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Research article

IN VITRO PROPAGATION OF*POLIANTHES TUBEROSA* L. CULTIVARS (CALCUTTA SINGLE)

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ABSTRACT: The tuberosa occupies a very selective and special position among the ornamental bulbous plants for its attractive beauty, elegance and sweet pleasant fragrance. It has a great economic potential for cut-flower trade and essential oil industry. Due to its great demand, it has been cultivated in most of the tropical and sub-tropical countries. The main objective of the present investigation was to develop a dependable protocol for large scale clonal multiplication of *Polianthes tuberosa* variety like *Calcutta Single* under *in-vitro* conditions by using different combination and concentrations of growth hormones in MS medium. Among the various hormones used for multiple shoot formation the best response was produced with MS medium containing 0.2 mg/l of BAP and Kinetin with an average of 3.5±0.2 shoots of *Calcutta Single*. Shoots excised from cultures of proliferating shoots were rooted in half-strength MS medium having 0.5 mg/l IAA and BAP. *In vitro* rooted shoots from plantlets grew luxuriantly under field conditions and came to flowering after 1 month of transplantation.

Keywords: *Polianthes tuberosa*, Propagation, Cultivars, Calcutta single

Abbreviations: IAA- Indole Acetic Acid, BAP- Benzyl amino purine, MS- MurashigeSkoog, LAF- Laminar Air Flow

INTRODUCTION

The common name of the plant used, derives from the Latin *tuberosa*, meaning swollen or tuberosa in reference to its root system. It comprises of about 12 species. *Polianthes* means "many flowers" in Greek language. Tuberosa is a native of Mexico from where it spread to different parts of the world during the 16th century. Its flower has a beautiful fragrance which is active at night when it blooms. Due to this it is sometime mistakenly known as “Night Queen” or “Mistress of the Night”. It grows well in Central America and India and is very much in demand in the countries of the Indian Subcontinent, Middle East, and Africa for making perfume. The main constituents of tuberose essential oil are benzyl alcohol, butyric acid, eugenol, farnesol, menthyl benzoate, geraniol and nerol used as chemical compounds [6, 2]. Due to their great demand, it is currently cultivated in most of the tropical and sub-tropical countries of the world [5, 1, 3]. 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 [8] and flowers in the cv. single. Date of planting plays an important role in regulating growth and quality of tuberose.

In India, the commercial cultivation of tuberosa is confined mainly to West Bengal, Karnataka, Tamil Nadu and Maharashtra. However, it is adapted to the North Indian climatic conditions and grows well in Uttar Pradesh. At present the total area .under tuberosa cultivation in the country is estimated to be about 20,000 hectare.

The genus contains about a dozen of species but they are not clearly distinct. Nine of the species have white flowers, one was white-and-red and two are red. The two well-known varieties are Single Mexican and the double pearl. The former is preferred for its enticing fragrance, while the double pearl is preferred in flower arrangements due to the density of flowers on its spike. The pearl also exudes fragrance characteristic of the tuberosa, but to a lesser extent than the Single Mexican variety. Several Indian hybrid varieties are also widely grown. They are as follows;

1. Calcutta Single
2. Phulerajni
3. Prajwal (Single)
4. Shringar
5. Calcutta double
6. Local double navsari
7. Local double navsari
8. Vaibhav (double)
9. Rajatrekha
10. Swarnarekha

The conventional method of propagation through bulbs is rather slow to meet the growing demand. Therefore, the main goal of the present study is to develop a dependable protocol for large scale clonal multiplication of *Polianthes tuberosa* variety. like *Calcutta Single.*

MATERIALS & METHODS

Explant Preparation and Sterilization

The plants were collected from Navsari Agricultural University, Navsari. The collected plants were washed thoroughly in running tap water for 15 min to remove soil particles. The bulbs were cut from the rhizome portion by the help of knife.

The explants were sterilized in two steps.

In step 1, the prepared explants were washed thoroughly with running tap water for 2-3 times to minimize the loss of culture due to microbial contamination and they were soaked with detergent for 10 min. It was followed by rinsing in running tap water to remove the detergent followed by D/W for 2-3 times. They were then transferred to another beaker containing bavistin with 2-3 drops of tween-20 solutions for 20 min.

In step 2, performed under the LAF unit, the explants were rinsed thoroughly with sterile D/W for 3-4 times in an aseptic condition. For the surface sterilization, explants were rinsed in 0.1% mercuric chloride ($HgCl_2$) solution for 2 min. They were rinsed properly with sterile D/W for 3-4 times inside the clean bench to remove all traces of $HgCl_2$ because it having carcinogenic nature [7]. Explants were inoculated in MS medium supplemented with different levels of auxins and cytokinins concentration.

Sterilized explants (bulbs) were excised from both ends, using a fine sterile 1forceps and scalpel. All the cultures were maintained in an air conditioned culture room at a temperature of 25 ± 4^0C with a light intensity of 2500-3000 lux (approximately) provides cool light. The maintain photoperiod was 16 hours light and 8 hours dark and relative humidity of 45-50%.

Shoot Induction and Multiplication

In this first set of experiment, the bulbs of rhizome of *Calcutta Single* were cultured in full strength MS medium supplemented with combination of two different hormones namely BAP and kinetin of concentration (0.2 to 2.5) mg/ l. The proliferating shoots were subcultured on fresh medium as single shoot or in a group of five and ten shoots to study the effect of initial inoculum on the rate of shoot multiplication.

Rooting of Shoots

For root induction, approximately 3-cm-long shoots, excised from cultures of proliferating shoots, were inoculated on MS medium supplemented with different auxins, namely, BAP and IAA with different combinationfrom 0.5 to 3.5 mg/l.

Acclimatization of *In-Vitro* Raised Plantlets

The rooted shoots—plantlets—were taken out from the culture tubes, their root system washed off under running tap water to remove traces of medium and transplanted in a potting mixture comprising garden soil and leaf mold (3:1) in 10-cm earthen pots and kept for 30 days in hardening room where they were acclimatized by growing them first in high humidity (99% RH) and gradually the humidity was reduced to 70% during a period of 30 days. The hardened potted plants were kept in glasshouse for about 2 months and finally transferred to field.

RESULTS

Effect of BAP and Kinetin

As compared to control, among the various hormones used for multiple shoot formation the best response was produced with MS medium containing 0.2 mg/l of BAP and Kinetin with an average of 3.5±0.2 shoots of *Culcutta Single* whereas 2.0 mg/l of BAP and kinetin with a shoot length of 6.2±0.3 cm.(Table-1 and Figure-1).

Effect of IAA and BAP

The best rooting appeared in combination of 0.5mg/l IAA and 05mg/l BAP with full strength MS medium; whereas control did not show any apparent result.These outcomes were shown in Table.2.

Table1: Effect of different concentrations of BAP and Kinetin in full strength MS medium on the shoot development and multiplication of *Calcutta Single* buds

Variety	Treatment (mg/lit)BAP + kinetin)	Shoot induction %	Days of initiation	Shoot length after 2 weeks (cm) Mean ± Standard deviation	No. of shoot Mean ± Standard deviation
Calcutta Single	**0.2+0.2**	**70**	**4**	**6.23±0.3**	**3.5±0.2**
	0.5+0.5	50	5	2.46±0.31	2.8±0.5
	1.0+1.0	60	5	2.31±0.29	2.5±0.4
	1.5+1.5	60	5	2.6±0.31	3±0.7
	2.0+2.0	40	7	1.66±0.298	1.90±0.2
	2.5+2.5	50	6	1.96±0.301	2.2±0.4
Control	-	20	9	1.0±0.31	1.8±0.2

Table 2: Effect of different concentrations of IAA and BAP in full strength MS medium on the root development and multiplication of *Calcutta Single*

Variety	Treatment (mg/lit) IAA+ BAP	Root induction %	Days of initiation	Root length after 2 weeks (cm) Mean± Standard deviation	No. of roots Mean± Standard deviation
Calcutta Single	**0.5+0.5**	**50**	**7**	**5**±0.25	**3±0.3**
	1.0+0.5	30	8	4±0.245	2±0.4
	2.0+0.5	20	8	3.4±0254	2.1±0.5
	2.5+1.5	20	10	2.8±0.234	2.6±0.3
	3.0+0.5	-	No response	-	-
	3.5+0.5	-	No response	-	-
Control	-	10	12	0.7±0.21	1

Figure-1: Multiple shoot formation of Calcutta Single in full strength MS medium supplemented with 0.2 mg/l BAP and 0.2 mg/l kinetin after first subculture

DISCUSSION

As growth concern, the endogenous hormone level is higher in the *Calcutta Single* variety as it necessitates only a small concentration of exogenous hormone that is 0.2 mg/l of BAP and 0.2 mg/l kinetin for its shoot induction and multiplication and simultaneously for root induction. Hutchinson et al evaluated the potential of thidiazuron (TDZ), a phenyl urea, BAP, a cytokinin and NAA, an auxin, on *in vitro* propagation of tuberose (*P. tuberosa* L.) from shoot tip explants [4]. Different urea derivatives are applied *in vitro* and *ex vitro* to enhance the growth and mass production in ornamental hybrid lily and other bulbous crops [10]. A gradual increase of bound ABA (abscisic acid) was observed during release of rest periods of *P.tuberosa* L. Acidic and bound phenols vary with the dormant phases. The levels of IAA remained low during the early stages of bulb sprouting in *P.tuberosa* L. but increased rapidly thereafter [9]. The dramatic reduction in the free ABA content in corm tissues are correlated with floral initiation and flower development in *P. tuberosa.* According to earlier investigations, we revealed that a specific secondary metabolite biosynthesis and accumulation in a plant is depending on the hormones involving in plant growth differentiations at different developmental stages. Therefore, incorporating specific plant growth regulators like IAA, kinetin and BAP in a growth medium (MS) can support the synthesis of chemical compounds of the interests, especially compounds related to perfumes and aroma in *P. tuberosa* L.

The present study is the first to describe a direct regeneration protocol for *Calcutta Single* employing buds. The micropropagation system has assured effective establishment, multiplication, rhizogenesis and acclimatization of this variety and could be exploited to multiply elite genotypes and develop in vitro strategies for the exploitation of this ornamental and medicinal herb.

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