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Assessment of genetic variability for biochemical traits in F_2 segregation population of Chilli (*Capsicum annuum* L.)

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Abstract
An experiment was conducted to estimate the genetic variability, heritability and genetic advance for seven qualitative characters viz., ascorbic acid, oleoresin content, capsaicin content, total extractable colour, red carotenoids, yellow carotenoids and total caroteniods. The phenotypic coefficient of variation was higher than genotypic coefficient of variation for all characters. High magnitude of PCV and GCV were observed for ascorbic acid, oleoresin content, yellow carotenoids and total caroteniods indicating the existence of wide range of genetic variability for these traits. High heritability coupled with high genetic advance as % of mean was observed for ascorbic acid, oleoresin content, capsaicin content, red carotenoids, yellow carotenoids and total caroteniods indicating the predominance of additive gene action making the selection more effective.

Keywords: *Capsicum annuum*, GCV, PCV, Heritability, Genetic advance

Introduction
Chilli *(Capsicum annuum* L. 2n = 24) is one of the most important commercial vegetable as well as spice crops grown all over the world. India is one of the leading chilli (*Capsicum annuum* L.) producing countries of the world. Chilli has diverse utilities as a spice, condiment, and culinary supplement, and medicine, vegetable and ornamental plant. In view of the changing of food habits and health conscious, food quality particularly perishables like fruits and vegetables is gaining importance since improved quality not only facilitates remunerative market price for the producer and also improves health of the consumer. Thus, the attempts towards improvement of quality characters in crop plants have lot of significance which can increase the income of the farmer through premium price.

Chilli besides imparting pungency and red colour to dishes, is also rich source of vitamin C, A and E and assists in good digestion. The vitamin C content (150-200 mg/100g) of chilli is the highest among all the vegetables. Capsicinoids and carotenoids, the major chemical constituents of chilli fruits add commercial value to the crop. The carotenoids which contribute fruit colour act as dietary precursors of vitamin A and among carotenoids 'capsanthin, capsorubin and capsanthin 5, 6-epoxide are responsible for the final red colour. The nature of pungency has been established as a mixture of seven closely related alkyl vanillyl amides, collectively referred as "Capsaicinoids". Among capsiacinoids, capsaicin (8-methyl-N-vanillyl-6-enamide) and dihydrocapsaicin accounts for more than 80% and determine the pungency. The degree of pungency varies widely with the genotypes (Kumar *et al.*, 2006) [13]. The 'capsaicin' is an alkaloid present in the placenta of the fruit, which can directly scavenge various free radicals (Bhattacharya *et al.*, 2010) [5]. The pharmaceutical application of capsaicinoids is attributed to its antioxidant, anticancer, antiarthritic and analgesic properties (Prasad *et al*., 2006) [20]. Chilli has also acquired a great importance because of the presence of 'oleoresin', which permits better color distribution and flavor in foods.

To improve the yield and other yield attributing characters, information on genetic variability present in the germplasm is pre-requisite. The improvement in any crop is proportional to the magnitude of its genetic variability present in germplasm. Greater the variability in a population, there will be the greater chance for effective selection for desirable types. Heritability is the portion of phenotypic variation which is transmitted from parent to progeny. Higher the heritable variation, greater will be the possibility of fixing the characters by selection. Hence, heritability studies are of foremost importance to judge whether the observed

variation for a particular character is due to genotype or due to environment. Heritability estimates may not provide clear predictability of the breeding value. Thus, estimation of heritability accompanied with genetic advance is generally more useful than heritability alone in prediction of the resultant effect for selecting the best individuals (Johnsen *et al*., 1955) [11]. Therefore, a study was carried out to estimate the genetic variability, heritability and genetic advance in F_2 segegation population of chilli in respect of qualitative traits.

Materials and Methods

The experiment was carried out at Horticultural Research Station, Lam farm, and Guntur during *Kharif* 2018-19 in F_2 population of two crosses namely viz., LCA-764 x LCA-315 and MS-276A x LCA-801. The site of the experiment at Lam is situated on 16.280 North latitude and 80.440 East longitude at an altitude of 31.5 m above mean sea level which falls under humid tropical climate. The soils of the experimental site are rich black cotton soils. The crop received timely management practices as per recommended package of practices. The crop was maintained properly till last harvest and observations on yield as well as yield contributing characters was noted on F_2 populations along with parents. From each cross 240 plants were studied and all the individual plants of segregating generations were selected for collecting the fruit samples to estimate qualitative traits *viz*. ascorbic acid (mg/100 g), oleoresin content (%), capsaicin content (%), total extractable colour (ASTA units), red carotenoids (%), yellow carotenoids (%) and total carotenoids (%).

Fruit samples were harvested at full ripe stage except for vitamin-C, for which mature green fruits were harvested. The red ripen fruits were sun dried for 6–7 days and ground in an electronic grinder and passed through a 0.5 mm sieve. By using chilli powder the following biochemical constituents were measured.

Ascorbic acid (mg/100 g)

Vitamin C content of mature green fruits was esti- mated by volumetric method described earlier (Sadasivam and Balasubramanian 1987) [25].

Oleoresin content (%)

The oleoresin content was estimated as per the standard procedure (Ranganna, 1986) [23]. Finely mashed 25 g chilli powder was transferred to a glass column, which was plugged by cotton plug on its narrow end. A thin layer of cot- ton 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 (W1 g) and was kept in water bath at 50–600C for 15–30 minutes so that acetone gets evaporated. Then, weight of the beaker along with contents was recorded as W2 g. The weight of the oleoresin content in the 25 g chilli powder was calculated and expressed in percentage using the given formula.

Oleoresin content (%) = ((W2—W1) ÷Weight of sample) × 100

Capsaicin content (%)

The capsaicin content of fruits was estimated by colorimetric method (Bajaj *et al*.,1980) [4]; 0.5 g dry chilli powder was weighed into glass-stoppard test tube ; 10 ml dry ac- etone (add 25 g anhydrous sodium sulfate to 500 ml 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 10 min to get clear supernatant. 1 ml of the supernatant was taken in to a test tube and evaporated to dryness in a hot water bath. Then, the residue was dissolved in 5 ml of 0.4% of NaoH solution and 3 ml of 3% phosphomolybdic acid was added. The contents were shaken and left undisturbed for 1 h. After 1 hr, the solution was quickly filtered into centrifuge tubes to remove any floating debris, and then centrifuged at 5000 rpm for 15 min. The clear blue colored solution was directly transferred into the cuvette and absorbance was read at 650 nm along with a reagent blank. A standard graph was prepared using 0–200 µg pure capsaicin. Simultaneously 0.2, 0.4, 0.6, 0.8 and l ml of working standard solution (stock standard capsaicin solution was prepared by dissolving 50 mg capsaicin in 50 ml of 0.4% NaOH solution (1000µg / ml) and working standard solution prepared by diluting the 10 ml of the stock standard to 50 ml with 0.4% NaOH solution (200 µg / ml) was taken into new test tubes and proceeded as mentioned above. Per cent capsaicin calculated using the formula mentioned below

$$\text{Capsaicin content (\%)} = \frac{(\mu g \text{ capsaicin} \times 100 \times 100) \div (1000 \times 1000 \times 1 \times 0.5)}{1\% = 1,\ 60{,}000 \text{SHU units}}$$

Total extractable colour (ASTA units)

Total extractable colour of fruits (ASTA–American Spice Trade Association units) was estimated as per the procedure given earlier (Rosebrook *et al.,* (1968) [24]. 100 mg 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 16 h at room temperature in dark and shaken intermittently. Solution was filtered using Whatman filter paper and final volume was made up to 100 ml. Absorbance of final extract was read at 460 nm using acetone as blank. ASTA color units were calculated as per the formula given below,

ASTA = (Absorbance at 460 nm×16.4) ÷ (Weight of sample in g)

Determination of yellow and red fractions in chilli powder

Total red (CR.; capsanthin, capsorubin and capsanthin-5, 6-epoxide) and yellow (CY.; zeaxanthin, violaxanthin, antheraxanthin, β-cryptoxanthin, β-carotene and cucurbitaxanthin A) carotenoid isochromic fractions were estimated following protocol of spec- trophotometric method (Hornero-Mendez and Minguez-Mosquera, 2001) [9].

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

CR (µg/ml) = ((A508 × 2144.0)–(A472 × 403.3)) ÷ 270.9
CY (µg/ml) = (A472×1724.3) – A508×2450.1)) ÷ 270.9

Total colour = $C^R + C^Y$

µg/ml values were converted into percentage on dry weight basis.

Analysis of variance was carried out as per the procedure given earlier (Panse and Sukhatme, 1985) [18]. The genotypic and phenotypic coefficients of variation were computed (Burton and Devane, 1953) [6] and categorized (Sivasubrahmanian and Menon, 1973) [28] while the heritability and genetic advance were calculated (Allard, 1960) [2] and categorized (Johnsen *et al.*, (1955) [11].

Results and Discussion

The extent of variability with respect to seven qualitative characters in different genotypes measured in terms of mean, genotypic coefficient of variation (GCV), phenotypic coefficient of variation (PCV) along with the amount of heritability (h), expected genetic advance and genetic advance as percent of mean (GAM) are presented in Table 1 & 2.

Table 1: Estimates of mean, components of variance, heritability and genetic advance for qualitative characters in in F_2 population of cross-1 (LCA-764 x LCA-315)

Character	Mean	GCV (%)	PCV (%)	h^2 (b) (%)	GA @ 5%	GAM @ 5%
Ascorbic Acid (mg/100mg)	53.62	32.90	33.34	97.41	35.87	66.90
Oleoresin (%),	9.48	11.41	14.31	63.59	1.78	18.74
Capsaicin (SHU),	0.58	17.24	24.38	50.00	0.15	25.11
Red carotenoids (%),	104.89	14.73	22.84	41.62	20.54	19.58
Yellow carotenoids (%),	65.47	13.79	24.31	32.18	10.55	16.11
Total carotenoids (%),	170.36	12.41	21.08	34.66	25.64	15.05
Total extractable color (ASTA units),	65.60	14.24	20.68	47.44	13.26	20.21

Where

GCV–Genotypic coefficient of variation, PCV–Phenotypic coefficient of variation, h2 (b)–Heritability at broad sense, GA–Genetic Advance, GAM–Genetic Advance as a per cent of mean.

The phenotypic coefficient of variation (PCV) was higher than the genotypic coefficient of variation for all the characters (Table 1&2) indicating the influence of environment on these characters and the considerable amount of variation was observed for all the characters. These observations are supported by earlier workers Arup *et al.*, (2011) [3], Farhad *et al*., (2008) [8], Rajya lakshmi and Vijayapadma (2012) [22], Naresh *et al.*, (2013) [17], Mahantesh *et al.*, (2015) [15] and Janaki *et al*., (2016) [10].

In the cross LCA-764 x LCA-315, results showed that the genotypic coefficient of variation was observed highest for ascorbic acid (32.90%) while moderate in capsaicin (17.24%) followed by red carotenoids (14.73%), total extractable color (14.24%), yellow carotenoids (13.79%), total carotenoids (12.41%) and oleoresin (11.41%).

The highest phenotypic coefficient of variation was observed highest for ascorbic acid (33.34%) followed by capsaicin (24.38%), yellow carotenoids (24.31%), red carotenoids (22.84%), total carotenoids (21.08%), total extractable color (20.68%), While moderate in oleoresin (14.31%).

Heritability in broad sense was high for ascorbic acid (97.41%), oleoresin (63.59%), While capsaicin (50.00%), red carotenoids (41.62%), yellow carotenoids (32.18%), total carotenoids (34.66%) and total extractable color (47.44%), recorded moderate broad sense heritability value.

Genetic advance as per cent mean was high for the characters like ascorbic acid (66.90%), capsaicin (25.11%) and total extractable color (20.21%) while moderate in oleoresin (18.74%), red carotenoids (19.58%), yellow carotenoids (16.11%), total carotenoids (15.05%).

Heritability estimates along with genetic advance are more useful than heritability value alone in predicting the selection of best individuals. In the present investigation Higher GCV, PCV and high estimates of heritability with high genetic advance as percent over mean were recorded for characters like ascorbic acid indicating higher magnitude of variability for this character. It might be assigned to be under the control of additive genes and phenotypic selection for their improvement could be achieved by simple breeding methods. These findings were similar to Farhad *et al*.,(2008) [8], Sharma *et al.*, (2010), Mahantesh *et al*.,(2013) [14], Patel *et al*., (2015) [19], Janaki *et al*.,(2016) [10] Priyanka and Naidu (2016) [21], Singh *et al*. (2017) [27], Zehra *et al*. (2017) [33], Nagaraju *et al*.(2018) [16] and Syed *et al*.(2020) [30] for ascorbic acid.

Table 2: Estimates of mean, components of variance, heritability and genetic advance for qualitative characters in in F_2 population of cross-2 (MS-276A x LCA-801)

Character	Mean	GCV (%)	PCV (%)	h^2 (b) (%)	GA @ 5%	GAM @ 5%
Ascorbic Acid (mg/100mg)	57.48	34.04	34.36	98.15	39.93	69.47
Oleoresin (%),	10.99	21.56	23.43	84.69	4.49	40.88
Capsaicin (SHU),	0.74	19.11	23.41	66.67	0.24	32.14
Red carotenoids (%),	142.70	18.62	20.25	84.61	50.35	35.29
Yellow carotenoids (%),	136.12	25.70	26.41	94.75	70.15	51.54
Total carotenoids (%),	278.82	21.41	22.35	91.78	117.80	42.25
Total extractable color (ASTA units),	118.41	12.84	16.81	58.30	23.91	20.19

Where

GCV–Genotypic coefficient of variation, PCV–Phenotypic coefficient of variation, h2 (b)–Heritability at broad sense, GA–Genetic Advance, GAM–Genetic Advance as a per cent of mean.

In the cross MS-276A x LCA-801, results showed that the genotypic coefficient of variation was observed highest for ascorbic acid (34.04%) followed by yellow carotenoids (25.70%),oleoresin (21.56%) and total carotenoids (21.41%) while moderate in capsaicin (19.11%) followed by red carotenoids (18.62%) and total extractable color (12.84%).

The highest phenotypic coefficient of variation was observed highest for ascorbic acid (34.36%) followed by yellow carotenoids (26.41%), oleoresin (23.43%), capsaicin

(23.41%), total carotenoids (22.35%), and red carotenoids (20.25%), while moderate in total extractable color (16.81%).

Higher GCV and PCV were recorded for characters like ascorbic acid, oleoresin, yellow carotenoids, total carotenoids indicating higher magnitude of variability for these characters. These findings were similar to Farhad *et al*.,(2008) [8], Sharma *et al.,* (2010), Mahantesh *et al*.,(2013) [14], Patel *et al*.,(2015) [19], Janaki *et al*.,(2016) [10] and Priyanka and Naidu (2016) [21] Singh *et al*. (2017) [26], Zehra *et al*. (2017) [33], Nagaraju *et al*.(2018) [16] and Syed *et al*.(2020) [30] for ascorbic acid : Singh *et al*., (2009) [27], Kumari *et al*., (2010) and Vijaya *et al*., (2014) [32] for oleoresin content: Naresh *et al*., (2013) [17] and Janaki *et al*. (2016) [10] for yellow : Naresh *et al*., (2013) [17] for total carotenoids.

Heritability in broad sense was high for ascorbic acid (98.15%), oleoresin (84.69%), capsaicin (66.67%), red carotenoids (84.61%), yellow carotenoids (94.75%), total carotenoids (91.78%) and while moderate in total extractable color (58.30%) broad sense heritability value.

Genetic advance as per cent mean was high for the characters like ascorbic acid (66.47%), oleoresin (40.88%), capsaicin (32.14%), red carotenoids (35.29%), yellow carotenoids (51.54%), total carotenoids (42.25%) and total extractable color (20.19%).

Heritability estimates along with genetic advance are more useful than heritability value alone in predicting the selection of best individuals. In the present investigation, Higher GCV, PCV and high estimates of heritability with high genetic advance as percent over mean were recorded for characters like ascorbic acid, oleoresin, capsaicin, red carotenoids, yellow carotenoids, total carotenoids indicating higher magnitude of variability for these character. It might be assigned to be under the control of additive genes and phenotypic selection for their improvement could be achieved by simple breeding methods. These findings were similar to Farhad *et al*.,(2008) [8], Sharma *et al.,* (2010), Mahantesh *et al*.,(2013) [14], Patel *et al*.,(2015) [19], Janaki *et al*.,(2016) [10] Priyanka and Naidu (2016) [21], Singh *et al*. (2017) [26], Zehra *et al*. (2017) [33], Nagaraju *et al*.(2018) [16] and Syed *et al*.(2020) [30] for ascorbic acid ; Singh *et al*., (2009) [27], Kumari *et al*., (2010) and Vijaya *et al*., (2014) [32] for oleoresin content: Kumari *et al*., (2010), Kumar *et al*., (2012), Datta and Das (2013), Vijaya *et al*., (2014) [32], Patel *et al*., (2015) [19], Ajith and Manju (2015) [1] and Janaki *et al*.,(2016) [10] for capsaicin content ; Naresh *et al*., (2013) [17] and Janaki *et al*.,(2016) [10] for red and yellow : Naresh *et al*., (2013) [17] for total carotenoids.

The findings indicate that there exists adequate genotypic variation in the genotypes for ascorbic acid, oleoresin content, capsaicin content, red carotenoids, yellow carotenoids, total caroteniods showing high values of PCV, GCV and high heritability coupled with high genetic advance as% of mean suggesting predominance of additive gene action and lower influence of environ- mental factors in the expression of these traits with possibility for improvement through selection.

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