

Genetics of anther culture capability traits and its association with seed yield traits in sesame

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Abstract

Successful development of heterotic hybrids in the autogamous sesame crop depends largely on the general combining ability of the parents as well as the specific combining ability of the hybrids produced from them. Homogeneity of V_r -W_r values and non-significance of regression coefficient (b) in a 6 parents half diallal cross were found in respect of capsule length, seeds per capsule, plant height, callus induction frequency, days to callus induction and response to somatic embryogenesis indicating validity of Hayman's assumptions. The estimates of D, H₁ and H₂ components indicated predominance of non-additive gene action in the inheritance of these six traits, and the ADD estimates indicated overdominance for all these characters. The estimates of F and $K_{\text{D}}/K_{\text{R}}$ indicated presence of more of dominant alleles in the parental population except for response to somatic embryogenesis. Strong positive association was recorded between seed yield and in vitro traits viz., callus induction frequency, callus fresh weight and somatic embryogenesis. This indicates that anther culture technique can assist in early screening for combining ability of parents as well as direct selection for heterotic crosses at an earlier stage of hybrid breeding programme.

Key words: Sesame, combining ability, correlation, anther culture, Hayman's approach

Introduction

Sesame (Gingelly) has gained importance as a specialty high value oilseed crop in the international market due to its high medicinal value seed oil. Due to bactericidal and antiviral properties of its oil it is used in many pharmaceutical and cosmetic formulations (Boer et al. 2017). As a result there is a higher demand for sesame seeds in the international market. At present, Asia contributes more than 33.3%

and 37% of total area and production, respectively of which India accounts for 44.3% and 33%, respectively. The steady demand for sesame seeds at a rate of approx. 2% per year decrees the need for enhancing the productivity of this crop by developing high yielding varieties (FAO statistics 2020).

For developing varieties with high yield potential in this autogamous crop, it is desirable to combine the genes from genetically diverse genotypes. But the selection of desirable genotypes depends mainly on the type of gene action operating on the inheritance of the traits associated with yield. The information on the gene content and prepotency of the parents to be used in crosses can be assessed from graphic analysis proposed by Hayman (1954a) as well as Griffing (1956a). However, identification of parents with superior cross performance is still the costlier and time consuming phase in hybrid development program. If superior parental lines could be identified and superior crosses predicted at an early stage, the efficiency of hybrid breeding programs could be greatly enhanced. With the advancement of in vitro culture techniques available anther culture technique is the most favored one for its usefulness in production of homozygous plants in one generation as compared to conventional breeding methods. Anther culture in sesame involves production of sufficient embryogenic calli from the microspores in less period of time, followed by regeneration of homozygous green plants from the calli. Studies on the anther culture capability can be used to generate different information on the prepotency of the parents for *in vitro* traits as suggested by many researchers. Any significant

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association between the morphological and in vitro traits thus can be exploited in direct selection of superior parents for heterosis breeding and development of useful germplasm. In vitro characters have been used along with other morphological traits in some crops. Early prediction of combining ability and heterosis for yield and component traits by measuring the in vitro characters like callus growth and regeneration potential of anther culture has been suggested in cereal crops (Haggag and El-Hennawy 1991; Abdel-Hady 2006; Khaled et al. 2013). But this approach requires information on the genetic nature of the anther culture capacity which will predict the response of these traits to selection (Powell et al. 1985). Griffing and Hayman's approach complement each other in data interpretation regarding the gene action as well as improving the efficiency of selection in small populations (Griffings 1956a, b). This knowledge can also be used in oilseed crops like sesame for generating information about the genetic potential of the parents to be used in breeding programme. However, information about the genetics of anther culture capability and its association with yield traits in sesame is scarce. Hence, the present study was undertaken to analyse the nature of gene action involved in inheritance of the morphological and in vitro traits and to evaluate the potential of using anther culture to predict combining ability.

Materials and methods

The experimental material comprised of six elite Indian sesame varieties along with a set of fifteen crosses (F_1) among selected parents in diallel fashion (no reciprocals). The field experiment was conducted in the Economic Botany farm at Odisha University of Agriculture and Technology. The hybridization was carried out using the fevicol method (Das 1990; Tripathy et al. 2016) suitable for this crop during *rabi* 2017-18. To avoid contamination the emasculated flowers were covered with butter paper bags before and after crossing. The F_1 seeds were harvested and stored in paper bags.

The F_1 hybrids along with their parents were grown in a randomized complete block design during 2018 *kharif.* Each entry was grown in a single row plot of 100 cm length, having 10 plants in each row. The rows were spaced at 50 cm apart. To ensure perfect stand, the genotypes were thinned to a single seedling per hill at a distance of 10 cm approximately. To remove the border effect non-experimental genotypes were planted all around the experimental plots. Normal agronomic practices including fertilizer application and necessary plant protection measures as recommended for sesame were followed. Observations on days to fifty per cent flowering (DF), height to first node (HFN), number of branches/plant (B/P), number of capsules/ plant (C/P), capsule length (CL), capsule breadth (CB), number of seeds/capsule (S/C), 100-seed weight (HSW), plant height (PH) and single plant yield (SPY) were recorded.

For anther culture studies small flower buds were collected in the early morning from the F1 plants. The developmental stage of the anthers was checked by acetocarmine staining procedure in the tissue culture laboratory. The medium size flower buds consisting of anthers at the uninucleate stage of microspore development were used for in vitro culture. Surface sterilization of the fresh anthers was done as suggested by Dash et al. (2017). The aseptic anthers were then plated on solid MS medium (Murashige and Skoog 1962) comprising of growth hormones viz., 0.5 mg I^{-1} 2,4-Dicholophenoxyaceticacid, 2.0 mg l⁻¹ Naphthalene acetic acid (NAA) and 3.0 mg l⁻¹ Benzyl amino purine (BAP) with 3% sucrose at pH 5.6 to 5.8 for callus induction. Before plating the anthers were slightly teased with pointed needle. Ten test tubes containing 25 ml MS solid medium with 10 anthers per test tube per genotype, were randomly kept in five groups. All cultures were incubated at 25±1°C in dark culture room for callus production. The callus was multiplied by repeated sub-culturing into fresh culture media with the same composition. For somatic embryogenesis one to two-week old callus of approximately 0.5 cm diameter were transferred to agar solidified MS medium containing 6.0 mg I^{-1} BAP, 0.5 mg I^{-1} NAA, 3.0 mg I^{-1} Kinetin and 10 mg I^{-1} Adenine sulphate with 3% sucrose at pH 5.6 to 5.8 and incubated under light intensity of 1000 lux at 16/8 hours (light/dark) photoperiod. Observations were recorded after one week for four in vitro traits viz., callus induction frequency, days to callus induction, callus weight and response to somatic embryogenesis.

Callus induction frequency (CIF) was calculated in terms of percentage of anthers producing calli. Days to callus induction (DCI) was recorded as the average number of days taken for callus initiation by the inoculated anthers. Callus fresh weight (CW) in gram was recorded for the fully developed callus. Response to somatic embryogenesis (SE) was calculated in terms of percentage of callus producing somatic embryos.

Statistical analysis

The analysis of variance for combining ability and estimation of various gene effects was done following Griffing (1956a,b). The genetic parameters were estimated only for those characters for which Hayman's assumptions were found valid (Hayman 1954a,b). To determine gene action the data was subjected to graphical analysis only for those characters for which the Hayman's assumptions were found to be valid (Jinks 1954). To examine the magnitude of association between the morphological and *in vitro* traits rank correlation was estimated using OPSTAT online free software (http://14.139.232.166/opstat/default.asp).

Results and discussion

Combining ability analysis

Highly significant differences among the test genotypes (parents and hybrids) was recorded for the ten morphological and four in vitro traits investigated. The variances due to GCA of the parents (σ^2_{gca}), SCA (σ^2_{sca}) of their crosses and error variances for all the characters was significant except for days to flowering. The SCA variance was higher than GCA variance for all the characters (Table 1). The relative magnitude of σ^2_{aca} and σ^2_{sca} indicated that non-additive gene action including dominance and epistasis played a part in the inheritance of the characters under study. The ratio of additive genetic variance (V_A) to dominance genetic variance (V_D) is less than unity, which indicated that all the characters were governed by non-additive gene action giving scope to heterosis breeding. This is the first report of gene action for in vitro traits in sesame. However, both the types of gene action for callus culture have been reported in crops like alfalfa, barley, wheat and sorghum (Wan et al. 1988; Dagustu 2008; Duy and Yoshida 1999), additive gene action in wheat callus culture (Abdel-Hady 2006) and for anther culture in barley (Dunwell et al. 1987). Similar results in sesame for morphological traits were observed earlier (Azeez and Morakinyo 2014; Raikumar 2018). The high estimates of broad sense heritability indicated that selection for these characters among crosses will be useful for rapid gain in seed yield as has also been reported earlier. (Sumathi and Muralidharan 2008, Anuradha and Reddy 2008b). For in vitro traits the observations were also in agreement with previous reports (Khaled et al. 2013; Baha 2018).

This study can aid in identification of superior parents and heterotic crosses at an early stage of breeding programme. Further, it will give information

 Table 1.
 Variance components for morphological and in vitro traits

Characters	σ^2_{gca}	σ^2_{sca}	σ ² e	h² _(bs) (%)	h² _(ns) (%)	V _A /V _D			
Morphologi	ical traits	;							
DF	-0.175	1.327	0.51	72.00	0.00	-0.26			
HFN	-0.085	1.376	0.01	98.71	0.00	-0.12			
B/P	-0.041	0.595	0.04	93.71	0.00	-0.14			
C/P	3.577	230.094	3.09	98.71	2.98	0.03			
CL	0.001	0.022	0.01	70.41	5.44	0.08			
СВ	0.00	0.001	0.00	83.20	0.00	-0.50			
S/C	12.926	44.169	7.37	90.48	33.40	0.59			
HSW	0.00	0.003	0.00	96.85	0.04	0.00			
PH	0.533	146.546	17.32	89.50	0.65	0.01			
SPY	-0.004	0.621	0.01	98.26	0.00	-0.01			
In vitro traits									
CIF	-0.522	25.966	0.02	99.89	0.00	-0.04			
DCI	0.085	4.826	0.19	96.19	3.28	0.04			
CW	-0.003	0.423	0.00	99.76	0.00	-0.01			
SE	-2.065	112.841	0.05	99.96	0.00	-0.04			

 σ^2_{gca} = GCA variance; σ^2_{sca} = SCA variance; V_A = Additive genetic variance; V_D = Dominance genetic variance

on the prospects and potentialities for genetic improvement of productivity and to identify suitable breeding methodology for accelerating the pace of improvement of this important oilseed crop in short time span.

Variance and covariance analysis

Regression of W_r on V_r was worked out for all the characters and the significance of deviation of regression coefficient (b) from unit tested by 't' test indicated validity of Hayman's assumptions and absence of epistasis only for three morphological traits, viz., CL, S/C, PH and three in vitro traits viz., CIF, DCI and SE. It indicated the absence of non-allelic gene interactions and their independent distribution among the six parents for these characters. Also as the regression line intersects the Wr-axis below the point of origin, it suggests the presence of overdominance for these six characters (Fig. 1). For rest of the traits it indicated failure of one or more of the assumptions, suggesting inadequacy of additive dominance model for these eight characters. However, in case of the in vitro trait days to callus induction, the relatively low magnitude and high deviation of



regression coefficient from unit suggested the importance of epistasis for this trait. For single plant yield the regression coefficient was significantly different from zero and unity hence suggesting a significant role of epistasis in the inheritance of yield. Nonadditive genetic variance in form of over dominance was present. Lack of epistatic interaction for in vitro traits has also been reported in many crops like rice, barley and rapeseed (Etedal et al. 2012).

The relative magnitude of additive component (D) was smaller than the dominance components $(H_1 \text{ and } H_2)$ for all the characters except for S/C indicating that non-additive gene action was more important for inheritance of these characters (Table 2). This conclusion supported by the estimates of heritability. For all of the six characters H1 is not equal to H_2 ($H_1 > H_2$) indicating asymmetric distribution of dominant and recessive alleles in the parents as also indicated in the graphs. The average degree of dominance (ADD) was greater than indicating unit over dominance for these six characters which corroborates the results of graphical analysis. The degree of dominance was higher for in vitro than characters for morphological characters.

Notations	CL	S/C	PH	CIF	DCI	SE
D	0.03 ± 0.02	197.2 ± 22.09**	259.33 ± 40.35**	19.13 ± 6.89	5.87 ± 2.59	66.43 ± 38.31
H ₁	0.08 ± 0.04	194.55 ± 56.06*	538.86 ± 102.43**	92.63 ± 17.50**	18.87 ± 6.58*	404.89 ± 97.25*
H ₂	0.08 ± 0.04	131.46 ± 50.08	436.01 ± 91.50**	87.2 ± 15.64**	17.56 ± 5.88*	389.37 ± 86.87*
F	0.02 ± 0.04	212.47 ± 53.95*	323.82 ± 98.57*	15.06 ± 16.84	5.14 ± 6.33	35.99 ± 93.59**
h²	0.05 ± 0.03	82.13 ± 33.71	581.64 ± 61.59**	81.38 ± 10.52**	6.40 ± 3.96	316.41 ± 58.47
E	0.01 ± 0.01	7.37 ± 8.35	17.32 ± 15.25	0.03 ± 2.61	0.20 ± 0.98	0.05 ± 14.48
ADD	1.82	0.99	1.44	2.20	1.79	2.47
	0.24	0.17	0.20	0.23	0.23	0.24
K _D /K _R	1.48	3.37	2.53	1.44	1.65	1.25
h ² _(bs)	71.60	88.50	88.09	99.89	96.46	99.95
h ² _(ns)	16.28	37.27	13.18	17.89	18.10	19.08

Table 2. Estimates of variance components and other genetic parameters for six traits

* and ** indicate significance at 5% and 1% levels of probability respectively.

D = Variance due to additive effect; H_1 = The component of variation due to dominance effect; H_2 = The component of variation due to dominance effect (where, $H_2 = H_1$ [1-(u-v)2)]) where, u and v are freq. of increasing and decreasing alleles and F = Mean of the covariances of additive and dominance effects over the arrays

 h^2 = Dominance effect (as the algebric sum over all the loci in heterozygous phase in all crosses); E = The expected environmental component of variation; ADD = Mean degree of dominance {(H₁ / D) ½}; *uv* = Proportion of genes with +ve and –ve effects in the parents (H₂/4H₁)

 $\frac{K_{D}}{K_{P}}$ Proportion of dominant and recessive genes in parents = {(4DH₁)^{1/2} + F/(4DH₁)^{1/2} - F};

h²_{(ns) =} Narrow sense heritability; h²_{(bs) =} Broad sense heritability

The positive direction of F indicates higher proportions of dominant alleles than the recessive alleles in the parental population. The ratio K_D/K_B , which is the relative proportion of dominant and recessive genes, corroborated the above conclusion as to the distribution of dominant and recessive alleles in the parental population. However, the estimate of K_D/K_B for SE being close to 1, indicated nearly equal proportions of dominant and recessive alleles for this character. The UV value was close to 0.25 for SE indicating symmetrical gene distribution of dominant and recessive genes in the parents for this character and asymmetrical for the rest of five characters. The allelic distribution was verified by the dominance order of parents based on Wr-Vr values (Fig. 1f). The findings of present study on morphological traits are in general agreement with previous results (Kamala 1999; Swain and Dikshit (2000); Abd El-Kader et al. 2017); and in vitro traits (He et al. 2006).

Some researcher have identified tissue culture technique as a beneficial tool for early selection for combining ability and best parents (Abdel-Hady 2006).

Hayman's approach estimates the prepotency of the parents without their progeny evaluation whereas Griffing's approach determines the potency of a parent based on the hybrid performances. The regression graph prospectively provided a useful means of measuring genetic relationship among the parents and also a test for absence of epistasis (Jinks 1954; Hayman 1954a, b). Thus both the methods supplement each other in generating genetic information in the present study. It was also reported earlier that with this kind of information on the combining ability for anther culture traits, prediction of heterotic hybrids can be done (Hou et al. 1994).

Correlation between morphological and in vitro traits

Seed yield displayed high positive correlation with morphological traits B/P, C/P and CL (Table 3). The seed yield also exhibited positive significant association with three *in vitro* traits i.e., CIF, CW and SE. Overall, SYP showed highest significant correlation with C/P followed by CIF and SE. The highest significant positive association among *in vitro* and

Characters	DF	HFN	B/P	C/P	CL	СВ	S/C	HSW	PH	CIF	DCI	CW	SE
HFN	0.039 ^{NS}												
B/P	-0.089 ^{NS}	0.512 [*]											
C/P	0.001 ^{NS}	-0.013 ^{NS}	0.416 ^{NS}										
CL	-0.520*	0.027 ^{NS}	0.248 ^{NS}	0.361 ^{NS}									
СВ	0.028 ^{NS}	0.337 ^{NS}	0.346 ^{NS}	0.068 ^{NS}	0.299 ^{NS}								
S/C	-0.404 ^{NS}	-0.034 ^{NS}	0.122 ^{NS}	0.457 [*]	0.785**	0.281 ^{NS}							
HSW	-0.109 ^{NS}	0.236 ^{NS}	0.175 ^{NS}	0.066 ^{NS}	0.227 ^{NS}	0.288 ^{NS}	0.162 ^{NS}						
PH	-0.253 ^{NS}	0.543 [*]	0.526 [*]	-0.090 ^{NS}	0.140 ^{NS}	0.080 ^{NS}	0.013 ^{NS}	-0.127 ^N	S				
CIF	-0.162 ^{NS}	0.353 ^{NS}	0.682**	0.363 ^{NS}	0.234 ^{NS}	0.033 ^{NS}	-0.029 ^{NS}	0.081 ^{NS}	0.395 ^{NS}				
DCI	-0.178 ^{NS}	-0.274 ^{NS}	0.189 ^{NS}	-0.050 ^{NS}	-0.101 ^{NS}	-0.316 ^{NS}	-0.047 ^{NS}	-0.246 ^{NS}	-0.055 ^{NS}	0.251 ^{NS}	6		
CW	-0.205 ^{NS}	0.371 ^{NS}	0.635**	0.114 ^{NS}	0.329 ^{NS}	0.344 ^{NS}	0.105 ^{NS}	0.251 ^{NS}	0.232 ^{NS}	0.666**	0.112 ^{NS}		
SE	-0.310 ^{NS}	0.161 ^{NS}	0.481 [*]	0.141 ^{NS}	0.234 ^{NS}	-0.288 ^{NS}	-0.125 ^{NS}	0.119 ^{NS}	0.317 ^{NS}	0.753**	0.261 ^{NS}	0.645	•
SPY	-0.139 ^{NS}	0.133 ^{NS}	0.493 [*]	0.659**	0.477 [*]	0.024 ^{NS}	0.305 ^{NS}	0.346 ^{NS}	-0.056 ^{NS}	0.558**	0.081 ^{NS}	0.443 [*]	0.542 [*]

Table 3. Spearman's rank correlation coefficient for morphological and in vitro traits

*, ** Significant at 0.05 and 0.01 probability level, respectively. NS=Non significant

morphological traits was recorded between CIF and B/P. CIF showed positive significant correlation with only one morphological trait i.e., B/P and positive correlation to other three in vitro traits. DCI was positively correlated with B/P and other three in vitro traits. CW had significant positive association with B/P and CIF. Similarly, response to somatic embryogenesis showed significant positive correlation with only B/P and CIF. Similar association of morphological traits with tissue culture traits were recorded in various crops and trees such as maize (Haggag and El-Hanna 1991), wheat (Li et al. 2003; Abdel-Hady 2006; Heidari et al. 2018; Dodig et al. 2008, Yildirim et al. 2008), rice (Emara et al 2013), soyabean (Huynh et al. 2017) and medicinal plant (Chand et al. 2019). Associations of seed yield with component traits has been earlier reported (Imran and Dash 2018).

As discussed earlier combining ability analysis and estimates of D, H₁ and H₂ components indicated predominance of non-additive gene action in the inheritance of six traits, while ADD estimates indicated overdominance for all these characters. The ratio of V_A/V_D less than unity indicated that the characters were governed by dominance part of non-additive gene action. Hence, hybrid breeding is ideal to improve these characters. It may also be useful to mention here that the information on positive association of seed yield with *in vitro* traits *viz.*, callus induction frequency, callus weight and somatic embryogenesis can assist in direct selection for heterotic crosses at an earlier stage of breeding programme. It is, therefore, suggested that early prediction of heterosis for seed yield and selection of desirable parents for use in hybrid breeding programme can be achieved by practising indirect selection for anther culture capability traits. This is the first report on establishing the existence of genetic control in anther culture capability traits and the association of seed yield with anther culture traits in sesame. Information generated in this study encourages for early screening based on in vitro traits, which will help in identification of superior parents, heterotic crosses in early stage and thus speeding up the selection work in a hybrid breeding programme. Further, it may help in generating information on the prospects and potentialities to identify suitable breeding methodology. However, it is advocated to use more number of sesame genotypes or oilseed species for better conclusion.

Authors' contribution

Conceptualization of research (MD); Designing oftheexperiments (MD, SN, BD); Contribution of experimental materials (MD); Execution of field/ labexperiments and data collection (SN); Analysis of data and interpretation (SN, MD, BD); Preparation of themanuscript (SN, MD).

Declaration

The authors declare no conflict of interest.

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