Journal of Tropical Agriculture 61(2): 227-236, 2023

Spatial and temporal gene expression pattern and taxonomic alignment at vegetative stage in cultivated *Capsicum* Spp.

Asish Edakkalathur I.*[1], Presannakumari, K.T.[1], Minimol, J.S.[1], Latha, M.[2] and Pradeepkumar T.[1]

[1] *Kerala Agricultural University, Thrissur 680 656, Kerala, India*
[2] *National Bureau of Plant Genetic Resources - ICAR, Regional Station, Thrissur 680 656, Kerala, India*

Received 19 March 2022; received in revised form 21 January 2023; accepted 11 November 2023

Abstract
One hundred and thirty five chilli landraces belonging to *Capsicum annuum*, *C. chinense* and *C. frutescens* species were analyzed for 20 unit characters. Temporal and spatial gene expression patterns of anthocyanin pigmentation and pubescence were analyzed which revealed species specific fixation of alleles and differential response of each species to the external environment. Relative fixation of alleles across species was used to frame taxonomic key based on seed and seedling characters which will be helpful for species alignment in the vegetative stage and will be complementary to the existing key. The relationship between *C. chinense* and *C. frutescens* was evident from many phenotypic character states with similar expression patterns in both the species.

Key words: Anthocyanin, Capsicum, Gene expression pattern, Pubescence,Taxonomic key.

Introduction

Chilli belongs to the genus *Capsicum* which originated in Central and South America. Genus *Capsicum* comprises around 40 species and exhibits huge variability which is attributed to peculiarities of its genome, multiple domestication events and introgressive hybridization. Large numbers of horticultural types, their transitory nature and phenotypic plasticity make taxonomic alignment in *Capsicum* a confusing exercise (Smith and Heiser, 1951). The high morphological variability of fruit as well as imperfectly understood inter-species relationships had drawn the attention of taxonomists even before the work of Linnaeus. Linnaeus described two species *viz*., *C. annuum* and *C. frutescens* in 1753 and later identified one more species *viz*., *C. baccatum* (Bosland and Votava, 2012).

Out of 40 species five *viz*., *Capsicum annuum* L., *C. chinense* Jacq, *C. frutescents* L., *C. baccatum* L. and *C. pubescence* Ruiz et Pavon are cultivated. Among the cultivated species, the first three are the members of 'the annuum complex' and form the major part of chilli cultivation and trade worldwide (Zonneveld et al*.,* 2015). Each of these cultivated species possesses one or a few unique characters mostly associated with reproductive stage. These key taxonomic characters include single flower per axil and white or purple corolla in *C. annuum*, two to five flowers per node with annular constriction of calyx for *C. chinense*, erect pedicel position at anthesis and greenish yellow corolla in *C. frutescens*, white corolla with paired yellow spots for *C. baccatum*, and lilac corolla with brown coloured seeds for *C. pubescens.* Apart from the above key taxonomic traits, corolla colour, calyx teeth, anther lobe colour, seed colour and texture, fruit shape and corrugation, life cycle, leaf shape,

* Author for correspondences: Phone: 9497407943, Email: asish.ei@kau.in

stem type, pedicel length and thickness could be used as supporting taxonomic characters for species alignment of cultivated *Capsicum* (Smith and Heiser, 1951; Smith and Heiser, 1957; Eshbaugh, 2012; Carvalho et al., 2014). Most of these key as well as supporting taxonomic features are associated with flowering and fruiting stages which make species alignment at vegetative stage a difficult task.

In the present investigation, we have evaluated cultivated *Capsicum* landraces belonging to 'the annuum complex' with unit characters mostly associated with anthocyanin pigmentation and pubescence at different stages of plant growth. Temporal and spatial gene expression patterns of these characters were analyzed to find out the existence of any species specific association. These species specific expression patterns were used to develop a taxonomic key mostly based on vegetative features. Similarity in expression patterns of unit characters across species is implemented to analyze the species relationship within the members of 'the annuum complex'.

Materials and methods

Present evaluation was carried out on 135 landraces (Table 1) of *Capsicum* belonging to three major cultivated species *viz*., *C. annuum*, *C. chinense* and *C. frutescens.* The landraces were collected from Kerala, India. Twenty unit characters as per *Capsicum* descriptor (IPGRI, 1995) representing the stages from seedling to seed were considered for evaluation. Evaluation was carried out at the regional station of National Bureau of Plant Genetic Resources (NBPGR), Thrissur ($10^0$33'12" N and $76^0$16'39"E).

The association of unit characters with species was assessed by Chi square analysis with SPSS 15.0. Temporal and spatial expression patterns of anthocyanin pigmentation were evaluated across species at nine growth stages ranging from hypocotyl to immature fruit colour. Similarly, pubescence intensity was evaluated on hypocotyl, leaves and stem.

Taxonomic key to align the accessions into major three cultivated species was framed based on mostly vegetative characters that are significantly associated with each type of species. A character was considered as a supporting taxonomic character, if major share of a descriptor state was constituted by a particular species or group of species. Similarity in phenotypic expression patterns was analyzed across species to evaluate the relationship between species.

Results and Discussion

i. Distribution of unit characters across species and relationship among cultivated species of the annuum complex

Distribution of unit characters across the major cultivated species and Chi square values depicting relationships of different unit characters with species are presented in Table 2. White coloured hypocotyl was observed in 6.38 and 11.77 per cent of *C. chinense* and *C. frutescens* accessions, respectively. None of the *C. annuum* accessions evaluated had

Table 1. Species particulars of accessions used in the study

Sl. No.	Species	No of genotypes	Accession number
1	*C. annuum*	54	10, 116, 9, 22, 36G, 23, 109, 36P, 111, 111-I, 111-II, 35, 112, 113, 226, 71, 110, 149, 217, 231, 235, 24, 27, 28, 33, 198, 200, 242, 179, 180, 222, 25, 26, 161, 30, 173, 243, 41,84,158, 178, 162, 197, 199,196, 234, 236, 86, 92, 91, 46,90, 210, 104
2	*C. chinense*	47	17, 144, 152,145, 147, 150, 244, 39, 137, 148, 153, 238, 190, 191, 195, 119, 194, 182, 61, 64, 53, 85, 59, 140, 141, 142, 143, 140G, 140P, 60, 63, 65, 66, 80, 99, 51, 54, 138, 139, 156, 177,176, 245, 246, 253, 254, 188
3	*C. frutescens*	34	11, 13, 14, 15, 20, 21, 114, 219, 225, 260, 1, 2, 31, 32, 40, 181, 184, 207, 211, 215, 216, 43, 56, 76, 97, 101, 103, 120, 131, 187, 221, 230, 252

Table 2. Species -wise descriptor state frequencies of unit characters and Chi square analysis of distribution of unit characters and species

Sl. No.	Descriptor	Descriptor state	Frequency			Chi square value	Asymp. Sig . (2-sided)
			C. annuum	*C. chinense*	*C. frutescens*		
1	Colour of hypocotyl	White	0.000	0.022	0.030	10.56	0.032
		Green	0.259	0.170	0.170		
		Purple	0.141	0.156	0.052		
2	Pubescence of hypocotyl	Sparse	0.148	0.037	0.007	40.595	0
		Intermediate	0.193	0.089	0.059		
		Dense	0.059	0.222	0.185		
3	Colour of primary leaf	Green	0.326	0.311	0.252	7.261	0.027
		Purple	0.074	0.037	0.000		
4	Stem colour	Green	0.259	0.193	0.222	21.187	0
		Green with purple stripes	0.081	0.022	0.000		
		Purple	0.059	0.133	0.030		
5	Stem pubescence (4^{th} node from tip)	Sparse	0.096	0.059	0.133	19.764	0.001
		Intermediate	0.148	0.089	0.081		
		Dense	0.156	0.200	0.037		
6	Nodal anthocyanin (whole plant- below 4^{th} node)	Green	0.178	0.259	0.252	31.093	0
		Purple	0.222	0.089	0.000		
7	Leaf colour	Green	0.363	0.348	0.252	7.788	0
		Purple	0.037	0.000	0.000		
8	Leaf pubescence (Upper side of 4^{th} leaf from tip)	Sparse	0.267	0.222	0.133	2.184	0.702
		Intermediate	0.052	0.052	0.037		
		Dense	0.081	0.074	0.081		
9	Leaf shape (4^{th} leaf from tip)	Deltoid	0.000	0.000	0.030	84.467	0
		Ovate	0.067	0.326	0.185		
		Lanceolate	0.333	0.022	0.037		
10	Type of leaf margin (4^{th} leaf from tip)	Entire	0.400	0.237	0.222	21.365	0
		Undulate	0.000	0.111	0.030		
11	Leaf density	Sparse	0.052	0.030	0.007	20.981	0
		Intermediate	0.311	0.252	0.119		
		Dense	0.037	0.067	0.126		
12	Pigmentation of calyx	Absent	0.326	0.319	0.207	2.283	0.319
		Present	0.074	0.030	0.044		
13	Petal shape	Rotate	0.331	0.220	0.237	11.548	0.003
		Companulate	0.034	0.136	0.042		
14	Petal colour	White	0.259	0.007	0.000	113.022	0
		Yellow-green	0.015	0.319	0.252		
		Purple	0.126	0.022	0.000		
15	Anther lobe colour	Yellow	0.008	0.110	0.025	16.465	0.002
		Blue	0.110	0.085	0.110		
		Purple	0.246	0.161	0.144		
16	Filament colour	White	0.267	0.044	0.015	61.565	0
		Yellow-Green	0.007	0.200	0.111		
		Purple	0.126	0.104	0.126		
17	Fruit colour at intermediate stage	White or yellow	0.015	0.015	0.104	38.927	0
		Green	0.311	0.319	0.148		
		Purple	0.074	0.015	0.000		
18	Anthocyanin spots / stripes on fruit	Absent	0.356	0.252	0.230	6.905	0.032
		Present	0.044	0.096	0.022		
19	Calyx margin of fruit	Entire	0.081	0.304	0.119	45.14	0
		Intermediate	0.319	0.044	0.133		
20	Seed surface	Smooth	0.119	0.007	0.007	29.33	0
		Rough	0.141	0.274	0.178		
		Wrinkled	0.141	0.067	0.067		

white coloured hypocotyl. Majority of *C. chinense* (63.87%) and *C. frutescens* (73.53%) accessions were characterized by the presence of densely pubescent hypocotyl. However, most of the *C. annuum* accessions were sparsely or intermediately pubescent. All the *C. frutescens* accessions exhibited green cotyledon colour. Purple pigmented cotyledon was observed in 10.64 and 18.52 per cent of *C. chinense* and *C. annuum* accessions, respectively.

Only 3.70 per cent of accessions evaluated exhibited anthocyanin pigmentation on the leaf. All these accessions belonged to *C. annuum*. Most of *C. annuum* accessions (83.33%) recorded lanceolate leaf. All the accessions with deltoid leaf shape belonged to *C. frutescens*. Among the *C. chinense* accessions, 93.62 per cent were characterized with ovate leaves. Leaf margin was observed to be entire in all the *C. annuum* accessions examined. The undulate leaf margin was exhibited by 31.92 per cent of accessions belonging to *C. chinense* and 11.77 per cent of accessions belonging to *C. frutescens*. Among the *C. frutescens* accessions, 97.06 per cent exhibited intermediate or high leaf density. Species-wise alignment of accessions having high leaf density revealed that 83.87 per cent of them belonged to either *C. chinense* or *C. frutescens*. There was no significant relationship between species type and leaf pubescence.

Purple coloured stem was rare (11.77%) among accessions belonging to *C. frutescens*. Accessions with purple coloured stem constituted 35.19 and 44.68 per cent of *C. annuum* and *C. chinense*, respectively. All the *C. frutescens* accessions studied were devoid of purple pigmentation on the nodes. However, nodal anthocyanin pigmentation was present in 55.56 and 25.53 per cent of *C. annuum* and *C. chinense* accessions, respectively. Sparsely pubescent stem was predominant in *C. frutescens*. Only 14.71 per cent of *C. frutescens* accessions were densely pubescent. About 90.57 per cent of accessions with densely pubescent stem belonged to either *C. annuum* or *C. chinense*.

No significant difference was observed among the species with respect to pigmentation on calyx. About 96.30 per cent of *C. annuum* types either had white or purple coloured corolla. Accessions of *C. frutescens* were exclusively greenish white or greenish yellow. Among the accessions with green or yellow coloured corolla, 97.47 per cent belonged to either *C. chinense* or *C. frutescens*. Rotate corolla was predominant across species. Companulate shaped corolla was present in 9.30, 38.10 and 15.15 per cent of accessions belonging to *C. annuum*, *C. chinense* and *C. frutescens* species, respectively. Across the species, purple coloured anther lobe was predominant. Most of the *C. annuum* accessions (97.67%) were characterized with either blue or purple anther lobe. *C. frutescens* also expressed similar trend with 90.91 per cent of accessions expressed either blue or purple anther lobe. Proportion of blue and purple anther lobe among *C. chinense* accessions were 23.81 and 45.24 per cent, respectively. Filament colour of *C. annuum* accessions were either white (66.67%) or purple (31.48%). Distribution of white filament colour was rare among *C. chinense* and *C. frutescens*, as only 12.77 and 5.88 per cent of accessions, respectively of the above two species expressed that trait.

Fruit calyx was having an entire margin in 87.23 per cent of *C. chinense* accessions and 20.37 per cent of *C. annuum* accessions. *C. frutescens* accessions had no inclination to any type of fruit calyx margin. Presence of anthocyanin pigmentation on immature fruits was observed among 38.24 per centof *C. chinense* accessions. However, only 12.50 per cent of *C. annuum* and 9.68 per cent of *C. frutescens* accessions expressed this trait on immature fruit. Rough or wrinkled seed surface was expressed by 97.87 and 97.06 per cent of accessions belonging to *C. chinense* and *C. frutescens*, respectively (Fig.1). *C. annuum* accessions did not show any specific relationship to kind of seed surface as accessions with smooth as well as rough and wrinkled type seeds were distributed in nearly equal proportions.

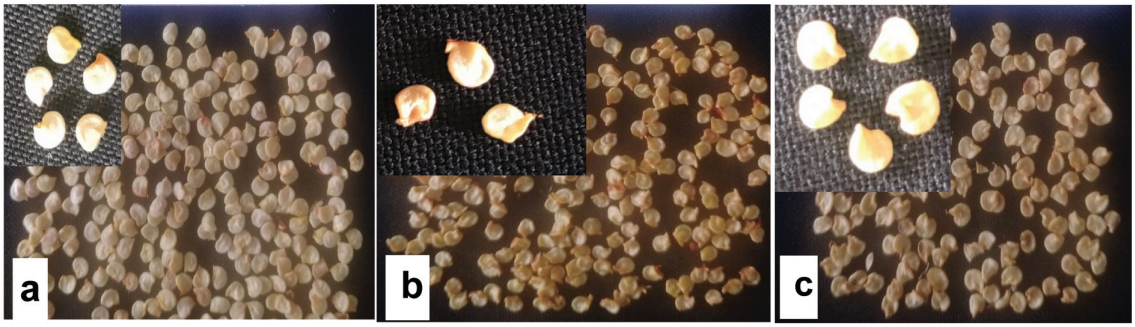

Figure 1. Seeds of *Capsicum* spp. Inset figure shows enlarged view. **a**. *C. annuum* **b**. *C. chinense* **c**. *C. frutescens*

Majority of *C. chinense* and *C. frutescens* accessions expressed similar phenotypic states for hypocotyl pubescence, leaf shape, filament colour and seed surface. All the accessions of *C. chinense* and *C. frutescens* were characterized with absence of anthocyanin pigmentation on leaves. White hypocotyl and undulate leaf margin were found to be restricted to the above two species. Similarity in the above characters among *C. chinense* and *C. frutescens* accessions points to the close genetic relationship of the two species. Earlier studies also reported that *C. chinense* and *C. frutescens* were sympatrically distributed in Peru, Brazil, Colombia and Bolivia (Baral and Bosland, 2004). Eshbaugh (1970) and Pickersgill (1971) postulated the origin of *C. chinense* from *C. frutescens* gene pool through repeated backcrosses. Genetic similarity between these two species was also evident from the gene introgressions and hybrid forms between them. The pepper variety 'Green leaf Tabasco' and world's hottest pepper 'Bhutjolokia' are the results of introgressive hybridization between the above two species (Baral and Bosland, 2004; Bosland and Baral, 2007). Previous studies also confirmed the genetic similarity between the two species at morphological (Borgohain et al., 2005; Sudre et al., 2010), biochemical (McLeod et al., 1979; Jensen et al., 1979; Figueroa et al., 1989) and DNA levels (Sanatombi et al., 2010; Thul et al., 2012; Carvalho et al., 2014).

Characters that shared similar phenotypic states among *C. annuum* and *C. chinense* were anthocyanin pigmentation on cotyledon and nodes, and intensity of pubescence on stem. These characters recorded varied tissue-specific expression governed by gene interaction and influenced by environmental factors (Shuh and Fontenot, 1990; Chaim et al., 2003; Gonzalez et al., 2012; Ying et al., 2018; Jung et al., 2019).

ii. Species-wise temporal and spatial gene expression pattern

Species-wise expression profile of anthocyanin pigmentation at nine different growth stages is depicted graphically in Fig. 2. Species level differentiation for anthocyanin expression was observed at all stages of growth, except in calyx pigmentation. At various growth stages, the proportion of *C. frutescens* accessions with anthocyanin pigmentation varied from zero to fifty per cent. None of the *C. frutescens* accessions exhibited any anthocyanin pigmentation in the cotyledon, leaf, node or petal. Petals of the *C. frutescens* were devoid of anthocyanin pigmentation whereas half of *C. frutescens* accessions had filaments with varying levels of deposition of the pigment. Except in mature leaves, all the other growth stages expressed anthocyanin pigmentation in a considerable proportion of accessions belonging to *C. chinense*. Proportion of anthocyanin pigmentation varied from 44.68 per cent on hypocotyl and stem, to zero per cent in mature leaf in *C. chinense*. Anthocyanin pigmentation was there at all stages of growth in *C. annuum* and the level of expression varied from 9.26 per cent in mature leaves to 55.56 per cent in nodes. Irrespective of species relationships, anthocyanin pigmentation was

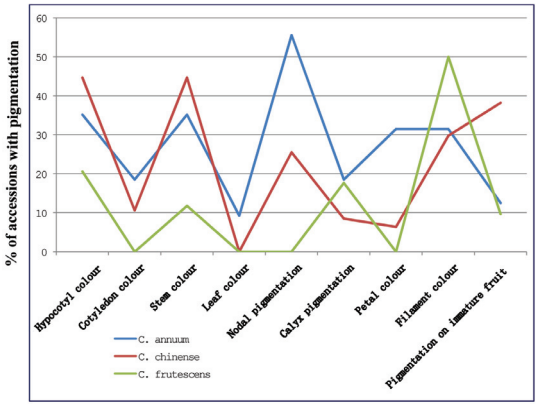

Figure 2. Anthocyanin pigmentation pattern in *Capsicum* accessions at nine growth stages

Figure 3. Various intensities of anthocyanin pigmentation in leaves of accession Ca180 at different growth stages: **a**. two leaf stage **b**. seedling stage **c**. flowering stage

least expressed by mature leaves. With respect to anthocyanin pigmentation on petal and immature fruits, *C. chinense* showed an increase in the proportion of accessions from flowering to fruiting stage. However, *C. annuum* accessions expressed a decreasing trend during this period.

Species level difference in the intensity of anthocyanin pigmentation was observed during growth stages. This change was more evident in *C. frutescens* as half of the accessions of that species having purple coloured hypocotyl failed to express purple pigmentation on the stem. The stem colour was evaluated at transplanting stage (one month after sowing). Accessions of *C. annuum* and *C. chinense* with purple coloured hypocotyl expressed anthocyanin pigmentation on the stem also, even though with reduced intensity. Among the *C. annuum* and *C. chinense* accessions having purple coloured stem, 57.90 and 14.29 per cent, respectively exhibited relatively lower deposition of anthocyanin compared to chloroplast pigment. Hence, these accessions had green coloured stem with purple stripes. The reduction in intensity of anthocyanin pigmentation on stem was the highest in *C. frutescens*, followed by *C. annuum* and *C. chinense*. All the *C. frutescens* accessions exhibited green cotyledon colour. Mature leaf colour observed two months after sowing (one month after transplanting) revealed that from cotyledon to normal leaf stage, anthocyanin expression was switched off in the majority of accessions. This switching off was more prevalent in *C. chinense* compared to *C. annuum*. All *C. chinense* accessions having purple cotyledon in the seedling stage produced green coloured leaves at vegetative phase. However, a similar changing trend was limited to half of *C. annuum* accessions (Fig. 3). *C. annuum* accessions having purple hypocotyl and purple pigmented stem, continued to express purple colour on nodes (Fig. 4). Accessions belonging to *C. chinense,* expressed further reduction in the intensity of anthocyanin pigmentation, as the percentage of accessions with nodal anthocyanin pigmentation was lesser than those having anthocyanin

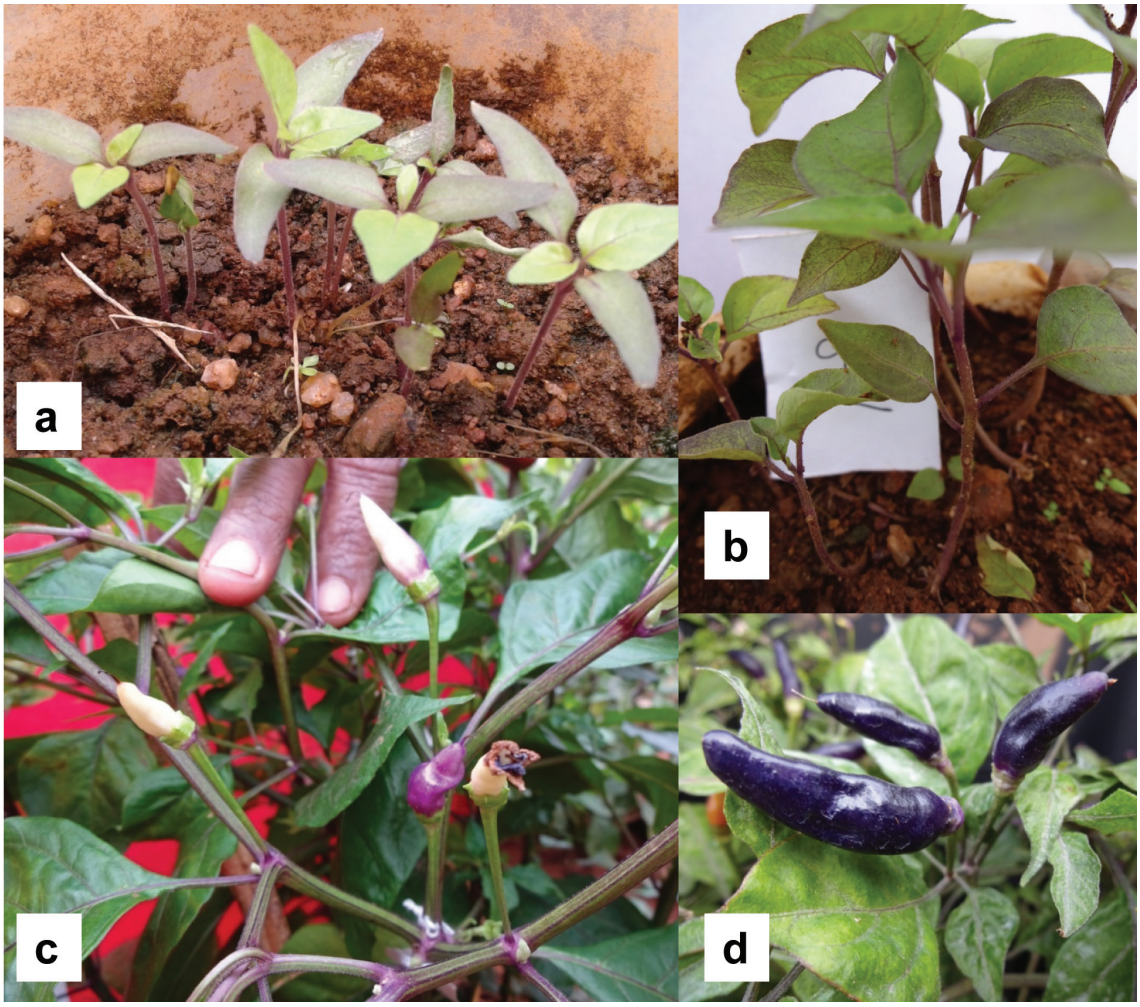

Fig. 4. Varying levels of anthocyanin pigmentation on stem, leaves, nodes, calyx and fruits in accession Ca 9. **a.** 4-leaf seedling **b**. one month old seedling ready for transplanting **c**. fruiting stage **d**. fruits just before ripening

pigmentation on stem.

Anthocyanin pigmentation in chilli is controlled by an incompletely dominant gene on chromosome 10 with two alleles *viz*., locus *A* and *Fc*. Locus *A* controls anthocyanin pigmentation on foliage, flower and fruit whereas locus *Fc* controls anthocyanin pigmentation of anther filament (Chaim et al., 2003). These alleles are influenced by regulatory genes, modifiers and transcription factors (TFs) which in turn are affected by light and temperature (Ying et al., 2018). Increased anthocyanin pigmentation was observed in tissues with increased expression of these TFs except in leaves (Gonzalez et al., 2012). Involvement of a large number of gene sequences and environmental influence makes anthocyanin expression a complex trait in chilli. Jung et al. (2019) identified non-LTR retrotransposon promoted anthocyanin pigmentation in chilli by activating the transcription of TFs. It is pertinent to mention here that 81 per cent of the chilli genome is composed of transposable elements (TEs) which are well known for chromosome breaks and interchanges (Qin et al., 2014). Regulatory genes, modifiers, TEs and TFs are responsible for varied tissue-specific expression patterns of anthocyanin pigmentation. Present study throws light on the fact that locus *Fc*

has more representation in *C. frutescens*. However, *C. annuum* and *C. chinense* had more representation of locus *A*. Ortiz et al. (2010) suggested purple coloured anther-filament as a distinguishable character of *C. frutescens*. Total absence of anthocyanin pigmentation in the leaves observed in the present study among *C. chinense* and *C. frutescens* accessions may be due to the leaf-specific negation of association between TFs and anthocyanin production (Gonzalez et al., 2012). Present study reveals that the above negation is less prominent in *C. annuum*.

Species-wise pubescence expression pattern was analyzed on hypocotyl, leaf and stem. Chi square analysis recorded significant association of pubescence expression and species at hypocotyl and stem. Most accessions of *C. frutescens* had highly pubescent hypocotyl but sparsely pubescent stem. Reverse trend was predominant in *C. annuum* as many accessions of that species having sparsely pubescent hypocotyl developed highly pubescent stems. Accessions belonging to *C. chinense* did not show any major change in the proportion of highly pubescent types at both stages.

Based on an inter-specific cross between *C. chinense* and *C. annuum*, Shuh and Fontenot (1990) postulated that pubescence is controlled by the interaction of two incompletely dominant genes in chilli and pubescence is dominant over non-pubescence. Present study indicated the species specific environmental influence on these genes in pubescence expression. Prevailing environmental factors increased the pubescence expression from hypocotyl stage to stem in *C. annuum*, whereas the same factors resulted in reduction in expression of same genes in *C. frutescens*. Accessions belonging to *C. chinense* were relatively unaffected by these factors. Even though the actual factors responsible for temporal and spatial change in pubescence pattern is not known, present study pointed out differential response of cultivated species towards these factors.

iii. Taxonomic key based on supporting vegetative characters

In the present study, white coloured hypocotyl and undulate leaf margin were restricted to *C. chinense* and *C. frutescens.* However, these traits were absent in *C. annuum*. Character states like densely pubescent hypocotyl, ovate or deltoid leaves and wrinkled seed surface were mostly associated with *C. chinense* and *C. frutescens* accessions. Earlier researchers also reported ovate leaves and wrinkled seed surface were associated with *C. chinense* (Smith and Heiser, 1957; Eshbaugh, 2012). All the accessions of *C. chinense* and *C. frutescens* are found to be characterized with the absence of anthocyanin pigmentation on leaves. These characters could be used to distinguish *C. chinense* and *C. frutescens* accessions from *C. annuum*. Similarly, anthocyanin pigmentation on the cotyledon, nodal anthocyanin pigmentation and densely pubescent stem could be used to demarcate *C. annuum* and *C. chinense* accessions from *C. frutescens*. In the present study, it was observed that most of *C. chinense* accessions had densely pubescent stem where as Smith and Heiser (1957) reported glabrous stem for that species. Lanceolate leaf, white coloured anther-filament and smooth seeds can be used as the augmenting characters to align the accessions into *C. annuum*. Smith and Heiser (1957) also reported smooth nature of *C. annuum* seeds. Deltoid leaves are limited to *C. frutescens* and can be used as a strong supporting character to identify that species. Among the *C. frutescens* accessions evaluated, anthocyanin pigmentation was totally absent in cotyledon, leaf, node and petal. Character states like yellow anther lobe and entire fruit calyx were closely associated with *C. chinense* in the present study.

Taxonomic key for species alignment into cultivated members of the annuum complex, based on the above supporting taxonomic characters pertaining to seed and seedling stages are furnished below.

1. Seedling with green or purple coloured and sparsely pubescent hypocotyl, lanceolate leaf, entire leaf margin, smooth seeds ———— *C. annuum*

2. Seedling with white or green or purple coloured and densely pubescent hypocotyl, ovate or deltoid shaped leaves, entire or undulate leaf margin, wrinkled seed surface

2a. Purple or green cotyledon, nodal anthocyanin present or absent, densely pubescent stem, ovate leaves, brown or yellow seeds —— *C. chinense*

2b. Green cotyledon, nodal anthocyanin pigmentation absent, sparsely pubescent stem, deltoid leaves, yellow seeds—— *C. frutescens*

Even though these supporting taxonomic characters are not foolproof or supplementary to the key taxonomic traits, they are useful for species alignment especially at seedling and vegetative stage. These traits are helpful when key taxonomic traits are confusing and misleading as in case of pseudo-annular constriction which arise from thickening of fruit base (Smith and Heiser, 1957). Edakkalathur (2018) also reported incidence of pseudo-annular constriction among *C. annuum* accessions.

Conclusion

Temporal and spatial phenotypic expression patterns of anthocyanin and pubescence indicated species specific relative fixation of alleles and differential response to external conditions. Taxonomic key developed based on seed and vegetative characters and similarity in the phenotypic expression pattern among cultivated members of 'the annuum complex' will be helpful in species alignment and understanding genetic structure of cultivated *Capsicum* germplasm.

Acknowledgement

We acknowledge funding received from University Grants Commission, India in the form of Junior Research Fellowship

References

Baral, J. B. and Bosland, P. W. 2004. Unravelling the species dilemma in *Capsicum frutescens* and *C. chinense* (Solanaceae): a multiple evidence approach using morphology, molecular analysis and sexual compatibility. J. Am. Soc. Hortic. Sci. 129 (6): 826-832.

Borgohain, R., Devi, J., and Sarma, R. N. 2005. Intra and interspecific genetic diversity exploration in chilli (*Capsicum* spp.) using morphological and randomly amplified polymorphic DNA markers. Indian J. Agric. Sci. 75 (9): 582-586.

Bosland, P. W. and Baral, J. B. 2007. Bhutjolokia – the world's hottest known chile pepper is a putative naturally occurring interspecific hybrid. Hortsci. 42(2): 222-224.

Bosland, P. W. and Votava, E. J., 2012. Peppers: Vegetable and Spice Capsicums. CABI publishing, New York, 198p.

Carvalho, S. I. C., Ragassi, C. F., Bianchetti, L. B., Reifschneider, F. J. B., Buso, G. S. C., and Faleiro, F. G. 2014. Morphological and genetical relationships between wild and domesticated forms of peppers (*Capsicum frutescens* L. and *C. chinense* Jacquin). Genet. Mol. Res.13 (3): 7447-7464.

Chaim, A.B., Borovsky, Y., Jong, D. W. and Paran, I., 2003. Linkage of the A locus for the presence of anthocyanin and fs10.1, a major fruit-shape QTL in pepper. Theor. Appl. Genet. 106: 889–894. doi:10.1007/s00122-002-1132-9.

Edakkalathur, A. I. 2018. Characterization and taxonomic evaluation of landraces of *Capsicum* spp. In Kerala. PhD (Ag) thesis, Kerala Agricultural University, Thrissur, 177p.

Eshbaugh, W. H. 1970. A biosystematic and evolutionary study of *Capsicum baccatum* (Solanaceae). Brittonia 22: 31-43.

Eshbaugh, W. H. 2012. The taxonomy of the genus *Capsicum*. In: Russo, V. M. (ed.), Peppers: Botany, Production and Uses. CAB International, New York, pp. 14-28.

Figueroa, L. F., Ritland, K., Cancino, L. J. A., and Tanksley, S. D. 1989. Patterns of genetic variation of the genus *Capsicum* (Solanaceae) in Mexico. Plant Syst. Evol. 165: 159-188.

Gonzalez, A. C., Palenius, N. H.G., Alejo, O. N., 2012. Molecular biology of chili pepper anthocyanin biosynthesis. J. Mexican Chem. Soc. 56(1): 93-98.

IPGRI (International Plant Genetic Resources Institute) 1995. Descriptors for Capsicum *(Capsicum spp.)*. International Plant Genetic Resources Institute,

Rome, 48p.

Jensen. R. J., McLeod, M. J., Eshbaugh, W. H., and Guttman, S. I. 1979. Numerical taxonomic analysis of allozymic variation in *Capsicum* (Solanaceae). Taxon 28: 315-327.

Jung, S., Venkatesh, J., Kang, M. Y., Kwon, J. K. and Kang, B.C., 2019. A non-LTR retrotransposon activates anthocyanin biosynthesis by regulating a MYB transcription factor in *Capsicum annuum*. Plant Sci. 287. doi:10.1016/j.plantsci.2019.110181.

McLeod, M. J., Eshbaugh, W. H., and Guttman, S. I. 1979. A preliminary biochemical systematic study of the genus *Capsicum*-Solanaceae. In: Hawkes, J. G., Lester, R. N., and Skelding, A. D. (eds), The biology and taxonomy of the Solanaceae. Academic Press, London, pp. 701-714.

Ortiz, R., Flor, F. D. D. L., Alvarado, G., and Crossa, J. 2010. Classifying vegetable genetic resources – a case study with domesticated *Capsicum* spp. ScientiaHorticulturae 126: 186-191.

Pickersgill, B. 1971. Relationships between weedy and cultivated forms in some species of chili peppers (Genus *Capsicum*). Evol. 25(4): 683-691.

Qin, C., Yu, C., Shen, Y., Fang, X., Chen, L., Min, J., Cheng, J., Zhao, S., Xu, M., Luo, Y., Yang, Y., Wu, Z., Mao, L., Wu, H., Ling-Hu, C., Zhou, H., Lin, H., Gonzalez-Morales, S., Trejo-Saavedra, D. L., Tian, H., Tang, X., Zhao, M., Huang, Z., Zhou, A., Yao, X., Cui, J., Li, W., Chen, Z., Feng, Y., Niu, Y., Bi, S., Yang, X., Li, W., Cai, H., Luo, X., Montes-Hernandez, S., Leyva-Gonzalez, M. A., Xiong, Z., He, X., Bai, L., Tan, S., Tang, X., Liu, D., Liu, J., Zhang, S., Chen, M., Zhang, L., Zhang, Y., Liao, W., Zhang, Y., Wang, M., Lv, X., Wen, B., Liu, H., Luan, H., Zhang, Y., Yang, S., Wang, X., Xu, J., Li, X., Li, S., Wang, J., Palloix, A., Bosland, P. M., Li, Y., Krogh, A., Rivera-Bustamante, R. F., Herrera-Estrella, L., Yin, Y., Yu, J., Hu, K., and Zhang, Z., 2014. Whole genome sequencing of cultivated and wild pepperrs provides insights into *Capsicum* domestication and specialization. PNAS 111(14): 5135-5140.

Sanatombi, K., Mandi, S. S., and Sharma, G. J. 2010. DNA profiling of *Capsicum* landraces of Manipur. Scientia Horticulturae124: 405-408.

Shuh, D. M. and Fontenot, J.F., 1990. Gene Transfer of Multiple Flowers and Pubescent Leaf from *Capsicum chinense* into *Capsicum annuum* Backgrounds. J. American Soc. Hortic. Sci. 115: 499–502.

Smith, G. P. and Heiser, C. B. Jr. 1951. Taxonomic and genetic studies on the cultivated peppers, *Capsicum annuum* L. and *Capsicum frutescens* L. Am. J. Bot. 38(5): 362-368.

Smith, G. P. and Heiser, C. B. Jr. 1957. Taxonomy of *Capsicum sinense*Jacq. and the geographic distribution of the cultivated *Capsicum* species. Bull. Torrey Bot. Club. 84(6): 413-420.

Sudre, C. P., Goncalves, L. S. A., Rodrigues, R., Junior, A. T. D., Riva-Souza, E. M., and Bento, C. D. S. 2010. Genetic variability in domesticated *Capsicum* spp. as assessed by morphological and agronomic data in mixed statistical analysis. Genet. Mol. Res.9(1): 283-294.

Thul, S., Darokar, M. P., Shasany, A. K., and Khanuja, S. P. S. 2012. Molecular profiling for genetic variability in *Capsicum* species based on ISSR and RAPD markers. Mol. Biotechnol. 51: 137-147.

Ying, L., Yury, T., Rob, S. E., Leo, M. F. M., Richard, V. G. F. and Arnaud, B. 2018. Anthocyanin Biosynthesis and Degradation Mechanisms in Solanaceous Vegetables: A Review. Frontiers in Chemistry 6: Available: https://www.frontiersin.org/article/10.3389/fchem.2018.00052

Zonneveld, M. V., Ramirez, M., Williams, D. E., Petz, M., Meckelmann, S., Avila, T., Bejarano, C., Rios, L., Pena, K., Jager, M., Libreros, D., Amaya, K., and Scheldeman, X. 2015. Screening genetic resources of *Capsicum* peppers in their primary center of diversity in Bolivia and Peru. PLoS ONE. 10 (9): Available: https://doi.org/10.1371/journal.pone.0134663.