The Bioscan 11(1): 33-36, 2016
AN INTERNATIONAL QUARTERLY JOURNAL OF LIFE SCIENCES
www.thebioscan.in

SELF AND CROSS COMPATIBILITY STUDIES IN TUBEROSE (*POLIANTHES TUBEROSA*)

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KEYWORDS
Tuberose-single
Types-
Self incompatibility
Cross compatibiliy

Received on :
18.09.2015

Accepted on :
21.01.2016

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ABSTRACT
An experiment on selfing and crossing techniques was conducted in the Department of Floriculture and Landscaping, TNAU, Coimbatore during the year 2012-13. The objective of this study was to evaluate self and cross compatibility in tuberose. Ten single genotypes of tuberose *viz*., Calcutta Single, Hyderabad Single, Kahikuchi Single, Mexican Single, Navsari Local, Phule Rajani, Prajwal, Pune Single, Shringar and Variegated Single were used for this study. By 48 hours after pollination, large numbers of germinating pollen grains were observed on most stigmas of both self and cross pollinated pistils but at 72 hours after pollination, none of the self pollinated pollen tubes had penetrated the style but in few crosses, pollen tubes reached the ovules. The present study thus reveals that the single genotypes of tuberose provide an evidence of a game tophytic self incompatibility system and are of cross compatible nature.

INTRODUCTION

Tuberose belongs to the family Amaryllidaceae (Bailey, 1939). It is native of Mexico and one of the most important cut flowers in tropical and subtropical areas. It occupies an important position among commercial ornamental bulbous crops, because of its highly fragrant florets which can be used in various ways and is essentially a florists flower (Sadhu and Bose, 1973; Benschop, 1993). It is commercially cultivated for cut flowers and loose flowers trade and also for extraction of its highly valued natural essential oil. The serene beauty of the flower spikes, bright white florets and delicacy of fragrance of this commercial ornamental crop, transform the entire area into a nectarine and joyous one. Fresh florets and value added products of tuberose are exported from India to USA, Germany, United Kingdom, Italy, The Netherland, Japan, United Arab Emirates and Saudi Arabia. It is cultivated on a large scale in Tamil Nadu, Karnataka, West Bengal and Maharastra and to a lesser extent in Andhra Pradesh, Haryana, Delhi, Uttar Pradesh and Punjab. Single type of tuberose is used for extraction of essential oil which is exported at an attractive price to France, Italy and other countries. There are only a few ornamental varieties of tuberose known *viz*., Calcutta Single, Calcutta Double, Hyderabad Single, Hyderabad Double, Kahikuchi Single, Mexican Single, Navsari Local, Pearl Double, Prajwal, Pune Single, Shringar and Suvasini (Sharge, 1976 and Kuang *et al.,* 2001). In India, tuberose is commercially cultivated over 3000 hectare area. Seed setting behaviour in tuberose is quite erratic in single flowered cultivars. Joshi and Pantulu (1941) stated that there is no defect or deformation in the development of pollen grains or embryo sac. However, complete self incompatability is in operation in tuberose hybrids and cultivars (Shen *et al*., 1987 and Uma and Gowda 1990). Cultivar development efforts with *Polianthes tuberosa* has primarily relied on the selection of superior plants from open - pollinated seedling populations (Airadevi and Archana. 2014. and Bhujbal *et al*., 2013). Continued improvement of this species, especially for triats such as growth habit and pest resistance, may require making controlled pollinations between selected individuals. The objective of this study was to evaluate self and cross compatible within and also between genotypes.

MATERIALS AND METHODS

The present study was carried out at Botanical gardens, Tamil Nadu Agricultural University, Coimbatore during the year 2011-2012. It is situated at 11º02" N latitude, 76º57" E longitude and 426.76 m above mean sea level. Experimental material consists of ten single genotypes of tuberose *viz*., Calcutta Single, Hyderabad Single, Kahikuchi Single, Mexican Single, Navsari Local, Phule Rajani, Prajwal, Pune Single, Shringar and Variegated Single. The experiment was laid out in randomized block design (RBD) with three replications. The soil was brought to a fine tilth by giving four deep ploughings. Weeds, stubbles, roots etc., were removed. At the time of last ploughing, FYM was applied at the rate of 25 t ha^{-1}. After levelling, raised beds of 1.5 x 1.5 m were formed and the medium sized bulbs of 3.0 – 3.5 cm diameter weighing about 25 grams were planted at a spacing of 45 x 30 m which accommodates 7 plants per m^2. Uniform cultural practices were followed throughout the experimentation.

Controlled pollinations

Prior to pollination, open florets were removed from inflorescences. For self- pollinations, pollen was obtained from a floret on the same plant as the emasculated flower. In case of cross pollination, florets that were to be used as sources of pollen were covered with a butter paper cover. For the maternal parent, petals and anthers were removed from flowers that appeared, based on size, color and position in the inflorescence, to be within one day before floret opening. Immature florets were then removed and the inflorescence covered with a pollination bag.

For pollinations, florets with newly dehisced pollen were removed and used immediately. The paper bag was removed from the inflorescence of the maternal parent and newly dehisced anthers were touched directly to the exposed stigmas of the emasculated flowers using a camel hair brush. The bag was then placed back over the female inflorescence.

Pollen tube staining and observations

Florets were placed into FAA fixative (70% FAA- 18 ethanol: 1 formalin: 1 glacial acetic acid) at 1, 2, 4, 8, 24, 48 and 72 hours after pollination immediately following collection. After 24 h at room temperature in fixative, flowers were transferred to 70% ethanol where they were stored for up to two months. Prior to staining, florets were rinsed in distilled water and then softened using 8 N NaOH for three hours. Florets were rinsed in distilled water for 30 min prior to tranfer to 0.1% (w/v) aniline blue in 0.3 N K_3PO_4 for one hour.

After staining, florets were placed on a glass slide containing a drop of glycerol and covered with 22 x 60 mm cover slip and pressed gently. The slides were observed under Olympus BX-60 microscope (Olympus America, Melville, N.Y.) equipped with fluorescent attachment using 390-420 nm barrier filter coupled with a 450 nm excitation filter. The pollen tube growth was observed and images were captured with an Olympus digital camera model E500. Pollen germination and growth of the pollen tubes through the styles were observed as bright yellow- green fluorescence. Percentage of florets in which germinated pollen was present in one or more of the stigmas was determined for each treatment. The longest pollen tube in each style was measured using ocular micrometer and an average pollen tube length estimated for each floret. Mean pollen tube length was calculated for each treatment using only those florets in which pollen germination was observed. Ovules were examined for evidence of pollen tube penetration.

Evaluation for self and cross compatibility

Fifty five self and cross pollinations were made on each of the six plants in this study three days after emasculation, which corresponded to two days after anthesis. All self and cross pollinations were made on an individual plant within a 15 minutes time period. Five florets from each set of self and cross pollinations were collected at 1, 2, 4, 8, 24, 48 and 74 h after pollination. Percent florets with germinated pollen, mean pollen tube length, percent florets in which pollen tubes had entered ovules and percent ovules that had been penetarted by pollen tubes were calculated.

Statistical analysis

For the evaluation of self and cross compatibility, ANOVA (analysis of variance) was used to compare pollen tube growth in both self and cross pollinations at each collection time.

RESULTS AND DISCUSSION

Germinated pollen was observed on a few stigmas collected at one to two hours after self and cross pollinated genotypes (Table 1). From 4 to 48 h pollination, germinated pollen was observed on stigmas in most of the florets in both sets of pollinations. Large numbers of germinating pollen grains were observed on most stigmas, making it impossible to quantify number of germinating grains (Table 2). The pollen tubes were easily detected in style, due to their bright fluorescence and minimal background flourescence. By 42 h after pollination, pollen tubes from cross- pollinations had grown to the base of the styles, but most of them had not yet reached the ovules. Pollen tubes from cross-pollination had reached the ovary by 48 h after pollination (Table 3) and were observed by entering of pollen tube in the ovules of the florets (Table 4). Pollen tubes were observed in 60% of the ovules examined at 48 h after cross-pollination. Differences in pollen tube length between self- and cross pollination became apparent by 8 hours after pollination.

From 24 to 48 h after pollination, self- pollen tubes were only 10 to 15% of the length of pollen tubes when comparing the cross pollinated pistils collected at the same time. Pollen tubes from self pollinations reached their maximum length by 24 h after pollination. None of the pollen tubes in the self-pollinated specimens grew to the base of the style. It is resulted because

Table 1: Pollen germination in the stigma of self and cross pollinated florets of *Polianthes tuberosa*

Type of pollination	Hours after pollination	Florets with germinated pollen (%)	Florets in which pollen tubes entered ovules (%)
Self	1	22	0
	2	28	0
	4	58	0
	8	71	0
	24	86	0
	48	91	0
Cross	1	20	0
	2	40	0
	4	80	0
	8	80	0
	24	93	0
	48	100	95

Table 2: Abundance of pollen grain germination on stigma upon selfing and crossing during 48 hours after pollination

	Genotypes	C.S	H.S	K.S	M.S	N.L	P.R	Pr	P.S	Sr	V.S
1.	C.S	3	3	3	3	3	3	3	3	3	3
2.	H.S	3	3	3	3	3	3	3	3	3	1
3.	K.S	3	3	3	3	3	3	3	3	3	3
4.	M.S	NA	NA	NA	3	NA	NA	NA	NA	NA	NA
5.	N.L	3	3	3	3	3	3	3	3	3	1
6.	P.R	3	1	1	1	3	3	3	3	3	1
7.	Prajwal	3	3	3	3	3	3	3	3	3	3
8.	P.S	3	3	3	3	3	3	3	3	3	3
9.	Sr	3	3	1	3	3	3	3	3	3	1
10.	V.S	1	3	1	1	1	1	3	1	3	3

1 = high(above 150)
2 = medium (76-150)
3 = low (1-75)
NA = Not Attempted

C.S - Calcutta Single
H.S - Hyderabad Single
K.S- Kahikuchi Single
M.S- Mexican Single
N.L.- Navsari Local

P.R- Phule Rajani
Pr- Prajwal
P.S- Pune Single
Sr- Shringar
V.S- Variegated Single

Table 3: Number of pollen tubes at stigmatic and stylar regions and entering ovary in tuberose genotypes upon crossing during 24 to 48 hours after pollination

S.No	Cross combinations	Stigmatic region	Middle of the style	Entry at ovary
1	Variegated Single x Calcutta Single	180- 250	116-192	46-65
2	Variegated Single x Kahikuchi Single	206- 216	106-159	40-52
3	Variegated Single x Mexican Single	198- 258	127-175	50-55
4	Variegated Single x Navsari Local	219- 278	127-173	41-61
5	Variegated Single x Pune Single	229- 265	138-170	37-64
6	Variegated Single x Phule Rajani	180- 250	116-192	46-65
7	Phule Rajani x Kahikuchi Single	212- 247	102-157	42-55
8	Phule Rajani x Mexican Single	229- 265	138-170	32-55
9	Phule Rajani x Hyderabad Single	202- 271	123-177	56-79
10	Phule Rajani x Variegated Single	296-318	175-212	73-95
11	Shringar x Kahikuchi Single	180- 250	116-192	46-65
12	Shringar x Variegated Single	198- 258	127-173	41-61
13	Hyderabad Single x Variegated Single	190- 290	135-182	55-82
14	Navsari Local x Variegated Single	197-247	133-172	37-64
	SE	8.94	8.44	2.07
	CD (5%)	28.16	27.52	6.96

Table 4: Number and percentage of ovules with pollen tube at micropylar end after 24 to 48 HAP in crosses

S.No	Cross combinations	Number of ovules in the ovary	Number of ovules with pollen tube at micropylar end	Percentage
1	Variegated Single x Calcutta Single	16	12	75
2	Variegated Single x Kahikuchi Single	14	8	57
3	Variegated Single x Mexican Single	16	13	81
4	Variegated Single x Navsari Local	16	8	50
5	Variegated Single x Pune Single	16	12	75
6	Variegated Single x Phule Rajani	17	15	88
7	Phule Rajani x Kahikuchi Single	15	12	80
8	Phule Rajani x Mexican Single	16	15	94
9	Phule Rajani x Hyderabad Single	16	12	75
10	Phule Rajani x Variegated Single	17	16	94
11	Shringar x Kahikuchi Single	15	8	53
12	Shringar x Variegated Single	16	12	75
13	Hyderabad Single x Variegated Single	14	8	57
14	Navsari Local x Variegated Single	14	9	64

of incompatible mechanism operating in self pollinated tuberose florets. This might be due to the dense accumulation of callose at the tips which prevents further growth of pollen tube. Apart from the arrest of pollen tubes in all self pollinated specimens, several abnormalities were noticed in the present investigation. The most common abnormality was the formation of knot like structures at the tip of the pollen tube. The other abnormalities include bulging at the tip, bursting of pollen tube tip and breakage of pollen tube. A similar type of malformation in pollen tube was observed in Rhododendron

(Williams *et al.*, 1982) although such results were not reported earlier in tuberose. The strength of callose thickening in crosses depends on the taxonomic distance between the pollen and pistil genotypes as was reported by de Nattancourt (1977).

Self- incompatibility is the inability of a plant with functional male and female gametes to set seed when self-pollinated (Brewbaker, 1957). Two homomorphic self- incompatibility systems are recognized (de Nattancourt, 1977). In the sporophytic system, incompatibility is determined by the genotype of the pollen parent and germination of incompatible pollen is inhibited at the stigmatic surface. Game tophytic self-incompatibility is determined by the genotype of the pollen and is manifested by the inhibition of pollen tube growth in the style.

In species with gametophytic self- incompatibility, self pollen tubes generally reach one-third to three-quarters the length of the style in the time required for compatible tubes to penetarte the entire style (Ascher, 1976). This study provides evidence of a self incompatibility system, which appears to be gametophytic nature in tuberose and are of cross compatible nature.

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