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Morphological and molecular characterization of interspecific cross between cultivated Sunflower (*Helianthus annuus* L. with wild annual diploid *H. argophyllus*.)

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Abstract
Sunflower breeding has reached a plateau for a number of important agronomic traits. The major limiting factor for further improvements of the genetic potentials for seed yield and oil quality is the susceptibility of the sunflower to a large number of pathogens. The interspecific hybrids produced from crosses between cultivated sunflowers (*H. annuus* L.)(2n = 34) and wild annual diploid *H. argophyllus* Acc No. 1317 (2n=34) was studied. The rate of cross-compatibility was good. The crossed plants had an annual growth cycle, branched stem and different anthocyanin coloration, but also showed morphological similarity with cultivated sunflower for several quantitative characters. The hybridity of cross was confirmed through SSR marker.

Key words: Diploid, *Helianthus argophyllus*, interspecific hybridization, morphological characteristics and SSR marker.

Introduction

Studies in the field of population genetics have shown that the genetic variability of the cultivated sunflower had been drastically narrowed. Molecular data on the origin and development of the cultivated sunflower are alarming, indicating that the possibility for further evolution of this economically important crop is limited (Rieseberg and Seiler, 1990). Wild *Helianthus* species constitute the basic genetic stock from which cultivated sunflower originated. The diverse species represent considerable genetic variability which can be utilized for the improvement of cultivated sunflower. The morphological variations in the interspecific crossed derivatives will help us to study barriers involved in crossing and find out diversified CMS source and source for disease and pest resistance along with abiotic tolerance and some agronomically important traits.

Material and Methods:

An interspecific cross was derived by involving an cultivated sunflower LSF-8 genotype (*H. annuus*) as female parent and an wild diploid species Acc. No 1317(H. argophyllus) as male parent. The investigation encompassed during the period 2013 – 2014. Hand emasculation was practiced to induce male sterility in cultivated species and artificial pollination was done by using pollens of *H. argophyllus*. Observations were recorded for various morphological characters on parents and interspecific cross. Hypocotyl anthocyanin pigmentation, days to 50% flowering, leaf size(cm), leaf colour, leaf hairiness, stem coloration, stem girth (cm), stem hairiness, number of leaves per plant, disc floret colour, head diameter (cm), head shape, days to maturity, plant height (cm) , plant type (branching/ non branching), number of branches, seed length (cm), test weight (g) and seed colour. Molecular characterization was done to confirm the hybrity of interspecific cross by using 45 SSR primers were used.

Results and discussion:

H. annuus and *H. argophyllus* are annual diploid species (2n = 34) and differ significantly for morphological traits such as hypocotyl anthocyanin pigmentation, days to 50% flowering, leaf size(cm), leaf colour, leaf hairiness, stem colouration, stem girth (cm), stem hairiness, number of leaves per plant, disc floret colour, head diameter (cm), head shape, days to maturity, plant height (cm) , plant type (branching/ non branching), number of branches, seed length (cm), test weight (g) and seed colour in spite of the differences in both the species, both species are easily crossable. In the present study cultivated sunflower LSF-8 was used as female parent and wild annual diploid *H. arogophyllus* was used as pollen donor. Hybridization was successful as it was found that there was good seed set in the female parent. Pollen fertility was studied on both the parents as the well as the cross and it was found that 92.48 per cent of pollen fertility in cultivated female and 91.24 in wild male parent. However the pollen fertility of cross was 87.15 per cent

indicating the good cross compatibility between *H. annuus* L. and wild annual diploid *H. arogophyllus*.

Interspecific hybrid, plants exhibited intermediate characters of either parent, with a completely new phenotype. This large genetic variability provides the basis necessary for successful plant breeding. (Encheva and Christov, 2006).

The list of characters recorded is presented in Table 1. The hypocotyl pigmentation differed in both the parents as it was absent in cultivated sunflower LSF-8, and pink pigmentation was present in wild annual diploid *H. argophyllus*. The cross showed the character as that of wild parent.

There is considerable difference in days to 50 per cent flowering. The cultivated sunflower LSF-8 showed early in flowering by 56 days and wild annual diploid *H. argophyllus* took 75 days to flowering. While the interspecific crossed plants showed intermediacy in flowering with mean 61days for 50 per cent flowering. Nikolova and Christov (2004) reported similar results of 50 per cent flowering in 84- 95 days in interspecific cross between *H. annuus* L. line LHA-300 x *H. argophyllus* (E-091).

The leaf size was medium in case of LSF-8 while in case of H. argophyllus was small but the cross had medium sized leaves. Valkova and Christov (2004) reported that the leaves had an intermediate type of inheritance in their study of interspecific cross obtained between wild *H. annuus* L. and several cultivated lines. Their results indicated that leaf size varied from short for the crosses 2607A x MXO-1 and 2607A x E-114, and medium for the cross 2607A x E-167 to large for the crosses 6075A x E-114 and 6075A x E-167. Encheva and Christov (2006) reported that data for the hybrids of the selected plants showed intermediacy with regard to the indices for leaf size in interspecific cross between *H. annuus* (hybrid Albena) × *H. salicifolius*. Hristova and Cherbadzi (2004) observed contradictory results in interspecific crosses involving annual diploid *H. bolanderi* Gray, *H. neglectus* Heiser and *H. petiolaris* that leaf size was smaller than cultivated parent.

The leaf colour of cultivated sunflower LSF-8 was dark green compared to wild annual diploid *H. argophyllus* which was ashy green but the cross exhibited leaf colour which was more towards female parent with light green colour indicating intermediacy. Encheva and Christov (2006) reported that cross possessed dark green leaves similar to female parent in interspecific cross between *H. annuus* (hybrid Albena) × *H. salicifolius*. In contrast Valkova and Christov (2004) observed dark green leaves in crossed plants involving wild *H. annuus* L. E-114, E-167 as male parent.

The leaf hairiness was absent in cultivated sunflower LSF-8 while dense pubescence was found in *H. argophyllus* but the cross had an intermediate hairiness. Encheva and Christov (2006) reported that data for the hybrids of the selected plants showed intermediacy with regard to the indices for leaf hairiness in interspecific cross between *H. annuus* (hybrid Albena) × *H. salicifolius* (Pic 2).

Stem colouration was green in case of cultivated sunflower LSF-8 and in wild annual diploid *H. argophyllus* the stem colouration was light green with dark green stripes the cross exhibited light green with dark green stripes as that of male parent which was dominantly from pollen parent. Valkova and Christov (2004) observed stem colour similar that of wild in crossed plants involving wild *H. annuus* L. E-114, E-167 as male parent.

The stem girth recorded in cultivated sunflower LSF-8 was lower (5 cm) while wild annual diploid *H. argophyllus* had stem girth of 8 cm. The cross showed mean stem girth of 8.2 cm. This character was vigorous than both the parents. Hristova and Cherbadzi (2004) observed in interspecific crosses involving annual diploid *H. bolanderi* Gray, *H. neglectus* Heiser and *H. petiolaris* that the central stem girth was higher in the direct crosses, i.e., 7 to 19 cm. Encheva and Christov (2006) reports were contradicting showing intermediacy with regard to stem diameter/girth in interspecific cross between *H. annuus* (hybrid Albena) × *H. salicifolius*.

The stem hairiness in cultivated sunflower LSF-8 was very sparse compared to wild annual diploid *H. argophyllus* which had dense hairs on its stem. Intermediate sparse amount of hairs were witnessed in the cross. Hristova and Cherbadzi (2004) observed in interspecific crosses involving annual diploid *H. bolanderi* Gray, *H. neglectus* Heiser and *H. petiolaris* has coarse hairs on the stem; these results are in contrast to results obtained in this study. Valkova and Christov (2004) observed stem covered with short, sharp hairs in their interspecific cross between cultivated and wild *H. annuus* L. E-114, E-167.

Number of leaves per plant varied on an average of 27 leaves recorded in case of cultivated sunflower *H. annuus* variety LSF-8, and 62 leaves in case of wild annual diploid *H. argophyllus* and the cross exhibited an average of 48 leaves. Encheva and Christov (2006) observed that there was negative transgression established for the number of leaves per plant, the investigated hybrid progenies of interspecific cross between *H. annuus* (hybrid

Albena) × *H. salicifolius* possessed mean arithmetic values lower than those of the two parents, i.e., the character was less expressed. Results of Valkova and Christov (2004) using wild *H. annuus* L. E-114, E-167 indicated number of leaves was of intermediate type.

The disc floret colour observed in cultivated sunflower LSF-8 was yellow and that of wild annual diplod *H. argophyllus* was purple. While the disc floret observed in the interspecific cross exhibited purple colour similar to that of wild parent. Hristova and Cherbadzi (2004) reported in interspecific crosses involving annual diploid *H. bolanderi* Gray, *H. neglectus* Heiser and *H. petiolaris* purple colour disc florets a character resembling to wild parent. Valkova and Christov (2004) observed that the disk flowers were yellow and strong anthocyanin coloration of the stigma was observed for crosses, derived from cultivated sunflower and wild *H. annuus* L. E-114, E-167.

As regard to head diameter the cultivated sunflower LSF-8 produces larger head (15 cm)compared to wild annual diploid *H. argophyllus*. The LSF-8 average head diameter was 15 cm and the head diameter of *H. argophyllus* (4 cm). The interspecific cross showed an average of six cm head diameter indicating intermediate type of head diameter. 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 head diameter. Nikolova and Christov (2004) observed that depression for head diameter with respect to wild parent which was established in hybrid combinations *H. argophyllus* (E-007) x L.1234, and L.2607 x H. argophyllus (E-091) and L.HA-300 x H. argophyllus (E-091), indicating similar results.

The head shape for wild annual diploid *H. argophyllus* was flat and that of cultivated sunflower LSF-8 was convex, the interspecific cross inherited the female parent character showing convex head. Nikolova and Christov (2004) observed convex head in hybrid combinations *H. argophyllus* (E-007) x L.1234, and L.2607 x H. argophyllus (E-091) and L.HA-300 x H. argophyllus (E-091), indicating similar results.

There exists much difference in case of days to maturity in between cultivated sunflower *H. annuus* and wild annual diploid *H. argophyllus.* The female parent LSF-8 matured in 90 days and male parent *H. argophyllus* took 144 days. But interspecific cross showed intermediate in maturity (110 days). Nikolova and Christov (2004) reported similar results of days maturity in 110-125 days in interspecific cross between *H. annuus* L. line LHA-300 x *H. argophyllus* (E-091).

Two species widely differed for plant height. Wild annual diploid *H. argophyllus* was taller (280 cm) than cultivated sunflower LSF-8 (150 cm). The interspecific cross recorded 217 cm. Encheva and Christov (2006) observed that there was negative transgression established for the plant height, the investigated hybrid progenies of interspecific cross between *H. annuus* (hybrid Albena) × *H. salicifolius* possessed lower values than those of the two parents. Hristova and Cherbadzi (2004) reported in interspecific crosses involving annual diploid *H. bolanderi* Gray, *H. neglectus* Heiser and *H. petiolaris* that all plants reached a height of up to 150 to 160 cm, and individual plants from the cross *H. neglectus x H. annuus* being as tall as 195 cm. Nikolova and Christov (2004) observed that depression for plant height with respect to wild parent which was established in hybrid combinations *H. argophyllus* (E-007) x L.1234, and L.2607 x H. argophyllus (E-091) and L.HA-300 x H. argophyllus (E-091), indicating similar results.

Branching is a dominant character for most wild species. Wild annual diploid *H. argophyllus* showed dominant branching where cultivated sunflower LSF-8 had no branching. The cross had branching indicating dominance of character. And the type of branching was as same as in that of wild parent. Results from study of Valkova and Christov (2004) using wild *H. annuus* L. E-114, E-167 indicated basal branching of plants from crosses 6075A x E-114 and 6075A x E-167 was observed and other crosses were distinguished by full branching.

Number of branches recorded in wild annual diploid *H. argophyllus* was 21 while branching was absent in cultivated sunflower LSF-8. The interspecific cross showed basal branching with an average of six branches per pant, number of branches were very less compared to wild but it can be said as intermediate inheritance. Hristova and Cherbadzi (2004) studied interspecific crosses involving annual diploid *H. bolanderi* Gray, *H. neglectus* Heiser and *H. petiolaris* to have braches up to 13 to 31 and indicated that they were less compared to wild.

Seed length of *H. argophyllus* recorded was very small (0.7 cm) while that of cultivated sunflower LSF-8 was medium (1.3 cm) and interspecific cross recorded intermediate size (1 cm) i.e. larger than wild annual diploid *H. argophyllus* and smaller than LSF-8. Encheva and Christov (2006) recorded intermediacy for seed length in interspecific cross between *H. annuus* (hybrid Albena) × *H.*

salicifolius. An intermediate heritability type seed length was observed in the results obtained by Hristova and Cherbadzi (2004) in their interspecific crosses between cultivated sunflower and wild annual diploid *H. bolanderi* Gray, *H. neglectus* Heiser and *H. petiolaris.*

The test weight in cultivated sunflower LSF-8 was an average five grams and wild annual diploid *H. argophyllus* was two grams. Interspecific cross had an average weight of four grams. Hristova and Cherbadzi (2004) studied interspecific crosses involving annual diploid *H. bolanderi* Gray, *H. neglectus* Heiser and *H. petiolaris* having intermediate heritability type seed length.

There was significant difference in seed coat colour, cultivated sunflower LSF-8 has black colour with shiny texture with no stripes while wild annual diploid *H. argophyllus* had brown colour with rough texture with stripes present on it and all interspecific crossed seeds showed light black seed coat colour indicating the character was intermediate. In contrast Hristova and Cherbadzi (2004) obtained pale gray-brownish to dark brown seed colour, in their interspecific crosses involving annual diploid *H. bolanderi* Gray, *H. neglectus* Heiser and *H. petiolaris.*

The results from the investigation gave the reasons for confirming the statement already established by different authors (Satziperov, 1916; Georgieva-Todorova, 1976; Bohorova, 1983; Christov, 1988) that after crossing between wild *Helianthus* species and cultivated sunflower creates diversity in the cross.

Molecular characterization of interspecific cross along with parents: In present study, both the parental species LSF-8, and *H. argophyllus* were analyzed for parental polymorphism using forty five SSR primers. Out of forty five sunflower SSR primers studied. Only one primer showed the polymorphism, twenty nine primers showed monomorphic bands and fifteen primers failed to amplify bands. The ORS-5 primer showed the polymorphism at 440 base pairs for parent one LSF-8, while for parent two *H. argophyllus* showed at 300 base pairs respectively .The same primer ORS-5 was used to screen polymorphism in all six interspecific cross, and all six interspecific cross produced both the bands present in the parents as represented in Fig.1.

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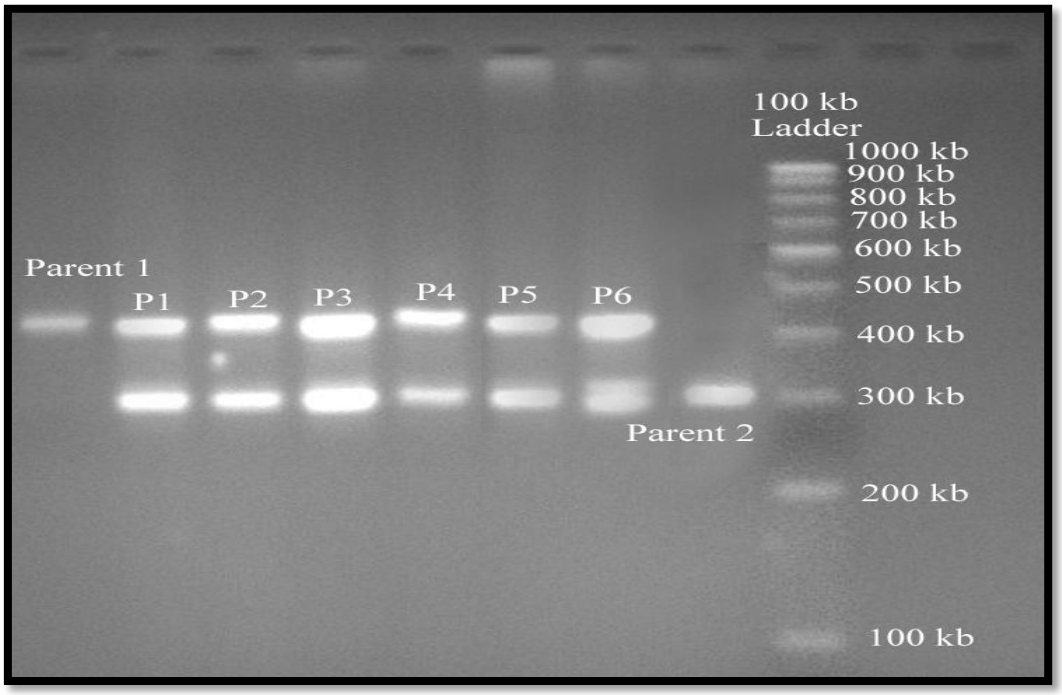

Fig. 1: SSR banding pattern in parents and interspecific cross.

Note – Parent 1 is LSF-8, Parent 2 is *H. argophyllus* P1, P2, P3, P4, P5, P6 – Six plants of interspecific cross (*H. annuus* × *H. argophyllus*)

Table 1. Comparative morphological features of parents and interspecific cross

Sl. No	Character	Female Parent	Male Parent	Interspecific cross (*H. annus* × *H. argophyllus*)	
		H. annuus LSF-8	*H. argophyllus* Acc No. 1317	Mean of six plants	Range
1.	Hypocotyl anthocyanin pigmentation	Absent	Present	Present	-
2.	Days to 50 *per cent* flowering	56	75	61	60-64
3.	Leaf size	Medium	Small	Medium	-
4.	Leaf colour	Dark green	Light green/ Ashy green	Light green	-
5.	Leaf hairiness	Absent	Present	Absent	-
6.	Stem colouration	Absent	Present	Present	-
7.	Stem hairiness	Very sparse	Present	Present	-
8.	Stem girth (cm)	5	8	8.2	7.8-8.5
9.	Number of leaves per plant	27	62	50	42-61
10.	Disc floret colour	Yellow	Purple	Purple	-
11.	Head diameter (cm)	15	4	6	5.4-6.3
12.	Head shape	Convex	Flat	Convex	-
13.	Days to maturity (days)	90	144	110	108-115
14.	Plant height (cm)	150	280	217	209-235
15.	Plant type	Non branching	Branching	Branching	-
16.	Number of branches	0	21	6	5-6
17.	Seed length (mm)	1.3	0.7	1	0.9-1.1
18.	Test weight (g)	5	2	4	-
19.	Seed colour	Black	Brown	Light black	-