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Identification of Autogamous and Self-Fertile Lines in Sunflower (*Helianthus annuus* L.) Over Seasons

Singh A.K. [a*], Dhuppe M.V. [b], Pole S.P. [b] and Meena P.K. [a]

[a] Department of Genetics and Plant Breeding, College of Agriculture, Latur, Vasantrao Naik Marathwada Krishi Vidyapeeth, Parbhani, 413512, Maharashtra, India.
[b] Oilseeds Research Station, Vasantrao Naik Marathwada Krishi Vidyapeeth, Parbhani, Nanded Road, Latur, 413512, India.

Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

This study aims to assess the autogamy and self-fertility percentage in sunflower (*Helianthus annuus* L.) across two distinct cropping seasons. Sunflower possesses self-incompatibility which can be overcome by exploring alternate pollination methods. Autogamy and self-fertility are two desirable traits of sunflowers that play an important role in reproductive success and yield stability. Through the examination of 46 genotypes, the research aims to determine the effects of seasonal variations on seed set, autogamy, and self-fertility across different sunflower genotypes during the *Kharif* and *Rabi* seasons. The study compared three modes of pollination: self-pollination (SP), self-pollination plus manual (S+M) pollination, and open pollination (OP). Results indicated that the *Rabi*

**Corresponding author: E-mail: aksashishsingh97@gmail.com;*

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season favoured a higher seed set and better seed filling compared to the *Kharif* season. The study revealed that the *Rabi* season's cooler temperatures, higher humidity, and reduced solar radiation positively influenced seed yield and quality. In contrast, *Kharif* season conditions, particularly the monsoon, led to reduced seed yield and fewer filled seeds due to factors like higher pollen movement and lower humidity inside pollination bags. The autogamy and self-fertility percentages during *Rabi* were higher at 79.01 percent and 85.01 percent, respectively. Genotypes PB-892, EC-198072, and PB-1444 showed the highest seed set under S+M pollination during Kharif, while CMS-107B, PB-1212, and CMS-47B excelled in SP. Across both seasons, EC-198078, EC-178168, and GMU-770 were identified as highly self-fertile, making them valuable for developing self-fertile hybrids and enhancing population development.

Keywords: Autogamy; genotypes, seasonal variation; self-fertility; sunflower.

1. INTRODUCTION

Oilseed crops hold a significant position in the agricultural economy, following food grains. Among these, Sunflower (*Helianthus annuus* L., 2n = 34) stands out as a prominent oilseed crop globally, including India. Belonging to the family Asteraceae, tribe Heliantheae, and sub-tribe Helianthae. Sunflower originated in North America. It ranks as the fourth most important oilseed crop worldwide, trailing behind soybean, groundnut, and rapeseed, as extensively noted by Shamshad et al. [1] and Yamgar et al. [2]. Its cultivation spans numerous countries, including Russia, Ukraine, Argentina, China, Myanmar, Spain, Kazakhstan, France, and India.

Poor seed set represents a significant constraint in sunflower productivity. While various factors such as physiological, genetic, nutritional, and environmental interactions influence seed filling, it has been determined that the ultimate seed set is largely dependent on the population of pollinators in the surrounding area, given that sunflower is primarily a cross-pollinated crop [3]. Moreover, sunflower exhibits sporophytic self-incompatibility [4], necessitating alternative pollination methods to overcome this constraint. Inbreeding programs aimed at heterosis, the development of inbred lines with high self-fertility is desirable, as it positively impacts hybrid performance.

The ability of sunflower genotypes to self-pollinate, known as autogamy, plays a crucial role in the crop's reproductive success and yield stability. Autogamy and self-fertility are particularly important in sunflower breeding programs, whereas self-fertility is a desirable trait. However, environmental factors such as seasonal variation can significantly impact self-fertility rates. This study aimed to assess the autogamy and self-fertility of various sunflower genotypes over two cropping seasons, providing insights into their stability and potential for breeding programs.

Sunflower is one of the important oilseed crops widely cultivated in different parts of India. It is a cross-pollinated crop and the percentage of seed set and oil content largely depend on the population of pollinators. It possesses self-incompatibility which can be overcome by exploring alternate pollination methods. Autogamy and self-fertility are two desirable traits of sunflowers that play an important role in reproductive success and yield stability. These traits can be affected by seasonal fluctuations. Knowledge of autogamy and self-fertility of genotypes in different seasons provides insights into their potential for breeding programs. So, it is important for the scientific community.

2. MATERIALS AND METHODS

The aim of the study was to determine how seasonal variations affected autogamy and self-fertility in sunflower genotypes for two separate growing seasons, *Kharif* and *Rabi*. There were forty-six different genotypes of sunflowers in the current study. Ten inbred lines, eleven Cytoplasmic Male Sterility (CMS) lines, seven restorer lines, seven population lines, seven germplasm lines, and four checks were among the genotypes obtained from Oilseeds Research Station, Latur. These genotypes provided a broad genetic base for the investigation.

Two replications of each genotype were conducted in the Randomised Block Design (RBD). Every genotype was sown in three rows, each row being 4.5 m long and separated by 60cm whereas the plant within a row was separated by 30cm. Two to three seeds were planted per hill and 15 days after sowing, the plant density was adjusted by thinning. Fifteen plants were randomly chosen for each treatment and replication. Five of these were covered with cloth bags as soon as the ray florets appeared, five more were covered and then manually pollinated by gently rubbing heads against the florets, and the other five were left to be pollinated in the open. We used the George and Shain [5] methods to determine seed set percentage, autogamy, and self-fertility.

To assess the influence of different pollination methods on autogamy and self-fertility, the study incorporated three distinct modes of pollination:

1. **Self-pollination (SP):** Self-pollination was facilitated by bagging individual flower heads before anthesis to prevent any foreign pollen from entering. This method aimed to determine the intrinsic ability of each genotype to self-pollinate without the assistance of external pollinators.
2. **Self + Manual Pollination (S+M):** Sunflower heads were bagged with cloth bags prior to flowering. During flowering, the heads were rubbed through the bags on alternate days to guarantee self-pollination. The cloth bags stayed on the sunflower heads until harvest. This method was used to ensure that pollen from the same flower or plant reached the stigma, stimulating self-fertility under controlled conditions and eliminating any potential barriers to pollen transfer.
3. **Open Pollination (OP):** In this mode, plants were left to naturally receive pollen from surrounding plants, allowing for cross-pollination. This method provided a baseline for comparing the natural reproductive success of the genotypes under typical field conditions

The percentage of seed set in the self-pollinated heads was used to assess autogamy. It is determined by the ratio of seeds set under autogamous pollination to those under open pollination.

By analyzing the seed set in the assisted pollination treatment, self-fertility was evaluated. It is determined by the ratio of the seed set under hand pollination (expressed as a percentage) to the seed set under open pollination (also expressed as a percentage). This method guaranteed that any variation in the seed set was caused by the genotype's natural capacity for self-fertilization rather than by outside influences.

3. RESULTS AND DISCUSSION

Significant seasonal variation in autogamy and self-fertility among the genotypes of sunflowers was found in this study. These genotypes inconsistent performance throughout the two seasons suggests that environmental variables were a major determinant of these features.

Genotypes during the *Rabi* season displayed higher levels of seed set, autogamy, and self-fertility compared to the *Kharif* season. In self-pollination, the average seed set of genotypes during *Kharif* was 50.30 percent; in S+M pollination, it was 68.81 percent. Out of all the pollination modes, open pollination produced the highest seed set (81.62%). During *Kharif*, the percentages of autogamy and self-fertility were 61.79 and 84.36 percent, respectively.

During *Rabi*, the average seed set of genotype in self-pollination was 68.01 percent; in S+M pollination, it was 73.43 percent. The highest seed set (86.14%) was obtained by open pollination out of all the pollination methods. The percentage of autogamy and self-fertility during *Rabi* was 79.01 percent and 85.01 percent, respectively. The season had a significant effect on genotypes because, compared to the *Kharif* season, the *Rabi* season favoured higher seed yields and better seed filling. Higher relative humidity, colder temperatures, less sun radiation, and less pollen movement inside the cloth bags may all be responsible for the decrease in seed yield and number of filled seeds, especially during the monsoon in the heads covered with the bags [6]. While PB-892, EC-198072, and PB-1444 had the maximum seed set with S+M mode of pollination during *Kharif* and the genotypes CMS-107B, PB-1212, and CMS-47B had the highest seed set via self-pollination. EC-178168, EC-198072, and CMS-107B were the genotypes with the highest seed set *via* self-pollination during *Rabi*, whereas EC-198078, EC-178168, and Morden were the genotypes with the best results under S+M pollination. Throughout two seasons, EC-198078, EC-178168, and GMU-770 were highly self-fertile lines, while PB-1212, CMS-47B, and EC-934477-1 were highly autogamous lines. The self-fertility of genotypes was evaluated using the seed set (Table 1), which is expressed as a percentage of filled seeds in proportion to total seeds. As a result, open pollination results in the highest seed set percentage, which is then followed by the S+M

Table 1. Percentages of seed set, autogamy, and self-fertility across a two-season period

Sr. No.	Genotypes	Seed set (%)						Autogamy (%)		Self-fertility (%)	
		Self		Self + Manual		Open					
		Kharif	*Rabi*	*Kharif*	*Rabi*	*Kharif*	*Rabi*	*Kharif*	*Rabi*	*Kharif*	*Rabi*
1	R-856	38.37	51.79	60.99	62.20	74.46	76.60	51.53	67.62	81.91	81.21
2	EC-279309-1	54.79	72.97	58.89	70.21	67.13	84.82	81.62	86.03	87.72	82.78
3	TSG-111	32.60	51.99	73.21	75.04	83.13	87.38	39.22	59.50	88.07	85.87
4	BK-R-1	59.88	74.34	62.39	76.39	73.46	79.85	81.52	93.10	84.94	95.66
5	EC-623008	50.90	69.23	71.24	70.17	80.31	84.41	63.39	82.02	88.72	83.13
6	EC-601951	64.52	77.66	72.08	77.19	78.75	94.80	81.94	81.93	91.53	81.43
7	RHA-138-2	57.49	80.64	73.97	84.22	86.75	96.18	66.27	83.85	85.26	87.57
8	PB-1444	25.03	51.86	79.45	71.45	84.45	94.61	29.64	54.81	94.08	75.52
9	PB-1445	24.90	52.40	63.00	70.26	74.79	90.81	33.30	57.70	84.25	77.37
10	PB-1435	50.49	64.35	66.79	62.45	75.74	74.86	66.67	85.97	88.19	83.42
11	PB-1212	71.50	75.98	75.55	73.74	79.31	80.14	90.16	94.80	95.27	92.01
12	PB-892	60.02	81.32	88.24	84.71	90.63	93.83	66.22	86.66	97.36	90.28
13	12-R-1	56.56	73.62	51.32	57.30	66.20	78.15	85.44	94.20	77.52	73.32
14	NO-1147-2	49.25	70.58	57.79	79.84	94.18	93.39	52.29	75.57	61.36	85.49
15	SCG-08	44.31	62.35	51.22	69.62	77.45	82.64	57.21	75.45	66.13	84.25
16	GMU-477	52.81	64.61	62.41	70.01	92.17	92.55	57.29	69.81	67.71	75.65
17	RHA-95C-10-1	49.03	67.26	51.06	60.33	75.17	79.12	65.23	85.01	67.92	76.25
18	EC-601766	23.34	36.44	46.11	42.70	57.51	58.95	40.59	61.83	80.18	72.44
19	EC-502036	52.96	70.37	62.41	65.66	76.46	77.18	69.26	91.18	81.63	85.08
20	GMU-481	27.80	48.18	53.43	50.84	63.17	74.84	44.01	64.37	84.59	67.93
21	GMU-258	56.28	80.48	70.08	75.00	85.39	95.79	65.92	84.02	82.08	78.30
22	GMU-249	61.96	79.98	76.30	87.44	92.20	96.11	67.21	83.21	82.76	90.97
23	EC-601924	33.91	59.65	67.65	75.51	82.63	89.16	41.04	66.90	81.87	84.70

Sr. No.	Genotypes	Seed set (%)						Autogamy (%)		Self-fertility (%)	
		Self		Self + Manual		Open					
		Kharif	***Rabi***	***Kharif***	***Rabi***	***Kharif***	***Rabi***	***Kharif***	***Rabi***	***Kharif***	***Rabi***
24	EC-934477	35.59	60.02	60.23	62.77	84.90	94.84	41.93	63.28	70.95	66.19
25	17B	33.88	59.05	59.01	67.11	78.63	90.06	43.08	65.57	75.04	74.51
26	CMS-103B	40.81	62.10	61.48	78.40	81.18	88.96	50.27	69.80	75.73	88.13
27	ARM-243B	50.51	71.01	78.67	73.31	81.96	84.90	61.63	83.64	95.99	86.35
28	CMS-10B	53.38	74.42	54.13	63.28	85.69	90.84	62.29	81.92	63.17	69.66
29	CMS 112B	51.69	78.79	76.73	86.88	90.77	95.67	56.95	82.36	84.52	90.81
30	CMS-249B	58.77	84.27	75.31	85.66	83.63	93.63	70.28	90.00	90.04	91.49
31	CMS-234B	59.70	81.50	70.71	82.39	81.37	88.40	73.37	92.20	86.89	93.21
32	CMS-47B	70.79	80.04	67.21	78.12	78.60	83.44	90.06	95.93	85.51	93.63
33	NDCMS-2B	53.20	75.88	72.15	89.55	90.11	96.00	59.04	79.04	80.07	93.28
34	CMS-107B	72.42	84.85	78.42	84.86	87.00	89.70	83.24	94.60	90.14	94.60
35	CMS-607B	61.27	79.70	75.66	87.90	89.70	94.31	68.30	84.51	84.35	93.21
36	GMU-520	61.16	32.71	79.15	35.31	93.11	39.68	65.69	82.42	85.01	88.98
37	EC-934477-1	69.42	84.21	70.02	81.29	79.24	86.46	87.60	97.40	88.36	94.02
38	EC-178168	67.66	88.06	72.81	90.47	76.10	94.78	88.91	92.91	95.68	95.45
39	EC-198072	64.78	87.99	82.02	82.48	92.48	94.15	70.04	93.46	88.69	87.61
40	EC-198078	61.30	81.31	77.51	91.83	79.52	95.42	77.09	85.22	97.47	96.24
41	GMU-770	56.58	74.73	76.88	84.16	78.96	89.97	71.66	83.06	97.37	93.55
42	CSFI-1509	41.23	61.17	78.08	73.74	86.80	84.52	47.51	72.38	89.96	87.25
43	DRSF-108	46.86	69.26	76.35	72.33	79.92	91.87	58.63	75.38	95.54	78.73
44	LSF-08	47.18	39.89	76.62	39.48	87.61	51.29	53.85	77.78	87.46	76.97
45	P. Bhasker	33.87	53.72	75.78	84.13	91.37	92.01	37.07	58.38	82.94	91.43
46	Morden	22.82	45.57	74.95	90.13	84.59	95.36	26.98	47.79	88.61	94.51
	Mean	**50.30**	**68.01**	**68.81**	**73.43**	**81.62**	**86.14**	**61.79**	**79.01**	**84.36**	**85.01**

mode of pollination and self-pollination. Comparable results were observed by Swamy & Giriraj [7], Janamma et al. [8], and Sumangala and Giriraj [9].

4. CONCLUSION

The study demonstrates the importance of seasonal variations while evaluating sunflower genotypes for autogamy and self-fertility. The self-fertility of genotypes was evaluated using the seed set (Table 1), which is expressed as a percentage of filled seeds in proportion to total seeds. As a result, open pollination results in the highest seed set percentage, which is then followed by the S+M mode of pollination and self-pollination. Similarly, high self-fertile lines *viz*., PB-892, PB-1212, CMS-249B, EC-178168, and EC-198072 offer valuable genetic resources for breeding programs aimed at improving sunflower yields. Further research should focus on the underlying genetic and environmental factors influencing autogamy and self-fertility in sunflower to enhance the development of high-yielding, self-fertile varieties, and hybrids.

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc) and text-to-image generators have been used during writing or editing of this manuscript.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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