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# Multiple Shoot Regeneration of *Polianthes tuberosa* Cultivars Phulerajni and Calcutta double

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**ABSTRACT**

*Polianthes tuberosa is an important ornamental plant and used in floriculture industry and cosmetic industry for its essential oils. Although tuberose propagates vegetatively in its natural state, but propagation rate is too slow to meet the demand of high quality planting material for commercial cultivation. Micropropagation method for two varieties of Polianthes tuberosaviz. Phule Rajni& Calcutta double by using bud as explant was standardized. Maximum shoot proliferation was achieved onmedium containing BAP 1.0mg/l with Kn1.0 mg/lin Phule Rajni and BAP( 2.5mg/l) and Kn (2.5mg/l) in Calcutta Double within 6 days of culture.Rooting of shoots was achieved on MS medium with NAA (0.5 mg/l) and IAA (0.5 mg/l) in Phule Rajni and NAA (3.5mg/l) and IAA (0.5 mg/l) in Calcutta Double within 7days. Plantlets were successfully transferred to pots for acclimatization after they had grown more than 3 roots and average root length exceeded 10 mm.*

**Keywords : BAP, Kn, NAA, IAA, Phule Rajni, Calcutta Double**

## INTRODUCTION

Among ornamental bulbous plants valued for their beauty and fragrance of the flowers, the tuberose (*Polianthes tuberosa* L.) occupies a very special and selective place (Sood and Nagar, 2005). Moreover, its flower is a very good source of essential oils which is used in the production of cosmetic and perfumery products (Hussain, 1986). The long spikes of flowers are excellent for cut flowers and people like their sweet fragrance (De Hertogh and Le Nard, 1993). Tuberose inflorescences (spikes) bear 10 to 20 pairs of florets which open from the base upward. Commercially, spikes 2 to 3 feet long are harvested when the basal florets are open. Unopened flower buds scarcely open after harvest, and thus display quality of tuberose spikes is limited (Reid, 1996). Plant hormones enable prolonging the vase life and delaying the onset of senescence. Flower senescence proceeds by coordinated regulation of plant hormones and response to them. Cytokinins and gibberellins tend to retard flower senescence (Halevy and Mayak, 1981). Cytokinins are plant hormones that plants produce naturally and regulate plant growth, including cell division and leaf senescence. There are several commercial plant growth regulators (PGRs) that contain benzyladenine, a synthetic cytokinin (Padhye et al., 2008).

Tuberose is an important ornamental plant belonging to family Amaryllidaceae. Micropropagation has been proven to be an extremely useful technique for clonally propagation of many species, especially ornamental plants. It is well known that several factors can affect in *vitro* micropropagation (George and Debergh, 2008). Most important of these parameters are the plant growth regulators content in the culture media (Gomes and Canhoto ,2000). Plant growth regulators act like signals to stimulate, inhibit or regulate growth in the developmental programs of plants (Mercier *et al*., 1997).

The conventional method of propagation through bulbs is rather slow to meet the growing demand and, therefore, *in vitro* multiplication shoot regeneration through tissue culture may be utilized for rapid and large-scale multiplication.

## MATERIALS & METHODS

**Choice of Material:**

*Polianthes tuberose* cv. Phule Rajni and Calcutta double, perennial plant of family *Amaryllidaceae* was selected as the experimental material and was collected from Agriculture University of Navsari, Gujarat.

The explants were employed 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), NAA and IAA (indol-3-acetic acid) in combination used for shoot and root proliferation. These media were accompanied 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,2.5 mg/l, 3.0 and 3.5) and adjusted to pH 5.8 prior to autoclaving at 120°C for 15 minutes. Culture media (25ml) were poured 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±2ºC) and 60% RH (Gajbhiye et al, 2011) .Shoot proliferation was observed after 5 to 6 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 and root proliferation.

## RESULTS

**Cultivar Phule Rajni**

**Effect of BAP and Kn on shoot induction**

The shoot induction of hybrid variety Phule Rajni of *Polianthes tuberosa L*. from bulb as explant with different combination 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).The maximum shoot induction was shown by the combination of 1.0 mg/l of both BAP and Kn.

**Effect of NAA and IAA on root induction**

The results of the different combination of growth regulators NAA (0.5 mg/l) and IAA(0.5 – 3.5 mg/l) on rootinitiation of hybrid variety Phule Rajni of *Polianthes tuberosa L*. from explant

(Bulb) and the results are presented in Table 2. It showed root growth at combined concentration of NAA (0.5mg/l) and IAA (2.5mg/l), when placed at 5$^0$C.

**Calcutta Double**

**Effect of BAP and Kn on shoot induction**

The multiplication of shoot along with induction of shoot was best observed in combination of growth regulators BAP (2.5mg/l) and Kn (2.5mg/l). The results were shown in Table.1 and shoot growth is shown in Plate 1 (B).

**Effect of NAA and IAA on root induction**

Almost all the growth regulators combination showed initiation of root but it was best shown by the combination of NAA (0.5mg/l) and IAA (3.5mg/l). It was represented in Table.2.

**DISCUSSION**

The goal of in vitroculture of tuberose is to develop persistent protocol for achieving plant regeneration in higher frequencies from bulb ( explants ) in order to use them for mass clonal propagation of desirable genotypes or cultivars .

In tuberose , various explants have been tried to produce regenerable plants 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; Rajasekaran*et al.,* 2000; Nazneen*et al*.,2003; Mishra *et al*., 2006 ) rhizome ( Sangavai and Chellapandi, 2008 ) , leaf disc ( Bindhani*et al*., 2004 ) , root ( Narayana Swamy and Prabhu Desai, 1979 ).However, the regeneration frequencies in all above experiments were found considerably moderate.

Even if , a few studies have been conducted in some laboratories in India and abroad to obtain prolific *in vitro*-culture system of tuberose with limiting regeneration potential , no work has been done so far in micropropagation . In view of this , an experiment was conducted to select the most responding cultivar to different growth hormones ratio and other physical factors exhibiting higher invitromorphogenesis by means of media standardized for two cultivars of tuberose viz*:* Phule Rajni and Calcutta double which has a high commercial value in Indian flower market.

**CONCLUSION**

As per the two varieties are concerned Phule Rajni shows better results as compared to Calcutta Double, which infers that Phule Rajni may possess higher endogenous hormone concentration that may lead to better growth.

Here, we have used semisolid media as the supporting material. In future we can use liquid media and study its effect. This may cause increase in growth as compared to semisolid media use because of proper aeration provided in liquid culture and also reduces the cost efficiently.

**Table.1: Effect of different concentration of BAP and Kn on shoot induction from bulb of *P. tuberosa* L. (P*hule Rajni*)**

| | | Phule Rajni | | | Calcutta Double | | |
|---|---|---|---|---|---|---|---|
| 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 | 3.4±0.2 | 35 | 7 | 6.0±0.08 | 60 |
| 0.5 | 0.5 | 6 | 4.0±0.11 | 40 | 8 | 5.1±0.09 | 50 |
| 1.0 | 1.0 | 6 | 7.1±0.07 | 80 | 8 | 4.1±0.11 | 48 |
| 1.5 | 1.5 | 5 | 5.4±0.09 | 60 | 8 | 3.7±0.09 | 47 |
| 2.0 | 2.0 | 4 | 5.2±0.08 | 70 | 8 | 5.9±0.11 | 55 |
| 2.5 | 2.5 | 6 | 4.4±0.11 | 41 | 6 | 7.5±0.2 | 80 |

**Table.2: Effect of different concentration of BAP and Kn on root induction from bulb of *P. tuberosa* L. (*Calcutta Double*)**

| | | Phule Rajni | | | Calcutta Double | | |
|---|---|---|---|---|---|---|---|
| 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 % |
| NAA | IAA | | | | | | |
| 0.5 | 0.5 | 05 | 4.5±0.02 | 80 | 11 | 3.0±0.07 | 38 |
| 0.5 | 1.0 | 06 | 3.9±0.11 | 70 | 09 | 3.5±0.08 | 40 |
| 0.5 | 2.0 | 08 | 3.2±0.09 | 60 | 08 | 3.9±0.11 | 49 |
| 0.5 | 2.5 | 08 | 3.1±0.09 | 50 | 07 | 4.2±0.09 | 63 |
| 0.5 | 3.0 | 09 | 3.0±0.08 | 42 | 07 | 4.6±0.11 | 69 |
| 0.5 | 3.5 | 10 | 2.9±0.071 | 39 | 06 | 5.1±0.08 | 80 |

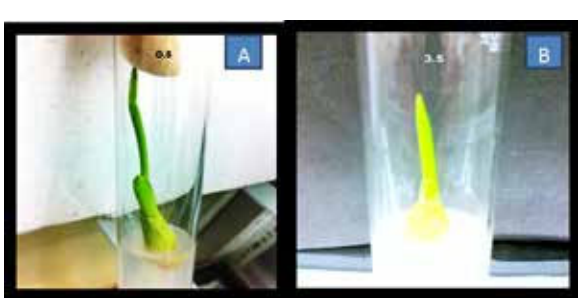


**Plate No.1:- (A) Effect of BAP and Kn on Phule Rajni, (B) Effect of NAA and Kn on Calcutta Double**

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