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Pollen studies in tuberose cultivars and hybrids

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Abstract

Pollen studies were conducted in ten single type genotypes of tuberose (*Polianthes tuberosa*) *viz.*, Shringar (Mexican Single x Pearl Double), Prajwal (Shringar x Mexican Single), Phule Rajani (Mexican Single x Shringar), Calcutta Single, Hyderabad Single, Kahikuchi Single, Mexican Single, Pune Single, Navsari Local and Variegated Single at TNAU, Coimbatore, India. Studies revealed that pollen grains were round in shape, pollen viability in acetocarmine stain was 96.73% in the genotype 'Variegated Single' and the germination was maximum (99.21%) in 15% sucrose solution with other chemicals. Pollen tubes grew to a length of 1234.949 microns 24 hours after the dehiscence of anther. Among the genotypes under study, the highest fruit set was 89% under natural open pollination and 0% under artificial self pollination.

Key words: Tuberose, single types, pollen, selfing

Introduction

The cut flowers like rose, carnation, gladiolus, tuberose, chrysanthemum, *etc.*, are commonly and frequently demanded in both local as well as international market. Among them, tuberose (*Polianthes tuberosa)* is one of the most important flowers used for both cut and loose flower purpose. It is an ornamental bulbous plant, native of Mexico and belongs to the family *Amaryllidaceae* Bailey (1939). Waxy white flowering spikes of single as well as double flower tuberose impregnate the atmosphere with their sweet fragrance and longer keeping quality of flower spikes (Sadhu and Bose, 1973; Benschop, 1993) and are in great demand for making floral arrangement and bouquets in major cities of India. 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, Suvasini (Sharge, 1976 and Kuang *et al.,* 2001). There is a need to develop varieties in tuberose for higher yield and essential oil content. Pollen germination and the growth of pollen tubes are, in principle, necessary for fertilization and seed formation in flowering plants. A good fruit set and high crop yield depend on healthy pollen grains of respective plants (Bhowmik and Datta, 2011; Bhowmik and Datta, 2012). Studies on *in vitro* pollen germination and pollen tube growth are very useful for explaining fertility (Pfahler *et al.,* 1997; Bhowmik and Datta, 2012). At present, studies on pollen viability and pollen germination in the existing cultivars of tuberose are lacking. Hence, the present pollen studies were undertaken in certain popular genotypes of tuberose to know their fruit set behaviour under selfing and open pollination conditions for understanding their possible use in effective hybridization programme.

Materials and methods

An investigation was undertaken to study the pollen size, equivalent diameter, radius, perimeter, pollen output, pollen viability, pollen germination, pollen tube length, seed setting behaviour of tuberose cultivars and hybrids maintained in the germplasm block at Tamil Nadu Agricultural University, Coimbatore. Three tuberose hybrids (Single x Double) developed by ornamental crop division of Indian Institute of Horticultural Research (IIHR) and Mahatma Phule Krishi Vidyapeeth (MPKV), Maharasthra and seven common cultivars (Single, Variegated) *viz.,* Shringar (Mexican Single x Pearl Double), Prajwal (Shringar x Mexican Single), Phule Rajani (Mexican Single x Shringar), Calcutta Single, Hyderabad Single, Kahikuchi Single, Mexican Single, Pune Single, Navsari Local and Variegated Single were used for the present study.

The parameters such as pollen size, equivalent diameter, radius, perimeter, pollen output and pollen tube length were measured by using microscope connected to a computer with 'Biowizard software'. Pollen output (pollen production per flower) was estimated using a Haemocytometer (Eti, 1990) and the pollen viability was estimated by using acetocarmine stain and the pollen germination status of 10 tuberose genotypes were estimated by using Brewbaker and Kwack media which was prepared by using sucrose (15%) as base medium supplemented with each 200 ppm of calcium nitrate, magnesium sulphate and 100 ppm each of potassium nitrate and boric acid (Boavida and McCormick, 2007). Artificial selfing was carried out on fully developed flower buds of hybrids and cultivars. The fully developed flower buds were bagged to prevent the entry of foreign pollen grains. The fresh pollen from bagged flowers was dusted on to the stigmatic surface after two, three and four days of flower opening. After pollination, flowers were bagged with butter paper cover. Seed set was observed a week after pollination and percentage of fruit set was estimated.

Significance of treatment means was tested using ANOVA and means were compared using LSD values (P=0.05).

Results and discussion

The data revealed that the pollen size, equivalent diameter, radius, perimeter, pollen output, pollen viability, pollen germination, pollen tube length, seed setting behaviour varied with genotype. Among the cultivars, Navsari Local excelled others in registering increased pollen size (17987.98 μm^2), equivalent diameter (161.94 µ), radius (80.06 µ) and perimeter (559.50 µ). This was followed by Hyderabad Single which registered more size (15961.53 μm^2), equivalent diameter (141.29 µ), radius (69.76 microns) and perimeter (546.07 µ) than others. Among the hybrids, Shringar showed its superiority with respect to pollen size (23464.24 μm^2), equivalent diameter (185.81 microns), radius (90.10 microns), perimeter (645.10 µ) followed by Prajwal in respect of above parameters (Table 1). The study revealed that the wide range of pollen size observed in the genotypes. This could be due to abnormal meiosis which occured during microsporogenesis. The diameter of the pollen grain was influenced primarily by the genotype with some effect of environment (Kumar and Sarkar, 1983). A significant variation in pollen grain size and perimeter was noticed between the genotypes. This is primarily due to differences in the genome constitution and variation within the genotype may be attributed to the environment. It has been found that the pollen grain size was controlled by polygenes which are sensitive to environment and pollen grain diameter was mainly controlled by additive components followed by dominance effect with greater influence of environmenal variations in maize (Kumar and Sarhar, 1984) and Sesame (Pfahler *et al.,* 1996). Pollen viability is ability of a pollen grain to germinate and develop as a pollen tube (Gerard, 1932). The growth of the pollen tube can be taken as the measure of pollen viability since the non-viable pollen could not attain the growth of a pollen tube. Good pod set cannot be achieved unless pollen is viable with high germination percentage. The frequency of getting fertile pollen is another factor for ensuring the fruit set. Fertile pollen along with viable pollen favour a better fruit set and consequently an acceptable yield. 'Variegated Single' showed its superiority over other cultivars with pollen output (10,000 anthers), pollen viability (96.73%), pollen germination (99.21%) and pollen tube length (1234.95 microns) . Next to this cultivar, 'Mexican Single' showed higher pollen output (9375 anthers) and pollen viability (89.11%), whereas 'Navsari Local' recorded increased pollen germination (78.52%) and pollen tube length (636.32 microns) (Table 2, 3). Similar results of a study on pollen viability was earlier reported in tuberose by Seetharamu *et al.* (2000).

Joshi and Pantulu (1941) stated that there was no defect or deformation in the development of pollen grains or embryo sac though seed setting behaviour in tuberose is quite erratic. Cultivar

Table 1. Size, equivalent diameter, radius and perimeter of the pollen grain in certain tuberose genotypes

Genotypes	Size (μm^2)	Equivalent diameter (microns)	Radius (microns)	Perimeter (microns)
Calcutta Single	15261.53	140.12	69.65	524.17
Hyderabad Single	15961.53	141.29	69.76	546.07
Kahikuchi Single	15142.46	138.71	68.40	528.18
Mexican Single	13193.75	136.13	68.71	490.30
Pune Single	13319.98	134.20	66.21	502.16
Navsari Local	17987.98	161.94	80.06	559.50
Variegated Single	17567.62	153.55	72.66	566.11
Shringar	23464.24	185.81	90.10	645.10
Prajwal	17557.53	152.55	71.64	565.10
Phule Rajani	11501.90	125.16	59.84	459.04
CD (0.05)	2.95	6.20	3.08	69.76

Table 2. Pollen output, pollen viability and pollen germination in certain genotypes of tuberose

Genotypes	Pollen output	Pollen viability (%)	Pollen germination (%)
Calcutta Single	6875	88.08	72.31
Hyderabad Single	5000	87.23	76.99
Kahikuchi Single	5625	79.93	63.08
Mexican Single	9375	89.11	71.27
Pune Single	7500	75.23	74.42
Navsari Local	5375	88.38	78.52
Variegated Single	10000	96.73	99.21
Shringar	8750	89.21	90.96
Prajwal	6250	79.83	52.12
Phule Rajani	8125	90.52	86.74
CD (0.05)	3	0.33	3.45

Table 3. Pollen tube length (microns) in certain tuberose genotypes

Genotypes	Hours after dehiscence of anther					
	1	5	10	15	20	25
Calcutta Single	43.84	160.93	242.61	383.00	473.52	500.78
Hyderabad Single	12.66	36.88	53.27	97.34	113.88	126.76
Kahikuchi Single	35.15	70.31	151.84	216.85	389.14	446.03
Mexican Single	4.98	18.64	25.00	46.27	78.36	72.55
Pune Single	4.73	16.95	26.68	49.81	76.72	78.25
Navsari Local	65.00	181.50	240.53	410.63	581.55	636.32
Variegated Single	76.82	98.18	210.44	452.23	625.76	726.07
Shringar	6.62	10.35	18.94	23.52	29.78	36.94
Prajwal	24.34	174.67	238.07	500.18	580.41	611.55
Phule Rajani	118.85	520.43	839.33	1020.63	1292.64	1234.95
CD (0.5)	0.17	12.66	36.88	53.27	97.34	113.88

Table 4. Fruit set (capsule retention) in certain hybrids and cultivars of tuberose (number of flowers=25)

Genotypes	Capsule retainion (%)			
	7 days	14 days	21 days	28 days
	Open pollination			
Calcutta Single	32.56	32.56	32.56	32.56
Hyderabad Single	22.90	22.90	22.90	22.90
Kahikuchi Single	25.69	25.69	25.69	25.69
Mexican Single	32.60	32.60	32.60	32.60
Pune Single	41.00	41.00	41.00	41.00
Navsari Local	37.50	37.50	37.50	37.50
Variegated Single	89.00	89.00	89.00	89.00
Shringar	18.50	18.50	18.50	18.50
Prajwal	35.30	35.30	35.30	35.30
Phule Rajani	50.00	50.00	50.00	50.00
	Artificial selfing			
Calcutta Single	42.56	0	0	0
Hyderabad Single	32.90	0	0	0
Kahikuchi Single	35.69	0	0	0
Mexican Single	35.60	0	0	0
Pune Single	46.00	0	0	0
Navsari Local	37.50	0	0	0
Variegated Single	92.00	0	0	0
Shringar	28.50	0	0	0
Prajwal	0.00	0	0	0
Phule Rajani	35.30	0	0	0

development efforts with tuberose have primarily relied on the selection of superior plants from open - pollinated seedling populations. In open pollination studies, the highest percentage of fruit set was reported in Variegated Single (89.00%) among cultivars and it is followed by 'Pune Single' (41.00%) and Phule Rajani (50%) among the hybrids (Table 4). Similar results were also obtained by Seetharamu *et al.* (2000) in tuberose. Under artificial selfing none of the genotypes set fruits. Hence the results of the present study showed that the complete self incompatability is in operation in tuberose hybrids and cultivars. The results of self-incompatability are in conformity with Shen *et al.* (1987), Uma (1990), Ueda and Akimoto (2001) and Sezai Ercisli (2007).

Based on the present research findings it may be concluded that pollen size, equivalent diameter, radius, perimeter, output, viability and germination capacity varied between tuberose genotypes. This could be due to abnormal meiosis which occured during microsporogenesis. Seed setting behaviour is quite erratic and this may be due to existence of self-incompatibility within the genotypes.

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