The Bioscan 11(3): 1407-1410, 2016
AN INTERNATIONAL QUARTERLY JOURNAL OF LIFE SCIENCES
www.thebioscan.com

MULTIPLE SHOOT REGENERATION OF TUBEROSE (*POLIANTHES TUBEROSA* LINN.) CV PRAJWAL BY USING BULB AS EXPLANT

NEHA KUMARI* AND A. K. PAL
Department of Floriculture and Landscaping,
Faculty of Horticulture, Bidhan Chandra Krishi Viswavidyalaya, Mohanpur , Nadia - 741 252, West Bengal, INDIA
e-mail: nehahorticoz86@gmail.com

KEYWORDS
Prajwal
Polianthes tuberosa L.
BAP
NAA
Bulb
In vitro

Received on :
02.03.2016

Accepted on :
21.06.2016

***Corresponding author**

ABSTRACT
In the present investigation, an experiment was conducted to standardise a protocol for higher shoot regeneration through bulb in tuberose (*Polianthes tuberosa* L.) cv. Prajwal. The growing medium (MS medium) was supplemented with various combination of growth regulators BAP (0.5, 1.0, 1.5, 2.0 mg l^{-1}) and NAA (0.5, 1.0, 1.5, 2.0 mg l^{-1}). Higher explant regeneration was observed in MS medium containing 2.0 mg l^{-1} BAP + 0.5 mg l^{-1} NAA. The highest shoot regeneration (2.12 / culture) was obtained by MS medium containing 0.5 mg l^{-1} BAP + 2.0 mg l^{-1} NAA this combination has also shown higher regenerated shoot length (6.42 cm / culture), number of leaves (3.23 /culture) and number of roots in regenerated shoots (6.62 / culture) followed by results obtained from the MS medium supplemented with 0.5 mg l^{-1} BAP + 1.5 mg l^{-1} NAA. The hardened plantlets were transferred successfully in the field.

INTRODUCTION

Tuberose (*Polianthes tuberosa* L.) belongs to family Amaryllidaceae is one of the important bulbous ornamental crop in India. Conventially it is propagated by blulb but looking at growing demand for disease free planting material *In vitro* propagation has been adopted in this crop, more number of shoots regenerated per explant will give more number of plants in this regard, the present study was carried out to see the effect of different combination and concentration of growth regulators BAP (6- benzylaminopurine) and NAA (1- Napthalene acetic acid) on shoot regeneration in single type tuberose cultivar Prajwal by using bulb as explant, it was found that the maximum shoot regeneration (2.12/ culture) was shown by MS medium containing growth regulators BAP (2.0 mg l^{-1}) and NAA (0.5mg l^{-1}).

Similar combination of growth regulators in different concentrations has been tried by Jitendriya and Sayad (2013) they reported best response for multiple shoot regeneration in tuberose variety Calcutta Single cultured on MS medium containing 0.2 mg l^{-1} of BAP and kinetin. Jyothi *et al*.(2008) also reported that the MS medium supplemented with 4 mg l^{-1} BAP induces shoot multiplication in both single (Prajwal) 3.3 and double (Vaibhav) 2.5 by using bulb scale as explant. Kadam *et al*. (2010) reported best medium for shoot regeneration was MS medium supplement with 6 mg/ l BAP + 0.5 mg/l NAA + 0.7 mg/ l 2,4-D + 0.5 mg/l TDZ for both petals and immature flower bud of tuberose increases shoot regeneration maximum roots was observed on half strength MS medium + 1 mg/l IBA. Toma and Petra (2005) reported the best regeneration of tuberose by using bulb as explant was recorded on MS medium supplimented with 0.2 mg l^{-1} NAA + 1.5 mg l^{-1} kinetin + 2.0 mg l^{-1} BAP.

MATERIALS AND METHODS

Present study on " Micropropagation of tuberose (*Polianthes tuberosa* L.) " was conducted at the Tissue culture laboratory of Faculty of Horticulture, Bidhan Chandra krishi Viswavidyalaya, Mohanpur, West Bengal. Single type tuberose cv. Prajwal bulb was selected as experimental material and was collected from Mondouri Horticulture Farm of the university. The collected explants were rinsed thoroughly in running tap water followed by washing with few drops of Tween-20 or Teepol (0.05%), then the explants were excised surface sterilised by soaking in Bavistin (0.4%) for 15 minutes to avoid any systemic contamination, then the explants were treated with $HgCl_2$ (0.1%) for 5-7 minutes and washed for 5-7 times with sterile distilled water and then culture the explants on freshly prepared solid MS medium (Murashige and Skoog, 1962) containing 0.8% (w/v) agar, and combination of growth regulators BAP (6-benzylaminopurine) and NAA (1- Napthalene acetic acid) in different concentrations for

shoot regeneration, adjust the medium pH to 5.7 prior to autoclaving at 121°C for 15 minutes**.** The exempted design was completely randomised design including 4 treatments and 5 replications. All the data were subjected to OPSTAT software for analysis of results.

Similar experiment was conducted by Jitendriya and Saiyad (2013) developed a protocol for large scale clonal multiplication of *Polianthes tuberosa* var. Calcutta Single under in vitro condition best response for multiple shoot formation was produced with MS medium containing 0.2 mg l-1 of BAP and kinetin with an average of 3.5 ± 0.2 shoots in Calcutta Single. Jyothi *et al*. (2008) also reported that the MS medium supplemented with 4 mg l^{-1} BAP induces shoot multiplication in both single (Prajwal) 3.3 and double (Vaibhav) 2.5 by using bulb scale as explant. Jyothi and Singh (2013) reported that single cultivar Prajwal exhibiting higher regeneration and multiplication of shoots and roots than double cv. Vaibhav when cultured on half strength MS medium + 1.0 mg l^{-1} IBA. Sangavi and Chellapandi (2008) reported that BAP at the rate of 1.5 mg l^{-1} showed higher explant regeneration and shoot differentiation frequency (2.2 ± 1.2 shoots/ explant) compare to control in tuberose *Polianthes tuberosa* L. By using rhizome as explant. Thus these experiments showed that appropriate proportion of BAP and IAA will be helpful for regeneration and cultivation of this plant in small scale nursery. Gajbhiye *et al*. (2011) established a protocol to propagate four cultivars of tuberose viz: Phule Rajni, Shringar, Prajwal, and Mexican Single through direct *in vitro* organogenesis from stem disc explants. During the investigation, culture medium containing (WH + 0.3 mg/l, TDZ + 0.5 mg/l NAA + 20.0 g/l sucrose + 7.5 g/l agar) was found to be more responsive for shoot proliferating (98.9%) and shoot (s) per explant (11.48).

RESULTS AND DISCUSSION

Effect of different concentrations of BAP and constant NAA concentration on shoot regeneration of tuberose

The effect of hormones on shoot regeneration was studied and effort was made to determine the appropriate hormone combinations for optimal shoot regeneration. Shoot initiation was observed after 5-6 days of inoculation of explant on MS medium.

The shoot regeneration of hybrid variety Prajwal of *Polianthes tuberosa* L. from bulb used as explant with different combination of growth regulators BAP (0.5-2.0 mg l^{-1}) and NAA (0.5 mg l^{-1}.) in MS medium was studied.

The maximum shoot initiation was shown by the medium containing 2.0 mg l^{-1} BAP + 0.5 mg l^{-1} NAA followed by 1.5 mg l^{-1} BAP + 0.5 mg l^{-1} NAA and minimum shoot initiation showed by 0.5 mg l^{-1} BAP + 0.5mg l^{-1} NAA. The effect of MS medium supplemented with different concentration of growth hormones was found to be statistically significant for regenerated shoot length of tuberose (*Polianthes tuberosa* L.) cv. Prajwal 15 days after inoculation (Table-1). The highest regenerated shoot length (4.63 cm/explant) and number of leaves (2.2/ explant) was obtained from the explants cultured on medium containing BAP (2.0 mg l^{-1}) and NAA (0.5mg l^{-1}), T4 followed by treatment T3 showed shoot length i.e 3.8 cm/ explant and number of leaves in regenerated shoots 1.9/explant containing BAP (1.5 mg l^{-1}) and NAA (0.5 mg l^{-1}) and the lowest regenerated shoot length (2.46 cm/ explant) and number of leaves (1.31/ explant) was obtained from treatment T1 containing BAP (0.5 mg l^{-1}) and NAA (0.5 mg l^{-1}). Similar observation has been reported in tuberose cv. Sringar (single type) and Suvasini (double type) by Chellapandi and Sangavai (2008). Panigrahi *et al*. (2013) reported maximum shoot regeneration in Calcutta double cultured on medium containing BAP 1.0 mg/ lit + Kn 2.5 mg/ lit. The size of callus was found statistically significant for tuberose cv. Prajwal. The largest size callus (0.81 cm) was shown by treatment T 2 containing BAP

Table 1: Effect of different concentration of BAP and NAA on various growth parameters of tuberose cv. Prajwal (15 days after inoculation):

Treatment	Shoot length(cm)	No. of leaves	Size of callus(cm)
T1	2.463	1.313	0.548
T2	3.278	1.58	0.813
T3	3.895	1.90	0.78
T4	4.63	2.20	0.74
SE(m)	0.3212	0.1287	0.0362
CD at 5%	0.9896	0.3965	0.1113

Treatment details: T1 = BAP (0.5 mg l^{-1}) + NAA (0.5 mg l^{-1}); T2 = BAP (1.0 mg l^{-1}) + NAA (0.5 mg l^{-1}); T3 = BAP (1.5 mg l^{-1}) + NAA (0.5 mg l^{-1}); T4 = BAP (2.0 mg l^{-1}) + NAA (0.5 mg l^{-1}).

Table 2: Effect of different concentration of NAA and BAP on various growth parameters of tuberose cv. Prajwal (15 days after third subculturing)

Treatment	Shoot length (cm)	No.of leaves	Size of callus (cm)	No. of roots	No. of shoots
T1	3.28	1.43	2.28	4.3	1.11
T2	4.52	1.62	2.88	4.68	1.25
T3	5.48	2.27	2.59	5.85	1.55
T4	6.42	3.23	2.84	6.62	2.12
SE(m)	0.36	0.21	0.25	0.15	0.11
CD at 5%	1.11	0.67	NS	0.48	0.34

Treatment details: T1 = NAA (0.5 mg l^{-1}) + BAP (0.5 mg l^{-1}); T2 = NAA (1.0 mg l^{-1}) + BAP (0.5 mg l^{-1}); T3 = NAA (1.5 mg l^{-1}) + BAP (0.5 mg l^{-1}); T4 = NAA (2.0 mg l^{-1}) + BAP (0.5 mg l^{-1}).

Figure 1: different stages of micropropagation of tuberose from explant (bulb) a. Inoculation, b. Greening, c. Shoot initiation, d. Shoot multiplication.

Figure 2: Micropropagation of tuberose through callus showed multiple shoot regeneration.

Figure 3: Tuberose plantlets obtained from in vitro propagation with varying shoot length, number of leaves and rootlets obtained from different culture media (treatments)

(1.0 mg l^{-1}) and NAA (0.5 mg l^{-1}) 15 days after inoculation. Similar observation was reported by Nazneen *et al*. (2003) that the bulb pieces showed maximum callus induction of 70 % at 0.5 mg l^{-1} each of BAP and 2,4- D. Shoot buds showed 100% callus induction at 1.0 mg l^{-1} each of BAP and 2,4- D.

Effect of different concentrations of NAA and constant BAP concentration on shoot regeneration of tuberose

In this set of experiment different concentrations of NAA (0.5, 1.0, 1.5, 2.0 mg l^{-1}) was taken in combination with constant BAP concentration (0.5 mg l^{-1}) and their effect was studied on the shoot regeneration of cv. Prajwal of *Polianthes tuberosa* L. after third subculturing result was presented in table 2.

The effect of MS medium supplemented with different concentration of growth hormones was found to be statistically significant for shoot regeneration in tuberose. It was observed that the maximum number of shoots regeneration (2.12/ culture) was obtained from T4 containing 2.0 mg l^{-1} NAA + 0.5 mg l^{-1} BAP followed by 1.55/ culture obtained from treatment T3 containing 1.5 mg l^{-1} NAA + 0.5 mg l^{-1} BAP and least 1.11/ culture was shown by treatment T1 containing 0.5 mg l^{-1} NAA + 0.5 mg l^{-1} BAP. Similar results were reported by Jitendriya *et al*. (2013) by using Calcutta Single var. of *Polianthes tuberosa* L. under *in vitro* condition gave on an average 3.5 $\pm$ 0.2 shoots when explant was on cultured MS medium containing 0.2 mg/ l BAP and Kn. Similar results has been reported in tuberose by Kadam *et al*. (2010) and Jyothi *et al*. (2008) in case of tuberose cv. Prajwal and Vaibhav.

The highest regenerated shoot length (6.42cm/culture), number of leaves (3.23/culture) and number of roots (6.62/ culture) 15 days after third sub culturing were observed in T4 containing 2.0 mg l^{-1} NAA + 0.5mg l^{-1} BAP followed by shoot length (5.48 cm/culture), number of leaves (2.27/ culture) and number of roots in regenerated shoots (5.85/ culture) obtained from treatment T3 containing 1.5 mg l^{-1}NAA + 0.5 mg l^{-1} BAP , Table-2. All parameters was found to be statistically significant in case of tuberose cv. Prajwal.

The size of callus was found to be statistically non-significant in case of *Polianthes tuberosa* L. cv. Prajwal, after third subculturing. The largest size callus (2.88 cm/ culture) was obtained from NAA (1.0 mg l^{-1}) and BAP (0.5 mg l^{-1} and the smallest size callus (2.28 cm/culture) was produced from NAA (0.5 mg l^{-1}) and BAP (0.5 mg l^{-1}) in MS medium 15 days after third subculturing.

Rooting and acclimatization

The influence of different concentrations of NAA and BAP on root induction from *in vitro* regenerated shoots after

one month of culture was also observed in this study. Rooting frequency was highest in the media containing NAA (2.0 mg l^{-1}) and BAP (0.5 mg l^{-1}) . Rooted plantlets were then transferred to a plug trays of sterile, moist coir dust. After four week of acclimatization, the plants grew vigorously and 80% survived in pot containing garden soil and sand after few weeks hardend plants were transferred to field with 100% survival rate. These results are in agreement with Nazneen *et al*. (2003) who found that the best root development was achieved on MS medium supplemented with 0.5 mg l^{-1} NAA.

REFERENCES

Bhatt, Sandhya, Sharma, S., Sharma, V. K., Pratap, P. S. and Kajla, S. 2015. Effect of concentration and application duration of EMS on *In vitro* root formation of Camarosa Strawberry (Frageria X Ananassa). *The Bioscan*. **10(1):** 187-191.

Gajbhiye, S. S., Tripathi, M. K., Shankar, M. V., Singh, M., Baghel, B. S. and Tiwari, S. 2011. Direct shoot organogenesis from cultured stem disc explants of tuberose (*Polianthes tuberosa* Linn.). *Intrl. J. Agri. Tech*. **7(3):** 695-709.

Jitendriya, P., Vaishali, D. R. and Patel, C. 2013. Multiple shoot regeneration of *Polianthes tuberosa* cv. *Phulerajni and Calcutta double*. *Indian J. Hort*. Volume: 3, Issue : 5.

Jitendriya, P. and Saiyad, M. L. 2013. In vitro propagation of *Polianthes tuberosa* L. cv. Calcutta Single. *Intrl. J. Plant, Animal and Environmental Sci*. Volume: 3, Issue : 3.

Jyothi, R., Singh, A. K. and K.P. Singh. 2008. *In vitro* propagation studies in tuberose (*Polianthes tuberosa* Linn.). *J. Ornamental Horticulture*.**11(3):** 196-201.

Kadam, G. B., Singh, K. P., Singh, A. K. and Jyothi, R. 2010. *In vitro* regeneration of tuberose through petals and immature flower buds. *Indian J. Hort*. **67(1):** 76-80.

Khan, N. H., Zaidi, N., Jabeen, S. and Javaid, I. 2000. Micropropagation potential of *Polianthes tuberosa* L. bulb scales and leaves. *Pak. J. Scientific and Ind. Res*. **43:** 118-122.

Kumari, K., Dhatt, K. K. and Kapoor, M. 2013. Induced mutagenesis in *Chrysanthemum morifolium* variety 'Otome Pink' through gamma irradiation. *The Bioscan*. **8 (4):** 1489-1492.

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

Nazneen, S., Jabeen, M. and Ilahi, I. 2003. Micropropagation of *Polianthus tuberosa* (tuberose) through callus formation. *Pakistan J. Botany*. **35(1):** 17-25.

Sangavai, C. and Chellapandi, P. 2008. *In vitro* propagation of tuberose plant (*Polianthes tuberosa* L.) . *Electronic J. Biology*. **4(3):** 98-101.