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Studies on Pollen Storage Conditions and Period on Seed Yield and Quality of Sunflower (*Helianthus annuus* L.) Hybrid KBSH-1

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ABSTRACT To know the influence of pollen storage conditions and period on seed set, seed yield and quality of sunflower hybrid KBSH-1conducted at STR Unit, NSP, UAS Bangalore in two seasons *Kharif* 2001 and *Rabi* 2001-02. Pollen stored under refrigerated condition for 24 h gave highest seed set and seed yield followed by pollen stored in an earthen pot placed in a tray containing water. The seed quality in terms of 100 seed weight and vigour index were found significant due to storage period and conditions, while germination was non significant.

Keywords: Sunflower, pollen viability, seed set

The practice of storing pollen goes back to the early eras of human civilization. A systematic study on the storage of pollen was initiated towards the end of the 19th century, when the longevity of pollen of more than 80 spp stored under air-dry conditions was investigated [1, 2]. Storage of viable pollen for longer period is essential for pollination, as it eliminates the laborious problems encountered when parent flowers at different times, pollen can be collected and stored successfully for pollination during hybrid seed production. For short and medium term storage, temperatures ranging from 5 to –20°C depending upon crops, with low relative humidity have been found most suitable [3]. The storage of pollen under controlled temperature and humidity condition is being practiced, however, ideal temperature and humidity for sunflower pollen storage has not been registered. Presently, the hybrid seed production is normally organized in rural areas where no facilities exist to regulate temperature and humidity. However, refrigerator, earthen pot with water at bottom is being used to store the pollen grains to maintain temperature and relative humidity to some extent. The information on different methods of storage of pollen grains in sunflower is scanty. Hence, the study was under taken.

MATERIAL AND METHODS

Two separate blocks of A and R lines of sunflower hybrid KBSH-1 were sown during *Kharif* 2001 and *Rabi* 2001-02 at STR Unit, NSP, G.K.V.K., Bangalore. Fresh pollen from 6D-1 was collected at 9.00 a.m and stored under different conditions viz., Pollen stored in refrigerator (T_1), Pollen stored in earthen pot and placed in a tray containing water (T_2), Pollen stored in earthen pot (T3), Pollen stored in plastic container (T_4) for 24, 48 and 72 h. The pollination was carried out every day till the completion of flowering in female line using the stored pollens with the help of camel hairbrush. At maturity, each pollinated capitulum was harvested and threshed separately. The observations on seed set per cent, seed yield, seed size, seed volume, germination, seedling length and vigour index was recorded [5] and analysed [6].

RESULTS AND DISCUSSION

Seed set percentage and seed yield per hectare were significantly high for pollen stored under refrigerated condition and stored in earthen pot placed in a tray containing water as compared to stored in plastic container. The seed set per cent was significantly higher when pollens used for pollination

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Table 1. Influence of pollen storage conditions and period on seed set per cent, pollen viability and seed yield in sunflower

Treatments	Seed set (%)			Pollen viability (%) by acetocarmine test			Seed yield (q ha^{-1})		
	Kharif 2001	Rabi 2001-02	Mean	Kharif 2001	Rabi 2001-02	Mean	Kharif 2001	Rabi 2001-02	Mean
T_1	76.4	84.2	80.3	78	78	78	4.2	6.4	5.3
T_2	75.3	79.5	80.3	77	75	77	3.9	5.5	4.7
T_3	70.0	71.4	70.7	75	72	73	3.1	4.3	3.7
T_4	65.4	63.8	64.6	61	59	60	2.7	2.9	2.8
SEm±	1.8	1.4		1.7	2.1		0.11	0.26	
CD (0.05P)	5.4	4.5		5.1	6.3		0.31	0.79	
D_1	80.2	77.3	78.9	75	76	75	4.2	5.0	4.6
D_2	70.2	74.0	72.7	73	71	72	3.4	4.7	4.1
D_3	64.9	72.8	70.3	71	65	68	3.0	4.6	3.8
SEm±	1.4	1.2		1.4	1.8		0.09	0.22	
CD (0.05P)	3.9	5.3		4.3	5.5		0.27	0.66	
T_1D_1	80.8	84.7	82.7	80	84	82	5.3	7.1	6.2
T_1D_2	75.2	85.0	80.1	78	77	77	3.5	6.1	4.8
T_1D_3	73.3	82.9	78.1	78	75	76	3.1	5.9	4.5
T_2D_1	79.1	84.7	82.4	80	81	80	4.6	5.6	5.1
T_2D_2	74.9	80.6	80.2	78	76	77	4.6	5.8	5.2
T_2D_3	72.0	73.2	78.5	79	70	74	3.3	5.1	4.2
T_3D_1	80.7	76.0	78.3	77	79	78	3.5	4.5	4.0
T_3D_2	67.3	67.7	67.5	77	76	76	2.8	3.7	3.3
T_3D_3	62.0	70.5	66.2	71	61	66	3.0	4.6	3.8
T_4D_1	80.3	64.0	72.1	63	63	63	3.4	3.0	3.2
T_4D_2	63.4	62.8	63.1	62	58	60	2.5	3.0	2.8
T_4D_3	52.6	64.7	58.6	59	56	57	2.3	2.7	2.5
SEm±	2.2	2.3		2.9	3.4		0.19	0.45	
CD (0.05P)	6.6	7.0		9.0	10.7		0.55	1.34	
CV (%)	8.7	6.7		8.9	11.7		12.78	21.42	

T_1= Pollen stored in refrigerator
T_2= Pollen stored in earthen pot and placed in a tray containing water
T_3= Pollen stored in earthen pot
T_4= Pollen stored in plastic container

D_1= Pollen storage for 24 h
D_2= Pollen storage for 48 h
D_3= Pollen storage for 72 h

from refrigerator storage (76.4%) followed by pollen stored in earthen pot placed in a tray containing water (75.3%) and highest seed yield of 4.2 and 6.4 q ha^{-1} in *Kharif* 2001 and *Rabi* 2001-02 respectively. This is due to availability of sufficient viable pollen during pollination, which was observed by acetocarmine test (Table 1). The viability of pollen was very well maintained under refrigerator condition and pollen stored in earthen pot placed in a tray containing water because of maintenance of low temperature which is ideal for pollen storage. Similar observations were noticed in pearl millet [7], rose [9] and citrus [10].

Among different duration of pollen storage, the pollen grains stored for 24 h recorded significantly highest seed set per cent over 48 and 72 h of storage.

Table 2. Mean temperature and relative humidity recorded during pollen storage

Containers	*Kharif* 2001		*Rabi* 2001-02	
	Temp. (°C)	R.H. (%)	Temp. (°C)	R.H. (%)
T_1= Pollen stored in refrigerator	6	55	7	53
T_2= Pollen stored in earthen pot and placed in a tray containing water	12	85	14	64
T_3= Pollen stored in earthen pot	23	82	25	57
T_4= Pollen stored in plastic container	26	78	27	54

Table 3. Influence of pollen storage conditions and period on seed number, 100 seed weight and seed size in sunflower

Treatments	Number of filled Seeds per capitulum			100 seed weight (g)			Seed size (cm^3)		
	Kharif 2001	Rabi 2001-02	Mean	Kharif 2001	Rabi 2001-02	Mean	Kharif 2001	Rabi 2001-02	Mean
T_1	340	350	345	3.12	3.04	3.08	0.53	0.54	0.53
T_2	319	302	310	3.19	3.13	3.16	0.59	0.55	0.57
T_3	219	202	210	3.32	3.49	3.41	0.60	0.56	0.58
T_4	283	155	219	3.64	3.56	3.60	0.62	0.58	0.60
SEm±	11.0	13.8		0.13	0.09		0.02	0.01	
CD (0.05P)	32.7	41.4		0.40	0.30		0.07	0.03	
D_1	373	270	321	3.08	3.22	3.15	0.57	0.56	0.56
D_2	296	247	271	3.17	3.15	3.16	0.58	0.56	0.57
D_3	238	239	238	3.43	3.52	3.48	0.60	0.59	0.59
SEm±	19.2	12.0		0.11	0.08		0.02	0.01	
CD (0.05P)	57.2	36.7		0.35	0.25		0.06	0.03	
T_1D_1	429	385	407	2.79	3.14	2.97	0.53	0.53	0.53
T_1D_2	412	335	328	3.10	3.18	3.14	0.53	0.54	0.53
T_1D_3	321	331	300	3.17	3.23	3.20	0.53	0.54	0.53
T_2D_1	270	331	371	2.95	3.32	3.14	0.58	0.57	0.57
T_2D_2	305	289	297	3.26	3.29	3.28	0.57	0.56	0.56
T_2D_3	240	287	263	3.29	3.37	3.33	0.56	0.57	0.56
T_3D_1	339	199	269	3.23	3.05	3.14	0.58	0.56	0.57
T_3D_2	280	213	246	3.13	3.18	3.16	0.56	0.56	0.56
T_3D_3	256	194	225	3.29	3.33	3.31	0.54	0.55	0.54
T_4D_1	315	166	240	3.40	3.52	3.46	0.58	0.58	0.58
T_4D_2	279	153	216	3.42	3.67	3.55	0.64	0.60	0.62
T_4D_3	186	146	166	3.59	3.62	3.61	0.66	0.61	0.63
SEm±	19.2	24.0		0.23	0.16		0.04	0.02	
CD (0.05P)	57.6	72.8		0.71	0.49		0.13	0.07	
CV (%)	14.0	21.2		16.9	10.8		4.20	3.60	

T_1= Pollen stored in refrigerator
T_2= Pollen stored in earthen pot and placed in a tray containing water
T_3= Pollen stored in earthen pot
T_4= Pollen stored in plastic container

D_1= Pollen storage for 24 h
D_2= Pollen storage for 48 h
D_3= Pollen storage for 72 h

Table 4. Influence of pollen storage conditions and period on germination and vigour index in sunflower

Treatment	Germination (%)			Vigour index		
	Kharif 2001	Rabi 2001-02	Mean	Kharif 2001	Rabi 2001-02	Mean
T_1	89.0	88.8	88.9	1426	1236	1331
T_2	89.3	88.4	88.8	1496	1327	1410
T_3	88.7	90.3	89.5	1732	1525	1628
T_4	90.6	91.2	90.9	1804	1746	1774
SEm±	0.84	1.20		92.1	93	
CD (0.05P)	NS	NS		277.1	280	
D_1	89.3	88.8	89.1	1508	1418	1463
D_2	88.8	89.4	89.1	1524	1568	1546
D_3	89.1	91.3	90.2	1762	1788	1775
SEm±	0.73	1.07		79.8	81.0	
CD (0.05P)	NS	NS		240	244	
T_1 D_1	90.0	88.6	89.3	1385	1451	1418
T_1D_2	88.0	87.0	87.5	1909	1275	1592
T_1 D_3	89.0	93.0	91.0	1904	1850	1877
T_2 D_1	89.0	87.3	88.2	1691	1283	1487
T_2 D_2	92.0	91.3	91.7	1483	1678	1580
T_2 D_3	87.0	86.6	86.8	1653	1969	1811
T_3 D_1	89.3	89.3	89.3	1544	1279	1411
T_3 D_2	88.7	90.0	89.6	1484	1047	1265
T_3 D_3	88.3	91.6	90.0	1588	1964	1776
T_4 D_1	91.0	90.0	90.5	1412	1863	1556
T_4 D_2	89.0	89.6	89.3	1250	1356	1420
T_4 D_3	92.0	94.0	93.0	1970	1862	1884
SEm±	1.46	2.15		159	114	
CD (0.05P)	NS	NS		478	344	
CV (%)	2.82	4.14		17.1	17.7	

T_1= Pollen stored in refrigerator
T_2= Pollen stored in earthen pot and placed in a tray containing water
T_3= Pollen stored in earthen pot
T_4= Pollen stored in plastic container

D_1= Pollen storage for 24 h
D_2= Pollen storage for 48 h
D_3= Pollen storage for 72 h

The reduction in seed set per cent at 48 and 72 h of pollen storage was due to reduction in viability of pollen because of aging, since pollen grains are very minute and sensitive, remain viable only for short period of time under ambient conditions [11]. Highest seed yield of 4.2 and 5.0 q ha^{-1} in *Kharif* 2001 and *Rabi* 2001-02 respectively was observed with 24 h of storage, while the per cent reduction was 28.5 and 8.6 in *Kharif* 2001 and *Rabi* 2001-02 respectively, when 72 h old pollen was used.

The interaction of pollen storage conditions and period on seed set percentage and seed yield per hectare were significant. Pollen stored under refrigerator condition and in earthen pot placed in a tray containing water for 24 h gave highest seed set per cent may be due to low temperature (<10°C

Table 5. Mean monthly meteorological data recorded during the crop growth period

Month	Rainfall (mm)	Temperature (°C)		Relative humidity (%)
		Maximum	Minimum	
MAY, 2001	5.0	32.9	21.2	62.5
JUNE	18.8	29.5	20.3	68.0
JULY	136.0	27.8	19.9	71.5
AUGUST	78.1	26.9	19.3	75.0
SEPTEMBER	347.6	28.2	19.6	73.5
OCTOBER	121.8	26.4	18.9	77.5
NOVEMBER	32.6	26.7	17.4	76.5
DECEMBER	13.8	25.3	14.3	75.0
JANUARY, 2002	-	27.3	15.0	70.5
FEBRUARY	-	28.7	15.4	64.5
MARCH	-	32.4	17.6	54.0
APRIL	1.8	34.3	20.7	56.0
MAY	133.6	32.1	21.0	61.0

and <12°C) in the containers. Further, the difference in RH between the pollen stored in earthen pot placed in water and pollen stored in earthen pot alone was only 3 to 7 per cent (Table 2), while RH in refrigerated condition is 53-55 per cent during the pollen storage. The pollen stored in earthen pot alone and under ambient condition for 24, 48 and 72 h recorded reduced pollen viability reflected in poor seed set. Similar reduction in pollen viability under ambient condition during long-term storage was observed in ornamental plants [12] and black berry [13].

Seed set per cent was highest with 80.8 and 84.7 per cent in *Kharif* 2001 and *Rabi* 2001-02 respectively when pollens were stored under refrigerator conditions for 24h. Under refrigerator condition low temperature (<10°C) was maintained which favours to prolong the viability of pollens. Where as pollen stored under ambient condition for 72 h the per cent reduction in seed set was highest (34.9 and 23.6 in *Kharif* 2001 and *Rabi* 2001-02 respectively). The highest seed per hectare (5.3 and 7.1 q ha^{-1} in *Kharif* 2001 and *Rabi* 2001-02 respectively) in the present study are in accordance with the findings in pearl millet [7].

The seed quality differed significantly due to different pollen storage conditions and period. Highest seed number per capitulum (340 & 350) was recorded (Table 3) when pollination was done by using pollens stored under refrigerator condition in *Kharif* 2001 and *Rabi* 2001-02 respectively, followed by (319 & 302) pollen stored in earthen pot placed in a tray containing water in *Kharif* 2001 and *Rabi* 2001-02 respectively because of highest seed set per cent under these conditions of storage. Highest 100 seed weight (3.64 g in *Kharif* 2001 & 3.56 g in *Rabi* 2001-02) was recorded under ambient storage over refrigerator and pollen placed in earthen pot kept in a tray containing water which may be due to more photosynthates available to the limited number of seeds per capitulum [14 and 15]. This leads to higher seed size of 0.62 & 0.58 cm^3 in *Kharif* 2001 and *Rabi* 2001-02 respectively. Although the seed germination (Table 4) was non significant due to pollen storage methods and periods, the highest seed germination per cent was recorded when pollens were stored under ambient condition (90.6 & 91.2) over refrigerated condition. Assessment of vigour index is also relevant since germination alone may be incomplete assessment of seed quality. In the present study the vigour index computed by multiplying germination percentage with seedling length was highly significant due to ambient pollen storage by recording 1804 and 1746 in *Kharif* 2001 and *Rabi* 2001-02 respectively. Similar results were noticed [16] in regal lily.

Among duration of storage, 24 h storage periods gave highest seed number per capitulum (373 in *Kharif* 2001 and 270 in *Rabi* 2001-02). The highest 100 seed weight (3.43 & 3.52 g) and seed size of 0.60 and 0.59 cm^3 was observed when pollen stored for 72 h and used for pollination. The less number of

viable pollen in case of pollen stored for 72 h leads to low seed set resulting in less number of seeds per capitulum which helps in efficient utilization of available nutrients by the limited number of seeds. Highest seed germination (89.1% in *Kharif* 2001 and 91.3 in *Rabi* 2001-02) was observed with healthy seedling growth resulted in highest vigour index (1762 & 1788) when pollination was done by using pollens stored for 72 h.

Among the different treatment combinations highest seed size (0.63 cm^3), 100 seed weight (3.61 g) and germination (93 %) was noticed when pollens were stored under ambient conditions for 72 h due to less number of seeds per capitulum since poor seed set. Similar observations were noticed [17] in sunflower.

The meteorological data during the crop growth period(s) is given in Table 5. The flowering in CMS-234A commenced between September 27 to October 5, 2001. The temperature during this period ranged from 19-28°C and RH from 74 to 78%. It is worthy to note that in T1 and T2, there was significant reduction in temperature (6-12°C) which helped in the improvement of pollen viability/seed set. Similarly, during rabi 2001-02 flowering commenced between February 6-14, 2002. During this period ambient temperature ranged from 15°C to 29°C while in T1 (7°C) and T2 (14°C) it was quite low influencing the pollen viability and seed set favourably.

It is concluded that during excess pollen production pollen grains can be stored in refrigerator or earthen pot placed in a tray containing water for a period of 72 h without affecting viability of pollen and these pollens can be used for pollination during hybrid seed production of KBSH-1.

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