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In Vitro Propagation of Hybrid Cultivars (Hyderabad Single & Local Double Navsari) of Ornamental Plant *Polianthes Tuberosa* L

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ABSTRACT

Tuberose, Polianthes tuberosa L. (Amaryllidaceae) is an herbaceous perennial commercially grown for its fragrant cut flowers and for the perfume industry. The present study includes: - 1. In vitro propagation of two hybrid variety of Polianthes tuberosa namely Hyderabad Single and Local Double Navsari from bulb as explant. 2. Effect of cytokinin and auxin of different concentration for shoot proliferation, to obtain their maximum growth and to standardize the optimum concentration of growth regulators for proper growth of this hybrid variety of Polianthes tuberosa. The rate of shoot proliferation frequency was gradually raised and reached maximum to 7.1 cm at 0.5mg/l concentration of both BAP and Kn I Hyderabad single as compared to 6.5cm at 2.0mg/l in Local Double Navsari. The plantlets were incubated in a solution of BAP (0.5 mg l^{-1}) and IAA (2 mgl^{-1}) and BAP (0.5mgl^{-1}) and IAA (3mgl^{-1}) at 5^{0}C to induce rooting of Hyderabad single and Local Double Navsari respectively.

KEYWORDS: BAP, Kn, Cytokinin, Auxin, Shoot proliferation

INTRODUCTION:-

Tuberose, *Polianthes tuberosa* L. (Amaryllidaceae) is an herbaceous perennial (Edwards, 2006), commercially grown for its fragrant cut flowers and for the perfume industry .Tuberose is a night blooming plant, belongs to the family Amaryllidaceae and is native to Mexico (Singh, 1995; Trueblood, 1973). Tuberose is not a popular name in the world of herbal medicines. Rather, it is very popular and priced among perfume manufacturers. Its flowers have beautiful fragrance which is active at night and this flower blooms at night. Due to this, tuberose is popularly known as "Night Queen" or "Mistress of the Night" or "Raat ki Raani" as it is known as in Hindi. *Polianthes tuberosa* is a day-neutral plant (Chang et al., 1998), floral induction and initiation of the inflorescence being brought about by relatively high temperatures after the corm has reached an appropriate size. The tuberose occupies a very selective and special position among the ornamental bulbous plants for its beauty, elegance and sweet pleasant fragrance. It has a great economic potential for cut-flower trade and essential oil industry.

Due to their great demand, it is currently cultivated in most of the tropical and sub-tropical countries of the world (Jowkar and Salehi, 2006; Benschop, 1993; Huang et al, 2001). Tuberose is generally planted in February-March in the plains and in April-May in the hills. On a medium fertile clay soil, the best time for planting was reported between 14 and 29 June. In southern parts of India, the bulb should be planted in the month of July-August. Planting of bulbs in the month of April has shown to record the highest yield of spikes (Mukhopadhyay & Banker, 1881) and flowers in the cv. single. Date of planting plays an important role in regulating growth and quality of tuberose. Vegetative growth and quality of tuberose is improved by early plantings (Khobragade et al., 1997).

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

MATERIALS & METHODS:-

Explant material:-

Two cultivars of *Polianthes tuberosa* were selected for the study of bulb culture. Two cultivars *viz:* Hyderabad Single and Local Double Navsari was collected from Navsari Agriculture University, Taluka Navsari and District Surat- Gujarat.

In vitro propagation

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, 2.5 mg/l) and adjusted to pH 5.8 prior to autoclaving at 120°C for 20 minute. 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).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.

Bulb excision

Bulbs were cut down from rhizome carefully where the leaf region is seen i.e. just slightly above the bulb portion. ¼th part of the bulb was placed in the medium. The upper part of the bulb must be in the direction facing the top of the tube.

Culture condition

Explants were subjected to photoperiod regime of 16 hours light/8 hour's dark cycle at an intensity of 2000-lux luminance provided by photosynthetically Active Radiation lamps at 25±2 °C and 60% RH. (Gajbhiye et al, 2011).

RESULT:-

Hybrid Variety Hyderabad Single

The experiments conducted to optimize the medium for the multiplication of Hyderabad single of Polianthes tuberosa L. have been summarized in Tables 1 and 2.

Effect of BAP and Kn

The shoot induction of hybrid variety ***Hyderabad single*** of *Polianthes tuberosa L*. from explant (Bulb) with different concentration of growth regulators BAP (0.5-2.5mg/l) and Kn (0.5 – 2.5mg/l) and the results are presented in Table 1 and shoot growth is depicted in Plate1(A).

Effect of BAP and IAA

The effects of the different concentration of growth regulators BAP (0.5 mg/l) and IAA (0.5 – 2.5mg/l) on root induction of hybrid variety ***Hyderabad single*** of *Polianthes tuberosa L*. from explant (Bulb) were presented in Table 2.

Hybrid Variety Local Double Navsari

Effect of BAP and Kn

The shoot induction of hybrid variety **Local Double Navsari** of *Polianthes tuberosa L*. from explant (Bulb) with different concentration of growth regulators BAP (0.5 - 2.5mg/l) and Kn (0.5 - 2.5mg/l) and the results are presented in Table 3 and shoot growth is depicted in Plate 1(B). The maximum shoot growth occurs at concentration of 2.0 mg/l of both BAP and Kn in combination.

Effect of BAP and IAA

The outcomes of the different concentration of growth regulators BAP (0.5 mg/l) and *IAA* (0.5 – 3.0 mg/l) on root initiation of hybrid variety ***Local Double Navsari*** of *Polianthes tuberosa L*. from explant (Bulb) and the results are presented in Table 4.

DISCUSSION:-

The comparative study of shooting and rooting in these two varieties might be due to high endogenous content of hormone in *Hyderabad Single* as compared to *Local Double Navsari*. Shoot length increased with the increase in BAP level. But growth got inhibited at concentration 1.0 mg/l of BAP and Kn of *Hyderabad Single* and at concentration of 2.5 mg/l of BAP and Kn, the shoot length growth of *Local Double Navsari* was inhibited. High concentration of hormones might hinder the shoot length as well as normal growth of normal shoot elongation.

The goal of *in vitro* culture of tuberose is to develop steadfast protocol for achieving plant regeneration in higher frequencies from bulb cultures in order to use them for mass clonal propagation of desirable genotypes or cultivars. After the *in vitro* multiplication, the plantlets were adapted to an *in vivo* environment. This acclimatization was done simultaneously with a rooting phase. The plantlets were incubated in a solution of BAP (0.5 mg l^{-1}) and IAA (2 mg l^{-1}) and BAP (0.5mgl^{-1}) and IAA (3mgl^{-1}) at 5^{0}C to induce rooting of Hyderabad single and Local Double Navsari respectively.

CONCLUSION:-

One of the main challenges facing production and marketing of good quality tuberose cut flowers is the lack of clean planting material as the resource. Another one noteworthy of this present work is that as India like warm countries we could not propagate *P. tuberosa* widely so that our preliminary attempt will perhaps be helpful for looking further research work and commercialization.

Table:1: Effect of different concentration of BAP and Kn on shoot induction
From bulb of *P. tuberosa* L. (*Hyderabad Single*)

Concentration (mg/l)	Concentration (mg/l)	Days of shoot initiation	Shoot length (cm) Mean ± standard deviation	Shoot Induction (%)
BAP	Kn			
0.2	0.2	5	5.9±0.23	50
0.5	0.5	4	7.1±0.21	70
1.0	1.0	4	6.6±0.24	50
1.5	1.5	5	6.0±0.22	40
2.0	2.0	5	5.6±0.24	40
2.5	2.5	6	4.9±0.25	30

Table2:- Effect of different concentration of BAP and IAA on root induction
from bulb of *P. tuberosa* L (*Hyderabad Single*)

Concentration (mg/l)	Concentration (mg/l)	Days of shoot initiation	Root length (cm) Mean ± standard deviation	Root Induction (%)
BAP	IAA			
0.5	0.2	10	1.2±0.231	50
0.5	0.5	8	2.2±0.234	70
0.5	1.0	5	3.0±0.232	50
0.5	1.5	5	3.2±0.235	40
0.5	2.0	4	3.5±0.237	80
0.5	2.5	6	2.4±0.223	30
0.5	3.0	8	1.9±0.245	20

Table 3: Effect of different concentration of BAP and Kn on shoot induction
From bulb of *P. tuberosa* L (*Local Double Navsari*)

Concentration (mg/l)	Concentration (mg/l)	Days of shoot initiation	Shoot length (cm) Mean ± standard deviation	Shoot induction (%)
BAP	Kn			
0.2	0.2	9	2.1±0.243	20
0.5	0.5	6	2.8±0.251	20
1.0	1.0	6	3.4±0.21	30
1.5	1.5	5	4.9±0.22	50
2.0	2.0	4	6.5±0.231	60
2.5	2.5	5	4.9±0.22	50

Table4. Effect of different concentration of BAP and IAA on root induction
from bulb of *P. tuberosa* L (*Local Double Navsari*)

Concentration (mg/l)	Concentration (mg/l)	Days of root initiation	Root length (cm) Mean± standard deviation	Root induction (%)
BAP	IAA			
0.5	0.2	15	0.3±0.001	10
0.5	0.5	12	0.9±0.004	10
0.5	1.0	6	1.5±0.004	20
0.5	1.5	6	2.1±0.006	20
0.5	2.0	5	2.5±0.005	30
0.5	2.5	4	3.0±0.006	40
0.5	3.0	5	3.8±0.007	50



Plate No.1:- (A) Effect of BAP and Kn on *Hyderabad Single,* (B) Effect of BAP and Kn on *Local Double Navsari*

NOTE:- Table 1 & 2 should be placed after the description of Effect of BAP and Kn in result.

Table 3 & 4 should be placed after the description of Effect of BAP and IAA in result.

Plate should be placed just above the heading of Local Double Navsari.

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