



Study of heterotic potential for important antioxidant and nutritional traits in okra (*Abelmoschus esculentus*)

ANJAN DAS¹, RAMESH KUMAR YADAV^{1*}, RAKESH BHARDWAJ², HARSHWARDHAN CHOUDHARY¹ and YOGESH P KHADE³

ICAR-Indian Agricultural Research Institute, New Delhi 110 012, India

Received: 11 February 2021; Accepted: 24 May 2022

ABSTRACT

Okra (*Abelmoschus esculentus*) is one of the most nutritious and economic vegetable crop, rich in vitamins, crude fibre, several minerals especially iodine and anti-oxidant compounds. In spite of its high nutritional value, little efforts have been done in the past for the development of the nutritionally rich F_1 hybrids. To address this issue, 10 lines and 3 testers were crossed in line $\times$ tester fashion to develop 30 F_1 hybrid combinations. All the hybrids, their parents and 1 leading private sector commercial hybrid (Shakti) were evaluated in Randomized Complete Block Design (RCBD) with 3 replications to measure average heterosis, heterobeltiosis, economic heterosis for 8 quality traits consecutively for two seasons (2018–19). Analysis of heterosis over commercial check (Shakti) revealed DOV-92 $\times$ Pusa Bhindi-5 as one of the best hybrid for chlorophyll a and b, CUPRAC and moisture (%). Concurrently, VRO-6 $\times$ Pusa A-4 was found best for ascorbic acid and sugar content, and DOV-62 $\times$ Pusa Bhindi-5 for ascorbic acid and total phenolic content. The check was found superior for total carotenoids content. On the basis of mean yield and quality traits, the best performing hybrid DOV-92 $\times$ Pusa Bhindi-5 may be recommended for the large scale evaluation for hybrid, can be released in future for commercial cultivation by the growers.

Keywords: Ascorbic acid, Heterosis, Line $\times$ tester, Nutritional content, Okra

Okra [*Abelmoschus esculentus* (L.) Moench.] is one of the most nutritious and popular vegetable crop in India. It is an annual crop and widely grown in tropical and sub-tropical parts of the world (FAOSTAT 2018). It is a rich source of vitamins (vit-A and vit-C), crude fibre, minerals (Ca, Mg, K, Fe) and iodine (Kumar *et al.* 2010, USDA National Nutrient Database 2019). A few studies have also confirmed its ethno-pharmacological (Agyare *et al.* 2009) and medicinal (Tseng *et al.* 2004) properties against cancer, high cholesterol and bacterial adhesion. Apart from this, okra fruits and seeds are rich in several phenolic compounds, carbohydrates, proteins and other dietary elements such as, dietary fibres. Natural carbohydrate content of okra mainly, polysaccharides cause a reduction in cholesterol and glucose level in blood which is good for both asthma and diabetic patient (Das *et al.* 2019a, Das *et al.* 2019b). Green okra vegetables are rich source of chlorophyll compounds and prevent the formation of hepatocellular (Kubickova *et al.* 2016), breast and lung cancer (Zhang *et al.* 2018) in human

cell and also possess anti-oxidant activity for scavenging free radicals.

In spite of its high nutritional value, very little effort has been done till now for its quality improvement (Das *et al.* 2021). But now on global scale the focus of any vegetable improvement programme has been shifted towards development of high yielding as well as nutritionally rich quality F_1 hybrids. In okra, hybrids are also popular among the farmers, and high heterosis for different quality traits like chlorophyll, vitamin-c and sugar has also been reported by several researchers (Senthilkumar *et al.* 2007, Hadiya *et al.* 2018). Still, very little information is available in the public domain in respect of heterosis for on total phenolic and antioxidant content in okra. The present experiment was formulated to evaluate the heterotic performance of 30 newly developed hybrids using line $\times$ tester mating design for different quality traits including antioxidant and carbohydrate content.

MATERIALS AND METHODS

An experiment was conducted at Vegetable Research Farm (latitude 28°40' N, longitude 77°12' E and at an altitude of 228.6 m amsl), Division of vegetable science, ICAR-Indian Agricultural Research Institute (IARI), New Delhi during spring-summer of 2018 to develop different

¹ICAR-Indian Agricultural Research Institute, New Delhi;

²ICAR-National Bureau of Plant Genetic Resources, New Delhi;

³ICAR-Directorate of Onion and Garlic Research, Rajgurunagar, Pune, Maharashtra. *Corresponding author email: rkydavneh@gmail.com

cross combinations followed by evaluation of F_1 hybrids in rainy (*kharif*) season 2018 and 2019. Ten lines (IC-685583, Arka Anamika, DOV-92, VRO-6, DOV-2, DOV-62, DOV-37, DOV-25, DOV-1, DOV-64) and 3 testers (Pusa Sawani, Pusa Bhindi-5, Pusa A-4) were crossed in line $\times$ tester mating design to develop 30 F_1 hybrid combinations. These hybrids along with their parents and 1 commercial check (Shakti) were sown in Randomized Complete Block Design (RCBD) with 3 replications. Seeds were sown in a spacing of 60 cm (row-to-row) and 30 cm (plant-to-plant) and all the recommended cultural practices were followed for successful raising of the crop.

For the analysis of quality traits, six-days old fresh and tender fruits were harvested which were tagged on day of first opening of flower. The harvested fruits were cleaned with double distilled water followed by treatment of 0.2% Teepol and 0.1 (N) HCl solution. Then, samples were weighed (W_1) and kept for drying in a hot air oven at 70°C for 7 days to calculate moisture percentage. After seven days, samples were again weighed (W_F) and moisture % (Mp) was calculated as: $Mp = (W_1 - W_F)/W_1 \times 100$. Fresh fruit samples were used for the analysis of other traits i.e. chlorophyll a and b, total carotenoids, ascorbic acid, total sugars, phenol and anti-oxidant contents. For estimation of chlorophyll a, b, and total carotenoid content, 100% acetone method as suggested by Lichtenthaler *et al.* (2001) was used, where the reading of final volume was taken at five different wavelengths (470 nm, 520 nm, 661.6 nm, 644.8 nm, and 750 nm).

$$\text{Chlorophyll a, } C_a \text{ (mg/100 g)} = (11.87A_{661.6} - 2.42A_{644.8}) \times \frac{EV \times DF \times 100}{SW}$$

$$\text{Chlorophyll b, } C_b \text{ (mg /100 g)} = (20.82A_{644.8} - 4.60A_{661.6}) \times \frac{EV \times DF \times 100}{SW}$$

$$\text{Total carotenoids, } C_c \text{ (mg/100 g)} = (1000A_{470} - 1.86C_a - 74.08C_b) \times \frac{EV \times DF \times 100}{SW}$$

where EV, extract volume (ml); DF, dilution factor; SW, sample weight (g).

Ascorbic acid content was determined as per method suggested by Jagota and Dani (1982) where ascorbic acid was taken as standard to prepare standard curve. Total phenol content was estimated following colorimetric technique as suggested by Singleton *et al.* (1999) using 20% Na_2CO_3 solution with folin-ciocalteu reagent where gallic acid was used to prepare standard curve. Total sugar content was determined using anthrone solution with glucose as standard for the preparation of standard curve and final reading of all the samples was taken at 630 nm in UV-VIS spectrophotometer (Hansen *et al.* 1975). Cupric Ion Reducing Antioxidant Capacity (CUPRAC) method was followed to estimate total antioxidant content of the samples using $CuCl_2$ and neocuproine solution, ammonium acetate buffer and gallic acid was taken to prepare standard curve (Apak *et al.* 2004). Final reading of the samples was taken at 450 nm in UV-VIS spectrophotometer. All the statistical analysis was done using freely available online tool OPSTAT.

RESULTS AND DISCUSSION

Mean performance of parents and testcross progenies:

Performance of top 3 parents and hybrids for different traits are presented in Table 1. Further, top three best F_1 hybrid combinations based on heterosis over mid parent (HMP), better parent (HBP) and commercial check (HSC) for 8 quality traits were selected (Table 1). For chlorophyll a content, 10 crosses over better parent (HBP), 21 crosses over mid parent (HMP) and 8 crosses over standard check (HSC) exhibited significant heterosis in positive direction. For this trait, hybrid VRO-6 $\times$ Pusa Sawani, DOV-37 $\times$ Pusa Sawani and VRO-6 $\times$ Pusa Bhindi-5 were considered as best hybrid on the basis of HBP, HMP and HSC, respectively. Similarly, for chlorophyll b content, 11 crosses showed significantly higher heterosis over better parent (HBP), 21 crosses over mid parent (HMP), and 12 crosses over standard check (HSC) in positive direction. Cross combinations, namely DOV-37 $\times$ Pusa A-4, DOV-37 $\times$ Pusa Sawani, and DOV-64 $\times$ Pusa Bhindi-5 were found as best hybrid over mid parent, better parent and check, respectively for chlorophyll b content. Positive heterosis for chlorophyll content was also reported by Hadiya *et al.* (2018) in Okra. Carotenoids act as a precursor of vitamin-A and also exhibits antioxidant property. The 13 crosses over better and 21 crosses over mid parent showed significant heterosis in desirable direction for total carotenoids. However, none of the cross combination exhibited significant heterosis in desirable direction over commercial check. Hybrid, DOV-92 $\times$ Pusa A-4, DOV-2 $\times$ Pusa A-4 and DOV-92 $\times$ Pusa Bhindi-5 exhibited highest heterotic value on the basis of HBP, HMP and HSC, respectively. Significant amount of heterosis in positive direction for total carotenoid content was also reported by Singh *et al.* (2018) in cauliflower. Total sugar as an indicator for carbohydrate content revealed that 16, 17, and 6 cross combinations showed positive and significant heterosis over better parent, mid parent and standard check, respectively. On the basis of HBP, HMP and HSP, hybrid IC-685583 $\times$ Pusa A-4, DOV-64 $\times$ Pusa A-4 and DOV-2 $\times$ Pusa Sawani were considered as top three best cross combinations for total sugar, respectively. These results are in close agreement with the findings of Rani *et al.* (2003) and Senthilkumar *et al.* (2007) in okra.

Apart from chlorophyll and carotenoid content, ascorbic acid and total phenolic content of fruit also enhances the nutritional value as well as increases tolerance under stress condition. Significant heterosis over better parent, mid parent and commercial check in desirable direction for ascorbic acid content was found in 8, 11, and 9 cross combinations, respectively. Hybrid, DOV-64 $\times$ Pusa Bhindi-5 was considered as best hybrid on the basis of both HBP and HMP while, VRO-6 $\times$ Pusa A-4 was regarded as best cross combination over commercial check. Significant heterosis in desirable direction was also observed by Senthilkumar *et al.* (2007) and Tiwari *et al.* (2015) in okra. Estimation of total phenol revealed that three crosses over better parent and four crosses each over average parent and commercial hybrid showed significant heterosis in desirable direction.

Table 1 Summary of top three F₁ hybrids based on heterosis per cent over BP, MP and CC

Parameter	Chl a (mg/100 g FW)	Chl b (mg/100 g FW)	Total carotenoids (mg/100 g FW)	Ascorbic acid (mg/100 g FW)
<i>Range of mean values</i>				
Parents	1.08 – 13.62	0.35 – 4.95	1.03 – 4.69	30.00 – 50.00
F ₁ Hybrid	2.25 – 11.09	0.82 – 3.65	1.09 – 4.02	16.00 – 60.00
Three top parents based on mean value	DOV- 25 (13.62), Pusa Bhindi-5 (11.05), DOV-92 (10.16)	DOV- 25 (4.95), Pusa Bhindi- 5 (3.58), DOV-92 (3.24)	DOV- 25 (4.69), Pusa Bhindi- 5 (4.03), DOV-92 (3.71)	DOV- 62 (50.00), Pusa Sawani (50.00), DOV- 2 (48.00)
Three top hybrids based on mean value	VRO-6 × Pusa Bhindi-5 (11.09), DOV-92 × Pusa Bhindi-5 (11.04), DOV-64 × Pusa Bhindi-5 (9.78)	DOV-64 × Pusa Bhindi-5 (3.65), DOV-92 × Pusa Bhindi-5 (3.64), Arka Anamika × Pusa Bhindi-5 (3.52)	DOV-92 × Pusa Bhindi-5 (4.02), VRO-6 × Pusa Bhindi-5 (4.00), DOV-1 × Pusa Bhindi-5 (3.58)	VRO-6 × Pusa A-4 (60.00), DOV-64 × Pusa Bhindi-5 (60.00), DOV-92 × Pusa Sawani (54.00)
<i>Three top F₁ hybrid based on heterosis (%)</i>				
BP	VRO- 6 × Pusa Sawani (88.53, 5.10)*, Dov-37 × Pusa A-4 (36.49, 7.18), DOV-37 × Pusa Sawani (29.46, 6.81)	Dov-37 × Pusa A-4 (45.69, 2.82), DOV- 37 × Pusa Sawani (34.85, 2.61), IC-685583 × Pusa Sawani (30.95, 1.04)	Dov-37 × Pusa A-4 (40.51, 2.74), Arka Anamika × Pusa A-4 (31.53, 2.52), DOV-62 × Pusa Sawani (29.82, 2.14)	DOV- 64 × Pusa Bhindi-5 (30.53, 60.00), VRO- 6 × Pusa A-4 (25.00, 60.00), DOV- 92 × Pusa Bhindi-5 (18.18, 52.00)
MP	DOV-37 × Pusa Sawani (71.03, 6.81), DOV-2 × Pusa A-4 (68.29, 5.67), DOV-64 × Pusa Sawani (46.10, 6.52)	DOV- 37 × Pusa Sawani (91.35, 2.61), IC- 685583 × Pusa Sawani (82.14, 1.04), IC- 685583 × Pusa A-4 (64.14, 1.81)	DOV-37 × Pusa Sawani (69.40, 2.54), IC-685583 × Pusa A-4 (65.22, 2.43), DOV-62 × Pusa Sawani (58.38, 2.14)	DOV- 64 × Pusa Bhindi-5 (53.85, 60.00), DOV- 62 × Pusa Bhindi-5 (41.46, 58.00), DOV- 92 × Pusa Bhindi-5 (36.84, 52.00)
CC	VRO-6 × Pusa Bhindi-5 (47.47, 11.09), DOV-92 × Pusa Bhindi-5 (46.81, 4.87), DOV-64 × Pusa Bhindi- 5 (30.10, 9.78)	DOV- 64 × Pusa Bhindi-5, - (43.62, 3.65), DOV- 92 × Pusa Bhindi-5 - (43.43, 3.64), Arka Anamika × Pusa Bhindi- 5 (38.68, 3.52)	- - -	VRO- 6 × Pusa A-4 (33.33, 60.00), DOV- 62 × Pusa Bhindi- 5 (28.89, 58.00), DOV- 92 × Pusa Sawani (20.00, 54.00)
<i>Number of hybrids exhibiting significant heterosis in positive direction</i>				
BP, MP, CC	10, 21, 8	11, 21, 12	13, 21, 0	8, 11, 9

For total phenol, DOV-62 × Pusa Bhindi-5 was found as best hybrid on the basis of HBP, HMP, and HSP. Total antioxidant content estimated through CUPRAC method revealed that 5, 13 and 10 crosses showed significant and positive heterosis over better parent, mid parent and check, respectively. Hybrid DOV-92 × Pusa A-4, DOV- 2 × Pusa A-4 and DOV-92 × Pusa Bhindi-5 were considered as top three best cross combinations on the basis of HBP, HMP, and HSC, respectively. For moisture percentage of fruit, 13, 17 and 16 crosses exhibited significant heterosis over better parent, mid parent and check in desirable direction, respectively. Cross combinations VRO-6 × Pusa Sawani, DOV-62 × Pusa Bhindi-5 and DOV-92 × Pusa Bhindi-5 showed maximum heterosis and were considered as top three best cross combinations for moisture content on the basis of HBP, HMP and HSP, respectively. Significant

heterosis in positive direction for total phenol and CUPRAC in cauliflower was also reported by Singh *et al.* (2018).

On over all basis, it was concluded that for heterosis over commercial check, DOV-92 × Pusa Bhindi-5 was found to be one of the best hybrid for the traits i.e. chlorophyll a and b, CUPRAC and moisture content. For chlorophyll a and b, DOV-64 × Pusa Bhindi-5 showed significant heterosis in desirable direction. Similarly, VRO-6 × Pusa A-4 considered as one of the best hybrid for both ascorbic acid and sugar percentage and DOV-62 × Pusa Bhindi-5 for both ascorbic acid and total phenolic content. Moreover, involvement of minimum one good combiner parent was found in all the above testcross combinations which reflected that heterosis was governed by the action of both additive and non-additive genetic components (Das *et al.* 2020a, Das *et al.* 2020b, Chandel *et al.* 2022). In future, these

Table 2 Cont.

Parameter	Sugar (%)	Total phenol (mg/100 g FW)	CUPRAC (mg/100 g GAE FW)	Moisture content (%)
<i>Range of mean values</i>				
Parents	1.05 – 2.24	28.37 – 106.67	123.08 – 307.69	87.40 – 91.92
Hybrid	0.80 – 2.02	22.70 – 98.67	130.77 – 313.00	87.48 – 92.20
Three top parents based on mean value	DOV- 25 (2.24), VRO- 6 (1.76), DOV- 2 (1.63)	Pusa A-4 (106.67), Pusa Sawani (96.00), DOV- 25 (92.00)	DOV- 64 (307.69), DOV- 25 (307.69), Pusa Sawani (307.69)	IC- 685583 (91.92), DOV-92 (91.54), Pusa A-4 (90.81)
Three top hybrids based on mean value	DOV-2 × Pusa Sawani (2.02), VRO-6 × Pusa A-4 (1.99), DOV-64 × Pusa A-4 (1.94)	DOV-62 × Pusa Bhindi- 5 (98.67), DOV-92 × Pusa Sawani (70.92), DOV-25 × Pusa Sawani (61.33)	DOV-92 × Pusa Bhindi-5 (313.00), DOV-62 × Pusa Sawani (300.00), DOV-2 × Pusa A-4 (276.92)	DOV-92 × Pusa Bhindi-5 (92.20), DOV-92 × Pusa A-4 (91.49), DOV-62 × Pusa Bhindi-5 (91.11)
<i>Three top F₁ hybrid based on herterosis (%)</i>				
BP	IC-685583 × Pusa A-4 (35.00, 1.53)*, DOV-2 × Pusa Sawani (23.53, 2.02), DOV-92 × Pusa Bhindi-5 (23.07, 1.78)	DOV-62 × Pusa Bhindi-5 (54.17, 98.67), DOV- 92 × Pusa Bhindi- 5 (2.00, 68.00), Arka Anamika × Pusa Bhindi- 5 (0.18, 61.11)	DOV-92 × Pusa A-4 (28.57, 207.69), DOV-2 × Pusa A-4 (20.00, 276.92), Arka Anamika × Pusa A-4 (15.28, 212.82)	VRO-6 × Pusa Sawani (3.05, 90.07), DOV-62 × Pusa Bhindi-5 (2.23, 98.67), DOV-2 × Pusa Sawani (1.32, 91.04)
MP	DOV-64 × Pusa A-4 (45.88, 1.96), DOV-92 × Pusa Bhindi-5 (42.21, 1.78), IC-685583 × Pusa A-4 (37.81, 1.53)	DOV- 62 × Pusa Bhindi- 5 (57.87, 98.67), Arka Anamika × Pusa Bhindi- 5 (28.60, 61.11), DOV-62 × Pusa Sawani (20.00, 96.00)	DOV- 2 × Pusa A-4 (56.52, 276.92), DOV- 92 × Pusa A-4 (45.95, 207.69), Arka Anamika × Pusa A-4 (38.33, 212.82)	DOV-62 × Pusa Bhindi-5 (2.41, 91.11), DOV- 92 × Pusa Bhindi-5 (2.25, 92.20), VRO- 6 × Pusa Bhindi-5 (1.96, 89.83)
CC	DOV-2 × Pusa Sawani (20.17, 2.02), VRO-6 × Pusa A-4 (18.46, 1.99), DOV-64 × Pusa A-4 (16.60, 1.96)	DOV-62 × Pusa Bhindi-5 (46.17, 98.67), DOV-62 × Pusa Sawani (42.22, 96.00), DOV-92 × Pusa Sawani (5.07, 70.92)	DOV- 92 × Pusa Bhindi-5 (28.98, 313.00), DOV-62 × Pusa Sawani (23.63, 300.00), DOV-2 × Pusa A-4 (14.12, 276.92)	DOV-92 × Pusa Bhindi-5 (2.63, 92.20), DOV-92 × Pusa A-4 (1.84, 91.49), DOV-2 × Pusa Sawani (1.34, 91.04)
<i>Number of hybrids exhibiting significant heterosis in positive direction</i>				
BP, MP, CC	16, 17, 6	3, 4, 4	5, 12, 10	13, 17, 16

*Values in parenthesis indicates heterosis % followed by mean value. BP, Better parent; MP, Mid parent; CC, Commercial check (Shakti).

best performing hybrids can be utilized for commercial cultivation after further evaluation at multiple locations for providing nutritionally rich okra hybrids.

ACKNOWLEDGEMENT

First author is grateful to the Indian Council of Agricultural Research (ICAR) for awarding ICAR-PG Fellowship (2017) during the entire period of this experiment.

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