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GENETIC VARIABILITY STUDIES FOR BIOCHEMICAL TRAITS IN CHILLI (*CAPSICUM ANNUUM* L.)

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ABSTRACT
Seventy one chilli genotypes comprising 54 F_1 hybrids, 15 parents and two commercial checks were evaluated at Horticultural Research Station, Lam, Guntur, Andhra Pradesh to estimate the genetic variability, heritability and genetic advance for seven biochemical characters. Significant differences were observed among the genotypes for all the traits. The phenotypic coefficient of variation was higher than genotypic coefficient of variation for all characters. All biochemical traits recorded high values of PCV, GCV and high heritability coupled with high genetic advance as % of mean indicated that there is an existence of wide range of genetic variability among the all 71 genotypes studied and suggested that predominance of additive gene action and lower influence of environmental factors in the expression of all biochemical traits making the selection more effective in their improvement.

KEYWORDS: Chilli, *Capsicum annuum*, GCV, PCV, genetic advance, heritability.

INTRODUCTION

Chilli, known as the universal spice of India and has diverse utilities as a spice, condiment, culinary supplement, medicine, vegetable and ornamental plant. The important chilli growing states are Andhra Pradesh, Telangana, Karnataka, Maharashtra, Orissa, Tamil Nadu and Madhya Pradesh. In recent years crop quality improvement is gaining more importance as quality produce improves human welfare and increased farm income. Thus, the improvement of quality characters in crop plants has great potential to alleviate problems related to poverty and malnutrition. Chilli is an important condiment used for imparting pungency, which is due to an active principle 'capsaicin', an alkaloid present in the placenta which can directly scavenge various free radicals (Bhattacharya *et al*., 2010 and Kogure *et al*., 2002). Chilli is a good source of vitamin C (ascorbic acid) used in food and beverage industries (Bosland and Votava, 2000). The carotenoids 'capsanthin, capsorubin and capsanthin 5,6 – epoxide are responsible for the final red colour (Davies *et al*., 1970) which act as dietary precursors of vitamin A and plays an important role in the regulation of vision, growth and reproduction. Chilli has also acquired a great importance because of the presence of 'oleoresin', which permits better distribution of color and flavor in foods. Importance of genetic variability in any breeding material is a pre-requisite as it provides not only a basis for selection but also provides some valuable information regarding selection of diverse parents to use in hybridization programme. The plant breeder has to identify the sources of favourable genes, incorporate them in breeding populations and aims at isolation of productive genotypes and cultivars. Thus, improvement in any crop is based on the extent of genetic variation and the degree of improvement depends upon the magnitude of available beneficial genetic variability. Genetic variability studies in chilli have also carried out by earlier workers *viz.* Gupta *et al.* (2009), Arup *et al.* (2011), Naresh *et al.* (2013) and Janaki *et al.* (2016). Hence, the present study was undertaken to analyse the extent of variability for seven biochemical traits in 71 genotypes of chilli comprising of 54 F_1 hybrids, 15 parents and two commercial checks.

MATERIALS & METHODS

An experiment was conducted at Horticulture Research Station, Dr. Y. S. R. Horticultural University, Lam farm, Guntur**.** The experimental material and their characters used in this experiment were presented in Table 1. The experimental material comprised of nine lines (LCA 504, LCA 615, LCA 446, LCA 466, LCA 442, LCA 654, LCA 607, LCA 655 and LCA 355) and six testers (G4, LCA 678, LCA 453, LCA 703-2, LCA 705-2 and LCA 315). These parents were crossed in Line × Tester fashion during *Kharif*, 2013-14 and developed 54 F_1 hybrids. The resulting 54 F_1 hybrids along with their 15 parents and two commercial checks (Tejaswini and Indam-5) were evaluated during *Kharif*, 2014-15 in a Randomized Block Design with three replications in two rows (one row of 4 m length) of each genotype at a spacing of 75 cm x 30 cm. The crop was raised as per the standard package of practices. The crop was maintained healthy till last harvest and fruit samples were collected from five randomly selected plants in each plot (one row of 4m length) to estimate the quality traits *viz.* ascorbic acid (mg/100g), oleoresin (%), capsaicin (%), red carotenoids (mg/100g),

yellow carotenoids (mg/100g), total carotenoids (mg/100g) and total color value (ASTA units).

The red ripe fruits were sun dried and ground in an electronic grinder and passed through a 0.5 mm sieve and the dry chilli powder was used to measure biochemical constituents *viz.* oleoresin, capsaicin, red carotenoids, yellow carotenoids, total carotenoids and total color value whereas mature green fruits were used for estimating the ascorbic acid content.

TABLE 1: Salient features of parents used in Line × Tester analysis of chilli

S.No	Parents	Features
Lines	LCA504	Drought resistant, highly pungent
	LCA615	High yielding line with parrot green fruits
	LCA446	Bold pod, high colour and oleoresin
	LCA466	Bold and long pod, high colour and oleoresin
	LCA442	Bold and long pod, high colour and mild pungent
	LCA654	Medium bold, shiny fruit surface, light green in colour
	LCA607	Light green pod, profuse branching
	LCA655	Dual purpose variety, bold light green pod
	LCA355	High colour with wrinkled surface
Testers	G4	Dark green (olive green) fruits, virus resistant
	LCA678	More primary branches, semi erect plant habit
	LCA453	Bold pod, erect growth habit
	LCA7032	Virus resistant, dark green fruits
	LCA7052	More no. of fruits, shiny dry pod
	LCA315	Virus resistant, fruits are long and dark green
Checks	Indam5	IndoAmerican Hybrid Seeds (India) Pvt.Ltd. (IAHS)
	Tejaswini	Maharashta Hybrid Seeds Co.Ltd. (MAHYCO)

The following procedures were used for estimating the biochemical constituents.

1. Ascorbic acid (mg 100g^{-1})

Ascorbic acid content of mature green fruits was estimated by volumetric method described by Sadasivam and Balasubramanian (1987). The dye solution was prepared by dissolving 42 mg of sodium bicarbonate in distilled water taken into 200 ml volumetric flask and 52 mg of 2-6 dichlorophenol indophenol added in it and then the volume was made up with distilled water. Stock solution was prepared by dissolving 100 mg ascorbic acid in 100 ml of 4% oxalic acid solution and 10 ml of this stock solution was diluted to 100 ml with 4% oxalic acid to get the working standard of 100 mg ml^{-1}. 5 ml of the working standard solution was pipetted into a 100 ml of conical flask to which 10 ml of 4% oxalic acid was added. The contents were titrated against the dye (V_1ml) to get a pink end point which persisted for a few minutes. The chilli sample (5 g) was extracted in 4% oxalic acid and the volume was made up to 100 ml and the contents were centrifuged. 5 ml of this supernatant was pipetted out, to which 10 ml of 4 per cent oxalic acid was added and titrated against the dye (V_2 ml). The ascorbic acid content was calculated using the formula given below.

$$\text{Ascorbic acid (mg } 100\text{g}^{-1}) = (0.5 \text{ mg} \div V1) \times (V2 \div 5\text{ml}) \times (100\text{ml} \div \text{Wt. of the sample}) \times 100$$

2. Oleoresin (%)

The oleoresin content was estimated as per the procedure given by Ranganna (1986). Finely mashed 25g chilli powder was transferred to a glass column, which was plugged by cotton plug on its narrow end. A thin layer of cotton was placed over chilli powder in the glass column and 25 ml of acetone was added. After all the acetone was decanted, 25 ml acetone was added each time till a total of 250 ml acetone was added to the contents. After decantatation, the resulting red colored liquid in beaker contains all the principle constituents of chilli. The collected filtrate was transferred to a 250 ml volumetric flask and the volume was made up with acetone.

The chilli extract was transferred to a 250 ml beaker of known weight (W_I g) and was kept in water bath at 50-60^{o}C for 15-30 minutes so that acetone gets evaporated. Then, weight of the beaker along with contents was recorded as W_2 g. The weight of the oleoresin content in the 25 g chilli powder was calculated and expressed in percentage using the given formula.

$$\text{Oleoresin content (\%)} = \left((W2 - W1) \div \text{Weight of sample}\right) \times 100$$

3. Capsaicin (%)

The capsaicin content of fruits was estimated by colorimetric method described by Bajaj *et al*. (1980). 0.5g dry chilli powder was weighed into glass-stoppard test tube; 10ml dry acetone (add 25g anhydrous sodium sulphate to 500ml of acetone at least one day before use) was added into the test tube and kept overnight for extraction. Next day samples were centrifuged at 10000 rpm for 10min to get clear supernatant. 1ml of the supernatant was taken into a test tube and evaporated to dryness in a hot water bath. Then, the residue was dissolved in 5ml of 0.4% of NaOH solution and 3ml of 3% phosphomolybdic acid was added. The contents were shaken and left undisturbed for 1hr. After 1hr, the solution was quickly filtered into centrifuge tubes to remove any floating debris, and then centrifuged at 5000rpm for 15min. The clear blue coloured solution was directly transferred into the cuvette and absorbance was read at 650nm along with a reagent blank. A standard graph was prepared using 0-200µg pure capsaicin. Simultaneously 0.2, 0.4, 0.6, 0.8 and 1ml of working standard solution (stock standard capsaicin solution was prepared by dissolving 50mg capsaicin in 50ml of 0.4% NaOH solution (1000µg ml^{-1}) and working standard solution prepared by diluting the 10ml of the stock standard to

50ml with 0.4% NaOH solution (200µg ml^{-1})) was taken into new test tubes and proceeded as mentioned above. Per cent capsaicin calculated using the formula mentioned below:

$$\text{Capsaicin content (\%)} = (\mu g \text{ capsaicin} \times 100 \times 100) \div (1000 \times 1000 \times 1 \times 0.5)$$

4. Total color value (ASTA units):

Total extractable colour of fruits (ASTA- American Spice Trade Association units) was estimated as per the procedure given by Rosebrook *et al.* (1968). 100mg of sieved fine chilli powder was weighed into a volumetric flask. Acetone was added and flask was closed tightly with stopper, then contents were kept for 16h at room temperature in dark and shaken intermittently. Solution was filtered using Whatman filter paper and final volume was made up to 100ml. Absorbance of final extract was read at 460nm using acetone as blank. ASTA color units were calculated as per the formula given below,

$$\text{ASTA} = (\text{Absorbance at 460 nm} \times 16.4) \div (\text{Weight of sample in g})$$

5. Determination of red and yellow fractions in chilli powder:

Total red (C^R; capsanthin, capsorubin and capsanthin-5, 6-epoxide) and yellow (C^Y; zeaxanthin, violaxanthin, antheraxanthin, β-cryptoxanthin, β-carotene and cucurbitaxanthin A) carotenoid isochromic fractions were estimated following protocol of spectrophotometric method (Hornero-Mendez and Minguez-Mosquera, 2001).

Dried chilli fruits were ground into a fine powder and 100mg of dried powder was extracted four times with 25ml acetone until the complete exhaustion of the color. The extract was filtered and transferred to 50ml volumetric flask and the volume was made up with acetone. The samples absorbance was read at two wavelengths *i.e.,* 472 and 508nm using acetone as blank. The red and yellow fractions were calculated using the following formulae.

$$C^R\ (\mu g\ ml^{-1}) = \big((A508 \times 2144.0) - (A472 \times 403.3)\big) \div 270.9$$

$$C^Y\ (\mu g\ ml^{-1}) = \big((A472 \times 1724.3) - (A508 \times 2450.1)\big) \div 270.9$$

$$\text{Total carotenoids} = C^R + C^Y$$

Analysis of variance was carried out as per the procedure given by Panse and Sukhatme (1985). The genotypic and phenotypic coefficients of variation were computed as per Burton and Devane (1953) and categorized as per Sivasubrahmanian and Menon (1973) while the heritability in broad sense and genetic advance were calculated as per Allard (1960) and categorized as per Johnson *et al.* (1955).

RESULTS & DISCUSSION

Analysis of variance (Table 2) revealed that significant differences for all the traits indicating the presence of wide range of variability among the genotypes and there is a considerable scope for their improvement through simple selection. Significant genetic variability for biochemical traits in chilli had also been reported by earlier workers Umajyothi *et al.* (2008) and Kumar *et al.* (2012).

TABLE 2. Analysis of variance for biochemical traits in chilli (*Capsicum annuum* L.)

S. No.	Characters	Mean Sum of Squares		
		Replications	Treatments	Error
1	Ascorbic Acid (mg/100g)	11.41	2418.73**	37.5
2	Oleoresin (%)	0.05	33.18**	1.76
3	Capsaicin (%)	0.00	0.05**	0.00
4	Red carotenoids (mg/100g)	2.86	4039.6**	140.97
5	Yellow carotinoids (mg/100g)	31.99	3072.99**	67.22
6	Total carotenoids (mg/100g)	5.04	10572.02**	273.67
7	Total colour value (ASTA units)	21.81	1717.09**	49.05

*: Significant at 5 % level; **: Significant at 1 % level

The extent of variability with respect to seven quality characters in different genotypes measured in terms of mean, range, genotypic coefficient of variation (GCV), phenotypic coefficient of variation (PCV), along with the amount of heritability (h), expected genetic advance and genetic advance as % of mean (GAM) are furnished in Table 3.

With respect to mean (Table 3), the characters *viz.* ascorbic acid, capsaicin, oleoresin, total colour value, red, yellow and total carotenoids had a range of 16.08 to 128.83 mg/100g, 0.08 to 0.74 %, 6.80 to 20.97 %, 51.54 to 163.68 ASTA units, 81.91 to 266.17 mg/100g, 28.12 to 180.62 mg/100g and 115.86 to 419.90 mg/100g respectively. Whereas, the ascorbic acid, capsaicin, oleoresin, total colour value, red, yellow and total carotenoids have been recorded the mean of 80.55 mg/100g, 0.36 %, 12.39 %, 105.71 ASTA units, 172.61 mg/100g, 97.53 mg/100g and 270.14 mg/100g respectively. Similar kinds of results are also noticed by earlier findings of Janaki *et al.* (2016).

The considerable amount of variation was observed for all the characters. The phenotypic coefficient of variation (PCV) was higher than the genotypic coefficient of variation for all the characters (Table 3) indicating the influence of environment on these characters. It is obvious because PCV includes variability due to genotype and genotype × environment interaction. These observations

are supported by earlier findings of Suryakumari *et al.* (2010) and Rajyalakshmi and Vijaya Padma (2012).

TABLE 3. Estimates of mean, range, components of variance, heritability and genetic advance for biochemical traits in chilli (*Capsicum annuum* L.)

Character	Mean	Range	GCV	PCV	h² (b)	h² (b) %	GA @ 5%	GAM @ 5%
Ascorbic acid (mg/100g)	80.55	16.08-128.83	34.98	35.79	0.95	95.48	56.71	70.41
Capsaicin (%)	0.36	0.08-0.74	37.33	38.38	0.94	94.44	0.27	74.79
Oleoresin (%)	12.39	6.80 - 20.97	26.11	28.22	0.86	85.60	6.17	49.77
Total Colour Value (ASTA Units)	105.71	51.54 - 163.68	22.31	23.27	0.92	91.89	46.56	44.05
Red Carotenoids (mg/100 g)	172.61	81.91 - 266.17	20.88	21.99	0.90	90.21	70.53	40.86
Yellow Carotenoids (mg/100 g)	97.53	28.12 - 180.62	32.45	33.52	0.94	93.71	63.12	64.72
Total Carotenoids (mg/100 g)	270.14	115.86 - 419.90	21.69	22.54	0.93	92.61	116.15	43.00

Where: GCV - Genotypic Coefficient of Variation, PCV - Phenotypic Coefficient of Variation, $h^2(b)$ - Heritability in Broad Sence, GA - Genetic Advance and GAM - Genetic Advance as % of Mean (GAM)

The estimates of PCV and GCV were high (>20%) and the difference between PCV and GCV was narrow for all the traits indicating the existence of wide range of genetic variability in the material studied. This also indicated that broad genetic base, less environmental influence and these traits are under the control of additive genes and hence there is a good scope for the further improvement of these characters through simple selection. High heritability coupled with high genetic advance as % of mean was observed for all the characters indicating the predominance of additive gene action and hence direct selection is useful with respect to these traits. These results are in conformity with earlier reports of Farhad *et al.* (2008), Arup *et al.* (2011) for ascorbic acid; Manju and Sreelathakumary (2002), Singh *et al*. (2009) for oleoresin; Gupta *et al*. (2009), Suryakumari *et al*. (2010) for capsaicin; Janaki *et al.* (2016) for total colour value; Naresh *et al.* (2013), Janaki *et al.* (2016) for red and yellow carotenoids who also had reported high PCV and GCV, high heritability coupled with high genetic advance as % of mean for respective characters in chilli.

The results of present study revealed that all traits reported high values of PCV, GCV and high heritability coupled with high genetic advance as % of mean which indicated that there is an existence of wide range of genetic variability among the all 71 genotypes studied. The results suggested that predominance of additive gene action and lower influence of environmental factors in the expression of all biochemical traits making the selection more effective in their improvement.

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