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A Casty Study :

Crop improvement and management strategies in paprika

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The condiment paprika is a variant of *Capsicum.* The widely used word 'chilli' is referring usually to the hot variant of capsicum. "Bell pepper " or "Bell capsicum" usually means the bell shaped, blocky and non-pungent capsicum types. The condiment or spice paprika refers to the type of capsicum, which when fully ripe, dried and milled and used as spice and colouring agent in cooking. The same paprika type is used for oleoresin (pigment) extraction for use in the food and cosmetic industries (Caselton, 1998). Paprika is one of the important natural colourants next to turmeric and grape colour extracts (Anon., 2001a). Paprika contains remarkable amount of the colouring material and is used as colourant in processed foods as they get the nod over synthetic products in the food colourant market (Anon., 2001b). Dried paprika powder and paprika oleoresin are the natural colour sources exempted from certification and can be used directly (Marmion, 1979). The commercial importance of paprika both as a spice and a vegetable with large scale cultivation in both tropical and sub tropical regions are increasing at an increasing pace.

Global scenario of paprika :

It is interesting to study the world condiment paprika trends. Table 1 shows condiment paprika production data of selected countries.

The world trade in paprika oleoresin is showing a growing trend in recent years. The world trade in paprika (powder form) is estimated to be around 25-28 thousand tonnes per annum, while oleoresin estimate touches about 400 to 500 tonnes per annum.

National scenario of paprika :

Though the area under paprika cultivation and export is meagre, there is an ample scope for spice paprika production in India. The present area under cultivation of paprika in India is almost negligible when compared to the production of chillies, 8.5 lakh tonnes. In Karnataka, the Byadagi Dabba chilli is only grown traditionally in the districts of Dharwad and Hubli and the tomato chilli is grown in Warangal in Andhra Pradesh are being used for the production of paprika oleoresin. The Byadagi chilli is very mild and has low pungency, but used for colour extraction (Balasubramanian, 2001). In India, there are few akin to paprika with less pungent, fleshy and large fruited with excellent colour suitable for export market (John, 2000). In India, Byadagi, Warangal chilli, Arka Abir and KtPl-19 are the important types of paprika under cultivation.

Crop improvement strategies :

Selection of spice paprika breeding lines :

In the breeding programme a large number of paprika germplasm was collected, evaluated, and-selfed. For paprika variety development, pungent and non-pungent types were- selected. Crossing was attempted successfully in the diallel sets of both pungent and non-pungent inbred. The observations are given for the most desirable horticultural traits required in the paprika varieties, developed by selection using single seed descent method, The colour value of' the grinded paprika was calculated as per Woodbury (1977). Fruit characteristics and yield of the lines selected given in the Table 2 revealed that genotype 'Kt-P1-19 is an ideotype which borne all the desired traits important in the sweet spice paprika. Its

Table 1: Paprika World Production (Mt)

Country	1997	1998	1999
Morocco	12,000	12,000	12,000
South Africa	11,000	10,000	9,500
Zimbabwe	10,000	23,000	22,000
China	200,000	200,000	200,000
Israel	2,600	2,600	2,600
Hungary	45,323	65,000	48,000
Slovenia	6,100	6,100	6,100
Spain	6,000	6,000	6,000
North America	56,000	50,000	50,000

Table 2 : Characteristics of the selected genotypes

Genotypes	Fruit size		Mature Fruit Yield (g/ha)	Pungency (ASTA)*		Color Unit (EOA)**
	Length (cm)	Diameter (cm)				
Kt-Pl-8	15.2	2.6	488.0	Sweet	178.35	66337.5
Kt-Pl-18	18.0	2.7	509.7	Sweet	174.25	64812.5
Kt-Pl-19	16.8	3.1	864.8	Sweet	233.70	86925.0
Kt-02	11.0	2.2	530.6	Mild	141.40	52612.5
Kt-03	13.0	1.3	340.4	High	95.30	35456.2
Kt-04	15.4	1.4	354.4	High	138.30	51468.7
NPKT-2	13.2	1.4	361.8	Mild	94.30	35075.0
Agni(F1)	10.6	1.3	222.7	Moderate	136.10	50630.0

*ASTA = American Spice Trade Association ** EOA = Essential Oils Association

'Kt-Pl-19' has also been identified by the Spice Board as a Standard genotype for taking up its production commercially

fruit has excellent firmness, pendent habit, two locules and 68.7% availability of high coloured skin for processing. These lines are being used to develop high coloured pungent and non-pungent varieties. It is being the first achievement in order to attend paprika cultivation within the country has tremendous potentialities of export (Joshj *et al*., 1993).

Inheritance of earliness :

Earliness which is determined by the date of first fruit set or flower opening is an important character in an intensive and multiple cropping system and is of particular importance in areas like Kashmir where growing seasons being short makes it necessary to develop varieties which should not only matures early but should fit well in short growing seasons and different cropping system. Inheritance of earliness was studied from six generations (P1 , P2 , F1 , F2, BC1 and BC2) of three intervarietal crosses *viz*. Shalimar Long x SPE-1 (SL x SPE-1),Shalimar Long x Selection-1 (SL x, Sel-1) SC1X SPE1. The F1 mean performance in all the three crosses indicated over - dominance of earliness over lateness. The significant chi-square value of simple additive-dominance model suggested the involvement of epistatic components. All the components in this model were significant with negative dominance component, which further supported dominance of early parent. In six parameter model, the additive component in all the crosses and dominance component in the cross SL x Sel-1 were significant. Among interaction components, the dominance x dominance in all the crosses and additive x additive in crosses SL x SPE-1 and SL x Sel-1 were significant. The magnitude - of non-additive gene effects were, however, large in compassion to additive effects. Further in cross SL x SP E-1 the dominance and dominance x dominance components were reinforcing each other leading to the complementary gene action which infact has been reflected in early fruiting of F1 hybrids **(**Nazeer Ahmad *et al*., 1993).

Breeding for quality in paprika :

The quality of paprika products is based on visual and extractable red color, pungency level, and to a lesser degree, nutrition. One of the obvious ways to improve the quality of processed products is to improve the quality of the raw material from which these products are derived. This usually means improved cultivars. Color is one of the most important attributes of red chilli and paprika. However, little attention has been given to Capsicum color control. Four different genes, with epistatic interactions have been reported to control color in mature fruit. Approximately 20 carotenoids contribute to the color of Capsicum powder. Unfortunately, the inheritance of the different carotenoids and the genetics of color intensity have not been elucidated. There is little, if any, research reported on the genetics or breeding for carotenoid content in chilli (Bosland, 1992).

Chemical characterization of cultivars and quantification of carotenoids from paprika varieties (Govindarajan, 1986) :

The content of total carotenoids of cultivars 1056 and 1057 was 134.0 and 124.4 mg/10g dry fruit weight (dfw), respectively. Compared with total carotenoids content of 77.6 mg/10g dfw characterizing the Lehava cultivar, the cultivars of the present invention exhibited a 72.7% (cv.1056) and 60.3% (cv.1057) increase in total carotenoids content. Furthermore, the beta-carotene content of cultivars 1056 and 1057, was 17.6 and 18.4 mg/10g dfw, respectively, compared with just 5.5 mg/10g dfw found in the Lehava cultivar, which translates to a 220% and 234.5% increase, respectively. The total carotenoids content and the beta carotene content of cultivars 1056 and 1057 are among the highest reported in paprika fruits.

Cultivar	Total carotenoids mg/10 g.d.w.	ASTA Units	Beta-carotene mg/10 g.d.w.	Capsanthin mg/10 g.d.w.	Vitamin E mg/10 g.d.w.
Lehava	77.6	233.1	5.5	28.0	3.4
cv. 1056	134.0	402.5	17.6	41.7	3.9
cv. 1057	124.4	373.6	18.4	36.5	3.0

Best performing hybrids:

Acc 12 × Acc 23 (P4 x P5)

Number of fruits per plant	: 110
Fresh yield per plant (g)	: 683.26
Dry yield /ha (t)	: 9.06
Ascorbic acid (mg/100g)	: 190.6
Total extractable colour (ASTA)	: 209
Oleoresin	: 15.3

Acc 13 × Acc 23 (P6 x P5)

Number of fruits per plant	: 94
Fresh yield per plant (g)	: 1043.15
Dry yield /ha (t)	: 8.49
Ascorbic acid (mg/100g)	: 77.14
Total extractable colour (ASTA)	: 204.9
Oleoresin	: 15.62

Ramar (2005) tried various cross combinations in order to obtain better hybrids with high yield with quality parameters. The cross combination Ktpl – 19 x Tomato chilli exhibited high mean and high variability for height of plant, number of branches per plant, number of fruits per plant, length of fruit, girth of fruit, weight of fruit, thickness of pericarb, length of placenta and yield per plant. While, the other cross combination Ktpl – 19 x Bydagi Kerala showed high mean and high variability for the characters number of branches per plant and number of fruit per plant. These two crosses offer scope for further selection. This study in paprika indicated that in a selection programme, progress could be made by first selecting on the basis of *per se* performance followed by combining ability effects. The study also suggested the merit of trying new hybrid combinations with successful parents which will have yield superiority over the currently available hybrids.

Biotechnological tools :

Molecular marker tagging of a male sterility gene :

Because the gene ms3 is widely used to develop

Development of f_1 hybrids with high yield, colourant and resistance to anthracnose (Prasath, 2005)
Disease reaction of parents and selected hybrids to *Colletotrichum capsici*

Sr. No.	Entry	Disease incidence (artificial screening)	*Response*	Anthracnose incidence (PDI) (field screening)	Response	Combined response
1.	P1 (Acc. 11)	4.33±0.36*	S	9.94	MR	S
2.	P2 (Acc. 22)	5.00±0.36	S	18.08	MR	S
3.	P3 (Acc. 5)	2.67±0.36	MR	5.54	R	MR
4.	P4 (Acc. 12)	4.33±0.36	S	26.73	S	S
5.	P5 (Acc. 23)	5.00±0.36	S	39.23	S	S
6.	P6 (Acc. 13)	5.00±0.36	S	14.26	MR	S
7.	P1 X P3	2.67±0.36	MR	5.37	R	MR
8.	P2 X P3	2.33±0.36	MR	5.16	R	MR
9.	P3 X P2	3.00±0.36	S	4.57	R	S
10.	P4 X P3	2.67±0.36	S	4.62	R	S
11.	P6 X P3	2.67±0.36	MR	4.78	R	MR
12.	P1 X P2	5.00±0.36	S	18.03	MR	S
13.	P2 X P6	5.00±0.36	S	25.86	MR	S
14.	P4 X P6	4.33±0.36	S	19.54	MR	S
14.	P5 X P1	5.00±0.36	S	31.59	S	S
16.	P6 X P2	2.67±0.36	MR	28.17	S	S
17.	Acc. 16	0.33±0.36	R	0.43	R	R

*Standard Error

hybrid *Capsicum* in other countries, an attempt was made to tag this gene to allow identification of sterile seedling plants. The amplified fragment length polymorphism (AFLP) technique identified a dominant marker co-segregating in repulsion with male sterility, using bulks of fertile and sterile plants in the cross Rapires (ms3)/ Conquistador. This marker mapped 2.7 cM from Ms3. Sterility was identified by the absence of the marker, when converted to a PCR format, has the potential for acceptable identification of male sterile individuals in segregating paprika populations at an early growth stage.

Possible vegetative production of male sterile lines :

Vegetative propagation of genetic male sterile lines provides an alternative way for economic production of hybrid seed without the necessity of phenotype identification, progeny testing, or marker screening. Moreover, vegetative propagation can be cost effective in the maintenance of cms lines. Male sterile *Capsicum* lines were successfully propagated during this study as cuttings in the greenhouse. Rooting was achieved in Jiffy propagation plugs after 4-5 weeks of culturing in misting chambers. A single Ethrel application (250 ppm) was sufficient to delay flowering and to induce vegetative growth. An attempt was made to propagate ms lines *in vitro*. A standard disinfecting procedure was established and applied in the studies to identify shoot induction medium. Despite some positive results, *in vitro* propagation was at so low a frequency that it could not be used on a large scale because of low reproducibility.

Doubl haploids as tool in paprika breeding :

- F1 hybrid production is in the focus of paprika breeding
- Production of F1 hybrids claim to homozygous parent lines
- Doubled haploids (DHs) represent the perfect homozygous state
- Double haploids can be produced by androgenesis and gynogenesis. The androgenetic or gynogenetic responsiveness of a given genotype is determined by the extent of haploid cell (microspore, macrospore) induction and plant regeneration. The main factors influence double haploid production are genotype, donor plant growth condition and *in vitro* and *in vivo* culture condition. The donor plants of paprika should be grown under greenhouse conditions.

Steps of improved haploid protocol in anther culture:

- Flow cytometry analysis of regenerated plants (PA-I, Partec, Münster) using method
- *In vitro* doubling of haploid regenerants with 400 mg/l colchicine for 6 days
- Acclimatization of regenerants step by step
- To guarantee the purity of the double haploid lines flowering plants were isolated and self-fertilised
- Check the homogenity of lines by microsatellite markers (in case when donors were F1 hybrid)

Outcome :

- 3.5% maltose in induction medium (1.5x more DHs production + recalcitrans genotypes work better)
- Efficient (95%) and economical genome duplication of haploids with 99% survival rate and no fertility problem in the trated lines

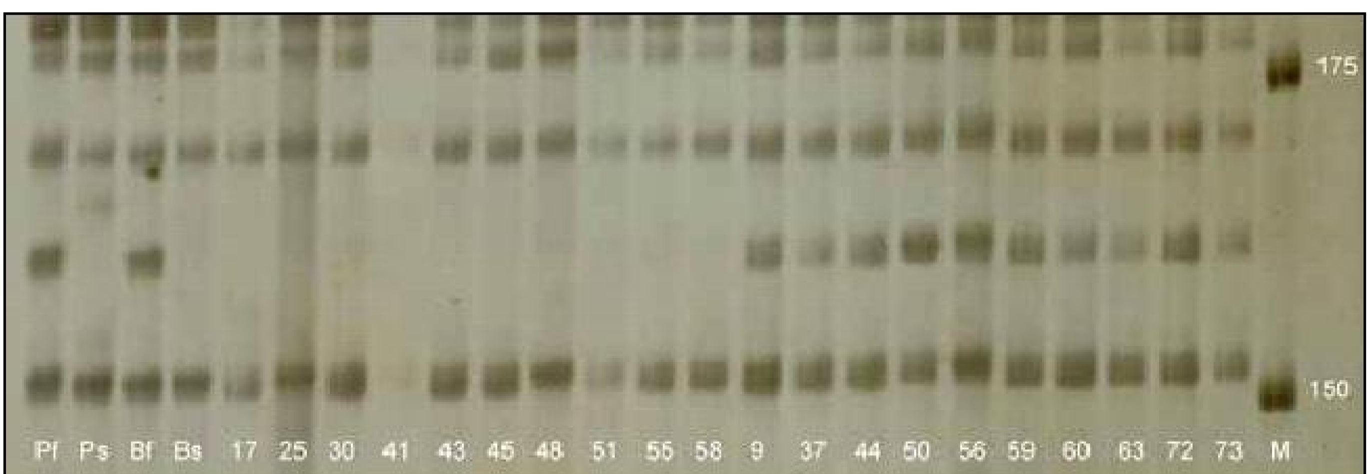

Fig. 1 : Amplification of the AFLP primer combination PgA/MagC gives a 160bp fragment present only in male fertiles in segregating populations of ms3. There is a segregating fragment at 160bp (between the 175 and 150bp maker on the right) that is present in the fertile parent (Pf), absent from the sterile ms3 parent (Ps), present in the bulk of 10 fertile lines (Bf, and the fertile lines 9-73), and absent from the bulk of 10 sterile lines (Bs and the 10 sterile lines 10-58)

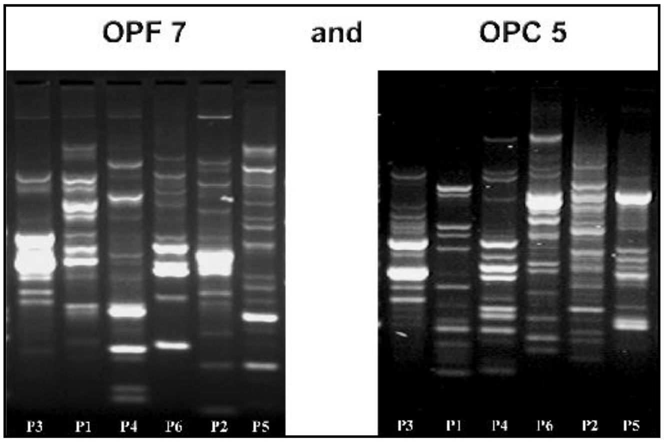

Fig. 2 : RAPD profile of parents for the primer

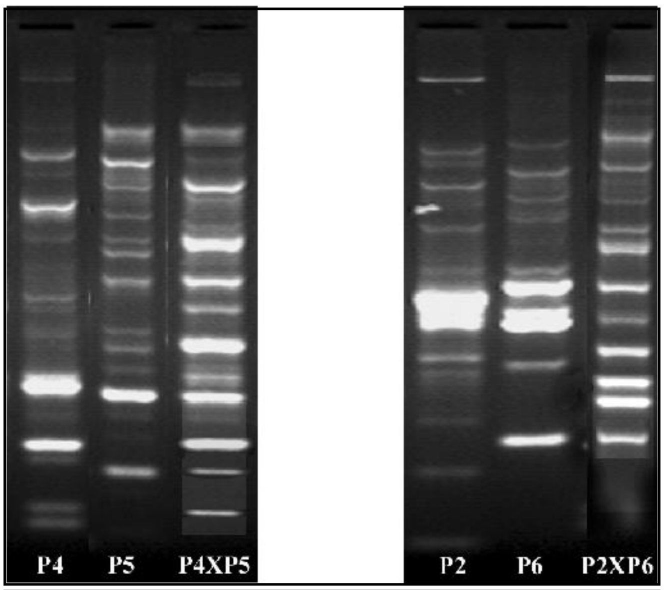

Fig. 3 : RAPD analysis of two F_1 hybrids amplified by primer OFP 7

– Microsatellite-based homozigosity control of spontaneous diploids

Parental diversity analysis and molecular marker (RAPD) (Prasath, 2005) :

RAPD analysis of six parents and two short listed hybrids of paprika type chilli with random primers amplified DNA fragments with different molecular weight, which ranged in size from approximately 50 to 1500 bp. All the primers tested successfully amplified the Capsicum DNA from all parents. A total of 66 bands were amplified across six parents with 12 RAPD primers revealing an average of 5.5 bands per primer in each parent. Ten and eight primers were able to detect the DNA variations between parents of P2 x P6 and P4 x P5, respectively. Of the 58 amplified products generated from the 10 primers used for, 29 were polymorphic and used for P2 x P6 purity test. There were 22 markers able to detect P4 x P5 hybridity. Interestingly, one RAPD primer (OPF 7) had detected hybridity of the two F_1 hybrids *viz*., P2 x P6 and P4 x P5.

Crop manangement strategies :

Effect of shade levels on paprika (Irene vethamoni, 2006) :

The paprika cultivar Kt-Pl-19 had better performance for growth and yield characters for yield and quality. The economics of shade net cultivation showed that paprika 'Kt-Pl-19' under 35 per cent shade registered the highest benefit cost ratio of 3.15. Ultimately, the study revealed the prospects of shade net cultivation of paprika (Kt-Pl-19) under tropical condition.

Fertigation in paprika under open and coconut shade conditions (Prabhu, 2006) :

The recommended dose N: P: K at 120: 100: 120 kg per hectare was applied through fertigation as water soluble fertilizers (Monoammonium phosphate, polyfeed and multi K). Fertigation was scheduled on alternate days starting from second week after planting. The imported grade micronutrient mixtures rexolin (0.1%) was used as a foliar spray. Two sprays were given at flowering and fruit development stage. Earliness in days to first flowering, 50 per cent flowering and the maximum number of flowers per plant were registered under open condition with the fertigation level of 100 per cent water soluble fertilizers in combination with micronutrients. The yield parameters like highest number of fruits per plant, girth of the fruit, number of seeds per fruit, weight of the seeds per fruit, 1000 seed weight, average fruit weight, yield per plant, yield per plot and yield per hectare were observed in the treatment with 100 per cent water soluble fertilizer with the application of micronutrients under open condition and under shade conditions. The fruit quality parameters *viz*., ascorbic acid, oleoresin and capsanthin were maximum with the application of 100 per cent water soluble fertilizer plus micronutrients under shade. The economic feasibility showed that application of 100 per cent water soluble fertilizer along with micronutrients recorded the highest benefit cost ratio during summer and *kharif* seasons.

Growth regulators on flowering and fruit set (Kannan, 2003) :

The increased fruit drop and decreased percentage of fruit set are the major issues lead to low productivity in paprika. Besides these reasons the delay in flowering also restricts the productivity, so that the yield and quality of paprika fruits were affected. Appreciable variation for the traits like plant height, number of branches, leaf area, days to first and 50 per cent flowering, number of flowers, fruit set and number of fruits per plant with different growth regulators (mepiquat chloride, NAA, 2,4-D and Nitrobenzene) during winter and summer seasons. The plants treated with 50 ppm NAA showed earliness for first flowering, fruit weight, yield /ha and capsanthin content during winter and summer, respectively. The comparison of different morphological, physiological and yield attributes revealed that NAA at 50 ppm treatment was effective in both the seasons in paprika variety Kt-Pl-19.

Biostimulants on paprika (Ashok Kumar, 2006) :

Among the different biostimulants tried as foliar spray at three stages on 25th, 45th and 65th days of crop growth such as panchagavya (2 and 4%), moringa leaf extract (2 and 4%), humic acid (0.1 and 0.2%), cytozyme (1%) and atonik (1%), the maximum fresh fruit weight (24.3t/ha) was obtained with panchagavya 4%. Similarly the same treatment produced maximum results for oleoresin (13.91%) and capsanthin (132.16 ASTA).

Pre-harvest spray of certain growth substances (Malarvizhi, 2007) :

Certain growth substances *viz.*, NAA, GA_3, BA, KCl, Boric acid, panchagavya, humic acid were sprayed thrice, first at 25 days after transplanting and the second and third sprays at 45th day and 65th day after transplanting. The plants treated with panchagavya at 2.0 per cent observed with earliness for first flowering in Kt-P1-19 and Arka abir cultivars. Panchagvya at 2.0 per cent was found effective for highest number of fruits per plant in Kt-P1-19 (88.56) and Arka abir (95.36) cultivars. Similarly the same treatment recorded the highest values for fruit weight (24.80 and 23.40 g), fresh fruit yield (19.88 and 20.52 t), capsanthin content (195.25 and 190.22 ASTA units) and ascorbic acid (123.67 and 133.81 mg/100 g) in both the cultivars. Fruits treated with different pre harvest treatments exhibits extended shelf life on storage over control. Among the treatments, panchagavya at 2.0 per cent sprayed fruits performed to extending the shelf life, better firmness, reduced PLW and minimum incidence of spoilage.

Methods of drying and packaging of paprika fruits (Malarvizhi, 2007) :

On comparing different drying methods and packaging materials were employed, maximum capsanthin content was obtained with combination of poly house single layer drying and polythene lined bags with pre harvest treatment of panchagavya at 2.0 per cent in Kt-P1-19 and Arka abir cultivars. From the economics of cultivation, it could be concluded that the treatment, panchagavya at 2.0 per cent followed by poly house single layer drying and polythene lined bag storage recorded the highest net return which could be recommended for commercial cultivation.

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