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GENETIC CONTROL IN CONFECTIONERY SUNFLOWER

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Abstract: The present study was carried out to understand the genetic control of achene colour and shape in confectionery sunflower. F_1 selfed seeds of three crosses were raised during off-season (August sowing) 2019 at the sunflower experimental area, Punjab Agricultural University, Ludhiana. In the first cross EC-734846 × EC-734849-II, the F_2 ratio of black seed to white seed approximated 3:1 showing typical monohybrid segregation while the back cross with recessive parent revealed typical 1:1 segregation. The F_2 progeny of the second cross EC-734863-II × EC-734846, comprised of grey seeds with white stripes, greyish black and white seeds with black stripes colour seeds exhibiting supplementary gene interactions (9:3:4). The results suggest that black seed colour is dominant over white seed colour and exhibits simple inheritance, while distinct markings on the seeds are two genes controlled. The F_2 progeny of a cross (EC-734864×EC-734876) was raised to understand the inheritance of seed shape, exhibiting ovoid-shaped to elongated- shaped seeds in a 15:1 ratio indicating duplicate dominant gene interaction with elongated seeds being a recessive trait.

Keywords: Confectionery sunflower, Inheritance, seed colour, seed shape, chi-square test.

INTRODUCTION

Sunflower (Helianthus annuus L.) is one of the most important cultivated species within the family Asteraceae. It ranks fourth among oilseeds globally concerning production after Palm oil, Soybean and Rapeseed and Mustard, owing to its wider adaptability across varied climatic conditions and multiple industrial uses (Pilorge, 2020). Sunflower oil is suitable for human consumption due to its light colour, no characteristic odour, higher mono and polyunsaturated fatty acids and lack of anti-nutritional factors. Apart from the consumption of sunflower oil, the kernels are directly consumed as a snack or added to confectionery products in form of a meal. Nearly, 10% of total sunflower production is utilized for non-oil purposes including confectionery purposes (National Sunflower Association, 2011). The confectionery (non-oilseed) sunflowers are different from oilseed sunflowers concerning seeds and quality characteristics, thus the breeding objectives for the development of superior confectionery sunflowers are significantly different from breeding for oilseed sunflowers. In general, oilseed sunflower genotypes have ovoid black seeds with high oil content and low protein content whereas, the confectionery type sunflower genotypes are characterized by low oil content (<30%), high protein (>25%) and loose hull for ease in dehulling (Fernandez-Cuesta et al. 2012). The white kernels with stripes have thick hull which is loosely held with the kernel making it easier for mechanical or manual de hulling of the kernels. The non-oil sunflower genotypes must have elongated seeds with a high seed length-width ratio while low length-width ratio genotypes are suitable for the extraction of oil (Soroka et al. 2017).

For any successful genetic improvement programme concerning a trait, the understanding of the underlying genetic mechanism is of utmost importance. Inheritance studies based on the evaluation of parental and segregating generations (F_2 or backcross generation) have been utilized by several researchers. The pigments present in different layers of sunflower seed hull impart variation for achne colour like grey, brown and black while white seed coat is caused due to absence of any pigmentations. Likewise, distinct stripes are also present on seeds due to variable pigmentation. Presence or absence of seed stripes are also a descriptive feature for conducting tests pertaining to distinctness, uniformity and stability of different sunflower inbreds (Nadkarni et al. 2017). In addition to this the colour of these stripes can also be used for morphological characterization of sunflower inbreds (Dhillon *et al* 2013). Similarly, elongated seeds are preferable as confectionery sunflower, as against ovoid kernels which are usually high in oil content. The seed length: width ratio is taken to study kernel shape in sunflowers since a lot of variation exists within a head for seed size however seed shape is more uniform. The seeds with seed length: width ratio <1.4 are categorized as ovoid seeds, 1.4 to 1.8 as intermediate and >2 are categorized as elongated seeds (Lofgren, 1997). Keeping in view the above the present study was carried out to understand the genetic control of achene colour and shape.

MATERIALS AND METHODS

In the present study, five genotypes of confectionery sunflower were utilized for investigating the inheritance of seed colour and seed shape parameters as mentioned in Table1.The parental inbreds for crossing were sown in February 2019 at the sunflower experimental area, Punjab Agricultural University (PAU), Ludhiana (30°54'N latitude, 75°48'E longitude). To understand the genetic control of seed colour, sunflower inbred EC-734846 was crossed with EC-734848-II representing a white seed × black seed cross.

Table 1: List of materials used for carrying out inheritance studies of seed colour along with their characteristics

S. No.	Genotype	Seed Characteristic
1	EC-734863-II	Black seeds with white stripes
2	EC-734846	White seeds with black stripes
4	EC-734849-II	Black seeds with grey stripes
5	EC-734864	Ovoid seed: Low seed length-width ratio (1.425)
6	EC-734876	Elongated seed: High seed length-width ratio (2.667)

Similarly, another cross was made representing grey kernels × white seed using inbreds EC-734863-II and EC-734846, respectively. Inheritance of seed shape was studied by crossing ovoid seeds (EC-734864) with elongated seeds (EC-734876). For the production of F_1 seed, the heads were covered with cloth bags before the emergence of anthers and then the male sterility was induced by applying Gibberellic acid (GA_3 @100ppm) at the button stage for three consecutive days. All the female plants were observed for pollen shedders during morning hours from 7-9 AM. All such plants which showed fertile anthers were discarded. Pollen from male parents was collected in fresh paper and gently applied on the female head using a camel hair brush. The F_1 seed was harvested from female and half of the seed lot was resown during August 2019 for advancing generation as well as making backcrosses. During February 2020, parents, F_1s, F_2s and backcross generations were raised in the sunflower experimental area, PAU, Ludhiana for inheritance studies. The row-to-row and plant-to-plant spacing was 60cm and 30cm, respectively. The standard agronomic practices were followed as per the package of practices of the PAU, Ludhiana to raise the healthy crop. The visible seed colour variation in seeds collected from each plant was observed and studied. The data was recorded separately for each genotype and these genotypes were further divided into different colour groups. Ten seeds were taken from each plant randomly and their length and width were measured manually by using a centimetre scale. Then their ratio was calculated by dividing the average seed length by the average seed width.

All the data obtained were statistically analysed to test the goodness of fit using chi-square test given by Karl Pearson (1900) as per the formula given below:

$$\chi^2 = \sum \frac{(O-E)^2}{E}, \text{ with (n-1) d.f.}$$

Where,

O =observed frequencies

E =expected frequencies

n =number of classes

d.f. =degree of freedom

The significance of the Chi-square was tested by comparing the calculated Chi-square value with the table value at 5% level of significance at appropriate degrees of freedom (n-1).

RESULTS AND DISCUSSION

In the cross, confectionery lines EC-734846 × EC-734849-II, representing white seeds × black seeds the F_1 plants exhibited black seeds colour with grey stripes as shown in Figure 1.In the F_2 generation, of the total 105 plants, 87 exhibited black seed colour while 18 plants exhibited white seed colour. Based on the chi-square test, the goodness of fit was observed for two ratios, one being typical monohybrid segregation of 3:1 (chi-square value = 3.457, p-value = 0.063) and the second being recessive gene interaction having a ratio of 13:3 (chi-square value = 0.178, p-value = 0.673). Furthermore, to resolve the issue, the observed ratios for backcross generation were used where F_1 was backcrossed with white- seeded parent EC-734846, of the total population of 65 plants, 27 were having white seeds while the remaining 38 were having black seeds (Table 2).The Chi-square test revealed the goodness of fit for the 1:1ratio with a chi-square value of 1.862 and a p-value of 0.172. The above observations suggest that seed colour is controlled by a single gene with a major effect. The allele controlling black seed colour (B) shows complete dominance over white seed colour which is controlled by a recessive allele (b).

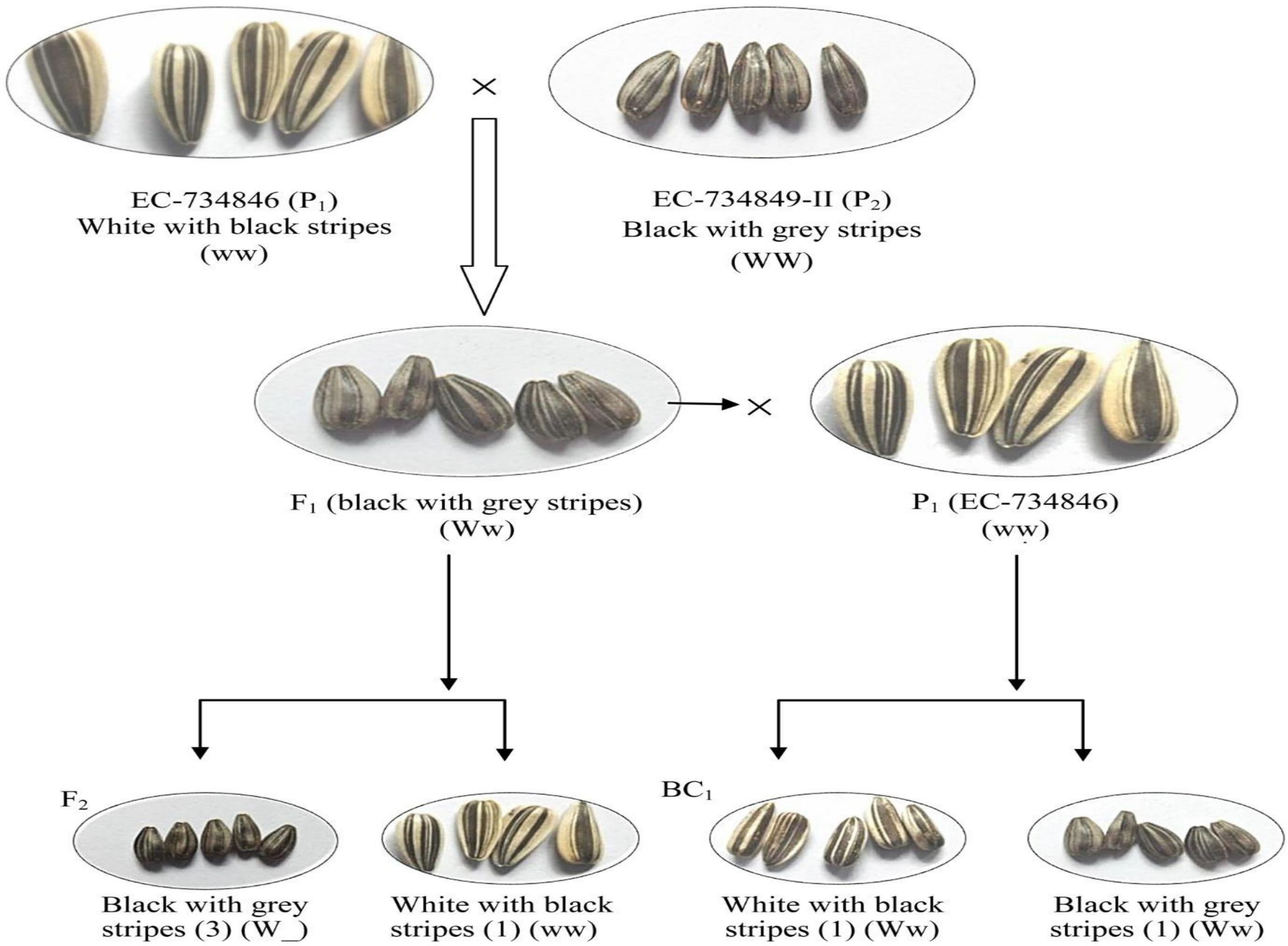

Figure 1: EC-734846 (White seeds with black stripes) × EC-734849-II (Black seeds with grey stripes)

Table 2: Segregation ratios of F_2 and BC_1 generation for white (EC-734846) × black (EC-734849-II) cross

Generations	Observed frequency		Expected frequency		Expected ratio	χ^2 values		p-value
	White	Black	White	Black		calculated	tabulated	
F_2	18	87	26.25	78.75	3:1	3.457	3.841	0.0630
	18	87	19.6875	85.3125	13:3	0.178	3.841	0.6731
BC_1	27	38	32.5	32.5	1:1	1.862	3.841	0.1724

In another cross, the female parent having grey seeds (EC-734863-II) was crossed to a male parent having white seeds (EC-734846). All the F_1 plants were having grey seeds with white stripes as shown in Figure 2. The F_2 generation was produced by selfing of F_1 plants and revealed that out of a total of 245 F_2 plants, 148 plants exhibited grey with white striped seeds, 40 plants exhibited greyish black seeds (or dark grey) and 57 plants exhibited white with black striped seeds as shown in Table3. With a calculated chi-square value of 1.815 and p-value of 0.403, the ratios of black seeds with stripes, black seeds without stripes and white seeds with black strips fit well into a 9:3:4 ratio. This suggests that two genes present in the dominant form are required to produce black seeds with white strips whereas when both genes are present in the recessive form they lead to the expression of white seeds with black strips. The allele B in absence of the dominant allele for a second gene (A) failed to express for white strips thus genotypes aaB_ resulted in black seeds with no stripes. In absence of a dominant allele for B black seed colour is not formed whereas A does not produce white stripes on its own thus producing a similar phenotype as that of aabb. This observation was also in concordance with results from the first cross that bb results in white kernels whereas BB results in black kernels. The results depicted in this study were contradictory to Nadkarni et al. (2017) who suggested that the inheritance of seed colour is controlled by complementary gene interaction whereas dominant alleles result in phenotype.

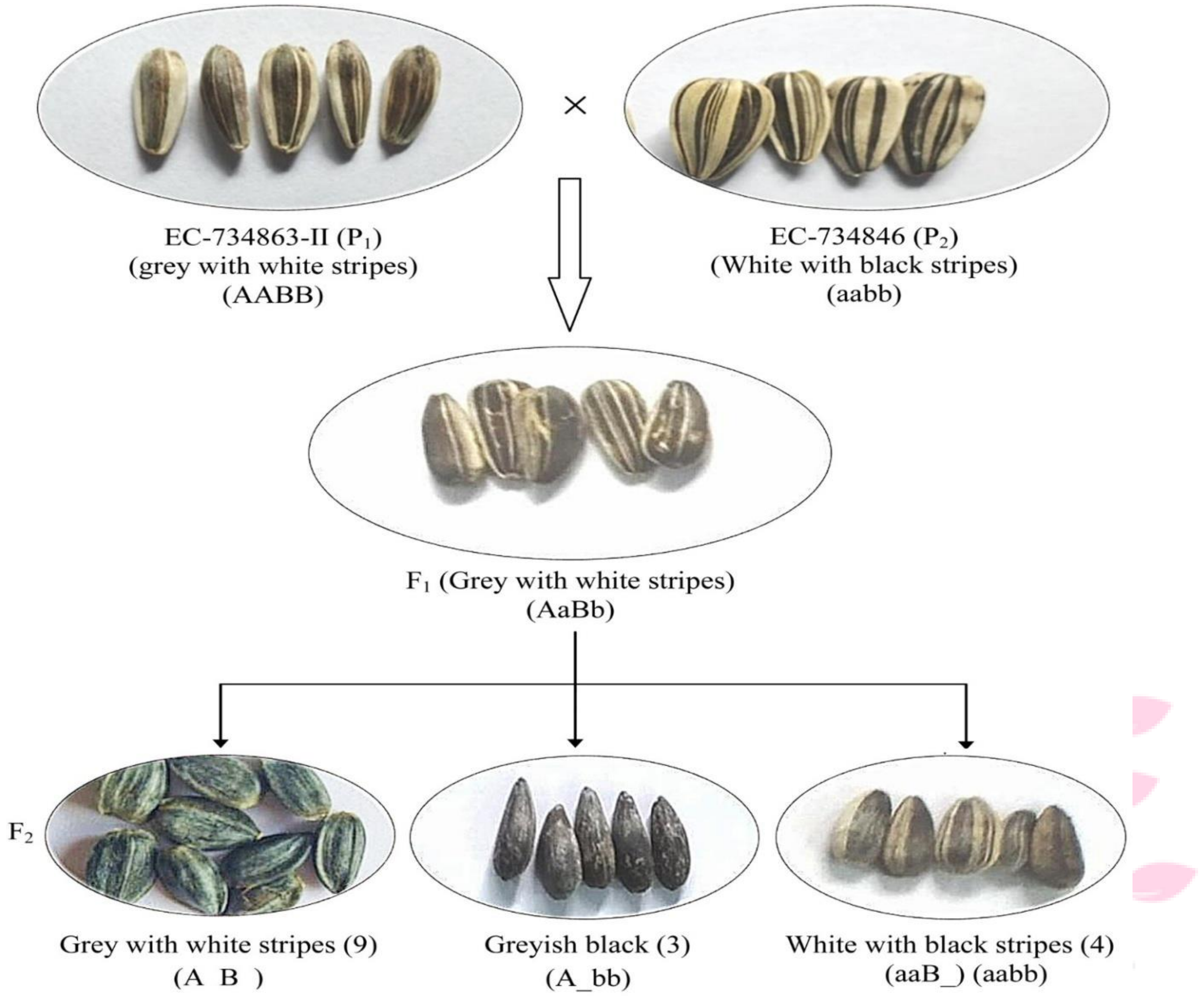

Figure 2: EC-734863-II (Grey seeds with white stripes) × EC-734846 (White seeds with black stripes)

Table 3: Segregation ratios of F_2 generation for stripped × white cross (EC-734863-II × EC-734846)

Generations	Observed frequency			Expected frequency			Expected ratio	χ^2 values		p-value
	Grey with white stripes	Greyish black	White with black stripes	Grey with white stripes	Greyish black	White with black stripes		Observed	Table	
F_2	148	40	57	137.813	45.937	61.25	9:3:4	1.815	5.991	0.4035

The inheritance of seed size was studied in the cross ovoid seeds (EC-734864) × elongated (EC-734876) as shown in Figure 3. All the F_1 plants showed an intermediate seed length-to-width ratio (1.709). F_2 plants were produced by selfing of F_1 plants. The F_2 population consisted of 78 plants, out of which, 68 plants were isolated with seeds where the length-to-width ratio did not exceed 2.0 cm representing an ovoid seed shape and the other 9 plants of this population had the length to width ratio is 2.0 cm or higher representing elongated seeds. The data shows the goodness of fit with a 15:1 ratio corresponding to duplicate dominant gene interaction with the calculated χ^2 value equal to 3.723 and p-value equal to 0.0537 (Table 4). This cross reveals that the ovoid seed shape shows incomplete dominance over elongated seeds. Furthermore, the trait is governed by two genes showing duplicate dominant gene interaction (15:1ratio) which means that the dominant alleles at both or either of the loci will give rise to an ovoid seed shape whereas for the development of elongated seeds both the alleles must be present in homozygous recessive state. Similar results were reported by Soroka et al. (2017) as they also studied the inheritance of seed shape in sunflowers and revealed that the shape of the seed was controlled by two or even three pairs of polymeric genes.

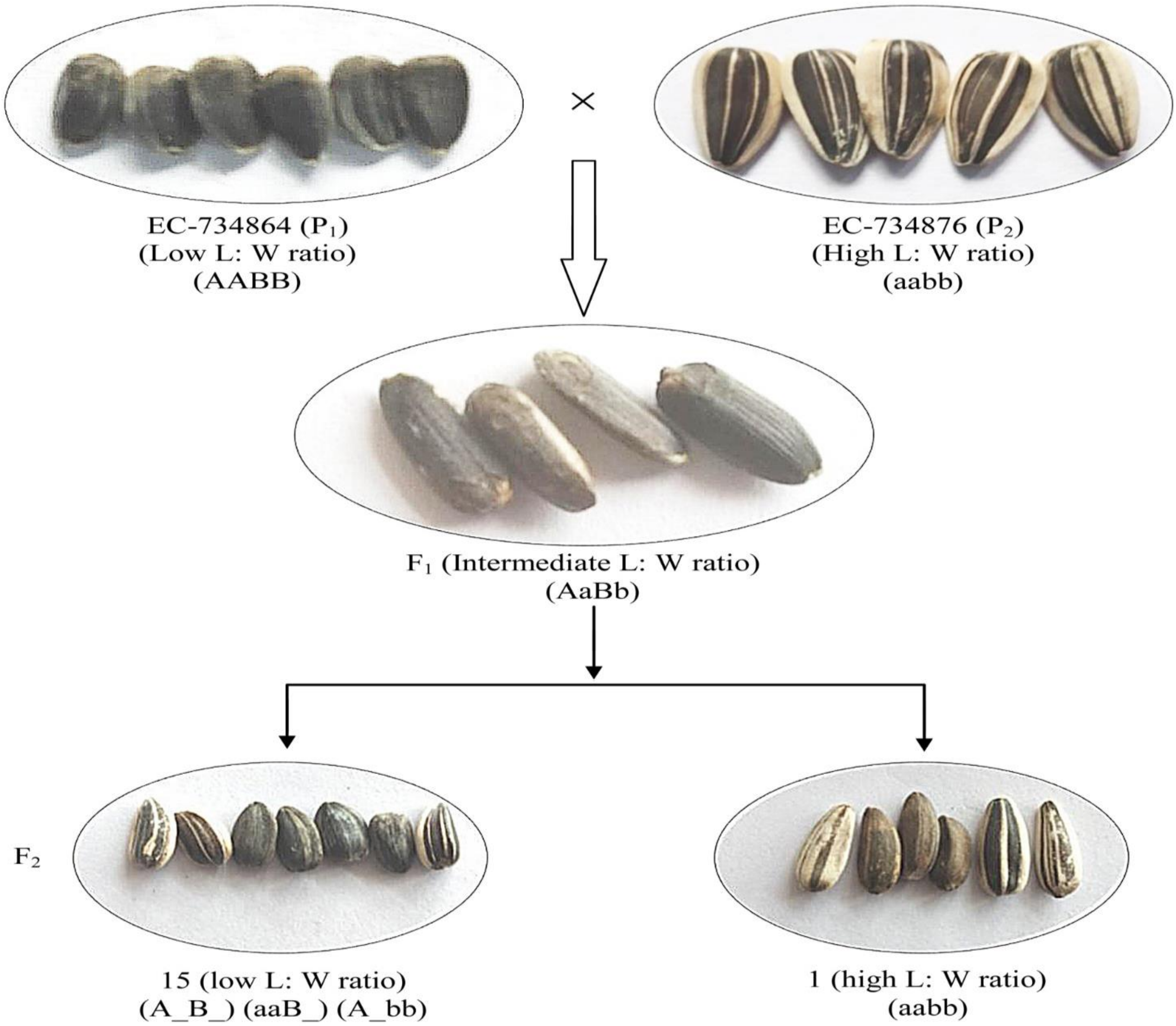

Figure 3: EC-734864 (Low L: W ratio) × EC-734876 (High L: W ratio)

Table 4: F_2 generation of low L: W ratio× high L: W ratio (EC-734864×EC-734876) of showing phenotypic segregation for seed size

Gen.	Observed		Expected		Expected ratio	χ^2 values		p-value
	<2.0	>2.0	<2.0	>2.0		Observed	Table	
F_2	69	9	73.125	4.875	15:1	3.723	3.841	0.0537

CONCLUSION

The results of the present investigation suggested that the desirable seed colour among confectionery sunflower, which is white background with distinct black stripes is controlled by recessive alleles. Based on the F_2 and backcross progeny ratios of the first cross and F_2 ratios of the second cross, it can be concluded that for developing hybrids for confectionary purposes with desirable seed colour and markings, we must select both the parents with white kernels. As far as seed shape is concerned which is a very important trait for confectionary sunflower, the desirable phenotype is only expressed when the two genes controlling the trait are present in a recessive state. From this, we can conclude that for the development of sunflower hybrids with elongated seeds both the parents of the cross must have elongated seed types.

ACKNOWLEDGEMENT

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