

Research Paper

DIALLEL ANALYSIS OF SEED OIL CONTENT IN SESAME (*Sesamum indicum* L.)

Aladji Abatchoua MMI¹, Noubissié TJB¹, Njintang YN^{1, 2}, Nguimbou RM² and Bell JM³

¹ Department of Biological Sciences,
Faculty of Science, University of Ngaoundéré,
P.O. Box 454 Ngaoundéré, Cameroon

² Department of Food Sciences,
Higher School of Agro-Industrial Science (ENSAI),
University of Ngaoundéré, P.O. Box 455 Ngaoundéré, Cameroon

³ Department of Plant Biology,
Faculty of Science, University of Yaoundé I,
P.O. Box 812 Yaoundé I, Cameroon.

Abstract

Development of high oil content varieties is one of the most important aims of current sesame programs. A set of 15 diallel F₁ hybrids, their parents and six additional cultivars were evaluated at Mora, in the sahelian savannah zone of Cameroon. The experimental design was a randomized complete block with three replicates. Analysis of variance revealed highly significant differences among the 12 pure lines for seed oil content. Broad sense heritability was high ($h^2=0.95$), suggesting that this character is controlled mainly by genetic factors. Narrow sense heritability value ($h^2_n=0.88$), the value of $\delta^2GCA / \delta^2SCA$ ratio ($r=10.93$), and the variance components showed the preponderance of GCA, indicating that this trait was largely controlled by additive gene effects. The combining ability analysis showed that the parents L2B (1.39), L1Y (2.19) and L2Y (4.39) were relatively good general combiners with high significant and positive GCA effects. Among F₁ progenies, L1B x L1Dj, L1B x L2Dj, L2B x L1Y, L1Dj x L1Y, L2Dj x L2Y and L1Y x L2Y were the best crosses based on SCA effects. Heterosis in F₁ over better parent was recorded only for L1Y x L2Y combination. High oil content is mainly associated with dominant genes. Improvement of oil content can be achieved through direct selection.

Key words: *Sesamum indicum* L., oil content, genetic improvement, diallel analysis.

INTRODUCTION

Sesamum indicum L. (Pedaliaceae) is an annual shrub with white bell-shaped flowers with a hint of blue, red or yellow with branches or without branches. It is grown for the production of seeds which is rich in oil content. Sesame has utmost economical importance and it is primarily grown by small farmers in tropical, subtropical, and southern temperate areas of the world. Sesame seeds contain phytochemicals which lead to the interest particularly in the area of antioxidant, antifungal, hypolipidaemic

and hypoglycemic actions [1]. The seeds contain two unique substances, sesamin and sesamol whence during refinement the two phenolic antioxidants, sesamol and sesaminol, are formed [2, 3]. Sesame contains about 35-60% seed oil [4, 5]. Sesame oil, otherwise also referred to as gingelly oil, is one of the major sources of edible oil. It is used widely in some drug formulations and is also useful in the industrial preparation of perfumery, cosmetics, pharmaceuticals, insecticides, paints and varnishes [6, 7]. *S. indicum* L. took the 9th position among the top 13 oilseed crops which make up 90% of the world production of edible oil [8]. The oil is highly resistant to oxidative deterioration due to the presence of antioxidant lignans [9]. Sesame seed and oil are widely used in food and nutraceutical industries in many countries [10]. It is widely known as one of the natural health promoting foods that has the potential to prevent various disorders such as hypertension, oxidative stress-associated and neurodegenerative diseases [11].

Despite its importance, sesame has not received adequate attention from the research world and more precisely for its oilseed [12]. In northern Cameroon, very few efforts have been made on sesame genetic improvement for oilseed. Therefore, it is important to upgrade the sesame program by selecting in northern Cameroon, superior cultivars for the oil that determine the nutritional and industrial qualities of this plant in order to promote sesame varieties adapted for production of oil. Moreover, the success of an improvement program for a crop essentially depends on the nature and the degree of variability in the attributes of that crop [13]. The diallel cross is a powerful tool to study the various variance components of the genetic systems controlling a quantitative trait. The diallel analysis, as outlined by Griffing [14], partitions variation into genotypic and error variation, and further divides genotypic variation into additive and dominance components. These values can then be used to calculate heritability estimates, draw inferences about genetic system, and determine the most efficient breeding procedures. Several studies Asghar and Majeed [2], Alyemeni *et al.*, [15] and Nzikou *et al.*, [16] recorded an important genetic variability for oil content in sesame. The purpose of the present study was to investigate in sesame the varietal differences and assess through diallel analysis the genetic control of oilseed; so that the oil production can be genetically improved.

MATERIALS AND METHODS

Experimental site

The research was conducted from 2011 to 2013 in a private farm at Mora, Far North region of Cameroon, which is intersected by 10.32° E East longitude and 09.30° N North latitude. This region belongs to the sahelian savannah agro-ecological zone. The climate is characterized by two seasons with an average annual rainfall of 1200 mm that is fairly distributed over the rainy growing period (June to September). The soil of experiments was sandy texture.

Plant material

The experimental material comprised of twelve sesame varieties (Table 1) including two popular lines from Chad namely Local 1 Djamena (L1Dj) and Local 2 Djamena (L2Dj); two registered genotypes originated from Nigeria namely Local 1 Banki (L1B) and Local 2 Banki (L2B), and eight local landraces from northern Cameroon: Local 1 Figuil (L1F), Local 1 Doulo (L1D), Local Podoko (LP), Local Mora (LM), Local 2 Doulo (L2D), Local 2 Figuil (L2F), Local 1 Yagoua (L1Y) and Local 2 Yagoua (L2Y) (Table 1). Six genotypes (L1B, L2B, L1Dj, L2Dj, L1Y and L2Y) which were chosen based on their genetic variation for oil content were planted in pots from July to October 2012 for

crossings. At flowering, manual crossings were made with emasculation to provide F_1 generation. At 6x6 half-diallel mating was obtained giving 21 combinations consisting of six pure lines and 15 F_1 hybrids.

Experimental Design

A preliminary field trial was conducted during the 2011 growing season to evaluate the genetic variability for oil content. The seeds of 12 entries were sown in a randomized complete block design (RCBD) with three replications. Sowing took place on July, 2011, on an experimental surface of 100 m² (20 m length x 5 m broad). Each plot unit consisted on one row of 2 m length x 0.5 m broad, spaced 30 cm apart. Five seeds of each variety were sown at an intra-row spacing of 30 cm and thinned to two per hill, 21 days after sowing (DAS). A mineral fertilizer (7% N; 14% P₂O₅; 7% K₂O) was applied to the seedlings four weeks after planting at rate of 60 kg per ha. The plots were manually weeded at 20 DAS, 45 DAS and at 65 DAS.

During the growing season 2013, all 21 genotypes obtained from diallel mating, were arranged in a duplicated randomized complete block design (RCBD) with three replications. Sowing took place on July, 2013, at the beginning of the rainy season on an experiment surface of 300 m² (30 m x 10 m). Each plot unit consisted on one row of 2 m length x 0.5 m broad, spaced 30 cm apart. Six seeds of each genotype were sown at an intra-row spacing of 30 cm and thinned to two per hill, 21 days after sowing (DAS). A mineral fertilizer (7% N; 14% P₂O₅; 7% K₂O) was applied to the seedlings three weeks after planting at rate of 60 kg per ha. The plots were manually weeded at 20 DAS, 45 DAS and at 65 DAS. At the maturity, the seeds obtained will be harvested and stored for oil extraction.

Oil extraction

Dried *Sesamum indicum* L. whole seeds were ground in Moulinex Model SeB PREP'LINE 850. For solvent extraction (Soxhlet method), 1g of ground seeds for each sample were placed into a cellulose paper cone in an I-L Soxhlet extractor for 8 h [17]. The oil was then recovered by evaporating of the solvent using rotary evaporator and residual solvent was removed by drying in an oven at 60°C for 1 hour and flushing with 99.9% nitrogen.

Statistical analysis

The means were subjected to analysis of variance (ANOVA) and using STATGRAPHICS PLUS statistical package program. The genotypic means were compared using Least Significant Difference at 5% level of probability (LSD 5%).

Diallel analysis

The diallel analysis was done using Dial 98 microcomputer package [18]. The Griffing's [14] method 2 (excluding reciprocal F_1 crosses), model 1 (fixed effects) was used to analyze the general combining ability (GCA) of lines and the specific combining ability (SCA) of crosses, supplemented by the analysis of variance by Walters and Morton [19]. With this approach, the components of variation were partitioned into the additive effects (a) and the dominance effects (b) which were further sub-divided into b_1 , b_2 and b_3 . The genetic parameters were estimated as per Hayman [20]. Heritability in broad sense (h^2) was measured as the proportion of genetic variance (δ^2_g) in the phenotypic variance between the parents (δ^2_p), while heritability in narrow sense (h^2_n) was calculated as the proportion of additive variance (δ^2_A) in the phenotypic variance between the parents (δ^2_p) [21, 22]. The correlation between parental values (Pr) and recessive factor (Wr+Vr) indicated the gene action for each trait [14].

Heterosis

F_1 value (F_1) from the better parent (BP) value as suggested by Foncesca and Patterson [23]. Heterobeltiosis (HB %) was quantified as deviation of F_1 value from the better parent (BP) as outlined by Foncesca and Patterson [23] as:

$$HB\% = [(F_1 - BP) / BP] \times 100$$

RESULTS AND DISCUSSION

The results of analysis of variance revealed that the 12 sesame lines exhibited highly significant differences ($P < 0.01$) for oil content (Table 1). Among the parents, the values of this trait ranged from 35.24 for L1B to 58.92% for L2B. Significant differences among sesame genotypes indicated the presence of diversity in the tested materials. Variability for seed oil content in sesame was also reported by Asghar and Majeed [2], Adeola *et al.*, [8], Nzikou *et al.*, [16], Pham [24], Manal [25], Shekhawat *et al.*, [26], Alege and Moustapha [27], Azeez and Morakinyo [28] and Bisen *et al.*, [29]. As pointed out by Were *et al.*, [30] black seed cultivars exhibited lower oil content compared with brown or white seed genotypes, indicating the possible linkage between oil content and seed coat color. Kamal-Eldind and Appelqvist [4] attributed the low oil content in black seed sesame to a high amount of crude fiber in the seed coats. Varietal differences, climate, soil type, ripening stage, the harvesting time of the seeds and the extraction method have an important effect on yield and quality of oil obtained from sesame [31].

Table 1: Origin and variability of 12 sesame genotypes for seed oil content

Lines	Origin	Seed colour	Oil content (%)
L1B	Local 1 Banki (Nigeria)	Black	35.24±3.74 ^g
L2B	Local 2 Banki (Nigeria)	White	58.92±1.34 ^a
L1D	Local 1 Doulo (Cameroon)	Brown	44.56±3.48 ^f
L2D	Local 2 Doulo (Cameroon)	Brown	49.07±1.50 ^{cde}
L1Dj	Local 1 Djamena Chad	White	46.90±3.32 ^{def}
L2Dj	Local 2 Djamena (Chad)	White	49.28±1.03 ^{cde}
L1F	Local 1 Figuil (Cameroon)	White	50.68±0.78 ^{cd}
L2F	Local 2 Figuil (Cameroon)	Brown	51.93±2.92 ^{bc}
LM	Local Mora (Cameroon)	Brown	44.19±2.50 ^f
LP	Local Podoko (Cameroon)	White	45.74±1.15 ^{ef}
L1Y	Local 1 Yagoua (Cameroon)	White	55.55±3.57 ^{ab}
L2Y	Local 2 Yagoua (Cameroon)	White	56.17±2.97 ^{ab}
Means			49.01±2.35
Least significant difference (LSD) at 5% level.			4.94

Means with the same subscript within the same column do not differ ($p > 0.05$).

Mean squares for general and specific abilities among genotypes and their crosses based on Griffing [14] method for the trait analyzed are presented in Table 2. Analysis of variance showed that, only the mean square of GCA was significant ($p < 0.05$) for oil content. The value of δ^2GCA/δ^2SCA ratio (10.93) and the variance components showed the preponderance of GCA, indicating that, this trait was largely controlled by additive gene effects. The significance of GCA showed that a large portion of variability among the F_1 hybrids in the current study was a result of gene actions with predominantly additive effects. This is a desirable phenomenon necessary for better crop improvement especially when quantitative traits are concerned. Hence, the crosses under investigation will result into effective selection in early generations of segregating

materials due to the small environmental effects and the poor contribution of dominance and/or epistasis to the total genetic variability. Since the additive gene effects are fixable through direct selection, the predominance of additive genes for this trait indicated that mass selection on the F₁ generation of the crosses would be worthwhile in the development of sesame genotypes with higher seed oil content. These observations are in conformity with the findings of Alege and Moustapha [27], Azeez and Morakinyo [28] and Bayoumi and El-Bramawy [32] for oil content. In contrast, the report of Goyal and Kumar [33], Praveenkumar *et al.*, [34] and Kavita *et al.*, [35] highlighted the predominance of non-additive or dominant gene action for this trait. According to Werle *et al.*, [36], both additive and non-additive effects were involved in the genetic control of this trait in maize. In *Brassica napus*, Sabaghnia *et al.*, [37] noted the preponderance of the dominance genetic variance for oil percentage.

Table 2: Mean squares for general and specific combining ability for oil content of sesame in a 6x6 diallel crosses

Source of variation	Degree of freedom	Mean squares for oil content
GCA	5	152.21**
SCA	9	3.51 ^{ns}
Error	28	3.64
Ratio δ^2 GCA/ δ^2 SCA		10.93

Df: Degree of freedom; GCA: Variation due to general combining ability; SCA: variation due to specific combining ability; Error: error variation or interaction between the replication and genotypes; δ^2 GCA: variance of general combining ability; δ^2 SCA: variance of specific combining ability; ns: not significant and ** indicates significance at $P \leq 0.01$.

Table 3 presents the mean squares from Walters and Morton [19] analysis of variance for additive (a) and dominance (b) effects and dominance components b₁, b₂ and b₃ for oil content. Both additive and dominant effects were all significant ($p < 0.01$). Within (b), the mean dominance effects (b₁), the additional dominance effects due to the parents (b₂) and the residual dominance effects (b₃) were also highly significant ($p < 0.01$) for this trait. The significance of b₁ component for this parameter indicated that, the dominance was unidirectional [19]. The significant b₂ item illustrated an asymmetrical distribution of dominant genes among the parents, reflecting that some parents harbored considerably dominant genes than others. Dominant and recessive loci are not harmoniously distributed among the parents. The significance of the residual dominance (b₃) for this characteristic confirmed the presence of specific dominance or combining ability in some crosses.

Table 3: Mean squares from analysis of variance for additive and dominance effects and dominance components for seed oil content

Source of variation	Degree of Freedom	Mean squares for seed oil content
Repetition	2	2.06 ^{ns}
Additive (a)	5	229.42**
Dominance (b)	15	56.78**
b ₁	1	59.74**
b ₂	5	21.78**
b ₃	9	75.95**
Error	40	4.61

Df: Degree of freedom; a = additive effects of genes; b = dominant effects of genes; b₁ = mean dominance effects; b₂ = additional dominance deviation due to the parents, b₃ = residual dominance effects, ns: indicates non significance at 5% and ** indicates significance at 1%.

For oil content, the genetic parameters (average degree of dominance, direction of dominance, correlation between the degree of dominance and parental value) as well as the broad and narrow sense heritability values were presented in Table 4. Broad and narrow sense heritabilities were 0.95 and 0.88 respectively for this character. Broad sense heritability value was high (0.95), indicating that this character is controlled mainly by genetic factors. High broad sense heritability values were also suggested for oil content in sesame by Alege and Moustapha [27], Bisen *et al.*, [29]. Narrow sense heritability value (0.88) compared to h^2 showed remarkably that, this trait is mainly controlled by additive genes. This observation is in agreement with findings of Alege and Moustapha [27], Bisen *et al.*, [29], El-Bramawy [38], Ramesh *et al.*, [39] and Wilson *et al.*, [40]. In maize inbred lines Werle *et al.*, [36] also noted that the oil content is much more related to the genotypes than the environment and is considered a trait with high heritability.

The mean degree of dominance $(H_1/D)^{1/2}$ was less than unity for oil content, indicating the presence of partial or incomplete dominance in the expression of this trait. Globally, the tested parents had a moderate proportion of dominant genes ($k_d = 68\%$). The positive estimation of average direction of dominance ($h = 3.59$) confirmed that the dominance was unidirectional and in the direction of the parent with the higher expression of the trait. The coefficient of regression of W_r on V_r was almost equal to one indicating that the simple additive-dominance model is adequate and the preponderance of additive effects according to the assumption of Hayman [20]. The value of this coefficient showed the absence of substantial non-allelic interaction. In peanut, Upadhyaya and Nigam [41] outlined that the inheritance of oil concentration is a more complex genetic system due to the presence of epistasis, which skews the relative contribution of the genotypic values associated with the parents. The coefficient of correlation between parental values (P_r) and recessive factor (W_r+V_r) was negative but not significant ($r=-0.55$) indicating the preponderance of positive dominant alleles for oil content in *S. indicum*. Similar findings were reported for this trait in rapeseed by Sabaghnia *et al.*, [37]. It was exactly reverse for this trait in safflower [42]. The association of high oil percentage and with dominant genes might present some advantages for selection during early generations.

Table 4: Genetic components estimates and heritability values for sesame oil content based on a 6 x 6 half diallel

Genetic parameter	Genetic components estimates for oil content
Average degree of dominance (H_1/D) ^{1/2}	0.44
Proportion of dominant genes Kd	0.68
Direction of dominance (h)	3.59
Broad sense heritability; h^2	0.95
Narrow sense heritability h^2_n	0.88
Difference $h^2-h^2_n$	0.07
Regression (Vr, Wr)	0.99Vr+15.29
r (Pr, Wr+Vr)	-0.55 ^{ns}
Regression (Pr,Wr+Vr)	-1.22 Pr+108.66

r (Pr, Wr+Vr): Correlation coefficient between the degree of dominance of the parents (Wr+Vr) and the parental value (Pr), Vr the variance of the rth array and Wr the covariance between the parents and their offspring in the rth array, ns: not significant, * Significant at the 5% level.

Based on GCA effects (Table 5), L2B, L1Y and L2Y with relatively large and positive GCA values could be used as parents in hybridization programs for genetic improvement of seed oil content in sesame. Selection of these parents should be important to develop superior recombinants in the segregating generation. This is in line with Ramalho and Andrade de Araujo [43] assuming that large and positive GCA estimates with significant additive gene effects could provide desirable genes for the improvement of the traits under consideration. L2Y, in particular, appears to be the most desirable genotype for the genetic improvement of seed oil content and therefore recommended for further use. These findings corroborated with those of Azeez and Morakinyo [28], El-Shakhess *et al.*, [44] and Banerjee and Kole [45] who reported large GCA effects of this character in sesame. Indeed, combining ability analysis is an important and powerful tool for selecting superior parents in hybridization program. In general, there was a fairly good relationship between the GCA effects and per se performance of the parents. In peanut, Wilson *et al.*, [40] also noted that the performance of parental lines was generally a good predictor of hybrid oil content. It is suggested that the per se performance of the parents could be reliable criterion for selecting parents for hybridization. The preponderance of additive genetic variance among the genotypes evaluated could be exploited to an advantage by using conventional breeding methods such as pedigree or pure line selections. Globally, the studied parents possessed high proportion of dominant genes for oil content except LIB and L2B (Table 5).

Table 5: Estimates of General Combining Ability effects (GCA) and proportion of dominant genes for oil content among six sesame genotypes.

Parents	General combining ability (GCA) effects and proportion of dominant genes (D) for oil content	
	GCA	% D
Local 1 Banki (L1B)	-5.35**	20
Local 2 Banki (L2B)	+1.39*	47
Local 1 Djamena (L1Dj)	-2.95*	84
Local 2 Djamena (L2Dj)	+0.33	82
Local 1 Yagoua (L1Y)	+2.19**	78
Local 2 Yagoua (L2Y)	+4.39**	99
Standard Error (SE)	2.35	

*Significant at $p = 0.05$ and ** Significant at $p = 0.01$; % D: proportion of dominant genes.

Positive and significant SCA effects observed for seed oil content in six combinations studied suggest that these crosses (*i.e.* L1B x L1Dj, L1B x L2Dj, L2B x L1Y, L1Dj x L2Y, L2Dj x L2Y and L1Y x L2Y) were the best specific combiners for this trait. Hybridization between two good general combiners (*i.e.* L2B, L1Y and L2Y) may be governed by additive x additive gene actions which might elicit transgressive segregants in the advanced generations, thus producing hybrids with good SCA. On other hand, the crosses exhibiting good SCA effect – though deriving from parents that are poor general combiners as was observed in crosses L1B x L1Dj and L1B x L2Dj, suggest the presence of dominance and epistatic gene actions and an indication of genetic interaction between favorable allele contributed by both parents. High SCA effects due to high x high combiners reflect additive x additive type of gene interaction and superiority of favorable genes contributed by their parents, while those involving high x low or low x low indicated the interaction of additive x dominance and dominance x dominance respectively [44, 46].

Results for heterosis over best parent (Table 6) revealed that only L1Y x L2Y recorded significant magnitude of heterobeltiosis for oil content. Success of hybridization program depends upon the magnitude of heterosis which also helps in the identification of potential cross combination to be used in the conventional breeding program. Like in many other crops, the magnitude of heterosis in sesame is related to the degree of genetic divergence of the parents. Azeez and Morakinyo [28] Yamanura [47] and Jawahar *et al.*, [48] obtained highest and significant positive heterosis over better parent for oil content in sesame. The superiority of hybrids over better parent indicated the parental combinations capable of producing the highest level of transgressive segregants [23]. These hybrids can be exploited as basic material for breeding purposes. According to Mather and Jinks [21], heterobeltiosis could be linked to one or two of the following situations: (i) the accumulated action of favorable dominant genes dispersed amongst two parents; (ii) the complementary interaction of additive dominant on recessive genes at different loci; (iii) favorable intra or inter locus interactions referred to as over dominance. Evidently, manifestation of heterobeltiosis might be due to the non-additive gene effects in the parents. Therefore, for all traits studied, hybrid vigour depends of the genotypes choice, thereby, it is necessary to cross the potentially complementary parents.

Table 6: Estimates of specific combining ability (SCA) effects and heterobeltiosis (HB) values among 15 F₁ hybrids for seed oil content

Crosses	SCA	HB (%)
L1B x L2B	-0.55	-18.51
L1B x L1Dj	0.45*	-4.73*
L1B x L2Dj	0.27*	-3.043*
L1B x L1Y	-0.19	-11.48
L1B x L2Y	0.02	-8.17
L2B x L1Dj	-1.38	-24.32
L2B x L2Dj	-0.29	-8.41
L2B x L1Y	2.17**	1.10*
L2B x L2Y	0.05	-0.96
L1Dj x L2Dj	0.17	1.62
L1Dj x L1Y	0.13	-6.57
L1Dj x L2Y	0.62*	-2.81*
L2Dj x L1Y	-0.78	-2.32
L2Dj x L2Y	0.63*	3.04*
L1Y x L2Y	0.33*	5.86**
Standard Error (SE)	0.63	2.02

* Significant at 0.05 probability level and ** Significant at 0.01 probability level.

CONCLUSION

Sesame genotypes were highly variable for seed oil content. Progress can be made toward developing seed with improved oil concentration since the vast majority of variation for this trait is genetic. This trait is controlled mainly by additive genes. The higher performance for oil content was due to the presence of dominant alleles. In general, there was a fairly good relationship between the GCA effects and per se performance of the parents. For this trait, conventional breeding methods such as pedigree or pure line selections might be useful breeding strategies. Improved methods to predict genetic gain and evaluate this trait without the environmental influence are also needed. Quantitative trait loci (QTL) may be targeted for future marker-assisted breeding strategies.

ACKNOWLEDGEMENT

Authors are thankful to Prof UKAI YASUO of the University of Tokyo for providing the DIAL computer program used to carry out the present work.

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