Quantification and Inheritance of Antioxidant Properties and Mineral Content in Ridge Gourd (*Luffa acutangula*)

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Abstract With a view to develop nutritious ridge gourd variety, a field as well as a laboratory study was conducted to quantify and enumerate the genetic control for antioxidant properties and mineral content in fruits. The hybrids were found to be superior over their parents for various nutritional traits such as ascorbic acid, total carotenoids and total phenolics content; in vitro antioxidant activity in the form of DPPH-RSA, ABTS-RSA and CUPRAC; and P, K, Ca, Na, S, Fe, Zn and Mn content in the fruits. All the nutritional parameters exhibited higher values of dominance variance over the additive genetic variance, more than unity value of average degree of dominance and low narrow-sense heritability (<50 %). The antioxidants and mineral content in the fresh fruits are predominantly attributed by the non-additive genetic component. Hence, the various tools of hybrid breeding would be useful to breed antioxidants- and minerals-rich genotypes of ridge gourd (*Luffa acutangula* Roxb.).

Keywords Antioxidants · Minerals · Inheritance · Dominance effect · Additive effect · Ridge gourd (*Luffa acutangula* Roxb.)

Introduction

Ridge gourd (*Luffa acutangula* Roxb.) is a cucurbitaceous summer vegetable of South-east Asia and few African countries. It is also known as ribbed gourd, angled loofah and Chinese okra. The young tender fruits of the non-bitter types are eaten fresh like cucumbers, cooked as a vegetable or used in the soups. Antioxidants and minerals are integral part of human and plant nutrition to support the various biological processes during various stages of growth and

developments. An appropriate plant-based diet can supply sufficient amount of nutrition to satisfy human dietary requirements. The chemical constituents of ridge gourd fruits include carbohydrates, carotene, fat, protein, phytin, flavonoids, saponin and amino acids. Kandlakunta et al. [15] reported that ribbed gourd contains about 300 µg β-carotene and 1,000 µg carotenoids per 100 g of fresh fruits. It has various pharmacological activities like hepatoprotective, antidiabetic, antioxidant, abortifacient and antifungal activity [10]. The ribosome-inactivating proteins (RIPs), luffins, have been isolated from *Luffa* and characterized [16]. A purified protein with molecular weight of 30 kD from seeds of *L. aegyptiaca* inhibited cell-free translation [24]. A recent report shows that the water extracts from fresh sponge gourds exhibited scavenging effect in terms of 1,1-diphenyl-2-picrylhydrazyl radical scavenging activity (DPPH-RSA) and butylated hydroxyanisole scavenging effect [5]. The fresh fruits of *L. acutangula* exhibited antioxidant activity [2]. Other than DPPH-RSA, in vitro antioxidant activity is also measured in terms of 2,2-azinobis-(3-ethylbenzthiazoline-6-sulphonic acid) radical scavenging

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activity (ABTS-RSA) and cupric ion reducing antioxidant capacity assay (CUPRAC assay).

Minerals including both macro- and micronutrients such as nitrogen (N), phosphorus (P), potassium (K), calcium (Ca), magnesium (Mg), sulphur (S), sodium (Na), iron (Fe), zinc (Zn), manganese (Mn), copper (Cu), cobalt (Co), boron (B) and molybdenum (Mo) are required by living organisms to support the various biological processes during different stages of life cycle. Billions of people in developing countries suffer from micronutrient malnutrition ‘hidden hunger’. The fruits of ridge gourd contain fair amount of minerals and fibre [14]. Tough sufficient amount of information related to inheritance of horticultural traits in ridge gourd had been reported by various researchers [17, 26], however, till now only limited information is available on the estimate of genetic component of variation for mineral content and antioxidant activity in this crop.

Being a one of the commonly used cucurbitaceous vegetables, it is essential to study the nutritional properties and the transmissibility to its progenies for identifying and developing superior genotypes. A detailed knowledge about the magnitude and nature of genetic variation in a specific population is of prime importance for the effective prediction of the suitable breeding programmes. The numerical analysis of diallel provides detailed account of additive and dominance components and the allied statistics. Till date only sparse information is available on nutritional contents and their inheritance in various vegetable crops, and none of the studies describe the gene action for antioxidants (ascorbic acid, total carotenoids, total phenolics, DPPH-RSA, ABTS-RSA and CUPRAC assay) and minerals (P, Ca, K, Na, S, Fe, Zn and Mn) in ridge gourd. Hence, the objectives of this experiment were to quantify antioxidant properties and mineral content, to enumerate the magnitude and nature of gene action, and also to suggest suitable breeding approaches for improving the nutritional quality of ridge gourd (*L. acutangula*) fruits.

Materials and Methods

Experimental Material, Field Experiment and Sampling

The seeds of seven parents, and 21 F_1 hybrids developed through half diallel fashion were sown during rainy season of 2008–2009 at Vegetable Research Farm, IARI, New Delhi, India. The experiment was laid out in RBD with three replications. Crops were raised as per the recommended package of practices followed for ridge gourd cultivation to have better phenotypic and morphological expression. The experimental plot was fed with FYM @ 20 t ha⁻¹, N @ 70 kg ha⁻¹, and P and K @ 25 kg ha⁻¹. At

edible fruit maturity, three fruits were harvested randomly from all 28 genotypes in each replication.

Determination of Antioxidant Properties

The collected fruit samples were chopped to homogenize and 5 g of it was taken as sample for estimation. The amount of ascorbic acid (mg 100 g⁻¹ fresh weight), total carotenoid (mg 100 g⁻¹ fresh weight) and total phenolics (gallic acid equivalent mg 100 g⁻¹ fresh weight) content were determined as described by Albrecht [1], Ranganna [25] and Singleton et al. [33], correspondingly. Moreover, the in vitro antioxidant activity (TEAC μ mol Trolox 100 g⁻¹) such as DPPH-RSA, ABTS-RSA and CUPRAC assay was measured according to the respective methods of Brand-Williams et al. [6], Re et al. [27] and Apak et al. [3].

Determination of Mineral Content

The sampled fruits were chopped and 100 g of fresh material was kept in hot air oven at 60–65 °C for drying. The dried samples were grounded, passed through 1 mm sieve and finally stored in airtight container until the digestion [30]. An amount of 0.5 mg dried tissues were pre-digested with 15 ml diacid mixture of perchloric acid and nitric acid in a ratio of 1:1 (w/w) overnight and then heated up to 250 °C until the solution turned colourless. The filtrate was used for determining concentration of Zn, Fe and Mn on AAS (AAS-4141). Furthermore, K, Na and Ca concentrations were measured in the filtrate using Flame Photometer (ELICO CL-361); while, P and S content were measured through Spectrometer. The content of P, Ca, K and Na was expressed as mg 100 g⁻¹; while S, Fe, Zn and Mn content was calculated in μ g 100 g⁻¹ on fresh weight basis.

Statistical Analysis

Data regarding content and in vitro activity of antioxidants, and mineral content in ridge gourd fruit were statistically analysed to study gene action using diallel method of numerical approach given by Hayman [12] through Statistical Package for Agricultural Research 2.0 (SPAR 2.0) software of Indian Agricultural Statistical Research Institute (IASRI), New Delhi, India.

Results and Discussion

Antioxidant Properties of Parents and Hybrids

The fresh fruits of ridge gourd were analysed for antioxidant properties viz., ascorbic acid, total carotenoids, total

phenolics content and in vitro antioxidant activity in terms of DPPH-RSA, ABTS-RSA and CUPRAC assay in seven parents and their 21 half diallel derived F_1 hybrids (Figs. 1, 2). The values of ascorbic acid, total carotenoids, total phenolics content, DPPH-RSA, ABTS-RSA and CUPRAC assay varied significantly in varieties used as parental lines and hybrids which were higher about 1.63-fold, 1.83-fold and 2.01-fold, 1.38-fold, 1.37-fold and 1.39-fold, respectively, in hybrids over their parents. The average value of 21 F_1 hybrids for ascorbic acid, total carotenoids and total phenolics content and in vitro antioxidant activity in terms of DPPH-RSA, ABTS-RSA and CUPRAC assay was higher than the average value of the seven parents. Nevertheless, the hybrids were superior over the parents with respect to antioxidant properties. This might be due to increased concentration of favourable allele in heterozygous locus in the hybrid progenies, showing heterosis for these traits, which agrees with the findings from luffa [36], tomato [35], safflower [9] and cabbage [29].

Genetic Component for Antioxidant Properties

Results pertaining to ascorbic acid, total phenolics, total carotenoids content, DPPH-RSA, ABTS-RSA and CUPRAC assay revealed that the estimates of D , H_1 , H_2 and h^2 were highly significant (Table 1). The value of H_1 was greater than D for all the traits related to antioxidant properties, suggesting the presence of dominant alleles in parents. The environmental influence (E) on the inheritance of these traits was negligible and non-significant. The negative values of F indicated more prevalence of recessive alleles for these traits in parents. The mean degree of dominance (H_1/D)^{1/2} being 1.7, 2.04, 2.37, 1.49, 1.5 and 1.7 for ascorbic acid, total phenolics, total carotenoids, DPPH-RSA, ABTS-RSA and CUPRAC assay, respectively. The mean degree of dominance greater than one for these traits

Fig. 1 Antioxidant content in parents and hybrids of ridge gourd. Standard error bars with $p < 0.05$

Fig. 2 Antioxidant activity in parents and hybrids of ridge gourd. Standard error bars with $p < 0.05$

related to oxidative defence mechanism indicated the role of over-dominance for these characters. The proportion of genes with positive and negative effects $H_2/4H_1$ in the parents was found to be less than 0.25 (0.17, 0.15, 0.17, 0.18, 0.19 and 0.18, respectively, for ascorbic acid, total phenolics, total carotenoids DPPH-RSA, ABTS-RSA and CUPRAC assay), denoting asymmetry at the loci showing dominance. The proportion of dominant and recessive gene in the parents as indicated by $[(4DH_1)^{1/2} + F/(4DH_1)^{1/2} - F]$ was 0.27, 18.25, 3.74, 0.30, 0.35 and 0.32 while the group of genes, which controlled the characters and exhibited dominance (h^2/H_2) was 2.33, 1.43, 2.95, 2.19, 2.85 and 3.22 for ascorbic acid, total phenolics and total carotenoids, DPPH-RSA, ABTS-RSA and CUPRAC assay, respectively. Narrow-sense heritability being 17.15, 12.87, 10.67, 20.88, 21.39 and 17.79 % for ascorbic acid, total phenolics, total carotenoids, DPPH-RSA, ABTS-RSA and CUPRAC assay, respectively, indicate that non-additive gene action has major role in the inheritance of antioxidant properties in ridge gourd fruits. The presence of more dominant genes than additive ones, minimal influence of environmental factors, preponderance of recessive alleles in parents, greater than unity value of average degree of dominance, asymmetry at loci showing dominance and low narrow-sense heritability indicate that the non-additive type of gene action played an major role in the inheritance of these traits. The present investigation, therefore, indicates that the predominance of non-additive gene action for antioxidants properties implies the importance of hybrid breeding approaches to realize the higher genetic gain. The presence of dominant component for vitamin C content reported in towel gourd and crossbreeding may have increased its content in the fruits of this gourd [36]. In contrast, Bo et al. [4], and Navazio and Simon [19] reported recessive inheritance for β -carotene content in cucumber. Additive, dominance, additive $\times$ dominance and dominance $\times$ dominance interaction were reported for

Table 1 Estimates of genetic components of variation and various statistical parameters for different characters

	Ascorbic acid (mg/100 g)	Total carotenoids ($\mu\text{g}/100\text{ g}$)	Total phenolics (mg/100 g)	DPPH-RSA ($\mu\text{mol Trolox}/100\text{ g}$)	ABTS-RSA ($\mu\text{mol Trolox}/100\text{ g}$)	CUPRAC assay ($\mu\text{mol Trolox}/100\text{ g}$)
Genetic components						
<i>D</i>	13.91** $\pm$ 1	352972.2* $\pm$ 163671.7	2.35** $\pm$ 0.51	2171.6** $\pm$ 11.74	9796.4** $\pm$ 729.09	1678.51** $\pm$ 103.7
<i>H</i> ₁	40.13** $\pm$ 2.40	474281.9* $\pm$ 394034.9	13.2** $\pm$ 1.22	4796.11** $\pm$ 282.65	22000.2** $\pm$ 1755.27	4833.5** $\pm$ 249.69
<i>H</i> ₂	27** $\pm$ 2.11	862475.2* $\pm$ 347200	8.75** $\pm$ 1.08	3424.78** $\pm$ 249.05	16406.9** $\pm$ 1546.6	3516.69** $\pm$ 220.01
<i>h</i> ²	63.1** $\pm$ 1.42	1236518** $\pm$ 233195.5	25.89** $\pm$ 0.72	7512.98** $\pm$ 167.28	46859.6** $\pm$ 1038.79	11353.38** $\pm$ 147.77
<i>F</i>	−26.8** $\pm$ 2.39	−913173* $\pm$ 392645.1	−6.44** $\pm$ 1.22	−3418.38** $\pm$ 281.65	−13985.5** $\pm$ 1749.07	−2890.7** $\pm$ 248.8
<i>E</i>	0.06 $\pm$ 0.35	53.65 $\pm$ 57866	0.01 $\pm$ 0.18	2.73 $\pm$ 41.51	5.02 $\pm$ 257.77	8.02 $\pm$ 36.67
Ratios of genetic components						
ADD	1.7	2.04	2.37	1.49	1.5	1.7
PPN	0.17	0.15	0.17	0.18	0.19	0.18
PDR	0.27	18.25	3.74	0.30	0.35	0.32
GD	2.33	1.43	2.95	2.19	2.85	3.22
Heritability % (ns)	17.15	12.87	10.67	20.88	21.39	17.79

** $P < 0.01$, * $P < 0.05$

D additive genetic variance, *H*₁ dominance variance, *H*₂, $H_1 \{1 - (u - v)^2\}$, where *u* and *v* are the proportions of positive and negative genes in the parents, h^2 dominance effect, as algebraic sum over all loci in heterozygous phase in all crosses, *F* gene distribution, *E* expected environmental component of variation, ADD average degree of dominance, $(H_1/D)^{1/2}$, *PPN* proportions of genes with positive and negative effects, $H_2/4H_1$, *PDR* proportion of dominant and recessive gene, $[(4DH_1)^{1/2} + F]/[(4DH_1)^{1/2} - F]$, *GD* group of dominant genes, h^2/H_2

Fig. 3 P, K, Ca and Na content in parents and hybrids of ridge gourd. Standard error bars with $p < 0.05$

Fig. 4 S, Fe, Zn and Mn content in parents and hybrids of ridge gourd. Standard error bars with $p < 0.05$

carotenoid content in carrot [28]. The genetic analysis showed significance of the presence of additive, dominance and epistatic genes for both phenolics and antioxidant activity in cowpea [20]. Breeding for high antioxidant activity in cowpea is possible by using highly pigmented parental lines as there is positive correlation between pigmentation and antioxidant activity [21].

Mineral Content of Parents and Hybrids

The minerals content such as P, K, Ca, Na, S, Fe, Zn and Mn were estimated in fresh fruits of seven parental lines and 21 F_1 hybrids (Figs. 3 and 4) and the magnitude of mineral content was significantly higher in F_1 hybrids than the parents. The average content of 21 F_1 hybrids for P, K, Ca, Na, S, Fe, Zn and Mn was greater as compared to the parental average, and the respective amount was calculated to be 21.7, 29.9, 25.9, 36.0, 71.1, 24.6, 50.7 and 67.9 % additionally. Therefore, higher value of hybrid average indicates the heterotic effect for mineral content in ridge gourd fruit. Raigon et al. [23] reported higher Fe and Zn

content in F_1 hybrids of brinjal over the parental mean. In rice, F_1 hybrids had increased content of grain Fe and Zn over standard variety [18]. In contrast, reduced content of mineral nutrients was reported in the F_1 hybrids as compared to the parental value in cabbage [31] and maize [7].

Genetic Component for Mineral Content

The D , H_1 , H_2 and h^2 estimates for all the mineral content except S content in fruit were highly significant (Table 2). The values of H_1 were more than D , which signifies that dominant genes were more than additive genes for P, Ca, K, Na, S, Fe, Zn and Mn content. The environmental influence (E) on the inheritance of these traits was negligible and non-significant. The negative values of F for these traits showed that recessive alleles were more frequent. The mean degree of dominance $(H_1/D)^{1/2}$ was 1.41, 1.38, 1.4, 1.85, 3.16, 1.47, 1.82 and 1.46 for P, Ca, K, Na, S, Fe, Zn and Mn, respectively. The mean degree of dominance greater than one for these mineral nutrients indicated the role of over-dominance for these traits. The proportions of genes with positive and negative effects $(H_2/4H_1)$ were 0.18, 0.19, 0.17, 0.22, 0.16, 0.18, 0.17 and 0.18, respectively, for P, Ca, K, Na, S, Fe, Zn and Mn. These values were less than 0.25, which implies asymmetry at the loci showing dominance. The proportion of dominant and recessive gene in the parents as indicated by $[(4DH_1)^{1/2} + F/(4DH_1)^{1/2} - F]$ was 0.30, 0.35, 0.27, 0.53, 0.26, 0.27, 0.29 and 0.32 while the group of genes, which controlled the characters and exhibited dominance (h^2/H_2) were 2.26, 2.16, 2.27, 1.11, 2.06, 2.06, 2.91 and 3.35 for P, Ca, K, Na, S, Fe, Zn and Mn, respectively. Narrow-sense heritability being 22.18 % (P), 22.58 % (Ca), 21.94 % (K), 17.88 % (Na), 6.8 % (S), 19.96 % (Fe), 15.3 % (Zn) and 21.48 % (Mn) for the macronutrients and micronutrients indicated that the non-additive type of gene action played an important role in the inheritance of these mineralogical traits. Highly significant D , H_1 , H_2 and h^2 estimate, non-significant value of E , higher magnitude H_1 over corresponding value of D , negative value of F , greater than unity value of $(H_1/D)^{1/2}$, <0.25 value for $(H_2/4H_1)$, and lower narrow-sense heritability (<50.0 %) for P, Ca, K, Na, S, Fe, Zn and Mn point towards the non-additive type of gene action in the inheritance of these nutritional traits. Over-dominance, lesser influence of environment on accumulation of minerals, the presence of more dominant genes than additive genes, preponderance of recessive alleles for these traits in parents and asymmetry at dominant loci played major role in the genetic control of mineral content in ridge gourd fruit. Hedau [13] also explored non-additive gene action to improve Ca and P content in ridge gourd fruits. Sit and Sirohi [34] reported over-dominance for P, Ca, Fe and vitamin C content in fruits of bottle gourd. Additive as

Table 2 Estimates of genetic components of variation and various statistical parameters for mineral contents

	P (mg/100 g)	Ca (mg/100 g)	K (mg/100 g)	Na (mg/100 g)	S (μg/100 g)	Fe (μg/100 g)	Zn (μg/100 g)	Mn (μg/100 g)
Genetic components								
<i>D</i>	39.95** ± 4.52	62.47** ± 3.79	81.46** ± 6.91	56.35** ± 16.93	64875 ± 70025.7	15591.3** ± 1098.3	55185** ± 3149.68	3550.6** ± 416.8
<i>H</i> ₁	79.33** ± 10.88	119.04** ± 9.12	159.81** ± 16.84	193.1** ± 40.05	647998.5** ± 168584.9	33498.6** ± 2644.1	182002.5** ± 22027	7573.5** ± 1003.5
<i>H</i> ₂	56.63** ± 9.59	88.32** ± 8.04	108.54** ± 14.67	166.89** ± 35.29	418526.2** ± 148574	24318.6** ± 2329.8	126998.9** ± 19409.4	5588.41** ± 884.2
<i>h</i> ²	128.21** ± 6.44	190.95** ± 5.40	246.54** ± 9.85	186.09** ± 23.70	862090.4** ± 99771	50317.7** ± 1564.8	369830.1** ± 10336.25	18772.5** ± 593.8
<i>F</i>	−59.66** ± 10.84	−82.27** ± 9.09	−128.85** ± 16.5	−64.06** ± 39.06	−239729.1 ± 167990.3	−25855** ± 2634.8	−108687.5** ± 21949.9	−5295.75** ± 999.9
<i>E</i>	0.29 ± 1.60	0.26 ± 1.34	0.29 ± 2.44	0.41 ± 5.88	315.4 ± 24757.8	783.6 ± 3883.1	133.54 ± 3234.9	27.18 ± 147.38
Ratios of genetic components								
ADD	1.41	1.38	1.4	1.85	3.16	1.47	1.82	1.46
PPN	0.18	0.19	0.17	0.22	0.16	0.18	0.17	0.18
PDR	0.30	0.35	0.27	0.53	0.26	0.27	0.29	0.32
GD	2.26	2.16	2.27	1.11	2.06	2.06	2.91	3.35
Heritability % (ns)	22.18	23.58	21.94	17.88	6.8	19.96	15.93	21.48

** $P < 0.01$, * $P < 0.05$

D additive genetic variance, *H*₁ dominance variance, *H*₂, $H_1 \{1 - (u - v)^2\}$, where *u* and *v* are the proportions of positive and negative genes in the parents, *h*² dominance effect, as algebraic sum over all loci in heterozygous phase in all crosses, *F* gene distribution, *E* expected environmental component of variation, *ADD* average degree of dominance, $(H_1/D)^{1/2}$, *PPN* proportions of genes with positive and negative effects, $H_2/4H_1$, *PDR* proportion of dominant and recessive gene, $[(4DH_1)^{1/2} + F]/[(4DH_1)^{1/2} - F]$, *GD* group of dominant genes, h^2/H_2

well as non-additive gene effects had been reported for the inheritance of K and protein content in pea [11]. Single dominant gene controls the high seed Zn concentration in French/navy bean [8]. Low level of narrow-sense heritability and partial dominance reported for K content in common bean seed [22]. Prevalence of non-additive variance and non-additive gene action for Fe, Zn, Mn, K and Ca accumulation in cabbage was reported by Singh et al. [32].

Conclusions

In conclusion, the hybrids were superior for nutritional quality over their parents. Thus F₁ hybrids provide greater opportunity for enhanced nutritional quality of ridge gourd fruits over open-pollinated varieties. Furthermore, the nutrient concentration of fruits was predominantly contributed by the non-additive genetic component; therefore, hybrid breeding approaches (heterosis, and crossbreeding and early generation selection) might be useful to improve antioxidant properties and mineral content in ridge gourd (*L. acutangula* Roxb.).

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