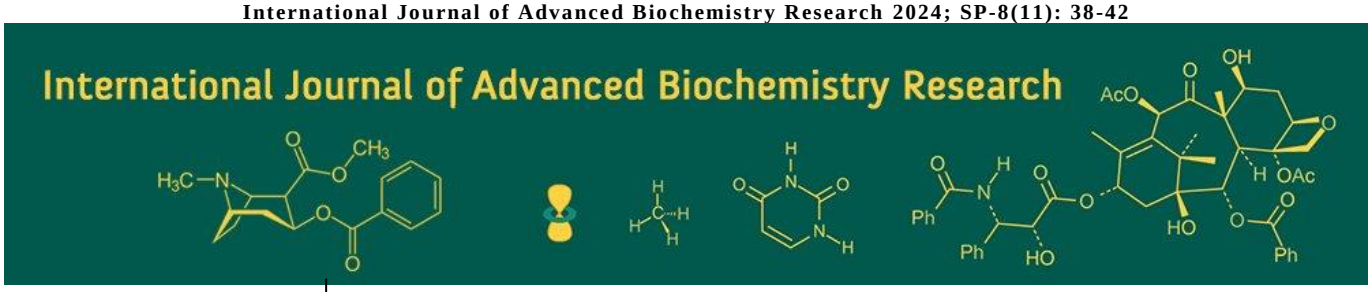

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Heterosis and combining ability analysis for quality traits in soybean [*Glycine max* (L.) Merrill]

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Abstract
Soybean [*Glycine max* (L.) Merrill] is "wonder crop" is richest, cheapest and easiest source of quality protein and oil quality. Heterosis and combining ability analysis were carried out in F_1's hybrids of 36 crosses derived from crossing of 12 soybean lines with 3 testers. Based on analysis of mean performance, heterosis and combining ability, the best soybean parents and crosses for quality traits were identified. For both seed oil and protein content, high heterosis and positive. Specific Combining Ability (SCA) effects were recorded in crosses RKS 113 x SL 958, JS 20-98 x NRC 138 RVSM 2011-35 x JS 20-34 and AUKS 212 x JS 20-34 could be further exploited. These crosses involved geographically diverse parents and at least on parent of high General Combining Ability (GCA) effects in combination of high x high, high x average and high x low. Therefore, these crosses could throw transgressive segregants in still advance generations. Future, the results of present study suggested that heterosis coupled with high SCA effects might be considered as a criteria for selecting crosses for further improvement with respect to quality traits.

Keywords: Soybean (*Glycine max*), wonder crop, quality protein, oil quality, heterosis

Introduction
Soybean [*Glycine max* (L.) Merrill.] is one of the most important oil seed crops. Its first rank in oil seed production and it is also contribution (38%) followed by Rapeseed-mustard, groundnut and sunflower among the oil seed crops of India (Joshi *et al.* 2016) [6]. Soybean is self-pollination crop and belongs to the family fabaceae and sub-family papilionace having 2n = 2x = 40 and originated in North Eastern China. Because of its multi uses, the soybean crop is called as "Golden Bean" or "Miracle crop of the world. Its cost-effectiveness is ensured through its biological nitrogen fixation and in rotation with exhaustive crops such as maize and sorghum. It helps replenish and maintain soil fertility. It provides a large amount of edible vegetable oil as well as soybean cake, which are used as high protein supplements in mixed feed ration for livestock.

Soybean is one of the best and cheapest sources of high-quality vegetable protein, calcium, phosphorus, iron, and vitamin A. The soy protein stands unique because it supplies all ten essential amino acids (EAAs) including lysine while most vegetable proteins are deficient in supplying all the essential amino acids. Today, soy foods such as Tofu, miso, shoyu, soy milk, tempeh and immature green bean are being consumed in daily dish by more than a billion people in China, Japan, Korea and Southeast Asia. Soybean plays a major role in the world food system. It has become the major source of edible vegetable oil and protein for feed and food supplement in the world. It is also known as an excellent source of good quality unsaturated fatty acids (about 80%) and is very high in the essential fatty acids such as oleic acids (23%) linoleic acid (53%) and linolenic acid (7%) (Fehr *et al*., 1992) [3], Soya foods not only provide a source of nutritionally balanced protein but they are rich in phytochemicals with proven benefits to prevent heart diseases, cancers, osteoporosis, diabetes and other chronic human diseases. Recently, soybean is found to be an industrially important crop used as anticorrosion agent, core oil and bio fuel to less or no nitrogen element in the oil, and as a disinfectant, in pesticides, printing ink, paints, adhesives antibiotics and cosmetics. The upsurge of interest in soybean is due to the presence of "isoflavones" which have been shown to inhibit the growth of cancerous cell, bone respiration and reduce the level of cholesterol.

Because of its high (20%) oil content, soybean is also classed as an oilseed crop among legumes, however, it also has high protein content of 38-45%. As compared to other legumes and cereals it has highest protein content of 40% where as other legumes have a protein content of 20-30% and cereals have protein content ranged approximately 8-15%. Soybean has second highest oil content among all food legumes, with peanut having oil content of approximately 48% on dry matter basis and chickpea as third highest (5% oil content) (Salunkhe *et al*., 1992) [8]. Other nutritionally important components found in soybeans include phospholipids, vitamins, and minerals. In addition to this, soybean also contains minor substance such as phytates, trypsin inhibitors, and oligosaccharides, in biologically active form.

To develop high yielding and better nutritional quality cultivars in soybean a systematic approach has to be adopted. Protein and oil are polygenic character and its improvement is basically based upon identification of quality traits as well as selection of parents or lines, which can be effectively used for developing superior quality genotypes for any breeding programme.

The knowledge of combing ability is useful to assess the nicking ability of parents and at the same time elucidating the nature and magnitude of different types of gene action involved. This can also be immense help to exploit hybrid vigor in the heterosis breeding, which indicates the ability of parents to combine well in hybrid combinations. One of the most important factors which determine the success of hybrid production is the nature and magnitude of heterosis over the better parent and the check. Latter a commercially exploitable heterosis exhibits by the cross commercial exploitation of heterosis has not become a reality in soybean mainly due to the self-pollinated nature of crop and it's not feasible to produce sufficient quantity of F_1 hybrid seed. The technique of Line x tester analysis (Kempthorne, 1957) [7] tends itself to detailed genetic analysis, identification of superior parents and cross combination on the basis of combing ability besides providing information pertaining to the nature and magnitude of gene action. This information can be utilized for adoption of appropriate breeding methods for improving the crop.

Materials and Methods

The experimental material consisted of F_1's hybrids of 36 crosses derived from crossing of 12 soybean lines with 3 testers in Line x Tester fashion during *Kharif*, 2020. The fifteen parents of soybean were selected on the basis of their diverse pedigree, growth habit, phenology and adaptation. The experimental material (36 F_1's hybrids, 15 parents along with the check variety JS 95-60) were raised in randomized complete block design with three replications at Research Field of AICRP on soybean, Agricultural Research Station, Ummedganj, Kota during *Kharif*, 2021. Each parent was sown in two rows along with a single row of F_1 hybrids of three-meter in length by adopting the spacing 45 × 10 cm. The recommended package and practices were followed to raise healthy crops during the crop period. Bulk seed of 10 randomly selected competitive plants of parents and F_1's hybrids in each replication was used for analysis of seed protein and oil content. The protein content was estimated by using the method described by Lowry *et al*. (1951) [16] while oil content in seed samples was analysed by Soxhlet extraction method at the central laboratory of Ummedganj Kota. Different types of heterosis *viz*.,heterosis over mid parent (MP), heterosis over better parent (Heterobeltiosis) and economic heterosis over standard check (JS 95-60) were estimated using mean values. Analysis of Line x Tester data for combining ability was done according to Kempthorne (1957) [7] and Arunachalam (1974) [1].

Results and Discussion

Analysis of variance was carried out for lines, tester, their crosses and that revealed highly significant differences for all the lines and crosses indicating sufficient variation in the material for both protein and oil content. However, mean squares due to testers were significant for only oil content. The study for mean and range revealed that among parents there was great variability in the lines than testers for both the characters. It was reflected that the hybrids superior in F_1'sinvolved both or at least one parent of high *per se* performance.

For protein content (Table-1), hybrids AUKS 212 x JS 20-34, JS 20-98 x JS 20-34 and RVSM 2011-35 x NRC 138 showed positive significant heterosis at all three levels of heterosis over mid parent, better parent and standard check (JS 95-60) in desirable direction. Out of 36 crosses, 26 hybrids exhibited positive significant economic heterosis over standard check (JS 95-60) and 10 exhibited positive significant heterosis over mad parent for protein content. The cross AUKS 212 x JS 20-34 was exhibited highest economic heterosis with high *per se* performance. Gadag and Upadhyaya (1995) [4] and Sabbouh *et al*., (1998) [10] also observed significant positive heterosis for protein content. Significant positive heterosis was also observed earlier by Sharma and Maloo (2009) [9], Samant *et al.* (2014) [11] and Indu *et al.* (2017) [5] for seed protein in soybean.

In case of oil content, revealed that 20 hybrids exhibited significant economic heterosis in desired direction for oil content. The cross RVSM 2011-35 x JS 20-34 showed significant heterosis at all the three levels such as over mid parent, better parent and standard check in desired direction while the cross combinations AUKS 212 x SL 958, AUKS 212 x JS 20-34 and RKS 18 x NRC 138 showed significant mid parent heterosis as well as economic heterosis in desired direction, while cross RKS 113 x SL 958 was exhibited positive significant average heterosis but non-significant heterobeltiosis as well as standard heterosis in desirable direction with high per se performance. Wehrman *et al.* (1987) [14], Fahmi *et al.* (1999) [12], Bhavani *et al.* (2017) [2] and Indu *et al*. (2017) [5] also reported earlier for oil content in soybean as observed in present study.

In autogamous crops like soybean, combining ability provides required information about the nature and magnitude of genetic variation along with nicking ability of genotypes. Over and above, the study become much more precise and reliable if the relative combining ability is analysed in F1, F2, and F3 generations so as to obtained a good degree of correspondence between these estimates which will help in identifying superior parents and crosses that could be effectively utilized in breeding progamme. Such study may also reflect the possibility of obtaining rare favourable combinations of characters in segregating generations.

Analysis of variance for combining ability for both the quality traits indicated that variance due to crosses, lines and line x tester interaction were significant for protein and oil content. Significance of lines and testers indicated higher

contribution towards general combining ability, while for lines x tester interaction towards specific combining ability for both the characters. Predominance of Specific Combining Ability (SCA) variance over General Combining Ability (GCA) indicated importance of non-additive genetic effects for protein and oil content. Contribution of lines was higher for oil content, whereas higher contribution due to interaction between line and tester was for protein content.

Table 1: Heterosis (MP), Hetero-beltiosis (BP) and Standard heterosis (SC) for quality traits in soybean.

Crosses	Protein content (%)			Oil content (%)		
	MP	BP	SC	MP	BP	SC
RKS 18 × JS 20-34	1.96**	1.52	6.00**	2.12	0.24	5.03**
RKS 113 × JS 20-34	0.20	-0.85	3.53**	-4.74**	-8.39**	-4.01**
AUKS 199 × JS 20-34	-3.52**	-5.62**	-1.45	1.15	0.57	5.38**
AUKS 200 × JS 20-34	1.48*	0.55	5.00**	-0.83	-3.09*	1.54
AUKS 212 × JS 20-34	4.28**	2.40**	6.93**	4.46**	0.53	13.91**
RVSM 2011-35 × JS 20-34	1.81*	-0.40	4.01**	8.55**	5.72**	10.77**
JS 20-98 × JS 20-34	1.83*	1.72*	6.46**	-10.31**	-11.48**	-7.25**
AUKS 202 × JS 20-34	-1.24	-1.59	3.49**	-6.36**	-7.65**	-3.24*
AUKS 208 × JS 20-34	-3.67**	-5.13**	-0.94	0.82	0.65	5.46**
AUKS 218 × JS 20-34	-0.91	-2.41**	1.90*	0.69	0.24	5.97**
JS 21-72 × JS 20-34	0.24	-1.92*	2.42**	1.28	-0.24	7.76**
NRC 165 × JS 20-34	0.15	-0.81	3.58**	-2.72*	-3.15*	2.39
RKS 18 × NRC 138	1.47*	1.17	4.72**	2.77*	-0.94	7.76**
RKS 113 × NRC 138	-3.57**	-3.88**	-1.09	-1.87	-7.29**	0.85
AUKS 199 × NRC 138	3.07**	1.55	4.49**	0.60	-1.80	6.83**
AUKS 200 × NRC 138	-0.82	-1.01	1.86*	-7.07**	-10.82**	-2.99*
AUKS 212 × NRC 138	2.23**	1.12	4.05**	2.19	0.15	13.48**
RVSM 2011-35 × NRC 138	3.99**	2.47**	5.44**	2.26	-2.20	6.40**
JS 20-98 × NRC 138	-6.89**	-7.67**	-3.37**	0.85	-2.27	6.31**
AUKS 202 × NRC 138	0.06	-1.02	4.09**	-1.82	-4.94**	3.41*
AUKS 208 × NRC 138	-1.06	-1.85*	1.00	1.56	-0.47	8.28**
AUKS 218 × NRC 138	-3.33**	-4.10**	-1.32	2.15	0.71	9.56**
JS 21-72 × NRC 138	-0.75	-2.18*	0.65	-0.83	-1.18	7.51**
NRC 165 × NRC 138	1.15	0.91	3.84**	-4.53**	-5.88**	2.39
RKS 18 × SL 958	-0.51	-0.75	3.22**	-0.13	-0.50	1.19
RKS 113 × SL 958	-0.55	-1.40	2.55**	3.35*	0.84	2.56
AUKS 199 × SL 958	-4.18**	-6.09**	-2.32**	2.41	1.48	5.12**
AUKS 200 × SL 958	-1.07	-1.79*	2.15*	-8.38**	-9.14**	-7.59**
AUKS 212 × SL 958	2.26**	0.61	4.64**	5.95**	0.53	13.91**
RVSM 2011-35 × SL 958	0.80	-1.19	2.77**	1.95	0.76	2.47
JS 20-98 × SL 958	-4.31**	-4.61**	-0.16	1.59	1.42	3.50*
AUKS 202 × SL 958	1.13	0.57	5.77**	-0.42	-0.50	1.37
AUKS 208 × SL 958	0.54	-0.79	3.19**	-1.16	-2.45	1.88
AUKS 218 × SL 958	1.30	-0.04	3.96**	-3.74**	-5.57**	-0.17
JS 21-72 × SL 958	-2.59**	-4.51**	-0.68	-0.16	-3.08*	4.69**
NRC 165 × SL 958	-0.65	-1.41	2.55**	-3.58**	-5.41**	0.00

*, ** Significant at 5% and 1% level of significance, respectively

On the basis of heterosis, it can be concluded that heterosis was displayed for almost all the characters. Seed yield is the sum total of contribution made by individual component and hence a complex character itself. The breeder has to simplify complex situation to breed for high yield by handling number of related traits simultaneously. A good number of crosses showed the presence of desirable heterotic response for the different characters over the standard cultivars. Among the parents lines AUKS 212, RKS 18, AUKS 202, RVSM 2011-35 and tester JS 20-34 exhibited highly significant GCA effects for protein content. While for oil content AUKS 212, JS 21-72, RVSM 2011-35, AUKS 199 and tester NRC 138 could be considered superior as these exhibited high GCA effects (Table 2). The per se performance of most of the parent was moderate to high for protein and oil content. Among the hybrids, positive significant SCA effects for protein content were exhibited by AUKS200 x JS 20-34, RVSM 2011-35 x JS 20-34, RKS 113 x SL 958, JS 20-98 x SL 958, JS 20-98 x NRC 138 and AUKS 218 x NRC 138. On the other hand total out of 36 crosses only one hybrid RKS 113 × NRC 138 was exhibited highly significant in positive SCA effect for oil content. However, sixteen crosses were also exhibited non-significant positive SCA effects in desired direction (Table 3).

Among these hybrids most of them had high *per se* and involving good general combiner parents, hence, could be used effectively in quality improvement of soybean. Therefore, on basis of combining ability effects (Table-3) for the quality traits *i.e.* protein and oil content, the cross combinations AUKS 200 x JS 20-34 (average × good), RVSM 2011-35 x JS 20-34 (good × good), RKS 113 x SL 958 (poor × average), JS 20-98 x SL 958 (poor × average), JS 20-98 x NRC 138 (poor x poor) and AUKS 218 x NRC 138 (poor × poor) were showed highly significant SCA effects as well as *per se* performance in desired direction for protein content and only one cross RKS 113 x SL 958 (poor x poor) was showed high *sca* effects as well as *per se* performance and identified as best hybrid for oil content. Moreover, a good correspondence between SCA effects and

heterosis was also observed in these cross combinations. Similar to present findings Gadag *et al.,* (1999) [4] also reported importance of non-additive gene effects for protein content. In case of oil content, as per present study Sharma *et al.,* (2009) [9] and Indu *et al.,* (2017) [5] recorded partial dominance and non-additive gene action.

Table 2: General Combining Ability (GCA) effects for quality characters.

S. No.	Parents	Protein content (%)	Oil content (%)
	Lines		
1	RKS 18	0.80**	0.14
2.	RKS 113	-0.35*	-0.81**
3.	AUKS 199	-0.91**	0.36**
4.	AUKS 200	0.16	-1.36**
5.	AUKS 212	1.02**	1.92**
6	RVSM 2011-35	0.58**	0.51**
7	JS 20-98	-0.62**	-0.61**
8	AUKS 202	0.73**	-0.67**
9	AUKS 208	-0.58**	0.24
10	AUKS 218	-0.41**	0.23
11	JS 21-72	-0.69**	0.53**
12	NRC 165	0.29	-0.46**
	Testers		
1	JS 20-34	0.32**	-0.06
2	NRC 138	-0.21**	0.36**
3	SL 958	-0.11	-0.30**

*, ** Significant at 5% and 1% level of significance, respectively

Based on various estimates viz., mean performance, heterosis and combining ability the best soybean parents and crosses for quality traits were identified. For both protein and oil content, high heterosis and positive SCA effects were recorded in cross RKS 113 x SL 958, AUKS 212 x JS 20-34, RVSM 2011-35 JS 20-34 and could be further exploited. These crosses involved geographically diverse parents and at least one parent of high GCA effects in combination of high x high, high x average and high x low. Majority of soybean breeding work is based on pedigree method, which exploits the fixable additive type of gene effects. However, this alone may be inadequate in view of the large non-additive component for both quality traits. Further, high heterosis for oil and protein along with positive SCA effects was observed in these crosses, which might be due to dominance or epistasis. Although heterosis breeding makes maximal use of the non-additive genetic effects appears to be difficult for improving soybean in view of the non-availability of mass pollination systems needed for hybrid seed production. Accordingly, the feasible alternative is to consider simultaneous exploitation of both additive and non-additive gene action by adopting recurrent selection procedures. Under similar situation Harer and Deshmukh (1991) [13] recommended biparental mating in soybean followed by recurrent selection. Since crossing is tedious in soybean, genetic male sterility and single seed or single pod descent method could be employed to facilitate recurrent selection schemes (Wilcox, 1998) [15]. Therefore, these crosses could throw transgressive segregants in still advance generations. Further, the results of present study suggested that heterosis coupled with high SCA effects might be considered as criteria for selecting cross for further improvement with respect to quality traits. To conclude, combining ability mean squares estimated revealed that both the quality traits displayed preponderance of non-additive gene effects. Often the unfavorable association between protein and yield generally met with are due to tight linkages, which could be broken by raising a large population. In the present study RKS 113 x SL 958 revealed superiority for protein content, oil content as well as yield might be considered a new favorable combination resulted due to breaking of tight linkages. Through the use of recurrent selection suggested that such negative relationship may be caused by linkage and improvements can be made for such traits by enforcing simultaneous selection in large segregation population.

Table 3: Specific Combining Ability SCA) for quality traits in soybean.

S. No.	Crosses	Protein content (%)	Oil Content (%)
1	RKS 18 × JS 20-34	0.13	-1.53
2	RKS 113 × JS 20-34	-0.68**	-2.05
3	AUKS 199 × JS 20-34	-0.02	1.24
4	AUKS 200 × JS 20-34	0.95**	-0.51
5	AUKS 212 × JS 20-34	0.09	2.01
6	RVSM 2011-35 × JS 20-34	0.89**	0.07
7	JS 20-98 × JS 20-34	-1.52**	1.85
8	AUKS 202 × JS 20-34	-0.67**	-1.66
9	AUKS 208 × JS 20-34	0.11	1.10
10	AUKS 218 × JS 20-34	0.23	-1.23
11	JS 21-72 × JS 20-34	0.28	-0.57
12	NRC 165 × JS 20-34	0.22	1.28
13	RKS 18 × NRC 138	0.24	1.10
14	RKS 113 × NRC 138	-0.16	-3.92**
15	AUKS 199 × NRC 138	-0.16	1.75
16	AUKS 200 × NRC 138	-0.36	1.02
17	AUKS 212 × NRC 138	-0.42	-1.32
18	RVSM 2011-35 × NRC 138	-0.39	1.62
19	JS 20-98 × NRC 138	0.70**	2.04
20	AUKS 202 × NRC 138	0.20	0.13
21	AUKS 208 × NRC 138	0.24	-0.12
22	AUKS 218 × NRC 138	0.50*	-0.14
23	JS 21-72 × NRC 138	-0.20	0.15
24	NRC 165 × NRC 138	-0.21	-2.31
25	RKS 18 × SL 958	-0.38	0.43
26	RKS 113 × SL 958	0.84**	5.97**
27	AUKS 199 × SL 958	0.17	-2.99*
28	AUKS 200 × SL 958	-0.59*	-0.51
29	AUKS 212 × SL 958	0.33	-0.69
30	RVSM 2011-35 × SL 958	-0.49*	-1.69
31	JS 20-98 × SL 958	0.82**	-3.90**
32	AUKS 202 × SL 958	0.47	1.54
33	AUKS 208 × SL 958	-0.35	-0.98
34	AUKS 218 × SL 958	-0.73**	1.37
35	JS 21-72 × SL 958	-0.08	0.42
36	NRC 165 × SL 958	-0.01	1.03

*, ** Significant at 5% and 1% level of significance, respectively

Conclusion

The study on combining ability and heterosis in soybean revealed significant variation in both protein and oil content across lines, testers, and their hybrids. The predominance of specific combining ability (SCA) effects over general combining ability (GCA) highlighted the importance of non-additive gene effects, particularly for oil content. High heterosis and positive SCA effects were observed in several crosses, including RKS 113 x SL 958, AUKS 212 x JS 20-34, and RVSM 2011-35 x JS 20-34, which could be exploited for quality improvement. These findings suggest that both additive and non-additive gene actions should be considered for future breeding strategies, with recurrent

selection offering a viable approach to overcoming the challenges in soybean breeding, particularly for traits like protein and oil content.

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