RESEARCH ARTICLE

Isolation of High Yielding Early Mutants of Sesame (*Sesamum indicum* L.) Based on the Relationship Between Traits Governing Yield and Earliness

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ABSTRACT

Sesame is a leading oilseed crop known for its quality edible oil and byproducts. Mutation breeding is an effective means of creating new genetic variability as certain desirable traits like uniform maturity, non-shattering capsules etc., do not exist in nature..The present study aimed to isolate high yielding early mutants of sesame on the basis of relationship between traits governing yield and earliness. Gamma-ray doses of 300 Gy, 350 Gy, and 400 Gy and combination treatments of gamma-ray + EMS: 300 Gy + 10 mM, 350 Gy + 10 mM, and 400 Gy + 10 mM were used in this study. In M2 generation, putative mutants with high yield and earliness were isolated and characterized individually to study the nature of the association of traits with single plant yield in early maturing lines. Two traits viz., increased height at the beginning of flowering and reduced days to the beginning of flowering were found to have a favorable effect on reducing crop period and increasing single plant yield. Therefore, these two traits should be used as a criterion for selection for achieving high yield with earliness in sesame. In addition, the 'tri-leaf' characteristic observed in the putative 'early mutants' led to increased single plant yield. Early lines of sesame with high yield and uniform maturity can enable mechanical harvesting in future sesame cultivation. The selected mutants may be forwarded to further generations and utilized in sesame breeding.

Keywords: *Sesame; Mutation; Earliness; Yield*

INTRODUCTION

Sesame (*Sesamum indicum* L.), a member of the Pedaliaceae family, is a traditionally cultivated oilseed crop called "Queen of oilseeds" because of its high oil content (45-55 %), quality edible oil with rich nutritional quality, industrial and medicinal values (Pathak *et al.,* 2017). Sesame originated from Africa and is now an important crop in the world. Globally sesame is cultivated in 8.8 million ha area with average productivity of 382 kg/ha. India, Sudan, and China are the leading producers of sesame with a large number of varieties. White seeded sesame fetches higher demand in the market for its higher shelf life and confectionary values (Jayaramachandran *et al*., 2020). The indeterminate nature of the crop makes it unsuitable for mechanical harvesting and hence the harvest of sesame is done manually. Early maturity in sesame is important for the crop to escape from biotic and abiotic stresses occurring in later stages of the crop, for efficient usage of nutrients, avoiding monsoonal problems and uniform maturity reduce shattering losses and makes it possible to adopt mechanical harvesting (Yousif and Babiker, 2018). Selection and pedigree breeding methods are commonly used in sesame for crop improvement but are helpful only when the desired traits are available in the existing germplasm.

To create new genetic variability, mutation breeding is an important tool. The key potential mutants reported in sesame with traits like good seed retention, shorter plant height, higher harvest index, uniform maturity, and reduced biomass were developed using mutational breeding. Physical mutagens (i.e., gamma-ray and X-ray) and chemical mutagens (i.e., Ethyl methane sulphonate (EMS) and sodium azide) are widely used to induce mutations in sesame. The combinational effect of physical and chemical mutagens has also been reported in sesame (Saha, 2018). In sesame, Phytophthora resistance, non-shattering capsule type, and determinate plant type were successfully developed using mutation breeding (Kumari *et al.,* 2019). The first determinate mutant (dt 45) was identified in Israel in 1981 (Ashri, 1981). Later a new determinate mutant (dt1) similar to dt 45was reported and found to be free of drastic changes present in dt 45 mutant (Cagirgan, 2006). Jayaramachandran *et al*. (2020) identified extra early mutants that matured in 70 days from the sesame variety, TMV 5 variety using 15 mM EMS treatment. Twenty-three early flowering mutants were identified from treatment with different doses of gamma-ray in three cultivars (Rajput *et al*., 2001). In the case of yield attributing traits, the number of capsules increased by 15 % and 32 % than the untreated control, the number of branches per plant improved by 17.5 % and 21.5 % than control upon treatment with gamma-ray and EMS, and the number of seeds per capsules increased by 6.98 % using EMS and thousand seed weight increased by 4.63 % using gamma-ray. Single plant yield improved by 24.8 % and 56.17 % using gamma-ray and EMS (Ganesan, 2001; Begum *et al*., 2010).

Isolation of high-yielding early mutants with synchronous maturity can change the plant architecture and make sesame suitable for modern farming practices like mechanical harvesting (Uzun and Cagirgan, 2006). Generally, the yield levels of early synchronous maturing sesame are expected to be lower than that of the naturally occurring indeterminate sesame. Hence, selection based only on earliness and determinate growth habits in sesame may lead to a plant with the following architecture: short plant height, low number of capsules bearing nodes, thin leaves, short flowering period, and low yield. Hence, the focus should be not only on the criteria of earliness and uniform maturity but, also other traits that increase single plant yield (Day *et al*., 2002). Poor branching in early lines due to lack of time for the development of multiple branches leads to fewer capsules and poor yield (Langham 2007). Earliness can be achieved by speeding up the following phases in the life cycle: vegetative, reproductive, ripening, and drying. With this backdrop, the study was carried out to isolate early maturing putative mutants with uniform maturity and high yield based on different selection criteria and to study the association of these traits with yield.

MATERIAL AND METHODS

Plant Genetic material

Genetically pure seeds of the white seeded sesame variety VRI 3 were obtained from Regional Research Station, Virudhachalam, and used for this study. VRI 3 was developed from the cross between SVPR 1 and TKG 22 and has an oil content of 49% and exhibits uneven maturity of capsules and has medium duration.

Mutation treatment

In this experiment, both gamma-ray treatment and gamma + EMS combinational treatments were used. Genetically pure and clean seeds of VRI 3 (5000 nos.) were subjected to irradiation with the following gamma-ray doses at the gamma chamber at BARC, Mumbai, followed by combinational treatment with 10 mM EMS. Totally six treatments were used and they are: T_1 -300 Gy, T_2- 350 Gy, T_3- 400 Gy, T_4 - 300 Gy + 10 mM EMS, T_5 - 350 Gy + 10 mM EMS and T_6 - 400 Gy + 10 mM EMS.

Development of mutants

The field experiments were conducted in the Experimental farm, Agricultural College and Research Institute, Madurai, Tamil Nadu, India (latitude and longitude of 9.9252° N, 78.1198° E). Mutagen-treated seeds were used to raise M_1 generation during Kharif, 2018, supplied with low nutrient input and closer spacing (40×10 cm) to avoid multiple branching. Only capsules from the main stem of individual plants were harvested. Five capsules from each plant of M_1 generation were forwarded to M_2 generation and raised during summer, 2019 with normal spacing (30 × 30 cm). Recommended nutrients were provided during the crop growth period.

Evaluation of agronomic traits

In M_2 generation the traits viz., height at the beginning of flowering (HTBF), height at the termination of flowering (HTTF), days to the beginning of flowering (DBF), days to termination of flowering (DTF), number of nodes at the beginning of flowering (NOBF), number of nodes at the termination of flowering (NOTF), degree of stem termination (DST), flowering period (FP), growth during flowering (GF), number of capsules (NOC), capsule length (CL), single plant yield (SPY) and crop period (CP) were recorded following Wongyai, 1997. Desirable qualitative trait variations associated with these putative mutants were also recorded.

Statistical analysis

The relationship between earliness and yield-related traits was assessed using correlation and path analysis. The software used for statistical analysis are as follows; Correlation and path analysis- GENRES Statistical software 7.01, Correlation plot drawing- 'R' version 3.6.0.

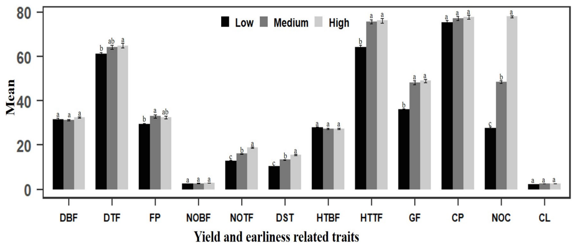
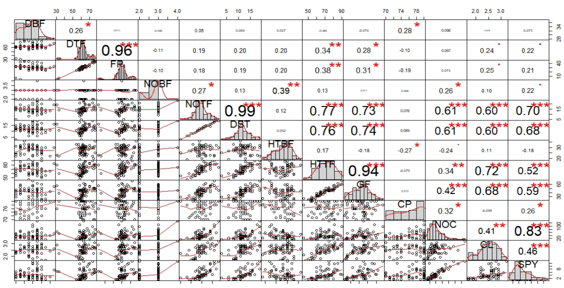


Figure 1. Trait values of low, medium, and high single plant yielding early mutants



(***, **, * indicate significant level on 0.001,0.01 and 0.05 %)

Fig 2. Correlation of earliness and yield-related traits

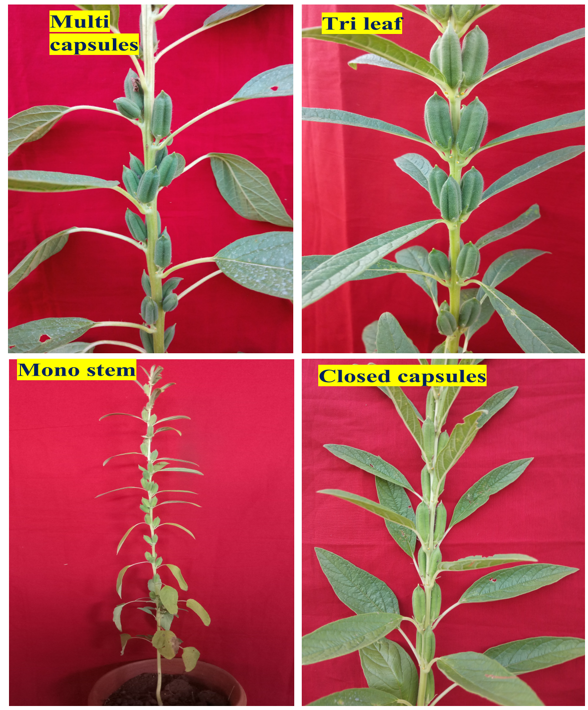


Fig 3. Qualitative traits associated with Early mutants

Table 1. observed value of early sesame mutants with high single plant yield

Mutant	DBF	DTF	FP	NOBF	NOTF	DST	HTBF	HTTF	GF	CP	NOC	CL	SPY	Qualitative trait
Control	35.5	76.75	41.25	3.5	18.1	14.6	32.25	83.58	51.33	90	79.85	2.53	7.1	Single capsule
T1_1	33	77	44	3	16	13	25	75	50	79	80	2.7	7.8	Parental type
T1_2	32	65	33	3	19	16	28	70	42	79	65	2.6	6.8	Mono stem + multi capsules
T1_3	32	70	38	3	19	14	32	85	55	73	19	2.6	12	Closed capsules
T1_4	32	58	26	3	21	18	23	81	58	80	110	2.47	11.7	Multi capsules
T2_1	32	74	42	2	21	19	22	90	68	78	96	2.83	13.2	Tri leaf
T2_2	33	69	36	3	19	16	28	75	47	77	70	2.87	11.3	Parental type
T3_3	31	58	27	3	15	12	28	73	45	78	60	2.8	8.6	Mono stem
T3_4	33	63	30	2	17	15	23	85	62	78	47	2.73	7.3	Parental type
T3_5	29	58	29	3	13	10	25	65	40	78	68	2.33	7.5	Parental type
T3_6	34	75	41	2	15	13	20	71	51	77	58	2.16	7.8	Multi capsules
T3_7	34	67	33	3	16	13	24	65	41	76	85	2.57	9.8	Tri leaf
T3_8	33	63	30	3	22	19	32	82	50	78	58	2.9	8	Parental type
T3_9	33	63	30	4	23	19	30	75	45	80	132	3.17	13	Tri leaf
T4_1	37	70	33	3	19	16	30	84	54	80	51	2.63	7.6	Mono stem

Table 2. Path coefficient of earliness and yield-related traits with single plant yield

Traits	DBF	DTF	FP	NOBF	NOTF	DST	HTBF	HTTF	GF	CP	NOC	CL
DBF	-1.224	1.211	0.110	0.015	0.137	-0.143	0.071	0.498	-0.575	0.021	0.070	0.000
DTF	-0.594	1.799	-1.733	0.035	0.426	-0.422	0.520	-1.263	1.216	-0.008	0.071	-0.001
FP	0.032	1.767	-1.763	0.032	0.402	-0.396	0.518	-1.287	1.240	-0.014	0.053	-0.001
NOBF	0.111	-0.914	0.796	-0.307	0.619	-0.270	1.046	-0.966	-0.086	0.001	0.192	0.000
NOTF	-0.136	1.009	-0.935	-0.084	1.227	-1.206	0.314	-1.587	1.567	0.006	0.447	-0.001
DST	-0.154	1.062	-1.050	-0.040	1.224	-1.208	0.139	-1.584	1.580	0.007	0.443	-0.001
HTBF	-0.060	1.056	-1.049	-0.121	0.268	-0.109	1.066	-1.033	-1.037	-0.020	-0.172	0.000
HTTF	0.146	1.275	-1.286	-0.039	1.074	-1.059	0.460	-1.766	1.734	-0.006	0.250	-0.002
GF	0.165	1.221	-1.235	0.003	1.046	-1.055	-0.465	-7.192	1.7814	0.001	0.307	-0.001
CP	-0.631	-0.836	1.446	-0.002	0.173	-0.185	-0.720	0.604	0.105	0.075	0.235	0.000
NOC	-0.216	0.777	-0.557	-0.081	1.394	-1.271	-0.630	-1.264	1.330	0.024	0.727	-0.001
CL	0.018	1.093	-1.092	-0.032	1.036	-1.026	0.280	-1.555	1.535	-0.007	0.299	-0.002

Bold values indicate direct effect; Residual effect: 0.324

RESULTS AND DISCUSSION

In the present study, the parental control registered days to maturity of 90 ± 4 days and single plant yield of 7.1 ± 0.50 g. In the M_2 generation, single plant yield ranged from 2.5 g to 15 g, and days to maturity varied from 68 days to 127 days across treatments (data not shown). Among them, 64 M_2 plants were earlier than the parent. Among the early mutants, 14 putative mutants were both earlier and had higher single plant yield than the parent. Out of 14 mutants, seven mutants were isolated from 400 Gy gamma-ray treatment, four from 300 Gy gamma-ray treatment, two from 350 Gy, and one from the 300 Gy + 10 mM gamma-ray and EMS combinational treatment (Table 1). Among the 14 putative early mutants, 13 were obtained through gamma irradiation and one from combinatorial treatment, suggesting the suitability of gamma-ray treatments in the induction of early mutants. Ashri (2001) also successfully used gamma-ray treatments for the induction of early and short flowering period mutants.

Based on single plant yield (SPY) the putative early mutants were grouped into low, medium, and high yield variants. All the above-mentioned traits were recorded for all the 14 putative early mutants. Wide variation was observed in the trait values for low, medium, and high SPY putative mutants. The high SPY group registered higher values than the low and medium SPY groups for most of the observed traits. The high SPY group recorded higher values for the traits viz., height at the termination of flowering, growth during flowering, number of nodes at the termination of flowering, degree of stem termination, number of capsules, and capsule length (Fig 1) than the low and medium SPY group. This suggests that due attention paid to these traits during selection could lead to the isolation of high-yielding early lines in sesame. Sheeba *et al.* (2003) isolated sesame mutants with single plant yields ranging from 4.03 g (700 Gy) to 6.75 g (500 Gy). Pawar and Monpara (2016) reported short flowering period could induce earliness with uniform maturity in sesame. In our study, one putative mutant (T1_4) with a flowering period of 26 days exhibited uniform maturity of capsules (Table 1) and appeared promising. However, the usefulness of this putative mutant depends on its true-breeding nature, which has to be confirmed in the next generation. Day (2000) reported a sesame cultivar with less than 25 days flowering period, which had determinate growth habit and reduced shattering loss.

Correlation and path analysis analyses were carried out to gather additional information on the association between these earliness-related traits and yield components. Based on the correlation study, it was observed that height at the beginning of flowering was negatively correlated with single plant yield. In the present study, the traits viz., days to termination of flowering, nodes at the termination of flowering, degree of stem termination, height at the termination of flowering, growth during flowering, number of capsules, and capsule length were found to be significantly positively correlated with single plant yield (Fig 2). Ismaila and Usman (2012) also observed that height at flowering negatively correlated with single plant yield. On the other hand, the number of capsules, capsule length, and height at maturity positively correlated with single plant yield in sesame mutants.

Days to maturity emerged as the most important earliness-related trait by registering a significant and positive correlation with days to the beginning of flowering and a negative correlation with height at the beginning of flowering. Height at the beginning of flowering was found to be negatively correlated with single plant yield (Fig 2). In this study, short flowering period variants had high yield and earliness and were identified as desirable. Long flowering periods tend to reduce the time available for seed maturation. However, Wongyai (1997) reported that a short flowering period reduced the capsule-bearing nodes, leading to poor yield. A subsequent study by Day *et al*. (2002) confirmed that short seed maturation period rather than short flowering period reduced yield. Hence, it is possible to get high-yielding early maturing sesame with a reduced flowering period.

The uniformity-related trait of the 'flowering period' was found to be positively correlated with nodes at the termination of flowering (NOTF) and growth during flowering (GF) and capsule length. Growth during Flowering (GF) was found to be positively correlated with nodes at the termination of flowering (NOTF), Degree of stem termination (DST), number of capsules (NOC), and capsule length (Fig 2). Day *et al.* (2002) reported that the flowering period positively correlated with capsule bearing nodes, capsule zonal length, and plant height, which agrees with the results of our study. Therefore, selection for the reduced flowering period and increased seed and capsule maturation period would increase seed yield in early lines of sesame.

The direct yield-related trait, number of capsules (NOC), was positively correlated with plant height at the termination of flowering, nodes at the beginning of flowering and Growth during flowering, degree of stem termination, and days to maturity. In addition, capsule length recorded a positive correlation with the following traits: height at the termination of flowering, nodes at the termination of flowering, degree of stem termination, number of capsules, and growth during flowering (Fig 2). Similar results were reported by Begum and Dasgupta (2011), who found the number of capsules to be positively correlated with plant height, duration of flowering, single plant yield, and capsule length associated with plant height and the number of capsules per plant.

From the above information, it is clear that there exists a very complex association between the traits. Many traits affect the desirable combination of earliness with a high single plant yield. Path analysis splits up the total correlation into direct and indirect effects. In our study, days to termination of flowering (DOTF), nodes at the termination of flowering (NOTF), height at the beginning of flowering (HTBF), growth during flowering (GF), and the number of capsules (NOC) were found to have a positive direct effect on yield. Hence, these traits can directly be used as selection indices for increasing seed yield in sesame.

Similarly, days to the beginning of flowering (DBF), flowering period (FP), nodes at the beginning of flowering (NOBF), degree of stem termination (DST), and height at the termination of flowering (HTTF) had a negative direct effect on single plant yield (SPY) (Table 2). So, during selection, plants with lower values for the traits viz., days to the beginning of flowering, flowering period, nodes at the beginning of flowering, degree of stem termination, and height at the termination of flowering can help to increase yield. Some of the traits viz., days to the beginning of flowering (DBF), flowering period (FP), nodes at the beginning of flowering (NOBF), degree of stem termination (DST), height at the termination of flowering (HTTF) and capsule length (Table 2) showed positive correlation but negative direct effect on single plant yield. This positive correlation might be due to the influence of other traits, and such traits should not be selected directly but improved through other traits. Navaneetha *et al*. (2019) reported similar results for plant height which showed a positive correlation and negative direct effect on single plant yield.

Height at the beginning of flowering (HTBF) was negatively correlated with SPY but had a high direct effect on yield. It affects the yield through the negative indirect effect of plant height and the number of capsules (Table 2). Our purpose should be to reduce days to maturity with increased yield to obtain early maturing sesame with high yield. In our study, the trait favouring reduced days to maturity and increased single plant yield was the height at the beginning of flowering (HTBF). Therefore, selection for increased height at the beginning of flowering

(HTBF) would lead to a high single plant yield in addition to reduced days to maturity.

In this study, high-yielding early putative mutants with the qualitative traits viz., mono stem, closed capsules, multiple capsules, and tri-leaf were also isolated (Fig 3). Among these traits, the tri- leaf trait resulted in higher single plant yield compared to others. These tri-leaf putative mutants had one capsule per leaf axil and three leaves in each node. Generally, in multiple capsules, three, four, or even eight capsules occur per leaf axil. Such multiple capsules are highly affected by the environment and produce small-sized capsules with low seed weight (Langham, 2018). Smaller capsules and smaller seeds of multiple capsules result from source limitation, which is overcome in tri-leaf variants. In tri-leaf variants, capsule size does not get affected as each leaf contributes photosynthates to the single capsule in its axil. Further, tri-leaf variants have more capsules as each node bears three leaves with three capsules, in contrast to normal plants bearing two leaves with two capsules in each node which further adds up to enhance the seed yield.

CONCLUSION

Finally, we conclude that for obtaining high yielding early sesame mutants/lines, selection should mainly be focused on the increased height at the beginning of flowering (HTBF), reduced days to the beginning of flowering (DBF) with the short flowering period (FP) in addition to other yield-related traits viz., a higher number of capsules (NOC) and long capsules. Also, it was observed that the tri-leaf trait increased single plant yield. So, selection based on the above-mentioned combination of traits will help to achieve high-yielding, early and uniform maturing sesame lines for future breeding programs.

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Ethics statement

No specific permits were required for the described field studies because no human or animal subjects were involved in this research.

Originality and plagiarism

Authors assure that they have written and submitted their original works and the work and/or words of others used has been appropriately cited.

Consent for publication

All the authors agreed to publish the content.

Competing interests

There were no conflict of interest in the publication of this content

Data availability

All the data of this manuscript are included in the MS. No separate external data source is required. If anything is required from the MS, certainly, this will be extended by communicating with the corresponding author through corresponding official mail; parameswari.c@tnau.ac.in

Author contributions

Conceptualization of research (CP, CV, EM); Designing of the experiments (GA, CP, EM, CV, PA); Contribution of experimental materials (CP); Execution of field experiments and data collection (GA, CP); Analysis of data and interpretation (GA, PA, CP); Preparation of the manuscript (GA, CP, CV, EM).

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