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Inheritance studies, detection and estimation of linkage of various important qualitative traits in safflower (*Carthamus tinctorius* L.)

Pratibha, Ankit Yadav and Rajeev Shrivastava

Abstract
Safflower (*Carthamus tinctorius* L.) is a versatile, herbaceous, annual thistle-like oilseed crop with lots of health benefits. Its seed oil is used for preventing various heart diseases and lowering the cholesterol level in blood. Safflower oil is richest in polyunsaturated fatty acid (PUFA). In the present study, nine qualitative contrasting parents were taken in consideration. These parents were RSS 2016-7, RVS 12-13, A-1, EC-755686, EC-755665, EC-755664, NARI-121, NARI-120 and NARI-119. The traits observed during the study were shape of capitulum, petal colour, faded petal colour, type of bracts and colour of stigma. Ten F_1's i.e RSS 2016-7 x RVS12-13, RSS 2016-7 x NARI-121, A-1 x EC-755686, A-1 x EC-755665, A-1 x EC-755664, A-1 x NARI-121, A-1 x NARI-120, EC- 755686 x RVS 12-13, EC- 755686 x A-1, EC- 755686 x NARI- 119 were generated from crossing these nine parents. Observations were taken for various qualitative traits from parents and F_1 generation. In F_2 generation pattern of segregation was observed and were analyzed using chi-square test. Further, test of independence for detection of linkage as suggested by Panse and Sukhatme (1967) [10] was calculated and linkage distance was estimated in terms of cross over value between two genes as suggested by the Minimum discrepancy method given by Haldane (1953) [4] as described by Murty (1954) [8]. A proper study of linkage present among these traits helps plant breeders for various crop improvement programmes.

Keywords: Safflower, linkage, qualitative traits

1. Introduction
Safflower (*Carthamus tinctorius* L.) is considered as one of the most important multipurpose oilseed crop. It is a member of Asteraceae family and grows as annual herb in hot and dry conditions (Li and Mundel 1996) [7]. Iran is one of the old world's epicentres for safflower cultivation, and diverse local populations of this crop can be found all around the nation (Knowles 1969) [6]. Several parts of Asia, including China and India, are used to cultivate safflower (Singh 2007) [14]. Safflower has historically been produced for its flower, which is used in food colouring and flavouring, dye production, and medicine. Yet over the past few years, there has been a significant increase in interest in the production of oilseed crops because of the rising demand for vegetable oil in human diets (Pahlavani *et al*., 2004) [9]. Safflower oil has a healthy amount of linoleic acid (78%), which lowers the level of cholesterol in the blood. The fatty acid composition of conventional safflower seed oil is composed of 6-8% palmitic acid, 2-3% stearic acid, 16-20% oleic acid, and 71-75% linoleic acid (Fernandez *et al*., 1993) [2]. In many regions of India, young seedlings of this plant are favoured by people as green leafy vegetables because its leaves are high in carotene, riboflavin, and vitamin C.

It is crucial for a breeder to understand the morphology of traits, their pattern of inheritance, linkage study and the proportion of genes responsible for that trait in any crop development effort. This information is crucial for a breeder. The objective of present study was to find out linkage between two or more loci controlling different desirable characters. Linkage study helps to know the distance between the two traits and to know whether the two traits are linked or not. A proper study of inheritance and finding out the presence of linked genes helps plant breeders to work on those desirable traits for further crop improvement programmes.

2. Materials and Methods
This experiment was conducted at Research cum Instructional Farm, IGKV, Raipur (C.G). In this research, a total of nine parents were taken in consideration during *rabi* 2017-18 named as RSS 2016-7, RVS 12-13, A-1, EC-755686, EC-755665, EC-755664, NARI-121, NARI-120

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and NARI-119. Ten F_1's were generated during *rabi* 2017-18 from crossing these parents namely RSS 2016-7 x RVS12-13, RSS 2016-7 x NARI-121, A-1 x EC-755686, A-1 x EC-755665, A-1 x EC-755664, A-1 x NARI-121, A-1 x NARI-120, EC- 755686 x RVS 12-13, EC- 755686 x A-1, EC-755686 x NARI- 119 and their inheritance study was investigated. During *rabi* 2018-19, F_2's were grown and taken in study to find out segregation pattern of various qualitative traits. The characters taken in consideration were shape of capitulum, petal colour, faded petal colour, type of bracts and colour of stigma. For study of genetics of quantitative traits, observations for contrasting characters were recorded separately for each trait in every parents, F_1 and F_2 populations. Chi square test was applied in this recorded data for goodness of fit. Observed value was compared with expected values of segregation ratio. If observed χ2 value is higher than expected χ2 value it is said to be significant. For detection of linkage, test of independence was calculated as suggested by Panse and Sukhatme (1967) [10]. Further, linkage distance was estimated in terms of cross over value between two genes as suggested by the Minimum discrepancy method given by Haldane (1953) [4] as described by Murty (1954) [8].

3. Results and Discussion
3.1 Inheritance studies
Inheritance studies gives out the information of the traits that are passed on from one generation to the next generation. Mendel and some succeeding workers believed that a single

gene controlled a character. Later, it was found that all organisms have two or more genes controlling many of their characters. Such genes have varying effects on how characters are developed, which results in different modifications of a typical dihybrid (9:3:3:1), trihybrid ratios (27:9:9:9:3:3:3:1) etc. in F_2 ratios. Based on how the interacted genes affect one another's expression, many types of gene interactions exist.

To study the gene interaction in this research, inheritance study was done for various characters in the above-mentioned crosses. In parents and F_1 generation, expression of various qualitative traits i.e shape of capitulum, petal colour, faded petal colour, type of bracts and colour of stigma were recorded (Pratibha *et al.*, 2018) [12]. In next generation, segregation pattern of F_2 for different traits were recorded. After that, statistical analysis was done with the help of goodness of fit. In this, chi square test was applied to find out how the observed value of a given phenomena was significantly different from the expected value. It is a valuable tool for the breeder to decide the observe data is according to an expected ratio or it agrees well with expected or theoretical frequency distribution. If observed χ^2 value is higher than expected χ^2 value, it is said to be significant. As few inheritance results (for 5 crosses i.e RSS 2016-7 x RVS12-13, RSS 2016-7 x NARI-121, A-1 x EC-755686, A-1 x EC-755665 and A-1 x EC-755664) of this research were already published (Pratibha *et al.*, 2018) [12], so the overall summary of it is presented in the table 1 and table 2.

Table 1: Observations of various qualitative traits in F_1 plants, F_2 segregating populations and their probable ratios (Pratibha *et al.*, 2018) [12]

S. No.	Name of cross	Trait	Expression in F_1	Observed value in F_2		Expected ratio	Expected value for F_2		Chi square value	P Value (0.05)
1	RSS 2016-7 X RVS 12-13	Petal colour	Red	93 Red	68 White	9:7	90.05 Red	70.43 White	0.149	3.84
		Bract type	Lanceolate	134 Lanceolate	13 Ovate	15:1	137.81 Lanceolate	9.18 Ovate	1.694	3.84
2	RSS 2016-7 X NARI-121	Petal Colour	Yellow	15 Yellow	180 White	15:1	12.18 Yellow	182.81 White	0.695	3.84
		Bract type	Narrow lanceolate	122 Narrow Lanceolate	73 Broad lanceolate	9:7	109 Narrow Lanceolate	85.3 Broad lanceolate	3.324	3.84
		Capitulum shape	Beak shape	184 Beak shape	11 Big flat	15:1	182.81 Beak shape	12.81 Big flat	0.121	3.84
3	A1 X EC-755686	Bract type	Long lanceolate	126 Long lanceolate	89 Short lanceolate	9:7	120.93 Long lanceolate	94.06 Short lanceolate	0.484	3.84
		Capitulum shape	Beak shape	200 Beak shape	15 Flat	15:1	201.5 Beak shape	13.4 Flat	0.202	3.84
		Stigma colour	Yellow	195 Yellow	20 Red	15:1	201.5 Yellow	13.4 Red	3.459	3.84
4	A1 X EC-755665	Petal colour	Red	202 Red	18 Yellow	15:1	206.25 Red	13.75 Yellow	1.401	3.84
		Faded petal colour	Dark red	203 Dark red	17 Faded yellow	15:1	206.25 Dark red	13.75 Faded yellow	0.819	3.84
5	A1 X EC-755664	Bract type	Lanceolate	151 Lanceolate	57 Extra narrow ovate	3:1	156 Lanceolate	57 Extra narrow ovate	0.641	3.84
		Faded petal colour	Dark red	201 Dark red	7 Off yellow	15:1	195 Dark red	13 Off yellow	2.953	3.84
6.	A1 X NARI-121	Faded petal colour	Faded yellow	266 Faded yellow	102 Dark red	3:1	276 Faded yellow	92 Dark red	1.449	3.84
		Stigma colour	Yellow	307 Yellow	61 Red	13:3	299 Yellow	69 Red	1.141	3.84
7.	A-1 X NARI-120	Bracts type	Broad lanceolate	118 Broad lanceolate	12 Long lanceolate	15:1	121.8 Broad lanceolate	8.12 Long lanceolate	1.972	3.84
		Faded petal	Faded	124	6	15:1	121.8	8.12	0.593	3.84

Sr. No.	Cross	Trait	colour	yellow	Faded yellow	Dark red		Faded yellow	Dark red		
		Stigma colour	Yellow		66 Yellow	64 Red	9:7	73.12 Yellow	56.87 Red	1.587	3.84
8.	EC-755686 X RVS 12-13	Petal colour	Yellow		81 Yellow	50 Red	9:7	73.6 Yellow	57.3 Red	1.674	3.84
9.	EC-755686 X A1	Bracts type	Long lanceolate		112 Long lanceolate	48 Short lanceolate	3:1	120 Long lanceolate	40 Short lanceolate	2.133	3.84
		Capitulum shape	Beak Shaped		155 Beak shaped	5 Flat	15:1	150 Beak shaped	10 Flat	2.666	3.84
		Stigma colour	Yellow		117 Yellow	43 Red	3:1	120 Yellow	40 Red	0.3	3.84
10.	EC-755686 X NARI-119	Petal colour	Yellow		98 Yellow	61 Red	9:7	89.4 Yellow	69.5 Red	1.866	3.84
		Stigma colour	Yellow		112 Yellow	47 Red	3:1	119.25 Yellow	39.75 Red	1.763	3.84

Table 2: Crosses showing their inheritance pattern and gene interactions present between the traits

Sr. No.	Crosses	Traits	Dominant trait	Inheritance pattern	Gene interaction
1.	RSS 2016-7 x RVS 12-13	• Petal colour • Bracts type	• Red • Lanceolate	• Digenic • Digenic	• Complementary • Duplicate
2.	RSS 2016-7 x NARI-121	• Petal colour • Bracts type • Capitulum shape	• Yellow • Narrow Lanceolate • Beak shape	• Digenic • Digenic • Digenic	• Duplicate • Complementary • Duplicate
3.	A-1 x EC-755686	• Bracts type • Capitulum shape • Stigma colour	• Long lanceolate • Beak shape • Yellow	• Digenic • Digenic • Digenic	• Complementary • Duplicate • Duplicate
4.	A-1 x EC-755665	• Petal colour • Faded petal colour	• Red • Dark red	• Digenic • Digenic	• Duplicate • Duplicate
5.	A-1 x EC-755664	• Bracts type • Faded petal colour	• Lanceolate • Dark red	• Monogenic • Digenic	• Duplicate
6.	A-1 x NARI-121	• Faded petal colour • Stigma colour	• Faded yellow • Yellow	• Monogenic • Digenic	Inhibitory
7.	A-1 x NARI-120	• Bracts type • Faded petal colour • Stigma colour	• Broad lanceolate • Faded yellow • Yellow	• Digenic • Digenic • Digenic	• Duplicate • Duplicate • Complementary
8.	EC-755686 x RVS 12-13	• Petal colour	• Yellow	• Digenic	• Complementary
9.	EC-755686 x A-1	• Bracts type • Capitulum shape • Stigma colour	• Long lanceolate • Beak shape • Yellow	• Monogenic • Digenic • Monogenic	• Duplicate
10.	EC-755686 x NARI-119	• Petal colour • Stigma colour	• Yellow • Yellow	• Digenic • Monogenic	• Complementary

3.2 Linkage detection between the traits

When two or more genes are linked together, they are always inherited in the same combination for more than two generations. Morgan observed that two genes failed to follow Mendel's law when they were crossed because they did not divide according to the law. When two genes are located on the same chromosome, there is a substantially higher chance than with non-parental combinations that a parental combination would result. Linkage is the term used to describe how genes are physically connected. When genes for several characters are found in the same chromosomes and are thus coupled to one another, the genes are said to be linked.

3.2.1 Test of independence among qualitative traits in Safflower

In the present study, test of independence was calculated for detection of linkage to know its presence or absence. In the first cross between RSS 2016-7 X RVS 12-13, the segregation ratio in F_2 population for the trait petal colour was 9Red:7White, whereas for bracts type was 15lanceolate:1ovate.

Test of independence was performed as suggested by Panse and Sukhatme (1967) [10].

$$\chi 2 = \frac{(ad-bc)^2\ (a+b+c+d)}{(a+b)\ (a+c)\ (b+d)\ (c+d)}$$

For 1 degree of freedom
a = 93 b = 68
c = 134 d = 13

$$\chi 2 = \frac{(93 \times 13 - 68 \times 134\ )^2\ (93+68+134+13)}{(93+68)\ (93+134)\ (68+13)\ (134+13)}$$

$\chi^2 = 44.20$

This value of chi square was compared against table value of chi square at 5% or 1% level of significance for 1 degree of freedom. If calculated value was greater than tabulated value, then the traits was not independent and linked.

Here calculated value of chi square was 44.20 which were higher than the table value which was 3.841. It concludes that these two traits i.e., petal colour and bracts type was not independent and was linked.

As same like this, test of independence for all the previously mentioned crosses i.e. RSS 2016-7 X NARI-121, A-1 X EC-755686, A-1 X EC-755665, A-1 X EC-755664, A-1 X NARI-121, A-1 X NARI-120, EC-755686 X A-1 and EC-755686 X NARI-119 was calculated. The calculated value was then compared with tabulated value as listed below (Table 3).

Table 3: Comparison of calculated values with tabulated values of the crosses for various characters from test of independence

| Crosses	Characters	Classes		Calculated value	Tabulated value
RSS 2016-7 X RVS 12-13	Petals colour	93	68	44.205*	3.841
	Bracts type	134	13		
RSS 2016-7 X NARI-121	Petals colour	180	15	49.366*	3.841
	Bracts type	122	73		
	Petals colour	180	15	0.659	3.841
	Capitulum shape	184	11		
	Bracts type	122	73	58.323*	3.841
	Capitulum shape	184	11		
A-1 X EC-755686	Bracts type	126	89	69.451*	3.841
	Capitulum shape	200	15		
	Bracts type	126	89	58.510*	3.841
	Stigma colour	195	20		
	Capitulum shape	200	15	0.777	3.841
	Stigma colour	195	20		
A-1 X EC-755665	Petal colour	202	18	0.031	3.841
	Faded petal colour	203	17		
A-1 X EC-755664	Bracts type	151	57	46.164*	3.841
	Faded petal colour	201	7		
A-1 x NARI-121	Faded petal colour	266	102	13.246*	3.841
	Stigma colour	307	61		
A-1 X NARI-120	Bracts type	118	12	2.1487	3.841
	Faded petal colour	124	6		
	Bracts type	118	12	50.274*	3.841
	Stigma colour	66	64		
	Faded petal colour	124	6	65.762*	3.841
	Stigma colour	66	64		
EC-755686 X A-1	Bracts type	112	48	41.811*	3.841
	Capitulum shape	155	5		
	Bracts type	112	48	0.383	3.841
	Stigma colour	117	43		
	Capitulum shape	155	5	35.392*	3.841
	Stigma colour	117	43		
EC-755686 X NARI-119	Petal colour	98	61	2.748	3.841
	Stigma colour	112	47		

"*" – significant characters having calculated value greater than tabulated value.

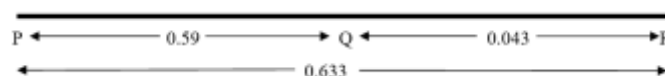### 3.3 Linkage estimation between the traits
In cross between RSS 2016-7 X RVS 12-13, the F_2 population shows segregation ratio for the trait petal colour was 9Red:7 White and for the trait bracts type it shows 15 Lanceolate: 1 Ovate. When test of independence was calculated among these two traits it was found that these traits were not independent and was linked. The linkage estimated among them was 0.573 map unit.
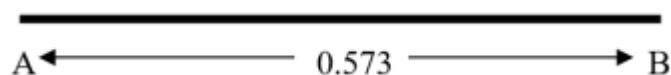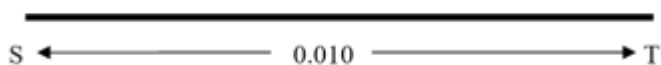

In cross between RSS 2016-7 X NARI-121, segregation ratio in F_2 population for the trait petal colour was 15 Yellow: 1 White and for the trait bracts type was 9Narrow lanceolate: 7 Broad lanceolate. Both two were linked characters and linkage between them was 5.63 map unit. In the same cross, segregation ratio for capitulum shape was 15 Beak shape: 1 Big flat. The linkage found among the two traits i.e bracts type and capitulum shape was 3.4 map unit.
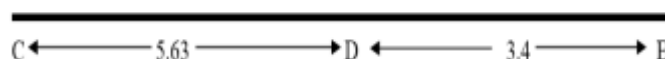
In cross between A-1 X EC-755686, the F_2 population shows segregation ratio for the trait bracts type was 9 Long lanceolate: 7 Short lanceolate and for the trait capitulum shape was 15 Beak shape: 1 Flat. Linkage estimated among them was 0.59 map unit. In the same cross, segregation ratio for the trait stigma colour was 15 Yellow: 1 Red. Linkage found among the traits bracts type and stigma colour was 0.633 map unit.

In cross between A-1 X EC-755664, F_2 population shows segregation ratio for the trait bracts type was 3 Lanceolate: 1 Extra narrow ovate and for the trait faded petal colour was 15 Dark red: 1 Off yellow. Linkage estimated between them was 0.010 map unit.
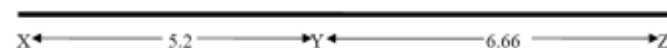
S ◄─────── 0.010 ───────► T

In cross between A-1 X NARI-121, F$_2$ population shows segregation ratio for the trait faded petal colour was 3 Faded yellow: 1 Dark red and for the trait stigma colour was 13 Yellow: 3 Red. Linkage estimated between them was 1.468 map unit.

K ◄─────── 1.468 ───────► L

In cross between A-1 X NARI-120, segregation ratio in F$_2$ population for the trait bracts type was 15 Broad lanceolate: 1 Long lanceolate and for the trait stigma colour was 9 Yellow: 7 Red. Both two were linked characters and linkage distance between them was 5.2 map unit. In the same cross, segregation ratio for faded petal colour was 15 Faded yellow: 1 Dark red. The linkage found among the two traits i.e faded

petal colour and stigma colour was 6.66 map unit.

X ◄─── 5.2 ──►Y ──── 6.66 ───►Z

In cross between EC-755686 X A-1, the F$_2$ population shows segregation ratio for the trait bracts type was 3 Long lanceolate: 1 Short lanceolate and for the trait capitulum shape was 15 Beak shape: 1 Flat. Linkage distance estimated among them was 0.045 map unit. In the same cross, segregation ratio for the trait stigma colour was 3 Yellow: 1 Red. Linkage distance found among the traits capitulum shape and stigma colour was 2.95 map unit.

U ─── 0.045 ───► V ◄─── 2.95 ───►W

The linked traits with its linkage distances in map unit have been summarized in the table 4.

Table 4: List of crosses showing linked traits and linkage distance between the traits

S. No.	Crosses	Linked traits	Linkage distance (map unit)
1.	RSS 2016-7 x RVS 12-13	• Petals colour • Bracts type	0.57
2.	RSS 2016-7 x NARI-121	• Petals colour • Bracts type	5.63
		• Bracts type • Capitulum shape	3.43
3.	A-1 x EC-755686	• Bracts type • Capitulum shape	0.59
		• Bracts type • Stigma colour	0.63
4.	A-1 x EC- 755664	• Bracts type • Faded petal colour	0.01
5.	A-1 x NARI-121	• Faded petal colour • Stigma colour	1.46
6.	A-1 x NARI-120	• Bracts type • Stigma colour	5.27
		• Faded petal colour • Stigma colour	6.66
7.	EC-755686 x A-1	• Bracts type • Capitulum shape	0.04
		• Capitulum shape • Stigma colour	2.95

The results of the current study were confirmed by Golkar *et al*. (2010) [3], Purkhyasta (2017), Purkayastha *et al*. (2017) [13] and Das (2018) [1] who observed that F$_1$ crosses between white and yellow petals produced offspring with yellow petals, but their F$_2$ generations were segregated in distinct ways, indicating two genes were responsible for the characteristic. Yellow petals crossed with white petals were controlled by a single gene and white petal colour was recessive relative to yellow petals, according to research by Joglekar and Deshmukh (1956) [5]. Paricharik and Kulkarni (1972) [11] observed dominance of red petal colour over white colour in a cross and it gets segregated in 9Red:7White ratio in F$_2$ generation. Present study also supports this finding. In all the crosses whichever were for capitulum shape, always beak shape was dominant trait over flat capitulum and in F$_2$ generation it gets segregated into 15 Beak shape: 1 Flat ratio being governed by duplicate gene action. For bracts shape, lanceolate bracts appeared dominant over ovate bracts always

and for stigma colour, yellow colour appeared as dominant trait over red coloured stigma in all the crosses. The findings of the linkage detection and estimation were supported by Purkhyasta (2017), Purkayastha *et al*. (2017) [13] and Das (2018) [1].

4. Conclusions
The present work greatly aids in understanding the Mendelian inheritance of the specified qualitative features and may be crucial to crop development programmes. Linkage detection was done in order to study linked traits in various above-mentioned crosses, test of independence was applied and linkage distance was estimated. The traits which were found to be linked were located in similar chromosome and always segregate together. These linked traits as for example in EC-755686 x A-1, the capitulum shape and stigma colour was linked, hence plant breeders can choose the colour of stigma according to need and along with that shape of capitulum also

comes along with. So, working on one desired trait can helps to get other desirable traits due to linkage. This can helps in various improvement programmes related to this oilseed crop.

7. Conflict of interest statement
The authors declare that they have no conflict of interest in the publication. All the information related to this article is well-known by all the authors.

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