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Screening of f₂ population in safflower for higher fatty acid content

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Abstract

The Present study was carried out at Research cum Instructional Farm, Department of Genetics and Plant Breeding, Indira Gandhi Krishi Vishwavidyalaya, Raipur (M.P.) during *rabi* of 2015-16. The present study is based on a cross between GMU 244 x GMU 1303 and its F₂ progeny study based on the segregation pattern of fatty acids and study of transgressive segregation. Over other allogamous oilseed crops, such as sunflower and rapeseed, safflower is characterized by high degree self-pollination which has greater advantage for commercial production of high oleic acid. It is a type of oilseed crop which consists of various composition of different fatty acids and this can be controlled by combination of various major genes and some changes in environment condition. The association of markers with traits of study is estimated by their positive average additive effect with different traits. In present study SSR marker SES 33 was found to be linked with number of seeds per capitulum and seed yield per plant. Similarly, SSR marker SF 18 was observed to be linked with plant height, number of branches per plant, number of capitulum per plant, seed yield per plant and oil %. SSR marker SES144 was found to be associated with yield contributing traits like plant height, number of capitulum per plant and oil %. SSR marker SES 99 was found to be associated with traits, plant height, and number of seeds per capitulum, seed yield per plant and oil %. In this study SSR marker SES 86 was observed to be linked with number of branches per plant and oil %. Marker SES 85 was found to be linked with number of branches per plant, oil %, number of seeds per capitulum and seed yield per plant. The SSR marker SES 81 was found to be linked with plant height.

Keywords: Safflower, fatty acids, oil%, MAS

Introduction

Safflower (*Carthamus tinctorius* L.) is one of the oldest domesticated crops. It has been grown since ancient times both for dye as well as oilseed crop in a wide range of geographical regions (Knowles, 1976)^[2]. It is a member of the family *Compositae* or *Asteraceae*, genus- *Carthamus*, tribe- *Tubiflorae*, sub division-*Angiosperm* of division- *Phanerogams*. It is the only cultivated type of safflower that contains 2n=24 chromosomes (Singh, 2007)^[7]. Safflower is a minor crop currently regarded as promising alternative for oilseed production in many areas of the world. The nutritional value of safflower oil is related to its high level of mono and polyunsaturated oils (Weiss, 2000)^[9]. Safflower oil contains about 75% linoleic acid that is essential for human nutrition (Weiss, 2000)^[9].

Oilseed crops are mostly known for its vegetable oil having nutritional value, its industrial importance or use and some pharmaceutical purposes. Safflower is one of the cultivated oilseed crop which contains various mono and polyunsaturated fatty acid which has high nutritional value especially for the cardiac patients as it helps in reducing the accumulation of cholesterol in blood veins. Its products in the world markets show great potential with contribution to edible oil which are highly nutritious. As olive oil also consists of large amount of mono and poly unsaturated fatty acid but our country has to import this from other different countries which make rise in foreign currencies. So for the same properties safflower production is enhanced in recent years. It consists of high oleic acid, linoleic acid and linolenic acid. Oleic acid and Linoleic acid account for about 90% of total fatty acids and remaining 10% are other fatty acids. High oleic acid containing vegetable oils are increasingly appreciated in edible oil markets combining hypocholesterolemic effect (Mensik and Katan, 1989)^[4]. Safflower seeds has shown great variability for fatty acid composition. Sim-et-al

(1961)^[8] reported, during first 30 days in developing seeds, oleic acid concentration increases slowly and then appeared level off in some cases. Over other allogamous oilseed crops, such as sunflower and rapeseed, safflower is characterized by high degree self-pollination which has greater advantage for commercial production of high oleic acid. It is a type of oilseed crop which consists of various composition of different fatty acids and this can be controlled by combination of various major genes and some changes in environment condition (Knowles, 1989)^[1]. There is negative correlation between oleic acid concentration and oil yield as if an improvement is done for oil yield in safflower, it could decrease oil content. Thus the purpose of present study was screening of safflower F_2 population between cross of two genetically diverse parents GMU 224 X GMU 1303 and if possible to find some transgressive segregants having high oleic acid content with average seed yield.

Materials and Methods

The Present study was carried out at Research cum Instructional Farm, Department of Genetics and Plant Breeding, Indira Gandhi Krishi Vishwavidyalaya, Raipur (M.P.) during *rabi* of 2015-16. The present study is based on a cross between GMU 244 x GMU 1303 and its F_2 progeny study based on the segregation pattern of fatty acids and study of transgressive segregation. These parents were diverse for many traits such as petal colour, spininess, leaf shape and oil content etc. In present study F_2 population was used to record the segregation pattern of different traits.

Parents, F_1 and F_2 populations were grown during the year 2015-16. In F_1 10 plants were raised along with the parents. F_2 population of cross (GMU 244 X GMU 1303) were raised in bulk. Observations of all individual plants of F_2 population were recorded, separately. The study observations for contrasting traits were recorded on 500 plants in F_2 population. In F_2 population each plant was tagged with a number and different observations i.e. plant height, number of capitulum per plant, number of branches per plant, number of seeds per capitulum, seed yield per plant (gm), days to flowering and oil (%) were recorded for each individual plants. The oil content (%) was measured on whole seeds (~ 20 g of sample) using nuclear magnetic resonance (NMR) spectroscopy as described by Yadav and Murthy (2016)^[6]. Morphological and fatty acid profiling dendrogram of F_2 generation were generated through SPSS software. Correlation and path analysis were done with the help of SPSS software.

Table 1: Distinguishing traits of parents

S. No.	Parents	Leaf characters	Petal colour	Oil content
1	GMU 224	Spiny leaves and bracts	Yellow petals	33%
2	GMU 1303	Non spiny leaves and bracts	Orange petals	24%

Fatty acid profiling

The fatty acid profiling of Parents and 246 plants of F_2 were carried out at laboratory of Bhabha Atomic Research Centre (BARC), Anushakti Nagar, Trombay, and Mumbai. Crushed seeds are mixed with 0.25 N 1 ml Na methylate and 1 ml methanol. After vortex, the samples were leave for 30 minutes in room temperature then 1 hour in water bath at 50 C, leave the samples at room temperature for 5 minutes so that the temperature were lower down. Add 2/3 petroleum ether (HPLC) then add 2 ml deionized water. The samples were vortex and pour 0.5 ml to 1 ml samples to GC vials to use in Gas Chromatography machine.

Table 2: Fatty acid profiling of parental lines

S. No.	Parents	Palmitic acid	Stearic acid	Oleic acid	Linoleic acid
1	GMU 224	5.9	2.5	13.2	78.3
2	GMU 1303	6.8	2.8	9.6	80.7

Molecular analysis for QTL study

The molecular study was conducted in Marker Assisted Selection (MAS) laboratory, Dr. R. H. Richharia Laboratory, Department of Genetics and Plant Breeding, College of Agriculture, Indira Gandhi Krishi Vishwavidyalaya, Raipur. In molecular marker studies, extracting DNA from a large number of plant accessions is difficult in plants that have high levels of polysaccharides and secondary metabolites (Pandya *et al.* 1996)^[5]. A simple, rapid, economical and effective DNA extraction method is highly desirable. In present investigation CTAB miniprep method was used to study the 94 randomly selected samples from F_2 population and parents with Simple Sequence Repeat makers. Very less molecular work in Safflower crop is so far done and trait related markers are also not much known.

Out of which genotyping was done only in 94 plants from F_2 , along with parents. For generating genotypic data, a set of 50 SSR markers of safflower were used in present study were specific to oil traits and these markers were taken from previously published research paper on Safflower (Kumari, 2010)^[3] and designed from Primer 3. Only eight markers (SF18, SES 33, SES 81, SES 85, SES 144, SES 143, SES 99 and SES 86) exhibited polymorphism between two parents. Further, these 8 markers were used to generate genotypic data of F_2 population of cross between GMU 224 and GMU 1303. Primers showing polymorphisms were further used for PCR amplification with all of the 94 lines along with parents using standardized PCR protocol.

The clear and unambiguous bands of markers were scored for the presence or absence of the corresponding bands among the genotypes. The score 1 and 0 indicates the presence and absence of the bands repeating.

Table 3: List of EST-SSR primers showing polymorphism

S. No.	EST-SSR Primers	Primer sequences	
		Forward (5' → 3')	Reverse (5' → 3')
1	SES-33	CGTTCTAGGACGACTACTCC	ACTGCTTTTGTCTCTTTCC
2	SES-81	GCAATACCATCATCATCTCAC	AGGAGGTGAAAGGGAAGAG
3	SES-85	GGGTTCACCTTCTTCTCTCTC	AGTACTCCTCCAGTGACATACAG
4	SES-86	ACCCTAGATTTCATTCATTC	GATTACAGTCTGAGAAACATCG
5	SES-99	TTCTCTACTCTTCACGATTGG	CCATCTGTCTTAAGCTGTTCC
6	SES-143	ACCACCTCATGCTCTTACC	AGCTATGAGTAGGAAGAATTGG
7	SES-144	CACCACCTCATGTTCTTACC	GAGGAGAAGAGAGTTTACAACC
8	SF -18	GCATATTTTGGATGTTGATATG	AATTAACAATGCAGCTCAATC

Results and Discussion

Table 4: Correlation matrix for variable of fatty acid recorded in F₂ population

Traits	Palmitic	Stearic	Oleic	Linoleic	Linolenic
Oil content (%)	-0.046	0.092	0.04	-0.045	0.093
Palmitic		0.119	-0.646**	0.627**	0.075
Stearic			-0.138*	0.103	0.138*
Oleic				-0.999**	-0.154*
Linoleic					0.145*

It was observed that oil has positive correlation with linolenic acid content. Palmitic acid showed positive correlation with stearic acid and significant positive correlation with linoleic acid whereas, significant but negative correlation with oleic acid content. Stearic acid exhibited significant positive correlation with linolenic acid whereas, significant negative correlation with oleic acid content. The oleic acid showed significant negative correlation with linolenic acid content and with linoleic acid. Linoleic acid showed significant positive correlation with linolenic acid content in safflower. This indicates that if oil content increased in safflower, linolenic acid content would increase simultaneously. Palmitic acid and stearic acid content shows significant negative correlation with

oleic acid, hence to develop a variety with high oleic acid needs to reduce palmitic acid and stearic fatty acid in it. Oil and oleic acid both exhibited negative correlation with linoleic acid content. This indicates that decreasing the quantity of linoleic acid will increase the oil and oleic acid content both in safflower.

Table 5: Path analysis among Oil content (%) and different fatty acids in F₂ population

Characters	Palmitic	Stearic	Oleic	Linoleic	Linolenic
Palmitic	0.045	0.003	-0.850	0.812	-0.001
Stearic	0.003	0.014	-0.100	0.073	-0.001
Oleic	-0.850	-0.100	38.155	-37.506	0.050
Linoleic	0.812	0.073	-37.506	36.942	-0.047
Linolenic	-0.001	-0.001	0.050	-0.047	0.003

The Morphological dendrogram of F₂ lines between parents GMU 224 and GMU1303 with 94 selected lines, shows that most of the plants in F₂ is of GMU 1303 and only 3 genotypes are of GMU 242 type.

The path analysis shows that oleic and linoleic acid has high direct effect, hence the lines showing high oleic and linoleic acid and the transgressive segregants can be further utilize as a parents in hybridization programme.

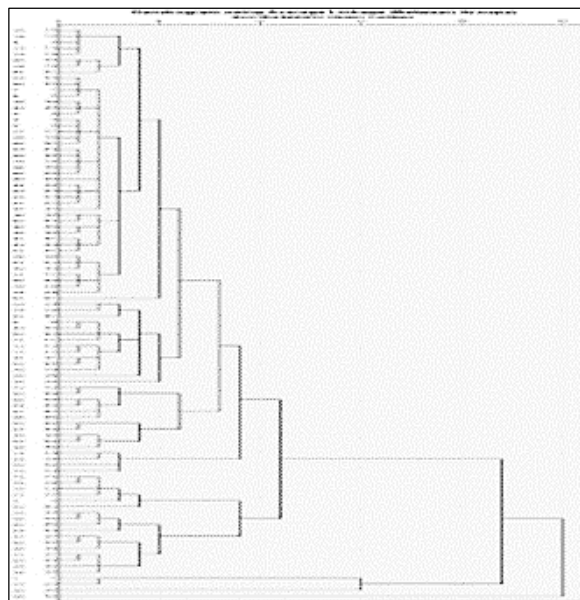


Fig 1: Morphological dendrogram of F₂ lines between parents GMU 224 and GMU1303. Line number 1 and 2 number denotes GMU 224 and GMU 1303 respectively.

The Morphological dendrogram of F₂ lines between parents GMU 224 and GMU1303 with 94 selected lines, shows that

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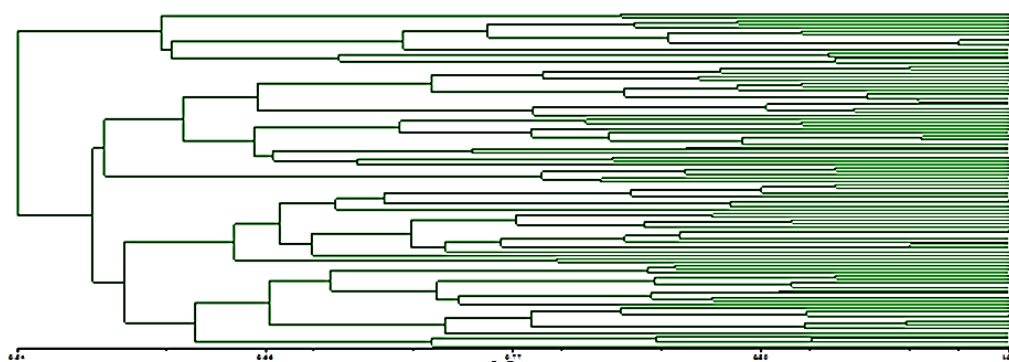


Fig 2: Molecular diversity analysis of 94 genotypes along with parents with the help of NTYSIS ver 2.2 software.

Molecular diversity analysis of 94 selected segregating lines along with parents were done. This also shows that only few lines were like GMU 224 type and others are of GMU 1303 type. Total 50 primers were designed from primer 3. out of 50

only 8 primers shows polymorphic between two parental genotypes which were further used in selected segregating genotypes.

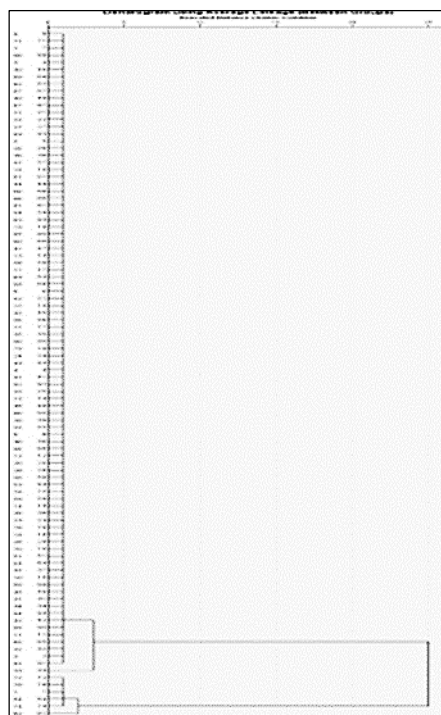


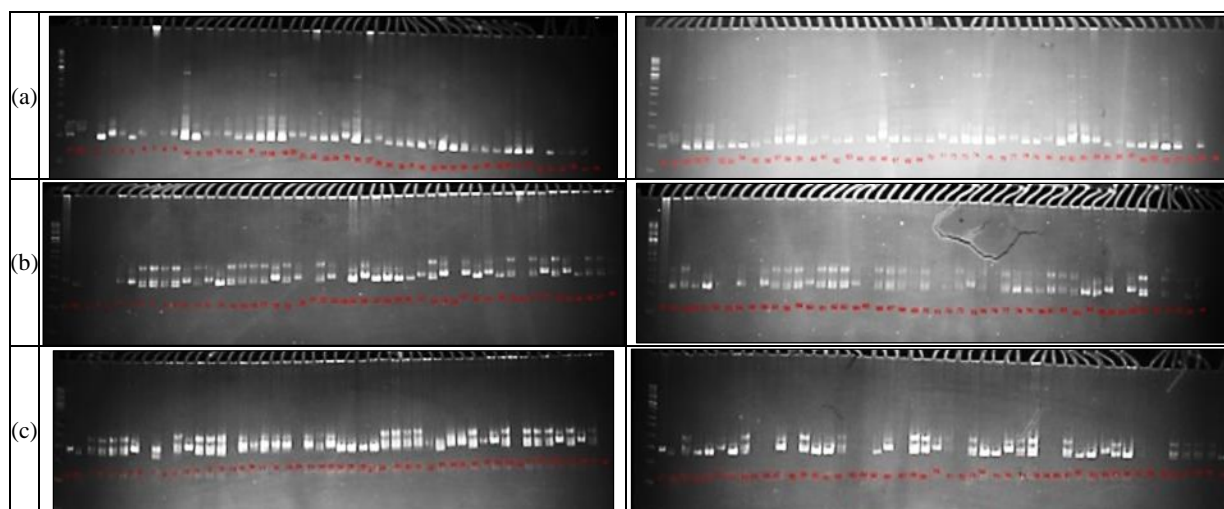
Fig 3: Dendrogram of different fatty acid of F₂ lines between parents GMU 224 and GMU1303. Line number 1 and 2 number denotes GMU 224 and GMU 1303 respectively.

Fatty acid diversity analysis shows that most of the F₂ plants is of GMU1303 and only 3 genotypes are of GMU 224 type. out of 500 lines in F₂ 237 plants were selected for fatty acid profiling with the help of GCMS machine and out of these only 94 were selected for molecular study. In line no. 73,74,76,79,110,115,119,137 there is vast increase in oleic acid and 43, 48, 62, 64, 66, 75, 96, 106, 112, 125, 135, 142, 158, 168, 198, 213, 231 lines has high linoleic acid this is due to the phenomena of transgressive segregation. There were some lines was also present showing moderate oleic and linoleic acid like line no. 40,55,78,85,109,111,120, 153. As in this, F₂ population shows variation from their range of parents. Therefore, the purpose of the study was to make the variety having high oleic acid content which can help especially cardiac patients in reducing the probability of accumulation of

Cholesterol in blood veins.

Single Marker Analysis (SMA)

The concept of detecting QTLs using linked major genes was given by Sax (1923). It is the earliest and simplest method of QTL analysis. It is based on the idea, that if there is an association between marker genotype and trait value, then it is likely that a QTL is close to that marker locus. Thus it finds association between marker genotype and trait value. It involves regression analysis. It is used for quick scanning of the entire genome to find out best possible QTL's. It is also used to identify missing or incorrectly formatted data. There are two main limitation of this method, first, it underestimates QTL number and second, it cannot determine QTL position precisely. The results obtained in this study are as follows:



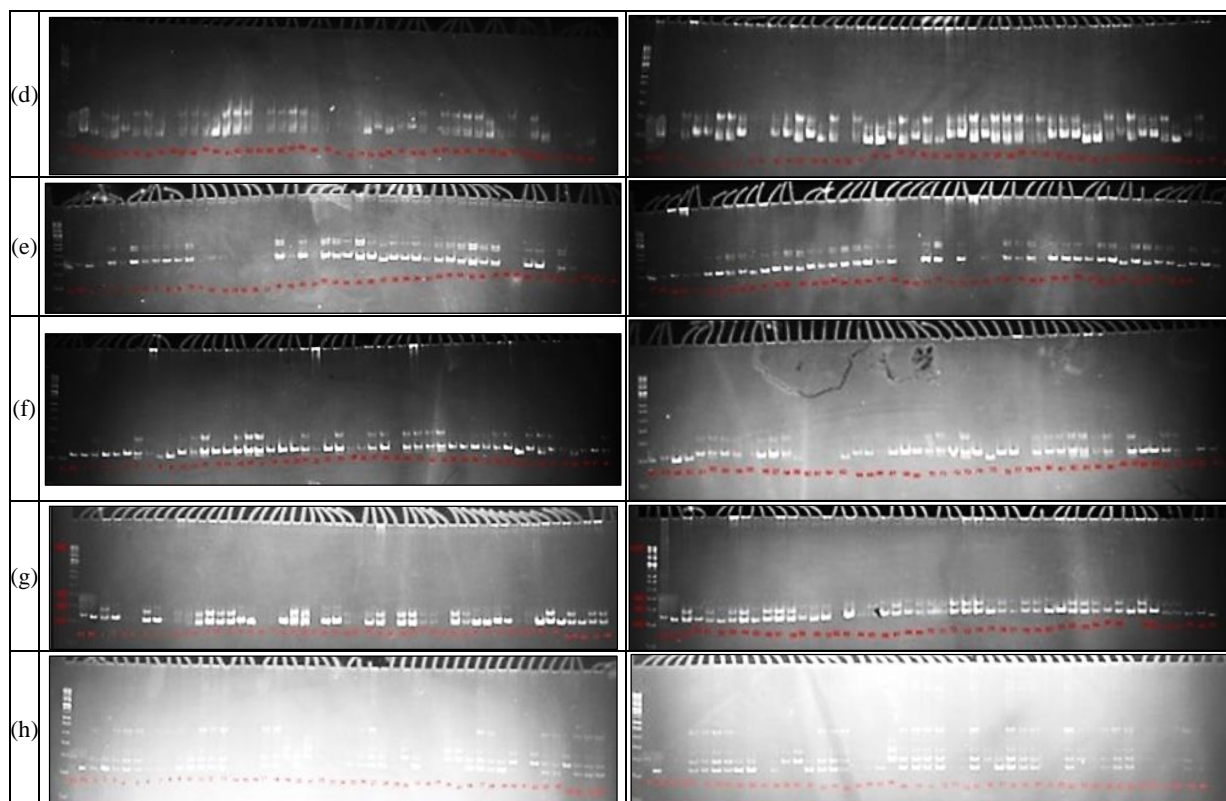


Fig 4: Page gel using microsatellite marker SES 81,SES 85,SES 86,SES 99,SES 143,SES 144,SF 18,SES 33 for 94 F₂ populations and parents.

Table 7: Association based on phenotypic variance of different traits

S. No.	SSR Marker	Associated trait
1	SES 33	Seeds per capitulum
2	SF 18	Plant height (oil specific)
3	SES 143	Number of branches, Number of capitulum per plants
4	SES 99	Days to flowering, Number of capitulum per plants
5	SES 81	Days to flowering

*0.06= significance level

The association of markers with traits of study is estimated by their positive average additive effect with different traits. In present study SSR marker SES 33 was found to be linked with number of seeds per capitulum and seed yield per plant. Similarly, SSR marker SF 18 was observed to be linked with plant height, number of branches per plant, number of capitulum per plant, seed yield per plant and oil %. SSR marker SES144 was found to be associated with yield contributing traits like plant height, number of capitulum per plant and oil

%. SSR marker SES 99 was found to be associated with traits, plant height, and number of seeds per capitulum, seed yield per plant and oil %. In this study SSR marker SES 86 was observed to be linked with number of branches per plant and oil %. Marker SES 85 was found to be linked with number of branches per plant, oil %, number of seeds per capitulum and seed yield per plant. The SSR marker SES 81 was found to be linked with plant height.

Table 8: Association analysis based on average additive effect

S. No.	SSR Marker	Associated trait
1	SES 33	Number of seeds per capitulum and seed yield per plant
2	SF 18	Plant height, number of branches per plant, number of capitulum per plant, seed yield per plant and oil %.
3	SES 144	Plant height, number of capitulum per plant and oil %
4	SES 99	Plant height, number of seeds per capitulum, seed yield per plant and oil %.
5	SES 86	Number of branches per plant and oil %.
6	SES 85	Number of branches per plant, oil %, number of seeds per capitulum and seed yield per plant
7	SES 81	Plant height

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