RESEARCH ARTICLE

SABRAO Journal
of Breeding and Genetics
50 (2) 180-191, 2018

COMBINING ABILITY AND HETEROSIS OF SESAMIN AND SESAMOLIN CONTENT IN SESAME

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SUMMARY

Combining ability is an important tool for the selection of desirable parents together with the information regarding nature and magnitude of gene effects controlling sesamin and sesamolin contents. The objective of this study was to determine the combining ability and heterosis of sesamin and sesamolin contents of seven parental genotypes and 21 hybrids of sesame. Seven different parental genotypes were crossed in a half diallel mating design to produce 21 hybrids. The hybrids and the parental lines were evaluated using a randomized complete block design with three replications in the early wet season of 2017 at the Kalasin University, Thailand. Data were measured for sesamin and sesamolin contents using HPLC. The result revealed that variance among genotypes, parents, hybrids, and parent vs. hybrid were significant. General combining ability (GCA) and specific combining ability (SCA) were significant ($P \leq 0.01$) for both sesamin and sesamolin contents, indicating that additive and dominance effects contributed to the genetic control of sesamin and sesamolin contents in the cultivars/lines used in this study. However, additive effects were more important than dominance effects since the mean square for GCA was greater than that for SCA.The parents KKU1 and C-plus 2 showed significant positive GCA effects for both traits, thus these genotypes were suitable parents for use in breeding programmes for improved lignan contents. The hybrid KKU1 × C-plus 2 showed significant positive SCA effects for sesamin content, while Mahasarakham 60 × White UB 2 and KKU1 × White UB 2 showed significant positive SCA effects for sesamolin content. The information could be used for development of hybrids or hybridization programs with high lignan contents.

Key words: *Sesamum indicum* L., lignans, antioxidant, diallel, gene action

Key findings: This study indicated that additive and dominance effects contributed to the genetic control of sesamin and sesamolin contents in sesame. Almost all of the hybrids had negative heterosis for both traits, may be due to outbreeding depression. The parent KKU1 and C-plus 2 showed excellent mean performance and

significant GCA effects for both traits, thus these genotypes are suitable parents for use in breeding programs for improved lignan contents.

Manuscript received: March 02, 2018; Decision on manuscript: March 30, 2018; Accepted: April 06, 2018.

Communicating Editor: Prof. Dr. Sanun Jogloy

INTRODUCTION

Sesame (*Sesamum indicum* L.) has been used as a traditional health food, cosmetic, and medicine in Thailand and Asian countries. It is cultivated in tropical and subtropical Asian and African countries (Al-Bachir, 2016). In 2016, the world had production quantity of about 6.11 million tons, and Tanzania was the most prolific sesame producer with about 0.94 million tons, followed by Myanmar (0.81 million tons), and India (0.79 million tons) (Food and Agriculture Organization of the United Nations, 2018). During the last 20 years, the world production of sesame seeds have substantially increased from 2.82 million tons in 1996 to 6.11 million tons in 2016 (Food and Agriculture Organization of the United Nations, 2018). The increased production of sesame seeds in recent years reflected its great application and consumer preferences of nutritious and healthy products (Hassan *et al*., 2018).

Sesame seed contains oil (57-63%), protein (22-24%), carbohydrates (10-12%), and crude fiber (4-7%) (Makinde and Akinoso, 2013). It is rich in unsaturated fatty acids, methionine, and tryptophan. Also, it is rich in micronutrients such as minerals, lignans, tocopherol, and phytosterol (Hassan *et al*., 2018). Sesamin and sesamolin have been considered as the major lignans in sesame seed (Osawa *et al*., 1985). They have been reported to have many pharmacological properties: e.g. decreasing blood lipids (Hirata *et al*., 1996), lowering cholesterol levels (Chen *et al*., 2005; Visavadiya and Narasimhachary, 2008), providing anti-proliferative activity (Pietinen *et al*., 2001), antioxidant activity (Suja *et al*., 2004), enhancing antioxidant activity of vitamin E in lipid peroxidation systems (Visavadiya and Narasimhachary, 2008), increasing hepatic fatty acid oxidation enzymes (Ashakumary *et al*., 1999), showing antihypertensive effects (Lee *et al*., 2004; Nakano *et al*., 2008), and neuroprotective effects against hypoxia or brain damage (Cheng *et al*., 2006).

Generally, breeding programs for sesame have been focused mostly on the crop production capacity and high oil content. Recently, the functional activities of lignans in sesame have become of major interest (Rangkadilok *et al*., 2010). Sesame cultivars with higher lignan content may allow farmer to produce sesame for various industry such as cosmetics and medicines or can be used directly as food. Previously, the studies mainly focused on variation of sesamin and sesamolin contents in sesame germplasm (Rangkadilok *et al*., 2010; Wang *et al*., 2013; Pathak *et al*., 2014).

To facilitate the development of new varieties with high lignan contents, it is necessary to understand the genetic basis of these traits (Ketthaisong *et al*., 2014). The combining ability is an important tool for the selection of desirable parents together with the information regarding nature and magnitude of gene effects controlling quantitative traits (Kumari *et al*., 2015). The studies on combining ability and heterosis for agronomic traits, yield components, yield, oil, and protein contents in sesame have been reported (Azeez and Morakinyo, 2014; Vimala and Parameshwarappa, 2017). However, the studies on combining ability and heterosis relating to quality traits such as sesamin and sesamolin contents are lacking. Therefore, the objective of this study was to determine combining ability and heterosis for sesamin and sesamolin contents.

MATERIALS AND METHODS

Basic plant materials and development of hybrid seed

Seven sesame cultivars/lines i.e. CM-07, Mahasarakham 60, KKU1, C-plus 2, Kanchanaburi, White UB 2, and MKS-I-84001 were used as genetic materials in this study (Table 1). These genotypes were selected based on their prior information. In 2016 season, a half diallel cross set was done among the seven parent using hand emasculation and pollination. After 50-60 days of pollinations, seed from pollinated plants were harvested.

Field experiment

The twenty-one hybrids and the seven parental lines were evaluated in a randomized complete block design with three replications in the early wet season of March to July 2017 at the Division of Plant Production Technology, Faculty of Agro-Industrial Technology, Kalasin University, Kalasin, Thailand (16°44'N, 103°53'E). The plot size was three rows with three meters in length and spacing of 50 cm × 10 cm. Recommended practices for production of sesame were followed (Department of Agronomy, 2004) and plant protection was performed uniformly to all the parents and hybrids during the experiment. Twenty-one hybrids and their parents in the experimental unit were harvested in July of 2017 and randomly obtained 10 g of grain from each plot for analysis of sesamin and sesamolin contents.

Sample extraction for sesamin and sesamolin determination

Samples were extracted according to the method previously described by Rangkadilok *et al*. (2010) with slight modifications. Briefly, 10 g of each sample was ground into powder and weighed (0.4 g) into 15 ml plastic tubes (two replications per plot). Eighty percent of methanol (5.0 ml) was added and the whole extracted for 30 min. The samples were then centrifuged at 2000 g, for 3 min at 25 °C. The supernatant was then transferred into a 10 ml volumetric flask and the residue was re-extracted with 4.0 ml of 80% methanol. All extracts were combined and volume adjusted with 80% methanol, and filtered through a 0.45 µm nylon membrane prior to HPLC analysis.

HPLC analysis for sesamin and sesamolin

The HPLC analysis of sesamin and sesamolin contents was performed using a Shimadzu SPD-M20A; Diode array detector. A reversed-phase

Table 1. Seven sesame parental used in the experiment.

Cultivars/lines	Descriptive features	Source[1/]
KKU1	rather high sesamin, white grain, many capsule per leaf axil	KKU
C-plus 2	rather high sesamin, white grain, large grain size	KU
Kanchanaburi	medium sesamin, black grain	UBU
WhiteUB2	medium sesamin, white grain, large grain, high adaptability	UBFCRC
MKS-I-84001	medium sesamin, black grain	UBFCRC
CM-07	low sesamin, black grain, large grain size, shatter resistance	KU
Mahasarakham 60	low sesamin, white grain, large grain size, high adaptability	UBFCRC

[1/] KKU = Khon Kaen University,KU = Kasetsart University, UBU = Ubon Ratchathani University and UBFCRC = Ubon Ratchathani Field Crops Research Center

Table 2. Analysis of variance for genotypes, parents, hybrids and orthogonal comparison for sesamin and sesamolin contents in sesame grains.

Sources of variation	df	Sesamin (mg/g grains)	Sesamolin (mg/g grains)
Replications	2	0.14	0.01
Genotypes	27	0.99**	0.14**
Parents (P)	6	0.92**	0.26**
Hybrids (H)	20	1.02**	0.10**
P vs H	1	0.82*	0.44**
C.V. (%)		18.63	13.14

* and **significant at $P \leq 0.05$ and $P \leq 0.01$, respectively

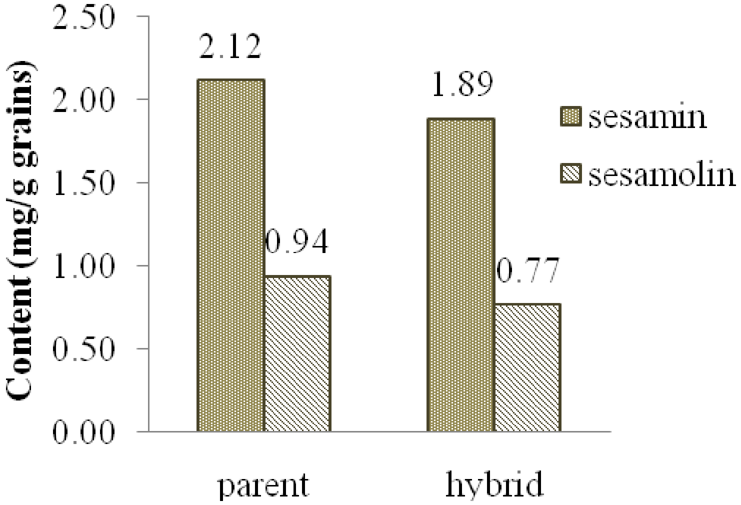

Figure 1. Sesamin and sesamolin contents among parent and hybrid.

column, Inertsil ODS-3 C_{18} 5 µm 4.6 × 250 (GL Sciences Inc, Japan) was used in this study. The composition of solvent and the gradient elution conditions used were those described by Rangkadilok *et al*. (2010) with slight modifications. The mobile phase consisted of water (solvent A) and methanol (Merck, KGaA, Germany) (solvent B) at a flow rate of 1 ml/min. Gradient elution was performed as follow: 0-5 min, 5-18% solvent B; 5-10 min, 18-35% solvent B; 10-15 min, 35-62% solvent B; 15-20 min, 80% solvent B; 20-25 min, 80% solvent B; 25-30 min, 80-5% solvent B. Operating conditions were as follows: column temperature 25 °C, injection volume 20 µl and detection at 280 nm. The standards for sesamin and sesamolin used this study were purchased from Sigma-Aldrich.

Data analysis

The data on sesamin and sesamolin contents were analyzed according to a randomized complete block design. Duncan's new multiple range test (DMRT) was used to compare means at 0.05 probability level. The orthogonal comparison was also used to compare the difference between of parent vs hybrid groups. Correlations between sesamin with sesamolin contents were also examined. Diallel analysis of combining ability was carried out using Method 2, Model I of Griffing (1956). Test of significant different from zero for GCA and SCA effects were performed using *t*-test. The percent of heterosis over the mid-parent (MP%) and better parent (BP%) were estimated by means of the formula:
[(mean of F_1-mean of parent) / (mean of parent) × 100] and [(mean of F_1-mean of better parent) / (mean of better parent) × 100], respectively (Islam *et al*., 2015).

RESULTS AND DISCUSSION

Analysis of variance

The analysis of variance showed significant differences ($P \leq 0.01$) among genotypes, parents, and hybrids for sesamin and sesamolin contents (Table 2). Orthogonal comparison among groups indicated significant differences between parent with hybrid for sesamin ($P \leq 0.05$) and sesamolin contents ($P \leq 0.01$). The significant variance among genotypes, parents, hybrids, and parent vs. hybrid for sesamin and sesamolin indicates presence of genetic variability for parents and their hybrids in this study (Abhishek *et al*., 2017). Experimental results revealed that the mean of sesamin and sesamolin contents for hybrids were lower than that of the parents (Figure 1). Significant positive correlation was found between sesamin with sesamolin contents ($r=0.72$; $P \leq 0.01$) (Figure 2). Similar results were observed by Yasumoto and Katsuta (2006); Wang *et al*. (2013), and Pathak *et al*. (2014). For positively related, improvement of sesamin content will result in a simultaneous improvement of sesamolin content.

Heterosis and Combining ability

Study on combining ability and heterosis is an important step of plant breeding to determine the best parents and the best hybrids (Worrajinda *et al*., 2013).

This study is the first report of combining ability and heterosis for sesamin and sesamolin contents in sesame. The range of heterosis over mid-parent (MP) and better parent (BP) for sesamin content were -46.60 to 21.21% and -50.25 to 14.58%, respectively and number of hybrids had heterosis over mid-parent and better parent in positive were 5 and 2, respectively (Table 5). Similarly, the range of heterosis over mid-parent and better parent for sesamolin content were -43.07 to 33.33% and -56.52 to 25.42%, respectively and number of hybrids had heterosis over mid-parent and better parent in positive were 3 and 2, respectively. The hybrids KKU1 x C-plus 2 and Mahasarakham 60 x White UB 2 had the highest positive heterosis for sesamin and sesamolin contents, respectively. The results revealed that most hybrids had negative heterosis for both traits, and mean performance for hybrids were lower than that of the parents. Our results were in agreement with Ogata and Kato (2016) and similar results were observed in other crops such as cabbage for cupric ion reducing antioxidant capacity, ferric reducing ability of plasma, β-carotene and chlorophyll-a (Parkash *et al*., 2017) and cauliflower for chlorophyll (Dey *et al*., 2014), may be due to outbreeding depression (Parkash *et al*., 2017). Ogata and Kato (2016) found that dominance gene effects reduced sesamin and sesamolin contents in sesame. In contrast, a very high positive heterosis had been found for ascorbic acid, anthocyanin, and carotenoids in cauliflower (Dey *et al*., 2014).

General combining ability (GCA) and specific combining ability (SCA) were significant ($P \leq 0.01$) for both sesamin and sesamolin contents (Table 3). The significant GCA and SCA implies that additive and dominance effects contributed to the genetic control of sesamin and sesamolin contents in the cultivars/ lines used in this study. Similar results were observed for antioxidant activity in cowpea (Nzaramba *et al*., 2005), for vitamin C and soluble solids in pepper (Geleta and Labuschagne, 2006), and tomato (Bhatt *et al*., 2001). A GCA/SCA ratio with a value greater than one indicates additive gene action, whereas a GCA/SCA ratio with a value lower than one indicates dominance gene action (Ketthaisong *et al*., 2014). The variances for GCA were larger than those of SCA indicating that additive effects were more important than dominance effects for both traits (Nzaramba *et al*., 2005; Geleta and Labuschagne, 2006). High additive gene action indicates higher heritability and fewer environment effects (Ketthaisong *et al*., 2014). Our results were in agreement with Ogata and Kato (2016). They found that sesamin and sesamolin contents can be selected during the early generation. Thus, based on these results, pedigree selection can be utilized for breeding for lignan contents in sesame, but it may use much labor, slow, time-consuming, and expensive, especially for the selection for lignan contents because the cost for determination of sesamin and sesamolin contents in sesame grains are very expensive and difficult.

The estimate of GCA of a parent is an important indicator of its potential for generating superior genotypes (Dey *et al*., 2014). Estimates of GCA effects for sesamin and sesamolin are presented in Table 4.

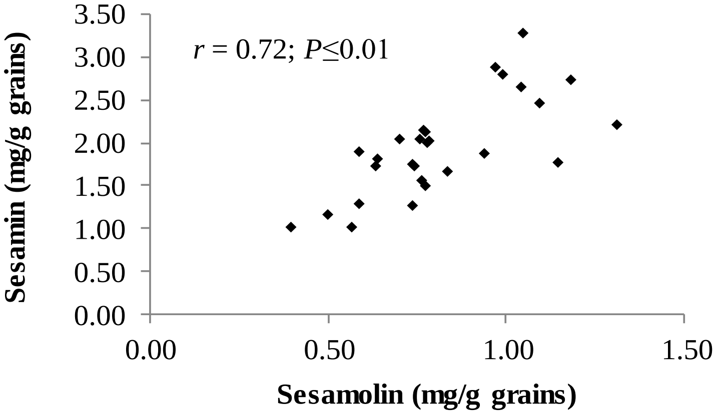

Figure 2. Correlation between of sesamin with sesamolin contents in sesame grains

Table 3. Mean squares for combining ability analysis for sesamin and sesamolin contents in sesame grains

Sources[1/]	df	Sesamin (mg/g grains)	Sesamolin (mg/g grains)
GCA	6	1.13^{**}	0.13^{**}
SCA	21	0.10^{**}	0.02^{**}
Error	54	0.04	0.004

[1/] GCA = general combining ability; SCA = specific combining ability

**significant at $P \leq 0.01$

Table 4. Means and general combining ability (GCA) for sesamin and sesamolin contents in sesame grains

Parents	Sesamin (mg/g grains)		Sesamolin (mg/g grains)	
	Means	GCA	Means	GCA
CM-07	1.77^{bc}	-0.30^{**}	1.15^{ab}	-0.01
Mahasarakham 60	1.29^{c}	-0.36^{**}	0.59^{d}	-0.16^{**}
KKU1	2.88^{a}	0.45^{**}	0.97^{bc}	0.07^{**}
C-plus 2	2.74^{a}	0.52^{**}	1.19^{ab}	0.14^{**}
Kanchanaburi	2.22^{ab}	-0.04	1.31^{a}	0.14^{**}
White UB2	1.89^{bc}	-0.01	0.59^{d}	-0.05^{*}
MKS-I-84001	2.04^{b}	-0.26^{**}	0.79^{cd}	-0.14^{**}

* and **significant at $P \leq 0.05$ and $P \leq 0.01$, respectively

Means in the same column with the same letters are not significantly different by DMRT ($P \leq 0.05$)

Table 5. Hybrid means, specific combining ability, heterosis mid-parent and better parent for sesamin and sesamolin contents in sesame grains

Crosses	Sesamin (mg/g grains)				Sesamolin (mg/g grains)			
	Mean	SCA	MP (%)	BP (%)	Mean	SCA	MP (%)	BP (%)
CM-07 × MKS-I-84001	1.02^{hi}	-0.36	-46.60	-49.75	0.57^{fgh}	-0.11^{*}	-40.93	-50.43
CM-07 × WhiteUB2	1.27^{f-i}	-0.36	-31.15	-33.33	0.74^{def}	-0.02	-14.94	-35.65
CM-07 × Kanchanaburi	1.67^{e-i}	0.07	-16.50	-24.77	0.84^{bcd}	-0.11^{*}	-31.71	-35.88
CM-07 × C-plus 2	2.13^{b-e}	-0.03	-5.75	-22.26	0.78^{cde}	-0.17^{**}	-33.05	-33.90
CM-07 × KKU1	2.06^{cde}	-0.04	-12.02	-28.82	0.76^{c-f}	-0.12^{*}	-28.30	-33.91
CM-07 × Mahasarakham 60	1.15^{ghi}	-0.14	-24.84	-35.39	0.50^{gh}	-0.15^{**}	-42.53	-56.52
Mahasarakham 60 × MKS-I-84001	1.01^{i}	-0.33	-39.16	-50.25	0.39^{h}	-0.13^{*}	-43.07	-50.00
Mahasarakham 60 × WhiteUB2	1.72^{e-h}	0.14	8.18	-8.99	0.74^{def}	0.13^{*}	25.42	25.42
Mahasarakham 60 × Kanchanaburi	1.75^{d-g}	0.19	0.00	-21.17	0.74^{def}	-0.06	-22.11	-43.51
Mahasarakham 60 × C-plus 2	2.01^{cde}	-0.09	0.00	-26.64	0.78^{cde}	-0.02	-11.86	-33.90
Mahasarakham 60 × KKU1	2.16^{b-e}	0.12	3.85	-25.00	0.77^{cde}	0.04	-1.28	-20.62
KKU1 × MKS-I-84001	1.82^{d-g}	-0.32	-26.02	-36.81	0.64^{d-g}	-0.10	-26.86	-34.02
KKU1 × WhiteUB2	2.66^{abc}	0.27	11.34	-7.99	1.04^{a}	0.20^{**}	33.33	7.22
KKU1 × Kanchanaburi	1.88^{def}	-0.48^{*}	-26.27	-34.72	0.94^{abc}	-0.08	-17.54	-28.24
KKU1 × C-plus 2	3.30^{a}	0.38^{*}	17.44	14.58	1.05^{a}	0.03	-2.33	-11.02
C-plus 2 × MKS-I-84001	2.04^{cde}	-0.16	-14.64	-25.55	0.71^{def}	-0.11^{*}	-28.57	-40.68
C-plus 2 × WhiteUB2	2.80^{ab}	0.35	21.21	2.19	0.99^{ab}	0.08	11.86	-16.10
C-plus 2 × Kanchanaburi	2.46^{bcd}	0.04	-0.81	-10.22	1.09^{a}	0.00	-12.45	-16.79
Kanchanaburi × MKS-I-84001	1.55^{e-i}	-0.10	-27.23	-30.18	0.76^{cde}	-0.05	-27.27	-41.98
Kanchanaburi × WhiteUB2	1.49^{e-i}	-0.41^{*}	-27.32	-32.88	0.77^{cde}	-0.14^{*}	-18.95	-41.22
WhiteUB2 × MKS-I-84001	1.74^{efg}	0.06	-11.22	-14.29	0.63^{efg}	0.00	-8.03	-19.23

* and **significant at $P \le 0.05$ and $P \le 0.01$, respectively

Means in the same column with the same letters are not significantly different by DMRT ($P \le 0.05$)

MP and BP = heterosis over the mid-parent and better parent, respectively

The parents KKU1 and C-plus 2 were good combiners for both traits. These parental lines were high on those traits and also showed significant positive GCA effects. The data indicates that these genotypes could be the best candidates for one of the parental lines to improve lignan contents. While the parent Kanchanaburi had high mean performance and also showed significant ($P \leq 0.01$) positive GCA effects for sesamolin (Table 4). In contrast, Mahasarakham 60 and MKS-I-84001 were significant ($P \leq 0.01$) negative GCA effects for both traits. A high GCA estimate indicated that the parental mean is superior or inferior to the general mean (Dey *et al*., 2014). Only two parents, i.e. KKU1 and C-plus 2 excelled for GCA effects and mean performance for both traits, which indicate the value and need for multiple crossing to develop lignans rich cultivars (Singh *et al*., 2009).

The SCA effects for sesamin and sesamolin contents were estimated in all the twenty-one hybrids. Estimates of SCA effects of hybrids for sesamin and sesamolin are presented in Table 5. Out of twenty-one hybrids, only one hybrid KKU1 x C-plus 2 had significant ($P \leq 0.05$) positive SCA effects for sesamin, while two hybrids i.e. KKU1 x Kanchanaburi and Kanchanaburi x White UB 2 had significant ($P \leq 0.05$) negative SCA effects for this trait. For sesamolin content, mostly hybrids had significant negative SCA effects while significant positive SCA effects were found in two hybrids (Mahasarakham 60 x White UB 2 and KKU1 x White UB 2).

Based on mean performance, SCA effects and heterosis, KKU1 x C-plus 2 had highest mean performance (3.30 mg/g grains), significant ($P \leq 0.05$) positive SCA effects (0.38), showed positive heterosis over mid-parent (17.44%), and better parent (14.58%) for sesamin content. Similarly, hybrid KKU1 x WhiteUB2 had high mean performance (1.04 mg/g grains), significant ($P \leq 0.01$) positive SCA effects (0.20), showed positive heterosis over mid-parent (33.33%), and better parent (7.22%) for sesamolin content.

The hybrid that showed highest mean performance and significant SCA effect was observed in crosses between parents with high GCA effects for sesamin content. Thus, the selection of parental lines for this trait should consider on GCA effects (Singh *et al*., 2009), and this cross could be used as a source population for pedigree selection for sesamin content since it had more additive genetic effects (Singh *et al*., 2014). In contrast, hybrids exhibited low SCA effects despite their parents having significant positive GCA effects for sesamolin content (KKU1 x C-plus 2, C-plus 2 x Kanchanaburi and KKU1 x Kanchanaburi). This is possible only in the absence of any interaction among favorable alleles contributed by the parents. Thus, it is evident that two parents with high GCA effects for this trait may not always result in a combination showing high SCA effects (Dey *et al*., 2014). In addition, parents with low GCA effects produced hybrids with high SCA effects (Mahasarakham 60 x White UB 2), which can be attributed to complementary gene action (Kumari *et al*., 2015).

CONCLUSION

The significance of both GCA and SCA implies that additive and dominance effects contributed to the genetic control of sesamin and sesamolin contents. The parents KKU1 and C-plus 2 showed significant GCA effects for both traits and that could be exploited through hybridization programmes. The crosses KKU1 x C-plus 2 and KKU1 x White UB 2 showed high heterosis and also showed positive SCA effects for sesamin and sesamolin, respectively. Almost all of the hybrids had negative heterosis for both traits, may be due to outbreeding depression. The results of the study can be helpful in sesame breeding for development of hybrids or hybridization programs with high lignan contents.

ACKNOWLEDGEMENTS

We are grateful to the Kalasin University. The Laboratory Equipment Center, Mahasarakham University is acknowledged for guidance for the sesamin and sesamolin analyses.

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