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GENETIC INTERRELATIONSHIP AMONG QUALITATIVE TRAITS AND YIELD IN CHILLI (*Capsicum annuum* L.) GENOTYPES

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ABSTRACT
The present investigation was conducted at Horticultural Research Station, Lam, Guntur, Andhra Pradesh to elucidate the interrelationship among six qualitative traits (ascorbic acid, oleoresin, capsaicin, total color value, red carotenoids, yellow carotenoids) and fruit yield and to estimate the direct and indirect effects of qualitative traits on yield in sixty three genotypes of chilli (*Capsicum annuum* L.). Analysis of variance revealed significant differences among the genotypes for all the traits. Fruit yield plant^{-1} showed significant positive association with ascorbic acid content (r_p and r_g) and oleoresin content (r_g) whereas it was negatively associated with total colour value and yellow carotenoids. The interrelationship among total colour value, red and yellow carotenoids were significant and positive. The path analysis revealed that ascorbic acid, oleoresin and red carotenoids had positive direct effect on yield plant^{-1} whereas capsaicin, total colour value and yellow carotenoids had negative direct effect on yield plant^{-1}.

KEYWORDS: *Capsicum annuum*, capsaicin, correlation, path analysis, carotenoids.

INTRODUCTION

India is one of the leading chilli (*Capsicum annuum* L.) producing countries of the world. Chilli has diverse utilities as a spice, condiment, culinary supplement, medicine, vegetable and ornamental plant. Chilli fruits are known for their flavour, pungency and are considered as one of the richest sources of vitamin C (Bosland and Votava, 4). Capsicinoids and carotenoids are the major chemical constituents of chilli fruits and add commercial value to the crop. The carotenoids contributing to fruit colour act as dietary precursors of vitamin A, and play an important role in the regulation of vision, growth and reproduction. Among carotenoids 'capsanthin, capsorubin and capsanthin 5, 6 – epoxide are responsible for the final red colour (Davies *et al*., 5). Pungency (heat) is an important quality attribute of hot pepper besides colour (carotenoids). The nature of pungency has been established as a mixture of seven closely related alkyl vanillyl amides, collectively referred as "Capsaicinoids" (Torabi, 25). Among capsiacinoids, capsaicin (8-methyl-N-vanillyl-6- enamide) and dihydrocapsaicin account for more than 80% and determine the pungency (Bosland and Votava, 4). The degree of pungency varies widely with the genotypes of five cultivated species (Kumar *et al*., 15) and range from less than 0.05% in the mildly pungent types to as high as 1.3% in the hottest chillies. The 'capsaicin' is an alkaloid present in the placenta of the fruit, which can directly scavenge various free radicals (Reddy and Lokesh, 20; Kogure *et al*., 12; Bhattacharya *et al*., 3). The pharmaceutical application of capsaicinoids is attributed to its antioxidant, anticancer, antiarthritic and analgesic properties (Prasad *et al*., 18). The oleoresins extracted from chilli fruits are extensively used in food and pharmaceutical industry. In view of changing life styles and health concerns quality improvement in crop plants has assumed greatly significance as quality not only improves human health but also adds to farm income. Thus, breeding programmes of late are targeted to improve quality along with yield and tolerance to biotic and abiotic stress.

Knowledge of interrelationship among characters is very important in plant breeding for indirect selection of characters that are not easily measured. For selection, it is essential to know the importance and association of various components and also their association with yield. The correlation coefficient analysis measures the mutual relationship between various characters and determines the component traits on which selection can be relied upon the effect of improvement. Assessing the direct and indirect effects of each component towards yield through path coefficient analysis would help in identifying the component traits contributing to yield. Farhad *et al*. (8), Sharma *et al*. (23), Arup *et al*. (1), Kumar *et al*. (14) were also studied the correlation and path analysis in chilli. But, the availability of data on pungency and colour was important for selection of genotypes from a gene bank for further use in crop improvement. However, data on pungency and carotenoids among the accessions in *Capsicum* gene banks are currently limited (Jarret *et al*., 11). Thus, the major objective of this study was to determine the nature and degree of association among the yield and qualitative characters and their direct and indirect effects on chilli yield. Based on this information an effective selection programme can be proposed for the genetic improvement of the crop.

MATERIALS & METHODS

Sixty three genotypes of chilli (Table 1) were evaluated in a Randomized Block Design with two replications at Horticultural Research Station, Lam, Guntur, and Andhra Pradesh, India. The site of the experiment at Lam is situated on 16^0 28' North latitude and $80^0$34' East longitude at an altitude of 31.5m above mean sea level which falls under humid tropical climate and the soils of the experimental site were rich black cotton soils. The nursery was raised during last week of July and the seedlings were transplanted at a spacing of 75 cm × 30 cm in a row of 4 m length (experimental unit) during first fortnight of September. Each row consisted of 12 plants, of which five competitive plants were selected at random for collecting the fruit samples to estimate qualitative traits *viz.* ascorbic acid (mg $100g^{-1}$), oleoresin (%), capsaicin (%), total color value (ASTA units), red carotenoids (%) and yellow carotenoids (%).

TABLE 1. List of chilli genotypes used in the experiment and their source

Treatment	Accession Number	Source
$T_{1.}$	G-3	HRS, Lam farm, Guntur
$T_{2.}$	G-4	HRS, Lam farm, Guntur
$T_{3.}$	G-5	HRS, Lam farm, Guntur
$T_{4.}$	LCA-206	HRS, Lam farm, Guntur
$T_{5.}$	LCA-235	HRS, Lam farm, Guntur
$T_{6.}$	LCA-305	HRS, Lam farm, Guntur
$T_{7.}$	LCA-315	HRS, Lam farm, Guntur
$T_{8.}$	LCA-353	HRS, Lam farm, Guntur
$T_{9.}$	LCA-357	HRS, Lam farm, Guntur
$T_{10.}$	LCA-424	HRS, Lam farm, Guntur
$T_{11.}$	LCA-436	HRS, Lam farm, Guntur
$T_{12.}$	LCA-620	HRS, Lam farm, Guntur
$T_{13.}$	LCA-625	HRS, Lam farm, Guntur
$T_{14.}$	LCA-702	HRS, Lam farm, Guntur
$T_{15.}$	LCA-703	HRS, Lam farm, Guntur
$T_{16.}$	LCA-704	HRS, Lam farm, Guntur
$T_{17.}$	LCA-705	HRS, Lam farm, Guntur
$T_{18.}$	LCA-706	HRS, Lam farm, Guntur
$T_{19.}$	LCA-707	HRS, Lam farm, Guntur
$T_{20.}$	LCA-708	HRS, Lam farm, Guntur
$T_{21.}$	LCA-709	HRS, Lam farm, Guntur
$T_{22.}$	LCA-710	HRS, Lam farm, Guntur
$T_{23.}$	LCA-711	HRS, Lam farm, Guntur
$T_{24.}$	LCA-712	HRS, Lam farm, Guntur
$T_{25.}$	LCA-713	HRS, Lam farm, Guntur
$T_{26.}$	LCA-714	HRS, Lam farm, Guntur
$T_{27.}$	LCA-715	HRS, Lam farm, Guntur
$T_{28.}$	LCA-716	HRS, Lam farm, Guntur
$T_{29.}$	LCA-718	HRS, Lam farm, Guntur
$T_{30.}$	LCA-720	HRS, Lam farm, Guntur
$T_{31.}$	LCA-722	HRS, Lam farm, Guntur
$T_{32.}$	LCA-724	HRS, Lam farm, Guntur
$T_{33.}$	LCA-726	HRS, Lam farm, Guntur
$T_{34.}$	LCA-728	HRS, Lam farm, Guntur
$T_{35.}$	LCA-730	HRS, Lam farm, Guntur
$T_{36.}$	LCA-732	HRS, Lam farm, Guntur
$T_{37.}$	LCA-734	HRS, Lam farm, Guntur
$T_{38.}$	LCA-736	HRS, Lam farm, Guntur
$T_{39.}$	LCA-738	HRS, Lam farm, Guntur
$T_{40.}$	LCA-740	HRS, Lam farm, Guntur
$T_{41.}$	LCA-742	HRS, Lam farm, Guntur
$T_{42.}$	LCA-744	HRS, Lam farm, Guntur
$T_{43.}$	LCA-746	HRS, Lam farm, Guntur
$T_{44.}$	LCA-748	HRS, Lam farm, Guntur
$T_{45.}$	LCA-750	HRS, Lam farm, Guntur
$T_{46.}$	LCA-752	HRS, Lam farm, Guntur
$T_{47.}$	LCA-754	HRS, Lam farm, Guntur
$T_{48.}$	LCA-756	HRS, Lam farm, Guntur
$T_{49.}$	LCA-758	HRS, Lam farm, Guntur
$T_{50.}$	LCA-760	HRS, Lam farm, Guntur
$T_{51.}$	LCA-762	HRS, Lam farm, Guntur
$T_{52.}$	CA-960	HRS, Lam farm, Guntur
$T_{53.}$	HC-28	HAU, Hisar
$T_{54.}$	KT-I	IARI, Katrain
$T_{55.}$	Aparna	HRS, Lam farm, Guntur

$T_{56.}$	Pandava	Local collection, Guntur
$T_{57.}$	Pant C-1	GBPUA&T, Pantnagar
$T_{58.}$	Phule Jyoti	MPKV, Rahuri
$T_{59.}$	Punjab Gucchedar	PAU, Ludhiana
$T_{60.}$	Pusa Sadabahar	IARI, New Delhi
$T_{61.}$	Super-10	Local collection, Guntur
$T_{62.}$	Warangal Chapata	Local collection, Warangal
$T_{63.}$	LCA-334	HRS, Lam farm, Guntur

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 whereas mature green fruits were used for estimating the Vitamin 'C' content. 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 (22). 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 (19). 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 50-60ºC 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*. (2). 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

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

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.* (21). 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 yellow and red 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, 10).

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.

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

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

Analysis of variance was carried out as per the procedure given by Panse and Sukhatme (17). Phenotypic and genotypic correlations were worked out by using formula suggested by Falconer (7). The direct and indirect effects were computed by using the procedure suggested by Wright (26) and elaborated by Dewey and Lu (6).

RESULTS & DISCUSSION

Analysis of variance (Table 2) revealed significant differences among the genotypes for all the traits indicating presence of significant variability in the genotypes which can be exploited through selection. These findings were in line with earlier reports of Singh and Singh, (24) and Krishnamurthy *et al*. (13).

TABLE 2. Analysis of variance for qualitative characters in chilli (*Capsicum annuum* L.)

Character	Mean sum of squares		
	Replications	Genotypes	Error
Ascorbic acid (mg $100g^{-1}$)	4.371	4326.548**	100.724
Oleoresin (%)	0.944	6.103**	0.572
Capsaicin (%)	0.000007	0.022**	0.0006
Total colour value (ASTA Units)	35.914	1234.578**	32.894
Red carotenoids (%)	0.000096	0.0032**	0.000046
Yellow carotenoids (%)	0.000179*	0.0020**	0.000032

*: Significant at 5 per cent level; **: Significant at 1 per cent level

The estimates of phenotypic and genotypic correlation coefficient (Table 3) revealed that the genotypic correlations were higher than the corresponding phenotypic correlations for most of the characters indicating high heritability of the traits under study as suggested by earlier reports of Farhad *et al*. (8) and Kumar *et al*. (14). Interrelationship among fruit yield $plant^{-1}$ and ascorbic acid was significant and positive at both phenotypic and genotypic levels. The genotypic association of oleoresin content was significant and positive with yield $plant^{-1}$. These findings suggested that selection for yield $plant^{-1}$ based on ascorbic acid content and oleoresin content is beneficial for further crop improvement programme. These results are in consonance with earlier observations of Arup *et al.* (1) and Kumar *et al*. (14). Yield $plant^{-1}$ showed significant, negative association with total colour value and yellow carotenoids at both phenotypic and genotypic levels. The inter relationship among total colour value, red and yellow carotenoids were significant and positive indicating that simultaneous selection of these traits is possible and also suggested that total colour value increases significantly with increase in red and yellow carotenoids. These findings are supported by the observations of Naresh *et al.* (16). Ascorbic acid content had significant and negative association with total colour value, red and yellow carotenoids at both phenotypic and genotypic levels indicating a significant decrease in ascorbic acid content leads to increase in total, red and yellow carotenoids and *vice-versa.* The inter relationship among oleoresin and capsaicin content was positive revealing that an increase in one trait leads to increase in another trait. These findings are supported by earlier reports of Gupta *et al.* (9). Phenotypic and genotypic association of total, red and yellow carotenoids with oleoresin content was non-significant and negative whereas association of carotenoids with capsaicin content was non-significant and positive.

TABLE 3. Phenotypic (P) and genotypic (G) correlation coefficients among six qualitative characters and yield per plant in chilli (*Capsicum annuum* L.)

Character		Ascorbic acid	Oleoresin	Capsaicin	Total colour value	Red carotenoids	Yellow carotenoids	Yield per plant
Ascorbic acid	P	**1.000**	0.0164	-0.0272	-0.2322**	-0.2307**	-0.2115*	**0.2495****
	G	**1.000**	0.0435	-0.0256	-0.2441**	-0.2431**	-0.2181*	**0.2946****
Oleoresin	P		**1.000**	0.1274	-0.0700	-0.0599	-0.1407	0.1516
	G		**1.000**	0.1477	-0.0801	-0.0554	-0.1657	**0.2426****
Capsaicin	P			**1.000**	0.0845	0.0546	0.0616	-0.1163
	G			**1.000**	0.0898	0.0508	0.0698	-0.1426
Total colour value	P				**1.000**	0.7881**	0.6859**	**-0.2108***
	G				**1.000**	0.8162**	0.7178**	**-0.2709****
Red carotenoids	P					**1.000**	0.5527**	-0.0784
	G					**1.000**	0.5678**	-0.0794
Yellow carotenoids	P						**1.000**	**-0.1780***
	G						**1.000**	**-0.2047***
Yield per plant	P							**1.000**
	G							**1.000**

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

The path analysis (Table 4) revealed that ascorbic acid, oleoresin and red carotenoids at both phenotypic and genotypic levels, yellow carotenoids at genotypic level had positive direct effect on yield plant^{-1} indicating that direct selection based on these traits may be helpful in evolving high yielding varieties of chilli whereas capsaicin, total colour value and yellow carotenoids (phenotypic level) had negative direct effect on yield plant^{-1}. These findings are in agreement with reports of Farhad *et al*. (8), Sharma *et al*. (23), Arup *et al*. (1) and Kumar *et al*. (14) who also reported positive direct effect of ascorbic acid content and negative direct effect capsaicin content on chilli yield. Ascorbic acid at both levels, red carotenoids at phenotypic level and oleoresin at genotypic level had moderate positive direct effect while oleoresin at phenotypic level had low positive direct effect on yield. Similar results were obtained by earlier observations of Arup *et al*. (1). Red carotenoids at genotypic level had high positive direct effect whereas total color value had high negative direct effect on yield. The positive indirect effects through capsaicin, total colour value and yellow carotenoids were responsible for high positive direct effect of red carotenoids on yield.

TABLE 4. Phenotypic (P) and genotypic (G) path analysis showing direct (diagonal) and indirect effects of qualitative characters on yield per plant in chilli (C*apsicum annuum* L.)

Character		Ascorbic acid	Oleoresin	Capsaicin	Total colour value	Red carotenoids	Yellow carotenoids	Correlation 'r' with Yield per Plant
Ascorbic acid	P	**0.2230**	0.0037	-0.0061	-0.0518	-0.0514	-0.0472	**0.2495****
	G	**0.2604**	0.0113	-0.0067	-0.0636	-0.0633	-0.0568	**0.2946****
Oleoresin	P	0.0025	**0.1523**	0.0194	-0.0107	-0.0091	-0.0214	**0.1516**
	G	0.0106	**0.2424**	0.0358	-0.0194	-0.0134	-0.0402	**0.2426****
Capsaicin	P	0.0031	-0.0146	**-0.1147**	-0.0097	-0.0063	-0.0071	**-0.1163**
	G	0.0038	-0.0216	**-0.1464**	-0.0132	-0.0074	-0.0102	**-0.1426**
Total colour value	P	0.0764	0.023	-0.0278	**-0.3290**	-0.2593	-0.2257	**-0.2108***
	G	0.1444	0.0474	-0.0531	**-0.5917**	-0.4829	-0.4247	**-0.2709****
Red carotenoids	P	-0.0596	-0.0155	0.0141	0.2038	**0.2586**	0.1429	**-0.0784**
	G	-0.1083	-0.0247	0.0226	0.3636	**0.4455**	0.2529	**-0.0794**
Yellow carotenoids	P	0.0042	0.0028	-0.0012	-0.0135	-0.0109	**-0.0196**	**-0.1780***
	G	-0.0162	-0.0123	0.0052	0.0533	0.0422	**0.0743**	**-0.2047***

'r' – Correlation coefficient , *: Significant at 5 per cent level; **: Significant at 1 per cent level

Yield is a complex character, contributed by many traits. In the present study, among the six traits, ascorbic acid content had positive significant correlation, moderate positive direct effect at both phenotypic and genotypic level on yield plant^{-1} indicating that selection could be effective through this trait for yield improvement. Oleoresin (moderate) and red carotenoids (high) had positive direct effect at genotypic level indicated that direct selection for yield plant^{-1} through these traits also will be effective.

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