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Direct shoot organogenesis from bulbs explants of *Polianthes tuberosa* cultivars (Prajwal and Shringar)

KEYWORDS: BAP, Kn, Prajwal, Shringar

Jitendriya Panigrahi	Nikita M Mistry	Illa C Patel
Asst. Prof, Shri A.N. Patel P.G. Institute, Anand, Gujarat- 388001	Dept. of Biotechnology, Shri A.N. Patel P.G. Institute, Anand, Gujarat- 388001	Asst. Prof in Botany, Dept of Botany, Hemchandracharya North Gujarat University, Patan, Gujarat

ABSTRACT *Ornamental plant Polianthes tuberosa (Amaryllidaceae) contains flowers which are used in wedding ceremonies, garlands, decoration and various traditional rituals. The present investigation given emphasis on: 1. In vitro propagation of two hybrid variety of Polianthes tuberosa namely Prajwal and Sringar from bulb as explant. 2. Effect of different combinations of BAP and Kinetin for its shoot proliferation, and to standardize the optimum concentration of growth regulators for proper growth of these hybrid varieties of Polianthes tuberosa. The rate of shoot proliferation frequency was gradually raised and reached maximum at 2.0mg/l concentration of both BAP and Kn in Prajwal as compared to 0.2mg/l in Shringar. The plantlets were incubated in a solution of IBA (0.5 mg l^{-1}) and IAA (2.0 mg l^{-1}) and IBA (0.5mgl^{-1}) and IAA (2.5 mgl^{-1}) at 5^{0}C to induce rooting of Prajwal and Shringar varieties respectively.*

INTRODUCTION

The tuberose (*Polianthes tuberosa*) is an herbaceous, perennial plant belongs to *Amaryllidacea* family. Tuberose is a popular flower in floral arrangements and their scent is used to produce perfumes the world over **(**Sangavai, 2008). The Tuberose is a night blooming plant thought to be native to Mexico and is mostly grown in southern hemisphere but can do nicely in the north if planted in a protected sunny location (Singh, 1995; Trueblood, 1973). The long spikes of flowers are excellent for cut flower and people like their sweet fragrance (Moazz Hassanpour Asil, 2011).

Tuberose inflorescences (spikes) bear 10 to 20 pairs of florets which open. Unopened flower buds scarcely open after harvest, and thus display quality of Tuberose spikes is limited (Michael Reid, 1996) .Its bloom time is August to September. The Tuberose exists only in cultivation. Flowers single, or double with twice or three times the number of petals. They are slow growers and one will need to have patience while waiting for them to pop out of the ground but these tropical beauties are well worth of time. It prefers to be kept on the dry side and needs rich well-drained, somewhat sandy, soil. The flowers are used in wedding ceremonies, garlands, decoration and various traditional rituals.$AgNO_3$, $CaCl_2 2H_2O$ and Tri-Miltox Forte treatments delayed flower opening and also extended vase-life. (Muhammad Akbar et al., 2001). Moreover Tri-Miltox Forte treatments maintained the fragrance of flowers for a longer period (Muhammad Akbar et al., 2001)**.** In tuberose, various explants have been tried to produce regenerable cultures via *in vitro* morphogenesis are shoot tips (Hutchinson et al., 2004), bulb scale (Muralidhar and Mehta, 1982; Bose et al., 1987; Khan et al., 2000; Rajeshkaran et al., 2000; Nazneen et al., 2003) rhizome (Sangavai and Chellapandi, 2008).

The traditional method of propagation through bulbs is rather slow to meet the growing demand and, therefore, direct shoot organogenesis through tissue culture may be utilized for rapid and large-scale multiplication.

MATERIALS & METHODS

Explant material & Inoculation

Two cultivars of *Polianthes tuberosa* were selected for the study. Two cultivars viz: *Prajwal* and *Shringar* were procured from Navasari Agriculture University, Navasari. (Navasari -Surat).

The explants were placed on solid Murashige and Skoog's (MS) basal medium (Murashige, T. and Skoog, F., 1962*)* containing 0.8% (w/v) agar, and various concentrations of BAP (6-benzylaminopurine), Kn (kinetin) and IAA (indol-3-acetic acid) in combination used for shoot proliferation and root proliferation. These media were supplemented with growth regulators of different concentration (0.2 mg/l, 0.5 mg/l, 1.0 mg/l, 1.5 mg/l, 2.0 mg/l, and 2.5 mg/l) and adjusted to pH 5.8 prior to autoclaving at 120°C for 20 minutes. Culture media (25ml) were dispensed into 50 ml test tubes and plugged with cotton. These were maintained under long day conditions (16 hours light/8 hours dark cycle at an intensity of 2000-lux luminance at 25±2 °C) and 60% RH (Gajbhiye et al, 2011) .Shoot proliferation was observed after 4 to 5 days of inoculation. The effect of hormones on shoot proliferation and root proliferation was studied and effort was made determine the appropriate hormone combinations for optimal shoot proliferation and root proliferation.

RESULT

Hybrid Variety *Prajwal*

The research conducted to optimize the medium for the multiplication of *Prajwal* of *Polianthes tuberosa L.* have been depicted in Tables 1 and 2.

Effect of BAP and Kn

The shoot initiation of hybrid variety *Prajwal* of *Polianthes tuberosa L.* from explant (Bulb) with different concentration of growth regulators BAP (0.2-2.5mg/l) and Kn (0.2 – 2.5mg/l) and the results are presented in Table 1 and shoot growth is depicted in Plate1(A).

Effect of IBA and IAA

The effects of the different concentration of growth regulators IBA (0.2 mg/l) and IAA (0.2 – 2.5mg/l) on root induction of hybrid variety *Prajwal* of *Polianthes tuberosa L.* from explant (Bulb) were presented in Table 2.The data showed that in Prajwal the combination of IBA (0.5mg/l) and BAP (2.0mg/l) gave best result for initiation of root at 5^{0}C.

Hybrid Variety *Shringar*

Effect of BAP and Kn

The shoot initiation of hybrid variety *Shringar* of *Polianthes tuberosa L.* from explant (Bulb) with different concentration of growth regulators BAP (0.2-2.5mg/l) and Kn (0.2 – 2.5mg/l) and the results are presented in Table 1 and shoot growth is depicted in Plate1(B).

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Effect of IBA and IAA
The results of the different combination of growth regulators IBA (0.5 mg/l) and IAA (0.5 – 3.0 mg/l) on root initiation of hybrid variety *Shringar* of *Polianthes tuberosa L.* from explant (Bulb) and the results are presented in Table 2. Analogous to Prajwal, it showed root growth at combined concentration of IBA (0.5mg/l) and IAA (2.5mg/l), when placed at 5^0C.

DISCUSSION

A protocol for the *in vitro* regeneration of shoot initiation has been suggested. BAP in combination with kinetin was found suitable for shoot induction. For shoot proliferation growth regulators especially cytokinin is one of the most important factors affecting the response. (Lane 1979, Stolz 1979,; Bhojwani 1980; Garland and Stolz, 1981).A wide range of cytokinin like kinetin, BAP, and zeatin has been employed in shoot proliferation (Bhojwani and Razdan, 1982). Murashige (1974), Hussey(1978) and Sharon and D'sauza (2000) described 2-ip as more effective than Kinetin or BAP. However, wide range survey of literature suggests that BAP is the most reliable and effective cytokinin.

Gajibhiye, S.S**.,** Tripathi (2011) has studied on the shoot organogenesis from explants of tuberose. In this study they reported the effect different auxin (alone), cytokinin (alone) and combination of auxin and cytokines on the proliferation of shoot. They reported that culture media fortified with auxin (2,4-D or 2, 4, 5-T) as alone in varying concentration performed poorly, as compared to media supplemented with NAA. This study suggests that NAA is more effective among three auxins. When they added cytokinins with varying concentration of BAP, Kinetin and TDZ supplemented in the media, they showed a higher response compared to culture fortified with auxin alone. They reported that TDZ more effective as compared to other media supplied with BAP and Kinetin.

The concentration of BAP above 4.0mg/l were not found effective for inducing higher number of shoot and length of shoots were inhibited due to inhibitor effect of BAP which results into bushy appearance due to excessive achlorophyllous tissue at the base. The results are conformity with Mishra et al.(2006). Huetteman and Preece (1993), who reported that TDZ at higher concentration inhibit shoot elongation in many species.

Medium supplemented with NAA in combination with cytokinins has shown to promote shoot differentiation, these result were reported by Mishra et al.(2006), Jyothi et al(2008), and Kadam et al (2009). The quality of shoots and overall growth response was better in these growth regulator combinations.

For induction of in vitro rooting, auxin like IBA, IAA and NAA are effective. In tuberose, IBA (Rajeshkaran et al, 2000; Krishnamurthy et al 2009) and NAA (Nazneen et al, 2003; Mishra et al 2006) were found effective for inducing in vitro rooting. The most effective auxin for rooting are IBA and NAA.(Perik,1987 ; Uddin et al,2005), likewise Amin et al (2002) reported that root initiation on in vitro raised on *Laxora fillgen*on half strength MS supplemented with 0.2mg/l IBA.

Among auxin, IBA was the most effective than any other synthetic auxin in the most of the case apparently because it is not destroyed by IAA oxidase or other enzymes and therefore persist longer. These results are in conformity with the earlier finding of Rajeshkaran et al (2000), Krishnamurthy et al (2001), Mishra et al (2006), and Kadam et al (2009) for in vitro rooting response of tuberose.

In present study, full strength MS medium supplemented with IBA and IAA with different concentration and sub-culturing them continuously, root initiation were observed. These results can be explained on the basis that different plants and even different organ of same plant are characterized not only by their unique intrinsic biochemical make –up but also by the sensitivity of the endogenously supplied chemical stimuli.

CONCLUSION

In conclusion, the present study, we established an efficient and reliable micropropagation protocol for *in vitro* regeneration of two varieties of *Polianthes tuberosa* from bulb as explant, which can ensure large scale propagation, as well as protocol can also be used for raising genetically uniform plants, which is important for the sustainable supply of plant materials to the pharmaceutical industries and for horticultural market.

Table.1. Effect of BAP and Kn on shoot induction from bulb of *Polianthes tuberosa*

		Prajwal			Shringar		
Concentration (mg/l)	Concentration (mg/l)	Days of shoot initiation	Shoot length (cm) Mean± standard error of mean	Shoot induction %	Days of shoot initiation	Shoot length (cm) Mean± standard error of mean	Shoot induction %
BAP	Kn						
0.2	0.2	8	3.5±0.2	30	4	7.0±0.08	70
0.5	0.5	6	4.0±0.11	40	5	5.1±0.09	60
1.0	1.0	6	4.1±0.09	40	6	3.9±0.11	46
1.5	1.5	5	5.4±0.09	60	6	3.7±0.09	45
2.0	2.0	4	7.2±0.08	70	6	3.6±0.11	30
2.5	2.5	6	4.2±0.11	41	8	3.5±0.2	27

Table.2. Effect of BAP and Kn on root induction from bulb of *Polianthes tuberosa*

		Prajwal			Shringar		
Concentration (mg/l)	Concentration (mg/l)	Days of root initiation	Root length (cm) Mean± standard error of mean	Root induction %	Days of Root initiation	Root length (cm) Mean± standard error of mean	Root induction %
IBA	IAA						
0.5	0.5	10	1.5±0.02	30	11	2.0±0.07	30
0.5	1.0	9	2.9±0.11	40	10	2.7±0.08	34
0.5	1.5	6	3.2±0.09	40	9	2.9±0.11	51
0.5	2.0	5	4.1±0.09	80	7	3.2±0.09	65
0.5	2.5	7	3.3±0.08	70	5	3.6±0.11	80
0.5	3.0	8	3.0±0.071	41	6	3.5±0.08	70

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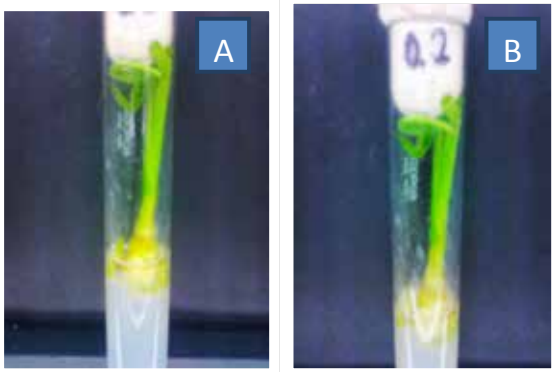

Plate1 (A) Effect of BAP and Kn on *Prajwal,* (B) Effect of BAP and Kn on *Shringar*
Note: - Table.1. Should be placed just below the description of Prajwal in result
Table.2. Should be placed just above the description of Shringar in result
Plate.1. Should be placed in the last part of result

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