ISSN 1023-1072

Pak. J. Agri., Agril. Engg., Vet. Sci., 2011, 27 (2): 143-149

EFFECT OF GROWTH REGULATORS ON MERISTEM TIP CULTURE OF LOCAL POTATO CVS DESIREE AND PATRONES

A. Yasmin[1], A. A. Jalbani[2] and S. Raza[3]

[1]Department of Biotechnology, Sindh Agriculture University, Tandojam, Pakistan
[2]Information Technology Center, Sindh Agriculture University, Tandojam, Pakistan
[3]Nuclear Institute of Agriculture Tandojam, Pakistan

ABSTRACT

This study was aimed to evaluate the effect of four different growth regulators (GRs) in two combinations on mass propagation of two potato cultivars cv. Desiree and Patrones. The first combination was Benzyl Amino Purine (BAP) with Naphthalene Acetic Acid (NAA) and the second was Pantothenic Acid with Gibberellic Acid (GA_3) The meristem tips of the selected cultivars were excised under aseptic conditions and cultured on Murashige and Skoog (MS) basal medium with nine different treatments of BAP (0.5, 1.0 and 2.0 mg L^{-1}) with NAA (0.5, 1.0 and 2.0 mg L^{-1}) and six different treatments of GA_3 (0.25, 0.5, 1.0 mg L^{-1}) with pantothenic acid (1.0 and 2.0 mg L^{-1}). The best regeneration of meristem tips was obtained when MS medium was supplemented with 1.0 mg L^{-1} pantothenic acid plus 0.5 mg L^{-1} gibberellic acid. This combination took minimum time for regeneration of multiple shoots and roots on meristem tips of variety Desiree. It is interesting to report that the combinations of BAP and NAA did not result in complete plantlets formation; most of the explants formed shoots and calli at the base without regenerating roots.

Keywords: GA_3, meristem tip culture, pantothenic acid, potato

INTRODUCTION

Potato (*Solanum tuberosum* L.) is the fourth most cultivated food crop after wheat, rice and maize (Moeinil *et al.,* 2011). It is a native of South America; in sixteenth century Spanish explorers introduced it in Europe and later it became an important food crop of the world (Khoso, 1988). In Pakistan during the year 2008 its production was 2.5 million metric tons (FAO, 2008). Conventionally, the crop is propagated asexually by tubers. However, this vegetative propagation contaminates tubers by different diseases resulting in poor quality and yields.

Alternatively, micropropagation methods are ideal for rapid multiplication of disease free material in masses. Meristem tip culture is an effective method for

Corresponding author: aneelayasmin@yahoo.com

the production of virus free plants (Badoni and Chauhan, 2009) as meristems are virus free. In addition, *in vitro* methods can be used for conservation, storage and easy distribution of potato germplasm in the form of breeding lines, new varieties and microtubers.

Although, there are many reports on potato micropropagation (Yousef *et al.*, 2001; Badoni and Chauhan, 2009; Rahman *et al.*, 2010), It is a well known fact that the regeneration potential of micropropagated plants is genotype dependent (Abe and Futsuhara, 1986). These researchers have standardized protocols for potato genotypes other than Desiree and Patrones. Thus, the present investigation was carried out to optimize the best combination of growth regulators (GRs) for the multiplication of local potato varieties Desiree and Patrones using meristem tips as explants; the most regenerative variety could be then efficiently micropropagated for commercial purposes and molecular studies.

MATERIALS AND METHODS

Plant material and mother culture initiation

Dormant tubers of two potato cultivars cv. Desiree and Patrones were surface sterilized using 10% commercial bleach (sodium hypochlorite- NaOCl) and treated with GA_3, at 50 ppm for 1 hour to break the dormancy as described by Quraishi *et al.* (1994). The treated tubers were stored at 10 ^{0}C which furnished the sprouts with distinct nodes and internodes within 12 days. Dissected segments of sprouts were used as the experimental plant material and sterilized as described by Yasmin *et al.* (2011a and b) with minor modifications. The explants were surface sterilized with 10% commercial bleach containing three drops of polyoxyethylene sorbitan monolaurate (Tween-20) for 10 minutes. The sprouts were rinsed 3-times with sterile distilled water under the clean bench. The surface sterilized sprouts were cut into one cm long cuttings carrying a single axillary bud and were planted on MS solid (8 g L^{-1}) basal media (Murashige and Skoog, 1962), 5% coconut water (CW) and 3% table sugar. The pH of the media was adjusted to 5.8 before autoclaving using 1M NaOH and HCl. After inoculations, the cultures were kept at the temperature 30$\pm$2 ^{0}C with a photoperiod of 16 hours light for two weeks.

Initiation of meristem culture

After raising the plantlets for two weeks at high temperature, the meristem tips measuring 0.3-0.5 cm were excised and cultured on fresh MS solid medium containing nine different combinations (C1-C9) of GRs i.e. Benzylamino Purine (BAP: 0.5, 1.0 and 2.0 mg L^{-1}) with Naphthalene Acetic Acid (NAA: 0.5, 1.0 and 2.0 mg L^{-1}) and six different treatments containing gibberellic acid (GA_3: 0.25, 0.5, 1.0 mg L^{-1}) and pantothenic acid (PA: 1.0 and 2.0 mg L^{-1}) such as T0: without growth regulators; T1: 0.25, 1.0 mg L^{-1}; T2: 0.25, 2.0 mg L^{-1}; T3: 0.5, 1.0 mg L^{-1}; T4: 0.5, 2.0 mg L^{-1}; T5: 1.0, 1.0 mg L^{-1}; T6: 1.0, 2.0 mg L^{-1}, respectively. The cultures were kept at 22$\pm$2 ^{0}C with a photoperiod of 16 hours light. This experiment was performed in three replicates and each replicate had 20 explants

per treatment. Data was collected during 6-weeks after meristem tip culture initiation. To evaluate the effect of different combinations of GRs on potato meristem tip culture, different *in vitro* growth traits as days took to initiate first shoot and root, plantlet height, number of shoots per explant and number of roots per explant were studied. The mean and standard deviation of different parameters were calculated as implemented in the R-software (R Development Core Team, 2010).

RESULTS AND DISCUSSION

Present investigation was carried out to evaluate the effect of different combinations of GRs on the *in vitro* regeneration potential of two important potato cultivars. The two cultivars Desiree and Patrones were propagated *in vitro* initially through shoot tips which were later used to start meristem tip culture. The best regeneration potential of the meristem tips of variety Desiree was observed when MS medium was supplemented with 1.0 mg L^{-1} pantothenic acid and 0.5 mg L^{-1} GA_3 (Table 1). This supplement of GRs took minimum time for regeneration of multiple shoots and roots on meristem tips of variety Desiree. Potato variety Patrones did not show consistent response to any single treatment.

Table 1. Effect of pantothenic acid and gibberellic acid on *in vitro* growth pattern of two potato cultivars regenerated from meristem tips.

Cultivars	Treatments* (mg L^{-1})			Mean days to shoot induction	Mean days to root induction	Mean plant height (cm)	Mean shoot plantlet^{-1}	Mean root plantlet^{-1}
	#	GA_3	PA					
Desiree	T0	0	0	11.5±1.1	21.1±1.5	7.3±0.3	5.1±1.0	2.1±1.9
	T1	0.25	1.0	6.5±0.7	30.8±0.1	5.0±0.9	7.1±0.3	7.6±0.3
	T2	0.25	2.0	7.3±0.8	32.3±0.3	6.5±1.2	7.1±0.5	9.2±0.7
	T3	0.5	1.0	4.3±0.5	16.7±0.9	11.1±0.9	10.5±0.1	15.7±0.9
	T4	0.5	2.0	7.1±0.3	22.5±0.3	8.1±0.1	6.1±0.9	8.5±1.1
	T5	1.0	1.0	6.3±0.2	29.7±0.1	5.9±0.3	8.1±1.0	7.9±0.5
	T6	1.0	2.0	6.1±1.3	30.6±0.4	6.3±0.5	7.9±0.5	6.1±0.7
Patrones	T0	0	0	15.1±2.0	25.9±1.6	4.2±1.5	3.0±1.2	1.4±1.3
	T1	0.25	1.0	8.4±0.3	35.1±0.1	5.1±1.0	6.3±0.3	5.9±0.2
	T2	0.25	2.0	6.9±0.1	39.2±0.5	5.9±0.5	5.9±1.2	4.2±0.1
	T3	0.5	1.0	5.1±0.6	33.7±0.5	6.0±0.1	8.2±0.9	10.1±0.8
	T4	0.5	2.0	6.5±0.9	29.3±0.9	8.3±0.3	4.9±0.1	8.9±0.3
	T5	1.0	1.0	7.9±1.1	35.1±1.3	7.2±1.0	5.0±0.1	7.0±0.5
	T6	1.0	2.0	7.1±1.3	31.5±1.1	6.7±0.5	5.8±1.5	5.8±1.3

Note: The data presented in Table 1 was collected during 6-weeks of *in vitro* culture. Each mean value represents 20 explants per treatment in 3-replicates.
Treatments*: Different concentrations of GRs (Gibberellic Acid- GA_3 and Pantothenic Acid-PA) are in mg L^{-1}.

Propagation of nodal cuttings on hormone free media

Initially potato *in vitro* culture was started from nodal cuttings and maintained on a hormone free media at 30 ^{0}C for 2-weeks. Although, media was free from synthetic GRs, 5% coconut water was used to provide undefined hormonal supplement for the rapid growth and to reduce the cost of protocol. In present study, the basic purpose of the mother culture initiation was to keep shoot tips in high temperature (30 ^{0}C) for the eradication and elimination of viruses. Usually virus free stocks are produced by meristem culture initiated in high temperature and or virus inhibitory chemicals are added in medium (Wang and Hu, 1985). There are several reports for the use of hormone free MS medium during potato proliferation (Ahsan *et al.,* 2003; Yasmin *et al.*, 2011c). However, the growth of explants is slow in such hormones free, cost effective media. Otherwise, the growth rate of explant can be improved by supplementing medium with growth regulators (Yousef *et al.*, 2001; Hoque, 2010). In our case, the growth of cultured shoot tips on MS solid media supplemented with CW was satisfactory. After 2-weeks the meristem tips from these mother cultures were excised and planted on MS solid medium with fifteen different treatments of GRs as described in materials and methods at 22$\pm$2 ^{0}C for 6-weeks.

Effect of PA and GA_3 on potato meristem tip culture

The two combinations of GRs i.e. BAP with NAA and GA_3 with pantothenic acid, evaluated in this study, were selected from previous reports on potato micropropagation (Quraishi *et al.*, 1994; Yousef *et al.*, 2001; Hoque, 2010) and optimized for two important local potato cultivars Desiree and Patrones. It is very obvious from the results shown in Table 1 and Figure 1 that the combination (T3) 1.0 mg L^{-1} pantothenic acid and 0.5 mg L^{-1} gibberellic acid induced the highest number of shoots and roots in studied varieties. Variety Desiree consistently responded the best to the all studied growth traits on T3, i.e. days to induce shoots (4.3), days to induce roots (16.7), plant height (11.1 cm), number of shoots (10.5 plantlet^{-1}) and roots (15.7 plantlet^{-1}). Although variety Patrones also revealed the lowest time for shoot induction (5.1) and highest number of shoots (8.2 plantlet^{-1}) and roots (10.1 plantlet^{-1}) on T3, lowest time for root induction (29.3) and highest length of plantlets (8.3) were achieved on T4. In addition, root induction was very early in T0- MS media without GR (control); whereas, the number of roots was the lowest in T0 as compared to other treatments. Genotypes were found detrimental for *in vitro* growth responses; it is not possible to micropropagate both cultivars on the same combination of GR. Pereira and Fortes (2003) reported MS liquid medium supplemented with 0.25 mg L^{-1} gibberellic acid and 5.0 mg L^{-1} pantothenic acid as the most suitable regime for potato micropropagation. Águila *et al.* (2001) cultured potato meristems on MS media supplemented with 1 mg gibberellic acid per liter in solid MS media. In agreement to above studies cultivars Desiree and Patrones also responded well when solid MS medium was supplemented with gibberellic acid (0.5 mg L^{-1}) and pantothenic acid (1.0 mg L^{-1}). However, the optimized concentration of GA_3 and pantothenic acid is different in this study as compared to reported studies possibly due to varietal differences. Moeinil *et al.* (2011) reported the maximum

growth of potato plantlets on MS solid medium with 0.25 mg L^{-1} GA_3 and 2 mg L^{-1} calcium pantothenate. Nevertheless, Hoque (2010) showed the best shoot and root regeneration on MS medium with 2 mg L^{-1} KIN and IAA, whereas Badoni and Chauhan (2009) detected lower concentration of NAA (0.01 mg L^{-1}) with Gibberelic Acid (0.25 mg L^{-1}) as the best combination for the regeneration of complete plantlets from meristem tips.

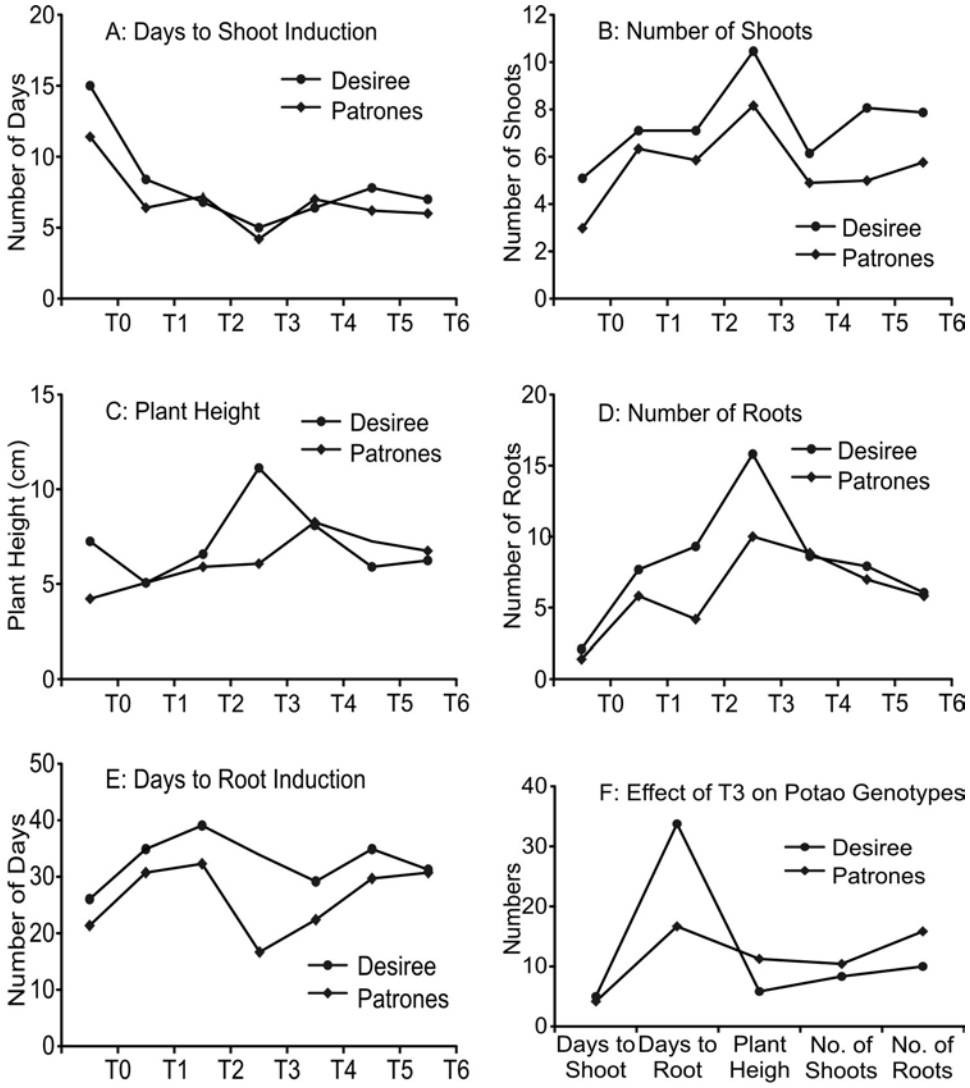

Figure 1. Effect of different treatments on mean performances of *in vitro* growth parameters of two potato cultivars (A - E). Graph F represents the response of two potato genotypes in T3 (1.0 mg L^{-1} pantothenic acid plus 0.5 mg L^{-1} gibberellic acid), treatment.

Effect of BAP and NAA on potato meristem tip culture

Nine different combinations of BAP (0.5, 1.0 and 2.0 mg L^{-1}) and the auxin NAA (0.5, 1.0 and 2.0 mg L^{-1}) were added to MS medium and evaluated for their effect on meristem tips. The results were interesting as shoots were regenerated but callus was formed at the base of explants. Two plantlets in the variety Patrones and three in the variety Desiree were regenerated having no calli at the basal end of explants when the combination of 0.5 mg L^{-1} BAP + 0.5 mg L^{-1} NAA was used. Otherwise, none of the observed explants formed complete plantlet with full root system. Although, Yasmin *et al.* (2011a) demonstrated the use of BAP for callus induction. These observations are in contrast to Yousef *et al.* (2001); they observed longest main shoot and highest node numbers in medium containing NAA and BAP. BAP stimulates apical dominance and the growth of lateral buds, whereas NAA decreases single nodes growth and rooting of potato plantlets (Moeinil *et al.,* 2011). It is difficult to speculate the reason for this difference; one factor could be the effect of potato genotypes.

Moeinil *et al.* (2011) demonstrated the effects of different combinations of NAA and BAP on both rooting and growth of Agria and Marfona cultivars of potato. In contrast to this study they supplimented MS medium with GA_3 (0.25 mg L^{-1}) and calcium pantothenate (2.0 mg L^{-1}) in addition to different concentration NAA and BAP. Even then they observed that the best medium for rooting and shooting is modified solid (MS) medium without NAA and BAP. In the presence of BAP and NAA shooting and rooting of single nodes was decreased. However, we also observed the negative effect of BAP and NAA on plant regeneration but it was severe as compared to the study of Moeinil *et al.* (2011). In our case callus was formed at the base of explants and rooting was not initiated. The only explaination for such differences seems to be genotypic difference as they have used the same concentrations of NAA and BAP as was used in this study.

CONCLUSION

A genotype dependent protocol for the micropropagation of our two local potato cultivars Patrones and variety Desiree was optimized. This protocol will provide the base for the mass production of studied cultivars through *in vitro* techniques. The authors would like to extend this study by investigating the factors inducing microtuberization in micropropagated potato plantlets and their virus indexing for the production of virus free potato plantlets and minitubers.

REFERENCES

Abe, T. and Y. Futsuhara. 1986. Genotypic variability for callus formation and plant regeneration in rice. Theor. Appl. Genet., 72: 3-10.

Ahsan, N., A. Hossain, M. F. Alam, M. M. Hossain, R. Islam and R. S. Sultana. 2003. Virus-free potato tuber seed production through meristem culture in tropical Asia. Asian J. of Plant Sci., 2 (8): 616-622.

Águila, G. L., S. Z. Hernández, P. T. Moya, T. and B. P. Mederos. 2001. Meristem culture for the elimination of the virus S of the potato in plants cultivated. Biotecnología Vegetal., 1 (2): 117-119.

Badoni, A. and J. S. Chauhan. 2009. Effect of growth regulators on meristem-tip development and *in-vitro* multiplication of potato cultivar 'Kufri Himalini'. Nature and Sci., 7 (9): 31-34.

FAO. 2008. Home page on internet. Available on the: http://www.FAO.org.

Hoque, M. E. 2010. *In-vitro* regeneration potentiality of potato under different hormonal combination. World J. of Agric. Sci., 6 (6): 660-663.

Khoso, A. W. 1988. Growing vegetables in Sindh (1st Ed.). M. Ismail Khoso Pub. Co., Tandojam, Pakistan.

Moeinil, M. J., M. Armin, M. R. Asgharipour and S. K. Yazdi. 2011. Effects of different plant growth regulators and potting mixes on micro-propagation and mini-tuberization of potato plantlets. Adv. Environ. Bio., 5 (4): 631-638.

Murashige, T. and F. Skoog. 1962. A revised medium for rapid growth and bioassays with tobacco tissue cultures. Physiologia Plantarum, 15: 473-497.

Pereira, J. E. S. and G. R. Fortes. 2003. Protocol for potato propagative material production in liquid medium. Pesquisa Agropecuária Brasileira, 38 (9): 1035-1043.

Quraishi, A., Z. Chaudhry, H. Rashid and P. Khaliq. 1994. Pre-basic seed potato production through tissue culture. Pak. J. Agric. Res., 15 (1): 8-13.

R Development Core Team. 2010. R: A language and environment for statistical computing. ISBN 3-9000051-07-0, URL: http://www.r project.org

Rahman, M. H., R. Islam, M. Hossain and M. S. Islam. 2010. Role of sucrose, glucose and maltose on conventional potato micropropagation. J. Agric. Tech., 6 (4): 733-739.

Wang, P. J. and C. V. Hu. 1985. *In-vitro* mass tuberization and virus free seed potato production in Tiwan. Amer. Pot. J., 59: 33-39.

Yasmin A., A. Jalbani and R. Kumar. 2011a. Regeneration potential of *benzyl amino purine* induced calli of *Solanum tuberosum.* Pak. J. Agri., Agril. Engg., Vet. Sci., 27 (1): 13-17.

Yasmin A., A. Jalbani, G. S. Mangrio and A. Nasreen. 2011b. Optimization of microtuberization in indigenous potato cv. Desiree. Pak. J. Biotech., 8 (2): 39-44.

Yousef, A. A. R., M. A. Suwwan, A. M. Musa and H. A. Abu-Qaoud. 2001. *In-vitro* culture and microtuberization of spunta potato (*Solanum tuberosum*). *Dirasat* Agric. Sci., 24: 173-181.

(Received 13 July, 2011; Revised 31 December, 2011)