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Cluster analysis based on biochemical constituents in paprika like chillies (*Capsicum annuum* L.)

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Abstract

Chillies and paprika like chillies (*Capsicum annuum* L.) belonging to *Solanaceae*, is a source of natural colour, widely used in the food industry and is in great demand in the international market. Twenty-four accessions belonging to Indigenous Collections of Byadagi Dabbi (ICBD) and others were analyzed for biochemical constituents from leaf. Biochemical constituents studied were total carbohydrate, starch, reducing sugars, total free amino acids, phenol and protein. The accessions were compared for the isozymes *viz*., peroxidase, super oxide dismutase and poly phenol oxidase in the leaf, besides, leaf protein using SDS-PAGE. The dendrogram showing the relationship among the accessions for leaf biochemical constituents and colour value of fruits formed six clusters. Colour value ranged from 109 to 353 ASTA units. The accessions ICBD-11 and ICBD-19 possessed highest colour value. Accession ICBD-24 was distinctly different from others. Accessions in each cluster had almost uniform colour value and uniform isozyme profile. The accession ICBD-24 was a Coorg collection and ICBD-11 and ICBD-19 were from Dharwad district of Karnataka. Significant positive correlation was observed between colour value and total free amino acids, total protein, total phenol and polyphenol oxidase in paprika like chillies.

Keywords: *Capsicum annuum,* colour, dendrogram, isozymes, paprika, paprika like chillies

Introduction

The genus *Capsicum* belonging to the family *Solanaceae* has five species *viz*., *Capsicum annuum, C. baccatum, C. chinense, C. frutescens* and *C. pubescens*. However, *C. annuum* is the most traded, which has two types, one is 'paprika'- defined as a ground, red powder derived from dried fruits with the desirable colour and flavour qualities and other one is with pungent fruits called 'chillies' (Berke & Shieh 2001). In India, there are a few indigenous types of

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chillies, which are akin to paprika with fruits having high colour and low pungency such as 'Byadagi chilli' grown in Dharwad district of Karnataka state and Warangal Chappatta (Tomato chilli) grown in Warangal and Khammam districts of Andhra Pradesh, which are identical in quality to paprika types that are grown in Spain and Hungary (Shiva *et al.* 2006, 2008). These chillies are much preferred by oleoresin manufacturers for extraction of paprika alike oleoresin (John 2000). The present investigation was aimed at studying the biochemical constituents and isozymes of leaf and analyzing its relationship with that of colour value of the fruits of chillies and paprika like chillies and grouping the accessions based on similarities existing among them.

Materials and methods

Twenty-four paprika like chillies i.e. Indigenous Collections of Byadagi Dabbi (ICBD) accessions and other types collected from various sources (Table 1) and maintained at Indian Institute of Spices Research, Kozhikode, Kerala (Table 1) were selected for the study during 2007–09. The plants were maintained in pots under rain-out shelter with uniform cultural practices. Youngest fully matured leaf samples were selected for the study. Biochemical constituents such as total carbohydrate, starch, reducing sugar, total free amino acids, protein and phenols and SDS-PAGE in leaf tissue were estimated following the standard methods as described by Sadasivam & Manickam (1991). Leaf samples prepared in phosphate buffer pH 7.1 were used for separating the isozymes of peroxidase, super oxide dismutase and polyphenol oxidase (Holstein *et al.* 1967; Ravindranath & Fridorich 1975).

Total extractable color expressed as colour value was estimated from powdered dry chilli samples using acetone and absorbance was read at 460 nm (ASTA 1995). Hierarchical cluster analysis was carried out using SPSS software version 11.5 and Jaccards Similarity Coefficient index was followed to group clusters.

Results and discussion

Twenty four paprika like chillies i.e., ICBD and other accessions were grouped into six clusters using Hierarchical cluster analysis (Table 2). Total carbohydrate among total accessions ranged from 1.1-5.2%, starch from 1.5-6.12%, reducing sugar from 0.15-2.1%, total free amino acids from 0.69-1.8%, phenol from 0.59-2.05% and protein from 0.4-1.4%. The total extractable colour value in chilli accessions ranged from 109-347 ASTA units. Such kind of variations in the biochemical constituents in chillies were also reported by Gupta & Tambe (2003) and Wall & Bosland (1993).

It is evident from Table 3 that the most important quality trait of paprika like chillies was colour value which had significant positive correlation with isozyme profile, particularly poly phenol oxidase and phenol and highly significant positive correlation with metabolites such as total free amino acids and protein.

Based on the dendrogram, the total extractable colour value, percentage similarity in isozymes, SDS PAGE and the level of biochemical constituents were compared (Fig. 1). The chilli accessions were grouped into six clusters based on biochemical characterization.

The accessions *viz*., ICBD-17, 18, 16, 3, and ICBD-22 falling under cluster-I contained relatively high total carbohydrate, high phenol, similar super oxide dismutase pattern and highly similar SDS pattern. Colour value of these accessions was > 250 ASTA units.

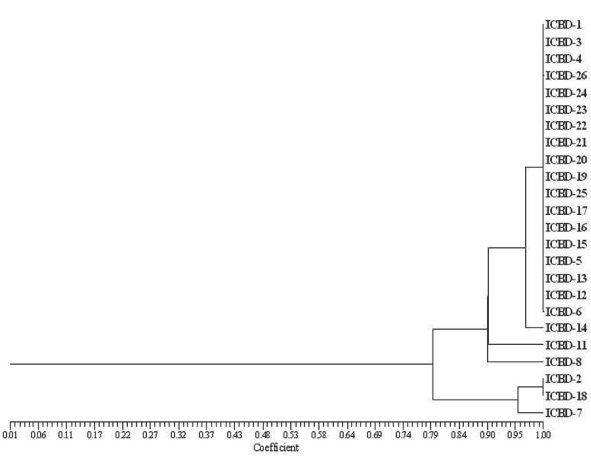

Fig. 1. Dendrogram of 26 accessions based on Jaccards Similarity Coefficient index using isozyme and SDS protein

Table 1. Genetic resources of paprika like chillies (indigenous)

Accession/ genotype	Source
ICBD-1	Noolvi Dabbi (ND), Dharwad District, North Karnataka
ICBD-2	Annekeri Delux (AD), Dharwad District, North Karnataka
ICBD-3	Anthur Dabbi (Dabbi deluxe), Dharwad District, North Karnataka
ICBD-4 (B/9-1)	Benthur Dabbi (Dabbi typical), Dharwad District, North Karnataka
ICBD-5 (B/9-3)	Benthur Dabbi (Dabbi typical), Dharwad District, North Karnataka
ICBD-6 (B/9-5)	Benthur Dabbi (Dabbi typical), Dharwad District, North Karnataka
ICBD-7 (B/9-7)	Benthur Dabbi (Dabbi typical), Dharwad District, North Karnataka
ICBD-8 (B/K-1)	Benthur Dabbi (Dabbi typical), Dharwad District, North Karnataka
ICBD-9 (B/K-4)	Benthur Dabbi (Dabbi typical) (Khubihal village),Dharwad District, North Karnataka
ICBD-10 (B/4-1)	Benthur Dabbi (Dabbi typical), Dharwad District, North Karnataka
ICBD-11 (B/K-11)	Benthur Dabbi (Dabbi typical) (Khubihal village), Dharwad District, North Karnataka
ICBD-12 (B/K-14)	Benthur Dabbi (Dabbi typical) (Khubihal village),Dharwad District, North Karnataka
ICBD-13 (B/K-16)	Benthur Dabbi (Dabbi typical) (Khubihal village),Dharwad District, North Karnataka
ICBD-14 (B/9-11)	Benthur Dabbi (Dabbi typical) (Khubihal village),Dharwad District, North Karnataka
ICBD-15 (B/7-5)	Benthur Dabbi (Dabbi typical) (Khubihal village),Dharwad District, North Karnataka
ICBD-16 (B/y-13)	Benthur Dabbi (Dabbi typical) (Yeliwal village),Dharwad District, North Karnataka
ICBD-17 (B/y-2)	Benthur Dabbi (Dabbi typical) (Yeliwal village),Dharwad District, North Karnataka
ICBD-18 (B/y-4)	Benthur Dabbi (Dabbi typical) (Yeliwal village),Dharwad District, North Karnataka
ICBD-19 (Arka Abhir)	Indian Institute of Horticultural Research, Bengaluru, Karnataka
Kt-Pl-19	Indian Agricultural Research Institute, Regional Station,Katrain, Himachal Pradesh
Kt-Pl-19 variant-1	Indian Agricultural Research Institute, Regional Station,Katrain, Himachal Pradesh
CC -1	Coorg, Karnataka
CC -2	Coorg, Karnataka
CC -3	Coorg, Karnataka
CC-4	Coorg, Karnataka
CC-5	Kozhikode, Kerala

Table 2. Percentage composition of biochemical constituents and isozyme pattern in chilli accessions

	Range in biochemical constituents						Isozyme pattern variation				Total extractable colour	Accession/ genotype
	T.C (%)	Starch (%)	R.S (%)	T.F. A.A (%)	Phenol (%)	Protein (%)	POX (%)	SOD (%)	PPO (%)	SDS Protein (%)	ASTA Units	
Cluster I	3.1-5.2	1.6-6.12	0.36-2.10	0.7-1.1	1.0-2.06	0.5-1.16	60-100	50-100	14-85	56-87	254-282	ICBD -17, 18, 16, 3, CC-1 & Kt-Pl-19 variant-1
Cluster II	1.1-4.8	1.83-2.4	0.31-1.19	0.73-1.18	1.44-1.67	0.37-0.68	60-100	50-100	28-71	50-81	144-173	ICBD - 8, 15, 7, 12
Cluster III	2.3-3.7	1.5-3.77	0.15-1.70	0.69-1.15	0.76-1.82	0.47-0.81	80-100	50-100	28-85	56-93	219-230	ICBD - 6, 13, 4, CC-2
Cluster IV	1.75-4.00	1.88-4.50	0.44-1.29	0.7-1.16	0.78-2.26	0.42-0.80	60-100	50-100	14.28-100	56-93	184-207	ICBD- 2, 5, 1, 14, Kt-Pl-19, CC-4 & CC-5
Cluster V	4.12	4.62	1.00	0.70	0.89	0.824	100	50	85	68	109	CC -3
Cluster VI	3.31-3.55	2.22-3.27	0.99-1.23	1.82-1.81	1.76-3.58	1.265-1.405	80	100	14.28	50-56	347-353	ICBD-11 and 19

Note: T.C=Total carbohydrate; R.S=Reducing sugar; T.F.A.A=Total free amino acids; POX=Peroxidase; SOD=Super oxide dismutase; PPO=Polyphenol oxidase

Table 3. Correlation matrix of biochemical constituents and colour of chilli accessions

	POX	PPO	SOD	SDS-Page	T.C	Starch	R.S	Protein	T.F.A.A	Phenol	Total extractable color
POX	1.000										
PPO	-0.576**	1.000									
SOD	-0.110	-0.008	1.000								
SDS-PAGE	0.616**	-0.509**	-0.290	1.000							
T.C	-0.508**	0.325	0.043	-0.414*	1.000						
Starch	-0.198	0.099	0.107	-0.241	0.482*	1.000					
R.S	-0.323	0.312	0.349	-0.510**	0.454*	0.367	1.000				
Proetin	0.054	0.014	0.126	-0.182	0.107	0.129	0.188	1.000			
T.F.A.A	0.202	-0.014	-0.416*	0.156	-0.307	-0.194	-0.281	0.635**	1.000		
Phenol	-0.375	0.362	-0.361	-0.276	-0.124	0.068	0.127	0.279	0.524**	1.000	
Total extractable color	-0.145	0.444*	-0.122	-0.264	0.275	0.050	0.204	0.629**	0.584**	0.447*	1.000

*Significant at CD (P<0.05); **Significant at CD (P<0.01)
POX=Peroxidase; PPO=Poly Phenol Oxidase; SOD=Super Oxide Dismutase; T.C=Total carbohydrate; R.S=Reducing sugar; T.F.A.A=Total free amino acids

The second cluster had paprika like chillies accessions (ICBD- 8, 15, 7, 12) possessing relatively low colour value (140-173 ASTA units). They were low in total carbohydrate, starch and phenol contents. The III cluster having accessions, ICBD-6, 13, 4 and ICBD-23 contained total extractable colour value of above 200 ASTA units. These accessions produced uniform carbohydrate content. The accessions (ICBD- 2, 5, 1, 14, 20, 25 & 26) falling under cluster IV revealed high carbohydrate content. The total extractable colour value in the cluster was in the range of 184-200 ASTA units. They possessed more similarity in isozyme pattern of super oxide dismutase and peroxidase.

The cluster V (ICBD-24) was distinct from other accessions. The accessions in this cluster expressed high total carbohydrate, high starch and low colour value. However, the accessions falling under Cluster VI (ICBD 11 and 19) had very high total extractable colour value (above 340 ASTA units). They were more similar in super oxide dismutase, peroxidase, polyphenol oxidase, and other biochemical constituents.

Cluster VI accessions contained high colour, relatively high total carbohydrate, starch, protein and total free amino acids. From this, it may be inferred that group distinction is mainly on the basis of colour value, which is supported by other biochemical constituents. Based on the coefficient value, accessions ICBD-11 and ICBD-19 belong to one group. High ASTA colour value, more similarity in isozyme pattern and SDS PAGE were observed in this group. Collection ICBD-3 is from Coorg and ICBD-19 and ICBD-11 are from Kubihal Village of Dharwad in Karnataka, India. Loaiza *et al.* (1989) also could establish genetic relationship of chilli accessions with geographic origin based on isozyme analysis. However, Anu & Peter (2003) associated the chilli germplasm with geographical origin based on protein profiles.

Based on biochemical characterization, the genotypes/accessions of paprika like chillies were grouped into six clusters. Cluster I and IV contained high number of accessions. ICBD-9 and ICBD-11 possessed high ASTA colour with great similarities with isozyme pattern, which indicated that the accessions are from the same group. ICBD-11 and ICBD-19 compositions can be mechanized to meet the consumer's high demand of natural food colours of additives. This data may provide a scientific basis for future selection and crop improvement.

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References

American Spice Trade Association (ASTA) 1995 Official Analytical Methods. American Spice Trade Association, New York.

Anu A & Peter K V 2003 Analysis of seed proteins of 29 lines of *Capsicum annuum* L. by polyacrylamide gel electrophoresis. Genet. Resour. Crop Evol. 50: 239–243.

Berke T G & Shieh S C 2001 Capsicum, chillies, paprika, birds eye chilli. In: Peter K V (Ed.) Handbook of herbs and spices (pp.111–122), Wood Head Publishing Ltd., Cambridge, United Kingdom.

Gupta D N & Tambe N G 2003 Physicochemical characteristics of some promising varieties of chilli grown in Konkan Region. J. Maharashtra Agri. Univ. 28: 327–328.

Holstein T, Burnett J & Quevedo W 1967 Genetic regulation of multiple forms of Tyrosinase in Mice; Action of alpha and beta Loci. Proc. Soc. Exp. Biol. Me. 126: 415.

John K 2000 Promotional activities of Spices Board on paprika. Indian J. Arec. Spices Med. Plants 2: 47–49.

Loaiza F, Ritland K, Cancino J & Tanksley S D 1989 Patterns of genetic variation of the genus *Capsicum* (Solanaceae) in Mexico. Plant Sys. Evol. 165: 159–188.

Ravindranath S D & Fridorich 1975 Isolation and characterization of manganese containing SOD from yeast. J. Bio. Chem. 250: 1607.

Sadasivam S & Manickam A 1991 Biochemical Methods for Agricultural Sciences. Wiley Eastern Limited publishers New Delhi.

Shiva K N, Prasath D & Parthasarathy V A 2008 Chilli and paprika. In: Parthasarathy V A, Kandiannan K & Srinivasan V (Eds.) Organic spices production (pp.477–522), New India Publishing Agency, New Delhi.

Shiva K N, Prasath D, John Zachariah T & Parthasarathy V A 2006 Chilli and paprika. In: Parthasarathy V A & Rajeev P (Eds.), Major Spices - Production and Processing (pp.228–272), Indian Institute of Spices Research, Kozhikode, Kerala.

Wall M M & Bosland P W 1993 The shelf-life of chillies and chilli containing products. In: Charalambous G (Ed.) Shelf life studies of foods and beverages, chemical, biological, and nutritional aspects (pp.487–500), Elsevier Publishing, New York.