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The Pharma Innovation

ISSN (E): 2277-7695
ISSN (P): 2349-8242
NAAS Rating: 5.23
TPI 2023; 12(6): 2505-2509

www.thepharmajournal.com
Received: 01-03-2023
Accepted: 07-05-2023

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Wide hybridization in sunflower to study backcross and selfed progenies

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Abstract
Sunflower (*Helianthus annuus* L.) is one of the important oilseed crop, with comparatively narrow genetic base. To broaden the genetic base wild species have been used through wide hybridization. Generally wide hybridization comes with linkage drags which contribute several undesirable traits to cultivated species. In the present study, comparative analysis of backcross (BC_2F_3) and selfed (BC_1F_4) progenies was carried out to know the extent of variability in desirable direction created in both the generation derived from wide hybridization. Results revealed that traits such as days to 50 percent flowering, days to maturity, head diameter, test weight, seed yield per plant exhibited more variability in backcross progenies as compared to selfed progenies. Hence backcrossing is an effective tool in early generation to derive stable inbred lines rather than selfing done.

Keywords: Sunflower, wide hybridization, backcrossing, selfing

Introduction
Sunflower (*Helianthus annuus* L.) is one of the important oilseed crops of India. Sunflower production continues to face challenges from both abiotic and biotic factors as well as from today's ever-changing market needs. However, the limited genetic variability in cultivated sunflower has slowed the future improvement of the crop and has placed the crop in a vulnerable position. The over- exploitation of a single CMS *PET1* cytoplasm and a few fertility restoration genes for worldwide sunflower production makes the crop extremely vulnerable with narrow genetic base. Diversity in CMS base and resistance to various diseases is strategically needed. Evaluation of wild species has provided information about useful genes for future sunflower improvement. However, there are still numerous genes in wild sunflower species yet to be identified and introgressed into cultivated sunflower (Jan *et al.*, 2006) [8]. Though crop improvement in sunflower has resulted in the development of many promising hybrids and populations, many biotic and abiotic stresses are still limiting the productivity. The assessment of *per se* utility of interspecific derived germplasm for their use in commercial plant breeding programs is an important prerequisite.

There is a need to broaden the genetic base of cultivar germplasm, break the yield stagnation, development of material for diverse location and situations and also for the introgression of specific characters from wild *Helianthus* species to cultivated species through pre-breeding programme. The backcross-derived inbred lines variability in terms of several distinct phenotypic characters not present in the cultivated species as a source of maintainer or restorers for different characters. (Sujatha *et al*., 2008) [15]. The process of creating variability in desirable direction can be achieved through wide hybridization and further selection of introgressed lines. The variability can be created by following several breeding methods *viz.*, hybridization, introduction, backcrossing and inter crossing between different species and genera. The present work was planned to compare and identify best breeding method *i.e.* Wheather backcrossing or selfing is prominent in generation advancement to create higher variability in desirable direction in pre-bred material of sunflower.

Material and Methods

The experiment was conducted during late *Kharif* 2021 at Main Agriculture Research Station, University of Agricultural Sciences, Raichur. The experimental material consisted of 24 introgressed family progenies of backcross and selfed generation along with parental CMS lines in which each entry was sown in two rows of 4 m length with spacing of 60 × 30 cm. introgressed families derived by crossing four wild accessions of *H. annuus* (ANN-61, ANN-98, ANN-19 and ANN-114) and three CMS lines (CMS 103B, CMS 104B and CMS 38B). Further BC_2F_3 generation was derived by backcrossing individual plants of BC_1F_3 to its respective cultivated genotype whereas the individual plants grown in BC_1F_3 were selfed to generate BC_1F_4. In further generation also while deriving backcross and selfed generation individual plant identity of different wild accession was maintained.

In the present investigation observations were recorded and analysed for seven traits *viz.*, plant height, head diameter, test weight, seed yield per plant, volume weight, days to 50 percent flowering and oil content (%).The data recorded were processed with statistical parameters *viz*., range, mean, standard error and coefficient of variation for all traits. The data was subjected to F-test or One way ANOVA to know the variability between the families and within the families of different crosses of backcross and selfed generation.

Results and Discussion

In present investigation, one way ANOVA was used to know the between family variance and within family variance for each trait with different backcross and selfed progenies. Four types of comparison were done for variance analysis. In first comparison backcross and selfed progenies were separately analyzed for each trait and each cross. While in second comparison backcross and selfed progenies were analyzed for each trait to know the variance between backcross and selfed progenies of each cross. Whereas, in third comparison, whole backcross (BC_2F_3) and selfed (BC_1F_4) progenies were analyzed separately to know variance among different crosses. In fourth comparison total population variance (includes both backcross and selfed progenies) was analyzed across different crosses.

In first comparison, significant difference was observed for both between family variance and within family variance of CMS-103B derived backcrosses and selfed progenies for the traits such as plant height and seed yield. However, some of the traits were shown significant difference only for selfed progenies of CMS-103B crosses *viz*., head diameter and test weight. While in CMS-104B crosses, plant height and seed yield recorded significant difference for both between family variance and within family variance of backcross and selfed progenies. Whereas, stem diameter and test weight recorded significant difference only in selfed progenies of CMS-104B crosses. While in CMS-38B crosses head diameter is the only trait showing significant difference of variance in both backcross and selfed progenies. However, three traits were shown significant difference of variance only in selfed progenies of CMS-38B cross *viz.*, plant height, test weight and seed yield (Table 1).

In second comparison, both backcross and selfed progenies of each cross were analyzed to know variance of between family and within family variance. All the crosses in CMS-103B and CMS-104B crosses, significant difference was observed for all the traits studied between backcross and selfed progenies. Whereas in CMS-38B crosses head diameter, plant height, days to 50 percent flowering, test weight, volume weight, yield and oil content revealed significant difference between backcross and selfed progenies (Table 2).

The backcross (BC_2F_3) and selfed (BC_1F_4) progenies comprising from all three 3 CMS lines were analyzed separately to know the variance between crosses and within crosses. In backcross progenies all the traits were shown significant difference except oil content *i.e.*, no difference was observed for oil content in backcross progenies across different crosses. While in selfed progenies head diameter and test weight recorded significant difference of variance and rest of the characters were shown non significance variance (Table 3).

In fourth comparison, total population variance (includes both backcross and selfed progenies) was analyzed among different crosses. Traits such as head diameter, test weight, volume weight and seed yield recorded significant difference of variance among different crosses irrespective of backcross and selfed progenies (Table 4).

Table 1: Analysis of variance for between and within families of backcross and selfed progenies derived from 103B, 104B and 38B in sunflower

Source of variation			Plant height (MSS)		Head diameter (MSS)		Test weight (MSS)		Volume weight (MSS)		Yield (MSS)		Oil content (MSS)	
		df	BC	Self	BC	Self	BC	Self	BC	Self	BC	Self	BC	Self
103B	Between families	7	942.65**	3301.73**	17.00	18.29**	3.11	2.77**	1.29	0.46	184.68**	144.68**	16.44	8.90
	Within families	32	116.40**	377.40**	7.66	3.55**	1.56	0.73**	0.79	0.26	42.57**	21.49**	13.49	8.90
104B	Between families	7	1952.5**	3070.86**	5.85	4.30	1.98	2.77*	1.06	0.50	89.58**	31.11*	15.37	7.71
	Within families	32	272.18**	311.09**	5.85	2.86	1.25	0.92*	0.95	0.45	22.75**	10.87*	9.70	4.45
38B	Between families	7	464.91	2601.42**	16.32**	68.54**	2.20	2.76**	2.36	0.56	73.45	79.55**	11.77	5.44
	Within families	32	313.12	323.56**	3.92**	4.40**	1.85	0.79**	1.17	0.33	36.87	14.11**	10.38	2.68

* = 5 percent level of significance ** = 1 percent level of significance

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Table 2: Analysis of variance backcross Vs selfed progenies of sunflower

Source of variation		df	Plant height (MSS)	Head diameter (MSS)	DFF (MSS)	Test weight (MSS)	Volume weight (MSS)	Yield (MSS)	Oil content (MSS)
			BC vs Self	BC vs Self	BC vs Self	BC vs Self	BC vs Self	BC vs Self	BC vs Self
103B	Between BC and self-generation	1	4708.61**	1058.54**	38.64**	5.832**	1122.38**	47.95*	729.00**
	Within BC and self-generation	78	583.49**	7.77**	1.47**	0.59**	55.84**	740.74*	8.05**
104B	Between BC and self-generation	1	41961.96**	784.37**	38.22**	5.85**	447.97**	100.68**	248.06**
	Within BC and self-generation	78	690.10**	4.49**	1.32**	0.71**	24.62**	7.87**	4.52**
38B	Between BC and self-generation	1	27937.81**	651.11**	15.75**	5.75*	2471.97**	139.92**	333.06**
	Within BC and self-generation	78	536.38**	11.03**	1.53**	0.88*	34.64**	6.90**	5.49**

* = 5 percent level of significance ** = 1 percent level of significance

Table 3: Analysis of variance between and within crosses for backcross and selfed progenies in sunflower

Source of variation		df	Plant height (MSS)	Head diameter (MSS)	DFF (MSS)	Test weight (MSS)	Volume weight (MSS)	Yield (MSS)	Oil content (MSS)
Backcross generation (BC_2F_3)	Between crosses	2	3204.74**	139.02**	9.10**	11.56**	526.10**	0.12	85.16**
	Within cross	117	392.95**	7.11**	1.41**	0.94**	48.75**	11.79	5.31**
Selfed generation (BC_1F_4)	Between crosses	2	2188.58	154.53**	9.37**	0.65	74.03	10.14	0.79
	Within cross	117	813.70	8.41**	1.16**	0.54	27.99	4.39	6.73

* = 5 percent level of significance ** = 1 percent level of significance

Table 4: Analysis of in segregating progenies derived from backcrossing and selfing in sunflower

Source of variation		df	Plant height (MSS)	Head diameter (MSS)	DFF (MSS)	Test weight (MSS)	Volume weight (MSS)	Yield (MSS)	Oil content (MSS)
			BC + self	BC + self	BC + self	BC + self	BC + self	BC + self	BC + self
Both backcross and selfed generation	Between crosses	2	445.56	280.92**	9.56**	5.26**	395.06**	4.11	51.06
	Within cross	237	910.49	18.19**	1.81**	0.79**	54.94**	9.21	34.73

Table 5: Mean *per se* performance of parents and crosses for different characters of backcross and selfed progenies of sunflower

Traits	CMS lines	Parents	Backcross progenies (BC_2F_3)		Selfed progenies (BC_1F_4)	
		Mean	Range	Mean	Range	Mean
Plant height	103B	89	105-175	138.5	61.5-170	123.2
	104B	118	105-200	154.2	62-163	108.4
	38B	117	120-190	108.4	72-172	116.5
Head diameter	103B	14.2	11-25	15.8	4-14.3	8.5
	104B	14.2	9-18.5	12.7	3.3-11.6	6.5
	38B	15	11-21	16.3	4-18.5	10.4
Test weight	103B	3.17	2-7	4.10	1.2-5.3	2.96
	104B	3.78	2.3-6.4	4.13	1.1-5.1	2.75
	38B	5.87	2.3-7	4.56	1.4-5.8	3.67
Seed yield per plant	103B	14.28	16-41.6	21.2	5-30.8	13.7
	104B	14.75	6.7-30.8	15.8	5-20.4	11.1
	38B	20.15	9.25-32.8	22.8	5.7-29.8	11.6
Volume weight	103B	3.23	2.1-4.8	3.9	3.6-5.6	4.5
	104B	3.69	3-7.6	4.9	3-5.4	4.9
	38B	3.61	3.1-8	5.0	3.2-5.4	4.4
Oil content	103B	32.26	25.1-39.8	32.37	25.7-34.7	30.82
	104B	36.60	23.9-39.6	32.40	25.6-39.3	30.20
	38B	33.49	23.4-37.1	32.40	26.5-32.7	28.80
Days to 50 percent flowering	103B	60	51-60	54	63-71	67
	104B	58	57-62	60	65-72	68
	38B	59	56-61	59	56-71	68

The test weight of parental lines was an average of 5.2 grams. The crosses of backcross progenies showed intermediate test weight to both parental CMS lines and wild accessions *ie.* 4.1-4.5 g. while in selfed progenies recorded test weight were similar to their wild accessions (2.9-3.6 g) as reported by Vishnutej *et al.* (2016) [18], Higher variability was observed for test weight in backcross progenies of CMS-103B, CMS-104B and CMS-38B crosses in comparison their selfed progenies. Similar results were found with Dudhe (2012) [4] reported hybrid derive from cross between ARM 243 A × RHA-6D-1 showed intermediate seed weight in F1 hybrids. Hristova and Cherbadzi (2004) [4] studied interspecific crosses involving annual diploid *H. bolanderi* Gray, *H. neglectus* Heiser and *H. petiolaris* having intermediate, these results are also in accordance with the results obtained by Whelan and Dorrell (1980) [19] for 100 seed weight in the interspecific derived hybrid between *H. annuus* × *H. maximiliani* with an intermediate test weight.

Seed yield per plant is less in wild accessions as compared to cultivated lines (Prashanth *et al.*, 2014) [14] parental CMS lines had an average yield per plant was 16.39 g. Backcross progenies showed yield per plant in the range of 15.8-22.8 g. Backcross progenies had higher yield per plant than both the parents. There is transgressive segregants appeared for this trait in backcross progenies. Higher variability was found in CMS-103B, CMS-104B and CMS-38B crosses of backcross progenies compare to their respective selfed progenies. Results were on par with Nikolova and Christov (2004) [12] for F1 derived from cross between *H. annuus* × *H. laevigatas*, reported high seed yield in the interspecific F1 hybrids. While in selfed progenies yield were lesser than parental CMS lines (11.1-13.6 g). But yield is not a simple character as it involves contribution more than one characters towards it based on the different yield contributing characters. Similar results were observed with Aswini (2018) [1] reported that lesser seed yield in interspecific hybrids than female cultivated lines.

The oil content of the female parental CMS lines were recorded 34.11 percent, In case of backcross progenies oil content was estimated as 32.37 to 32.4 percent. While in selfed progenies oil content varied from 29.8 to 30.8 percent. Backcross progenies CMS-103B and CMS-38B crosses exhibited higher variability for oil content as compare to their selfed progenies. While in CMS-104B crosses both backcross and selfed progenies varied in a similar fashion. Comparatively backcross progenies had higher oil content than selfed progenies. This indicates the possibility of improving hybrid performance in backcross progenies more prominent than selfed progenies through further breeding and selection process in oil content trait. The results were contradictory with the results obtained by Whelan (1978) [20] reported high oil content in the interspecific hybrids derived from cross between *H. annuus* and *H. maximiliani*. The performance of interspecific hybrids was either superior or nearer to their parents in respect of all yield contributing traits.

There is considerable difference in days to 50 percent flowering. The parental CMS lines flowered early in 59 days. The backcross progenies showed 50 percent flowering in the range of 54 to 60 days. While in selfed progenies days to 50 percent flowering recorded from 67-68 days which indicates intermediacy in flowering in comparison to both parents. With respect to flowering duration backcross progenies of CMS-103B, CMS-104B and CMS-38B crosses were early types as compare to their respective selfed progenies. The results were on par with Vishnutej *et al.* (2016) [18] interspecific crosses showed intermediacy in days to 50% flowering in comparison their parents. Meena *et al.* (2017) [9] reported similar results in interspecific cross between *H. annuus* × *H. argophyllus.* The cultivated species inbred ARM243B flowered early (48.5 days) while the *H. argophyllus* was late in flowering (79.4 days), the F_1 was intermediate and flowered in 74.6 days.103B crosses of backcross progenies showed early flowering than female parent these result were in accordance with Encheva and Christov (2006) [5] results showed that in the hybrid progenies of the interspecific cross *H. annuus* (hybrid Albena) × H. *salicifolius* recorded two to three days earlier flowering than that of the female parents. Results obtained by Atlagic (1996) [3] for days to 50 percent flower in the interspecific derived hybrid between *H. annuus* and *H. occidentalis* were late in flowering compared to the parent.

There exists much difference in case of days to maturity in between cultivated sunflower and wild annual diploid *H. argophyllus* (Meena *et al*., 2017) [9]. The parental CMS lines matured in 87 days. But backcross progenies were matured in 88-96 days which implies intermediate duration to both parents. While selfed progenies took 103-106 days for maturity which indicates the lateness of the crosses over female parent. Backcross progenies of CMS-103B, CMS-104B and CMS-38B crosses matured early as compare to their selfed progenies. These results are in accordance with Prashanth *et al.* (2014) [14] in which the interspecific hybrids (M-106 × OCC 52and M-106 × MAX 1631) were late (90.4 and 95.4 days) in maturity compared to than the female parent (76.7 days), Similar results were reported by Hristova *et al.* (2011) [7]. They reported that interspecific hybrids of *H. annuus* × *H. maximiliani* had more days to maturity. Nikolova and Christov (2004) [11] reported similar results of days to maturity in 110-125 days in interspecific cross between *H. annuus* L. line LHA-300 x *argophyllus* (E-091).

Conclusion

The mean performance and spectrum of genetic variation helps to identify superior crosses among backcross (BC_2F_3) and selfed progenies (BC_1F_4). The cross or families with high mean and variability could be effectively utilized to identify superior segregates. The study revealed that variability created in head diameter, test weight, seed yield per plant and days to 50 percent flowering were at desirable direction in backcross progenies *ie.*, the mean of all these traits were shown higher end of the range as compare to their selfed progenies. So that to create variability in these traits backcrossing is recommended as it reduce undesirable traits. The study also gave a clear demarcation between backcross and selfed progenies derived from wide hybridization indicating the possibility to develop more stable and promising lines through one or more of progenies of backcrossing rather than selfing for progenies advancement.

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