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ASSESSMENT OF GENETIC DIVERSITY OF SELECTED *Capsicum chinense* AND *C. frutescens* ACCESSIONS DERIVED THROUGH MORPHOLOGICAL AND MOLECULAR CHARACTERIZATION

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ABSTRACT

***Capsicum chinense* and *C. frutescens* are common cultivated and consumed chilli species in some parts of Sri Lanka. Thirteen *Capsicum* accessions were characterized by morphological and molecular means to assess genetic diversity in plants by randomized complete block design with two replicates during *yala* 2016 and *maha* 2016/17 at the Field Crops Research and Development Institute, Mahailluppallama. Twelve morphological characters were analysed using analysis of variance (ANOVA) and multivariate methods. ANOVA revealed significant differences among genotypes for most of the tested traits. Principal component (PC) analysis explained more than 71% of total variability for the first 3 components among the traits of genotypes evaluated. Plant height, width, days to 50% flowering, pods per plant and yield were positively correlated with PC1. Dendrogram based on morphological and SSRs analyses showed two and three clusters respectively at 0.1 similarity levels and both analysis showed comparable results. A total of 45 alleles were detected in 15 microsatellite markers (M1 to M15) across the 13 *Capsicum* accessions. Out of these 15 SSR loci, 14 loci showed polymorphism. Genetic diversity ranged from 0.00 to 0.75 with an average of 0.51. High allelic richness was observed in M 10 and M 14. The PIC value varied from 0.13 to 0.70 with an average of 0.44. To date molecular characterization data of *Capsicum* accessions in Sri Lanka is limited. Therefore, this study will pave the way for a detailed characterization of *C. chinense* and *C. frutescence* accessions using morphological descriptors and SSR molecular markers.**

Key words: *Capsicum* accessions, Molecular markers, Morphological descriptors

INTRODUCTION

Capsicum chinense and *C. frutescens* are other commonly cultivated and consumed chili species next to *Capsicum annuum* as a spice crop in Sri Lanka for their specific flavour, aroma and pungency. Jing *et al* (*2013*) have explained that the genus *Capsicum* is a member of the Solanaceae family and consists of five domesticated species*: Capsicum annuum, C. baccatum, C. chinense, C. frutescens and C. pubescens* with approximately 33 species. Perez *et al* (2014) have explained that through domestication process *C. annuum* was the most successful. *C. chinense* and *C. frutescens* became also popular in Africa and Asia. Whereas *C. baccatum* and *C. pubescens* mostly remain in South America. Ahamed *et al* (2000) have shown that during secondary diversification, different species were selected by farmers to fit the diverse agro-climatic environments showing the great phenotypic diversity found at present. It is essential to investigate the relationships among the species at the different secondary diversification centres. Germplasm can be characterized based on morphological descriptors, agronomic traits, and molecular markers. Standardized *Capsicum* descriptors have developed by the Plant Genetic Resources Centre (PGRC) of the Department of Agriculture. The PGRC descriptors include phenotypic traits for vegetative, flower, fruit, seed and yield characters.

Molecular markers became of preference because they are not under the influence of

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environmental conditions or plant development factors. Presently, several groups of *Capsicum* microsatellites, both genomic and EST-based markers, came to be available for diversity studies. Different types of DNA markers, such as RFLPs, RAPDs, AFLPs or microsatellite (SSRs), have been developed and used in *Capsicum* spp to determine the relationships and levels of genetic variation in wild and domesticated *Capsicum* spp. Perez, (2014) has pointed out that SSR markers have emerged as widely-used genotyping markers over the last decade, in plants because of their co-dominance, stability, capacity of multi-allelic detection, easy application and excellent sensitivity, especially between species. Those markers make better understanding of the genetic diversity.

Results of this study will be contributed towards the understanding of the knowledge in genetic diversity pattern in *C. chinense* and *C. frutescens* accessions using morphological descriptors and SSR molecular markers.

MATERIALS AND METHODS

This experiment was conducted in Field Crops Research and Development Institute, Mahailluppallama during *yala* 2016 and *maha* 2016/17. A total of thirteen *Capsicum* accessions including *Capsicum chinense* and *C. frutescens* (accession KH1 to KH13) were evaluated using morphological and molecular markers to study the genetic diversity of germplasm (Table 1). The experiment was laid out in Randomized Complete Block Design (RCBD) with two replicates. The unit plot size was 6 m x 1.8 m consisting of 3 rows with spacing 60 cm x 60 cm. Twelve morphological characters recorded were plant height and breadth (cm), mature leaf length and width (cm), number of days to 50% flowering, corolla colour, stem colour, pod length and girth (cm), pericarp thickness (mm), number of pods per plant and pod yield (t/ha).

DNA isolation

DNA was isolated from tender leaves of each accessions using CTAB method and stored in a -20 ℃ refrigerator. Molecular characterization was done using fifteen microsatellite markers (M1-M15 SSR markers) according to their broad transferability and high polymorphism in different *Capsicum* spp. PCR amplification and detection of microsatellite markers were performed.

Statistical analysis

Analysis of variance (ANOVA) was performed to test variations among genotypes for twelve morphological traits using SAS 9.1. Morphological data were analysed using Statistical software package MINITAB 17 with Multivariate data analytical methods *viz*. principal component and cluster analysis. For molecular analysis POWERMARKER V 3.25 software resulting matrix was employed to generate a dendrogram based on Unweighted Pared Group Method with Arithmetic Average (UPGMA).

RESULTS AND DISCUSSION

Analysis of variance revealed significant differences among genotypes for plant height,

Table 1: Accessions of *Capsicum chinence and C. frutescence:*

No	Source	Accessions	No	Source	Species
1	KH 1	*Capsicum frutescence*	8	KH12	*Capsicum chinence*
2	KH2	*Capsicum frutescence*	9	KH15	*Capsicum chinence*
3	KH 3	*Capsicum frutescence*	10	KH16	*Capsicum chinence*
4	KH 6	*Capsicum chinence*	11	Ho KH	*Capsicum chinence*
5	KH 7	*Capsicum chinence*	12	Hen KH	*Capsicum frutescence*
6	KH 8	*Capsicum frutescence*	13	KH 18	*Capsicum chinence*
7	KH10	*Capsicum chinence*			

plant breadth, mature leaf length and breadth, plant height and breadth, pericarp thickness, and pod yield at 0.05% probability level.

Genetic relationships among *Capsicum* spp. was further investigated using principal component analysis which helps in describing grouping of variables. The first principal component (PC1) is related to morphological characters such as pods per plant, pod weight, pericarp thickness and yield explained 37% of total variability. The second principal component (PC2) is related to stem colour, pod weight and mature leaf breadth explained 21% of total variability. The third principal component (PC3) is related to yield, plant height and days to 50% flowering. The eigenvalues revealed that the first three principal components accounted for 71% of the total genetic variability. Pod characters (pods per plant, pod weight and pericarp thickness), plant height, plant width and yield were recorded higher positive magnitudes (above 0.30) for the PC1. Furthermore, leaf length, leaf width, pod length, width and pericarp thickness was negatively correlated with PC1. While, stem colour and pods weight were recorded high positive magnitude for PC2 and leaf length and leaf breadth recorded high negative magnitude for PC2.

The dendrogram derived based on Pearson distance displays the relative positions of *Capsicum* genotypes scored on morphological traits. There were two clusters at 0.1 similarity level. Accessions of *Capsicum chinense* and *Capsicum frutescence* were grouped in cluster 1 and cluster 2 respectively. It is confirmed that morphological data supported to differentiate accessions at species level. Genetic diversity within closely located accessions is lower than that of distantly located ones. The distance parents would be assisted to different genetic constitution that can be utilized for future breeding programmes. For inter-specific crosses the accessions of cluster 1; *C. chinense*, could be combined with accessions in cluster 2 belonging to *C. frutescence* to have new genetic makeup.

According to molecular analysis a total of 45 alleles were detected at 15 microsatellite markers across the 13 *Capsicum* accessions. The accessions of same species were clustered together and this indicated that ability to use of SSR markers to distinguish *Capsicum* accessions. Out of these 15 SSR loci, 14 loci showed polymorphism with a total of 45 alleles, with an average of 3.0 alleles per locus. High allelic richness was observed in M 10 and M 14 (Figure 1). Less allelic richness was observed in M 7. Allele richness varied from 1 to 6 alleles across tested accessions. A high level of genetic diversity existed among 15 loci studied across 13 *Capsicum* accessions. It ranged from 0.00 to 0.75 with an average of 0.51. The PIC value of each marker could be evaluated on the basis of the allele frequencies. It varied from 0.13 to 7.0 with an average of 0.44.

In the dendrogram based on molecular analysis (Figure 3), all the accessions were grouped into 3 main clusters at 0.1 similarity level. In

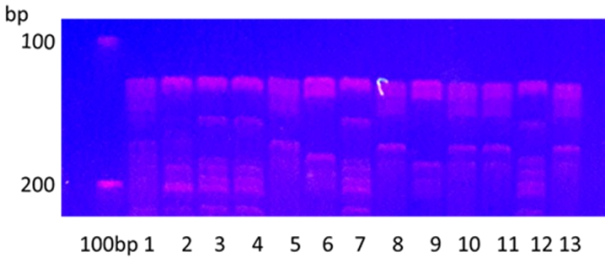

Figure 1: Acrylamide Gel Electrophoresis for 13 Capsicum accessions for Markers 10

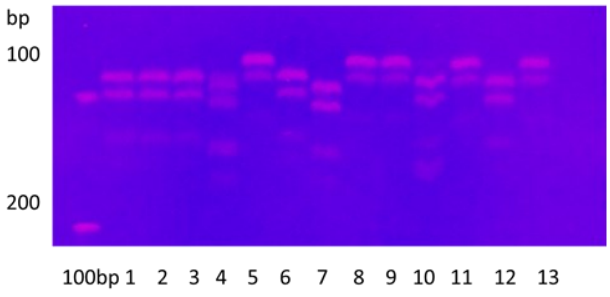

Figure 2: Poly Acrylamide Gel Electrophoresis for 13 Capsicum accessions for Markers 14

the first cluster, accession KH2 which belong to *Capsicum chinense* and KH3 which belong to *C. frutescence* grouped together showing close genetic relationship though those belong to different species. Most of the *Capsicum chinense* accession except KH 10 were grouped in cluster 2. Most of the *C. frutescence* except KH 1 was grouped in cluster 3. Only a few displacements were observed, some accessions not being grouped into clusters that corresponded to their morphological classification. Perez *et al* (2014) have shown that the cultivated *C. annuum* accessions were clustered together in a single group. The other domesticated species *C. chinense, C. frutescens, C. pubescens* and *C. baccatum* were separated clearly into distinct branches supported by high values of bootstrap (>80%). The *C. chinense* accessions were visibly separated from *C. frutescens* accessions. Jing *et al* (2013) have shown that multivariate and model-based analyses partitioned the collection in seven clusters comprising the ten different *Capsicum* spp analysed*: C. chinense, C. frutescens*, the data clearly showed the close relationships between *C. chinense* and *C. frutescens*.

Dendrogram based on morphological analysis (Figure 4) confirms the pattern that is found in the molecular analysis except for few accessions. Even though accessions identified with some similarities as they evolve through a long process under different environmental condition hence genetic divergence can occur. Morphological characteristics were essential for the evolution process. There was also an association between the morphological descriptors and SSR markers. This study suggests that both characterization steps are important for understanding and differentiating the *C. chinense* and *C. frutescence* accessions.

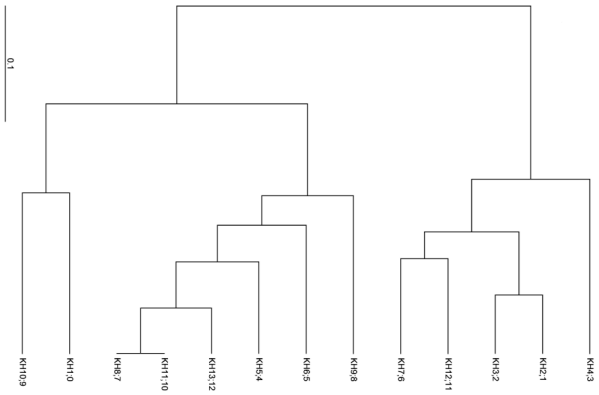

Figure 3: Dendrogram based on molecular analysis

CONCLUSION

Assessment of diversity with respect to quantitative traits such as pods per plant, pod weight, pericarp thickness and pod yield will help for identifying parental materials. SSR markers with high allelic richness (M 10 and M 14) can be used for future *Capsicum* characterization programs. Dendrogram derived based on molecular analysis confirmed the result of morphological analysis. The combination of morphological and molecular analyses is suggested for understanding and differentiating the *C. chinense* and *C. frutescence* accessions.

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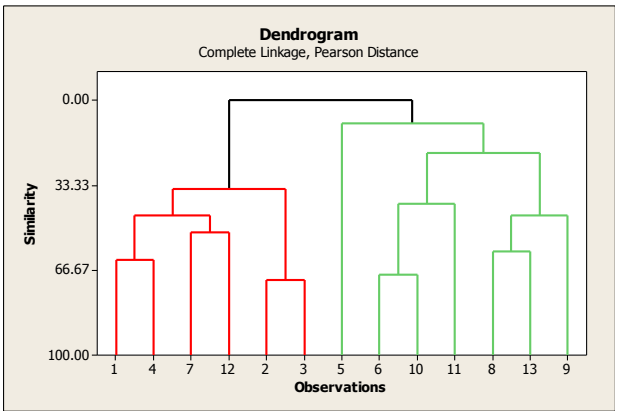

Figure 4: Dendrogram based on 12 morphological characters in 13 *Capsicum* genotypes

Table 2: List of primers, sequences and references

Primer (SSR)	Pack Size	Nucleotide	Sequence (5'-3')	References
M1. CAMS -405	25nmole	20	TTCTTGGGTCCCACACTTTC	Tilahun *et al*.,Asian Journal of Agricultural Sciences 5(2) : 25-31,2013
	25nmole	20	AGGTTGAAAGGAGGGCAATA	
M2. CAMS -864	25nmole	21	CTGTTGTGGAAGAAGAGGACA	Tilahun *et al*.,Asian Journal of Agricultural Sciences 5(2) : 25-31,2013
	25nmole	22	GCTTCTTTTTCAACCTCCTCCT	
M3. GPMS -113	25nmole	20	GCACAAGTCAATCCAAACGA	Tilahun *et al.*,Asian Journal of Agricultural Sciences 5(2) : 25-31,2013
	25nmole	23	CAAAAAGATGATGATGATGGATGAGA	
M4. GPMS -161	25nmole	23	CGAAATCCAATAAACGAGTGAAG	Tilahun *et al*.,Asian Journal of Agricultural Sciences 5(2) : 25-31,2013
	25nmole	22	CCTGTGTGAACAAGTTTTCAGG	
M5. GPMS -197	25nmole	22	GCAGAGAAAATAAAATTCTCGG	Tilahun *et al.,*Asian Journal of Agricultural Sciences 5(2) : 25-31,2013
	25nmole	20	CAATGGAAATTTCATCGACG	
M6. CAMS -142	25nmole	21	GAGCGCTTAAGTGGTCATAGG	Patel *et al* ., Electronic Journal of Plant Breeding, 2 (1): 67-76 (Mar 2011)
	25nmole	20	CTACAACGCCCCAAAACAAT	
M7. CAMS -153	25nmole	22	TGCACAAATATGAATCCCAAGA	Patel *et al* ., Electronic Journal of Plant Breeding, 2 (1): 67-76 (Mar 2011)
	25nmole	23	AAGTCAGCAAACACATCTGACAAA	
M8. CAMS -403	25nmole	20	TTCTTGGGTCCCACACTTTC	Patel *et al* ., Electronic Journal of Plant Breeding, 2 (1): 67-76 (Mar 2011)
	25nmole	20	AGGTTGAAAGGAGGGCAATA	
M9. GPMS 1	25nmole	18	CCCTAATGCTTGACGTGG	Nagy *et al.,* Agricultural Biotechnology Center , SZent -Gyo rgyi Albert u. 4, H-2100 Go do IIo", Hungary
	25nmole	17	GGTTAAGGGGGTTGGGG	
M10. GPMS6	25nmole	20	CAGAGCACTTGACATGCCTT	Nagy *et al.,* Agricultural Biotechnology Center , SZent -Gyo rgyi Albert u. 4, H-2100 Go do IIo", Hungary
	25nmole	24	GATCTTTATAGTAGCTCATCAATA	
M11. GPMS166	25nmole	20	AAAACCGACACACCAAAAGC	Nagy *et al.,* Agricultural Biotechnology Center , SZent -Gyo rgyi Albert u. 4, H-2100 Go do IIo", Hungary
	25nmole	20	CCCTAGTTTCCGTTGCAGAG	
M12. GPMS178	25nmole	24	GATTTTTGACATGTCACATTCATG	Nagy *et al.,* Agricultural Biotechnology Center , SZent -Gyo rgyi Albert u. 4, H-2100 Go do IIo", Hungary
	25nmole	25	AACGTTGAAAAATAAAGTAAGCAAG	
M13. GPMS194	25nmole	20	AGGTGGCAGTTGAGGCTAAG	Nagy *et al*., Agricultural Biotechnology Center , SZent -Gyo rgyi Albert u. 4, H-2100 Go do IIo", Hungary
	25nmole	20	GTTCTAGGTCTTTGCCCTGG	
M14. EP-MS331	25nmole	20	AACCCAATCCCCTTATCCAC	Nagy *et al*., Agricultural Biotechnology Center , SZent -Gyo rgyi Albert u. 4, H-2100 Go do IIo", Hungary
	25nmole	20	GCATTAGCAGAAGCCATTTG	
M15. CAK24	25nmole	20	AAACGTCATCACAGCCATCA	Kong *et al.,*American Journal of Botany : 59- e61.2012
	25nmole	20	CGTAACGCACCCTCTAGGAA	

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