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Pollen viability and stigma receptivity in sunflower (*Helianthus annuus*)

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ABSTRACT Pollen viability is a critical factor determining the success of sunflower seed production. Fresh pollen-grains without blending of filler material recorded maximum viability. The pollen-grains stored in refrigerator recorded higher viability (%) compared to those stored in moist earthen pots. This may be attributed to low temperature of 4ºC and 75% RH prevailing in refrigerator. Storage of pollen grains in earthen pots surrounded with moist sand was adjudged as the next best treatment since their viability could be maintained through in a lower scale up to fifth day. Irrespective of filler material and storage methods, viability (%) and stigma receptivity decreased with increased age of pollen-grains.

Key words: Pollen viability, sunflower, stigma, seed production

Sunflower (*Helianthus annus* L.) is an important edible oilseed crops, cultivated in different parts of the world. It occupies about 1.88 m ha of the area, with a total production of 1.46 m tonnes and productivity of 777 kg/ha [1]. In India, sunflower is mostly grown in Karnataka, Andhra Pradesh, Maharashtra, Tamil Nadu, Punjab, Haryana and Uttar Pradesh. In sunflower, seed setting is major problem for seed production of hybrids and parental lines. The seed filling in sunflower is largely dependent on proper pollination, which is normally inadequate and improper, leading to non-availability of pollen-grains on to the stigma, thereby resulting in poor seed setting. The successful transfer of pollen-grains to stigmatic surface before pollen-grains lose their viability, is important. Several researchers reported that seed setting and productivity were affected due to non-synchrony in flowering, inadequate availability of pollen-grains at stigma receptivity period, inadequate production of pollen-grains by the restorer line and adverse climatic factors. Under such situations pollen-grains can be collected and stored for pollination during hybrid seed production. For short-term and medium-term storage, temperatures ranging from 5ºC to –20°C depending upon crops, along with low relative humidity were found most suitable. Generally hybrid seed production of sunflower is concentrated mainly in rural areas, wherein it is difficult to regulate temperature and relative humidity. Therefore, to avoid wastage of pollen-grains, wherever excess quantity of pollen-grains is available from the male parent, pollen-grains may be stored and can be subsequently used for pollination. In members of graminae, pollen viability is lost rapidly, often within minutes, however in sunflower (Asteraceae) researchers reported differential longevity of pollen-grains, throughout the day [2], 12 hours [3], 22 hours [4] and 34 hours [5]. Hence, there is a need to increase seed setting and productivity for newly-released hybrids of sunflower. Keeping this in view present investigation was taken up to determine the viability (Shelf-life) of pollen-grains and impact on seed setting and find out the appropriate time (Period) for optimum seed setting and seed yield.

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MATERIALS AND METHODS

Parental lines of sunflower hybrid, DRSH-1, ARM-243A (A-line) and 6D-1 (R-line), were sown in block method during *Rabi* 2008-09 at Seed Research and Technology Center, Rajendranagar, Hyderabad. All the recommended package of cultural practices were followed to raise a healthy crop. The experiment was laid in 2^3 FRBD with storage methods (C_1 : moist earthen pot, C_2 : refrigerator (4±1°C) and C_3 : earthen pots surrounded with moist sand), pollen with filler material (F_1 : fresh pollen-grains without blending of filler material, F_2 : fresh pollen-grains + borax @ 5% (75:25) and F_3 : fresh pollen-grains + borax @ 5% + sucrose @ 5% (50:25:25) and age of pollen-grains (D_1 : one day aged pollen-grains, D_2 : two days aged pollen-grains, D_3 : three days aged pollen-grains, D_4 : four days aged pollen-grains and D_5 : five days aged pollen-grains) as 3 different factors along with three controls (Fresh pollen with and without blending of filler materials of same day pollen-grains) and replicated thrice.

Pollen-grains were collected from restorer line in plastic trays and transferred to butter paper bags which were then stored in moist earthen pots, refrigerator and earthen pots surrounded with moist sand for respective periods. Later, pollen-grains stored in butter paper bags were transferred to respective plastic containers treatment-wise to store for a period of 5 days *i.e.* from 1st day to 5th day.

Acetocaramine was prepared by mixing 0.5 g of caramine powder in 50 ml acetic acid (45%) and heating gently with reflux condenser. A drop of saturated ferric acetate was added as mordant so that viable pollen-grains take red stain, whereas non-viable pollen remain in original yellow. In this test, for each treatment, 3 slides were prepared and for each slide 3 microscopic fields were identified and the total numbers of viable and non-viable pollen-grains (In each field) were recorded and percentage is calculated.

The reliable method for determining endosperm/embryo ratio is tetrazolium chloride test. For each treatment, 50 seeds were taken randomly and soaked overnight in water. Then the seed coat was removed carefully and the seed was cut longitudinally without damaging the seed. Later, seeds were placed in Petri plates containing 0.5% tetrazolium chloride solution. Petri plates along with seeds were kept in darkness at room temperature for 2-3 hr. After staining was completed, viable part of seed *i.e.* embryo which had turned red was excised carefully and weighed (mg). Similarly unstained portion *i.e.* endosperm was also weighed (mg) and ratio was computed using the following formula.

$$\text{Endosperm/embryo ratio} = \frac{\text{Weight of the endosperm (mg)}}{\text{Weight of the embryo (mg)}}$$

From 15 rows, ten plants were selected per row and bagged before flowering. Pollination was done by maintaining the pollen starvation *i.e.* on first day, only ten plants were pollinated in first row. On second day, plants in both first row and second row were pollinated. Thus pollination was done by increasing one row per day. Later, the rows were harvested individually and the seed set per cent was calculated.

The data was subjected to statistical analysis by following 2^3 Factorial Randomized Block Design to study various treatments and their effects as per the procedure outlined by Panse and Sukhatme [6].

RESULTS AND DISCUSSION

The results indicated that pollen viability (%) and endosperm/embryo ratio were significantly different due to filler material, storage methods, age of pollen and their respective interactions.

Maximum pollen viability (F_1: 74.41%) was recorded when fresh pollen-grains were used without blending of filler material followed by blending of fresh pollen-grains with borax + sucrose (F_3: 73.53%) and fresh pollen-grains blending with borax (F_2: 73.42%). Pollen-grains stored in refrigerator had shown better performance (C_2: 76.34%) compared to those stored in earthen pots surrounded with moist sand (C_3: 72.89%) and pollen-grains stored in moist earthen pots (C_1: 72.13%). As the age of pollen-grains increased from first day D_1: 79.70% to fifth day D_5: 63.33%, the pollen viability decreased. Interaction effect was

Table 1. **Effect of filler material (F), storage methods (C) and age of pollen (D) on endosperm/embryo ratio in sunflower**

Treatment	C_1	C_2	C_3	Filler material mean (F)
F_1D_1	45.9	38.8	56.1	42.21
F_1D_2	43.5	37.7	51.1	
F_1D_3	41.6	37.2	47.5	
F_1D_4	41.5	36.7	44.3	
F_1D_5	40.3	35.4	44.6	
F_2D_1	46.7	39.6	57.4	44.22
F_2D_2	45.8	38.0	57.3	
F_2D_3	45.6	37.0	50.3	
F_2D_4	43.3	36.0	45.8	
F_2D_5	41.8	35.1	44.0	
F_3D_1	42.9	38.8	49.1	38.98
F_3D_2	40.4	35.0	47.0	
F_3D_3	39.6	34.6	42.6	
F_3D_4	38.6	34.5	38.9	
F_3D_5	36.2	32.8	33.7	
Control				
	$F_1C_0D_0$	-	-	46.2
	$F_2C_0D_0$	-	-	47.2
	$F_3C_0D_0$	-	-	46.3
Storage methods mean (C)	42.24	36.48	47.29	

Aged pollen mean (D)	D_0	D_1	D_2	D_3	D_4	D_5
	46.57	46.08	43.98	41.79	39.95	38.23

	F	C	D	F x C x D
SEm	0.29	0.29	0.39	1.16
CD (5%)	0.58	0.58	0.76	2.27

C_1, Moist earthen pot; C_2, Refrigerator; C_3, Earthen pot surrounded with moist sand

F_1, Filler material; F_2, Filler material : borax; F_3, Filler material: borax : sucrose

D_1, One day; D_2, Two days; D_3, Three days; D_4, Four days; D_5, Five days (Age of pollen)

maximum in pollen-grains without blending of filler material stored under refrigerator for one day ($F_1C_2D_1$: 80.99%). The possible cause for decline in pollen viability with other storage methods or with increased storage period may be due to deficiency in respiratory substances, inactivation of enzymes and growth hormones as observed by Shivanna and Johri [9]. Pfahler and Linskens [11] reported that effect of pollen-grains viability during storage is influenced by internal alteration of the amino acids present in the pollen.

The pollen-grains stored in the refrigerator recorded higher filled seeds (265.62) compared to other treatments. This can be attributed to low temperatures of 4ºC and 75% relative humidity prevailing in this method. The present findings were in agreement with the results of Pokhriyal and Mangth [7] and Patil and Goud [8] in sorghum. The seed set (%) results of refrigerated pollen are in accordance with findings of Jones and Newell [12] in buffalo grass and corn. The pollen of buffalo grass stored at 40ºC with 90% relative humidity remained viable up to ninth day and good number of filled seeds were noticed up to third day, whereas in corn, seed setting (%) under similar environment was recorded up to seventh day. The lowest seed setting (%) observed after fourth day in buffalo grass and eighth day in corn may be explained by the fact that extremely high temperatures and low relative humidity prevailed in later period of storage.

Seed setting gradually decreased with increase in period of stigma starvation from same day (98.00%) or one day starvation (96.00%) to 14 days starvation (4.00%). The seed setting drastically reduced from 8th day starvation onwards. A similar decrease in seed set with increased starvation was also noticed in sorghum which may be due to decrease in non-structural carbohydrates level in floral tissue which influences the seed set [10].

The pollen stored in earthen pot surrounded with moist sand was adjudged as next best treatment after refrigerator treatment, since the pollen viability could be maintained, though in a lower scale up to fifth day. Appreciable seed set (71.69%) was noticed up to three days only, later due to the decline in pollen viability, the seed set (39.90%) was also reduced.

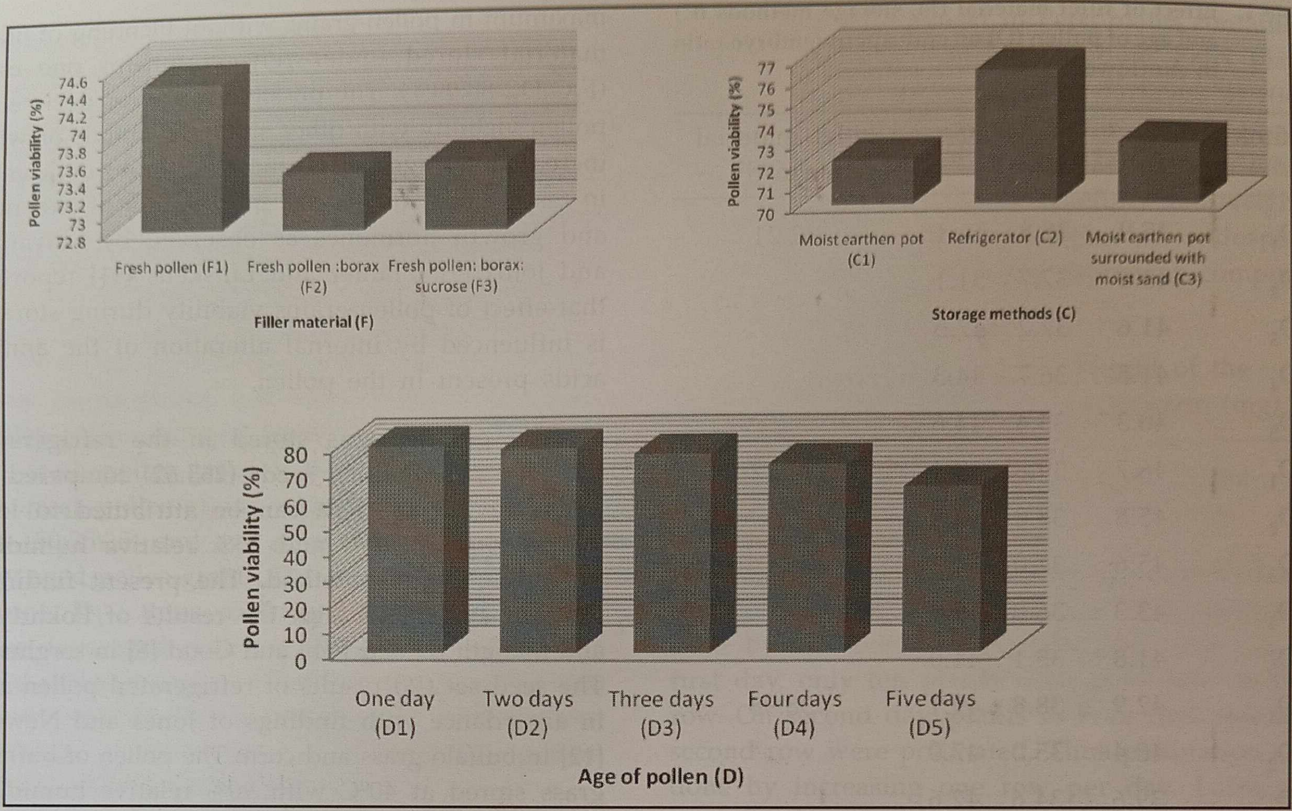

Fig. 1. Effect of filler material (F), storage methods (C) and age of pollen (D) on pollen viability (%) in sunflower

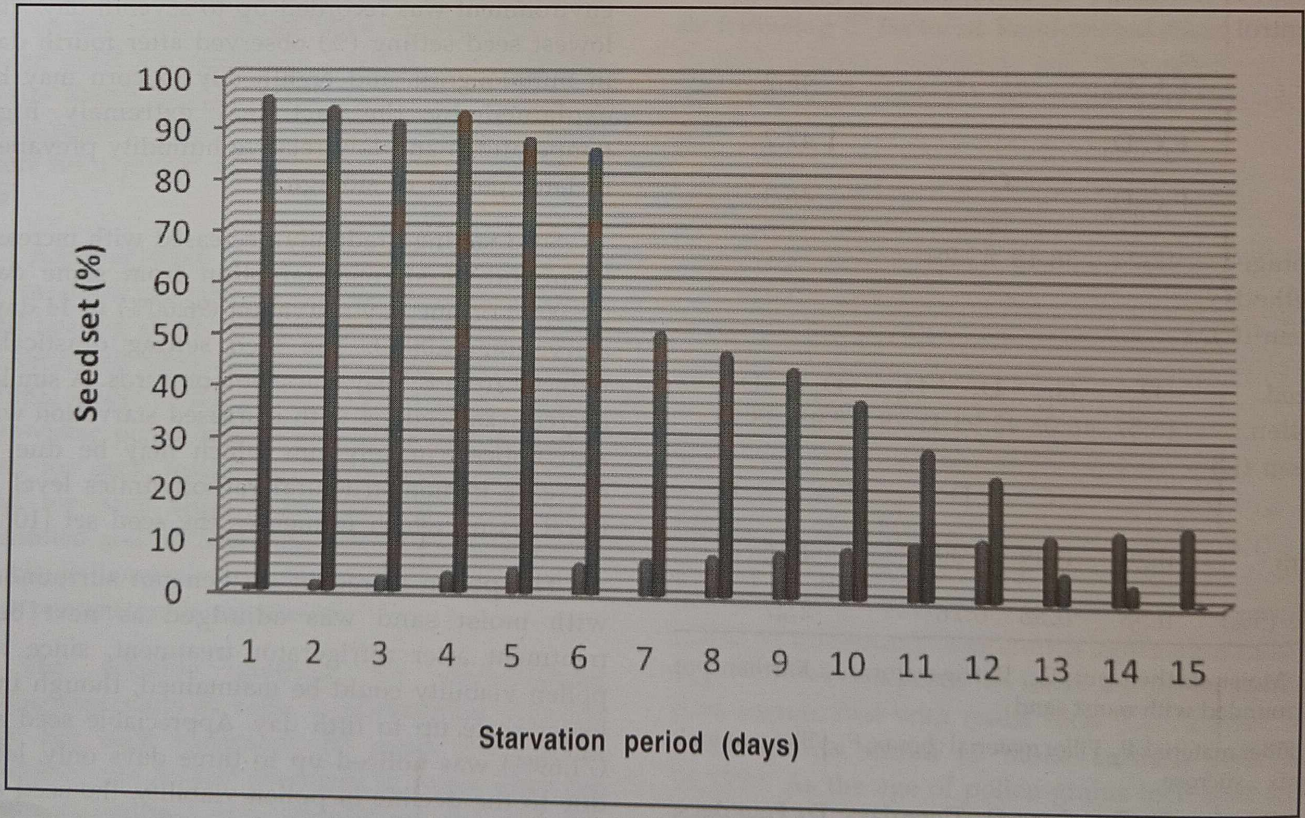

Fig. 2. Effect of starvation period (days) on stigma receptivity in sunflower

Fresh pollen-grains blended with borax (F_2: 44.22) were found superior in relation to endosperm/embryo ratio followed by fresh pollen-grains without blending of filler material (F_1: 42.81) and fresh pollen-grain blended with borax + sucrose (F_3: 38.98). Pollen-grain stored in earthen pot surrounded with moist sand had greater influence on endosperm/embryo ratio (C_3: 47.29). Maximum endosperm/embryo ratio was observed in pollen blended with borax (75:25) stored under earthen pot surrounded with moist sand for one day ($F_2C_3D_1$: 57.4). Further, it is interesting to note that endosperm/embryo ratio gradually decreased with increased age of pollen (D_1: 46.08 to D_5: 38.23).

The pollen-grains stored in moist earthen pot gave satisfactory results up to three days only. The reasons may be due to continuous wetness of cloth, temperatures were maintained around 20ºC with 60-70% relative humidity. These results were comparable to the findings of Beasley and Yermanous [13] in Simmondsia wherein the pollen stored at 24ºC showed considerable viability up to one week and reduced to zero level of viability by two months storage. The results of the present investigations were similar to Hoekstra and Bruinsma [14] in chrysanthemum (Trinucleate pollen) wherein better viability was noticed up to seven days at 20ºC with 60% relative humidity.

Pollen-grains viability gradually decreased with age of pollen-grains starting from same day, first day to fifth day from pollen collection, as adjudged by seed set. Pollen-grains stored in refrigerator had shown better viability to that of moist earthen pots and earthen pots surrounded with moist sand. Alternatively farmers in rural areas can store excess pollen-grains under refrigerator and can be further used for hand pollination for enhanced seed setting and increased seed yield.

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