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Comparative Analysis of Backcross and Selfed Progenies in Sunflower (*Helianthus annuus* L.)

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ABSTRACT

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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 (BC₂F₃) and selfed (BC₁F₄) 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 per cent flowering, days to maturity, head diameter, test weight, seed yield per plant exhibited more variability in backcross progenies as compared to selfed progenies. Hence minimum of two generations of backcrossing is an effective in early generation to derive stable inbred lines rather than selfing alone in pre-breeding programmes of sunflower.

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). 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). 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.* whether backcrossing or selfing is prominent in generation advancement to create higher variability in desirable direction in pre-bred material of sunflower.

Materials and Methods

The experiment was conducted during late *Kharif* 2018 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 BC₂F₃ generation was derived by backcrossing individual plants of BC₁F₃ to its respective cultivated genotype whereas the individual plants grown in BC₁F₃ were selfed to generate BC₁F₄. 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 eleven traits *viz.*, number of leaves, plant height, head diameter, stem girth, test weight, seed yield per plant, volume weight, leaf length, days to 50 per cent flowering, days to maturity, 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 (BC₂F₃) and selfed (BC₁F₄) 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 103B derived backcrosses and selfed progenies for the traits such as number of leaves, plant height, leaf length and yield. However, some of the traits were shown significant difference only for selfed progenies of 103B crosses *viz.*, head diameter, stem diameter and test weight. While in 104B crosses, plant height, leaf length and seed yield were recorded significant difference for both between family variance and within family variance of backcross and selfed progenies. Traits like number of leaves, stem diameter and test weight were showed significant difference only in selfed progenies of 104B crosses. Whereas in 38B crosses head diameter is the only trait showed significant difference of variance in both backcross and selfed progenies.

However, four traits were shown significant difference of variance only in selfed progenies of 38B cross *viz.*, number of leaves, plant height, test weight and seed yield. Leaf length is the only character which expressed significant difference of variance in backcross progenies of 38B crosses (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. In 103B crosses, significant difference was observed for all the traits except leaf length *i.e.* there was no difference existing between backcross and selfed progenies of 103B crosses of Leaf length. While in 104B crosses also all traits were shown significant difference backcross and selfed progenies except stem diameter. Whereas in 38B crosses number of leaves, head diameter, plant height, days to 50 per cent flowering, days to maturity, test weight, volume weight, yield and oil content were revealed significant difference between

backcross and selfed progenies except stem diameter and leaf length (Table 2).

In third comparison, whole backcross (BC₂F₃) and selfed (BC₁F₄) progenies 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 *ie.*, No difference was observed for oil content trait in backcross progenies across different crosses. While in selfed progenies head diameter, stem diameter and test weight were recorded significant difference of variance and rest of the characters were shown non significance (Table 3).

In fourth comparison, total population variance (includes both backcross and selfed progenies) was analyzed among different crosses. Traits such as head diameter, stem diameter, test weight, volume weight and yield were revealed significant difference of variance among different crosses irrespective of backcross and selfed progenies. Remaining all traits were shown non-significant variance (Table 4).

A comparative summary of means and ranges of yield and yield attributing traits across different crosses of backcross and selfed progenies summarized in table 5.

Head diameter in cultivated sunflower produces larger head compared to wild annual diploid *H. argophyllus* (Vishnutej *et al.*, 2016). The cultivated lines had average head diameter was 14.4 cm. The backcross progenies showed head diameter of 12.7-15.8 cm Indicating dominance of parental CMS lines whereas selfed progenies recorded intermediate (8.5-10.4 cm) head diameter. Variability observed for head diameter was more in backcross progenies of 103B, 104B, 38B crosses as compared to their selfed progenies.

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		df	No of leaves (MSS)		Plant height (MSS)		Head diameter (MSS)		Stem diameter (MSS)		Test weight (MSS)	
			BC	Self	BC	Self	BC	Self	BC	Self	BC	Self
103B	Between families	7	159.48*	1860.8**	942.65**	3301.73**	17.00	18.29**	3.18	9.27**	3.11	2.77**
	Within families	32	53.67*	313.21**	116.40**	377.40**	7.66	3.55**	1.14	0.59**	1.56	0.73**
104B	Between families	7	96.32	981.36*	1952.5**	3070.86**	5.85	4.30	6.00**	2.37	1.98	2.77*
	Within families	32	49.91	314.7*	272.18**	311.09**	5.85	2.86	1.55**	1.22	1.25	0.92*
38B	Between families	7	44.22	1706.04**	464.91	2601.42**	16.32**	68.54**	1.34	3.06	2.20	2.76**
	Within families	32	26.82	228.65**	313.12	323.56**	3.92**	4.40**	1.27	2.17	1.85	0.79**

* = 5 per cent level of significance ** = 1 per cent level of significance

Contd...

Source of variation		df	Leaf length (MSS)		Volume weight (MSS)		Yield (MSS)		Oil content (MSS)	
			BC	Self	BC	Self	BC	Self	BC	Self
103B	Between Families	7	35.80**	41.49**	1.29	0.46	184.68**	144.68**	16.44	8.90
	Within Families	32	9.42**	8.69**	0.79	0.26	42.57**	21.49**	13.49	8.90
104B	Between Families	7	49.44**	50.45**	1.06	0.50	89.58**	31.11*	15.37	7.71
	Within Families	32	8.97**	5.51**	0.95	0.45	22.75**	10.87*	9.70	4.45
38B	Between Families	7	24.97*	22.57	2.36	0.56	73.45	79.55**	11.77	5.44
	Within Families	32	9.03*	13.28	1.17	0.33	36.87	14.11**	10.38	2.68

* = 5 per cent level of significance ** = 1 per cent level of significance

Table.2 Analysis of variance backcross Vs selfed progenies of Sunflower

Source of variation		df	No of leaves (MSS)	Plant height (MSS)	Head diameter (MSS)	Stem diameter (MSS)	DFF (MSS)	DM (MSS)
			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	17052.8**	4708.61**	1058.54**	32.00**	729.00**	1332.25**
	Within BC and self generation	78	331.82**	583.49**	7.77**	1.83**	8.05**	15.55**
104B	Between BC and self generation	1	6462.01**	41961.96**	784.37**	0.68	248.06**	529.00**
	Within BC and self generation	78	246.30**	690.10**	4.49**	1.89	4.52**	23.07**
38B	Between BC and self generation	1	6037.81**	27937.81**	651.11**	2.01	333.06**	132.25*
	Within BC and self generation	78	261.88**	536.38**	11.03**	1.81	5.49**	15.53*

* = 5 per cent level of significance ** = 1 per cent level of significance

Contd...

Source of variation		df	Leaf length (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
103B	Between BC and self generation	1	22.36	38.64**	5.832**	1122.38**	47.95*
	Within BC and self generation	78	14.37	1.47**	0.59**	55.84**	740.74*
104B	Between BC and self generation	1	139.12**	38.22**	5.85**	447.97**	100.68**
	Within BC and self generation	78	14.90**	1.32**	0.71**	24.62**	7.87**
38B	Between BC and self generation	1	10.22	15.75**	5.75*	2471.97**	139.92**
	Within BC and self generation	78	13.42	1.53**	0.88*	34.64**	6.90**

* = 5 per cent level of significance ** = 1 per cent level of significance

Table.3 Analysis of variance between and within crosses for backcross and selfed progenies in Sunflower

Source of variation		df	No of leaves (MSS)	Plant height (MSS)	Head diameter (MSS)	Stem diameter (MSS)	DFF (MSS)	DM (MSS)
Backcross generation (BC ₂ F ₃)	Between crosses	2	180.92*	3204.74**	139.02**	30.90**	85.16**	124.04**
	Within cross	117	53.6*	392.95**	7.11**	1.97**	5.31**	14.13**
Selfed generation (BC ₁ F ₄)	Between crosses	2	648.67	2188.58	154.53**	20.88**	0.79	43.62
	Within cross	117	486.56	813.70	8.41**	1.96**	6.73	21.97

Source of variation		df	Leaf length (MSS)	Test weight (MSS)	Volume weight (MSS)	Yield (MSS)	Oil content (MSS)
Backcross generation (BC ₂ F ₃)	Between crosses	2	70.91**	9.10**	11.56**	526.10**	0.12
	Within cross	117	14.09**	1.41**	0.94**	48.75**	11.79
Selfed generation (BC ₁ F ₄)	Between crosses	2	21.35	9.37**	0.65	74.03	10.14
	Within cross	117	14.37	1.16**	0.54	27.99	4.39

* = 5 per cent level of significance ** = 1 per cent level of significance

Table.4 Analysis of segregating progenies derived from backcrossing and selfing in Sunflower

Source of variation		df	No of leaves (MSS)	Plant height (MSS)	Head diameter (MSS)	Stem diameter (MSS)	DFF (MSS)	DM (MSS)
			BC + self	BC + self	BC + self	BC + self	BC + self	BC + self
Both backcross and selfed generation	Between crosses	2	298.58	445.56	280.92**	20.88**	51.06	11.08
	Within cross	237	398.77	910.49	18.19**	1.96**	34.73	61.15

Source of variation		df	Leaf length (MSS)	Test weight (MSS)	Volume weight (MSS)	Yield (MSS)	Oil content (MSS)
			BC + self	BC + self	BC + self	BC + self	BC + self
Both backcross and selfed generation	Between crosses	2	36.00	9.56**	5.26**	395.06**	4.11
	Within cross	237	14.77	1.81**	0.79**	54.94**	9.21

* = 5 per cent level of significance ** = 1 per cent level of significance

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

Traits	Crosses	Parents	Backcross progenies (BC ₂ F ₃)		Selfed progenies (BC ₁ F ₄)	
		Mean	Range	Mean	Range	Mean
Number of leaves	103B	24	22-58	36	22-114	66
	104B	30	27-56	41	26-116	59
	38B	34	28-53	38	14-95	56
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
Stem diameter	103B	6.2	4.4-9.5	6.5	4.1-9.8	7.8
	104B	5.3	3.5-11	6.3	4.1-8.6	6.1
	38B	5.5	4.2-9.2	6.4	4.2-11.5	6.7

Traits	Crosses	Parents	Backcross progenies (BC ₂ F ₃)		Selfed progenies (BC ₁ F ₄)	
		Mean	Range	Mean	Range	Mean
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
Leaf length	103B	15.6	15.5-32	21.6	12.5-31.2	20.5
	104B	16.8	15.3-33	21.7	12.4-26.5	19.1
	38B	16.9	12.5-26.5	19.4	14.6-30	20.1
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

Traits	Crosses	Parents	Backcross progenies (BC ₂ F ₃)		Selfed progenies (BC ₁ F ₄)	
		Mean	Range	Mean	Range	Mean
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 per cent 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
Days to maturity	103B	89	84-93	88	98-112	106
	104B	86	82-100	92	100-113	103
	38B	88	92-100	96	96-110	102

Similar results were line with Encheva and Christov (2006) showed intermediacy with regard to head diameter in interspecific cross between *H. annuus* (hybrid Albena) × *H. salicifolius* and also observed that positive transgression was present for diameter. Hristova *et al.*, (2011) reported intermediate type of head diameter in the F1 hybrid derived between *H. annuus* × *H. maximiliani*. Prabakaran and Sujatha (2004) reported intermediate head type for the interspecific hybrids developed from *H. annuus* × *H. simulans*.

The test weight of parental lines was an average of 5.2 grams. The 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), Higher variability was observed for test weight in backcross progenies of 103B, 104B and 38B crosses in comparison with their selfed progenies. Similar results were found with Dudhe (2012) reported hybrid derive from cross between ARM 243 A × RHA-6D-1 showed intermediate seed weight in F1 hybrids. Hristova and Cherbadi (2004) studied interspecific crosses involving annual diploid *H. bolanderi* Gray, *H. neglectus* Heiser and *H. petiolaris* having intermediate test weight, these results are also in accordance with the results obtained by Whelan and Dorrell (1980) 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) parental CMS lines had an average yield per plant of 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 103B, 104B and 38B crosses of backcross progenies compare to their respective selfed progenies. Results were on par with Nikolova and Christov (2004) for F1's 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) 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 per cent, In case of backcross progenies oil content was estimated as 32.37 to 32.4 per cent. While in selfed progenies oil content varied from 29.8 to 30.8 per cent. Backcross progenies 103B and 38B crosses exhibited higher variability for oil content as compare to their selfed progenies. While in 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 for oil content. The results were contradictory with the results obtained by Whelan (1978) where he 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 per cent flowering. The parental CMS lines

flowered early in 59 days. The backcross progenies showed 50 per cent flowering in the range of 54 to 60 days. While in selfed progenies days to 50 per cent flowering recorded from 67-68 days which indicates intermediacy in flowering in comparison to both parents. With respect to flowering duration backcross progenies of 103B, 104B and 38B crosses were early types as compare to their respective selfed progenies. The results were on par with Vishnutej *et al.*, (2016) interspecific crosses showed intermediacy in days to 50 % flowering in comparison their parents. Meena *et al.*, (2017) 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 F₁ was intermediate and flowered in 74.6 days. The 103B backcross progenies showed early flowering than female parent these result were in accordance with Encheva and Christov (2006) 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) for days to 50 per cent 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). 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 103B, 104B and 38B crosses matured early as compare to their selfed progenies. These results are in accordance

with Prashanth *et al.*, (2014) in which the interspecific hybrids (M-106 × OCC 52 and M-106 × MAX 1631) were late (90.4 and 95.4 days) in maturity compared to female parent (76.7 days), Similar results were reported by Hristova *et al.*, (2011). They reported that interspecific hybrids of *H. annuus* × *H. maximiliani* had more days to maturity. Nikolova and Christov (2004) reported similar results of days to maturity in 110-125 days in interspecific cross between *H. annuus* L. line LHA-300 x *H. argophyllus* (E-091).

In conclusion the mean performance and spectrum of genetic variation helps to identify superior crosses among backcross (BC₂F₃) and selfed progenies (BC₁F₄). 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, days to 50 per cent flowering and days to maturity 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. Earlier reports also confirmed that these traits were utilized for sunflower crop improvement by the earlier findings of Nikolova and christov(2004), Encheva and Christov (2006), Hristova *et al.*, (2011), Dudhe (2012), Prashanth *et al.*, (2014), Vishnutej *et al.*, (2016) and Meena *et al.*, (2017) in pre-breeding programmes. So that to create variability in these traits backcrossing is recommended as it reduces undesirable linkage drag rapidly as compared to advancement through selfing. It 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 two generation of backcrossing at

early generation rather than progenies advancement only through selfing in sunflower pre-breeding programme.

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