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Studies on floral biology of Tuberose (*Polianthes tuberosa* L.) under *Tarai* regions of Uttarakhand

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ABSTRACT

Ten varieties of Tuberose i.e Prajwal, Shringar, GKTC-4, Mexican Single, Sikkim Selection, Kalyani Single, Single, Sikkim Selection, Phule Rajani and Arka Nirantara were studied for their floral biology. Anther dehiscence started simultaneously with anthesis in most of the varieties except Phule Rajani, Arka Nirantara and Single. The peak time of anthesis ranged from 9.00 to 10.00 hrs. The duration of stigma receptivity was for 48 hours for all the varieties except Arka Nirantara. Variety Phule Rajani had highest pollen sterility while Arka Nirantara had lowest pollen sterility. The pollen germination was found to be higher when 60 ppm boric acid + 15% sucrose solution was used and the highest germination (51.83%) was observed in variety Single. The pollen diameter of the varieties ranged from 145.83 µm to 212.50 µm and number of pollens was found to be maximum (271.30 X10^4) in Mexican Single and minimum (87.20 X10^4) in Phule Rajani of the large bud.

***Keywords*:** Anthesis, anther dehiscence, floral biology, pollen, stigma receptivity

Tuberose (*Polianthes tuberosa* L.) is a bulbous perennial plant perpetuating through bulbs. Among different flower crops, tuberose is an important commercial flower crop in India and is popular due to its sweet fragrance and long keeping quality of spikes. It is mainly propagated vegetatively by bulbs but seed propagation is also followed to evolve new varieties (Bhattacharjee, 1995). The genetic variability available in tuberose is very limited and available named varieties are very few in India. Before taking up any systematic breeding programme, a thorough knowledge of floral biology in existing varieties and newly developed hybrids is a prerequisite in order to find out parents with desirable characters, *viz*., time and duration of flowering, anthesis, anther dehiscence, stigma receptivity, pollen sterility and pollen germination.

MATERIALS AND METHODS

The present study was undertaken in open field conditions at the Model Floriculture Centre, G.B. Pant University of Agriculture and Technology, Pantnagar during June, 2012 to July, 2014. Ten Tuberose varieties *viz*. Mexican Single, Sikkim Selection, Shringar, Phule Rajani, Prajwal, Single, Arka Nirantara, Kalyani Single, GKTC- 4 and Hyderabad Single were selected for investigation. The experiment was laid out in Randomized Block Design with three replications for recording time of anthesis (hrs), anther dehiscence (hrs) and stigma receptivity while Completely Randomized Design (CRD) with three replications was used for recording observations on pollen sterility, pollen germination, pollen diameter and number of pollens in a floret. The stigma receptivity was recorded by pollinating the florets on the same day, at one day, two days, three days and four days after anthesis. The pollen sterility (%) of ten varieties were estimated by using acetocarmine solution. Pollen germination status of the varieties were estimated by using hanging drop technique *in vitro* (Stanley and Linskens, 1974). For estimation of pollen diameter, ocular micrometer / oculometer and stage micrometer were used. The number of pollens was counted using haemocytometer.

RESULTS AND DISCUSSION

Highly significant differences were observed for all the characters studied in different varieties of tuberose. The results of the analyzed data are presented in table 1 to 5. The data on anthesis and anther dehiscence are presented in table 1 which envisages significant variations were found regarding time of anthesis among the different varieties. Anthesis was completed on the same day. Anthesis started from 7.30 hrs and continued till 11.00 hrs during the observation. However the peak time of anthesis was from 9.00 to 10.00 hrs. The pooled data for both the years 2012 and 2013 revealed that anthesis was observed earliest in the variety Phule Rajani (7.41 hrs) while Shringar took the longest time (10.52 hrs) for anthesis. The differences in the time of anthesis among the varieties might be due to their genetic factor and environmental effects. Anther dehiscence started

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simultaneously with anthesis in most of the varieties except Single, Arka Nirantara and Phule Rajani. Anther dehiscence took place one hour after anthesis in Single, 1-2 hours after anthesis in Arka Nirantara and 2-3 hours after anthesis in Phule Rajani. The data in table 1 revealed that anther dehiscence varied significantly within the varieties of tuberose. The pooled data for both the years 2012 and 2013 revealed that anther dehiscence was observed earliest in the variety Mexican Single (9.09 hrs) while Arka Nirantara took the longest time for anther dehiscence (11.43 hrs). The results of the present investigation was in accordance with the findings of Srivastava and Sridhara (1999) who reported that in tuberose the time of anthesis and dehiscence were similar. Mahawar and Misra (1993) also reported in gladiolus that anthesis and anther dehiscence both coincided very closely with flower opening. The differences in the time of anther dehiscence among the varieties might be due to their genetic factor and environmental effects.

The data on stigma receptivity is also presented on table 1. Stigma receptivity was recorded by observing the fruit set when pollination was done on the first, second, third and fourth day after anthesis. The duration of stigma receptivity ranged from 48 hrs to72 hours *i.e* for 2-3 days. The duration of stigma receptivity was for 48 hours for almost all the varieties except Arka Nirantara which had a duration of 72 hours *i.e* 3 days. The data on stigma receptivity was found to be non-significant. This finding is in close conformity with the finding of Singh and Singh (1985) as they reported that stigma became receptive one day before anthesis and remained up to three days after anthesis in gladiolus. The finding is also in accordance with Shen *et al.* (1987) who observed that in tuberose there was no pollen tube production if stigmas were pollinated when flowers were at the bud stage, the day before anthesis, or 1 day after opening. However there was considerable pollen tube growth when flowers were pollinated three days after opening and some pollen tubes were seen at 2 days. The difference in stigma receptivity of Arka Nirantara from the rest of the varieties might be due to the fact that it is a hybrid which shows vigour in many of the floral and vegetative characters.

The pollen sterility of the ten tuberose varieties was estimated by using acetocarmine solution. Three different sizes of buds *i.e* small, medium and large buds were used for the study. The data in table 2 revealed that pollen sterility test of small, medium and large buds varied significantly within the varieties and also between the type of buds. For the small bud, the pooled data for both the years 2012 and 2013 revealed that the minimum pollen sterility was observed in Mexican Single (7.38%) while the maximum sterility of the pollen was observed in variety Phule Rajani (73.06%). For the medium bud, the pooled data for both the years 2012 and 2013 revealed that the minimum pollen sterility (7.88%) was observed in Arka Nirantara while the maximum sterility of the pollen was observed in variety GKTC-4 (93.17%) which was closely followed by Kalyani Single (91.87). For the large bud, the pooled data for both the years 2012 and 2013 revealed that the minimum pollen sterility (6.80%) was observed in Arka Nirantara while the maximum sterility of the pollen (77.16%) was observed in variety Phule Rajani.

However, the comparision of the three sizes of buds from the pooled data showed that the pollen sterility was maximum in the medium bud (93.17%) in the variety GKTC-4 which was followed by large bud (77.16%) in variety Phule Rajani and small bud (73.06%) in Phule Rajani while the pollen sterility was minimum in large bud (6.80%) in Arka Nirantara followed by small bud (7.38 %) in Mexican Single and medium bud (7.88%) in Arka Nirantara (Table 4.2). In general, variety Phule Rajani had highest sterility while Arka Nirantara had lowest sterility. Generally high sterility of pollens is observed in all the varieties which might be due to the genetic makeup of the crop and also environmental effects while the differences in sterility of pollens among the varieties might be due to their varietal character. Seetharamu (1993) observed that tuberose hybrids IIHR-3 had 11.17 per cent pollen sterility while IIHR-2 had 17.91 per cent while in the present study higher pollen sterility was observed in most of the varieties which might be due to their genetic factor and environmental effects. The present results were similar to the findings of Gurumurthy (1991) and Lata (1971) in rose who observed high percentage of pollen sterility. Poon *et al.* (2010) observed that in gladiolus, the lowest percentage of sterile pollen was noticed in genotype Sapna (2.82%) followed by Poonam (4.00%), Hybrid selection 88-10-22 (4.82%) and Hybrid selection 82-11-27 (5.22%).

The pollen germination of the ten tuberose varieties were estimated by using hanging drop technique *in vitro* (Stanley and Linskens, 1974). Two types of media were used for the test. A 15 per cent sucrose (7.5 g sucrose in 50 ml distilled water) solution and a 15 per cent sucrose + 60 ppm boric acid (7.5 g sucrose + 3 mg boric acid in 50 ml distilled water) solution were used. The percentage of germinated and non-germinated pollen grains in all the varieties are presented in table 3. The data presented in table 3 indicated that there was significant variation in pollen germination of the varieties and also between the types of media used. In the year 2012, no pollen germination was observed in both the media. The reason for the non – germination of the pollens might be due to the loss of viability or decrease in vigour. In the year 2013, pollen germination was observed in both the

media. When 15% sucrose solution was used, the maximum pollen germination was observed in variety Mexican Single (22.93%) followed by Single (10.40%) while minimum pollen germination was observed in Kalyani Single (0.83%) followed by Phule Rajani (1.33%). However, when 60 ppm boric acid was added in 15% sucrose solution, the pollen germination was found to be still higher and the highest germination (51.83%) was observed in variety Single followed by Mexican Single (41.83%) and lowest (0.67%) in GKTC-4 followed by Kalyani Single (2.17%) and Shringar (2.67%). The germination of pollen is comparatively lower in all the varieties which might be due to the genetic makeup of the crop. The differences in germination of the pollens among the varieties was due to their varietal character while the differences between the two media might be due to the addition of boron which helps in better growth of pollen tubes. The results are similar with the findings of Gilissen (1978), Duric (1990), Aswath *et al*. (1990) and Sreekala and Pandurangan (2004) who reported good pollen germination with 15% sucrose, 10% sucrose + 100 ppm boric acid, 5% and 20% sucrose + 150 ppm boric acid respectively. Seetharamu (1993) recorded the percentage of pollen germination of nine genotypes of tuberose ranged from 43.51 to 53.90 %. In general, variations in pollen germination exists in various genotypes of bulbous ornamental crops. Additionally, the pollen germination in a crop is not high and uniform as a number of factors, viz., genotype, constituents of pollen germination medium, floret and anther stage, moisture content of the pollen, incubation period and the purity during pollen germination would have an effect on pollen germination. Poon *et al*. (2010) observed that the highest pollen germination (76.41%) was in genotype 'Hybrid selection 88-10-22, and the lowest non- germinated pollen (10.47%) was in genotype *Gladiolus callianthus* while using the media consisting of 15% sucrose supplemented with 300 ppm calcium nitrate, 200 ppm magnesium sulphate, 100 ppm potassium nitrate and 100 ppm boric acid..

A suitable carbohydrate source in the pollen germination medium is required for adequate pollen germination and pollen tube growth. A carbohydrate source serves two functions (i) maintains the required osmotic potential of the medium and (ii) serves as a suitable substrate for pollen metabolism. Most of the investigators have used high sucrose concentration in medium for the pollen germination as well as tube growth in *Brassica* (Shivanna and Sawhney, 1995). The result are in agreement with the results of Chaudhary (1991) and Jisha (1999) who reported the best pollen germination of gladiolus under 15 % sucrose + 75 ppm boric acid in germination medium. Yuxin *et al.* (2005) also found that sucrose and boron have great effects on the germination of lily pollen. Assessment of pollen viability has direct relevance in hybridization as pollen of male parent takes part in the fertilization process. Therefore, pollen germination study is an important activity in order to determine the potentiality of male parent for fertilization and seed setting after crossing (Shivanna *et al*. 1991).

The measurement of pollen diameter of the ten varieties was done by using oculometer and stage micrometer. The data for pollen diameter are presented in Table 4. The data revealed that the pollen diameter in the ten varieties range from 145.83 µm to 212.50 µm. Pollen diameter showed significant differences among the varieties of tuberose. For the small bud, in the pooled data of both the years, the maximum diameter of pollen (197.92 µm) was observed in Prajwal and GKTC-4 while the minimum pollen diameter (147.92 µm) was recorded in Arka Nirantara. For the large bud, in the pooled data of both the years, the maximum diameter of pollen (212.50 µm) was recorded in Shringar while the minimum (160.42 µm) was recorded in Arka Nirantara. In general, variety Shringar has bigger pollen diameter while Arka Nirantara has smaller pollen diameter. Prativa *et al.* (2012) observed that the pollen diameter in rose range from 25.25 µm in variety Pusa Ajay to 51.22 µm in variety Dr. Bharat Ram. The differences in the pollen diameter among the varieties might be due to their genetic makeup. The comparison of pollen diameter between the small bud and large bud showed that the large bud had bigger pollen diameter than the small bud. The reason for bigger pollen diameter of large bud might be due to the bigger size of bud and maturity.

In the present study, the number of pollens in a floret was counted by using haemocytometer. This method was also used by Eti (1990) to determine the number of pollens per anther in fruit crops. The average number of pollens was counted for an anther first and then counted for a floret. The data presented in Table 5 showed significant variations among the varieties and also between the type of buds for the number of pollens in a floret.

For the small bud, the pooled data for both the years showed that the maximum number of pollens (141.50 $X10^4$) was in Kalyani Single and the minimum (13.10 $X10^4$) was recorded in Hyderabad Single. For the medium bud, the pooled data for both the years showed that the maximum number of pollens (254.50 $X10^4$) was observed in Mexican Single and the minimum (30.00 $X10^4$) was recorded in Single. For the large bud of the floret, the pooled data for both the years showed that the maximum number of pollens (271.30 $X10^4$) was

Table 1: Time of anthesis, anther dehiscence and stigma receptivity (hrs) in different varieties of tuberose.

Treatment	Time of anthesis (hrs)			Anther dehiscence (hrs)			Stigma receptivity (hrs)
	2012	2013	Pooled	2012	2013	Pooled	Pooled
Prajwal	9.15	9.30	9.23	9.15	9.30	9.23	48.0
Shringar	10.43	11.00	10.52	10.43	11.00	10.52	48.0
Kalyani Single	9.25	10.00	9.43	9.25	10.00	9.43	48.0
GKTC-4	8.53	9.30	9.12	8.53	9.30	9.12	48.0
Mexican Single	8.58	9.00	9.09	8.58	9.00	9.09	48.0
Hyderabad Single	9.27	9.50	9.38	9.27	9.50	9.38	48.0
Sikkim Selection	9.00	10.00	9.30	9.00	10.00	9.30	48.0
Single	8.43	9.00	8.52	9.43	10.30	9.52	48.0
Arka Nirantara	10.00	11.00	10.30	10.43	12.43	11.43	72.0
Phule Rajani	7.53	7.30	7.41	9.53	12.00	10.57	48.0
SEm (±)	**0.383**	**0.462**	**0.340**	**0.151**	**0.477**	**0.250**	**2.40**
LSD(0.05)	**1.138**	**1.373**	**0.975**	**0.449**	**1.417**	**0.717**	**NS**

Table 2 : Pollen sterility in different varieties of tuberose

Treatment	Pollen sterility (%)								
	Small bud (2.5 cm)			Medium bud (4.0 cm)			Large bud (5.5 cm)		
	2012	2013	Pooled	2012	2013	Pooled	2012	2013	Pooled
Prajwal	42.35	41.81	**42.08**	39.30	37.45	**38.38**	47.61	47.88	**47.74**
Shringar	10.04	9.95	**10.00**	53.31	52.97	**53.14**	27.20	28.03	**27.61**
Kalyani Single	57.99	57.96	**57.97**	89.02	94.71	**91.87**	49.78	50.04	**49.91**
GKTC-4	38.69	40.53	**39.61**	91.00	95.33	**93.17**	39.74	42.67	**41.21**
Mexican Single	8.08	6.67	**7.38**	15.42	12.16	**13.79**	22.18	20.82	**21.50**
Hyderabad Single	20.18	22.33	**21.25**	19.25	20.83	**20.04**	16.70	14.55	**15.63**
Sikkim Selection	33.02	31.56	**32.29**	42.54	42.09	**42.31**	48.77	48.27	**48.52**
Single	15.02	15.79	**15.40**	16.82	16.82	**16.82**	63.41	64.57	**63.99**
Arka Nirantara	12.30	10.92	**11.61**	8.95	6.81	**7.88**	7.85	5.75	**6.80**
Phule Rajani	71.85	74.27	**73.06**	69.89	74.73	**72.31**	78.55	75.77	**77.16**
SEm (±)	**2.673**	**2.277**	**1.220**	**2.960**	**1.591**	**1.717**	**2.218**	**1.070**	**1.277**
LSD(0.05)	**7.885**	**6.717**	**3.487**	**8.732**	**4.693**	**4.908**	**6.543**	**3.156**	**3.650**

Table 3 : Pollen germination of different varieties of tuberose

Treatment	Pollen germination A (%)			Pollen germination B (%)		
	2012	2013	Pooled	2012	2013	Pooled
Prajwal	-	2.43	2.43	-	4.00	4.00
Shringar	-	3.67	3.67	-	2.67	2.67
Kalyani Single	-	0.83	0.83	-	2.17	2.17
GKTC-4	-	3.00	3.00	-	0.67	0.67
Mexican Single	-	22.93	22.93	-	41.63	41.63
Hyderabad Single	-	2.50	2.50	-	25.53	25.53
Sikkim Selection	-	5.67	5.67	-	7.00	7.00
Single	-	10.40	10.40	-	51.83	51.83
Arka Nirantara	-	2.00	2.00	-	5.00	5.00
Phule Rajani	-	1.33	1.33	-	4.00	4.00
SEm (±)	-	**2.43**	**2.43**	-	**4.00**	**4.00**
LSD(0.05)	-	**2.145**	**2.145**	-	**1.622**	**1.622**

A – 15% sucrose (7.5 g sucrose in 50 ml distilled water);B - 15% sucrose + 60 ppm boric acid (7.5 g sucrose + 3 mg boric acid in 50 ml distilled water)

Table 4 : Pollen diameter (µm) in different varieties of tuberose

Treatment	Small Bud (2.5 cm)			Large Bud (5.5 cm)		
	2012	2013	Pooled	2012	2013	Pooled
Prajwal	195.83	200.00	197.92	205.83	210.00	207.92
Shringar	187.50	187.50	187.50	212.50	212.50	212.50
Kalyani Single	161.67	175.00	168.33	175.00	175.00	175.00
GKTC-4	195.83	200.00	197.92	204.17	200.00	202.08
Mexican Single	190.00	195.00	192.50	200.00	200.00	200.00
Hyderabad Single	166.67	162.50	164.58	170.83	175.00	172.92
Sikkim Selection	192.50	187.50	190.00	212.50	200.00	206.25
Single	179.17	200.00	189.58	187.50	200.00	193.75
Arka Nirantara	145.83	150.00	147.92	158.33	162.50	160.42
Phule Rajani	175.00	175.00	175.00	187.50	175.00	181.25
SEm (±)	**7.759**	**1.155**	**3.503**	**6.112**	**1.155**	**2.678**
LSD(0.05)	**22.889**	**3.407**	**10.012**	**18.030**	**3.407**	**7.654**

Table 5. Number of pollens in a floret in different varieties of tuberose

Treatment	Small bud (2.5 cm)			Medium bud (4.0 cm)			Large bud (5.5 cm)		
	2012	2013	Pooled	2012	2013	Pooled	2012	2013	Pooled
Prajwal	84.27	86.33	85.30	145.40	146.40	145.90	172.30	174.30	173.30
	X 10^4	X 10^4	X 10^4	X10^4	X10^4	X10^4	X10^4	X10^4	X10^4
Shringar	104.27	106.43	105.35	172.33	146.67	159.50	183.70	185.70	184.70
	X10^4	X 10^4	X 10^4	X10^4	X10^4	X10^4	X10^4	X10^4	X10^4
Kalyani	142.00	141.00	141.50	164.00	165.00	164.50	203.80	205.80	204.80
Single	X10^4	X10^4	X10^4	X10^4	X10^4	X10^4	X10^4	X10^4	X10^4
GKTC-4	28.00	32.00	30.00	47.80	46.80	47.30	88.00	92.00	90.00
	X10^4	X10^4	X10^4	X10^4	X10^4	X10^4	X10^4	X10^4	X10^4
Mexican	58.00	62.00	60.00	254.00	255.00	254.50	270.80	271.80	271.30
Single	X10^4	X10^4	X10^4	X10^4	X10^4	X10^4	X10^4	X10^4	X10^4
Hyderabad	13.00	13.20	13.10	135.70	136.70	136.20	155.50	157.50	156.50
Single	X10^4	X10^4	X10^4	X10^4	X10^4	X10^4	X10^4	X10^4	X10^4
Sikkim	14.20	16.30	15.25	98.50	97.50	98.00	217.30	219.30	218.30
Selection	X10^4	X10^4	X10^4	X10^4	X10^4	X10^4	X10^4	X10^4	X10^4
Single	22.70	20.70	21.70	28.00	32.00	30.00	120.80	121.80	121.30
	X10^4	X10^4	X10^4	X10^4	X10^4	X10^4	X10^4	X10^4	X10^4
Arka	39.50	37.50	38.50	148.40	146.40	147.40	180.80	181.80	181.30
Nirantara	X10^4	X10^4	X10^4	X10^4	X10^4	X10^4	X10^4	X10^4	X10^4
Phule	20.50	22.50	21.50	36.60	35.70	36.15	86.20	88.20	87.20
Rajani	X10^4	X10^4	X10^4	X10^4	X10^4	X10^4	X10^4	X10^4	X10^4
SEm (±)	**2.233**	**1.115**	**29.934**	**8.239**	**8.305**	**14.304**	**1.017**	**1.017**	**41.743**
LSD(0.05)	**6.587**	**3.289**	**85.558**	**24.305**	**24.500**	**40.884**	**3.000**	**3.000**	**119.311**

observed in Mexican Single and the minimum (87.20 x10^4) was recorded in Phule Rajani. Among the types of buds, the maximum number of pollens were observed in the large buds followed by medium bubs and the minimum was in the small buds. The variation in the number of pollens among the varieties might be due to their genetic makeup. While the variation in the number of pollens between the three types of buds might be because of their different sizes and maturity. Ercisli (2007) observed that the highest average number of pollens per flower was in *Rosa dumalis* (18.82 x 10^4) and the lowest was in *Rosa villosa* (10.24 x 10^4) while studying four genotypes of rose. Eti (1991) found that a lot of factors such as species, cultivars, age of plants, nutritional conditions, culture environment affect the amount of pollen production in fruit species. In the present study also, the differences in number of pollens in a floret among the varieties might be due to nutritional conditions, varietal character and age of the plants.

Recommendation: Variety Arka Nirantara has the potential to be used both as female parent and pollen parent since it has maximum duration of stigma receptivity and lowest pollen sterility among these varieties.

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