Inheritance of Mature Fruit Colour in Capsicum

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An experiment was conducted at Indian Agricultural Research Institute, Regional Station, Katrain (HP), India during 2001-2008 to study the inheritance of mature fruit colour in capsicum (*Capsicum annuum* var. *grossum*). Two inbreds each of 4 different mature fruit colours (red, yellow, orange and yellow orange) were crossed in complete diallele fashion to determine the dominance relationship. Red colour was found dominant over others whereas yellow-orange was recessive to all others. From analysis of a series of F_2 generations and test cross of all possible F_1 progeny it was established that when the *c* locus is silent (*i.e*., homozygous recessive) the *y* locus controls the appearance of orange colour (*Y_cc*) vs. yellow orange (*yycc*). On the other hand when the *y* locus is homozygous recessive, the *c* locus controls the expression of yellow (*yyC_*) vs. yellow-orange (*yycc*) colour. Dominant genes at both the loci resulted in red colour while recessive *yycc* led to appearance of yellow-orange colour in the mature fruit of capsicum. No linkage between the loci was found and cytoplasmic inheritance of this trait was absent.

Key Words: Capsicum, Fruit colour, Carotenoids

Introduction

Fruit colour of sweet pepper has attained commercial importance. Of late there has been increasing demand of coloured capsicum resulting in a pressure on vegetable breeding institutions and seed companies to launch such products in market. At vegetable stage varied colours, *viz*., green, yellow, cream, white, chocolate, purple etc. are known whereas at mature stage a great assortment of shades of red, orange, yellow and yellow-orange have been observed. The colour of ripe sweet pepper fruit is due to presence of various pigments. In the light of above facts, it has become imperative for breeders to obtain insight into the genetics and inheritance of colour. Lang *et al.* (2004) found on TLC (thin layer chromatography) of red and orange fruits that capsanthin-one of the major carotenoids was absent in orange fruits. Further observation through PCR (polymerase chain reaction) polymorphism of *CCS* (capsanthin-capsorubin synthase) gene and TLC pattern of carotenoid composition co-segregated completely in the F_2 population suggesting that the *CCS* gene, responsible for capsanthin synthesis, controls the orange and red colour of capsicum by changing the carotenoid composition. The orange colour is caused by lack of expression of *CCS* gene. There have been many reports which throw only limited light on the matter. Lefebvre *et al.* (1998), Popovsky and Paran (2000) reported the *CCS* gene to control the segregation of yellow and red colour. Thorup *et al.* (2000) have localized the structural genes from carotenoid pathway on F_2 genetic map of capsicum.

Materials and Methods

Studies were carried out at Indian Agricultural Research Institute, Regional Station, Katrain during 2001 to 2008 with a view to develop superior hybrids of capsicum with different mature fruit colours. Lines with different fruit colours were obtained from various sources and selfed and selected for desired colours repeatedly for five years. Only single plant progenies were brought forward each year. The homozygosity of these lines for character under study was confirmed before effecting crossing by growing large populations of their selfed single-plant progenies to observe any variation. In order to work out the inheritance of mature fruit colour, the purelines used are presented in Table 1.

These lines were crossed in complete diallel fashion and the first filial generation (F_1) was grown out to observe the dominance relationship of colours. A few plants of each F_1 were selfed to produce the F_2 seeds. The F_2 generations were analyzed for segregation of colour in the mature fruit. The F_1 of each cross was also test crossed for confirming the outcomes of the F_2 analysis. In the test crosses, the homozygous recessive line, *i.e*., light orange D-20 was taken as test cross parent. For avoiding confusion leading to wrong conclusions the experiments were conducted in parts and progenies of different crosses were grown over many years so that there was enough population size of each progeny to make observations. The goodness of fit to Mendelian ratios was calculated by the chi-square method.

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Table 1. Material used in the experiment

Line/inbred	Mature fruit colour	Source
Yolo Wonder	Red	IARI, RS, Katrain
HC-201	Red	DRDO, Pithoragarh, Uttarakhand
Golden Pepper-3	Orange	A line purified from a collection obtained from Indo-Israel project, IARI, New Delhi in 2000
Goldmine (AC-49)	Orange	A line obtained from GBPUAT, Pantnagar in 1999, maintained since then at IARI, RS, Katrain
Yellow Capsicum	Yellow	EC113372
DP-2	Yellow	Pure-breeding, yellow, non-pungent Paprika line obtained from segregation of selfed progeny of DARL-204 developed from DRDO, Pithoragarh, Uttarakhand, maintained at IARI, RS, Katrain
Golden Lion	Light orange (yellow-orange)	EC203600, maintained as a germplasm line at IARI, RS, Katrain
D-20	Light orange (yellow-orange)	EC203602

This investigation aims to provide answers to following questions: (i) Whether the carotenoid pigmentation in mature fruit in capsicum is controlled by a gene with 2 alleles, a gene with more than two alleles or more than one genes? (ii) What is the dominance relationship between the colours? (iii) Does any linkage exist between the genes governing this trait? (iv) Is this character controlled by cytoplasmic genes? (v) What are the contrasting colours produced by first locus in case the 2nd locus is homozygous recessive and *vice-versa*?

Table 2. F_1 progenies of crosses between purelines with different fruit colours

	Red	Orange	Yellow	Yellow-Orange
Red	Red	Red	Red	Red
Orange	Red	Orange	Red	Orange
Yellow	Red	Red	Yellow	Yellow
Yellow-Orange	Red	Orange	Yellow	Yellow-Orange

Results

The observations were recorded on plants grown in field at fruit maturity stage. From Table 3 it is clear that red is dominant over all other colours and yellow-orange is recessive to all others. The complete diallele among the four colour types did not show any difference in reciprocal crosses, thus ruling out any possibility of maternal (or cytoplasmic) control of this character. A cross between red and yellow-orange segregates in a dihybrid ratio of 9:3:3:1 in the F_2 generation (Table 3). The red phenotype appeared in a proportion of 9/16 and the yellow-orange phenotype appeared in a proportion of 1/16. Therefore, it can be inferred that a pure breeding line with red mature fruit colour contain the homozygous dominant alleles on both the loci and the yellow-orange phenotype has homozygous recessive alleles on both the loci. The F_2 progeny of the cross between the purelines with red and yellow orange fruit colours segregated in dihybrid ratio of 9:3:3:1 which is possible when there are at least two loci

Table 3. Segregation pattern of progeny of different crosses in second filial generation (F_2)

	Genotype of F_1 hybrid	Total plants	F_2 dihybrid ratio				Ratio	χ^2
			Red	Orange	Yellow	Yellow-Orange		
Red x Orange (*YYCC* x *YYcc*)	*YYCc*	37	28	9			3:1	0.009
Red x Yellow (*YYCC* x *yyCC*)	*YyCC*	42	32		10		3:1	0.03
Red x Yellow-Orange (*YYCC* x *yycc*)	*YyCc*	62	33	12	13	4	9:3:3:1	0.28
Orange x Yellow (*YYcc* x *yyCC*)	*YyCc*	66	35	14	12	5	9:3:3:1	0.53
Orange x Yellow-Orange (*YYcc* x *yycc*)	*Yycc*	26		19		7	3:1	0.05
Yellow x Yellow-Orange (*yyCC* x *yycc*)	*yyCc*	31			22	9	3:1	0.27

controlling the carotenoid differences. This was proved by the observations presented in Table 3. As may be expected, the F_2 progeny of the two intermediate phenotypes (*i.e.*, yellow and orange) also exhibited segregation in the same ratio. From Table 2, it clearly appears that the F_1 progeny of the cross between yellow and orange purelines are red and the F_2 generation shows segregation as stated above (Table 3) suggesting that these two colours are controlled by dominant genes on either of the loci concerned and exhibit a complementary action for producing red phenotype. The second filial generation (F_2) of the cross between red and orange segregates in a ratio of 3 red: 1 orange, similarly the F_2 of the cross between red and yellow segregates in 3:1. This indicates that these two colours (orange and yellow) behave as monogenic recessive to red. In the same manner, when yellow is crossed with yellow-orange the F_2 generation of this cross shows a ratio of 3 yellow:1 yellow-orange. A similar segregation is observed when orange pureline is crossed with yellow-orange.

The test cross progenies, *i.e.*, between the F_1 generation and the recessive parental pureline (yellow-orange fruit colour) were grown and fruit colours were recorded. The test cross progeny of the cross red X orange and red X yellow segregated in a ratio of 1:1 (Table 4, cross serial nos. 1 and 2). Similarly, the test cross of the F_1s of yellow X yellow-orange and orange X yellow-orange showed a segregation of 1:1 (Table 4, cross serial nos. 5 and 6). In case of red X yellow-orange and yellow X orange, the segregation of the test cross progeny was found to fit satisfactorily in the ratio of 1:1:1:1 (Table 4, cross serial nos. 3 and 4).

Discussion

Colours, besides appealing the consumer preference, have effect on other characters. In onion, coloured bulbs have been reported to be highly resistant to onion smudge organism, *Colletotrichum circinans* where as white bulbs were susceptible (Clarke *et al.*, 1944). In case of capsicum also, carotenoid differences have been shown to be affecting nutritional properties.

Lang *et al.* (2004) observed a Mendelian ratio of 3:1 (red:orange) suggesting dominance of red over orange. Since there are four alternate colours, it suggests that the character is not controlled by one locus. The F_2 progeny of the cross between the lines with red (homozygous recessive at both loci) and yellow-orange (homozygous recessive at both loci) fruit colours segregated in dihybrid ratio of 9:3:3:1 which can be accounted for by assuming that there are at least two loci.

Since in many studies the locus or the gene has been referred to as capsanthin-capsorubin synthase gene (Lang *et al.*, 2004), this gene is being designated *C-c*. The other locus controlling the expression of yellow colour in mature fruits has been repeatedly named as *Y-y* locus, therefore, for the purpose of analysis we term this locus *y*. Thus the pureline with red fruit colour has *YYCC* constitution and the one with yellow orange has *yycc* constitution. The progeny of cross between red and yellow-orange phenotypes was test crossed with the parent homozygous recessive at both the loci, *i.e.*, yellow-orange.

Since there is no distortion in the normal Mendelian dihybrid ratio of 9:3:3:1, it is clear that the genes controlling the carotenoid differences are not linked. Yellow and orange

Table 4. Segregation pattern of progeny of different crosses in second filial generation (F_2)

S.No.	Cross	F_1	Total plants	Test cross progeny				Ratio	χ^2
				Red	Orange	Yellow	Yellow-Orange		
1.	Red x Orange (*YYCC* x *YYcc*)	Red *YYCc*	81	42	39			1:1	0.11
2.	Red x Yellow (*YYCC* x *yyCC*)	Red *YyCC*	47	22		25		1:1	0.19
3.	Red x Yellow-Orange (*YYCC* x *yycc*)	Red *YyCc*	37	9	8	10	10	1:1:1:1	0.3
4.	Orange x Yellow (*YYcc* x *yyCC*)	Red *YyCc*	47	12	11	11	13	1:1:1:1	0.23
5.	Orange x Yellow-Orange (*YYcc* x *yycc*)	Yellow *Yycc*	43		23		20	1:1	0.21
6.	Yellow x Yellow-Orange (*yyCC* x *yycc*)	Orange *yyCc*	41			19	22	1:1	0.22

genotypes are the intermediate ones with *yyC_*and *Y_cc* constitution, respectively. The pure breeding yellow and orange genotypes would be *yyCC* and *YYcc*. The red colour of mature fruit appears when the dominant allele is present on both the loci (*Y_C_*). Orange and yellow colours are recessive to red. From the results presented in Table 3 is apparent that the F_1s of red x orange and red x yellow are red while the selfed progeny of these F_1s produced a ratio of 3 red: 1 orange and 3 red: 1 yellow, respectively. Consequently it can be hypothesized that in these crosses, orange and yellow behave as simple monogenic recessive characters with respect to red. The relation between orange and yellow colour can be easily understood from the observations. When purebred orange and yellow lines were crossed, the progeny was red (Table 2). This F_1 progeny when selfed resulted in a population segregating in a ratio of 9:3:3:1 (red: orange: yellow: yellow-orange) (Table 4). Therefore, it can be postulated that these two loci are complementary and red colour appears when dominant alleles are present on both the loci (*C_Y_*). The cross between orange and yellow orange resulted in an orange hybrid which on selfing showed a segregation fitting well into the ratio of 3:1 (orange: yellow-orange). Similarly, the cross between yellow and yellow-orange gave a yellow hybrid in F_1 which segregated almost clearly in a ratio of 3:1 (yellow: yellow-orange). It follows from here that yellow-orange appears when both the loci are homozygous recessive and when the *c* locus is silent (*i.e.*, homozygous recessive) the *y* locus controls the appearance of orange colour (*Y_cc*) vs. yellow orange (*yycc*). On the other hand, when the *y* locus is homozygous recessive, the c locus controls the expression of yellow (*yyC_*) vs. yellow-orange (*yycc*) colour. The segregation pattern of the test cross progenies (Table 4) confirmed that there are two loci involved; there is no linkage between the genes and their two alternative alleles on both the loci.

A similar two gene system (*CCRR*) has been reported by Clarke *et al.* (1994) controlling the 3 colour scheme in onions. They also hypothesized an additional 3rd locus I responsible for appearance of intermediate red colour between red and white which modifies the appearance of red, white and yellow in clear classes. Tomes *et al.* (1953), too, has demonstrated two genes (*RRTT*) controlling the carotenoid pigment system in tomato. This system bears more resemblance to the results obtained from the present investigations. According to their report, the red colour is controlled by *R_T_*, yellow by *rrT_* and tangerine, *i.e.*, orange by *R_tt*. They also found a third gene *b* responsible for appearance of beta-orange colour due to synthesis of beta-carotene. Taking this study further Jenkins and McKinney (1953) on crossing yellow (*rrTT*) and tangerine (*RRtt*) tomatoes found that the F_1 hybrid was red and the F_2 segregated 9 red: 3 yellow: 3 tangerine: 1 yellow-tangerine.

In some cases the yellow-orange (light orange) may be enough deep coloured to be mistakenly classified as orange due to environmental effects or various loci affecting the intensity of colour expression or variously the amount of pigments. This may modify the observed ratio to 9 red: 3 yellow: 4 orange. MacArthur has reported this ratio in tomato first in 1934.

Although brown (or chocolate coloured) pepper fruits are also found, it is not due to any separate pigment but is a result of the combination of the normal red pigments and undecomposed chlorophyll. The retention of chlorophyll is governed by *cl* gene (Smith, 1950). While observing the effect on phenotypes of red, yellow, orange and light orange types may be of some interest, the *cl* gene has nothing to do with carotenoid pigment system of capsicum.

Kormos and Kormos (1960) reported that there were three independent genes, *viz.*, *y*, *c*-1 and *c*-2. According to their analysis *c*-1 and *c*-2 in recessive expression reduced the pigmentation of *y*. However, this report has been questioned severely by Hernandez and Smith (1985) on account of reporting only 6 phenotypes instead of normal 8 that are expected from interaction of 3 pairs of genes and use of only 2 gene symbols to identify each of the 6 phenotypes. Moreover, Kormos (1954) has reported yellow colour as recessive to orange which has neither been found anywhere else in literature nor been reported by any of the subsequent workers. In the present study, the two traits were found complementary. Report of Hernandez and Smith (1985) describes 3 pairs of genes corresponding with 8 phenotypic classes in the F_2 generation of a cross between white and red. A careful perusal of their findings indicates that they have found *y* locus governing the expression of red vs. yellow colour and *c*-2 locus determining the segregation of red and orange fruit colour (corresponding to the *y* locus and *c* locus of the present investigation, respectively). This report is in close conformity with the findings of the present investigation. A third locus mentioned by Hernandez and Smith (1985), *viz.*, *c*-1 is a modifier of expression of *y* and *c*-2 loci. The *c*-1 locus in recessive configuration reduces the pigmentation of *y* and *c*-2 loci. Similar observation about the *c*-1 locus was made by Thorup *et al.* (2000).

According to a report by Lefebvre *et al.* (1998), the candidate gene *y* for yellow pigmentation is present on chromosome 6 whereas according to a study by Romer *et al*. (1993) *c*-locus is located on chromosome 4. Huh *et al.* (2001) found that the polymorphism of *Psy* (phytoene synthase) gene co-segregated with the yellow and red fruit colour where as Lang *et al.* (2004) reported similar co-segregation of *CCS* gene with red and orange fruit colour. It corroborates the suggestion of Lang *et al.* (2004) that there are a series of genes responsible for synthesis of different pigments which finally determine the colour of mature fruit. The vast range found among the paprika genotypes by several investigations (Anu *et al.,* 2002) stand testimony to the fact that there must be several other factors including QTLs, modifier genes and environment which affect the intensity of colour development in ripened fruits of capsicum.

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