EFFECT OF DIFFERENT PHYTOHORMONES ON PLANT REGENERATION OF *AMARYLLIS HIPPEASTRUM*

F. Aslam, S. Habib and S. Naz

Department of Biotechnology &Microbiology, Lahore College for Women University, Lahore, Pakistan

Corresponding Author E-mail: drsnaz31@hotmail.com

ABSTRACT: Tissue culture of bulbous plants has been successfully established and plantlets are regenerated in large number from this technique. An efficient method for rapid propagation of *Amaryllis hippeastrum* L. by *In Vitro* technique has been developed in this study by using micropropagation practice. In this technique explant is inoculated on medium fortified with different nutrients and growth hormones as well. Scales and meristem of underground bulb used as explants were cultured on MS medium supplemented with different concentrations and combinations of cytokinins and auxins. Effect of these phytohormones on plant formation of *Amaryllis hippeastrum* was studied. Best results for shoot initiation were obtained by using Thidiazurone (TDZ) 10 µM/l + Alpha-Naphthalene acetic acid (NAA) 1.0 mg/l i.e. (90%) frequency of shoot initiation for scales explants and 80% frequency of shoot initiation for meristems explants with 3.6 ± 0.0632 cm of shoot length for both explants. The best results for multiple shoot production were obtained from the combination of 6-benzyl amino purine (BAP) 5 mg/l + Alpha-Naphthalene acetic acid NAA 0.1 mg/l with 80% frequency of shoot formation Bulblet formation was promoted in MS basal medium fortified with BAP 4 mg/l and NAA 0.1 mg/l NAA + 60% sucrose. The regenerated plantlets were transferred to different potting media in green house for acclimatization. Best hardening response was obtained in (Vermicompost + Sand). A completely randomized design was used for the experiment. Analysis of data was carried out by using COSTAT V.63 software following Duncan's New Multiple Range Test.

Key words: *Amaryllis hippeastrum*; Bulb; Shoot initiation; acclimatization.

INTRODUCTION

Amaryllis, an ornamental bulbous flowering plant belongs to the family Amaryllidaceae, it has large and showy flowers with many bright colors (Jana *et al.*, 1995). It is native to Central and South America, and is easily grown in the tropical and subtropical regions (Okubo, 1993). Propagation can be accomplished by using seed, offset bulblets and twin scaling (Siddique *et al.,* 2006; Vijverberg, 1981). Plants of *Amaryllis hippeastrum* are suitable for planting in the bed, pot, rookery, shrubbery and greenhouse garden and also in landscaping. It is usually a spring planting bulb (Okubo, 1993; Jana *et al.,* 1995).

Worldwide the Amaryllidaceae have greatest economic value as ornamentals. In addition, huge numbers of plants are traded for traditional medicines. Africans use the bulbs and leaves as poultices and decoctions for treating sores and digestive disorders, but in large dosages they are extremely poisonous (Sanijman, 2004).

Multiplication of plant from seed showed wide variation in flower colour, plant shape and time of flowering. Monocotyledonous tissues, in general, are characterized by slow growth and sometimes are recalcitrant to growth hormones in culture media. It was thought interesting, to explore the morphogenetic potential of explanted tissues of family Amaryllidaceae, namely *Amaryllis*, hybrid specie with red flowers under culture conditions.

The main aim of the present study was to establish protocols for micropropagation of disease free and high quality plants of Amaryllis by using *in vitro* technique.

MATERIALS AND METHODS

Explants source: The under ground bulbs used as explants were procured from nursery in Lahore.

Preparation of explants: Meristems and inner scales obtained from bulbs were used as explants. Bulbs were cut-off into different sections of scales of 1x1 cm^2.

Surface sterilization: Explants of *Amaryllis hippeastrum* L. were washed thoroughly under running tap water. Washed explants were further treated with household detergent for 3-4 minutes and were again rinsed with distilled water to remove traces of detergent. Explants were then immersed in commercial bleach (80% sodium hypochlorite) for sterilization for 25 minutes to remove surface contaminants. Then these were washed three times with autoclaved distilled water to remove all the traces of sodium hypochlorite.

Culture media and conditions: Explants were cultured on MS medium supplemented with various concentrations and combinations of plant hormones (TDZ µM, BAP mg/l, NAA mg/l & TDZµM + NAA mg/l Table 1&2). Effects of these hormones on shoot initiation, multiple shoot formation, root formation and bulblet formation have been studied. The basal medium used for all the experiments was Murashige & Skoog (1962) mineral formulation containing micronutrients, macronutrients and vitamins, 30g/l sucrose, 1.0 ml/l PPM (plant preservative mixture) and 1.5g/l phytagel. The pH of each medium was adjusted to 5.5±0.2 before adding phytagel and medium was autoclaved at 15 $Ib/inch^2$ for 20 min at 121ºC. Cultures were incubated at 22±2ºC with a photoperiod of 16 hour at 2000-3000 lux light intensity of cool white fluorescent light.

Shoot initiation: Meristems and scales excised from bulbs of *Amaryllis hippeastrum* L. were implanted on nutritional media supplemented with different hormones (TDZ µM & TDZ µM + NAA mg/l). To get the standard medium used for shoot initiation, different concentrations of cytokinins TDZ (Table 1) alone and in combinations with auxins i.e. TDZ + NAA (Table 1) in MS medium were used.

Multiplication, root formation and bulblet formation: To induce multiple shoots as well as roots in regenerated plants, different concentrations and combinations of cytokinins and auxins (TDZ µM & TDZ µM + NAA mg/l, BAP mg/l + NAA mg/l) were tested. Observations on frequency of shoot formation, multiple shoot formation (%), shoot length (cm), root formation (%), root length (cm) and bulblet formation were recorded at 30 days after establishing the culture.

Acclimatization of micro plants: After rooting, regenerated plants were transferred to small pots containing different combinations of sand, vermicompost & peat under greenhouse conditions for 1-3 month.

Statistical analysis: A completely randomized design with 3 replicates was used for the experiment. The data for each parameter were subjected to analysis of variance using the COSTAT V.63: statistical software (Cohort software, Berkely, California). The mean values were compared by applying Duncan's new multiple range test at 5% level.

RESULTS AND DISCUSSION

Effect of different concentration of TDZ (µM) and TDZ (µM) + NAA (mg/l) on shoot initiation from scales of *Amaryllis hippeastrum*: Scales were inoculated on MS media containing different concentrations of TDZ (8.0, 10.0, 14.0, 16.0, 22.0 µM) alone and TDZ (2.0 + 5.0 +, 8.0, + 10.0, 12.0) µM + NAA mg/l (0.5, 1.0, 0.1, 1.0,

3.0) mg/l. These all combinations were selected on the basis of prior work and then the combination which showed better results are to be selected for further study. It is evident that among the different media used, the medium containing TDZ 22 µM and TDZ 10.0 µM + NAA 1.0 mg/l showed the best shoot induction response. Highest response of shoot induction was 90% (Table 1) with 3.6^{c}±0.0632 cm of shoot length followed by 70% of shoot induction (Table 1) in MS + TDZ 22 µM (Fig. 1b). The effect of TDZ alone or combination with NAA was also well documented by other scientists.

Xiao-Ming *et al*., (2009) reported highest frequency of shoot formation i.e. 87.12% by using bulb as explant in MS MS+TDZ 0.5 mg/l+ NAA 0.1 mg/l.

Effect of different concentration of TDZ (µM) and TDZ (µM) + NAA (mg/l) on shoot initiation from meristems of *Amaryllis hippeastrum* : Meristems were inoculated on MS media containing varying concentrations of cytokinins and auxins for 4 weeks to get optimal medium. Percentage of response of the explant to all concentrations was shown in Table-1. It is evident that among the different media used, the medium containing TDZ 10.0 µM + NAA 1.0 mg/l showed the best shoot induction response with 80% frequency & 3.6^{c}±0.0632 cm shoot length. (Fig. 1d) Guozheng & Cheng Chang (2005) studied the effect of TDZ on bulblet formation and shoot formation of Amaryllis and found that higher concentrations of TDZ promoted shoot as well bulblet formation but reduced root foramtion.

Effect of different concentrations of BAP + NAA (mg/l) on *In vitro* shoot multiplication and bulblet formation: For *In vitro* multiple shoot formation, MS medium was supplemented with different concentrations of BAP + NAA (mg/l). It was noticed that when concentration of BAP was increased, frequency of multiple shoot formation was also increased but by increasing concentration of NAA, negative response was obtained. Highest frequency of multiple shoot formation i.e. 80% was observed shown in (Table-2). *In vitro* bulblet formation (Fig. 1i) was obtained with highest frequency of 80% in MS + BAP 4.0 mg/l + NAA 0.1 mg/l (Table-2). The higher concentration and combination with NAA stimulated high frequency of regeneration particularly the formation of multiple shoots (Nasirujjaman *et al*., 2005; Panda *et al*., 2007; George, 1993). **Huang (1990)** regenerated Protocorm-like bodies from single scales of *A. hippeastrum* L. Cultured *in vitro*, which ultimately formed bulblets. Bulblets were directly produced from twin scales. De-Bruyn (1992) multiplied *Amaryllis hippeastrum* L. plants successfully by means of tissue culture techniques in which twin-scale explants had the highest multiplication rate when a medium with 22.2 µM benzyladenine and 0.54 µM naphthaleneacetic acid was used and sucrose concentration played an important role in the initiation of new plantlets, and the best results were obtained when a sucrose concentration of 2-3%.

Acclimatization of plants under *ex vitro* conditions: In order to maximize the survival of *In vitro* derived plants, it is routine practice to acclimatize them under high levels of relative humidity (Short, 1991). *In vitro* regenerated plantlets of *Amaryllis hippeastrum* L. were grown by using different media and were also treated with different nutrient solution's to get the information about the best potting media as well as nutrient solution. Best potting media used for acclimatization of *A. hippeastrum* L. was (Vermicompost + Sand) and best nutrient solution observed was macronutrient (Fig. 1j-K). Similar findings were also observed by Song *et al.*, (2002) in which plantlets with roots were transplanted into vermiculite and were sprayed with MS mineral elements once every day with the survival rate of more than 98%.

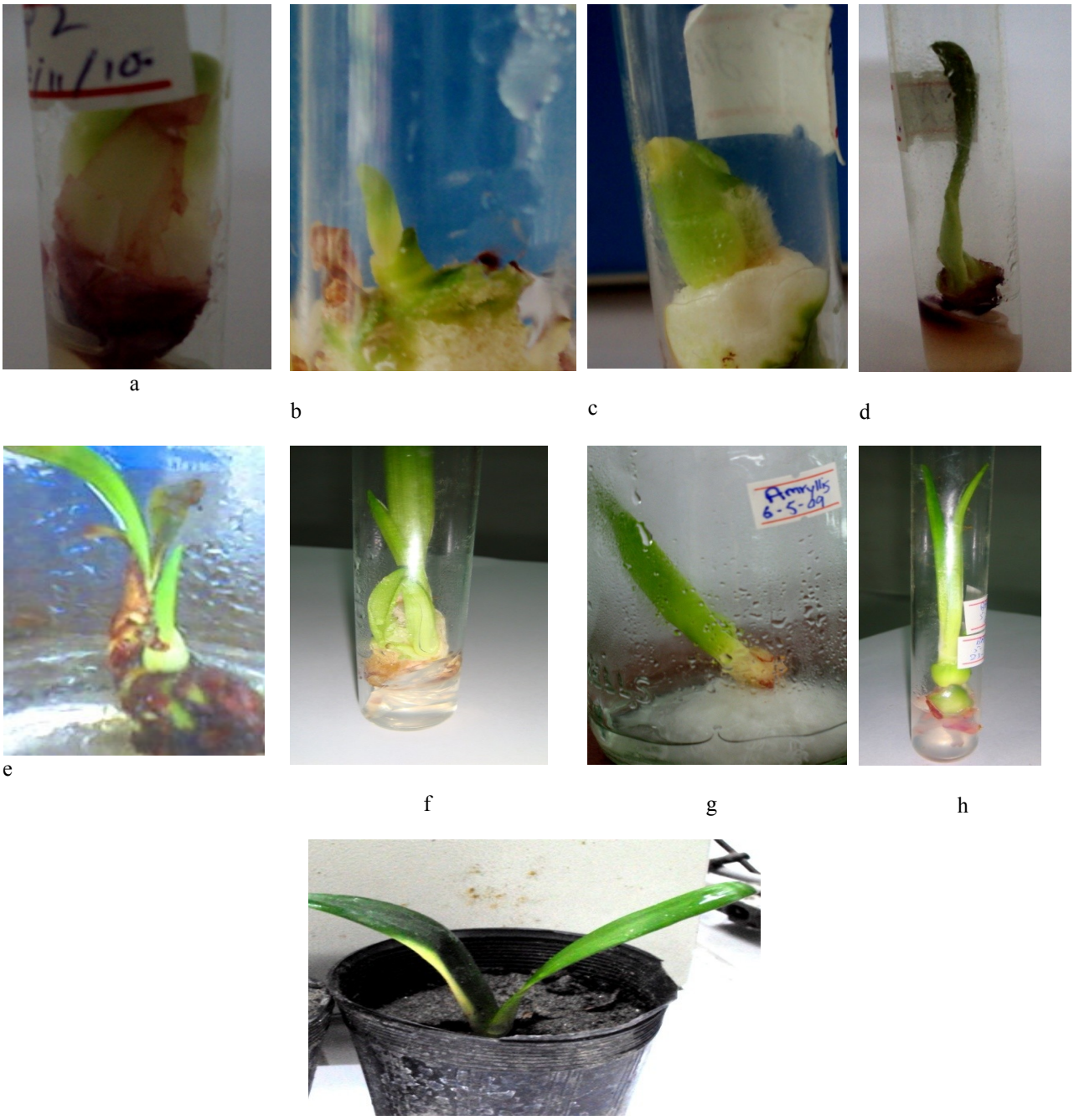

Fig-1: Micropropagation of *Amaryllis* a) Scale used as explant. b) Shoot initiation from scale in MS + TDZ 22 µM/l. c) Meristem used as explant. d) Shoot initiation from meristem. e) Multiple shoot formation in MS with BAP 5 mg/l + NAA 0.1 mg/l. f) Shoot elongation from scale of bulb. g) Shoot elongation along with root initiation from meristems of the bulb. h) Plantlet formed by using meristem as explant. i) Bulblet formation. J) Hardening of plantlet.

Table-1. Effect of different concentrations of TDZ and TDZ + NAA on shoot formation from scales and meristems explants of *Amaryllis hippeastrum* L.

S. NO	Plant hormone	Shoot length (cm)	Frequency of shoot Formation (%)	Plant hormone	Shoot length (cm)	Frequency of shoot formation

	TDZ (µM)					TDZ (µM) + NAA (mg/l)				(%)
		For Scales	For Meristems	For scales	For meristems		For Scales	For meristems	For scales	For meristems
1.	8.0	1.5±0.08[b]	2.0±1.17[a]	20	10	2 + 0.5	1.0±0.10[c]	1.2±0.04[b]	40	40
2.	10.0	1.0±0.06[c]	1.0±0.06[c]	50	30	5.0 + 1.0	1.5±0.23[b]	0.8±0.06[c]	20	30
3.	14.0	1.0±0.06[c]	1.0±0.05[c]	40	30	8.0 + 0.1	1.0±0.10[c]	0.9±0.10[a]	20	10
4.	16.0	1.0±0.10[c]	1.5±0.23[b]	30	20	10.0+1.0	3.6±0.0632[a]	3.6±0.0632[c]	90	80
5.	22.0	2.0±1.17[a]	1.0±0.06[c]	70	30	12.0+3.0	1.5±0.23[b]	2.0±0.17[a]	60	60

Table-2. Effect of different concentrations of BAP + NAA on *in vitro* multiplication and bulblet formation of *Amaryllis hippeastrum* L.

S. No	Plant hormones		Frequency of multiple shoot formation (%)	Frequency of bulblet formation (%)
	BAP (mg/l)	NAA (mg/l)		
1.	1.0	0.5	40	-
2.	2.0	0.3	50	-
3.	3.0	0.5	30	50
4.	4.0	0.1	70	80
5.	5.0	0.1	80	-

REFERENCES

Bapat, V. J. and S. Narayanaswamy. Growth and organogenesis in explanted tissue of *Amaryllis* in culture. Bulletin of the Torrey Botanical Club, 103: 53-56 (1976).

De-Bruyn, M. H., D. I. Ferreira, M. M. Slabbert and J. Pretorius. *In vitro* propagation of *Amaryllis hippeastrum*. Plant Cell, Tissue and Organ Culture, 31(3): 179-184 (1992).

Duncan, W. G.2002. A theory to explain the relationship between corn population and grain yield. *Crop Sci*., 24: 1141-1145.

George, E. F. *Plant Propagation by Tissue Culture*. Part I. The Technology, Exegetics Ltd., Edington, Wilts, UK (1993).

Guozheng, Z. and K. Cheng-Chang. Studies on *in vitro* morphogenesis from ovary and pedicel explant of Amaryllis. NDLTD, (2005).

Huang, C. W., H. Okubo and S. Uemoto. Comparison of bulblet formation from twin scales and single scales in *Hippeastrum hybridum* cultured *in vitro*. Scientia Horticulturae, 42: 151-160 (1990).

Jana, B. K., K. L. Chadha and S. K. Bhattacharjee. Cultural requirements of Hippeastrum. *In*: Advances in Horticulture. 12 Malhotra publishing House, New Delhi, India (1995).

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

Nasirujjaman, K., M. Salah-Uddin, S. Zaman and M.A. Reza. Micropropagation of turmeric (*Curcuma longa* Linn.) through *in vitro* rhizome bud culture. Journal of Biological Sciences, 5(4): 490-492 (2005).

Okubo, H. Hippeastrum (Amaryllis). In: The physiology of flower bulbs. A. DE Hertogh and M. LE Nard (Eds). Elsevier, 321-324 (1993).

Panda, M. K., S. Mohanty, E. Subudhi, L. Acharya and S. Nayak. Assessment of genetic stability of micropropagated plants of *Curcuma longa* by cytophotometry and RAPD analyses. International Journal of Integrative Biology, 1(3): 189-195 (2007).

Short, K. C. The physiology of cultured plantlets and methods for facilitating their transfer to field condition. Conservation of plant genetic resources through *In vitro* methods, ISBN, pp. 967-991 (1991).

Siddique, M. N. A., N. Sultana, M. A. Haque, M. M. Hossain and J. U. Ahmed. Effects of Twin Scale Size and Hormones on *In Vitro* Propagation of Hippeastrum (*Hippeastrum hybridum*). Plant Tissue Cult. & Biotech, 16(2): 105-110 (2006).

Siddique, M. N. A., J. Sultana, N. Sultana and M. M. Hussain. *Ex Vitro* establishment of *in vitro* produced plantlets and bulblets of Hippeastrum (*Hippeastrum Hybridum)*. Int. J. Sustain. Crop Prod, 2(3): 22-24 (2007).

Sanijman, D. "Amaryllidaceae Family". Plantzafrica, *South African National Biodiversity Institute* (2004).

Song, Z., D. Kedong, C. Chenxing, J. Luyan, Z. Ruifu and W. Luping. Rapid Micropropagation System Via *in Vitro* Culture in *Amaryllis vittata* and Its Embryogenesis. Acta Horticulturae Sinica, (2002).

Vijverberg, A. J. Growing Amaryllis. Grower Book, London. 57 (1981).

Xiao-ming, L. 2009. Study on adventitious bud induction of *Hippeastrum hybridum* bulb. Journal of Anhui Agricultural Science, (2009).