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COMBINING ABILITYSTUDIES IN OKRA (*ABELMOSCHUS ESCULENTUS* L.) MOENCH) FOR PROTEIN, TOTAL DIETARY FIBRE AND MINERAL CONTENT

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ABSTRACT: Okra is an important vegetable crop cultivated across the warmer parts of the world and has high nutritive and medicinal values. Since little information is available with respect to the combining ability for nutritional traits, like protein, total dietary fibre, phosphorus, potassium, calcium, iron and copper, therefore, the present study was undertaken to estimate the combining ability of eight parental lines for mineral, protein and total dietary fibre content. The general combining ability (GCA) and specific combining ability (SCA) variances were highly significant for all the characters, but the magnitude of the GCA variance were more preponderant (σ^2gca/ σ^2sca ratio < 1) indicating that the additive gene effect was important in the control and inheritance of most of the traits, except total dietary fibre and calcium. DOV-1-2 was found to be a good combiner for phosphorus, calcium and iron, DOV-2 for total dietary fibre, phosphorus and copper, Pusa A-4 for calcium and iron, and DOV-62 for total dietary fibre, phosphorus. Significant SCA effect in positive direction was recorded in twoF_{1s} (DOV-1-2 × IC-090491and Pusa A4 × DOV-2) for total dietary fibre, phosphorus, potassium, calcium, iron and copper and another hybrid WB Selection × IC-090491 for phosphorus, potassium, calcium, iron and copper. Not a single parent or cross combination showed significant GCA or SCA for all the characters under study, indicating to the need of utilising population improvement methods for biofortification in okra without compromising yield and other horticultural traits.

KEYWORDS: Okra, nutritional quality, Protein, Total Dietary Fibre, Minerals, Combining Ability

After having achieved food security, nutritional security is a major challenge for India. As we know that due to deficiency of various nutrients in our diet, peoples are facing problem of malnutrition, stunting and underweight especially children and women. Okra being a rich source of minerals, vitamins and fibre will be helpful in overcoming these problems.The dry fruit shell and stem containing crude fibre are used in paper making and dried stems and roots are used for clarification of sugarcane juice. Okra polysaccharides are also used as egg white substitute; fat substitute in chocolate bar cookies and in chocolate frozen dairy dessert (Sengkhamparn et al. 2009). India ranks first in the world with a production of 6.0 million tonnes (72% of world production) from over 0.5 million ha land (NHB, 2016-17). The improvement in its nutritional status coupled with yield can effectively contribute to reduction of micronutrient malnutrition existing especially in developing countries.

The combining ability analysis i.e. GeneralCombining Ability (GCA) and Specific Combining Ability (SCA) effects aid in identification of superior parents and best hybrids, respectively. The half diallel analysis is the biometric technique used in this study to evaluate the GCA and SCA estimates for important nutrients. The estimates of combining ability also evaluate the relative magnitude of additive and non-additive gene effects and relevant breeding techniques for involving potential and nutrient rich cultivars and hybrids in okra. Little orientation is accessible on the genetic variability and combining ability of protein, TDF and mineral content in okra, based on the available literature. Thus, this study was undertaken to identify potential genotypes and crosses based on GCA and SCA performance, also to suggest suitable breeding approaches for nutrient enhancement in okra fruits for bio fortification.

MATERIALS AND METHODS: The study was carried out at the Research Farm of Division of Vegetable Science, Indian Agricultural Research Institute, New Delhi witheight genetically divergent parental lines *viz.*, Pusa A4, DOV-1-2, DOV-2, DOV-12, WB selection, IC-090491, DOV-62 and C-10 whose mean nutrient content is depicted in Table 1 were selected and crossed in diallel fashion without reciprocal (Simmonds 1990; Fehr et al.1987) to obtain 28 F_1 hybrids. Twenty eight hybrids were developed and sown along with their parents at a spacing of 60cm x 30cm in a completely randomised block design with three replications and recommended cultivation practices were followed to raise a good crop and data were recorded for the quality traits.

For nutrient analysis, fresh fruits after 6th day of anthesis were collected from each replication after washing thoroughly in tap water to remove adhering dust and soil, decontaminating using 0.2 per cent teepol solution, 0.1 N HCl solutions and double distilled water in series were cut and packed. Then these samples were dried in a hot air oven at temperature of 70-72^{0}C in labelled paper bags. The dried samples were ground with the help of Wiley mill, passed through 1mm mesh sieve and stored in air tight containers for digestion for tissue nutrient analysis. Each sample was digested by using di-acid mixture (nitric acid and perchloric acid in 9:4 ratio) (Prasad 1998)and assayed on GBC Avanta PM 904 AA model atomic spectrophotometer (GBC, Australia) for Ca, Fe and Cu using nitrous oxide–acetylene flame, air-acetylene flame respectively at 422nm, 279.5nm 324.7nm wavelength, at current 5 mA. Phosphorous content was determined by Vando-molybdate method given by measuring per cent transmittance of diluted di acid digested sample at 420 nm with the help of methodUV-VIS spectrophotometer DR5000 model(Hach company, USA) (Olsens and Sommers 1982). Potassium was estimated by using Systronic Flame Photometer Type 128 (Systronics, India) using specific filter for potassium and LPG flame according to method given by Jackson 1973and expressed as particle per million. Protein was determined by multiplying nitrogen content estimated through Micro Kjeldhal method with conversion factor (6.25) expressed in grams per 100 gram sample. Total dietary fibre content was estimated using the Megazyme TDF Kit KTDFR (Bray, Co.Wicklow, Ireland) based on AOAC enzymatic gravimetric method 985.29 and AACC method 32-05.01 expressed as milligram per gram sample (AOAC 1987).

STATISTICAL ANALYSIS: Statistical analysis of data generated was done following the Method II under Model I as suggested by (Griffing 1956) and by using OPSTAT developed by Chaudhary Charan Singh Haryana Agricultural University, Hisar, and Haryana, India.

RESULTS AND DISCUSSION: The estimates of variance components for various nutrients, namely, protein, total dietary fibre, P, K, Ca, Fe and Cu content were depicted in Table 2. For protein, P, K and Cu, the value of $\sigma^2_{gca}$was lower than σ^2_{sca}, while for TDF, Ca and Fe content, variance due to SCA (σ^2_{sca})was lower than variance due to GCA (σ^2_{gca})(Table 3). The $\sigma^2_{gca}/\sigma^2_{sca}$ ratio was less than unity for protein, P, K, Cu and more than unity in TDF, Ca and Fe content. In all nutrient traits studied, dominance variance (σ^2D) was greater than additive variance (σ^2A).

Table- 1: Performance of eight parental lines of okra for the various nutritional traits

Parents	Protein (g)	TDF (mg/g)	Phosphorus (ppm)	Potassium (ppm)	Calcium (ppm)	Iron (ppm)	Copper (ppm)
PUSA A4	1.02	62.33	24.80	227.00	42.49	10.34	3.66
DOV-1-2	2.17	62.00	28.67	190.67	65.20	22.30	3.34
DOV-2	1.52	70.67	26.77	250.33	43.71	14.81	4.79
DOV-12	2.08	55.67	26.40	205.33	38.64	10.23	4.63
WB Selection	1.96	80.67	23.50	196.33	35.85	7.27	3.18
IC-090491	1.85	70.67	23.27	212.00	25.08	8.43	3.98
DOV-62	2.33	97.33	23.30	205.33	36.73	15.59	4.31
C-10	1.46	70.67	27.33	306.00	33.23	15.73	3.50
MEAN	1.80	71.25	25.51	224.12	40.12	13.09	3.92
S.E.	0.09	3.69	0.76	1.61	2.45	0.37	0.12
C.D. at 5%	0.23	9.78	2.02	4.25	6.48	0.97	0.31

Table-2: Analysis of variance for protein, total dietary fibre (TDF), phosphorus (P), potassium (K), calcium (Ca), iron (Fe) and copper (Cu) in okra fruits

Source of variation	d.f.	Protein	TDF	P	K	Ca	Fe	Cu
Replication	2	0.325	1792**	5.55**	242.12**	1439.5**	33.64**	22.12**
Treatment	35	0.315	657.68**	13.73**	3672.52**	263.30**	50.86**	0.316
Error	70	0.024	20.45	0.869	3.87	8.98	0.199	0.017

**= Significance at 1% level

Table- 3: Estimates of variance components for protein, total dietary fibre, phosphorus, potassium, calcium, iron and copper in okra fruits

Source of Variation	d.f.	Mean sum of squares						
		Protein	TDF	P	K	Ca	Fe	Cu
Due to GCA	7	0.10**	1293.11**	12.77**	2956.86**	614.53**	87.90**	0.13**
Due To SCA	28	0.51**	498.83**	13.96**	3851.43**	175.50**	41.60**	0.36**
Error	70	0.011	20.45	0.869	3.87	8.98	0.20	0.02
$\sigma^2_{gca}/\sigma^2_{gca}$		0.20	2.59	0.91	0.77	3.50	2.11	0.73
σ^2A		0.002	1.2	0.04	0.22	0.52	0.012	0.002

σ^2D	0.007	5.6	0.24	1.06	2.46	0.05	0.005

**= Significance at 1% and * = Significance at 5% level

ESTIMATION OF GENERAL COMBINING ABILITY (GCA)

The positive GCA of the parental line for a trait indicates, its contribution to the high concentration of that trait, while the negative GCA indicate its contribution to low concentration of that trait.The estimates of GCA effects (Table 4) revealed that among the eight parental lines, DOV-2 is a good combiner for only TDF while WB Selection showed poor general combining ability for all the traits studied. general combiner for TDF, P and Cu but a poor general combiner for protein, K, Ca and Fe. DOV-1-2 is a good general combiner for K, Ca and Fe, while being a poor general combiner for other traits like protein, TDF and K. Pusa A4 was a good general combiner forCa and Fe while DOV-62 was best general combiner for TDF. Similarly IC-090491 was a good general combiner for protein and Cu, while being a poor general combiner for all other traits. DOV-12 showed poor general combining ability for most of the traits except protein. C-10 had been good general

Table- 4: Estimates of general combining ability (GCA) effects for protein, total dietary fibre, phosphorus, potassium, calcium, iron and copper in okra fruits

Parent	Protein	TDF	P	K	Ca	Fe	Cu
PUSA A4 (P_1)	-0.06**	-8.31**	-0.09	4.79	2.82**	3.15**	0.01
DOV-1-2(P_2)	-0.003	-0.91	0.82**	17.79**	9.49**	1.76**	-0.03
DOV-2(P_3)	0.01	8.19**	0.48**	-8.37**	-1.04**	-0.20**	0.11**
DOV-12(P_4)	0.09**	-4.11**	0.09	-13.94**	-3.18**	-0.01	-0.03
WB Selection (P_5)	-0.03	-6.44**	-1.09**	-1.14	-5.45**	-2.14**	-0.08**
IC-090491(P_6)	0.05**	-0.01	-0.69**	2.76	-2.01**	-0.46**	0.05**
DOV-62 (P_7)	-0.08**	10.22**	0.61**	-7.07**	-0.57	-1.52**	0.04
C-10 (P_8)	0.01	1.36*	-0.12	5.19	-0.06	-0.57**	-0.07**
SE	0.02	0.77	0.16	0.34	0.51	0.08	0.03

**= Significance at 1% level

ESTIMATION OF SPECIFIC COMBING ABILITY (SCA) : Among the 28 crosses studied for SCA effects (Table 5), positive significant SCA was obtained in twelve crosses for TDF and K, nine crosses for protein and Cu, thirteen crosses for P, eight crosses for Ca and ten crosses for Fe content. The crosses DOV-1-2 × IC-090491 showed significantly positive SCA for all traits estimated except protein. Significant positive SCA effects for P, K, Ca, Fe and Cu were obtained in the crosses WB Selection × IC-090491 and Pusa A4 × DOV-2. The cross DOV-12 × WB Selection showed positive significant SCA for protein, TDF and K content. Significantly positive SCA effects for nutrients were obtained in four crosses, i.e., Pusa A4 × DOV-1-2 for protein, P, Ca and Fe; Pusa A4 × DOV-62 for protein, TDF, Fe and Cu; DOV-1-2 × DOV-62 for P, K, Ca and Cu, DOV-12 × IC-090491 for TDF, P and K. Three crosses revealed significantly positive SCA in three traits DOV-1-2 × C-10 for protein, TDF and Ca, Pusa A4 × DOV-12 for protein, P and Cu, DOV-2 × C-10 for Caand Fe.

Table- 5: Estimates of specific combining ability (SCA) effects for protein, total dietary fibre, phosphorus, potassium, calcium, iron and copper

Sr no	Cross	Protein	TDF	P	K	Ca	Fe	Cu
1	Pusa A4 x DOV-1-2	0.41**	-8.27**	2.41**	-51.04**	15.64**	8.11**	-0.49**
2	Pusa A4 x DOV-2	-0.19**	-8.71**	0.85*	34.80**	4.69**	2.58**	0.73**
3	Pusa A4 x DOV-12	0.28**	-11.41**	0.88*	-4.64**	1.75	-2.19**	0.72**
4	Pusa A4 x WB Selection	0.27**	15.93**	-0.84*	-26.44**	1.23	-3.02**	-0.59**
5	Pusa A4 x IC-090491	0.09	-0.51	-1.48**	-14.67**	-12.97**	-3.54**	0.03
6	Pusa A4 x DOV-62	0.64**	15.93**	-2.75**	-11.50**	-2.77*	4.68**	0.36**
7	Pusa A4 x C-10	-0.26**	-1.87	2.02**	76.90**	-6.77**	3.87**	-0.30**
8	DOV-1-2 x DOV-2	-0.14*	-6.11**	-4.05**	-47.87**	-4.05**	0.19	0.65**

9	DOV-1-2 x DOV-12	-0.17**	16.19**	2.94**	67.70**	-4.26**	-1.42**	-0.15**
10	DOV-1-2 x WB Selection	0.27**	24.19**	-1.15**	19.90**	-11.59**	-3.70**	-0.02
11	DOV-1-2 x IC-090491	-0.15*	15.43**	2.41**	23.10**	12.56**	10.18**	0.15**
12	DOV-1-2 x DOV-62	-0.69**	1.86	1.28**	22.50**	4.13**	-1.78**	0.24**
13	DOV-1-2 x C-10	0.33**	0.73**	-0.39	-21.10**	2.68*	-2.29**	-0.10
14	DOV-2 x DOV-12	0.11	7.09**	-2.39**	-20.47**	-4.79**	-0.54**	-0.21**
15	DOV-2 x WB Selection	-0.72**	14.09**	-2.11**	7.06**	3.00*	1.05**	0.29**
16	DOV-2 x IC-090491	0.13	4.33*	-1.58**	-28.50**	-1.36	-0.92**	-0.08
17	DOV-2 x DOV-62	0.42**	-1.91	2.72**	51.66**	-1.59	-1.27**	-0.27**
18	DOV-2 x C-10	-0.04	1.63	1.45**	-2.60**	11.26**	3.14**	-0.55**
19	DOV-12 x WB selection	0.31**	11.73**	0.01	16.30**	0.62	-0.87**	0.05
20	DOV-12 x IC-090491	-0.10	15.29**	1.21**	5.40**	1.01	-1.62**	-0.01
21	DOV-12 x DOC-62	-0.17**	5.06**	-1.83**	-69.77**	-6.45**	-2.31**	-0.29**
22	DOV-12 x C-10	-0.15*	-16.07**	1.04**	34.63**	-8.31**	-1.56**	0.04
23	WB selection x IC-090491	-0.06	-17.71**	3.96**	34.60**	9.11**	1.34**	0.12*
24	WB selection x DOV-62	-0.62**	-12.61**	0.15	1.10	-4.49**	2.17**	-0.23**
25	WB selection x C-10	0.001	-3.74	1.06**	1.83	1.59	-0.06	0.17**
26	IC-090491 x DOV-62	-0.17**	-9.04**	-2.08**	-6.14**	6.56**	-0.13	-0.07
27	IC-090491 x C-10	0.18**	-10.17**	0.96**	23.93**	-6.13**	-2.21**	0.04
28	DOV-62 x C-10	0.10	5.93**	-0.79	-15.24**	-7.60**	0.83**	0.06
	SE	0.05	2.36	0.05	1.03	1.57	0.23	0.07

Combining ability studies help in the identification of suitable parents for hybridisation and superior cross combinations for improving a trait (Sprague and Tatum1954).The combining ability is measured by diallel cross analysis in which performance of single crosses made in definite fashion are evaluated. Since, the reciprocal differences were not significant in this crop, diallel mating (without reciprocal) design was followed in studying the genetic components of variance in okra. The high GCA is an indication of preponderance of additive gene effects and if epistasis is present it also include additive × additive gene effects, which can be very effectively utilised for improvement through hybridisation and selection programmes. Parents with high GCA effects can contribute to the favourable gene flow to offspring and also can provide information about concentration of predominantly additive genes.The estimates of GCA effects of eight parental lines for various nutritional trait indicates that none of the parents was superior for all the nutritional traits studied in positive direction which points to the importance of utilising population improvement methods for nutrient rich varietal or hybrid development (Singh et al. 2012). GCA variance (Table 3) was found higher than SCA in TDF, Ca, Fe showing that there was preponderance of additive gene action and selection of progeny will be effective. In contrast to this SCA variance (Table 3) was higher in protein, P, K and Cu, therefore, non-additive gene action was playing important role hence ----- breeding will be effective for these traits. The parents identified with high mean value and positively significant GCA can be utilized by crossing in appropriate combination and utilisationof high SCA crosses for heterosis. The $\sigma^2_{gca}/\sigma^2_{sca}$ less than unity indicates the role of non-additive gene action being more important in concentrating P, K , protein and Cu content in okra fruits. Similar results were obtained in cabbage by (Singh et al.2012) and in cauliflower by (Deyet al. 2014) and in okra for yield and contributing traits by (Reddy et al.2013; Wammandaet al.,2010).Several superior parents having good general combining abilities were identified for various nutritional traits, such as DOV-12 for protein content, DOV-62 for TDF, DOV 1-2 for K and Ca, Pusa A-4 for Fe and DOV-2 for Cu. These parents can be utilized in various cross combinations for improvement in the above traits.

The SCA effect denotes the dominance and all the three types of epistatic gene effects which aids in determination of superior cross combinations for exploitation through heterosis breeding and hybridization programme. On the basis of SCA performance, the superior cross combinations were identified which can be used as hybrid. For high protein content, Pusa A-4 × DOV-62 followed by DOV-2 × DOV-62. It is also evident from table (5) that most of the crosses where Pusa A-4 and IC-090491 were one parent showed high value of SCA effects for protein content. It was correlated with high seed content of these two lines showing that lines with high seed content will have better protein content.For TDF content, maximum SCA value was recorded in DOV-1-2 × WB Selection showing its higher fibre content which is good for digestion.Similarly, other cross combinations namely, WB Selection × ic-090491 for P, Pusa A-4 × C-10 for K, PusaA4 × DOV-1-2 for Ca, DOV-1-2× IC-090491 for Fe and Pusa A-4 × DOV-2 for Cu had high *per se* performance and good general combiner parents with significant SCA effects suggesting the role of cumulative effect of additive and additive × additive gene. Similar results were obtained for yield and its contributing traits in okra. (Reddy et al.2013; Mehta et al. 2007). At least one good general combiner parent was involved in many of the crosses with positively significant SCA effects. The combination of one good and one poor combiner produced crosses with significantly positive SCA effects viz., Pusa A4 × DOV-2 and DOV-1-2 × IC-090491 for P, Ca and Fe. The additive and non-additive gene effects in these crosses can be effectively employed in heterosis breeding and recurrent selection. Similar results were obtained in okra for fruit yield and contributing traits. (Reddy et al. 2013, Khatiket al.2013, Pal and Sabesan 2009). In the crosses DOV-2 × DOV-62 for TDF and P, DOV-12 × IC-090491 for protein, and DOV-62 × C-10 for TDF, even though good combiner parents were involved, the consequent crosses were of low SCA effects. Absence of any interaction among favourable alleles contributed by the parents can only the workable reason for this situation. This points to the assumption that two parents with high GCA effects for a trait may not always result in a cross depicting high SCA effects. Similar findings for different minerals content in cabbage head was reported by (Singh et al. 2012), for yield and its contributing traits in okra by (Wammandaet al 2010,Obiadalla-Ali et al. 2013, Mehta et al.2007).On over all basis, the most prominent hybrids for various traits were Pusa A-4 × DOV-62 for protein content, DOV-1-2 × WB Selection for total dietary fibre (TDF), WB Selection × IC-090491 for Phosphorus (P), Pusa A-4 × C-10 for Potassium (K), Pusa A-4 × DOV-1-2 for Calcium (Ca), DOV-1-2 × IC-090491 for Iron (Fe) and Pusa A-4 × DOV-2 for Copper (Cu) content in okra. Therefore, these cross combinations can be utilized for obtaining high nutritional content in okra. Three parents, namely Pusa A-4, DOV-1-2 and IC-090491 showed superior performance in various cross combinations for different quality traits studied. It was mainly because the better general combining ability of DOV-1-2 and Pusa A-4 for various nutritional traits.

These results points to the fact that, for a more reliable prediction of hybrid with good nutrient content in okra fruits, the role of SCA is over and above to that of GCA.This study further emphasizes the significance of assessment of the genetic combining ability for protein, total dietary fibre and mineral elements, recognition of exceptionable parents and relevant breeding techniques for involving potential and nutrient rich okra cultivars, synthetics and hybrids, and to encounter micronutrient malnutrition in human beings through bio-fortification.

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