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Pollen germination and stigma receptivity in China aster (*Callistephus chinensis* Nees.).

Chetna Jyoti[1]*, R Kumar[1], P E Rajasekharan [2], S A Nair[1] and T U Bharathi[1]
[1]Division of Flower and Medicinal Crops, [2]Division of Plant Genetic Resources ICAR-Indian Institute of Horticultural Research, Hesaraghatta Lake PO, Bengaluru, 560089, Karnataka, India.
**Corresponding Author - Email address: chetna_jyoti28@yahoo.co.in*

***ABSTRACT**: China aster is botanically called as Callistephus chinensis Nees, It is native to China and belongs to family Asteraceae. It is a half hardy annual ornamental flower crop grown for its flowers. In china aster, stamens and pistils do not mature simultaneously in the individual flower. The stigma of the individual flower unfolds after the pollen is discharged from the flower., In present study, a simple and reliable pollen germination medium was developed as modified Brewbaker and Kwack (BK) medium. Different pollen germination media as BK modified media with PEG and sucrose in different combinations was used. Modified BK media + 15% sucrose + 30% PEG 6000 recorded maximum pollen germination in Arka Kamini (70%) followed by Arka Archana (80%) and Arka Aadya (46.83%), whereas BK media 5% Sucrose + 15% PEG6000 recorded maximum pollen germination in variety Arka Shashank (45%). However, pollen germination was not observed in Arka Poornima in lab condition. Stigma receptivity was recorded by H_2O_2 effervescence method. It was lowest in Arka Kamini in 1st stage (bud stage) increased up to 5th stage of anthesis, Arka Archana and Arka Aadya was highest on 5th and 6th stage of anthesis. While in variety Arka Shashank it was constantly increases up till 5th stage then decreases. The variety Arka Poornima showed maximum stigma receptivity on 5th and 6th stage of flower anthesis. This study is important for breeders to help in developing new varieties in China Aster.*

***Key words:** China aster, pollen, stigma receptivity, pollen, pollen germination.*

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I. INTRODUCTION

China aster is a self-pollinated with 10% of natural cross pollination. It belongs to Asteraceae and chromosome number 18. It is most widely grown for loose flower, bedding, pot and boarders. Pollination is very important for seed setting in China aster. Therefore, pollen germination and stigma receptivity plays important role. A flower head consist of outer ray floret that act as pure female and central disc floret hermaphrodite. Pollen often discharge earlier after that stigma became receptive. Pollen grains that fail to germinate frequently have weak pollen tube growth and are therefore ineffective during sexual fertilisation. Therefore, modified Brewbaker and Kwack media was tried with different concentration of sucrose and PEG 6000. Stigma receptivity can be determine cytochemical testing for the existence of enzyme activity. For measuring stigma receptivity Hydrogen peroxide 6% was used to see the bubble intensity for measuring the receptiveness. Therefore, this study aimed to provide scientific information on pollen germination and stigma receptivity in order to determine the best pollination timing for breeders.

II. MATERIALS AND METHODS

The present study was carried out in the Division of Floriculture and Medicinal Crops, ICAR-Indian Institute of Horticultural Research, Hessaraghatta Lake Post, Bengaluru during 2022-2023. The experimental material consisted of 5 varieties Arka Aadya, Arka Archana, Arka Shashank and Arka Kamini and Arka Poornima. Pollen germination was estimated by using Modified Brewbaker and Kwack's (1963) medium which was prepared by using following protocol Boric acid (100 ppm), Calcium nitrate (300 ppm), Potassium nitrate (100 ppm), Magnesium sulphate (200 ppm) with varying concentration of Sucrose 5%, 10%, 15% and Polyethylene Glycol (15% and 30%). In hanging drop technique (Stanley and 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 twenty four hours time intervals to know the germination percentage and pollen tube length. 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). The pollen tube growth was observed and images were captured with a photomicroscope. Florets were isolated by transparent paper bag at 1 day of

anthesis to 7 Days after anthesis. The pistils of the five cultivars were collected at 9 am and tested by H2O2 (6 % H2O2 hydrogen peroxide) respectively (Dafni and Motte, 1998). The collected pistils were stained for 10 min at room temperature and a pistil was regarded as receptive with some amount of bubbles. Each treatment was carried out with ten pistils in three replicates. The data analysis was done with WASP 2.0 Statistical package for pollen germination CRD (Alviano, et. al., 2015).

III. RESULTS AND DISCUSSION

Table 1 Pollen Germination.

	Treatments	Arka Aadya	Arka Archana	Arka Shashank	Arka Kamini	Arka Poornima
T1 -	5% Sucrose + 15% PEG + BK medium	0.00	0.00	30.33	0.00	0.00
T2 -	10% Sucrose+ 15% PEG +BK medium	45.33	47.00	0.00	0.00	0.00
T3-	10% Sucrose+ 30% PEG +BK medium	0.00	0.00	27.33	0.00	0.00
T4 -	15% Sucrose + 15% PEG +BK medium	0.00	44.00	0.00	0.00	0.00
T5 -	15% Sucrose + 30% PEG +BK medium	0.00	0.00	0.00	72.67	0.00
	Mean	9.07	18.20	11.53	14.67	0.00
	CD (0.05)	3.08	18.11	13.21	3.08	0.00
	SeM±	9.07	11.16	11.16	14.53	0.00
	CV %	18.67	54.72	62.96	11.65	0.00

In Arka Poornima in all treatments pollen germination was zero. In arka Aadya there was maximum germination in treatment T2 taht is 45%.Also in Arka Archana 47 percent germination was recorded, 30 percent germination was recorded in first treatment in Arka Shashank. In Arka Kamini maximum germination was there i.e. 72 percent. This may be due to genetic makeup of the variety, Type of season dehydration of pollen in desiccation for complete pollen germination (Božič and Šiber, 2020).

Table 2 Stigma Receptivity.

S.No.	Stages of Stigma Receptivity	Varieties Arka Aadya	Arka Archana	Arka Shashank	Arka Kamini	Arka Poornima
1	Bud Stage	+	+	+	+	+
2	Semi open Stage	++	++	++	++	+++
3	Half open stage	+++	++++	++	+++	++++
4	Fully open stage	++++	+++++	++	++++	+++++
5	Fully expended ray	+++++	++++++	++++	++++	+++++
6	Semi open tubular disc	-	-	+++	-	+++
7	Withering stage	-	--	-	-	-

A	Bud stage	-	No reactivity
B	Full bloom stage	+	Very less reactivity
C	Flower withering stage	++	Less reactivity
		+++	Moderate reactivity
		++++	High reactivity
		+++++	Very high reactivity

The assessment of stigma receptivity is important as this represents the best time for pollination, which assists in successfully controlled pollination. The small buds did not show receptiveness (Pio et al., in 2004). Stigma became fully receptive after opening of flower bud. Stigma of older flowers which tested positive for peroxidase activity showed a varying level of reaction. Maximum stigma receptivity was found at fully

expanded ray. For successful fertilization, it is desirable that the pollen is transferred to the receptive stigma of another flower. In many cases, fertilization can occur when the pollen grain is deposited before the receptive period as long as it remains viable long enough to be able to germinate as soon as the flower becomes receptive (Thomson and Barret, 1981). It is shown that stigma receptivity was more on third day onwards. In the hydrogen peroxide test, the number of bubbles recorded on the stigma of flowers up to 7 d after the commencement of bloom stage, which indicates the degree of receptivity of stigma. The maximum number of bubbles was observed on third and fourth day after flower opening (Gupta et. al., 2015) in rice and wheat crop.

IV. Conclusion

The results indicate that both differences in duration of stigma receptivity and interference from self-pollen deposition may contribute to observed variation in seed production and pollen limitation among style morphs in natural populations of China aster. An association between stigma position and duration of stigma receptivity should constrain the evolution of flower morphology.

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