

Ovarian Incompatibility in Tuberose (*Polianthes Tuberosa L.*) Cultivars and Breeding Lines

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Abstract

Background: Incompatibility occurs frequently in the plant system upon intra- or interspecific crosses resulting in several pollination barriers. The modest understanding on the breeding behaviour and mechanisms governing tuberose (*Polianthes tuberosa* L.) through this study will direct us to appraise the pollination and post pollination events on self- and cross- incompatibility and seed set behaviour for cross breeding programme.

Results: Self-pollination of tuberose cultivars Arka Prajwal, Mexican Single and Arka Sugandhi failed to produce pods upon autogamy and geitonogamy. Pollen-pistil interaction study of incompatible cultivar Arka Prajwal substantiates that pollen tube enters the ovule within 24 hours after pollination. It discharges its contents into the embryo on the 1st day after pollination and polysaccharide granules evident upon fluorescence microscopic study. The pollen tube growth rates of self and controlled pollination were similar; however, sterility was expressed in the ovary. The female gametophytic development in self and cross incompatible cultivar Arka Prajwal showed normal development initially after pollination whereas embryosac degeneration was observed at later stage. Complete degeneration of the integument and nucellar cells was also observed.

Conclusions: This study is the first to elucidate the occurrence of pseudo self-incompatibility in tuberose by identifying tuberose cultivars and breeding lines which produce pods upon geitonogamy. A positive pollen-pistil interaction with the degeneration of embryosac, integument and nucellar cell and pod shrinkage, abortion and fall confirms the prevalence of the 'Ovarian Incompatibility' or 'Late-Acting Incompatibility' in tuberose.

Background

Tuberose (*Polianthes tuberosa* L.) belonging to the family 'Asperagaceae' is a bulbous ornamental crop grown in most of the tropical and sub-tropical regions of the world and is a native of Mexico (Bailey, 1919). It is cultivated widely due to its great demand for floral decorations, its pleasant fragrance, wide adaptability and long-lasting vase life (Sadhu and Bose, 1973). Tuberose is commercially cultivated in India in an area of about 16,190 ha, with a loose flower production of 1,07,910 metric tons and cut flower production of 89.29 lakh numbers of cut stems (Anon, 2016). Economic impact assessment study conducted at ICAR-IIHR, Bengaluru on tuberose variety Arka Prajwal indicated a net economic benefit of Rs. 880 crores during the period of 2009 to 2015. Tuberose absolute is one of the most expensive of the fragrant oils used in perfumes and has high demand in international market. Essential oil market in India is anticipated to grow at 5.92% during the predicted period 2017-2023. India exports tuberose essential oil worth US \$ 2,908,748 to other countries and sixteen top exporting ports in India which trade Tuberose essential oils and Sharjah port of UAE holds largest share in importing (Anon, 2018).

Crop improvement work in tuberose had been done through mutation and hybridization and few commercial hybrids had been released with improved characters and performance (Howard, 1978). ICAR-

Indian Institute of Horticultural Research, Bangalore has released six superior tuberose hybrids and working on the development of suitable varieties for processing with high concrete content. Tuberose possesses the characters of self-incompatibility and dichogamy, thereby preventing the fruit set upon selfing (Shen et al., 1986; Seetharamu, 1993; Uma, 1990). Earlier studies on tuberose also stated that the seedlessness in tuberose, especially single flowered cultivars is due to sterility (Joshi and Pantulu 1940), at present found to be owing to self-incompatibility (Shen et al., 1986 and Karihaloo, 2019).

Self-incompatibility involves the dynamic rejection of male gametophytes that contains the same S-alleles as the female sporophyte (Nettancourt, 2001). This rejection stated that it happens in the stigma or style region; however, in late-acting self-incompatibility the rejection arises in the ovary (Seavey and Bawa, 1986). Late acting self-incompatibility or self-sterility involves prezygotic and post-zygotic embryo sac degeneration following self-pollination (Sage et al., 2006 and Bittencourt et al., 2003). Pollen germination on stigma and pollen tube growth through the pistil and into the ovule are regulated by pollen-pistil interactions (Linskens, 1986). Pollen-pistil interaction has been used to study the compatibility and incompatibility relationships in many crops including *Lilium longifolium* (Dickinson et al., 1982) and sweet cherry (Radunic et al., 2017).

Tuberose cultivars have also been observed to show incompatibility issues during hybridization resulting in no seed set (Hemanta, 2015). The exact cause of sterility in tuberose is not known and investigations carried out showed that sterility is not due to any defects or deformation in formation of the pollen grains and development of the embryo sac (Joshi and Pantulu, 1940). However, detailed information regarding the source and mechanism of the self and cross incompatibility within these cultivars had not been reported. There is a need to understand the mechanisms and problems associated with hybridization and incompatibility within the varieties. The crossing barrier existing thus could be identified through a systematic study on the interaction between the pollen and pistil of the genotypes and histological analysis. The investigation would help to identify the site of inhibition of pollens in the incompatible cross and thus methods to overcome such barriers could be developed in order to use a certain superior variety as a parent for further improvement of tuberose.

Materials And Methods

Plant material

Seven single floret type genotypes of tuberose were selected from the experimental fields of ICAR-Indian Institute of Horticultural Research (ICAR-IIHR), Bengaluru (Karnataka, India). The experimental site was located at an altitude of 930 m above MSL 12° 58' North latitude and 78° 35' East longitudes in the Eastern Dry Zone (zone 5) of Karnataka. The soil type is red loamy with the E.C. 0.26 dSm⁻¹ and pH of 7.35. The tuberose genotypes of single type (Fig. 1) viz., Arka Nirantara, IIHR-6, Variegated, Arka Shringar, Arka Sugandhi, Arka Prajwal and Mexican Single were used for the study.

Pollination And Seed Set

Eighteen genotypes of tuberose were subjected for autogamy, geitonogamy and allogamy using 25 flowers each and pod set was observed 90 days after pollination. Pollinations were carried out during July 2017 and repeated during July 2018. The flowers were bagged for autogamy to observe seed set. For geitonogamy, viable pollen grains were collected from the flowers on 1st day after anthesis and pollinated on the receptive stigma of flowers 2 days after anthesis. Natural out crossing was observed for the allogamy. For the intercrossing the matured buds were emasculated to be used as the female parents by removing the anthers manually and bagged. Continuous crossing for two to three days was practiced observing the seed set. Cut style pollination was carried out by removing the style and pollinated just above the ovary.

Fixing And Softening Of Pistil

The pollinated pistils of crosses were collected six and 24 hours after pollination. Pistils were fixed in FAA fixative (70% FAA- 18 ethanol: 1 formalin: 1 glacial acetic acid) for 24 hours at room temperature. Samples were prepared after 24 hours, or pistils were transferred to 70% ethanol where they can be stored for up to two months. The fixed pistils were removed from the fixative before sample preparation and rinsed in distilled water thoroughly; they were then transferred to 8 N NaOH solutions for 4-6 hours for softening of the tissues. Optimum softening of the pistil is required for proper staining of the sample.

Staining And Slide Preparation

The softened pistils were then rinsed in distilled water and let it stand for 24 hours to remove all the adhering chemicals prior to transfer to 0.1% (w/v) aniline blue in 0.3 M K_2PO_4 for one hour. Aniline blue (0.1%) stain was prepared by adding 0.1 g of aniline in a beaker containing 100 ml of 0.3 M sodium phosphate (K_2PO_4), stirred and transferred to amber bottles for storage. The stained pistils were then carefully mounted on glass slides using glycerin, covered with cover slips and pressed gently to keep the cover slips intact. The prepared slides were observed for the pollen tube growth and behaviour under the fluorescence microscope (Leica DM LB2, Wetzlar, Germany). The pollen-pistil interaction studies were observed for the compatible crosses of tuberose.

Histological Study

A comparative histological study was done on the self and cross compatible as well as the self and cross incompatible genotypes of tuberose under controlled pollination. The pods after 1st, 2nd, 3rd, 4th, 5th, 6th, 7th, 8th, 9th and 10th day of pollination were fixed in FAA (Formalin: acetic acid: alcohol) for at least 24 hours and subjected to ethanol-butanol series. The samples were subjected to paraffin infiltration and embedded with paraffin wax (60°C), longitudinal sections of 8µm were made and affixed on the slide

using gelatin adhesive (Jensen, 1962). The sections were hydrated with alcohol series and deparaffinised with xylol-butanol series and stained with haematoxyline. The sections were dehydrated and mounted with DPX and observed under microscope.

Statistical analysis

The data were analysed using SAS procedures (SAS, 1990). The variables expressed as percentages were normalized by a root square transformation. The significant differences between means were determined by the least significant difference (LSD) method at 5%.

Results

Self-pollination and fruit set

Self-pollination especially autogamy between the Arka Nirantara, IIHR-6, Variegated, and Mexican Single produced pod set. Arka Shringar showed fruit set upon geitonogamy with 20.00% of fruit set which was failed to set pod upon autogamy (Table 2). Pollen donor and type of pollination affected the fruit set percentage of Arka Shringar. The varieties under study, Arka Prajwal, Mexican Single and Arka Sugandhi had failed to produce fruits upon autogamy and geitonogamy. The percentage of pod set was noticed higher in all self-compatible varieties upon geitonogamy than the autogamy. Autogamy recorded the highest percentage of pod set in Variegated (26.19%) and in case of geitonogamy, percentage of pod set was the maximum in IIHR-6 with 80.00%.

Table 1

Percentage of fruit set in intercrossing and self-pollination by autogamy and geitonogamy of tuberose

Controlled pollination	Arka Nirantara	IIHR-6	Variegated	Arka Shringar	Arka Sugandhi	Arka Prajwal	Mexican Single
Arka Nirantara	5.58* 52.00**	68.75	58.67	61.25	64.00	0	55.56
IIHR-6	72.00	23.13* 80.00**	68.00	62.67	64.00	0	57.33
Variegated	80.00	76.00	26.19* 36.00**	68.00	65.00	0	60.00
Arka Shringar	53.33	54.29	63.00	0* 20.00**	45.33	0	62.35
Arka Sugandhi	61.33	82.67	70.67	44.71	0* 0**	0	62.35
Arka Prajwal	0	0	0	0	0	0* 0**	0
Mexican Single	25.56	29.33	53.75	26.67	37.33	0	0* 0**
* autogamy, **geitonogamy							

Table 2
Effect of different methods to improve seed set upon self-pollination on Arka Nirantara

Treatments	No. of flowers selfed	Percentage of fruit set	No of seeds per pod	No. of viable seeds/pod
Autogamy	251	5.58	12.00	10.76
Geitonogamy	75	52.00	26.00	25.20
Bud pollination	75	0	0	0
Bud pollination + Sucrose 2%	75	0	0	0
Cut style method	75	6.67	16.00	12.33
Cut style method 2% sucrose	45	8.89	16.33	15.00
Cut style method +Boric acid 50 ppm	70	0.00	0.00	0.00
Cut style method + NaCl 1%	75	10.67	13.33	12.33
Cut style method + NaCl 3%	45	8.89	18.00	15.67
Self pollination of 1st day of anthesis	70	0	0	0
Self pollination of 2nd day of anthesis	75	0	0	0
Self pollination of 3rd day of anthesis	75	50.00	15.00	12.68
Self pollination on 4th day of anthesis	75	0	0	0
Self pollination on 5th day of anthesis	70	0	0	0
NaCl-Sodium chloride				

Methods To Improve Pod Set Upon Self-pollination

Studies were conducted to improve the seed set upon self-pollination and percentage of fruit set (Table 3). Cut style method + NaCl 1% recorded the highest percentage of fruit set (10.67%) than the autogamy *i.e.*, natural self-pollination (5.58%). Number of seeds per pod (26.0), number of filled seeds per pod (21.20) and number of ill filled seeds per pod (5.00) were also found to be the highest in geitonogamy. The other treatments namely bud pollination, bud pollination + Sucrose 2%, cut style method + boric acid

50 ppm failed to produce seed set upon self-pollination. Artificial self-pollination (geitonogamy) was carried out in five different stages of flower *viz.*, 1st day of anthesis, 2nd day of anthesis, 3rd day after anthesis, 4th day after anthesis and 5th day after anthesis and fruit/seed set was observed on the 3rd day of anthesis.

Table 3

Influence of chemicals and different methods of pollination on post pollination pod retention (days) of the intercross Arka Prajwal with compatible pollens of Arka Nirantara

Treatment	Cut style pollination	Stigmatal pollination	Pod set (%)
Control	6.90	6.90	0
1% Sucrose	7.30	6.10	0
2% Sucrose	8.70	7.10	0
1% NaCl	5.90	5.30	0
2% NaCl	7.40	6.70	0
50% Boric acid	7.40	6.50	0
50 ppm NAA	10.30	9.50	0
50 ppm GA ₃	7.50	8.10	0
50 ppm BAP	8.10	9.00	0
Mean	7.72	7.24	
CV%	12.81	14.11	
SE (d)	0.82	0.84	
CD (p=0.05)	1.72	1.76	
NaCl- Sodium chloride, NAA-1- Naphthalene Acetic Acid, GA ₃ -Gibberellicacid, BAP – Benzylaminopurine			

Self-incompatibility And Pollen Pistil Interaction

The pistils of the self-incompatible and compatible varieties of tuberose after 6, 24, 48 and 72 hours of selfing was used for the fluorescence microscopic study to understand the pollen pistil interaction and mechanism of incompatibility. The stigma of tuberose found reflex at maturity, wet upon receptivity and surrounded by fringe of papillae with tapering cylindrical shape protruding from the stigmatic lobes. A very few pollen grains adhered on the stigmatic surface, pollen tube growth and entry on stigma and stylar regions were limited in self-incompatible pistils of tuberose on 6 hours after pollination of Mexican Single and Arka Prajwal (Fig. 2a). Pollen tubes entered the stylar region after 6 hours of selfing and were seen coiled in the midway after reaching 3/4th of style and further growth was arrested in some of the

pistils of Mexican Single (Fig. 2g and 2h). Lesser number of the pollen tubes was observed to reach 1/3rd of the length of the stigma at this stage as maximum of the tubes were observed to be inhibited or terminated in the upper styler region. In contrary to that, majority of the tuberose genotypes exhibited pollen tube germination, growth and entry through the entire length of the style, base of the styles and converged into ovary region and penetrated the ovule (Fig. 2i, 2j and 2k). Pollen tube entry into the ovule via micropylar end was observed after 24 and 48 hours of self-pollination of Arka Prajwal (Fig. 2k and 2l). The selfed pollen tube growth in the style is more active with the pollen tubes reaching the ovary within 24 hours and effecting the fertilization and after 7th and 10th day of pollination the pods shrunk after attaining pea size and abscised.

Cross Pollination And Fruit Set

Arka Prajwal, as female and pollen parent failed to set seed/fruit upon intercrossing and its reciprocally cross incompatible. The compatible cross Arka Sugandhi x IIHR-6 recorded the highest percentage of pod set (82.67%). The crossed flowers of the compatible cross Arka Sugandhi with IIHR-6 as pollen donor reported highest fruit set and the pistils were collected for fluorescence microscopy study to observe the pollen-pistil interaction in the normal compatible pollination. The pollens of IIHR-6 were observed to germinate abundantly in the stigma of Arka Sugandhi (Fig. 3a, 3b and 3c) and grow within the styles 6 hours after pollination (Fig. 3d and 3e). The pollen tubes entered through the entire length of the style and finally penetrated the ovary (Fig. 3h and 3i) and entered into the ovules (Fig. 3j, 3k and 3l) within 24 hours of pollination and leads to fertilization of the egg cells. Callose, a specialized polysaccharide which is an important cell wall component can be identified due to the emittance of secondary fluorescence with aniline blue. The growth of the pollen tubes down the style were characterized by a series of callose plugs sealing off the pollen tube transversely at regular distance dividing the pollen tube into many compartments as observed in Fig. 3f and 3g. It was not found to be deposited at the tip of the growing pollen tube revealing that the pollen tube grows without inhibition within the style.

Cross Incompatibility And Pollen Pistil Interaction

The cross combinations involving the genotype Arka Prajwal as either female or male that did not resulted in pod set were taken up for fluorescence studies to observe the interaction between the pollen and pistil following incompatible pollinations. The incompatible crosses with Arka Prajwal as female parent exhibited profuse pollen grains on the stigmatic surface. The pollen tubes were seen penetrating the stigma and extending into the styler region within 6 hours, penetrated ovary and ovule upon 24 hours after pollination. The bulging of ovary was noticed initially however subsequent pod abscission was observed at pea nut stage after 7-15 days of pollination.

Post Pollination And Pod Retention

The pod retention following stigmatal pollination was observed as the number of days pods were retained on the spike after pre-treatment followed with pollination. In the cross combination Arka Prajwal x Arka Nirantara, the pod retention ranged from 5.30 days (1% NaCl) to 9.50 days (50 ppm NAA) days with a mean of 7.24 days. The maximum pod retention was observed with the stigmatal application of 50 ppm NAA (9.50 days) as compared to control (6.90 days). Among the various treatments applied in the cut style pistils, the pod retention ranges from 5.90 days (1% NaCl) to 10.30 days (50 ppm NAA) in cross between Arka Prajwal x Arka Nirantara with mean of 7.72 days. The maximum pod retention was observed in the treatment with 50 ppm NAA (10.30 days) compared to control (6.90 days). Application of different growth regulators recorded 0% fruit set and growth regulators alone helped extending the pod retention and subsequently resulted in pod abscission.

Post Pollination Events And Zygotic Barriers

The post pollination events were studied using the pods of self and cross incompatible genotype Arka Prajwal. The ovary of tuberosa is observed as trilocular in Arka Prajwal and each locule contains number of ovules. The ovules were attached to the central axis of the ovary by axile placentation. The longitudinal sections of the ovules revealed that the ovules are not in same stage of development and both the mature and immature ovules are observed on the 1st day of pollination and mature ovules with the microspore mother cell and other ovules with initial stage of embryo sac formation (Fig. 4a and 4b). The irregular development of ovules was similar in case of self- and cross-pollinated ovules under study.

Pollen tube entry through micropylar end of the ovule and intracellular growth of the self-pollen tube between the nucellus tissue in Arka Prajwal was observed (Fig. 5a, 5b and 5c). Bitegmic ovules with organized nucellar tissue and vacuoles which later enlarges into an embryo sac were observed on 2nd day after pollination (Fig. 6a). The embryo sac containing two synergids, a central cell and an egg cell were also observed, and immature embryos showed continuous development. The 3rd day after pollination showed the matures ovules with following stages such as syngamy with the fusion of egg cell and male nuclei, zygote of resting phase and central cells without triple fusion. The observations on 4th day after pollination showed syngamy and formation of zygote (Fig. 7a). The triple fusion was also seen which later develops into endosperm which is $3n$ in nature (Fig. 7b and 7c). At the 5th, 6th and 7th day after pollination the ovule has not shown any division or zygote development (Fig. 7d). The volume of embryo sac was reduced as compared to previous stage after 6th day of pollination (Fig. 8a, 8b and 8c). Irrespective of self and cross pollination, ovule development was not seen on 8th day after pollination, in turn it showed degeneration of nutritive tissue nucellus before the formation of the embryo (Fig. 8d and 8e) which resulted in the immature pod fall and no seed set in tuberosa cultivar Arka Prajwal.

Discussion

Self-pollination, incompatibility and pollen-pistil interaction

Pod set observed from the cultivars upon selfing indicates their compatibility. The results derived supports the findings of Karihaloo (2019) and Uma and Gowda (2000) that the tuberose variety Variegated is self-compatible. Apart from that, the other single-type tuberose lines like IIHR-6, Arka Nirantara, Arka Shringar were also found to be self-compatible with the highest seed set percentage upon self-pollination. Self-pollination is restricted due to dichogamy and in the case of tuberose protandry, where the anther matures earlier than the gynoecium which takes 2 to 3 days for receptivity. Pollen viability study indicated that some of the genotypes of tuberose continued to retain viability up to four days after dehiscence, though the percentage of viability decreased over time. The stilar polymorphism at three different levels of stigma (L-morph, M-morph and S-morph) with constant stamen is also noticed in tuberose which did not have any influence on self-incompatibility. Species that exhibits stilar polymorphism shows ovarian self-sterility as in *Narcissus triandrus* (Barret et al., 2000). However, in depth research work on this aspect is required to elucidate the mechanism involved. *Polyanthes tuberosa* with stilar polymorphism produces cymose (multi-flowered) inflorescence which reduces the geitonogamous self-pollination by evolution of herkogamous polymorphisms as mentioned by Barret et al. (2000) in *Narcissus*. The influence of stilar polymorphism in reproductive biology of tuberose is unclear. Despite of all the differences, the results of this study provide evidence that the self-compatibility is present in tuberose and tuberose genotypes IIHR-6, Arka Nirantara and Arka Shringar produced seed upon self-pollination. The results are in controversy with the findings of Karihaloo (2019), Ranchana and Kannan (2016), Seetharamu (1993), and Shen et al (1987) that all the single type tuberose are self-incompatible. Among the tuberose genotypes available, few are self-fertile and most of the genotypes are self-incompatible, and some are cross-incompatible as found in sweet cherry (Choi et al., 2002). The genotype Mexican Single is self-incompatible but cross-compatible whereas Arka Prajwal is self and cross-incompatible. Self and cross pollination is essential within and among the genotypes for fruit set and for creation of variability in breeding programme.

Observation of fruit set indicated that the stigma is receptive on the 3rd day of anthesis, and early and delayed pollinations do not have any effect on overcoming the self-incompatibility. None of the methods were found to temporarily suppress the self-incompatibility in other self-incompatible genotypes of tuberose. Cut style had been successfully reported to increase pollen tube penetration (Van Creij et al., 1999 in *Tulipa* and Van Tuyl et al., 1982 in *Lilium*). However, cut style pollination failed to produce seed upon self-pollination in other genotypes of tuberose thus indicating that there is no stilar inhibition in tuberose. Bud pollination also failed to produce pods upon self-pollination with matured pollen grains and this result can be ascribed with the pistil immaturity as was observed in Easter cactus by Boyle et al. (1994).

Florescence microscopic study on the pollen-pistil interaction indicated the presence of 'gametophytic self-incompatibility' in operation as reported by Karihaloo (2019); Ranchana and Kannan (2016) in tuberose. Stilar inhibitions were also reported by Ghosh and Shivanna (1980) in *Linum grandiflorum*, Karihaloo (2019) in tuberose and Shimizu and Okada (2000) in *Arabidopsis*. However, the observation of pollen tube germination, growth and entry through the style and into the ovule in other cultivars raises the possibility that negative pollen tube stilar interactions may be triggered in the late-acting self-

incompatibility (LSI) species even though selfed pollen tubes continue to grow and enter the ovule for fertilization (Sage et al.1999). Slower incidence of ovule penetration in selfed pistils and consequently, a slower rate of development with the embryo sac was noted in *Spathodia companulata*. Moreover, in some of the cases the selfed pollen tube growth is depressed severely, and the tube never reaches the ovary (Bittencourt et al., 2003). The occurrence of embryogenic malformation, degeneration and delayed abscission of young fruits irrespective of the pollen tube entry into the ovary explains the prevalence of ovarian self-incompatibility (OSI) or late-acting self-incompatibility (LSI) in tuberose. It derives support from Uma and Gowda (2000) who stated that the pollen tube growth was uninhibited in the style confirming that the inhibiting factor was located at the ovary of tuberose. Few studies elucidates that the OSI is due to genetic control (LaDoux, 2014 and Lipow and Wyatt, 2000). In the ovarian self-incompatibility, selfed pistils are rejected though the selfed pollen tubes grew to the ovary and penetrated ovules (Bittencourt et al., 2003).

Cross pollination, incompatibility and pollen pistil interaction

Studies on pod set in several cross combinations of tuberose were also experimented by Hemanta et al. (2016), Krishnamoorthy (2014), Shen et al. (1986) and Uma (1990) with different genotypes resulting in different pod set percentage upon cross pollination. Ranchana (2013) also observed high rate of growth of pollen tube in all successful crosses of tuberose. According to Uma and Gowda (2000), the uninhibited pollen tube growth in the style substantiated that the inhibiting feature was present in the ovary. Pollen hydration, tube emergence and ovary penetration were also observed within 24 hours in the compatible pollination of *Petunia sp.* (Herrero and Dickinson, 1981), Rhododendron (Williams et al., 1982) and European globeflower (Antkowiak et al., 2017). The proper guidance of the pollen tube towards the female gametophyte was due to the positive coordination between the pollen and pistil of the compatible cross combination.

Pod abscission observed at pea nut stage might be due to the post zygotic barriers prevailing in the tuberose genotype Arka Prajwal. Arka Prajwal was used as the pollen parent showed the cross-incompatibility reaction and pollen germination and pollen tube growth was not observed in the pistils of female parent except Arka Nirantara. The pollen viability and pollen germination of the cultivar, Arka Prajwal has been reported to be very low and Arka Prajwal has not produced seed set upon direct or indirect crosses thus suggesting the prevalence of sterility and incompatibility (Ranchana and Kannan, 2016). In the cross using Arka Nirantara as female parent, the pollens of Arka Prajwal were not germinated within six hours but showed slow pollen germination after 24 hours as compared to compatible cross. This showed that although the genotype Arka Nirantara showed positive interaction and selectivity to the sterile pollens as compared to the other genotypes, it showed slower germination as compared to compatible cross and eventually restriction of the pollen tubes within the style. Herrero and Dickinson (1981) had reported that incompatible pollen tubes of *Petunia sp.* showed slower growth as compared to the compatible one. Similar finding was also reported in *Lilium* by Niizeki (1959) and hazelnut by Hampson et al. (1993). The difference in the length of the pollen tube growth of direct and indirect crosses may be due to the difference in the pollen viability and germinability among the

genotypes which is governed by their genetic makeup as well as the genotype specificity for a particular pollen or specific interaction between the pollen and pistils of different genotypes. Schmidt and Timmann (1997) suggested that the pollen tube growth through the style and ovule penetration alone does not determine the cross compatibility of cultivars.

Post Pollination And Pod Retention

The increase in the number of days in cut style pollination as compared to stigmatal pollination may be due to the removal of stylar barrier as maximum of the pollen tubes were inhibited in the upper stylar region in case of stigmatal pollination. However, in case of tuberose genotypes, in spite of the increase in the days of pod retention, abortion of pods was reported before the maturation. This could be due to the post-fertilization barrier governing the genotypes which resulted in abortion. Uma (1990) had treated the incompatible crosses of tuberose with IAA, IBA and bud pollination, but recorded no fruit set, mentioning that the inhibition factor was located at the ovary after fertilization. The pollen-pistil interaction study using aniline blue staining technique was conducted to observe the post-pollination events occurring in the pistil. The interaction between the pollen and pistil in the various stages showing pollen germination, pollen tube growth and penetration within the styles and ovary were observed under the fluorescence microscope. The pods were retained for 8.47 days and after that the pods started changing into yellow colour, it shrunk and fell which confirms that the post pollination incompatibility in tuberose. The results derived support from the study from Shao and Wang (2020) in Jujube where regardless of self and cross pollination, the pollen tube enters into ovary and cause swelling of spherical area of the stigma.

Post Pollination Events And Zygotic Barriers

Post pollination studies revealed abnormalities in ovule development following self- and cross-pollination of Arka Prajwal. Regen (1941) in *Agave virginica* and Gutiérrez and Garay (2016) in tuberose also described the occurrence of unproductive ovules where embryo sac was not formed due to the degeneration of nucellar tissues. Similar observations were illustrated by Krishnamurthy and Srinivas (2005) in tuberose where the fusion nucleus did not show any signs of division whereas the symptoms of degeneration of egg apparatus in selfed ovule were observed. The results of this study indicated that the late-acting gametophytic- incompatibility exists in tuberose, where the tissue in the nucellus and the embryo sac is not supporting the zygote development and further embryogenesis is not observed (Fig. 8a, 8b, 8c and 8d). Abnormalities in the embryo sac development in tuberose variety 'Simple' was stated by Gutiérrez and Garay (2016) where three kinds of defects were seen in embryo sac development such as retarded embryo sac development, collapsed embryo sac with degradation of embryo sac and nucellar tissue, abnormal thickening of nucellar layer in the micropylar end without egg apparatus. Krishnamurthy and Srinivas (2005) furthermore described that the fusion nucleus has not gone through division upon selfing and crossing after 1st and 4th day of pollination and exemplified degeneration of embryo sac, egg apparatus and fruit wall on 7th day after pollination in tuberose. Uma (1990) also recorded degeneration

of embryo sac in all the ovules of tuberosa in shorter period after anthesis and concurrent degeneration of antipodals, egg apparatus and polar nuclei. The results derived support from Sage et al. (1999) in *Narcissus* where the absence of seed set might be due to the lack of vital stimulus for normal seed development upon self and cross pollination.

The current study established that the characteristic of pollination, fertilization and embryo development are associated to differences in tuberosa cultivars. The recognition of self-compatible genotypes of tuberosa provides a way to produce inbred lines with effective exploitation of rare resistance traits and expression of hidden recessive genetic potential. The successful fruit set is the indicator for the self and cross-compatible genotypes, despite the pollen viability, pollen pistil interaction and fertilization the ovary has the influence on fruit set. The degeneration of embryo sac after the zygote formation was observed in the self and cross incompatible tuberosa cultivar Arka Prajwal indicating that the reproductive incompatibility stimulates the metabolic activities in the associated tissues and causes disintegration of embryo sac. It is also evident that normal development of pollen pistil interaction, fertilization, double fertilization and zygote development in tuberosa and abrupt degeneration of surrounding embryo sac and zygote which ultimately leads to the seed abortion in tuberosa is mainly due to the self and cross incompatibility. As per Liang et al. (2005) any phase of embryo formation *viz.*, pollen viability, pollen pistil interaction, embryo sac, zygote, nucellar tissue, embryo and endosperm might lead to seed abortion. The study on pollination, pollen pistil interaction, fertilization, zygote development and subsequent degeneration of embryo sac and zygote would be beneficial to understand the degree of compatibility for the selection of appropriate compatible parents in tuberosa for the future cross breeding programme. This study elucidated the prevalence of self and cross incompatibility of tuberosa genotypes and post zygotic barriers that causes late acting self-incompatibility. The current research paved the way for the detailed systematic study on the rationale of embryo sac degeneration, gene and molecular mechanism related to seed abortion in tuberosa.

Declarations

Consent for publication: Not applicable

Availability of data and material: Data is available in the form of tables

Competing interests: The author(s) declare(s) that they have no competing interests”

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Authors' contributions:

T.Usha Bharathi: Conceived and designed the study, Conducted the experiments, collected data, performed analysis and wrote the paper

Rosalind Lallawmzuali: Collected data and performed data analysis, wrote the paper

Subramanyamrao Prabhakar Rao Kirthishree: Conducted the experiments, Collected data

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References

1. Anonymous. 2016. Horticulture Crops Estimates for the Year 2016-17, National Horticulture Board, Indian Horticulture Database. Min. Agri., Govt. of India. 8 March 2021.
2. Anonymous. 2018. Analysis of export of tuberose essential oil. 6 May 2021.
3. Antkowiak W, Niemann J, Wojciechowski A. 2017. The assessment of incompatibility and cross-compatibility in three populations of European globeflower (*Trollius europaeus* L.) by observing the pollen tubes growth. *Acta Societatis Botanicorum Poloniae* 86 (2): 3553. <https://doi.org/10.5586/asbp.3553>
4. Bailey LH. 1919. The standard cyclopedia of horticulture Vol.2. Macmillan. New York. <https://doi.org/10.1038/101241a0>
5. Barrett SCH, Jesson LK, Baker AM. 2000. The evolution and function of stylar polymorphisms in flowering plants. *Annals of Botany* 85: 253-265. <https://doi.org/10.1006/anbo.1999.1067>
6. Bittencourt NS, Gibbs PE, Semir J. 2003. Histological study of post pollination events in *Sapthodea campanulata* Beauv.(Bignoniaceae), a species with late-acting self-incompatibility. *Annals of Botany* 91: 827–834. <https://doi.org/10.1093/aob/mcg088>
7. Boyle T H, Menalled F D, Maureen CO. 1994. Occurrence and physiological breakdown of self-incompatibility in Easter cactus. *J. Amer. Soc. Hort. Sci.* 119 (5): 1060-1067. <https://doi.org/10.21273/JASHS.119.5.1060>
8. Brewbaker JL, Kwack BH. 1963. The essential role of calcium ion in pollen germination and pollen tube growth. *Am. J. Bot.* 9: 859-865. <https://doi.org/10.1002/j.1537-2197.1963.tb06564.x>
9. Choi CPA, Wiersma PT, Kappel F. 2002. Fruit growth, firmness and cell wall hydrolytic enzyme activity during development of sweet cherry fruit treated with gibberellic acid (GA₃) *J. Hort. Sci. Biotechnol.* 77(5): 615-621. <https://doi.org/10.1080/14620316.2002.11511547>
10. Nettancourt D. 2001. Incompatibility and incongruity in wild and cultivated plants Springer-Verlag, New York, USA. <https://doi.org/10.1007/978-3-662-04502-2>
11. Dey K, Mondal S, Mandal S. 2016. Flower visitor interaction and fruit production of *Grewia asiatica* L. *Int. J. Curr. Microbiol. Appl. Sci.* 5: 761-767. <http://dx.doi.org/10.20546/ijcmas.2016.501.077>
12. Dickinson HG, Moriarty J, Lawson J. 1982. Pollen–pistil interaction in *Lilium longiflorum*: the role of the pistil in controlling pollen tube growth following cross-and self-pollinations. *Proceed. Royal Soc. London. Series B. Biol. Sci.*, 215(1198): 45-62. <https://doi.org/10.1098/rspb.1982.0027>
13. Ghosh S, Shivanna KR. 1980. Pollen-pistil interaction in *Linum grandiflorum*; stigma-surface proteins and stigma receptivity. *Proceed. Ind. Nat. Sci. Acad.* 2: 177-183. <https://doi.org/10.1007/bf00384562>

14. Gutiérrez AGG, Garay BR. 2016. Embryogenesis in *Polianthes tuberosa* L. var. Simple: from megasporogenesis to early embryo development *Springer Plus*. 5 (1): 1-13.
<https://doi.org/10.1186/s40064-016-3528-z>
15. Hampson CR, Azarenko AN, Soeldner A. 1993. Pollen-stigma interactions following compatible and incompatible pollinations in hazelnut. *J. Am. Soc. Hort. Sci.* 118: 814-819.
<https://doi.org/10.21273/JASHS.118.6.814>
16. Hemanta L. 2015. Studies on floral biology, pollination and crossability in tuberose (*Polianthes tuberosa* L.). PhD Thesis, GB PUAT, Pantnagar, India.
17. Hemanta L, Srivastava R, Kumar A, Chand S. 2016. Studies on Seed Set by open Pollination in tuberose (*Polianthes tuberosa* L.) under Tarai Regions of Uttarakhand. *J. Agri. Technol.* 3 (1): 71.
18. Herrero M, Dickinson HG. 1981. Pollen tube development in *Petunia hybrida* following compatible and incompatible intraspecific matings. *J. Cell Sci.* 47, 365-383. <https://doi.org/10.1242/jcs.47.1.365>
19. Howard TM. 1978. *Polianthes* hybrids Plant Life. 126-129.
20. Joshi AC, Pantulu, JV. 1940. A morphological and cytological study of *Polianthes tuberosa* L. *J. Ind. Bot. Soc.* 20: 37–71.
21. Karihaloo JL. 2019. Cytological and hybridization studies in three cultivars of tuberose (*Polianthes tuberosa* L.). *Cytologia*. 84 (1): 47-52. <https://doi.org/10.1508/cytologia.84.47>
22. Krishnamoorthy V. 2014. Assessment of tuberose (*Polianthes tuberosa* L.) varieties for growth and yield characters. *As. J. Hort.* 9 (2): 515-517. 10.15740/HAS/TAJH/9.2/515-517
23. Krishnamurthy KB, Meenakshi S. 2005. Histological and histochemical studies of ovule development in tuberose (*Polianthes tuberosa* L.). *J. Orn. Hort.* 8 (2): 81-85.
24. LaDoux T. 2014. Self-incompatibility in Polemoniaceae. PhD diss., The Claremont Graduate University. <https://doi.org/10.1086/500985>
25. Linskens HF. 1986. Recognition during the progamic phase In: Cresti, M., Dallai, R. (Eds.), *Biol. Reprod. Cell Motility Plants*. Anim. Univ. of Siena, Siena, pp. 21–32.
26. Liang CL, Liu MJ, Zhao J. 2005. Research progress on plant abortion. *Mol. Plant Breeding*. 3(1):117-122.
27. Lipow SR, Wyatt R. 2000. Single gene control of postzygotic self-incompatibility in Poke milkweed, *Asclepias exaltata* L. *Genetics*. 154 (2): 893-907. <https://doi.org/10.1093/genetics/154.2.893>
28. Martins C, Oliveira R, Filho CVM, Lopes LT, Silveira RA, de-Silva JAP, Aguiar L.M, Antonini Y. 2016. Reproductive biology of *Cipocereus eminensis* (Cactaceae)-A columnar cactus endemic to rupestrian fields of a Neotropical savannah Flora-Morph. Dist. Func. *Ecol. Plants*. 218: 62-67.
<https://doi.org/10.1016/j.flora.2015.11.010>
29. Niizeki S. 1959. Cross experiments in *Lilium* I: Pollen tube behaviors in the crosses of *Lilium henryi* with some species of *Lilium*. *Dept. Bulletin Paper*, Ochanomizu Univ., Tokyo, Japan.
30. Ranchana P. 2013. Evaluation of tuberose genotypes for development of F₁ hybrids for quantitative, qualitative characters and nematode resistance. PhD. Thesis, TNAU, Coimbatore, India.

31. Ranchana P, Kannan M, Jawaharlal M. 2015. Evaluation of pollen viability under in vitro conditions in tuberose (*Polianthes tuberosa* L.). *Suppl. Gen. Plant Breed.*10: 807-812.
32. Ranchana P, Kannan M. 2016. Self and cross compatibility studies in tuberose (*Polianthes tuberosa* L.). *The Bioscan.* 11(1):33-36.
33. Radunic M, Jazbex A, Ercisli S, Cmelik Z, Ben S.G. 2017. Pollen -pistil interaction influence on the fruit set of sweet cherry. *Sci.Hortic.* 224: 358–366. [https:// doi. org/ 10.1016 /j. scienta. 2017. 06.010](https://doi.org/10.1016/j.scienta.2017.06.010)
34. Regen L. 1941. The development of the embryo sac in *Agave virginica*. *Bulletin of the Torrey Botanical Club.* 229-236. <https://doi.org/10.2307/2481501>
35. Sadhu M K, Bose TK. 1973. Tuberose for most artistic garlands. *Ind. Hort.* 18: 17-20.
36. Sage TL, Price MV, Ware NM. 2006. Self-sterility in *Ipomopsis aggregata* (Polemoniaceae) is due to prezygotic ovule degeneration. *Am. J. Bot.* 93(2): 254-262. [https:// doi.org/ 10.3732/ ajb.93.2.254](https://doi.org/10.3732/ajb.93.2.254)
37. Sage TL, Strumas F, Cole WW, Barrett SCC. 1999. Differential ovule development following self-and cross-pollination: the basis of self-sterility in *Narcissus triandrus* (Amaryllidaceae). *Am. J. Bot.* 86 (6): 855-870. <https://doi.org/10.2307/2656706>
38. SAS Institute, Statistical Analysis System Institute (Cary, and NC). 1990. SAS Language: Reference: Version 6.
39. Schmidt H, Timmann E. 1997. On the genetics of incompatibility in sweet cherries. Pt.1. Identification of S alleles in self-incompatible cultivars. Gartenbauwissenschaft, Germany.
40. Seavey SR, Bawa KS. 1986. Late-acting self-incompatibility in angiosperms. *Bot. Rev.* 52: 195–218. <https://doi.org/10.1007/BF02861001>
41. Seetharamu GK. 1993. Hybridization Studies in Tuberose (*Polianthes tuberosa* Linn.). PhD diss., UAS, Bangalore.
42. Shao F, Wang S, Liu Z, Hong R, Zhao T. 2020. Pollination, fertilization and embryo development in southern China Fresh eating Jujube. *Hort Sci.* 55(8): 1315-1322. <https://doi.org/10.21273/HORTSC15144-20>
43. Shen TM, Huang KL, Huang TS. 1986. Study of tuberose hybridization. Symposium on Development of New Floricultural Crops XXII IHC. 205, 71-74. <https://doi.org/10.17660/ActaHortic.1987.205.11>
44. Shen TM, Huang KL, Huang TS. 1987. Study on hybridization and sexual propagation of tuberose. Symposium on the Improvement for Flower Production. *Tau-Yuan Dis. Agric. Improv. Sta.* 167-175. <https://doi.org/10.17660/actahortic.1987.205.11>
45. Shimizu KK and Okada K. 2000. Attractive and repulsive interactions between female and male gametophytes in Arabidopsis pollen tube guidance. *Development.* 127: 4511-4518. <https://doi.org/10.1242/dev.127.20.4511>
46. Stanley RG, Linskens HF. 1974. Viability tests. In Pollen. *Springer* 67-86. Berlin, Heidelberg. https://doi.org/10.1007/978-3-642-65905-8_6
47. Uma S.1990. Studies on causes of non-seed setting in some ornamental bulbous crops, PhD. Thesis, UAS, Bangalore, India.

48. Uma S, Gowda JVN. 2000. Self-incompatibility studies in *Polianthes tuberosa* L. *Res. Crops*. 1 (3): 418-420.
49. Van Creij MGM, Kerckhoffs DMFJ, Van Tuyl JM. 1999. The effect of ovule age on ovary-slice culture and ovule culture in intraspecific and interspecific crosses with *Tulipa gesneriana* L. *Euphytica*.108 (1): 21-28. <https://doi.org/10.1023/A:1003644009572>
50. Van Tuyl JM, Clara MM, Visser T.1982. Pollen and pollination experiments. VII. The effect of pollen treatment and application method on incompatibility and incongruity in *Lilium*. *Euphytica*. 31 (3): 613-619. <https://doi.org/10.1007/BF00039199>
51. Williams EG, Knox BR, Rouse JL. 1982. Pollination sub-systems distinguished by pollen tube arrest after incompatible interspecific crosses in *Rhododendron* (Ericaceae). *J. Cell Sci*.53: 255-277. <https://doi.org/10.1242/jcs.53.1.255>

Figures

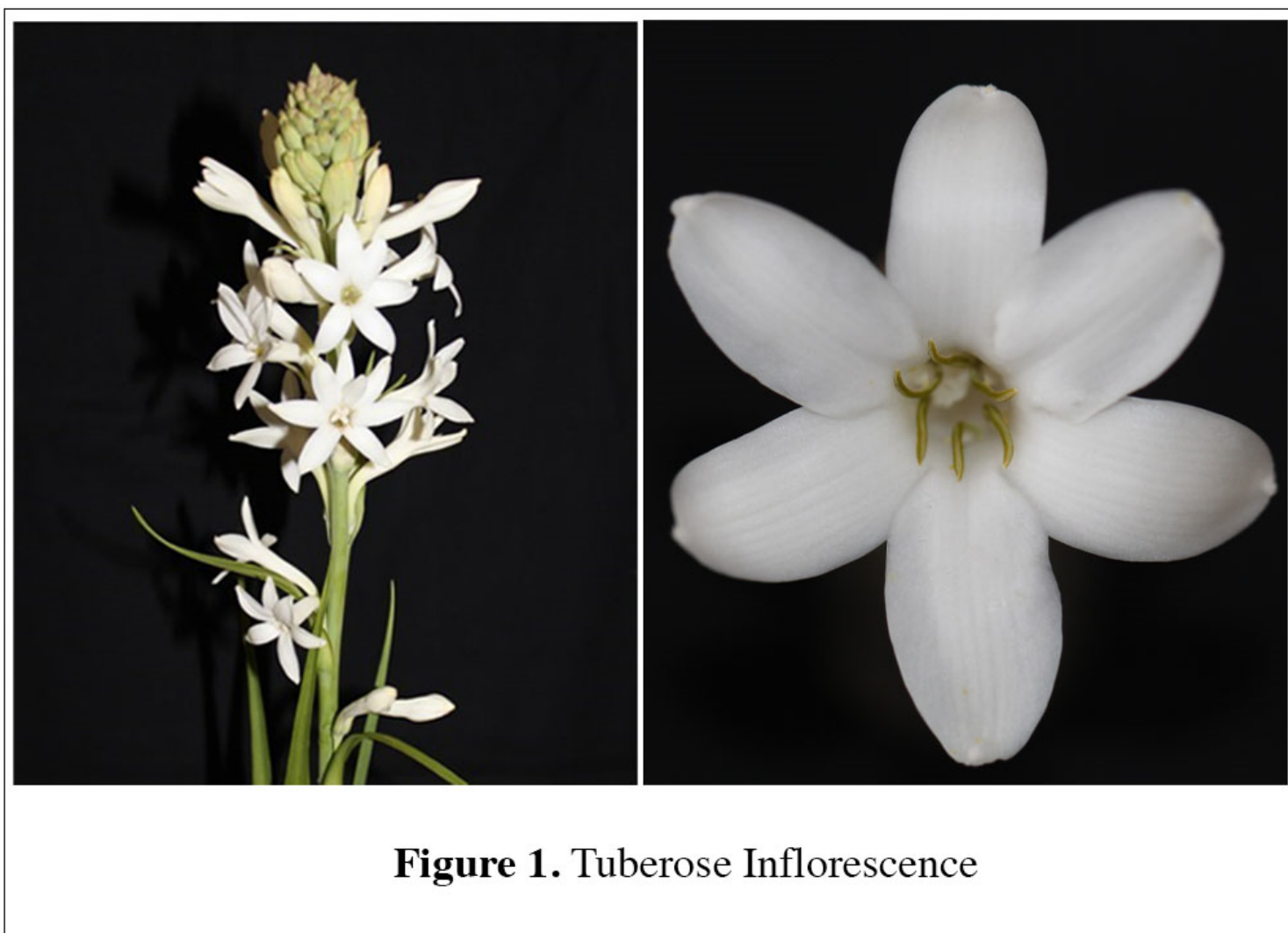


Figure 1

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