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The Effects of Different Concentrations and Combinations of Growth Regulators on the Callus Formation of Potato (*Solanum tubrosum*) Explants

[1]Shahab-ud-din, [1]I.N. Sultan, [1]M.A. kakar, [1]A. Yousafzai, [1]F.A. Sattar,
[1]F. Ahmmad, [2]S.M. Ibrahim, [3]M. Hassanullah and [4]B. Arif
[1]Balochistan University of Information Technology Engineering and Management Sciences (BUITEMS), Quetta, Pakistan
[2]National Centers of Excellence in Molecular Biology, University of the Punjab, Lahore Pakistan
[3]Quaid-i-Azam University Islamabad, Pakistan
[4]Directorate General Agriculture Extension Balochistan, Pakistan

Abstract: The main objective of the present study was to investigate the effects of different concentrations of plant growth regulators and their combinations on callus induction of potato (*Solanum tuberosum* L.). The explants of potato tuber were cultured on Modified Murashige and Skoog medium which was supplemented with different concentrations of 2,4-Dichlorophenoxy acetic acid (2,4-D), α-naphthalene Acetic Acid (NAA), Benzyl Adenine (BA), 2,4-D in combinations with BA and NAA in combination with BA for callus induction. The Concentration of sucrose was 3% W/V level and the pH of the media was adjusted to 5.7 before the addition of agar 8% W/V. The explants were first dissected out aseptically and then inoculated to the media (with various levels of hormones), then incubated at 27±2ºC in the culture room. Among the treatments 2, 4-D at different concentrations produced different degree of callus but comparatively a massive amount of callus was formed on MS medium supplemented with 2, 4-D alone at 3.0 mg/L. Also NAA and BA with different concentrations produced considerable degrees of callus but the degree of callus was best at higher concentrations of NAA and BA. 2, 4-D in combination with BA at 2.0 mg/L both produced considerable amount of callus. In case of NAA in combination with BA the degree of callus formation was best at concentration 1.0 mg/L each. So according to the above findings it was concluded that 2, 4-D is the best option for induction of callus among the other hormones used in the study.

Key words: Callus, explants, plant growth regulators, potato

INTRODUCTION

Cultivated potato (*Solanum tuberosum* L.) is one of the most important vegetable crops in the world (Solmon-Blackburn and Baker, 2001).

Potato (*Solanum tuberosum*) is one of the most economically important annual vegetable crops of Solanaceae family. It is the fourth most important crop by volume of production; it is high yielding, having a high nutritive value and gives high returns to farmers. Pakistan is the seventh largest potato producing country in the world. (Humera and Iqbal, 2010).

Potato is a crop of worldwide importance. It supplies at least 12 essential vitamins, minerals, proteins, carbohydrates and iron (Gray and Hughes, 1978; Thornton and Sieczka, 1980). Potato tubers give an exceptionally high yield per acre many times that of any grain crop (Burton, 1969) and are used in a wide variety of table, processed, livestock feed and industrial uses (Feustel, 1987). It is the fourth most cultivated food crop exceeded only by wheat, rice and maize in world production for human consumption (Ross, 1986). In Pakistan, it is grown on an area of 104.5 thousand ha with 1684.7 thousand tons production per annum (MINFAL, 2004). Potato (*Solanum tuberosum* L.) is cultivated as a vegetable and cash crop in Pakistan. It produces the largest quantity of carbohydrates per day and per unit area among food crops (Zaag and Harton, 1983). Methodologies for in vitro culture have contributed to vegetative propagation of many plant species (Murashige, 1977; Abo-el-Nil, 1977), and have emerged as an alternative to reduce the cost of production of virus free seed needed to be produced year around (Abbot and Belcher, 1986).

Plant tissue culture is recognized as a source to generate useful genetic variability (somaclonal variation) for crop improvement (Larkin and Scowcroft, 1981; Karp and Bright, 1985; Evans *et al*., 1989; Brar and Jain, 1998). Biotechnology can contribute to solution of these problems and realize great benefit to potato farmers. The

Corresponding Author: Shahab-ud-din. Balochistan University of Information Technology, Engineering and Management Sciences (BUITEMS), Quetta, Pakistan. Tel.: +92-334-2384766.

regeneration of plants from cell and tissue culture represent an essential component of biotechnology and have the potential not only to improve the existing cultivars, but also for the generation of novel plants in a comparatively short time compared to conventional breeding (Khadiga *et al*., 2009)

Potato tubers give an exceptionally high yield per acre many times that of any grain crop (Burton, 1969) and are used in a wide variety of table, processed, livestock feed and industrial uses (Feustel, 1987).

Tissue culture offers an excellent technique for rapid multiplication of potato plant (Tovar and Dodds, 1986). Majority of commercial potato cultivar in uses e.g. Ultimus, Desiree, Malta, Cardinal and Diamon, are being imported in Pakistan, every year in the form of virus free seed. The same seeds could be prepared in Pakistan by tissue culture techniques. Much works has been carried out on potato to enhance callus induction, improve the frequency of plant regeneration from the callus and investigate the factors affecting plant regeneration. Both callus induction and plant regeneration from explants require the presence of appropriate concentrations and combinations of plant growth regulators in the culture media. Many researchers work to standardize the optimum concentrations of growth regulators for regeneration of potato and consequently great progress has been made in potato callus induction and plant regeneration (Ahloowalia, 1982; Dobranszki *et al*., 1999; Hansen *et al*., 1999; Ehsanpour and Jone, 2000; Fiegert *et al*., 2000; Khatun *et al*., 2003; Yasmin *et al*., 2003; Shirin *et al*., 2007). Therefore, it is important to develop a protocol through the callus of potato varieties that are grown in Pakistan. It is because of the fact that the plants regenerated through callus are free from pathogens and genetically identical. The callus culture also make possible to produce a huge number of plantlets in short interval of time.

The main objective of the study was to conduct a protocol for callus induction of the potato cultivar widely grown in Pakistan. The study was done to show the influence of auxins and cytokinin on callus induction of potato. A comparative analysis of the effects of growth regulators on callus induction was also observed and discussed.

MATERIALS AND METHODS

This research work was carried out in Balochistan University of Information Technology Engineering and Management Sciences Quetta, at the Faculty of life Sciences and informatics. The duration of the research work was from Jun 2009 to December 2009. The internodal explants of potato were used for the purpose of callus induction.

Preparation of plant material: Potato explants were cut at the nodal section of stems, about 2-3 cm in length. Plant materials (explants) were prepared by washing in detergent-water mixture for about 20 min. This will help remove fungi etc. The washed plant materials were later transferred to sodium hypochlorite solution in which few drops of tween-20 were also added. The explants material was kept in this bleach solution for 20 min and then the bleach was poured off. Then the explants were placed in 70% alcohol solution for 10 min. After rinsing and washing, the potato explants were washed with sterilized distilled water and kept in laminar air flow chamber for further process.

Transfer of plant material to tissue culture medium: The ultra violet light was turned off just before the work started. All the culturing dishes, prepared plant material, scalps and forceps were kept in the laminar airflow chamber. All the equipments like the gloves, scalps and forceps were continuously sprayed with 70% alcohol solution and hands rubbed together to spread the alcohol. The sterilized explants were removed from the sterile water and placed on the paper or sterile Petri dish. The explants were cut in a size of 2 to 3 cm. Take the prepared section of plant material in sterile forceps and place into the medium. Potato explants were partly submerged in the medium. The nodal portion was facing up. The caps of the dishes were replaced tightly.

Growing the explants: The jars that contain explants were placed in the shelves of culture room where favorable temperature was provided. To fulfill the requirement of sunlight, grow lights were kept on for 16 h/day. The temperature of the culture room was up to 28ºC. The jars were daily checked so that if there was any contamination, it could be discarded on time. As the callus formed the data was taken. The color and texture of callus were also recorded.

Inoculation and callus induction: Explants were cultured in Petri dishes containing MS (Murashige, 1977) basal media supplemented with different concentrations of auxins (2, 4-D and NAA), cytokinins (BA), Combinations of 2, 4-D with BA and NNA with BA for callus induction. The explants were inoculated on callus induction media for 4-6 weeks. The callus was transferred to the fresh media every 21 days interval for further proliferation and maintenance.

RESULTS AND DISCUSSION

The internodal plant materials (explants) of potato were cultured on MS medium that contains different concentrations of Auxins (2, 4-D, NAA) and cytokinines (BA) alone, 2, 4-D in combination with BA (Cytokinine)

Table 1: Effect of different concentrations of α-naphthalene acetic acid (NAA), 2, 4-dichlorophenoxy acetic acid (2, 4-D) and benzyl adenine (BA) on the callus induction of potato.15 explants were inoculated in each treatment

Growth regulator	Concentration of growth regulator	Days to callus initiation	% of explants formed callus	Callus color	Degree of callus formation
2,4-D	1.0	21	10	C	+
	1.5	19	20	B	+
	2.0	18	40	LB	++
	2.5	18	50	LB	++
	3.0	17	70	B	+++
	3.5	16	55	B	++
	4.0	14	45	B	+
NAA	1.0	20	-	-	-
	1.5	20	10	W	+
	2.0	20	30	LY	+
	2.5	18	20	W	+
	3.0	15	40	YL	++
BA	1.0	16	2	G	+
	2.0	18	20	G	+
	3.0	19	50	G	+
	4.0	19	30	G	++
	5.0	19	30	G	++

B = brown, LB = light Brown, C = creamy, Gr B = Greenish Brown, LG = light green, W = white, Y = yellow, LY = light yellow, - = No callus, + = slight callus, ++ = Moderate callus, +++ = Massive callus

Table 2: Effects of α-naphthalene acetic acid (NAA) in combination with benzyl adenine (BA) on callus induction of potato

Growth regulator	Concentration of growth regulator		Days to callus initiation	% of explants formed callus	Callus color	Degree of callus formation
Naa & Ba	0.5	0.5	13	20	W	+
	0.5	1.0	11	20	W	+
	1.0	0.5	10	40	W	++
	1.0	1.0	23	50	W	+++
	1.5	0.5	11	30	W	+
	1.5	1.0	12	20	W	+

B = Brown, C = creamy, Gr B = Greenish Brown, LG = light green, W = white, Y = yellow, LY = light yellow, - = No callus, + = slight callus, ++ = Moderate callus, +++ = Massive callus

and BA (Cytokinine) in combination with NAA (Auxin). The data was anatomized after six weeks of the inoculation of the explants. During the analysis of data, days to callus initiation, the percentage of explants that formed callus, days to callus initiation, callus color and degree of callus formation were examined. (Table 1, 2 and 3).The callus initiation of invitro cultured explants could be observed at all the hormones combinations after 8-21 days. But with out any growth regulator, the explants did not show any callus formation on MS medium.

Among all the concentrations and combinations, 3.0 mg/L 2, 4-D either used alone or in combination with BA was found to be the most effective auxin concentration for callus induction in all the explants that formed callus. The percentage of explants that formed callus at this concentration was found to be 70% (Table 1 and Fig. 2a). These results are in support of (Khadiga *et al.*, 2009; Shirin *et al*., 2007; Castllo *et al*., 1998). They reported that Auxin 2, 4-D by itself or in combination with cytokinine has been widely uses to enhance callus induction and maintenance. Many researchers complied 2, 4-D as the most effective auxin for callus induction as common as in monocot and even dicot (Evans *et al*., 1989; Ho and Vasil, 1983; Jaiswal and Naryan, 1985; Chee, 1990; Mamun *et al*., 1996). 2, 4-D which is an Auxin, when used alone at different concentrations, the highest degree of callus was formed when the MS medium was supplemented with 3.0 mg/L of the growth regulator (2, 4-D) (Table 1 and Fig. 2a). The above results are in convention with (Shirin *et al*., 2007) in which 2, 4-D was used for the induction of callus from leaf and internodal explants. These explants were obtained from four potato cultures including Diamant. It was concluded that 3.0 mg/L was found to be the most effective auxine concentration for callus induction.

Among the different concentrations of NAA (Auxin) the highest callus forming rate was recorded in the MS medium that was supplemented with 3.0 mg/L. the percentage of explants that formed callus was 40% (Table 1 and Fig. 2b).

On the other hand a combination of NAA (Auxin) and BA (cytokinine) the highest callusing rate i.e. 50% was examined for potato explants in medium containing 1.0 mg/L NAA and 1.0 mg/L of BA (Table 2). These results are in agreement with (Shirin *et al*., 2007) (Table 2 and Fig. 2c).

The BA (cytokinine) is also an eminent growth regulator for callus induction, when used alone or in combination with 2, 4-D. The MS medium when supplemented with different concentrations and combinations of BA and 2, 4-D, the best result was observed when 2, 4-D at 2.0 mg/L was used in

Table 3: Effects of α-naphthalene acetic acid (NAA) in combination with benzyl adenine (BA) on callus induction of potato

Growth regulator	Concentration of growth regulator		Days to callus initiation	% of explants formed callus	Callus color	Degree of callus formation
2,4-Dδ Ba	0.5	0.5	-	-	-	-
	1.0	1.0	15	30	Y	++
	2.0	2.0	13	40	G	+++
	3.0	3.0	12	50	G	++

B = brown, LB = light Brown, C = creamy, Gr B = Greenish Brown, LG = light green, W = white, Y = yellow, LY = light yellow, - = No callus, + = slight callus, ++ = Moderate callus, +++ = Massive callus

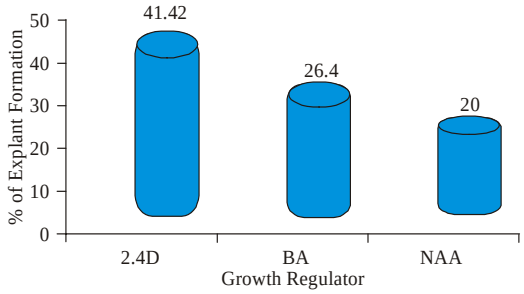

Fig. 1: A comparative analysis amongst the effects of auxins (2, 4-D and NAA), and cytokinin (BA) on potato explants callus formation

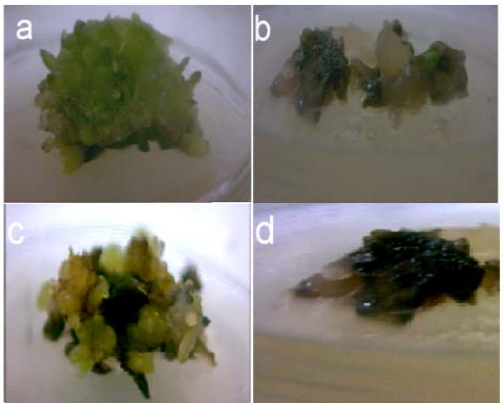

Fig. 2: Callus Formation of from explants of potato (*Solanum tuberosum*) in MS medium supplemented with different concentrations of 2, 4-D, NAA, BA

combination with BA at the same concentration i.e., 2.0 mg/L. (Table 3 and Fig. 2d). These results are in support of (Khadiga *et al*., 2009). They recorded that the MS medium when supplemented with different combinations of BA and 2, 4-D, Callus initiation was observed between 7-20 days depending on concentrations and combinations. The best result for degree of callus formation was obtained when 2, 4-D at 2.0 mg/L was used in combination with BA at the same concentration. Figure 1 shows a comparative analysis of Auxins and cytokinin. This simple bar chart amongst the effects of auxins (2, 4-D and NAA), and cytokinin (BA), shows that 2, 4-D is the most effective growth regulator for callus initiation in potato explant (41.42/46.5 %), however BA with 27.60/31.0 % is comparatively a better growth regulator than NAA with 20.00/22.5.

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REFERENCES

Abbot, A.J. and A.R. Belcher, 1986. Potato Tuber Formation *in vitro*. In: Wither, L.A. and D.G. Alderson, (Eds.), Plant Tissue Culture and its Agriculture Application, Butterworths, London. pp: 113-122.

Abo-el-Nil, M.N., 1977. Plant cell tissue culture as a mean for the development of horticultural varieties for the arid zone. Proc. 1st Agric. Conf. Muslim Scientists, Riyadh, Saudi Arabia, pp: 307-323.

Ahloowalia, B.S., 1982. Plant regeneration from callus culture in potato. Euphytica., 31: 755-759.

Brar, D.S. and S.M. Jain, 1998. Somaclonal variation: Mechanism and application in crop improvement. In: Jain, S.M., D.S. Brar and B.S. Ahloowalia, (Eds.), Somaclonal Variation and Induced Mutations in Crop Improvement. Kluwer Academic Publishers, London, pp: 15-37.

Burton, W.G, 1969. Potato. Encyclopaedia Britannica, Benton, Chicago, pp: 95-134.

Chee, P.P., 1990. High frequency of somatic embryogenesis and recovery of fertile cucumber plants. Hort. Sci., 25: 792-793.

Dobranszki, J., H.A. Takacs, T.K. Magyar and A. Ferenczy, 1999. Effect of the medium on the callus forming capacity of different potato genotyes. Acta Agron. Hungarica., 47: 59-61.

Ehsanpour, A.A. and M.G.R. Jone, 2000. Evaluation of direct shoot regeneration from stem explants of potato (*solanum tuberosum* L.) cv. Delaware by thidiazuron TDZ. J. Sci. Tech. Agric. Natl. Res., 4: 47-54.

Evans, D.A., 1989. Somaclonal variation-genetic basis and breeding application. Trends Genet., 5: 46-50.

Feustel, I.C., 1987. Miscellaneous Products from Potatoes. In: Talburt, W.F. and O. Smith, 4th Edn., Potato Processing. Van Nostrand, New York, pp: 727-746.

Fiegert, A.K., W.G. Mix and K.D. Vorlop, 2000. Regeneration of *Solanum tuberosum* L. Tomensa, Induction of somatic embryogenesis in liquid culture for the production of artificial seed. Landbauforsch. Volk., 50: 199-202.

Gray, D. and J.C. Hughes, 1978. Tuber Quality. In: Harris, P.M., (Ed.), The Potato Crop Halsted Press, New York, pp: 511.

Hansen, J., B.S.V. Nielsen and S. Nielsen, 1999. In vitro shoot regeneration of Solanum tuberosum cultivars interactions of medium composition and leaf, leaflet and explant position. J. Natl. Sci. Foundat. Srilanka., 27: 17-28.

Ho, W.O. and I.K .Vasil, 1983. Somatic embryogenesis in sugarcane (*Saccharum officinarum* L.) the morphology and physiology of callus formation and the ontogeny of somatic embryos. Protoplasm., 118: 169-180.

Humera, A. and J. Iqbal, 2010. *In vitro* techniques and mutagenesis for the genetic improvement of potato cvs. Deseree and diament. Pak. J. Bot., 42: 1629-1637.

Jaiswal, V.S. and P. Naryan, 1985. Regeneration of plantlets from the callu of stem segment of adult plants of *Fucus religosia* L. Plant Cell Reports., 4: 256-258.

Karp, A. and S.W.J. Bright, 1985. On the causes and origins of somaclonal variation. Oxford Surv. Plant Molecu. Cell Biol., 2: 199-234.

Khadiga, G.A.E., S.M. Rasheid and M.M. Khalafalla, 2009. Effect of plant growth regulators on callus induction and plant regeneration in tuber segment culture of potato (*Solanum tuberosum* L.) cultivar Diamant, Afr. J. Biotechnol., 8: 2529-2534.

Khatun, N., M.A. Bari, R. Islam, S. Huda, N.A. Siddque, M.A. Rahman and M.U. Mullah, 2003. Callus induction and regeneration from nodal segment of potato cultivar Diamant. J. Biol. Sci., 3: 1101-1106.

Larkin, P.J. and W.R. Scowcroft, 1981. Soamclonal variation-a novel source of variability from cell cultures for protoplast improvement. Theor. Appl. Genet., 60: 197-214.

Mamun, A.N.K, R. Islam, M.A. Reza and O.I. Joadar, 1996. In vitro differentiation of plantlet of tissue culture of Samonea saman. Plant Tissue Cult., 6: 1-5.

MINFAL, 2004. Agriculture Statistics of Pakistan. Government of Pakistan, Ministry of Food, Agriculture and Livestock Economic wing, Islamabad.

Murashige, T., 1977. Plant cell and organ cultures as horticultural practices. Acta Hort., 78: 17-30.

Ross, H., 1986. Potato Breeding-Problems and Perspectives. J. Plant Breed. Supplement., 13: 1-132.

Shirin, F., M. Hossain, M.F. Kabir, M. Roy and S.R. Sarker, 2007. Callus induction and plant regeneration from internodal and leaf explants of four Potato (*Solanum tuberosum* L.) cultivars. World J. Agric. Sci., 3(1): 01-06.

Solmon-Blackburn, R.M. and H. Baker, 2001. Breeding resistance virus potatoes (*Solanum tuberosum* L.) a review of traditional and molecular approaches. Heredity., 86: 17-35.

Thornton, R.E. and J.B. Sieczka, 1980. Commercial potato production in North America. Am. Pot. J., 57: 534-536.

Tovar, P. and J.H. Dodds, 1986. Tissue Culture Propagation of Potato. CIP Slide Training Series 1-5 Int. Potato Center, Department of Training and Communications, P.O. Box. 5659, Lima, Peru.

Yasmin, S., K.M. Nasiruddin, R. Begum and S.K. Talukder, 2003. Regeneration and establishment of potato plantlets through callus formation with BAP and NAA. Asian J. Plant Sci., 2(12): 936-940.

Zaag, D.E. and D. Harton, 1983. Potato prospective with special reference to the tropics and sub-tropics. Potato Res., 26: 323-328.