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Studies on combining ability of okra genotypes for protein, total dietary fibre and mineral content

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ABSTRACT

Okra is an important traditional vegetable crop cultivated across the warmer parts of the world and has high nutritive and medicinal values. Though there is lot of information available in respect of yield traits but a little information are available with respect to the combining ability for nutritional traits like protein, total dietary fibre and minerals like 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 studied. The magnitude of the GCA variance appeared 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. Among parents, DOV-1-2 was a good combiner for phosphorus, calcium and iron, DOV-2 for total dietary fibre, phosphorus and copper, Pusa A4 for calcium and iron and DOV-62 for total dietary fibre and phosphorus. Significant SCA effect in positive direction was recorded in the crosses DOV-1-2 × IC-090491 and Pusa A4 × DOV-2 for total dietary fibre, phosphorus, potassium, calcium, iron and copper; 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 utilizing population improvement methods for biofortification in okra without compromising yield and other horticultural traits.

Key words: *Abelmoschus esculentus*, bhindi, protein, total dietary fibre, minerals.

INTRODUCTION

Nutritional security is a major challenge for country after achieving the food security. Due to deficiency of various nutrients in our diet, peoples are facing problem of malnutrition, stunting and underweight especially children and women. As we know that vegetables are rich source of minerals and vitamins, therefore its intake in required quantity may overcome their problem. Okra being a rich source of minerals, vitamins and fibre will be helpful in overcoming these problems. The tender fruits are used for culinary purposes. 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. The dried seeds are used as a caffeine free substitute for coffee beans and also a mucilaginous food additive (Gemede *et al*., 3). Okra polysaccharides are also used as egg white substitute; fat substitute in chocolate bar cookies and in chocolate frozen dairy dessert (Sengkhamparn *et al*., 11). India ranks first in the world with a production of 6.1 million tons (72% of world production) from over 0.50 million ha land (Anonymous, 1). Although, more than 99% okra farming is solely done in Asian and African countries, the productivity is low in African countries (1.82 tons per ha) compared with other parts of the world. The improvement in its nutritional status coupled with yield can effectively contribute to reduction of micronutrient deficiencies existing especially in developing countries.

The combining ability analysis i.e. General Combining 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 such as protein, total dietary fibre (TDF) and minerals, like phosphorous (P), potassium (K), calcium (Ca), iron (Fe) and copper (Cu). The estimates of combining ability also evaluate the relative magnitude of additive and qnon-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 to suggest suitable breeding approaches for nutrient enhancement in okra fruits for biofortification.

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MATERIALS AND METHODS

The study was carried out at the Research Farm of Division of Vegetable Science, Indian Agricultural Research Institute, New Delhi which is located at latitude 28°40' N, longitude 77°12' E with an altitude of 228.6 m above mean sea level (MSL). The climate is semi-arid with hot summers and cool winter's average received annual rainfall of 661mm. Eight 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 to obtain 28 F_1 hybrids.

For hybridization, the floral buds of the female parents were emasculated one day prior to flower opening with the aid of pointed forceps, a circular cut was given around the fused calyx including epicalyx at about 1mm from its base, then the corolla was removed gently without injuring the gynoecium and all the anthers were removed. The emasculated flowers were bagged immediately with butter paper covers. Similarly, the flower buds of male parents from which the pollen grains need to be collected were also covered with butter paper covers to check contamination in the previous day of flower opening. On the next day morning, the butter paper covers were removed and dehisced anthers were collected from the bagged male flowers were gently rubbed on the stigma of female flowers. The covers were replaced immediately after pollination and put a proper tag having the name of cross and the date of pollination. To ensure selfing of parental genotypes, flowers were covered with butter paper bags one day prior to opening. Seeds were extracted from dried fruits after 35 days of pollination. Twenty eight hybrids along with their parents were sown at a spacing of 60cm × 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 fruit quality traits.

For nutrient analysis, fresh fruits after 6^{th} 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 solution 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) and assayed on GBC Avanta PM 904 AA model atomic spectrophotometer 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 method UV-VIS spectrophotometer DR5000 model. Potassium was estimated by using Systronic Flame Photometer Type 128 using specific filter for potassium and LPG flame according to method given by Jackson (5) and 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

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

per 100 gram sample. Total dietary fibre content was estimated using the Megazyme TDF Kit KTDFR based on AOAC enzymatic gravimetric method 985.29 and AACC method 32-05.01 expressed as milligram per gram sample. Statistical analysis of data generated was done following the Method II under Model I as suggested by (Griffing, 4) and by using OPSTAT (Sheoran *et al*., 12) developed by Chaudhary Charan Singh Haryana Agricultural University, Hisar, Haryana, India.

RESULTS AND DISCUSSION

The estimates of variance components for 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 σ^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).

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 general combiner for TDF, P and Cu but a poor general combiner for protein, K, Ca and Fe. DOV-1-2

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 * = Significancwe at 5% level

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

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 for Ca 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 combiner for only TDF while WB Selection showed poor general combining ability for all the traits studied.

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

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 × DOV-1-2	0.41**	-8.27**	2.41**	-51.04**	15.64**	8.11**	-0.49**
2	Pusa A4 × DOV-2	-0.19**	-8.71**	0.85*	34.80**	4.69**	2.58**	0.73**
3	Pusa A4 × DOV-12	0.28**	-11.41**	0.88*	-4.64**	1.75	-2.19**	0.72**
4	Pusa A4 × WB Selection	0.27**	15.93**	-0.84*	-26.44**	1.23	-3.02**	-0.59**
5	Pusa A4 × IC-090491	0.09	-0.51	-1.48**	-14.67**	-12.97**	-3.54**	0.03
6	Pusa A4 × DOV-62	0.64**	15.93**	-2.75**	-11.50**	-2.77*	4.68**	0.36**
7	Pusa A4 × C-10	-0.26**	-1.87	2.02**	76.90**	-6.77**	3.87**	-0.30**
8	DOV-1-2 × DOV-2	-0.14*	-6.11**	-4.05**	-47.87**	-4.05**	0.19	0.65**
9	DOV-1-2 × DOV-12	-0.17**	16.19**	2.94**	67.70**	-4.26**	-1.42**	-0.15**
10	DOV-1-2 × WB Selection	0.27**	24.19**	-1.15**	19.90**	-11.59**	-3.70**	-0.02
11	DOV-1-2 × IC-090491	-0.15*	15.43**	2.41**	23.10**	12.56**	10.18**	0.15**
12	DOV-1-2 × DOV-62	-0.69**	1.86	1.28**	22.50**	4.13**	-1.78**	0.24**
13	DOV-1-2 × C-10	0.33**	0.73**	-0.39	-21.10**	2.68*	-2.29**	-0.10
14	DOV-2 × DOV-12	0.11	7.09**	-2.39**	-20.47**	-4.79**	-0.54**	-0.21**
15	DOV-2 × WB Selection	-0.72**	14.09**	-2.11**	7.06**	3.00*	1.05**	0.29**
16	DOV-2 × IC-090491	0.13	4.33*	-1.58**	-28.50**	-1.36	-0.92**	-0.08
17	DOV-2 × DOV-62	0.42**	-1.91	2.72**	51.66**	-1.59	-1.27**	-0.27**
18	DOV-2 × C-10	-0.04	1.63	1.45**	-2.60**	11.26**	3.14**	-0.55**
19	DOV-12 × WB selection	0.31**	11.73**	0.01	16.30**	0.62	-0.87**	0.05
20	DOV-12 × IC-090491	-0.10	15.29**	1.21**	5.40**	1.01	-1.62**	-0.01
21	DOV-12 × DOC-62	-0.17**	5.06**	-1.83**	-69.77**	-6.45**	-2.31**	-0.29**
22	DOV-12 × C-10	-0.15*	-16.07**	1.04**	34.63**	-8.31**	-1.56**	0.04
23	WB selection × IC-090491	-0.06	-17.71**	3.96**	34.60**	9.11**	1.34**	0.12*
24	WB selection × DOV-62	-0.62**	-12.61**	0.15	1.10	-4.49**	2.17**	-0.23**
25	WB selection × C-10	0.001	-3.74	1.06**	1.83	1.59	-0.06	0.17**
26	IC-090491 × DOV-62	-0.17**	-9.04**	-2.08**	-6.14**	6.56**	-0.13	-0.07
27	IC-090491 × C-10	0.18**	-10.17**	0.96**	23.93**	-6.13**	-2.21**	0.04
28	DOV-62 × 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

for P, K, Ca and Cu, DOV-12 × IC-090491 for TDF, P and K. Three crosses revealed significantly positive SCA effects 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 Ca and Fe.

Combining ability studies helps in the identification of suitable parents for hybridization and superior cross combinations for improving a trait (Sprague and Tatum, 14). 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 hybridization 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 nutritional traits 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.*, 13). 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 was higher in protein, P, K and Cu, therefore, non-additive gene action was playing important role. The parents identified with high mean value and positively significant GCA can be utilized by crossing in appropriate combination and utilisation of 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.*, 13) and in cauliflower by (Dey *et al.*, 2) and in okra for yield and contributing traits by (Reddy *et al.*, 10; Wammanda *et al.*, 15). 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, Pusa A4 × 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.*, 10; Mehta *et al.*, 7). 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.*, 10; Khatik *et al.*, 6; Pal and Sabesan, 9). 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.*, 13), for yield and its contributing traits in okra by (Wammanda *et al.*, 15; Obiadalla-Ali *et al.*, 8; Mehta *et al.*, 7). 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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