Plant Environment Development, Vol. 9(1): 20-27, 2023 (June)
Department of Botany, University of Rajshahi,
Rajshahi 6205, Bangladesh
E-mail: plant.environ.dev@ru.ac.bd

Print ISSN 1994-1501
Online ISSN 2311-3529
http://plantenvdev.com

Research Paper

Indirect organogenesis of potato (*Solanum tuberosum* L.) cultivars Japanese Red, Cardinal and Asterix

Umme Qulsum*, M Monzur Hossain and AKM Rafiul Islam
Department of Botany, Faculty of Biological Sciences, University of Rajshahi, Bangladesh
*Corresponding author: qulsum@ru.ac.bd, uqulsum31@gmail.com

Abstract

The study was conducted to investigate the effects of different concentrations and combinations of growth regulators on callus induction and plant regeneration of three potatoes (*Solanum tuberosum* L.) cultivars viz. Japanese Red, Cardinal and Asterix. *In vitro* grown leaf segments were cultured on Murashige and Skoog (MS) medium supplemented with different concentrations of 2,4-Dichlorophenoxy acetic acid (2,4-D), in addition different combinations and concentrations of naphthalene acetic acid (NAA) with benzyl adenine (BA) for callus induction. The best degree for callus formation (80%) was obtained on MS medium supplemented with 2,4-D (2.50 mg/l) in Japanese Red followed by Asterix (70%) and Cardinal (50%) in 3.0 mg/l. For NAA + BA combinations highest callus induction showed in Japanese Red (70%) in leaf explants followed by Asterix, (60%) in 1.0 mg/l NAA + 1.0 mg/l BA. In case of Cardinal (30%) was observed in 1.5 mg/l NAA + 0.5 mg/l BA combination. The calli from the leaves of all three potato cultivars produced multiple shoots in media with 3.0 mg/l BA + 0.1 mg/l NAA and 4.0 mg/1 KIN + 0.5 mg/1 NAA.

Keywords: Potato, Plant growth regulator, Leaf explants, Callus induction.

INTRODUCTION

Potato (*Solanum tuberosum* L.) of the family Solanaceae is one of the economically valuable vegetables worldwide (Solmon-Blackburn and Baker 2001). It is an economically important food crop of Bangladesh and all over the world. It is cultivated in winter season. At present potato is grown in about 243 thousand hectares of land in Bangladesh (DAE, 2002). It is now one of the most important vegetables in Bangladesh (Ahmad, 1977). In Bangladesh, it is the third most important crop after rice and wheat in respect of nutrient (Siddique *et al*., 2015). In the developing countries, good quality potato production is hampered by limited potato production, inadequate supply of high quality seeds, insufficient storage facilities and short growing season (Hajare *et al*.,2021; Azad *et al*., 2020; FAO 2009; Islam and Alsadon, 2003). The average production of potato in Bangladesh is only 13.32-18.08 ton/ha (BBS, 2011; BBS, 2012). Potato production can be increased upto 30 - 40 ton/ha by selecting high quality seeds, improved irrigation system, utilization of modern agricultural support and modern production technology (Chowdhury and Hasan, 2013; Siddique *et al*., 2015; Azad *et al*., 2020). The success of plant biotechnology relies on several factors which include an efficient tissue culture system for regeneration of plants from cultured cells and tissues (Hajare *et al*.,2021; Pua *et al.*, 1996). Shoot generation and rooting are important in the realization of the potential of the cell and tissue culture techniques for plant improvement (Purnhauser *et al.*, 1987). Plant regeneration in potato has been progressed a lot in recent years (Shirin *et al.*, 2007). Successful *in vitro* plant regeneration has been achieved from explants of different organs and tissues of potato such as leaf (Cearley *et al.*, 1997) stem (Garcia and Martinez 1995), tuber discs (Mozafri *et al.*, 1997) and unripe zygotic embryos (Pretova and Dedicova 1992).

The aim of the present study was to establish an effective protocol and analysis of different results for callus induction and rapid plant regeneration from leaf segment explants of three potato cultivars Japanese Red, Cardinal and Asterix for future study on somaclonal variation and gene transformation programme.

MATERIALS AND METHODS

Experiments were carried out in the Plant Breeding and Gene Engineering Laboratory of the Department of Botany, Faculty of Biological sciences, University of Rajshahi. The leaves (21 days old *in vitro* plant) of three exotic cultivars (Japanese Red, Cardinal and Asterix) of potatoes were used in this study.

Explants were cultured in culture tubes containing MS (Murashige and Skoog, 1962) basal media supplemented with different concentrations of 2,4-D (1.0, 1.5, 2.0, 2.5, 3.0, 3.5 mg/l) and combinations of NAA+BA (0.5+0.5, 0.5+1.0, 1.0+0.5, 1.0+1.0, 1.5+0.5, 1.5+1.0 mg/l) for callus induction and proliferation. The explants were inoculated on callus induction media for 4 - 6 weeks. The color and texture of callus were also recorded. The calli were transferred to the fresh callus inducing media every 21 days interval for further proliferation and maintenance. After 6 – 8 weeks of incubation in the dark, the callus induction frequency was determined and well developed calli were selected and sub-cultured on regeneration media. MS medium was supplemented with different concentrations and combination of BA–NAA (2.0+1.0, 2.0+1.5, 3.0+0.1, 3.0+0.5, 3.0+1.0, 3.0+1.5 mg/l) and KIN-NAA (3.0+0.1, 3.0+0.5, 3.0+1.0, 4.0+0.5, 4.0+1.0, 4.0+1.5 mg/l) for shoot regeneration at 25 ± 2℃ with a 16 h photo- period.

RESULTS

Leaf segments of *in vitro* grown three potato cultivars were cultured in MS medium supplemented with different concentrations of 2,4-D as well as various combinations and concentrations of NAA and BA. Different concentrations and combinations of plant growth regulator affect degree of callus induction, callus color and number of callus formation. The results are presented in **Table 1**.

The best degree of callus formation in Japanese Red (80%) was achieved on MS medium supplemented with 2,4-D alone at 2.5.0 mg/l (**Fig. 1A**).; in Cardinal (50%) and Asterix (70%) (**Fig. 1B**) on MS medium supplemented with 2,4-D alone at 3.0 mg/l. The lowest 30% and 20% callus formation were recorded in medium containing 1.0 mg/l 2,4-D in Japanese Red and Asterix respectively. In Cardinal lowest (10%) callus induction was recorded in medium containing 1.5 mg/l 2,4-D. The leaf explants of Cardinal produced no callus in medium with 1.0 mg/l 2,4-D. For NAA+BA combinations, the highest callus induction was found in Japanese Red (70%) and Asterix (60%) in medium having 1.0 mg/l NAA + 1.0 mg/l BA (**Fig. 1D**). The highest (30%) callus induction from leaf explants of cv. Cardinal was observed in media having 1.5 mg/l NAA + 0.5 mg/l BA (**Fig. 1C**). No callus was observed in the medium having 0.5 mg/l NAA + 0.5 mg/l BA. The lowest callus induction was recorded on MS media supplemented with 0.5 mg/l NAA + 0.5 mg/l BA and 0.5 mg/l NAA +1.0 mg/l BA.

The results of shoot regeneration from leaf explants derived callus is shown in **Table 2**. The highest 70%, 50%, and 60% calli were shown to stimulate shoot regeneration in the medium supplemented with 3.0 mg/l BA + 0.1 mg/l NAA (**Fig. 2A**) in Japanese Red, Cardinal, and Asterix respectively. In this study, we observed the lowest number of shoot regeneration in Asterix (10%) on the MS medium supplemented with 3.0 mg/l BA + 1.5 mg/l NAA followed by Japanese Red (20%) and Cardinal (10%) on MS media supplemented with 2.0 mg/l BA + 1.0 mg/l NAA. The highest number of shoot regeneration was found on MS media supplemented with 4.0 mg/l KIN + 0.5 mg/l NAA in Japanese Red (50%), Cardinal (30%) and Asterix (40%) (**Fig. 2B**). The lowest (10%) calli were induced to shoot regeneration in medium having 3.0 mg/l KIN + 0.5 mg/l NAA in Japanese Red followed by Cardinal and Asterix in 3.0 mg/l KIN + 0.1 mg/l NAA.

The combination of BA+NAA and KIN+NAA showed better performance for shoot regeneration of all potato cultivars for leaf explants-derived callus. Between these two combinations BA+NAA proved to be more effective than KIN+NAA for maximum shoot induction from leaf explant derived calli. The medium fortified with 3.0 mg/l BA+0.1 mg/l NAA was found to be the best formulations among the various treatments, where the multiple shoots development from leaf explants in Japanese Red, Cardinal, and Asterix were identified as 70%, 50%, and 60%, respectively. (**Table 2**).

Table 1. Effect of different concentrations of 2, 4-D and combinations of NAA with BA in MS medium on callus induction from leaf explants of different potato cultivars. In each treatment 10 explants were inoculated. Data were recorded after 5 weeks of culture.

Plant growth regulators (mg/l)	Cultivars								
	Japanese Red			Cardinal			Asterix		
	% of callus induction	Callus color	Degree of callus formation	% of callus induction	Callus color	Degree of callus formation	% of callus induction	Callus color	Degree of callus formation
2,4-D									
1.0	30	LB	+	-	-	-	20	G	+
1.5	40	LB	+	10	G	+	30	LG	+
2.0	50	GB	+	20	G	+	30	LG	+
2.5	80	GB	+++	30	LG	+	60	LG	+++
3.0	50	LB	+++	50	LG	++	70	G	+++
3.5	40	LB	+	20	G	+	40	G	++
Mean	**48.33**			**26**			**40**		
NAA+BA									
0.5+0.5	20	LB	+	-	-	-	30	G	+
0.5+1.0	30	LB	+	10	LG	+	40	LG	++
1.0+0.5	40	LB	+	20	LG	+	50	LG	++
1.0+1.0	70	GB	++	20	LG	+	60	LG	++
1.5+0.5	30	GB	+	30	LG	+	30	LG	+
1.5+1.0	40	B	+	20	G	+	20	G	+
Mean	**38.33**			**20**			**38.33**		

LB = Light Brown, G = Green, LG=Light green, B = Brown, - = No callusing, + = Trace callus, ++ = Moderate callus, +++ = Massive callus, GB=Greenish Brown.

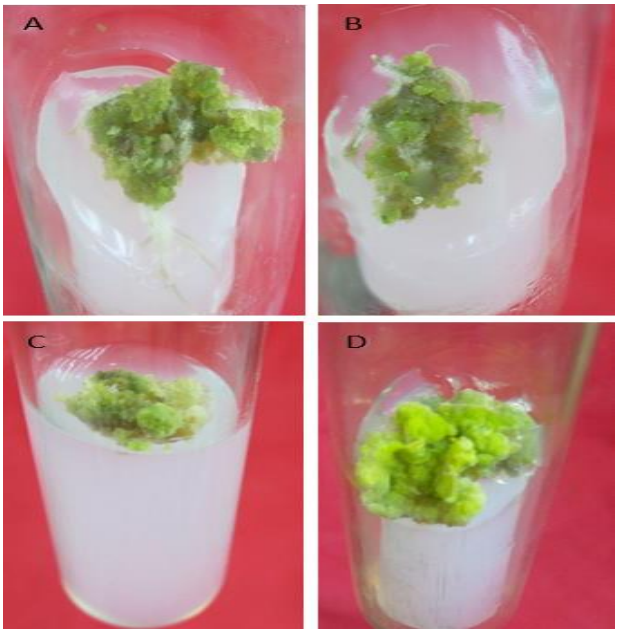

Fig 1. Different concentration of growth regulator on callus induction from leaf explants of three potato cultivars after 5 weeks of culture. A. MS medium supplemented with 2.5 mg/l 2,4-D in Japanese Red. B. MS medium supplemented with 3.0 mg/l 2,4-D in Asterix. C. MS medium supplemented with 1.0 mg/l NAA+1.0 mg/l BA in Japanese Red. D. MS medium supplemented with 1.0 mg/l NAA+1.0 mg/l BA in Asterix.

Table 2. Effect of different concentrations and combinations of BA with NAA and KIN with NAA in MS medium on shoot regeneration from leaf explant-derived calli of different potato cultivars. In each treatment 10 calli were inoculated. Data were recorded after 30 days of culture.

Plant growth regulators (mg/l)	Cultivars					
	Japanese Red		Cardinal		Asterix	
	No. of calli induced in shoot regeneration	% of calli regenerated shoots	No. of calli induced in shoot regeneration	% of calli regenerated shoots	No. of calli induced in shoot regeneration	% of calli regenerated shoots
BA+NAA						
2.0+1.0	02	20	01	10	02	20
2.0+1.5	03	30	02	20	02	20
3.0+0.1	07	70	05	50	06	60
3.0+0.5	04	40	03	30	04	40
3.0+1.0	03	30	02	20	03	30
3.0+1.5	03	30	03	30	01	10
Mean % of regeneration ability **(X±SE)**		**30.33±0.45**		**25±0.41**		**30±0.38**
KIN+NAA						
3.0+0.1	02	20	01	10	01	10
3.0+0.5	01	10	02	20	02	20
3.0+1.0	02	20	02	20	02	20
4.0+0.5	05	50	03	30	04	40
4.0+1.0	02	20	02	20	02	20
4.0+1.5	02	20	02	20	02	20
Mean of % regeneration ability **(X±SE)**		**21.67±0.47**		**20±0.32**		**23.33±0. 33**

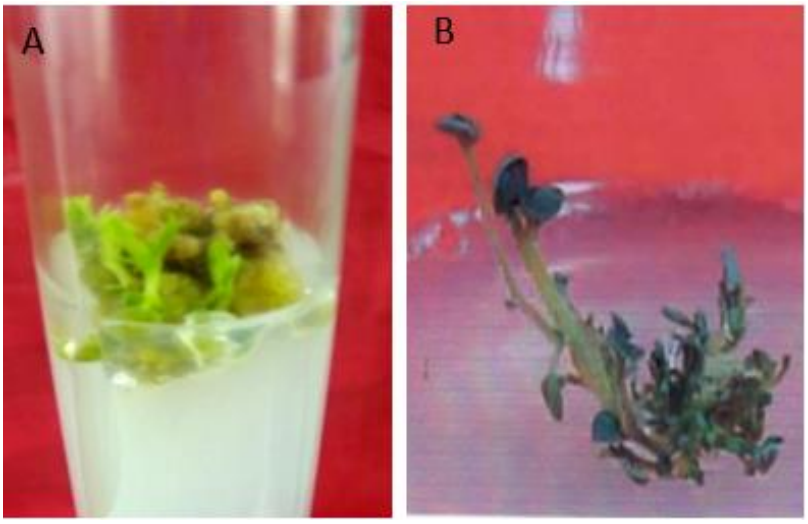

Fig. 2. Effects of NAA, BA and KIN on shoot regeneration from leaf explant derived calli of potato cultivars. A. Shoot regeneration in MS + 3.0 mg/l BA+0.1 mg/l NAA after five weeks of culture of Japanese Red. B. Shoot regeneration in MS + 4.0 mg/l KIN+ 0.5 mg/l NAA after five weeks of subculture of Asterix.

DISCUSSION

Tissue culture technique is the classical approach for callus induction and plant regeneration from potato (*Solanum tuberosum* L.) (Qureshi, *et al.,* 2023; Reddy and Reddy 1993). In the present investigation MS medium was found to be effective for plant-regenerating callus induction. In Cardinal and Asterix among all treatments of 2,4-D highest callusing rate was found in medium having 3.0 mg/1 from leaf explants. In Japanese Red highest callusing rate was recorded in media having 2.5 mg/1 2,4-D from leaf explant. In previous study it is reported that, 2,4-D for callus induction from internodes and leaf explants of four potato cultivars and found that at concentrations 3.0 mg/1 was found to be the most effective concentrations for all cultivars (Mutasim *et al*.,2010; and Shirin *et al.,* 2007). In general, 2,4-D formulations was found more effective in callus induction then those of NAA. Many researchers observed that 2,4-D was the best plant growth regulator for callus induction (Qureshi, *et al.,* 2023; Abu Zeid, *et al.,* 2022; Metwali, *et al*., 2020; Khatun, 2004; and Alsadon *et al.,* 2004).

According to the findings of the current investigation, different auxin and cytokinin concentrations and combinations (BA+NAA) were also shown to produce callus from leaf explants of various potato varieties. This result was identical to that obtained in potato by Nasrin *et al*., 2003. Khatun *et al*., (2003) also found same result when using 1.0 mg/1 BA+1.0 mg/1 NAA in potato callus induction. Similar results were also reported by Kathari and Chandra (1984) in African Merigold. From the above experimental results, it was clearly focused that MS medium supplemented with 2.5-3.0 mg/l 2,4-D was the best formulation for callus induction in potato. However, Mamun *et al*., (1996) reported that 2,4-D proved less effective when used alone for other plant species. Sultana (2001) and Sultana (2008) used 2,4-D alone for callus induction from internode and leaf explants of potato. She also obtained same results as Mamun *et al*., (1996) in three potato cultivars. However, Malamug *et al*., (1991) found better results when using 2,4-D as a callus inducing plant growth regulator in potato callus induction, Khatun *et al*., (2003) and Nasrin *et al*., (2003) used 2,4-D alone for callus induction and found better results in potato.

Calli derived from leaf segments were subcultured for shoot regeneration in MS medium supplemented with different concentrations and combinations of BA+NAA and KIN+NAA. After 20-25 days of inoculation shoot regeneration started. The media with different concentrations and combinations of BA with NAA and KIN with NAA both were found effective for shoot regeneration of potato cultivars from leaf explants derived callus. In some research reported that two successive phases of culture are required for inducing multiple shoots (Nahirnak *et al.,* 2022; Ohbayashi *et al.,* 2022; Litz and Conover, 1981). Between two combinations used BA+NAA was proved to be more effective than KIN+NAA for multiple shoot regeneration from leaf explants derived calli of potato cultivars.

Among the different combinations, media fortified with 3.0 mg/1 BA+0.1 mg/1 NAA were found optimum for maximum proliferation of shoot from leaf explants. The maximum numbers of shoots per callus was recorded in the same medium formulation. Khatun *et al*. (2003) also found the same results in potato. Sultana (2001) used BA+NAA for multiple shoots proliferation and obtained maximum shoots when cytokinin and auxin were used in MS medium in *Chrysanthemum morfoliam*. Sultana (2001) was also found same results in potato. The effect of BA with NAA on multiple shoots proliferation has also been demonstrated by Malamug *et al.* (1991) in *Ctydsnyhrmum motigolism.* The highest calli induced multiple shoots in media having 4.0 mg/1 KIN +1.0 mg/1 NAA from leaf derived calli of all three cultivars of potato. Similar results have been obtained by Sultana (2001) and Nasrin *et al*. (2003). The results of this study demonstrate that calli produced from leaf segments of *in vitro* grown plantlets of the three potato cultivars Japanese Red, Cardinal, and Asterix could be used to regenerate plants.

CONCLUSION

The present study was undertaken to evaluate the efficacy of the growth regulator through *in vitro* techniques and to justify its application in potato breeding. We found different concentration and combination of plant growth regulator affecting the callus induction and shoot regeneration in the abovementioned potato cultivars. Combination of BA+NAA was proved to be more effective than KIN+NAA for multiple shoot regeneration from leaf explants derived calli. Growth media fortified with 3.0 mg/1 BA+0.1 mg/1 NAA provided best result for maximum proliferation of shoot from leaf explants. We think that our research output will be applicable to grow these potato cultivars in large scale for plantlets production and propagation.

ACKNOWLEDGMENT

The authors are grateful to the Plant Breeding and Genetic Engineering Lab., Dept. of Botany, Faculty of Biological sciences as well as Ministry of Science and Technology, Bangladesh for their financial support.

REFERENCES

Abu Zeid, I.M., Soliman, H.I.A., and Metwali, E.M.R. 2022. *In vitro* evaluation of some high yield potato (*Solanum tuberosum* L.) cultivars under imposition of salinity at the cellular and organ levels. Saudi J Biol Sci. 29(4):2541-2551.

Ahmad, K.U. 1977. Potato for the tropics. *West of agricultural laboratory*, Farm Gate, Dacca-15, Bangladesh. pp 240.

Alsadon, A.A., Al-Mohaaideb, M., Rahaman, M.H. and Islam, R. 2004. Evaluation of vegetative growth traits of eight potato cultivars. *Bangladesh Journal of Genetics and Biotechnology*, 5(1&2):61-64.

Azad, Md.A.K., Khatun, Z., El-Jaoual Eaton, T., Hossen, Md.I., Haque, Md.K. and Soren, E.B. 2020. Generation of Virus Free Potato Plantlets through Meristem Culture and Their Field Evaluation. *American Journal of Plant Sciences*, 11, 1827-1846.

BBS 2011. Vegetables Production Statistics, 2011. Bangladesh Bureau of Statistics, Dhaka.

BBS 2012. Statistical Year Book of Bangladesh. Bangladesh Statistics Division, Ministry of Planning Govt. of the People's Republic of Bangladesh, Bangladesh Bureau of Statistics, Dhaka.

Cearley, T.A. and Bolyard, M.G. 1997. Regeneration of *Solanum tuberosum* cv. Katahdin from leaf explants *in vitro*. *American Potato Journal*, 74: 125-129.

Chowdhury, M.A.H. and Hasan, M.S. 2013. Handbook of Agricultural technology. Bangladesh Agricultural Research Council, Farmgate, Dhaka, 230.

DAE. 2002. Annual report. Department of Agricultural Extension. *Ministry of Agriculture*, Bangladesh.

FAO. 2009. Sustainable potato production: Guidelines for developing countries. Food and Agriculture Organisation of the United Nations. ISBN 978-92-5-106409-2.

Garcia, E. D., and Martinez, S. 1995. Somatic embryogenesis in *Solanum tuberosum* L. cv. Desireefrom stem nodal sections. *Journal of Plant Physiology*, 145: 526-530.

Hajare ST, Chauhan NM, Kassa G. 2021. Effect of Growth Regulators on *In Vitro* Micropropagation of Potato (*Solanum tuberosum* L.) Gudiene and Belete Varieties from Ethiopia. ScientificWorldJournal. doi: 10.1155/2021/5928769. PMID: 33628138; PMCID: PMC7884170.

Islam, R. and Alsadon, A.A. 2003. Successful application of biotechnology in potato: A review paper. *Bangladesh Journal of Genetics and Biotechnology*, 4(1&2): 1-6.

Kathari, S.L. and Chandra, N. 1984. *In vitro* propagation of African Marigold. *Horticultural Science*,19: 703-705.

Khatun, N. 2004. Micropropagation and genetic transformation in potato (*Solanum tuberosum* L.). Ph.D. Thesis, *Institute of Biological Sciences. Rajshahi University*, Bangladesh.

Litz, R.E. and Conover, R.A. 1981. Effect of sex type, season and other factors on *in vitro* establishment and culture of *Carica papaya* L. explants. Journal of the American Society for Horticultural Science106: 792-794.

Malamug, J.J.F, Inden, H. and Asahira, T. 1991. Plantlet regeneration and propagation from ginger callus. *Scientia Horticulturae*, 48: 89-97.

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

Metwali, E.M.R., Kadasa, N.M.S., Soliman, H.I.A., Almaghrabi, O.A., and Fuller, M.P. 2020. *In vitro* propagation of date palm cultivars Magdoul and Safwai through somatic embryogenesis. *Int. J. Agric. Biol*, 24: 1745-1753.

Mozafri, J., Wolyn, D.J. and Ali, K.S.T. 1997. Chromosome doubling via tuber disc culture in dihaploid potato as deterimined by confocal microscopy. *Plant Cell Reports*, 16: 329-333.

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

Mutasim M. Khalafalla, Khadiga G. Abd Elaleem and Rasheid S. Modawi. 2010. Callus formation and organogenesis of potato (Solanum tuberosum L.) cultivar Almira. J phytol 2/5: 40-46.

Nahirnak, V., Almasia, N.I., Gonzalez, M.N., Massa, G.A., Decima Oneto, C.A., Feingold, S.E., Hopp, H.E. and Vazquez Rovere, C. 2022. State of the Art of Genetic Engineering in Potato: From the First Report to Its Future Potential. *Front Plant Sci*, 12:768233.

Nasrin, S., Hossain, M.M., Khatun, A., Alam, M.F. and Mondal, M.R.K. 2003. Induction and evaluation of somaclonal variation in potato *(Solanum tuberosum* L.*). Online Journal of Biological Sciences*, 3(2): 183-190.

Ohbayashi, I., Sakamoto, Y., Kuwae, H., Kasahara, H. and Sugiyama, M. 2022. Enhancement of shoot regeneration by treatment with inhibitors of auxin biosynthesis and transport during callus induction in tissue culture of *Arabidopsis thaliana*. *Plant Biotechnol*, 39(1):43-50.

Pretova, A. and Dedicova, B. 1992. Somatic embryogenesis in *Solanum tubersom* L. cv. Desiree from unripe zygotic embryos. *Journal of Plant Physiology*, 139: 539-542.

Pua, E. C., Sim, G. E., Chi, G. L. and Kong, L. F. 1996. Synergistic effect of ethylene inhibitors and putrescine on shoot regeneration from hypocotyls explants of Chinese radish (*Raphanus sativus* L. var. longipinnatus Bailey) *in vitro*. *Plant Cell Reports*, 15:685–690.

Purnhauser, L., Medgysey, P., Czako, M., Dix, J. P. and Marton, L. 1987. Stimulation of shoot regeneration in *Triticum aestivum* and *Nicotiana plumbaginifolia*Viv tissue cultures using the ethylene inhibitor silver nitrate. *Plant Cell Reports*, 6 (1) :1-4.

Qureshi, M.A., R. Ahmad, B. Ahmed, T.U. Khan and M. Yasin. 2023. Effect of growth regulators on callus induction and plant regeneration in potato (*Solanum tuberosum* L.) explants. *Sarhad Journal of Agriculture*, 39(1): 140-146.

Reddy, I.R. and Reddy, G.M. 1993. Factors

affecting direct somatic embryo-geneses and plant regeneration in grrundnut (*Arachis hpogaca* L.). *Indian Journal of Experimental Biology*, 31:57-60.

Shirin, F., Hossain, M., Kabir, M.F., Roy, M. and Sarker, S.R. 2007. Callus Induction and Plant Regeneration from Internodal and Leaf Explants of Four Potato (*Solanum tuberosum* L.) cultivars. *World Journal of Agricultural Science*, 3 (1): 01-06.

Siddique, M.N.A., Sultana, J., Huda, M. S., Abdullah, M. R. and Chowdury M. A. 2015. Potato Production and Management with Preference to Seed Potato Supply Chain, Certification and Actors Involve in Bangladesh. International Journal of**ES** Business, Management and Social Research 01(01): 01-13.

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

Sultana, M.R. 2008. Production of Therapeutic Calcitonin Protein in Transgenic Potato, M.Sc thesis. *Plant Breeding and Gene Engineering Lab., Department of Botany*, Rajshahi University.

Sultana, R.S. 2001. Callus induction and evaluations in potato (*Salanun tuberosum* L.). M.Sc. Thesis, *Department of Botany*, Rajshahi University, Bangladesh.