87^{b} \pm 0.24$	$0.67^{ab} \pm 0.22$	$0.51^{bc} \pm 0.11$	$0.82^{b} \pm 0.06$	$0.61^{e} \pm 0.03$	$0.52^{e} \pm 0.07$	15.03^{ab}	26.13^{cd}	34.20^{cd}
	1.00	$1.11^{a} \pm 0.16$	$0.71^{a} \pm 0.23$	$0.57^{a} \pm 0.19$	$0.86^{a} \pm 0.08$	$0.69^{c} \pm 0.10$	$0.58^{d} \pm 0.06$	12.27^{a}	23.40^{c}	26.87^{c}
	1.50	$0.98^{a} \pm 0.23$	$0.68^{ab} \pm 0.18$	$0.55^{b} \pm 0.17$	$0.68^{e} \pm 0.06$	$0.55^{ef} \pm 0.05$	$0.41^{f} \pm 0.07$	13.45^{a}	25.33^{cd}	35.20^{cd}
	2.00	$0.94^{ab} \pm 0.12$	$0.59^{b} \pm 0.23$	$0.52^{bc} \pm 0.23$	$0.79^{c} \pm 0.08$	$0.59^{e} \pm 0.07$	$0.43^{ef} \pm 0.03$	15.53^{ab}	28.87^{d}	37.67^{d}
Kin. (mg/l)	0.50	$0.69^{cd} \pm 0.19$	$0.31^{cd} \pm 0.18$	$0.15^{f} \pm 0.12$	$0.45^{f} \pm 0.07$	$0.47^{f} \pm 0.05$	$0.53^{e} \pm 0.03$	15.93^{ab}	15.47^{a}	18.40^{bc}
	0.75	$0.54^{d} \pm 0.17$	$0.27^{d} \pm 0.21$	$0.13^{fg} \pm 0.21$	$0.58^{d} \pm 0.10$	$0.59^{e} \pm 0.07$	$0.56^{de} \pm 0.06$	18.73^{bc}	18.67^{bc}	16.87^{b}
	1.00	$0.79^{bc} \pm 0.13$	$0.28^{d} \pm 0.20$	$0.17^{e} \pm 0.16$	$0.79^{c} \pm 0.06$	$0.81^{a} \pm 0.03$	$0.82^{a} \pm 0.04$	18.80^{bc}	20.20^{c}	21.07^{bc}
	1.50	$0.88^{b} \pm 0.21$	$0.29^{d} \pm 0.23$	$0.25^{de} \pm 0.17$	$0.60^{d} \pm 0.05$	$0.67^{cd} \pm 0.05$	$0.78^{b} \pm 0.06$	17.87^{b}	17.20^{b}	15.27^{a}
	2.00	$0.94^{b} \pm 0.12$	$0.33^{c} \pm 0.15$	$0.28^{d} \pm 0.23$	$0.76^{d} \pm 0.07$	$0.76^{b} \pm 0.12$	$0.73^{c} \pm 0.04$	17.60^{b}	18.07^{c}	20.20^{bc}

Means with the same letter in a column are not significant and are separated using DMRT.

It was observed that increasing the incubation period from 4 to 10 weeks significantly enhanced the number and weight of microtubers but it was insignificant at 10 to 12 weeks. The present findings are consistent with Hossain *et al.* (2017) who have reported that increasing the incubation period from 28 to 70 days significantly increased the number and fresh weight of microtubers. Leclerc *et al.* (1994) also reported that increasing the incubation period from 28 to 56 days significantly increased the fresh weight of microtubers in potato. It can be assumed that within this period the growth of microtuber is completed. The effect of incubation period on *in vitro* microtuberization of three potato cultivars demonstrated positive correlation of incubation period with increased number and fresh weight of microtubers (Fig. 1). It was observed that increasing the incubation period from 4 to 10 weeks significantly enhanced the number and weight of microtubers but it was insignificant at 10 to 12 weeks. The present findings are consistent with Hossain *et al.* (2017) who have

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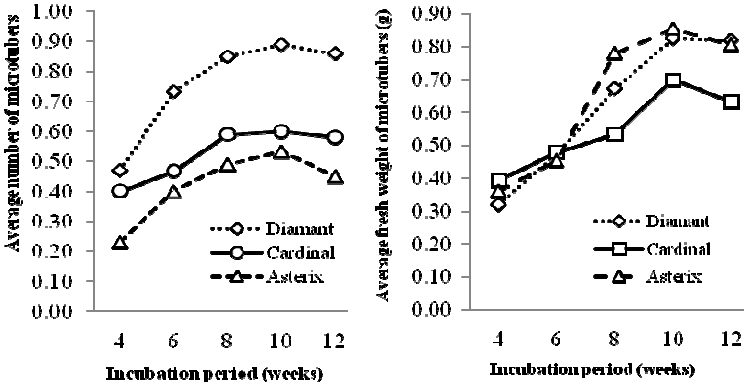

Fig. 2: Effect of various incubation periods (weeks) on average number and average fresh weight of microtubers in Diamant, Cardinal and Asterix.

In nature, microtubers generally occur in darkness, so it may be assumed that *in vitro* microtuber induction could be better in such conditions. Lawrence and Barker (1963) found enhanced *in vitro* microtuber induction in complete dark which was later supported by Schilde-Rentschler *et al.* (1982) and Abdelaleem *et al.* (2015). In the present investigation, dark condition (0/24 h) produced highest number of microtubers in three genotypes i.e. 0.93, 0.53 and 0.40, respectively than the two other light conditions (Table 3).

Average fresh weight of microtubers i.e. 0.84, 0.62 and 0.86 g, respectively was also highest in dark condition. Whitish shoot and microtuber was also observed in this condition. Similar to our findings, Slimmon *et al.* (1989); Dobranszki and Mandi (1993); Levy *et al.* (1993); Akita and Takayama (1994) and Zarrabeitia *et al.* (1997) also observed enhancement in number and weight of microtuber with dark condition. A Possible reason is that light condition has increased endogenous gibberellins levels in potato which are reported to have inhibitory effect on *in vitro* microtuberization (Sakha *et al.,* 2004); beside this, dark condition may have triggered physiological responses to starch biosynthesis in microtubers

Table 3. Effect of photoperiodic regimes on *in vitro* microtuberization of three potato cultivars (Mean ± SE)

Characteristics	Photoperiod (light/dark)	Cultivars		
		Diamant	Cardinal	Asterix
Number of microtubers	0/24 h	$0.93^{a} \pm 0.12$	$0.53^{d} \pm 0.13$	$0.40^{e} \pm 0.13$
	8/16 h	$0.80^{b} \pm 0.17$	$0.40^{e} \pm 0.13$	$0.33^{f} \pm 0.13$
	16/8 h	$0.73^{c} \pm 0.13$	$0.33^{f} \pm 0.14$	$0.20^{g} \pm 0.12$
Fresh weight of microtubers (g)	0/24 h	$0.84^{a} \pm 0.04$	$0.62^{de} \pm 0.07$	$0.86^{a} \pm 0.03$
	8/16 h	$0.67^{cd} \pm 0.08$	$0.54^{e} \pm 0.08$	$0.79^{ab} \pm 0.04$
	16/8 h	$0.73^{bc} \pm 0.08$	$0.60^{de} \pm 0.05$	$0.80^{ab} \pm 0.02$

Means with the same letter are not significant and are separated using DMRT.

In a previous study, Estrada et al. (1986) investigated the genotypic effects on microtubers production and later studies by Gopal *et al.* (1998) and Hossain (2005) have confirmed that different potato cultivars, under the same culture conditions, have a wide range of variation in their growth pattern. In our study, Diamant showed the highest microtubers production compared to other two cultivars. The cultivars Cardinal and Asterix also showed fast growth at the initial phase of micropropagation; but it was not satisfactory like Diamant. The reason Diamant exhibited the best performance to produce microtubers may be due to its inherent genetic potential or due to the degree of cell sensitivity towards growth regulators, which depends on cultivar and endogenous levels of growth regulators (Uranbey, 2005; Nistor *et al*., 2010; Harun-Or-Rashid *et al.* 2017).

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