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Inheritance of Pollen Colour in Sunflower (*Helianthus annuus* L.)

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ABSTRACT

This investigation was carried to study the inheritance of pollen colour in sunflower (Two crosses for pollen colour). F_1 selfed seeds of two crosses were raised during kharif season 2013 at Main Agricultural Research Station, Raichur (Karnataka). Inheritance of pollen colour in sunflower (Helianthus annuus L.) using two distinct colour morphs, yellow and white pollen colour. The two parents used in the study are 104B and 17B had yellow and white pollen respectively. The study consists of two crosses direct and reciprocal cross CMS17A x 104B (white pollen x yellow pollen) and CMS104A x 17B (yellow pollen x white pollen). The F_1 plants showed yellow pollen in all the plants in both direct and reciprocal crosses which revealed that yellow pollen colour is dominant over white. The F_2 generation of direct cross CMS17A x 104B exhibited 726 yellow colour pollen plants and 239 white colour pollen plants out of 965 total number of plants scored and in the reciprocal cross CMS104A x 17B out of 1017 F_2 plants scored, 761 plants had yellow pollen and 256 plants had white pollen. In both the crosses the data showed good fit into 3:1 ratio indicating with dominance of yellow pollen over the white pollen with monogenic inheritance.

Key words: *Pollen colour, Inheritance, Chi-square Test*

INTRODUCTION

Sunflower (*Helianthus annuus* L.) known as "Golden Girl of American Agriculture", belongs to the genus 'Helianthus', family 'Asteraceae' (Compositae). The name has its origin in Greek "Helios" means "Sun" and "Anthois" means flower. It is native to southern parts of USA and Mexico[5]. Sunflower is an important oil seed crop in India popularly known as "Surajmukhi" as it follows the movement of the sun, always turning towards its direct rays. It has many small flowers aggregated together on the capitulum. The rear side of the head is covered with small bracts called phyllaries. Above them are the familiar yellow petal-like structures, which are called rays or ray flower.

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They don't play any role in the reproduction of the plant except serving as a signal for bees and other pollinating insects. Inside the rays are many small complete flowers known as disk florets. Each floret has the potential to develop an achene or seed[1]. In India, it is an important source of edible and nutritious oil. It is a rich source of edible oil (40-52%) and considered as good quality oil from health point of view due to polyunsaturated fatty acids with 55-60 per cent of linoleic acid and 25-30 per cent of oleic acid which are known to reduce the risk of cardiac related problems.

Inheritance studies are important both from theoretical and applied point of view. Yield levels can be stepped up by eliminating the undesirable genes. The knowledge of inheritance of various qualitative characters is of paramount importance to achieve success in plant breeding. Sunflower is an important oilseed crop, which normally having yellow or yellowish pollen colour and rarely white pollen colour which serve as a gene marker in identifying the parents in hybrid seed production plots. The main objective of this study was to know the inheritance pattern of pollen colour and to determine the number of genes involved in the inheritance of this trait. This investigation of characters in any crop was a pre-requisite to plant breeding. Although improvement work in sunflower has been in progress at a number of places, the studies on inheritance of pollen in sunflower are reported sporadically.

Pollen is a fine to coarse powder containing the micro gametophytes of seed plants, which produce the male gametes (sperm cells). Pollen grains have a hard coat that protects the sperm cells during the process of their movement from the stamens to the pistil of flowering plants or from the male coneto the female cone of coniferous plants. When pollen lands on a compatible pistil or female cone (i.e., when pollination has occurred), it germinates and produces a pollen tube that transfers the sperm to the ovule (or female gametophyte). Pollen colour acts as a gene marker for identifying the parental lines, inbreds and hybrids in hybrid seed production, to identify the role of pollen colour in transmitting the economically important traits like oil content, seed colour and other yield attributing traits. Hence studies on inheritance of seed colour and pollen colour is of atmost importance in determining the seed quality. White pollen color reported in the mutant of a male sterile restorer line C 8711 of sunflower was found to be under the control of a monogenic recessive gene. White pollen could probably be due to flavonoid deficiency and serves not only as a genetic marker but also as a useful model in gene regulation studies[7].

MATERIALS AND METHODS

This field study was undertaken at Main Agricultural Research Station, Raichur campus of the University of Agricultural Sciences, Raichur. The campus is being geographically situated in the North Eastern Dry Zone (Zone 2) of Karnataka state at 16^{o} 12' N latitude and 77^{o} 21' E longitude with an altitude of 389.37 meters above mean sea level. The plant material used in the study was comprising of F_1 selfed seeds of two crosses *viz,*. CMS17A x 104B and CMS104A x 17B. The plant was done in separate plots during *kharif* season. Sufficient plant population was maintained by sowing all the available selfed F_1 seeds to raise the F_2 generation to study the inheritance pattern of pollen colour and other traits in the segregating population observations. Plant populations in each plot were ranged from 965 to 1017. A spacing of 60 cm between rows and 30 cm between plants was followed. The recommended dose of fertilizer was applied at the rate of 60:75:60 NPK kg per ha. Half of the recommended dose of nitrogen along with the entire dose of phosphorus and potassium was applied at the time of sowing in the furrows. The remaining 50 per cent of nitrogen was top dressed at 30 days after sowing, crop was grown under irrigated condition and all the recommended package of practices was followed to raise the crop.

All the data obtained were statistically analysed by using chi square test proposed by Karl Pearson to test the goodness of fit. Karl

Pearson[6] developed χ^2 (Chi-square) test and it is defined as "The sum of square of the deviations from observed to expected frequencies divided by expected frequencies". The general formula for χ^2 is as follows:

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

Where,

O = Observed frequencies
E = Expected frequencies
n = Number of classes
d.f. = Degrees of freedom
$\sum$ = Summation

Thus, application of χ^2 requires observed and expected frequencies. The expected frequencies can be calculated from the observed frequencies assuming a particular hypothetical segregation ratio. Each deviation (O-E) is squared and each squared deviation is then added together to give a single value of χ^2. This value is tested against table χ^2 at five per cent for (n-1) d. f., where 'n' is the number of segregation classes. The degrees of freedom are '1' for two-class segregation (3:1) and it is '3' for four class segregation (9:3:3:1) and each class of data should have at least about 50 observations.

The significant value of χ^2 suggests that the deviation of expected frequencies from the observed values are large hence the observed frequencies are not in agreement with the expected or hypothetical frequencies and the assumed segregation value is not valid and was considered as poor fit. The non significant value of χ^2 on the other hand indicates that the deviation of expected frequencies from the observed values are small, χ^2 tends to be zero and it is less than table value at five per cent level for (n-1) d.f., and hence observed values are close agreement with the expected frequencies and the deviation is due to chance factor only and the segregation value is valid and was considered as good fit.

Apart from fitting the ratios, the chi-square test is also a useful tool for testing the association of characters. Therefore, it was again employed to test the independent assortment of genes or linkage.

Pollen colour was scored in the plants based on visible physical appearance of yellow and white pollen colour in all fertile plants in F_2 segregating population. Mendelian Genetics deals with qualitative or oligogenic characters, which exhibit discontinuous variations. It is very easy to classify the plant population into distinct classes for such characters. These traits are interpreted in terms of frequencies or ratios.

RESULTS AND DISCUSSION

This study was carried out on the inheritance of pollen colour was studied on two crosses *viz.,* CMS 17A x 104B (White pollen Vs Yellow pollen) and CMS104A x 17B (Yellow pollen Vs White pollen). The contrasting characters of parents and their expression in F_1 are presented in Table 1. In the first cross CMS 17A (white pollen) x 104B (yellow pollen) all the F_1 plants had normal yellow pollen grains and none of the F_1 plants had white pollen. Apart from the two pollen colours, no other pollen colour was found. And in the second cross CMS 104A (yellow pollen) x 17B (white pollen) also showed that all the F_1 plants had normal yellow pollen grains and none of the F_1 plants had white pollen. Apart from the two pollen colours, no other pollen colour was found. The details of observed frequencies of phenotypic segregation in F_2 generation for the character, the expected frequencies of phenotypic ratios, and the calculated chi-square with their levels of probability indicating the goodness of fit of observed frequencies to the assumed ratios are presented in Table 1.

1) DIRECT CROSS: CMS 17A x 104B (White pollen x Yellow pollen)

The pollen colour in F_2 generation for the cross CMS 17A (white pollen) x 104B (yellow pollen), the F_2 segregation showed that, out of 965 plants, 726 plants exhibited yellow pollen, whereas the remaining 239 showed white pollen. The ratios of yellow pollen to the white pollen plants approximated 3:1 for the cross. The calculated χ^2 value was 0.027, which is in

the close agreement with expected frequencies and hence the deviation is due to only chance factor and the segregation value is valid and the probability value is 99 per cent. F_2 generation is controlled by a pair of single genes as confirmed from the chi-square test of non significant χ^2 value ($\chi^2 = 0.027$.)

2) RECIPROCAL CROSS: CMS104A x 17B (Yellow pollen x White pollen)

While in the reciprocal cross of the CMS 104A (yellow pollen) x 17B (white pollen) showed that out of 1017 plants, 761 plants exhibited yellow pollen, whereas the remaining 256 had white pollen. The ratios of yellow pollen to the white pollen plants approximated 3:1 for the cross. The calculated χ^2 value was 0.016, this indicated that the deviations are due to chance factor and the segregation value is valid and the probability value is 99 per cent. Segregation of pollen colour in F_2 generation is controlled by a pair of single genes as confirmed from the chi-square test of non significant χ^2 value. ($\chi^2 = 0.016$.).

The ratio to white pollen remained 3:1 irrespective of the use of white pollen parent (17B) as a male or female. This indicates that there is no reciprocal effect for the expression of pollen colour. The pollen colours of F_2 generation plants of the two crosses were recorded during flowering stage. The results obtained are presented in Table 1. Among 965 F_2 plants of the cross CMS17A x 104B (white pollen x yellow pollen), 726 plants had yellow pollen, whereas the remaining 239 plants had white pollen. Similarly, in the reciprocal cross CMS104A x 17B (yellow pollen x white pollen) a total of 1017 plants are raised in F_2 and the segregation for pollen colour found to be 761 yellow colour pollen plants and 256 plants with white pollen colour. The segregating ratio of yellow pollen to white pollen plants were approximated to 3:1 respectively for these two crosses.

Majority of sunflower genotypes have yellow pollen with genotype '**PP**' whereas white pollen with genotype '**pp**'. When white and yellow pollen colour plants are crossed, they form genotype '**Pp**' which also showed yellow pollen, because recessive gene '**p**'. Thus, white pollen is controlled by a pair of recessive genes '**pp**' and this character is expressed only when the recessive genes are in homozygous condition. Genotypes '**PP**' or '**Pp**' will have yellow pollen colour, while the genotype '**pp**' will have white pollen.

Qiao *et al*[8]., Qiao *et al*[8]., Wang and Wang[9], Devaraja and Shanker Goud[2], Fambrin *et al*[4]., and Dhillon and Bajaj[3] also reported that yellow pollen was dominant over white pollen and controlled by a single pair of gene with a monogenic inheritance. The results obtained from the present study are in agreement with previous reports. The white colour pollen sunflower helps to enrich the gene pool of sunflower germplasm and might have special importance in sunflower breeding programme, it acts as a very good gene marker in detecting admixtures and contaminants in sunflower hybrid seed production.

Table 1: The F_2 generation of direct and reciprocal crosses of showing phenotypic segregation for pollen colour in sunflower

Cross	Observed frequency		Expected frequency		Total observed frequency	Ratio of yellow to white colour pollen plants	χ^2	P- value
	No. of Yellow colour pollen plants	No. of White colour pollen plants	No. of Yellow colour pollen plants	No. of White colour pollen plants				
CMS17A x 104B (Direct cross)	726	239	723.75	241.25	965	3:1	0.027	0.99
CMS104A x 17B (Reciprocal cross)	761	256	762.75	254.25	1017	3:1	0.016	0.99

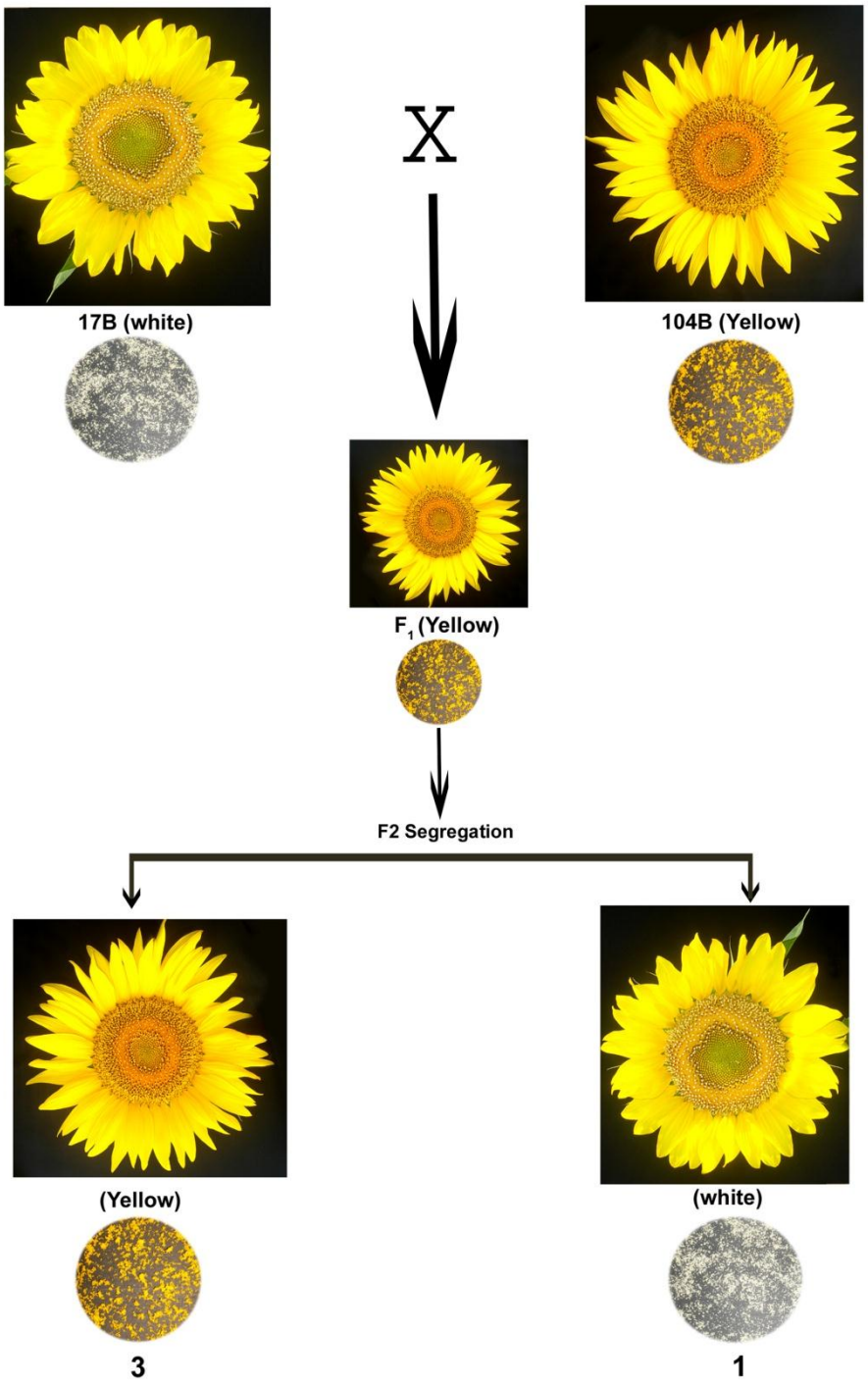

Plate 1: Inheritance of pollen colour in Sunflower

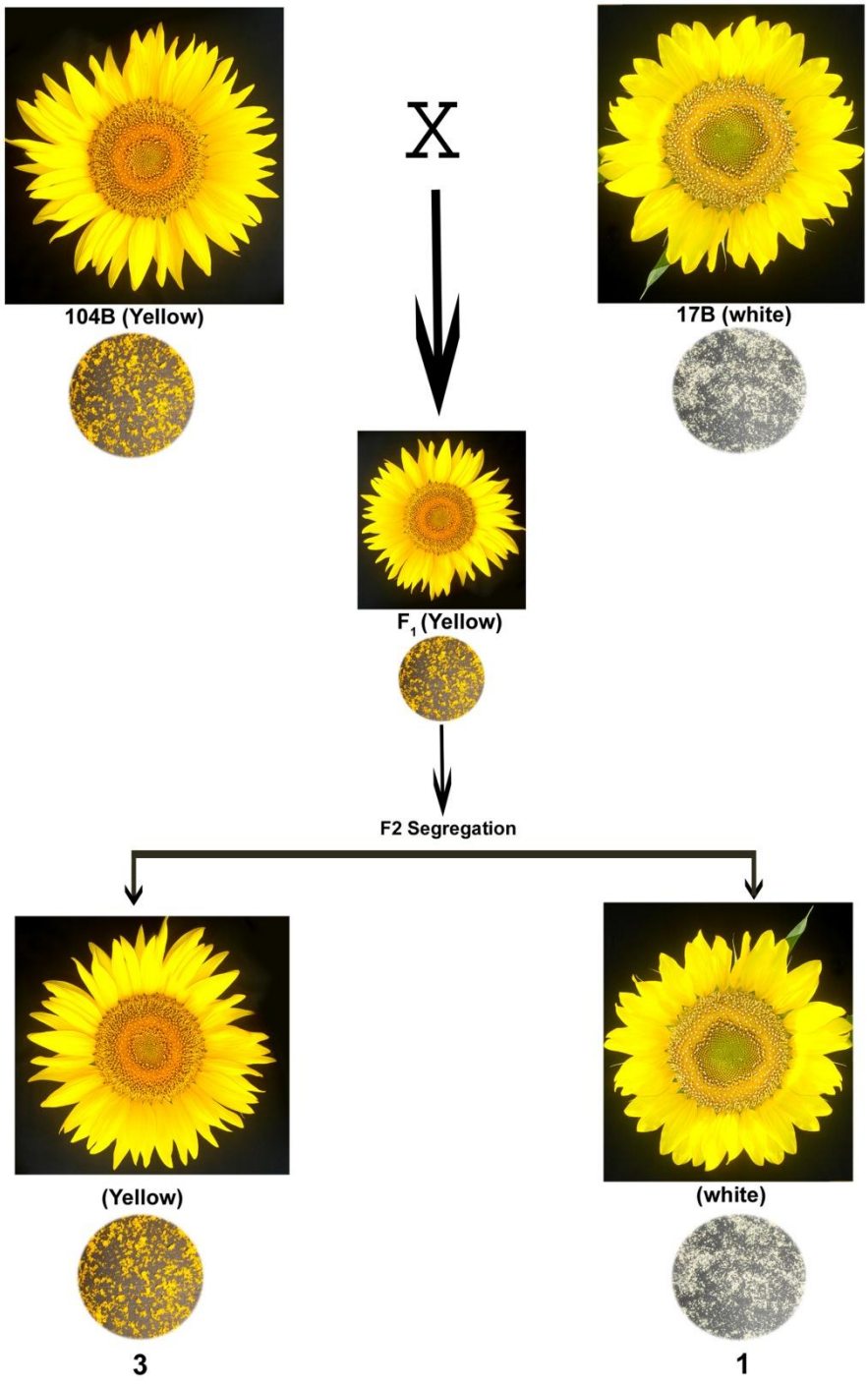

Plate 2: Inheritance of pollen colour in Sunflower

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