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Genetic Association Analysis for Qualitative Traits in Paprika (*Capsicum annuum* L.) Genotypes

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The present study on inter relationship among seven qualitative traits (ascorbic acid, oleoresin content, capsaicin content, total extractable colour, red carotenoids, yellow carotenoids, total carotenoids) and fruit yield and the direct and indirect effects of qualitative traits on yield of forty four genotypes of paprika (*Capsicum annuum* L.), revealed that the analysis of variance exhibited significant differences among the genotypes for all the traits. Dry fruit yield per plant showed significant negative association with ascorbic acid content (r_p and r_g) and total carotenoids (r_p and r_g) at both phenotypic and genotypic level and showed significant and negative with red carotenoids and yellow carotenoids at genotypic level only. The path analysis revealed that ascorbic acid, oleoresin content, capsaicin content and total extractable colour had negative direct effect on yield per plant.

Introduction

Paprika (*Capsicum annuum* L.), 2n = 24 is one of the important commercial vegetable as well as spice crops grown all over the world. Paprika, a form of chilli is mainly valued for its high colour, low or no pungency and oleoresins. 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 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 color act as dietary precursors of vitamin A and among carotenoids 'capsanthin, capsorubin and capsanthin 5, 6-epoxide are responsible for the

final red color. The nature of pungency has been established as a mixture of seven closely related alkyl vanillylamides, collectively referred as "Capsaicinoids". Among capsiacinoids, capsaicin (8-methyl-N-vanillyl-6-enamide) and dihydrocapsaicin accounts for more than 80% and determine the pungency (Bosland and Votava, 2000). The degree of pungency varies widely with the genotypes (Kumar *et al.,* 2006). The 'capsaicin' is an alkaloid present in the placenta of the fruit, which can directly scavenge various free radicals (Reddy and Lokesh, 1992; Kogure *et al.,* 2002; Bhattacharya *et al.,* 2010) and has wide applications in the food, medicine and pharmaceutical industries. Chilli has also acquired a great importance because of the presence of 'oleoresin', which permits better color distribution and flavor in foods. The demand for paprika oleoresin as a colouring agent has increased in international market especially in Europe and USA due to ban on artificial colouring substances (Joshi *et al.,* 1995). There is considerable demand for paprika powder in the western countries. There is a great demand for the natural colour from paprika fruits and is used in processed foods in place of synthetic colours.

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.,* (2008), Gupta *et al.,* (2009) Sharma *et al.,* (2010), Arup *et al.,* (2011), Kumar *et al.,* (2012), Vikarm *et al.,* (2014), Vijaya *et al.,* (2014), Shiva *et al.,* (2015), Rinchan *et al.,* (2015) and Janaki *et al.,* (2016) 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.,* 2003). 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 paprikai yield. Based on this information an effective selection programme can be proposed for the genetic improvement of the crop.

Materials and Methods

Forty four genotypes of paprika (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 160 28' North latitude and 80034' 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 first week of August 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 content (%), capsaicin content (%), total extractable colour (ASTA units), red carotenoids (%), yellow carotenoids (%) and total carotenoids (%). The red ripen 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.

Ascorbic acid (mg/100g)

Ascorbic acid content of mature green fruits was estimated by volumetric method (Sadasivam and Balasubramanian, 1987). Dye solution was prepared by dissolving 42 mg of sodium bicarbonate in distilled water taken into 200 ml volumetric flask, to which 52 mg of 2-6 dichlorophenol indophenol was added and the volume was made up to 200 ml 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 per ml.

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. 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

Ascorbic acid (mg/100 g) = (0.5 mg ÷ V_1) × (V_2 ÷ 5ml) × (100ml ÷ Wt. of the sample) × 100

Oleoresin content (%)

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_1 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.

Oleoresin content (%) = ((W_2–W_1) ÷ (Weight of sample)) × 100

Capsaicin content (SHU)

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) and working standard solution prepared by diluting the 10ml of the stock standard to 50ml with 0.4% NaOH solution (200µg/ ml)) was taken into new test tubes and proceeded as mentioned above. Per cent capsaicin calculated using the formula mentioned below

Capsaicin content (%) = (µg capsaicin × 100 × 100) ÷ (1000 × 1000 × 1 × 0.5)

Where, 1 % = 1,60,000 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 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,

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 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 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.

CR (µg/ml) = (A508×2144.0) — (A472× 403.0) ÷ 270.9

CY (µg/ml) = (A472× 1724.3) — (A508 × 2450.1) ÷ 270.9

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

Analysis of variance was carried out as per the procedure given by Panse and Sukhatme (1985). Phenotypic and genotypic correlations were worked out by using formula suggested by Falconer (1964). The direct and indirect effects were computed by using the procedure suggested by Wright (1921) and elaborated by Dewey and Lu (1959).

Table.1 List of paprika genotypes used in the experiment and their source

Treatment	Accession Number	Treatment	Accession Number	Source
T_1	LCA 445	T_{23}	LCA 465	HRS, Lam farm, Guntur
T_2	LCA 447	T_{24}	LCA 475	HRS, Lam farm, Guntur
T_3	LCA 439	T_{25}	LCA 488	HRS, Lam farm, Guntur
T_4	LCA 442	T_{26}	LCA 499	HRS, Lam farm, Guntur
T_5	LCA 430	T_{27}	LCA 506	HRS, Lam farm, Guntur
T_6	LCA 457	T_{28}	LCA 503	HRS, Lam farm, Guntur
T_7	LCA 443	T_{29}	LCA 490	HRS, Lam farm, Guntur
T_8	LCA 437	T_{30}	LCA 501	HRS, Lam farm, Guntur
T_9	LCA 453	T_{31}	LCA 504	HRS, Lam farm, Guntur
T_{10}	LCA 450	T_{32}	LCA 510	HRS, Lam farm, Guntur
T_{11}	LCA 441	T_{33}	LCA 510	HRS, Lam farm, Guntur
T_{12}	LCA 425	T_{34}	LCA 511	HRS, Lam farm, Guntur
T_{13}	LCA 440	T_{35}	LCA 512	HRS, Lam farm, Guntur
T_{14}	LCA 446	T_{36}	LCA 513	HRS, Lam farm, Guntur
T_{15}	LCA 470	T_{37}	Warangal chappatta single patti	HRS, Lam farm, Guntur
T_{16}	LCA 436	T_{38}	Warangal chappatta double patti	HRS, Lam farm, Guntur
T_{17}	LCA 466	T_{39}	Byadagikaddi	HRS, Lam farm, Guntur
T_{18}	LCA 472	T_{40}	Byadagidabbi	HRS, Lam farm, Guntur
T_{19}	LCA 476	T_{41}	Kt-1	HRS, Lam farm, Guntur
T_{20}	LCA 480	T_{42}	Jangareddygudem local	HRS, Lam farm, Guntur
T_{21}	LCA 482	T_{43}	LCA 436	HRS, Lam farm, Guntur
T_{22}	LCA 498	T_{44}	LCA 424	HRS, Lam farm, Guntur

Table.2 Analysis of variance for qualitative characters in paprika (*Capsicum annuum* L.)

Character	Mean sum of squares		
	Replications	Genotypes	Error
Ascorbic acid (mg/100g)	145.89	4674.56**	151.61
Oleoresin content (%)	0.53	16.66**	0.66
Capsaicin content (SHU)	1784670.75	36198080.00**	157919.3
Total extractable colour (ASTA units)	22.99	2376.67**	115.58
Red carotenoids (%)	0.0005	0.02**	0.0007
Yellow carotenoids (%)	0.00004	0.015**	0.0009
Total carotenoids (%)	0.0003	0.03**	0.0019
Dry fruit yield per plant (g)	0.1	2316.47**	178.91

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

Table.3 Phenotypic (above diagonal) and genotypic (below diagonal) correlation coefficients among qualitative characters and yield per plant in paprika (*Capsicum annuum* L.)

Character	Ascorbic acid (mg /100g)	Oleoresin content (%)	Capsaicin content (SHU)	Total extractable colour (ASTA Units)	Red carotenoids (%)	Yellow carotenoids (%)	Total carotenoids (%)	Dry fruit yield per plant (g)
Ascorbic acid (mg /100g)	**1.0000**	0.0505	-0.0575	-0.3234**	0.2261*	0.0231	0.2065	**-0.3482****
Oleoresin content (%)	0.0361	**1.0000**	-0.1764	0.0338	-0.2308*	0.0591	-0.1599	**-0.1793**
Capsaicin content (SHU)	-0.0705	-0.1913	**1.0000**	-0.1621	-0.0039	-0.1024	-0.0682	**-0.1720**
Total extractable colour (ASTA Units)	-0.3656**	0.0442	-0.2010	**1.0000**	0.0005	0.0139	0.0105	**-0.0752**
Red carotenoids (%)	0.2227*	-0.2477*	-0.0093	-0.0120	**1.0000**	-0.1023	0.7753**	**-0.2066**
Yellow carotenoids (%)	0.0254	0.0368	-0.1257	-0.0064	-0.1221	**1.0000**	0.5484**	**-0.1879**
Total carotenoids (%)	0.2091	-0.1950	-0.0866	-0.0110	0.7835**	0.5211**	**1.0000**	**-0.2956****
Dry fruit yield per plant (g)	**-0.3953****	**-0.2040**	**-0.1800**	**-0.1101**	**-0.2383***	**-0.2235***	**-0.3469****	**1.0000**

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

Table.4 Phenotypic path analysis showing direct (diagonal) and indirect effects of qualitative characters on yield per plant in paprika (C*apsicum annuum* L.)

Character	Ascorbic acid(mg /100g)	Oleoresin content (%)	Capsaicin content (SHU)	Total extractable colour (ASTA Units)	Red carotenoids (%)	Yellow carotenoids (%)	Total carotenoids (%)
Ascorbic acid (mg /100g)	**-0.3660** **-0.5532**	-0.0185 -0.0200	0.0211 0.0390	0.1184 0.2023	-0.0827 -0.1232	-0.0085 -0.0140	-0.0756 -0.1157
Oleoresin content (%)	-0.0129 -0.0069	**-0.2549** **-0.1920**	0.0450 0.0367	-0.0086 -0.0085	0.0588 0.0476	-0.0151 -0.0071	0.0408 0.0374
Capsaicin content (SHU)	0.0171 0.0271	0.0523 0.0735	**-0.2966** **-0.3843**	0.0481 0.0772	0.0012 0.0036	0.0304 0.0483	0.0202 0.0333
Total extractable colour (ASTA Units)	0.0727 0.1622	-0.0076 -0.0196	0.0365 0.0891	**-0.2249** **-0.4435**	-0.0001 0.0053	-0.0031 0.0029	-0.0024 0.0049
Red carotenoids (%)	0.7472 -3.3473	-0.7629 3.7233	-0.0129 0.1400	0.0018 0.1810	**3.3048** **-15.0307**	-0.3382 1.8347	2.5622 -11.7773
Yellow carotenoids (%)	0.0564 -0.2811	0.1440 -0.4074	-0.2497 1.3916	0.0338 0.0712	-0.2495 1.3514	**2.4377** **-11.0709**	1.3369 -5.7686
Total carotenoids (%)	-0.8627 3.6040	0.6682 -3.3618	0.2847 -1.4922	-0.0437 -0.1897	-3.2391 13.5077	-2.2911 8.9826	**-4.1778** **17.2391**
'r' with dry fruit yield per plant (g)	**-0.3482**** **-0.3953****	**-0.1793** **-0.2040**	**-0.1720** **-0.1800**	**-0.0752** **-0.1101**	**-0.2066** **-0.2383***	**-0.1879** **-0.2235***	**-0.2956**** **-0.3469****

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

Results and 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, (2011) and Krishnamurthy *et al.,* (2013).

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.,* (2008), Kumari *et al., (*2011), Kumar *et al.,* (2012), Vikarm *et al., (*2014), Shiva *et al., (*2015) and Janaki *et al., (*2016). Interrelationship among dry fruit yield plant-1 and ascorbic acid, total carotenoids was significant and negative at both phenotypic and genotypic levels. The genotypic association of red and yellow carotenoids was significant and negative with yield plant-1. These findings suggested that selection for yield plant-1 based on ascorbic acid content, total, red and yellow carotenoids is not beneficial for further crop improvement programme. These results are in accordance with previous reports of Farhad *et al.,* (2008).

The inter relationship among red, yellow and total carotenoids were significant and positive indicating that simultaneous selection of these traits is possible and also suggested that red and yellow carotenoids increases significantly with increase in total carotenoids. These findings are supported by the observations of Naresh *et al.,* (2013). Ascorbic acid content had significant and negative association with total extractable colour at both phenotypic and genotypic levels indicating a significant decrease in ascorbic acid content leads to increase in total extractable colour and *vice-versa.* Oleoresin content showed non-significant and positive association with ascorbic acid (0.0505 and 0.0361), total extractable colour (0.0338 and 0.0442) and yellow carotenoids (0.0591 and 0.0368) at both phenotypic and genotypic levels. These results are in line with earlier findings of Gupta *et al.,* (2009). Phenotypic and genotypic association of total extractable colour, red, yellow and total carotenoids with capsaicin content was non- significant and negative.

The path analysis (Table 4) revealed that ascorbic acid, oleoresin content, capsaicin content and total extractable colour had negative direct effect on yield plant-1 at both phenotypic and genotypic levels indicating that direct selection based on these traits may be not be helpful in evolving high yielding varieties of paprika. These findings are in agreement with reports of Singh *et al.,* (2009), Arup *et al.,* (2011), Vijaya *et al.,* (2014), Rinchan *et al.,* (2015) and Janaki *et al., (*2016). Red and yellow caroteniods at phenotypic level had high positive direct effect and total carotenoids at genotypic level had high positive direct effect whereas total extractble colour had moderate negative direct effect on yield.

Yield is a complex character, contributed by many traits. In the present study, among the seven traits, ascorbic acid content, oleoresin content, capsaicin content and total extractable colour had negative direct effect at both phenotypic and genotypic level on yield plant-1 indicating that selection could be not effective through this trait for yield improvement. Red and yellow caroteniods at phenotypic level had high positive direct effect and total carotenoids at genotypic level had high positive direct effect indicated that direct selection for yield plant-1 through these traits will be effective.

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