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Research Article

Genetic studies on sesamin and sesamolin content and other yield attributing characters in sesame (*Sesamum indicum L.)*

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Abstract
Sesamum (*SesamumindicumL.*) is an important ancient oilseed crop known to mankind, which is considered as Queen of Oilseed crops. Lignans a group of secondary metabolites present in seeds which are responsible for the therapeutic value, especially sesamin and sesamolin possess various biological properties attributing to its health promoting effects. Four low lignan lines and four high lignan lines were selected and hybrids synthesized in Line x Tester mating design. These hybrids were raised during Kharif 2019 in RCBD design with three replications. The genotype JLS-57 was identified as good general combiner for seed yield, the total number of capsules, plant height, the number of primary branches and the number of secondary branches, sesamin and sesamolin content showing a significant GCA effect. The tester GUN-18 and PKDS-12 shows significant GCA effects towards seed yield, plant height, sesamin and sesamolin content. The cross (KRISHNA x PKDS-12) shows high and negative SCA effects towards sesamin and sesamolin content. The cross (JLS 57 x PKDS 12) was high and positive SCA effects towards earliness and 1000 seed weight, but low towards yield and sesamin and sesamolin content. The crosses (JLS-57 x GUN-18), (SI-3099 x PKDS 12) and (IC-205071 x GUN-18) shows high SCA effects towards sesamin and sesamolin content. These crosses will be evaluated in further generations for isolating high sesamin, sesamolin content and high yield.

Keywords
Sesame, Lignan, Sesamin and Sesamolin.

INTRODUCTION

Sesame (*SesamumindicumL.*) is an important, ancient and underexploited oilseed crop known to mankind, locally called as til, gingelly, simsim, ellu, benniseed etc., which is also considered as a Queen of Oilseed crops. Sesame is a member of the order Tubiflorae and family Pedaliaceae which consists of 16 genera and 36 species. It is grown in the tropical and subtropical regions in all over the world. Sesame seeds contain high oil and protein accounting to about 50% and 25% respectively. The fatty acid profile of sesame oil reflects Linoleic acid (37-47%), Oleic acid (35-43%), Palmitic (9-11%) and Stearic acid (5-10%) with the trace amount of Linolenic acid(Latif and Anwar, 2011). The sesame oil has a prolonged shelf life than the other oils as it consists of a higher proportion of linoleic-oleic acid (Thakur *et al.,*2018).

The term Lignan was coined by Howarth in 1948 to describe the group of dimeric phenyl propanoids, which are responsible for the therapeutic value. Sesame lignans especially sesamin and sesamolin possesses various biological activities attributing to its health promoting effects. These two compounds have been reported multiple pharmacological properties, such as antioxidant activity (Shahidi *et al.*2006), anti-proliferative activity

(Yokotaet al., 2007) and anti-inflammatory function (Hsu et al.,2005). In addition, anti-cholesterolemic and antihypertensive properties of sesamin (Penalvo et al.2006). Lignans, the natural anti-oxidants in sesame oil prevent oxidative rancidity and provide a high stability and improved the shelf life of the oil (Suja et al., 2005). The decreasing LDL oxidants, inhibiting lipid oxidants, Lowering LDL levels in humans and guarding against DNA mutating toxins.

The most significant findings about the sesame lignans is their unique ability to increase tissue levels of vitamin E (including gamma tocopherol) *via* several different mechanisms. The elevation of gamma tocopherol, quenches a particularly dangerous type of free radical (peroxynitrite radical) that plays a major role in the development of age related disorders. The primary purpose for taking vitamin E is to supress free radicals (Wei X, et al,2015).In India, every year edible oils are imported due to self-insufficiency to overcome this problem the superior varieties are eagerly needed. Sesame shows normally 90% autogamous. However, the degree of out crossing to the extent of 65%, availability of male sterility due to its epipetalous nature outcrossing shows a massive manual hybridization technique. This caught the attention of breeders for studying the extent of heterosis in sesame and for developing commercial hybrids in sesame. Breeders often wish to improve several traits of sesame simultaneously.

The knowledge on combining ability helps in the selection of appropriate parents for a hybridization program for evaluating superior F_1 hybrids as well as elite segregants with high seed yield in the segregating generations (Kumar et al., 2009). Studies on combining ability and gene action on seed yield and other quantitative traits in advanced generation are very limited. Hence, this study was undertaken to understand the genetics of sesamin and sesamolin content along with various biometrical characters contributing to the yield.

MATERIALS AND METHODS

Four low lignan lines PKDS-12, IC-131651, SI-3478 and GUN-18 used as a Lines and four high lignan lines were used as a Tester are IC2050-71, JLS-57, SI-3099 and KRISHNA were collected from Department of Oilseeds, TNAU, Coimbatore, and they were crossed in Line x Tester mating design (4x4) and developed 16 hybrids. The experiment was conducted in Department of Oilseeds TNAU CBE, during Kharif 2019 in Randomized Complete Block Design (RCBD) design with three replications and also with 2 checks TMV-7and VRI-3. The parents, crosses and checks were raised in a row length of 4 meters. Package of practices were followed as per the crop production guide recommendations for sesamum cultivation. Biometrical observations were recorded for ten characters like Days to 50% flowering, Days to maturity, Plant height (g.), the number of primary branches, the number of secondary branches, the number of capsules per plant, 1000 seed weight (g.), yield per plant (g.), Sesamin (mg.) and Sesamolin (mg.) content. The data obtained for all characters were analysed through TNAUSTAT software and the results were partitioned further into the sources of general combining ability and specific combining ability components in accordance with the procedure suggested by Kempthorne (1957).

Sesamin and Sesamolin standards (97% purity) and Methanol (HPLC grade) were purchased from Sigma-Aldrich. Calibration curves were prepared by injecting varying concentration of working standard in HPLC system as reported by (GhaneAjit et al., 2019) for the sample preparation 300mg. of seeds were crushed in pestle and mortar with 5ml. of Methanol (HPLC grade). The extract was shaken vigorously for 5 minutes using a vortex mixer then centrifuged at a speed of 10,000rpm for 5min. The supernatant was collected into test tube and the residues were re-extracted with another 2ml. and centrifuged again. The supernatants were pooled. It was filtered through 0.45-ìm nylon membrane prior to HPLC analysis. Quantification of sesamin and sesamolin in seeds was achieved by HPLC-DAD system (Agilent 1220 Infinity II LC). The separation was carried out on a reversed-phase C18 column under the following chromatographic conditions. The injection volume is 10ìL, column temperature was maintained at 30°C, wavelength of UV detection was set at 290nm and mobile phase consisted of Methanol and Milli-Q water (70/30, v/v) with a flow rate of 1.0 mL/min.

RESULTS AND DISCUSSION

The analysis of variance for both F_1's and parents were significant for all the ten characters. This revealed that there was a significance difference observed between parents and crosses for all characters, especially sesamin and sesamolin content are highly significant in crosses and also in parent vs. crosses (**Table 1**).

Table 1. Analysis of variance

Sources	DF	DFF	DM	PH	NPB	NSB	TCPP	TSW	YPP	Sesamin	Sesamolin
Genotypes	23	17.96**	35.94**	173.54**	2.13**	2.7**	1613.14**	0.23**	6.28**	1.01**	1.62 **
Parents	7	33.09**	35.593**	342.22**	1.5**	1.54**	2110.1**	0.14**	8.6**	2.06**	2.83 **
Crosses	15	11.45**	36.8**	105.42**	2.47**	3.47**	1475.2*	0.21**	5.55**	0.56**	1.15 **
P Vs C	1	9.76 **	25.17**	14.53	1.29**	0.05	202.8*	0.97**	1.13**	0.5 **	0.26 **
Error	46	2.29	1.48	19.85	0.41	0.80	147.49	0.03	0.22	0.01	0.01

*, ** indicates significant at 5% and 1% respectively.

The mean performances of the parents were given (**Table 3**). None of the parents used in the study were not significant for all the characters. The two lines JLS - 57 and KRISHNA were early matured lines with 40 and 43 days. These lines can be used for developing early maturing varieties and hybrids in breeding programme. The line JLS -57 shown taller and also yields more with a greater number of capsules per plant than other parents studied. The tester SI-3478 shows high 1000 seed weight (2.9mg). The mean performances of the hybrids were given (**Table 4**). The cross KRISHNA x SI-131651 was significant for yield and yield attributing characters and also lignan content especially sesamin and sesamolin. The cross KRISHNA x GUN-18 shows high total lignan (sesamin and sesamolin) content 3.4 mg/g *(***Fig 1***)* followed by SI-3099xPKDS-12 (3.3mg/g), JLS-57xPKDS-12 (3.2mg/g), JLS-57xGUN-18 (3.1mg/g) and KRISHNAxPKDS-12 (2.8mg/g). The cross JLS-57x IC-131651 recorded low sesamin and sesamolin content (0.3mg/g and 0.2mg/g) followed by cross SI-3099 xSI-3478 (0.6mg/g and 0.4mg/g) (**Fig 2**) respectively. The range of sesamin content in all the crosses from 0.3mg/g JLS-57 x SI-131651 to 1.7mg/g in KRISHNA x GUN-18 *(***Fig 1***)* and sesamolin content from 0.2mg/g in JLS-57 x SI-131651 to 1.9 mg/g in SI 3099 x PKDS-12 among all the hybrids under the study, are based on the mean performance of crosses the tester PKDS-12 contribute superior towards the sesamin and sesamolin content.

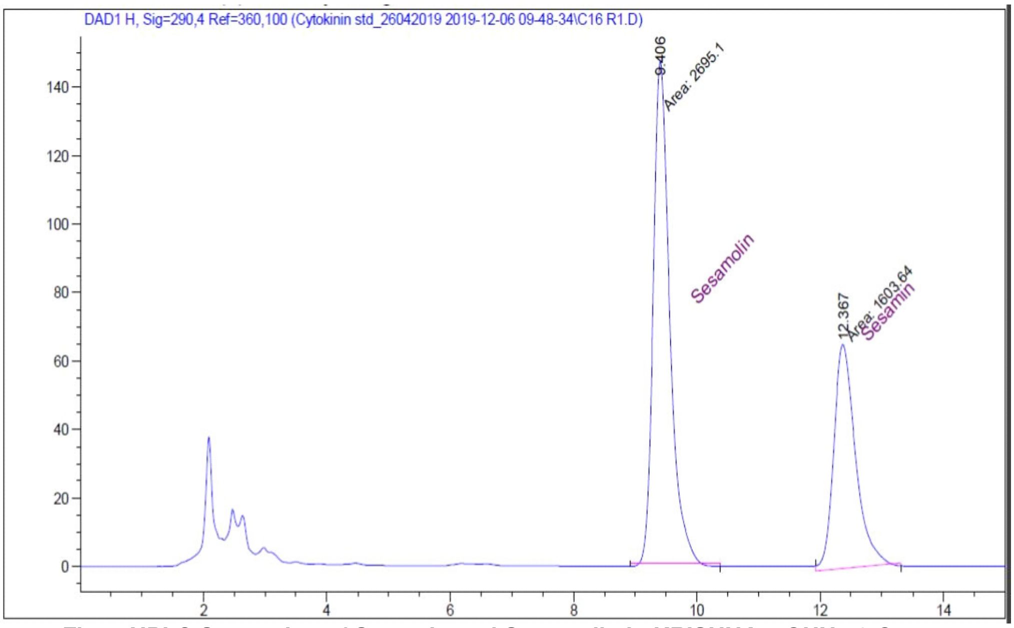

Fig. 1 HPLC Separation of Sesamin and Sesamolin in KRISHNA x GUN-18 Cross.

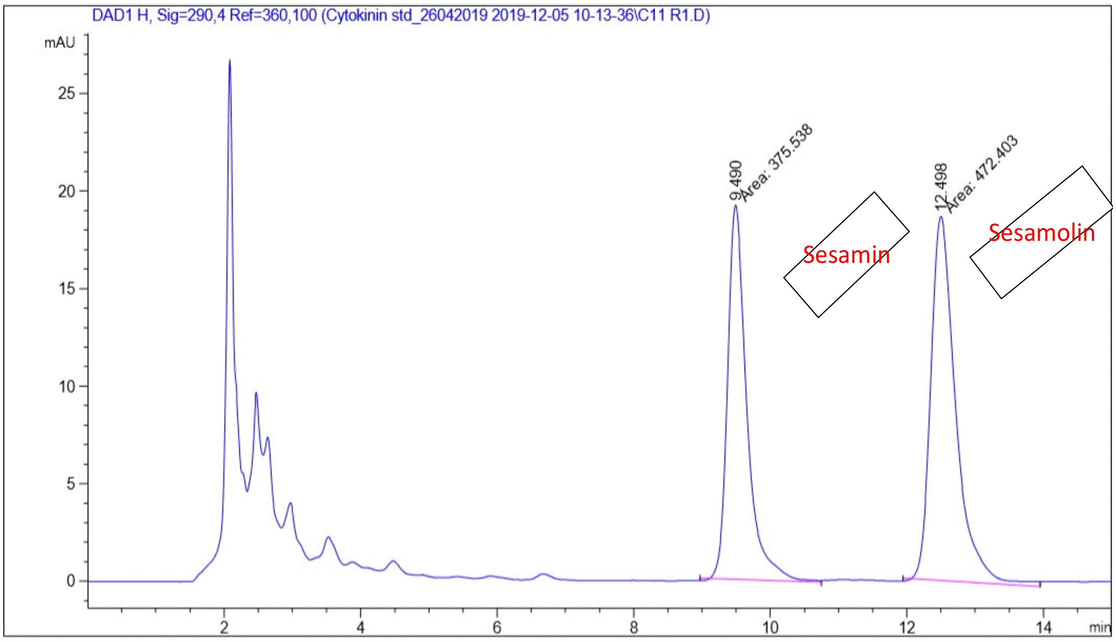

Fig. 2 HPLC Separation of Sesamin and Sesamolin in SI-3099XSI-3478.

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Table 3. Mean performance of parents

Parents	DFF	DM	PH	NPB	NSB	TCPP	TSW	YPP	Sesamin	Sesamolin
IC-205071	47.67	106.33	92.45	3.50	2.67	63.33	2.78	3.19	0.28	0.20
JLS-57	39.17 **	95.5 **	100.42 **	3.60	2.80	102.87 *	2.70	6.15 **	0.24	0.26
SI-3099	47.33	103.67	98.41	4.70	4.33	102.93 *	2.65	5.62 **	0.23	0.45
KRISHNA	43.83 **	101.33	69.93	4.93	4.20	41.13	2.41	2.36	0.65	0.41
PKDS 12	47.50	104.67	76.03	4.13	3.83	86.49	2.57	3.56	1.56 **	0.99
SI-131651	49.33	99.33 **	85.65	4.67	3.93	67.65	2.23	2.16	1.77 **	2.82 **
SI-3478	48.67	103.00	89.61	3.12	2.58	109.53 *	2.89 *	5.44 **	2.25 **	2.16 **
GUN-18	47.33	104.00	80.75	3.29	3.78	47.40	2.41	1.98	1.78 **	1.38 **
Check	44.08	94.67	88.39	4.35	4.28	80.80	2.95	3.93	1.51	1.47
Mean	46.35	102.30	86.66	3.99	3.52	77.67	2.58	3.81	1.09	1.08
SE	0.87	0.78	2.57	0.37	0.52	7.01	0.10	0.27	0.05	0.06
CD(P=0.05)	2.48	2.23	7.31	1.05	1.46	19.93	0.29	0.78	0.14	0.18
CD(P=0.01)	3.31	2.97	9.75	1.40	1.95	26.57	0.39	1.04	0.19	0.24

*, ** indicates significant at 5% and 1% respectively.

Abbreviations: DFF=Days to 50% flowering, DM=Days to maturity, PH=Plant height (cm.), NPB=Number of primary branches, NSB=Number of secondary branches, TCPP= Total Capsules per plant, TSW=1000 seed weight (g.), YPP=Yield per plant (g.).

Table 4. Mean performance of 16 hybrids

Hybrids	DFF	DM	PH	NPB	NSB	TCPP	TSW	YPP	Sesamin	Sesamolin
IC-205071 X PKDS 12	45.2	102.6	85.1	3.7	3.0	74.8	3.22 **	4.6 **	0.8	1.0
IC 2050-71 X SI-131651	48.2	104.33	79.5	4.9	3.6	49.4	2.5	1.8	0.6	0.3
IC 2050-71 X SI-3478	46.7	97.67 **	93.9 *	6.3 **	5.9 **	73.6	3.21 **	2.7	0.8	0.8
IC 2050-71 X GUN-18	46.5	98.33	90.3	3.9	3.5	86.3	2.7	3.6	0.6	0.3
JLS 57 X PKDS 12	53.0	107.67	77.3	3.9	3.4	34.7	3.39 **	3.1	1.3 **	1.86 **
JLS 57 X SI-131651	46.3	97.33 **	82.7	4.1	4.2	66.3	2.8	2.4	0.3	0.2
JLS-57 X SI-3478	47.0	97.67 **	76.9	2.8	2.0	52.7	2.6	2.4	0.5	0.6
JLS 57 x GUN-18	46.7	99	91.5	4.4	2.8	88.1	2.8	3.3	1.52 **	1.61 **
SI 3099 x PKDS 12	46.8	104.33	76.9	4.9	2.6	45.6	2.5	1.5	1.43 **	1.9 **
SI 3099 x SI-131651	49.2	99.67	85.6	4.8	3.1	64.9	2.7	3.7	0.6	0.3
SI 3099 x SI-3478	44.7	98.67 *	82.8	5.18 *	5.2 **	71.8	2.9	2.8	0.6	0.3
SI 3099 x GUN-18	48.3	105	89.2	3.9	4.6	83.2	2.7	4.8 **	0.7	0.4
KRISHNA x PKDS 12	46.8	106	90.2	3.3	3.4	76.3	2.6	3.5	1.2 **	1.38 **
KRISHNA x SI-131651	45.3	96.67 **	92.7	5.2 *	4.7	114.2 **	3.1 **	6.5 **	1.2 **	1.15 **
KRISHNA x SI-3478	46.1	100	84.7	3.1	2.4	92 *	2.8	5.5 **	1.05 **	1.5 **
KRISHNA x GUN-18	47.5	100.67	92.1	4.0	2.8	111.8 **	2.8	4.6 **	1.73 **	1.64 **
Check	44.1	94.6 **	88.4	4.4	4.3	80.8	2.9 *	3.9	1.51 **	1.465 **
Mean	47.1	102.5	85.7	4.3	3.6	74.1	2.8	3.5	0.9	1.0
SE	0.9	0.8	2.5	0.3	0.4	6.2	0.1	0.3	0.0	0.1
CD(P=0.05)	2.5	2.2	7.2	1.0	1.2	17.6	0.3	0.7	0.1	0.2
CD(P=0.01)	3.3	3.0	9.7	1.3	1.6	23.5	0.3	0.9	0.2	0.2
CD(P=0.01)	3.31	2.97	9.75	1.40	1.95	26.57	0.39	1.04	0.19	0.24

*, ** indicates significant at 5% and 1% respectively.

Abbreviations: DFF=Days to 50% flowering, DM=Days to maturity, PH=Plant height (cm.), NPB=Number of primary branches, NSB=Number of secondary branches, TCPP= Total Capsules per plant, TSW=1000 seed weight (g.), YPP=Yield per plant (g.).

The mean performance gives individual data of the parents and crosses whereas combining ability estimates their usefulness of parents and crosses in the breeding programme. The Analysis of variance for combining ability for different characters reveal that the lines, testers and LxT were highly significant for all the studied characters except days to maturity (**Table 2**). This indicates the predominance of both additive and non-additive gene action. The predominance of non-additive gene action for the seed yield and its component traits were also reported by Manivannan and Ganesan *(2001)* for capsules/plant, days to maturity and seed yield/plant, Babu *et al.*,(2004) Gawade *et al.*, (2007) El-Shakhess and Khalifa, *(2007)* Kumar and Vivekanandan *(*2009), Shekhat *et al. (*2009) and Yamanura *et al* (2009). for days to 50 per cent flowering, and for 1000-seed weight by Prajapati *et al* (2010), Mandal *et a*.,(2010) Sakhiya (2013), Ramesh (2014), Pawar and Monpara (2016), and Dela *et al* (2019).

Table 2. Anova for combining ability

Source	DF	DFF	DM	PH	NPB	NSB	TCPP	TSW	YPP	Sesamin	Sesamolin
Lines	3	8.02 *	5.1078	148.9**	2.88**	2.196*	3418.73**	0.13**	12.03**	0.89 **	1.61 **
Tester	3	6.99 *	103.13	153.94**	1.56**	1.78 *	2406.28**	0.10 *	1.82 **	0.891**	2.236 **
L x T	9	14.07**	25.2893	74.76 **	2.64**	4.46**	517.07 **	0.28**	4.62 **	0.338**	0.64 **
Error	30	2.21	1.8319	20.27	0.38	0.52	128.79	0.03	0.21	0.0059	0.01

*, ** indicates significant at 5% and 1% respectively.

The line JLS – 57 was significant and shows negative GCA effects for all most all the characters except days to maturity and 1000 seed weight. This was found to be a good general combiner for the yield and yield attributing characters likes days to 50% flowering, plant height, the number of primary branches, the number of secondary branches and capsules per plant, and also good combiners for sesamolin content but non-significant for sesamin content (**Table 5**). The tester GUN-18 was significant and shows high and positive GCA effects towards yield and plant height, negative effects towards 1000 seed weight. The tester PKDS-12 was significant and shows negative GCA effects towards yield, total capsules, secondary branches and plant height, positive GCA effects for days to maturity, sesamin and sesamolin (**Table 5**). The entire tester included in the study was good general combiners towards sesamin and sesamolin content. None of the crosses are significant for SCA effects for all the characters. The cross IC-205071xPKDS-12 was found high and positive SCA effects towards seed yield per plant, but low towards sesamin and sesamolin content. The cross JLS-57xPKDS-12 was high and positive SCA effects towards earliness and 1000 seed weight, but low towards yield and sesamin and sesamolin content. The cross IC-205071xSI-131651 was high and negative SCAeffects towards plant height and total capsules, but

Table 5. Estimates of GCA effects

Parents	DFF	DM	PH	NPB	NSB	TCPP	TSW	YPP	Sesamin	Sesamolin
Lines										
IC-205071	0.51	-0.24	1.47	0.41 *	0.43 *	-3.07	0.09	-0.39 **	-0.26 **	-0.35**
JLS-57	1.11*	-0.56	-3.62 **	-0.47 *	-0.47 *	-13.66 **	0.06	-0.75 **	-0.02	0.12 **
SI-3099	0.10	0.94 *	-2.06	0.43 *	0.28	-7.73 *	-0.15 **	-0.34 *	-0.10 **	-0.23**
KRISHNA	-0.70	-0.14	4.21 **	-0.38 *	-0.24	24.47 **	-0.01	1.48 **	0.38 **	0.46 **
Testers										
PKDS 12	0.81	4.18 **	-3.35 *	-0.34	-0.48 *	-16.29 **	0.09	-0.37 **	0.25 **	0.57 **
SI-131651	0.11	-1.48**	-0.58	0.47 *	0.31	-0.40	-0.04	0.04 NS	-0.27 **	-0.46**
SI-3478	-1.04 *	-2.48**	-1.14	0.08	0.31	-1.59	0.06	-0.2	-0.20 **	-0.14 **
GUN-18	0.11	-0.23	5.06 **	-0.22	-0.15	18.27 **	-0.11 *	0.53 **	0.22 **	0.03
SE of Lines	0.4293	0.3907	1.8381	0.178	0.2092	3.2761	0.0474	0.1324	0.0222	0.0297
CD(P=0.05)	1.2385	1.1272	3.7497	0.5159	0.6034	9.4514	0.1368	0*382	0.064	0.0856
CD(P=0.01)	1.6696	1.5195	5.0548	0.6954	0.8135	12.7409	0.1844	0.515	0.0862	0.1154
SE of Tester	0.4293	0.3907	1.8381	0.1788	0.2092	3.2761	0.0474	0.1324	0.0222	0.0297
CD(P=0.05)	1.2385	1.1272	3.7479	0.5159	0.6034	9.4514	0.1368	0.382	0.064	0.0856
CD(P=0.01)	1.6696	1.5195	5.0548	0.6954	0.8135	12.7409	0.1844	0.515	0.0862	0.1154

*, ** indicates significant at 5% and 1% respectively.

less significant towards yield and lignan content. The cross IC 205071xSI 3478 was highly significant towards the number of primary and secondary branches and also plant height, but non-significant towards yield (**Table 6**). The cross KRISHNAxSI-131651 has a significant yield and yield attributing characters but less significant towards sesamin and sesamolin content. The cross KRISHNAxPKDS-12 and SI-3099xPKDS-12 were found to be good and negative SCA effects towards yield and high and positive SCA effects towards sesamolin and sesamin content. The crosses JLS-57xGUN-18, SI-3099xGUN-18 and IC-205071xGUN-18 were found to be good SCA effects towards sesamin and sesamolin content, but non-significant towards yield and yielding contributing characters (**Table 6**).

Table 6. Estimates of SCA effects

Hybrids	DFF	DM	PH	NPB	NSB	TCPP	TSW	YPP	Sesamin	Sesmolin
IC-205071xPKDS 12	-2.26*	-2.31**	1.23	-0.67	-0.53	20.05**	0.21 *	1.81 **	-0.16 **	-0.19 **
IC 2050-71 x SI-31651	1.43	5.07 **	-7.10*	-0.28	-0.73	-21.2**	-0.3**	-1.39**	0.16 **	0.15 *
IC 2050-71 x SI-3478	1.08	-0.59	7.83**	1.56**	1.63**	4.16	0.23 *	-0.30	0.31 **	0.36 **
IC 2050-71 x GUN-18	-0.24	-2.18 **	-1.96	-0.61	-0.37	-2.97	-0.11	-0.12	-0.31 **	-0.33 **
JLS 57 x PKDS 12	3.94**	3.07 **	-1.48	0.44	0.78	-9.49	0.41**	0.68 *	0.14 **	0.21 **
JLS 57 xSI-131651	-2.03*	-1.61 *	1.16	-0.19	0.81	6.29	-0.04	-0.45	-0.33 **	-0.39 **
JLS-57 x SI-3478	-0.21	-0.27	-4.05	-1.09 **	-1.41 *	-6.19	-0.20	-0.19	-0.21	-0.32
JLS 57 x GUN-18	-1.70	-1.19	4.37	0.84 *	-0.17	9.39	-0.01	-0.05	0.40 **	0.50 **
SI 3099 x PKDS 12	-1.27	-1.76 *	-3.39	0.53	-0.78	-4.54	-0.3**	-1.34**	0.37 **	0.59 **
SI 3099 x SI-131651	1.82 *	-0.77	2.57	-0.35	-1.10*	-1.07	0.05	0.43	0.00	0.04
SI 3099 x SI-3478	-1.53	-0.77	0.30	0.38	0.99 *	7.01	0.15	-0.18	-0.05	-0.27 **
SI 3099 x GUN-18	0.99	3.31 **	0.53	-0.56	0.89 *	-1.41	0.09	1.09 **	-0.31 **	-0.36 **
KRISHNA x PKDS 12	-0.41	0.99	3.64	-0.30	0.53	-6.02	-0.3**	-1.16**	-0.35 **	-0.61 **
KRISHNAx SI-131651	-1.21	-2.69 **	3.37	0.83 *	1.02 *	16.02*	0.32**	1.41 **	0.17 **	0.19 **
KRISHNA x SI-3478	0.67	1.64 *	-4.08	-0.85*	-1.2**	-4.99	-0.03	0.66*	-0.04	0.23 **
KRISHNA x GUN-18	0.95	0.06	-2.93	0.32	-0.35	-5.01	0.03	-0.92**	0.22**	0.19 **
SE	0.8586	0.7814	2.5995	0.3576	0.4183	6.5521	0.0948	0.2648	0.0444	0.0593
CD(P=0.05)	2.4771	2.2545	7.4994	1.0318	1.2069	18.9028	0.2735	0.764	0.128	0.1712
CD(P=0.01)	3.3392	3.0391	10.1095	1.3909	1.6269	25.4817	0.3687	1.0299	0.1725	0.2308

*, ** indicates significant at 5% and 1% respectively.

Abbreviations: DFF=Days to 50% flowering, DM=Days to maturity, PH=Plant height (cm.), NPB=Number of primary branches, NSB=Number of secondary branches, TCPP= Total Capsules per plant, TSW=1000 seed weight (g.), YPP=Yield per plant (g.).

In this study, we concluded that the tester PKDS-12 was found to be good general combiner for seed yield, capsules per plant, plant height, days to maturity, the number of secondary branches, along with high sesamolin and sesamin content. The cross combinations *viz.*, KRISHNAxPKDS-12, SI-3099xPKDS-12, JLS-57xGUN-18 were found to be good specific combiners for sesamin and sesamolin content. These crosses will be further exploited for isolating high sesamin and sesamolin content with high yield.

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