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EVALUATION OF IN VITRO TUBER INDUCTION ABILITY OF TWO POTATO GENOTYPES

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The *in vitro* effect of growth regulators under cool white fluorescent light (for 16 h at 50 µmol. m^{-2} s^{-1}) and dark conditions to accelerate microtuberization in potato cvs. Desiree and Cardinal were investigated. The axillary buds were selected as an explant, inoculated on MS medium augmented with and without growth regulators (BAP (6-Benzylaminopurine) and NAA (α-Naphthaleneacetic acid) to initiate microtuberization. Combination of growth regulators (NAA + BAP) in media produces callus or had no effect on culture. NAA in any concentration, alone or in combination with BAP promote callus formation only. BAP alone in low concentration (0.5 mg/l) proliferate shoot regeneration and while at high (1.0 mg/l) concentration it induces microtuberization in the dark by 63 percent of control. It was found that nutrient media, without growth regulator had higher yield and greater number of microtubers under both light (16 h) and dark conditions as compared to those cultured on media with growth regulator. However, study revealed that MS_0 medium with vitamins and solidified agar free of growth regulators can be utilized for mass propagation and microtuberization under the optimized culture conditions.
Keywords: *Solanum tuberosum* L., potato diseases, plant tissue culture, 6-Benzylaminopurine, α-naphthaleneacetic acid, photoperiod, microtuber induction.

INTRODUCTION

Main reason behind reduction of *Solanum tuberosum* L. (potato) yield in Pakistan is basically lack of healthy and certified seed. The low productive cultivars, invalid agronomic decisions, inadequate management practices and inefficient land usage further aggravate the issue (FAO, 2009; Qasim *et al*., 2013). Furthermore, Momena *et al*. (2014) reported that physiological quality and health of seed tubers also influenced yield. Per unit average yield increases up to 30 to 50% with the use of high quality vigorous seed as compared to infected ones (Wang, 2008). Traditional potato seed production method is symbolized by low multiplication rate, loss of a large amount of food material, lack of uniformity, exposure to infective diseases/pests and gradual aggregation of degenerative viruses during asexual propagations (Kaur *et al*., 2015).

Production of potato microtubers through tissue culture technique offers several advantages, because of their storability and direct plantation in the field even without a weaning phase. Its care and transportation also facilitated in marketing and global exchange of germplasm (Jimenez-Gonzales, 2005). The production of micro tubers can be done throughout the year (Ranalli, 1997). Tissue culture is an excellent choice to produce uniform, free of pathogen micro tubers to get healthy vigorous crop leading to better yield.

Studies have shown that potato tuberization is determined by both genomic (Madhu *et al*., 2014) and environmental elements (Kittipadukal *et al*., 2012) along with sucrose concentration (Coleman *et al*., 2001). According to Lentini and Earle (1991) cytokinins (BAP) support tuber formation; while auxins have a role in triggering tuberization (Roumeliotis *et al*., 2012).

Therefore, the present study was planned to recover the potential yield of potato crop in Pakistan. The objective of this study was to evaluate micro tuber induction ability and an attempt towards developing an efficient *in vitro* protocol for production of disease free micro tuber as a healthy seeding material.

MATERIALS AND METHODS

Certified virus free plant material of two important red skin potato *(Solanum tuberosum* L.) cultivars Desiree and Cardinal was acquired from Four Brothers Agri Services Pakistan. The company is working for the introduction of high yielding vegetable & crop varieties in Pakistan.

Surface sterilization of explants (nodal sections) was accomplished by washing in running tap water for 1 hour. Then explants were disinfected with 0.1% $HgCl_2$ for five min supplemented with washing in a solution of two drops of

Table 1. The combinations of growth regulators used in MS media tested for micro-tuber production.

	MS 0	MS 1	MS 2	MS 3	MS 4	MS 5	MS 6	MS 7	MS 8
BAP mg/l	0	0	0.5	0	1	0.5	0.5	1	1
NAA mg/l	0	0.5	0	1	0	0.5	1	0.5	1

surfactant 0.1% (v/v) Tween 20 in a 15% Na-hypochlorite for 15-25 min. Finally, explants were properly rinsed 3 times in a double distilled sterile deionized water to thoroughly remove sterilant (disinfectant and detergent).

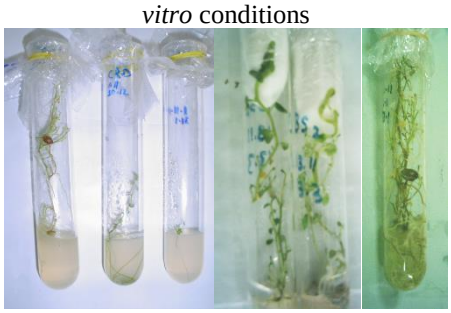
Surface sterilized single explant was inoculated aseptically in 150 x 25 mm test tubes on a MS nutrient medium for shoot and root formation. The media was supplemented with BAP (0.5-1 mg/l) and NAA (0.5- 1 mg/l) (Table 1). For 20 min and at 121°C, MS (Murashige and Skoog, 1962) nutrient media (pH at 5.74) was autoclaved. The cultures were nurture in a growth room in supporting environment of 25±2°C temp and cool white fluorescent light (for 16 h at 50 µmol. m^{-2} s^{-1}) in a photoperiod treatment of 16 and 8 hours, light and dark respectively with five replicates. After 6 weeks, single nodal segments (0.5 - 1.0 cm) axillary buds as an explant were taken from *in vitro* grown plantlets and placed in two separate growing conditions for evaluating its impact on microtuberization.

The experiment was executed in a RCB Design (two genotypes, eight media compositions and two growth/light conditions) along with 5 replications. *In vitro* grown plantlets were taken out, once the foliage showing senescence and data pertaining number of micro tubers/ plantlet and average micro tuber weight (mg) were recorded.

Table 2. Effect of growth regulator on *Solanum tuberosum* L. varieties Desiree and Cardinal.

Media	BAP mg/l	NAA mg/l	Light	Dark
MS0	0.0	0.0	micro-tubers formation	micro-tubers formation
MS1	0.0	0.5	Callus	Callus
MS 2	0.0	1.0	Callus	Callus
MS3	0.5	0.0	Short and healthy Shoot regeneration	Long and week Shoot regeneration
MS 4	0.5	0.5	Callus	Callus
MS 5	0.5	1.0	Callus	Callus
MS 6	1.0	0.0	micro-tubers formation	micro-tubers formation
MS 7	1.0	0.5	Callus	Callus
MS 8	1.0	1.0	Callus	Callus

Microtubers of *S.tuberosum* L. produced under *in-vitro* conditions



In vitro culture showing microtubers *S. tuberosum* L.

In vitro multiplication of culture of *S. tuberosum* L

Table 3. Number of microtubers per plant and their fresh weight (mg) of *Solanum tuberosum* L. cultured under light and dark photoperiod.

Media	Growth Regulator		Potato cultivar Desiree and Cardinal			
			Light	Dark	Light	Dark
	BAP mg/l	NAA mg/l	Microtuber /plantlet	Microtuber /plantlet	Fresh weight (mg) Microtuber	Fresh weight (mg) Microtuber
MS 0	0	0	11.7±0.965	13±0.945	470.6±10.11	490.2±10.78
MS 6	1	0	6.8±0.735	8.55±0.655	303.9±16.60	373.3±16.51

RESULTS

The cultivars Desiree and Cardinal showed comparatively similar response under *in vitro* conditions. Data was taken after 4-6 weeks when maximum plantlet`s growth was achieved and senescence starts. The impact of growth regulators under different photoperiod conditions on explants were recorded (Table 2). It was found that media without growth regulator was the best for multiplication and microtuber formation, BAP alone support both multiplication and tuber formation, at low concentration it promotes shoot regeneration in 3-5 weeks and in high concentration (1 mg/l) promote microtuber formation in the dark conditions. NAA in any concentration and combination with BAP promote callus formation only. Production of microtubers in dark photoperiod is better as compared to those exposed to light (Table 2). Number of micro-tuber per plant in dark condition was higher 13±0.945 as compared to light condition 11.7±0.965 in media without growth regulator. The other media found effective was supplemented with BAP 1 mg/l produces 8.55±0.655 microtubers in dark and 6.8±0.735 microtubers in light. The fresh weight of microtubers per plant was also influenced by photoperiod. It was higher in dark condition 490.2±10.78 as compared to light condition 470.6±10.11 in media without growth regulator (Table 3).

DISCUSSION

Axillary buds are frequently used as an explants for the purpose of *in vitro* multiplication of healthy seed tubers, germplasm transportation and conservation (Gopal *et al*., 1998; Prematilake and Mendis, 1999; Salih *et al*., 2001; Akhtar *et al*., 2018). Suitable *in vitro* environment helps plantlets to yield microtubers (size 2-10 mm). Research on microtuberization in potato had mainly been focused on the incorporation of growth regulators and there were noticeable dissimilarity in the outcomes of those investigations, the responses achieved, banked on a number of aspects including amount of sucrose, temperature, photoperiod light intensity and cultivar (Kumlay *et al*., 2014). This study is about evaluating microtuber induction ability of a genotype and also an attempt towards rapid induction of healthy microtubers in medium with or without growth regulators. Study reveals the effect of photoperiod and growth regulator (BAP and NAA) on yield and number of micro-tubers in two varieties Desiree and Cardinal. The results showed that the varieties Desiree and Cardinal respond in a similar fashion under *in vitro* condition with the application of different growth regulators and photo period.

The effect of different concentration of growth regulators along with light and dark conditions on explants showed that media without growth regulator was more suitable for multiplication and microtuberization. The outcome of the study supported by Seabrook *et al*. (1993) who commented that the maturity group of the potato cultivars and photoperiodic treatments *in vitro* have a great influenced on the induction of microtubers. This was also observed that experiment placed in the dark produces more tubers as compared to those exposed to light. Study findings regarding light duration and impact on microtuber formation are in agreement with Hoque (2010), Ghavidel *et al*. (2012) and Setayesh *et al.* (2017).

Current study reveals that in an attempt to evaluate the effect of growth regulator 6-Benzylaminopurine (BAP 0.5 - 1 mg/l) and α-Naphthaleneacetic acid (NAA 0.5 - 1 mg/l) for the induction of microtuberization resulted in highest number and weight of microtubers were obtained in a media without growth regulator under both light and dark conditions. BAP alone in low concentration (0.5 mg/l) proliferate shoot regeneration while at high (1.0 mg/l) concentration it induce microtuberization in the dark conditions. Kane (2000) had also reported noticeable microtuberization in shoot culture with the support of cytokinin. However, NAA in any concentration alone and in combination with BAP promote callus formation only.

Current investigation endorse the study of Ranalli (1997) who concluded that microtuberization can be initiate even in the absence of growth regulators, our findings are further in agreement with Motallebi and Kazemiani (2013) and Al-Hussaini *et al*. (2015), who communicated that high concentrations of sucrose (80 g/l) had a documented beneficial on *in vitro* microtuberization. Gopal *et al*. (1998) concluded that absence of growth regulators in media provide excellent environment to evaluate microtuber induction ability of a genotype and to eliminate the incident of any objectionable carry over impact of growth regulators on morphology, dormancy or sprouting. Study finding was further endorsed by Fawzia *et al*. (2015) who reported that

MS medium with no growth regulators can be utilized for mass propagation of disease free plants. Kaur *et al*. (2015) concluded that ½ strength MS basal medium free of growth regulator can be used for finishing rooting phase. Study results are also in an agreement of Vinterhalter *et al*. (1997), who concluded that nodal explants of potato does not wanted external hormone for root initiation because this species is easy to root. It was also observed that the average weight of micro tuber was higher in MS medium without growth regulator as compared to the media with growth regulators that might increase the rate of survival of healthy microtubers in the field.

Current investigation suggested that continuous darkness had a great impact on microtuberization phase. This phase was achieved in 16 weeks (under dark) compare to cultures placed in light which took 6-8 months. These findings authenticated by Gopal *et al*. (1998) who reported short days have a greater influence on microtuber induction.

Conclusion: Potato microtuberization can be initiated *in vitro* in the absence of external plant growth regulators and under complete darkness. Furthermore, MS media without growth regulator can be used for commercial production of microtubers. It will be a feasible solution to produce disease free high quality potato microtubers, as a seeding material for potato growers. Healthy, certified micro tubers will enable farmers to achieve higher and sustainable yield; however higher yield targets are also dependent on improved agronomic decisions along with appropriate and timely crop inputs.

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