

Original Research Paper

Floral biology and pollen germination studies in China aster [*Callistephus chinensis* (L.) Nees]

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ABSTRACT

An investigation on floral biology and pollen germination in China aster [*Callistephus chinensis* (L.) Nees] was carried out during 2020 to 2022. Results revealed that, anthesis was observed in afternoon hours from 2:15 p.m.-3:15 p.m. in China aster genotype Arka Aadya, whereas, in Arka Archana, Arka Kamini and Arka Poornima, it was 10:15 a.m. to 11:30 a.m. Among genotypes, anther dehiscence was observed from forenoon till afternoon. Number of ray florets per flower head ranged from 29.53 (Arka Shashank) to 132.37 (Arka Poornima), while, disc florets per flower head ranged from 128.93 (Arka Shashank) to 238.93 (Arka Poornima). Hundred per cent pollen viability was recorded in Arka Aadya, Arka Archana, Arka Kamini, and Arka Shashank with seed setting ranged from 24.55% (Arka Poornima) to 54.13% (Arka Kamini). Increased stigma receptivity among the genotypes was observed from bud stage to fully expand floret stage and declined in semi expanded tubular disc stage. Among genotypes, maximum pollen germination was observed in Arka Archana (17.67%) followed by Arka Kamini (14.10%). Among different media, maximum pollen germination ranged from 5.13% (10% sucrose + 15% PEG + BK medium) to 17.07% (10% sucrose + 15% PEG + BK media). Among interaction effect, maximum pollen germination was recorded in 15% sucrose + 30% PEG + BK medium x Arka Kamini (70.50%) followed by 10% sucrose + 15% PEG + BK medium x Arka Archana (44.33%) and 10% sucrose + 15% PEG + BK medium x Arka Aadya (44.00%).

Keywords: China aster, floral biology, pollination, stigma receptivity, seed setting

INTRODUCTION

China aster, family Asteraceae, is a semi-hardy, annual flower crop commercially grown for cut flower, loose flower and pot culture purposes. It has derived its name '*Callistephus*' two Greek words '*kalistos*' meaning 'most beautiful' and '*stephos*', a crown, referring to the flower head of China aster. Its flowers are used for flower arrangement, interior decoration, garland making, and also in worshipping. The present-day China aster has been developed from a single form of wild species, *Callistephus chinensis* L. Nees. (Bhargav et al., 2018). Fleming (1937) estimated approximately 10 per cent natural crossing in China aster. Strube (1965) described that China aster flower head consists of both pistillate ray florets and perfect disc florets. The proportion of ray florets and disc florets is a measure of doubleness of the flower. The stamens and pistils do not mature simultaneously in the individual flower. The stigma of the individual floret unfolds after the pollen is dehisced from the disc floret. However, sufficient pollen remains in the

capitulum. The China aster is therefore, in the geitonogamous condition.

The domain of reproductive biology includes floral biology, pollination dynamics, fertilization and embryogenic, seed development and germination (Marbaniang et al., 2018). Information on various aspects of floral biology such as time of anthesis, anther dehiscence, pollen fertility, stigma receptivity etc. are essential pre-requisite of any hybridization program, which will lead to the development of improved varieties/hybrids. With the above facts, the present study was conceived with objective to study the floral biology and pollen germination in China aster.

MATERIALS AND METHODS

The present investigation on floral biology and pollen germination studies in China aster [*Callistephus chinensis* (L.) Nees], was carried out in the experimental block of Division of Flower and



Medicinal Crops, ICAR-Indian Institute of Horticultural Research, Hesaraghatta Lake Post, Bengaluru, India during 2020 to 2022, geographically located at 13° 58' N latitude, 78°E longitude at an elevation of 890 m above mean sea level. An experiment with 5 China aster genotypes viz., Arka Aadya, Arka Archana, Arka Shashank and Arka Kamini and Arka Poornima as experimental material were laid out in randomized complete block design, with four replications, at spacing 30 cm x 30 cm during winter season of 2020-21 to 2021-22. Five flower heads were taken per replication for recording observations on anthesis and anther dehiscence, number of ray & disc florets per flower head, length & width of ray florets and shape of ray florets, pollen viability (%), seed set (%), number of seeds obtained from disc & ray florets, stigma receptivity (days) and pollen germination.

The pollen viability (%) was estimated by using Alexander staining solution (Alexander 1969). Freshly dehiscent pollen grains were taken on a slide with one to two drops of Alexander stain on which cover slip was placed and kept for two to three minutes for proper and uniform staining. The pollen grains which were round and deep purplish pink colour stained were recorded as viable. The average viable pollen grains were estimated and expressed in percentage.

In in vitro pollen germination studies, five genotypes (Factor 1) namely Arka Aadya, Arka Archana, Arka Kamini, Arka Poornima and Arka Shashank were evaluated with five pollen germination media (Factor 2) consisting T₁: 5% sucrose + 15% PEG + BK medium, T₂: 10% sucrose + 15% PEG + BK medium, T₃: 10% sucrose + 30% PEG + BK medium, T₄: 15% sucrose + 15% PEG + BK medium and T₅: 15% sucrose + 30% PEG + BK medium, replicated thrice in factorial completely randomised design. In hanging drop technique (Stanley & Linskens, 1974), small drop of medium was placed on cover glass and dusted with pollen. Cover glass was slowly and gently tilted to correctly fix on to the cavity of slide. Cavity slides then kept in Petri dishes lined with moist filter paper and examined under an Olympus-BX43 microscope at low magnification (10X) at one, two, three, four- and twenty-four-hours' time intervals to know the germination percentage. The pollen grain was considered as germinated when its pollen tube length becomes equal to or larger than the pollen diameter (Chagas et al., 2010).

RESULTS AND DISCUSSION

Anthesis and anther dehiscence

The variation was observed among genotypes for their anthesis (Table 1), which was observed from forenoon to afternoon. The genotypes Arka Aadya observed anthesis in afternoon hours, ranged from 2:15 p.m.-3:15 p.m., whereas, other genotypes such as Arka Archana, Arka Kamini and Arka Poornima, anthesis was observed in forenoon hours (10:15 a.m. to 11:30 a.m.). However, Arka Shashank recorded anthesis from 10:45 am to 12.15 pm. Kulloli et al. (2015) also found that anthesis occurs between 3:00-5:00 h in *Impatiens grandis*, whereas, Messar et al. (2016) reported that in *Gladiolus* sp., anthesis completed on the same day, early 8.02 a.m. (American Beauty) to late 10.28 a.m. (Jester). Layek et al. (2022) revealed that flower opening from 5.00 to 8.00 a.m. on a hot day (April to May) than on cold day (10.00 a.m. to 12.00 p.m.) was observed during December to January in *Justicia betonica*. In *Senecio macrophyllus* according to Czamecka & Denisow (2014) disc florets open diurnally with intensive anthesis in early afternoon which attract insect for nectar and pollen.

The genotypes showed variation in their anther dehiscence as depicted in Table 1. The genotypes Arka Kamini (10:15 a.m.-12:15 p.m.), Arka Poornima (11:00 a.m.-12:15 p.m.), Arka Shashank (11:15 a.m.-3:00 p.m.) and Arka Archana (11:15 a.m.-2:00 p.m.) observed anther dehiscence from forenoon till afternoon, while in Arka Aadya anther dehiscence was observed from 12:00 p.m. to 12:45 p.m. Gupta et al. (2004) observed pollen dehiscence between 10-12 am in *Dianthus barbatus*. Poon et al. (2009) noticed that duration of initiation and completion of anther dehiscence was recorded highest in Psittacinus hybrid (5:19 h) and H.S. 82-11-27 (5:7 h) in gladiolus. Kalaiyarasi et al. (2019) observed anther dehiscence from 4:00 to 7:00 p.m. in different genotypes of jasmine. Yadav et al. (2022) reported that anther dehiscence in German chamomile (*Matricaria chamomilla* L.) started 1 to 2 days before style branches flushes showed protandry and overlapped later with style branches flushes.

Number of ray and disc florets per flower head

Significant differences were observed among genotypes for number of ray and disc florets per flower head (Table 1). Maximum number of ray florets per

Table 1 : Anthesis, anther dehiscence, number of ray and disc florets per flower head, length and width of ray florets in different genotypes of China aster

Genotype	Anthesis (a.m./p.m.)	Anther dehiscence (a.m./p.m.)	No. of ray florets/ flower head	No. of disc florets/ flower head	Length of ray florets (cm)	Width of ray florets (cm)
Arka Aadya	2:15 p.m. - 3:15 p.m.	12:00 p.m. - 12:45 p.m.	111.63	171.41	2.280	0.61
Arka Archana	10:15 a.m. - 11:30 a.m.	11:15 a.m. - 2:00 p.m.	126.05	168.23	2.318	0.48
Arka Kamini	10:15 a.m. - 11:15 a.m.	10:15 a.m. - 12:15 p.m.	119.05	164.10	2.220	0.59
Arka Poornima	10:30 a.m. - 11:15 a.m.	11:00 a.m. - 12:15 p.m.	132.37	238.93	2.348	0.59
Arka Shashank	10:45 a.m. - 12:15 p.m.	11:15 a.m. - 3:00 p.m.	29.53	128.93	1.755	0.64
SEm±			1.86	2.46	0.020	0.003
CD at 5%			4.05	5.36	0.050	0.107
CV (%)			2.53	1.99	1.45	3.56

flower head was recorded in Arka Poornima (132.37), followed by Arka Archana (126.05), Arka Kamini (119.05) and Arka Aadya (111.63), whereas, it was recorded minimum in Arka Shashank (29.53) as it has one or one and half row of ray florets. Ray florets in China aster are mostly female flower which open from outside to inside. It is measure of doubleness or singleness of flower type (Khangjarakpam et al., 2014) in China aster. Allen et al. (2011) found that inflorescence disc florets develop sequentially, while, ray florets matures before centrally located disc florets in *Senecio squalidus*.

Significantly highest number of disc florets were recorded in Arka Poornima (238.93), followed by Arka Aadya (171.41), Arka Archana (161.23) and Arka Kamini (164.10), whereas, lowest number of disc florets per flower head was recorded in Arka Shashank (128.93) (Table 1). Wist & Davis (2006) reported that protandrous disc florets mature from periphery to the centre, with one whorl of florets reaching anthesis in the morning in *E. purpurea*. Mature buds pass through a staminate phase on the first day of anthesis followed by a pistillate phase. Allen et al. (2011) found that inflorescence disc florets develop sequentially, while, ray florets matures before centrally located disc florets in *Senecio squalidus*. As the pistil matures and grows past the anthers, sterile pseudo-papillae at the ends of the stigmatic lobes collect pollen from the anthers and present this to pollinators. Disc florets are mostly of hermaphrodite in nature, produces abundant pollen and attract pollinator especially honey bees. Disc florets

produce more seed set than ray florets in China aster (Bhargav et al. 2016).

Length and width of ray florets

On the perusal of data presented in Table 1 showed significant differences for length and width of ray florets among genotypes studied. Maximum length of ray florets was recorded in Arka Poornima (2.35 cm), which was on par with Arka Archana (2.32 cm) and Arka Kamini (2.22 cm), while, it was varied significantly with Arka Aadya (2.28 cm). However, minimum length of ray florets was recorded in Arka Shashank (1.75 cm). Length of ray florets decides the size of flower head, and helps in fixing the position of fertile pistil in the whole capitulum. Over lapping of ray florets within the flower makes it less viable to set seed (Miyajima, 1995) in zinnia. Thus, the position of ray florets and out crossing of female flower for fertilization and seed setting is most important. Significantly, maximum width of ray florets was recorded in Arka Shashank (0.64 cm) which was on par with Arka Aadya (0.61), Arka Kamini (0.59 cm) and Arka Poornima (0.59), while, it was recorded significantly minimum in Arka Archana (0.48 cm).

Shape of ray florets

The genotypes showed variation for their shape of ray florets (Table 2 & Fig. 1). The genotypes Arka Aadya and Arka Kamini showed pointed tip, while, Arka Archana, Arka Poornima and Arka Shashank observed blunt ray florets at the tip. The shape of ray florets was observed to be narrow in Arka Aadya, Arka Archana and Arka Kamini, whereas, broad shaped ray

Table 2 : Shape of ray florets in different genotypes of China aster

Genotype	Shape of tip	Shape of ray florets	Shape of cross section
Arka Aadya	Pointed	Narrow	Concave
Arka Archana	Blunt	Narrow	Convex
Arka Kamini	Pointed	Narrow	Concave
Arka Poornima	Blunt	Broad	Concave
Arka Shashank	Blunt	Broad	Convex

florets were observed in Arka Poornima and Arka Shashank. With respect to shape of cross section of ray florets, the genotypes Arka Aadya, Arka Kamini and Arka Poornima showed concave shape, while, genotype Arka Archana and Arka Shashank observed convex shape.

It is important for pollinator to provide a surface to sit and collect the pollen and thus perform fertilization to form seed. Also, it helps to hold water within the ray florets to take pollen to the inner most zone of ray florets to do fertilization within same flower. It is helpful in self-pollination in semi-double type of capitulum. Similar results were also reported by Miyajima (1995) in zinnia, and Bhargav et al. (2016) in China aster.

Pollen viability, seed set, number of seeds obtained in disc and ray florets

The data presented in Table 3 showed significant variation in pollen viability, seed set, number of seeds obtained in disc and ray florets in different genotypes of China aster. Hundred per cent pollen viability was recorded in Arka Aadya, Arka Archana, Arka Kamini, and Arka Shashank, while, Arka Poornima (88.25%) recorded minimum pollen viability. Messar et al. (2016) found that maximum pollen viability in cv. Nathan White (64.95%) closely followed by

cv. Nathan Red (64.45%), while, least in cv. Red Beauty (56.69%) in *Gladiolus* sp. He et al. (2017) observed that Asiatic hybrids Tiny pudhye, exhibited low pollen viability (from 0 to 1 day after anthesis), while, in other genotypes, pollen germination rates were highest on anthesis, 0-1 day (*L. sulphureum*), or 0-2 days (Longiflorum hybrid), and then gradually decreased with days after anthesis. Selvarasu & Kandhasam (2019) studied reproductive biology of *Gloriosa superba* and found that pollen viability and fertility were recorded on the day of anthesis. According to Zhang et al. (2021), *N.* hybrid pollen viability was highest between 9:30 a.m. and 11:30 a.m. on the 2nd day of anthesis.

Maximum seed set was obtained in Arka Kamini (54.13%), which was followed by Arka Archana (41.12%), Arka Shashank (31.48%) and Arka Aadya (29.83%), whereas, minimum seed set was observed in Arka Poornima (24.55%) (Table 3). Esmacili et al. (2020) reported variation in flowering period of *S. atropatana* and *S. virgata* (15 days to 41 days), affecting seed set, as flowering periods were overlapping and the sharing of pollen among different plants coincides. Liu et al. (2021) reported low seed setting rate in *N. insignis* under natural condition. Neither pollinator nor pollen causes the low seed setting rate of *N. insignis*.


Fig. 1 : Shape of ray florets in different genotypes of China aster

Table 3 : Pollen viability, seed set, number of seeds obtained in disc and ray florets in different genotypes of China aster

Genotype	Pollen viability (%)	Seed set (%)	No. of seeds obtained	
			Disc florets	Ray florets
Arka Aadya	100.00 (1.57)	29.83 (0.84)	66.38	1.00 (1.22)
Arka Archana	100.00 (1.57)	41.12 (1.06)	106.88	1.50 (1.41)
Arka Kamini	100.00 (1.57)	54.13 (1.57)	123.88	1.63 (1.46)
Arka Poornima	88.25 (1.22)	24.55 (0.74)	72.38	0.87 (1.17)
Arka Shashank	100.00 (1.57)	31.48 (0.87)	47.00	1.38 (1.37)
SEm±	0.030	0.030	1.74	0.130
CD at 5%	0.070	0.070	3.79	0.280
CV (%)	2.87	4.56	2.98	13.23

Significant variation was observed for number of seeds obtained in disc florets and ray florets (Table 3). Maximum number of seeds from disc florets were obtained in Arka Kamini (123.88), followed by Arka Archana (106.88), Arka Poornima (72.38) and Arka Aadya (66.38), whereas, it was recorded minimum in Arka Shashank (47.00). Seed set increased with number of disc florets following cross pollination, whereas, it decreased with increasing number of disc florets in autonomously self-pollinated capitula. Indeed, the number of disc florets, or inflorescence size, may also reflect individual variation in flower allometry, which is known to translate into reproductive differences between flowers (*i.e.* herkogamy, differential stigma size and into decreased pollen receipt and fertilization efficiency. This variation might thus reflect different reproductive strategies: small capitula, which are also probably less attractive to pollinators, would still ensure reproduction via selfing, when pollination service is insufficient or uncertain. On the other hand, the larger and more attractive capitula would result in efficient outcross reproduction under good pollination conditions. Similar observations were also reported by Penet et al. (2012) in *Centaurea cyanus*.

Negligible seeds were obtained from ray florets, among genotypes. Arka Kamini recorded maximum number of seeds from ray florets (1.63) followed by Arka Archana (1.50), Arka Shashank (1.38), Arka Aadya (1.00) and minimum number of ray florets were observed in Arka Poornima (0.87). Poor seed from ray florets may be attributed to the lack of pollen in ray florets. Similar results are in conformity with findings of Yu et al. (2011) in *Petasites tricholobus*

Stigma receptivity

In general, the increased stigma receptivity among the genotypes was observed from bud stage to fully expand floret stage and declined in semi-expanded tubular disc stage (Table 4). No stigma receptivity was observed at withering stage. Among genotypes, Arka Shashnak recorded stigma receptivity from bud stage to semi-expanded tubular disc stage. Stigma receptivity is the critical stage of maturation of flower that may greatly influence the success of pollination. It is the ability to accept pollen from landing till fertilization for formation of ovule. Liu et al. (2021) reported low seed setting rate in *N. insignis* under natural condition. The obstacles in sexual reproduction

Table 4 : Stigma receptivity in different genotypes of China aster

Stages of stigma receptivity	Genotype				
	Arka Aadya	Arka Archana	Arka Kamini	Arka Poornima	Arka Shashank
Bud stage	+	+	+	+	+
Semi expanded stage	++	++	++	+++	++
Half expanded stage	+++	++++	+++	++++	++
Fully expanded stage	++++	+++++	++++	+++++	++
Fully expanded florets	+++++	+++++	++++	+++++	++++
Semi expanded tubular disc	-	-	-	-	+++

Table 5 : Effect of pollen germination media and genotypes on pollen germination

Treatment	Arka Aadya	Arka Archana	Arka Kamini	Arka Poornima	Arka Shashank	Mean
T ₁ : 5% Sucrose + 15% PEG + BK medium	0.00	0.00	0.00	0.00	36.17	7.23
T ₂ : 10% Sucrose+ 15% PEG +BK medium	41.00	44.33	0.00	0.00	0.00	17.07
T ₃ : 10% Sucrose+ 30% PEG +BK medium	0.00	0.00	0.00	0.00	25.67	5.13
T ₄ : 15% Sucrose + 15% PEG +BK medium	0.00	44.00	0.00	0.00	0.00	8.80
T ₅ : 15% Sucrose + 30% PEG +BK medium	0.00	0.00	70.50	0.00	0.00	14.10
Mean	8.20	17.67	14.10	0.00	12.37	10.47
				SEm±	CD (0.01)	CV
Genotype				0.04	0.11	9.32
Media				0.04	0.10	8.45
Interaction				0.04	0.10	9.67

process may be attributable to poor pollen viability and stigma receptivity. Navarro & Guitian (2002) reported that close stigma-anther proximity and only slight temporal separation between the pollen grain germinability and stigma receptivity peak, so that autonomous self-pollination is enabled, providing reproductive assurance in the absence of pollinators in *Petrocoptis viscosa*.

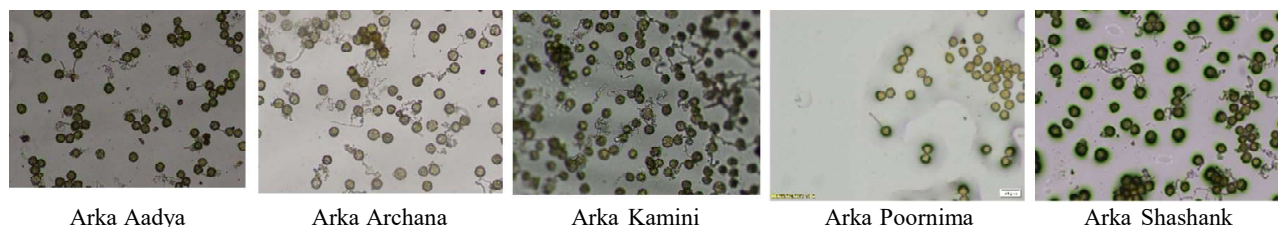
Pollen germination

On the perusal of data presented in Table 5 indicated significant variation among genotypes, media and their interaction for pollen germination. Among the genotypes, maximum pollen germination was observed in Arka Archana (17.67%) followed by Arka Kamini (14.10%), Arka Shashank (12.37%) and Arka Aadya (8.20%), however, no pollen germination was recorded in Arka Poornima. Among the different media, maximum pollen germination was observed in medium 10% sucrose + 15% PEG + BK media (17.07%) followed by 15% sucrose + 30% PEG + BK media (14.10%), 15% sucrose + 15% PEG + BK media (8.80%) and 5% sucrose + 15% PEG + BK media

(7.23%), while, minimum pollen germination was recorded in medium 10% sucrose + 15% PEG +BK medium (5.13%).

Among interaction effect, maximum pollen germination was found in 15% sucrose + 30% PEG + BK medium x Arka Kamini (70.50%) followed by 10% sucrose + 15% PEG + BK medium x Arka Archana (44.33%), 10% sucrose + 15% PEG + BK medium x Arka Aadya (44.00%), 10% sucrose + 30% PEG + BK medium with x Arka Shashank (41.00%) and 5% Sucrose + 15% PEG + BK medium x Arka Shashank (36.17%).

In *Lilium*, pollen germination rates were highest on anthesis (five of seven genotypes), 0-1 day after anthesis (*L. sulphureum*), or 0-2 days after anthesis (one *Longiflorum* hybrid), and then gradually decreased with days after anthesis (He et al., 2017). *P. edmundoi* showed highest pollen germination and viability percentages and tube length, makes it better parent in interspecific crosses for obtaining hybrids with good agronomic and/or ornamental potential (dos Santos Ferreira et al., 2021).


Fig. 2 : Pollen germination in different genotypes of China aster

In vitro and *in vivo* pollen germination, has no significant correlation between the self-compatible and germination rate of pollen. In seven chrysanthemum genotypes with self-pollinated progenies, only pollen tube of 'Hongguan' could elongate, penetrate its own stigma and reach the base of the style, which was key to successful self-pollination in chrysanthemum mutants (Pu et al., 2021).

CONCLUSION

The study on floral biology and pollen germination in China aster revealed variation in time of anthesis, anther dehiscence, number of ray and disc florets per flower head among the genotypes studied. Increased stigma receptivity among the genotypes was observed from bud stage to fully expand floret stage and declined in semi expanded tubular disc stage. Among genotypes, maximum pollen germination was observed in Arka Archana, while, among different media, maximum pollen germination ranged from 5.13% (10% sucrose + 15% PEG +BK medium) to 17.07% (10% sucrose + 15% PEG + BK media). Among interaction effect, maximum pollen germination was recorded in 15% sucrose + 30% PEG + BK medium x Arka Kamini (70.50%) followed by 10% sucrose +15% PEG + BK medium x Arka Archana (44.33%) and 10% sucrose + 15% PEG +BK medium x Arka Aadya (44.00%). This study reveals significant variation in floral and reproductive traits in different China aster genotypes, which can be valuable for breeding programs, improving floral and seed production.

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