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Full Length Article

Assessment of Genetic Diversity in Local Chilli (*Capsicum annuum*) Varieties in Mauritius

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Abstract

The genetic diversity of five chilli (*Capsicum annuum* L.) varieties cultivated in Mauritius was evaluated using morphological and molecular techniques. Morphological assessment was done on fruit, leaf, height and canopy size of the plant. Morphological characters, namely from the fruit can be used as reliable morphological markers to distinguish between the different varieties. The DNA was amplified using the RAPD technique. Primer OPW04 showed the highest degree of polymorphic bands. Overall, RAPD markers can be used to differentiate between the local chilli varieties.

Keywords: Chilli; RAPD; Genetic diversity; Polymorphism

Introduction

Capsicum annum L. (commonly known as hot pepper or chilli pepper) is a dicotyledonous flowering plant which belongs to the family Solanaceae (Knapp, 2002). Chilli is an important commercial crop cultivated exclusively in tropical and temperate zones of the world and grown on more than 1.5 million hectares worldwide (FAO, 2007). Chilli are usually classified based on fruit characteristic, including pungency, color, shape, flavor, size and use (Bosland, 1992, 1994).

Long term sustainable yield and buffering capacity of a crop species to the spread of pest and diseases depends on the infield, genetic divergence of the various cultivars. Breeding for the cultivars that share similar pedigree, morphological traits or molecular variability tends to narrow the genetic diversity (Rauf *et al.,* 2010; 2012).

Traditionally, the characterization and evaluation of chilli varieties has been established by a combination of morphological and agronomic traits. However, this approach is prone to environmental influences. Cytological or biochemical markers have also been used to evaluate the genetic diversity within germplasm (Kaur and Kapoor, 2001).

Recent developments in DNA based technologies have brought about a radical change in the utilization of molecular markers in genetics and breeding studies. Present research was carried out to analyze the genetic diversity in *Capsicum* germplasm using Randomly Amplified Polymorphic DNA (RAPD) primers. The use of RAPD was easier, cheaper, quicker more user friendly, and no prior sequence information on the target genome was required (Williams *et al*., 1990).

Prince *et al.* (1995) used RAPD analysis to assess the fingerprinting of pepper cultivars and allowed the discrimination of closely related *Capsicum annum* genotypes, while the same technique was used to assess genetic diversity among Capsicum genotypes (Akbar *et al.*, 2010). Wang *et al*. (1996) surveyed 14 diverse pepper (*Capsicum* spp.) accessions by RAPD analysis and this technique was useful for differentiating these species. Pawar (2000) studied the genetic diversity among and within three cultivars of chilli at the morphological level and polymorphism was observed at the molecular level. Genetic diversity among commercial chilli varieties has also been carried out (Makari *et al*., 2009).

No molecular study has been reported on the chilli varieties cultivated in Mauritius. The aim of this study was to distinguish between the chilli varieties in Mauritius based on their morphological characteristics. Genetic relatedness among these chilli varieties was assessed using RAPD markers to find out if this technique was sensitive enough to capture the existing heterogeneity within the local chill germplasm. A molecular study could contribute in characterizing and evaluating genetic diversity among chilli varieties in Mauritius since plant breeders are willing to distinguish between their varieties and to validate their uniqueness. During this study, comparison based on morphology and molecular characterization was carried out using five selected local chilli varieties available in different chilli cultivation fields in Mauritius (mainly the northern region and in the region of PlaineWilhems). The varieties used were ‘Long chilli’, ‘Small chilli’, ‘Piment carri’, ‘Piment blanc’ and ‘Piment petard’.

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Materials and Methods

Plant Material

Young, fresh and tender leaves of the five different selected chilli varieties were collected in the Northern region of the country and in the region of Plaine Wilhems. 'Long chilli' was collected at St François, 'Piment carri' at Vale, 'Piment blanc' at Rose-hill and 'Piment petard' and 'Small chilli' at Petit Raffray, respectively. Once collected, the leaves were wrapped with moist tissue paper and placed in labeled plastic bags and kept away from sunlight. All experiments were carried out from August 2010 to March 2011 in the Molecular Biology laboratory, Faculty of Agriculture, The University of Mauritius.

Morphological Characterization

The morphological characterization was carried out based on field observations at different collection sites. The data of the different parameters assessed was collected from 5 different plants for each variety and morphological characterization was done according to the Descriptive Terminology by Agarwal (1996). The parameters used were plant height, plant canopy width, leaf length, leaf width, leaf color, leaf shape, fruit color (young and ripen), fruit length and fruit shape.

DNA Isolation and Purification

Fresh leaf tissue (0.075 g) was ground in liquid nitrogen to form a thin powder, which was transferred to 750 µL of cetyl methyl ammonium bromide (CTAB) extraction buffer (2% CTAB, 5M NaCl, 2% polyvinylpyrrolidone (PVP), 0.5M ethylene diamine tetra acetic acid (EDTA) pH 8, Tris-HCL (Trizma base HCl) pH 8 and 2% β-mercaptoethanol in a centrifuge tube. The tube was then incubated in a water bath at 65°C for about 30 min with occasional swirling. 2/3 volume of chloroform:isoamyl alcohol (24:1, v/v) was added to the tube, which was tilted several times and was centrifuged at 10,000 rpm for 10 min. The aqueous phase was transferred to a new tube. DNA was precipitated by the addition of 2/3 volume of ice-cold isopropanol and the tube was incubated at - 20°C overnight. The tube was centrifuged at 13,000 rpm for 30 min and the precipitated DNA was washed in 70% ice cold ethanol by centrifugation at 13,000 rpm for another 15 min. DNA pellets were then dried and re-suspended in 100 µL of sterile distilled water. RNAse treatment was carried out by adding 1 µL of RNAse to dissolved DNA and kept overnight at 37°C.

RAPD Protocol

The RAPD reactions were always carried out on ice to prevent degradation of reagents used. The master mixes for the total number of tubes were prepared in a 1.5 mL eppendorf. Each 25 µL master mix consisted of a final molarity of 1 X reaction buffer, 1.5 mM $MgCl_2$, 0.2 mM dNTPs, 0.5 µM primer, 40 ng DNA template and 0.016 U *Taq* DNA polymerase and made up to the required final volume with nanopure water. The master mixes were aliquoted in their respective labeled PCR tubes to which the diluted DNA samples of all the varieties were already added, except the one used as negative control. The PCR tubes were centrifuged at 2000 rpm for a few seconds using the quick run function to ensure proper mixing. The RAPD–PCR reaction was carried in a BioRad thermal cycler. The cycle conditions consisted of 1 cycle involving an initial denaturation step at 95°C for 90 sec, 40 cycles including a denaturation process at 92°C for 30 sec, a primer annealing step at 35°C for 1 min and a step for DNA amplification at 72°C for 3 min. Thereafter, a final delay cycle for primer extension was run at 72°C for 10 min and 15°C for 5 min. These reaction products were then run on 1.5% agarose gel at 90 V and viewed under ultra violet light after staining with ethidium bromide.

RAPD Profile Analysis

Sixty primers were used to evaluate genetic diversity and maximum polymorphism was observed with 12 random primers including OPA10, OPA18, OPP20, OPB11, OPL05, OPD13, OPK05, OPW04, OPC03, OP003, OPC16, and OPC08. Each genotype was characterized by its banding pattern using the DNA hyperladder 2 (Bioline) as base pair ladder. The RAPD markers as viewed from the gels after electrophoresis and staining were converted into a matrix of binary data, where the presence of the band corresponded to value 1 and the absence to value 0. The statistical software NTSYS-PC (Rohlf, 2005) and DARwin 5 software (Perrier and Jacquemoud-Collet, 1996) were used to construct a UPGMA dendrogram using hierarchical clustering. Using NTSYS software, a dissimilarity matrix was calculated utilizing Jaccard (1908) coefficient. The matrix was converted to a dissimilarity matrix corresponding to the complement (dissimilarity = 1 − similarity). Cluster analysis based on the dissimilarity matrix, was performed using un-weighted pair group method arithmetic averages (UPGMA) (Sneath and Sokal, 1973) of the NTSYS–PC version 2.2 (Rohlf, 2005).

Results and Discussion

Morphological Characterization of Five Different Chilli Varieties

A high degree of similarity was observed among the chilli varieties and these include plant characteristics and leaf characteristics. In contrast, there appeared to be greater variation in the fruit traits (Fig. 1–5). Each fruit character had a large number of character states with many intermediate gradations between extremes.

'Small chilli', 'Piment carri' and 'Piment blanc' share

Table 1: Assessment of the morphological features of the five selected chilli varieties

Parameters	'Long Chilli'	'Small Chilli'	'Piment Carri'	'Piment Blanc'	'Piment Petard'
Plant height (cm)	90-100	32-45	40-45	35-40	30-43
Plant canopy width (cm)	70-75	45-55	50-55	40-45	42-48
Leaf length (cm)	8.0-10.5	4.5-6.5	5.5-6.0	2.5-8.0	4.0-6.5
Leaf width (cm)	2.5-3.5	2.5-3.0	2.0-3.0	2.0-4.0	2.5-3.0
Leaf color	Green	Light Green	Green	Dark green	Green
Leaf Shape	Lanceolate	Ovate	Ovate	Ovate	Lanceolate
Fruit color (young)	Green	Green	Green	White	Green
Fruit color (Ripen)	Red	Red	Red	Reddish pink	Red
Fruit length (cm)	8.0-10.0	4.0-5.0	6.0-8.0	2.8-3.4	5.0-6.0
Fruit shape of longitude section	Narrow triangular	Elongated	Rectangular	Triangular	Elongated

Fig. 1: Fruits of 'long chilli'

Fig. 2: Fruits of 'small chilli'

Fig. 3: Fruits of 'pimentcarri'

Fig. 4: Fruits of 'pimentblanc'

the same leaf shape. 'Long chilli', 'Piment carri' and 'Piment petard' share the same leaf color. 'Long chilli' plant is the highest among all the five chilli varieties and can reach up to one meter, thus making harvesting easier. In accordance with Table 1, the 'Small chilli' variety and the 'Piment petard' variety may share a few similarities in terms of morphological characteristics. It has been noted that these two varieties are almost similar in terms of plant height, plant canopy width, leaf length, leaf width, and fruit color (young and ripen) but differ in their leaf color, leaf shape, fruit length and fruit shape.

Table 2: RAPD markers used for the generation of polymorphism among local chilli varieties

Primer	Primer sequence (5' - 3')	Number of markers	Number of polymorphic markers	% polymorphism
OPW04	CAGAAGCGGA	12	6	50
OPD13	GGGGTGACGA	15	5	33.33
OPB11	GTAGACCCGT	7	2	28.57
OPL05	ACGCAGGCAC	17	5	29.41
OPC03	GGGGGTCTTT	21	5	23.80
Total		72	22	31.94

Table 3: Dissimilarity matrix based on the proportion of shared RAPD fragments among different chilli varieties

	'Long Chilli'	'Small Chilli'	'PimentCarri'	'Piment Blanc'
'Small Chilli'	0.444			
'PimentCarri'	0.994	0.553		
'Piment Blanc'	0.8694	0.597	0.82	
'Piment Petard'	0.994	0.761	0.994	0.8694

Molecular Characterisation

The CTAB extraction protocol successfully yielded DNA (500-600 ng/µL) from all the chilli varieties with a A260/A280 ratio of 1.800–1.900.

Out of the 60 primers used, maximum polymorphism was observed using OPA10, OPA18, OPP20, OPL05, OPK05, OPW04 (Fig. 6), OPD13 (Fig. 7), OPB11 (Fig. 8), OPL05 (Fig. 9), OPC03 (Fig. 10), OP003, OPC16, and OPC08. OPW04 yielded the highest percentage of polymorphism (54.55%), followed by OPC03 (36.84%), OPL05 (23.60%), OPD13 (20%) and OPB11 (14.29%) respectively (Table 2). The primers, which are most suitable to differentiate the varieties from each other, are OPW04 and OPC03 (Table 2).

The 'Piment carri' and 'Piment blanc' variety

Fig. 5: Fruits of 'piment petard'

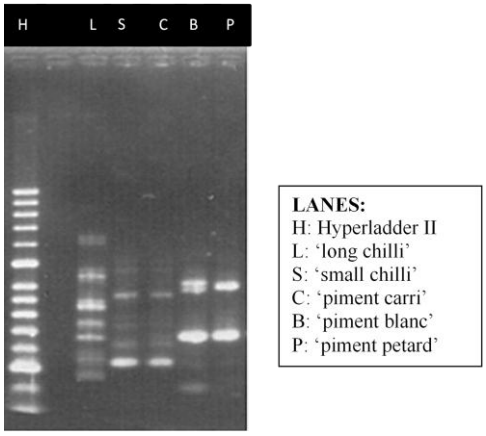

Fig. 6: Amplification products from all five chilli varieties with primer OPW04

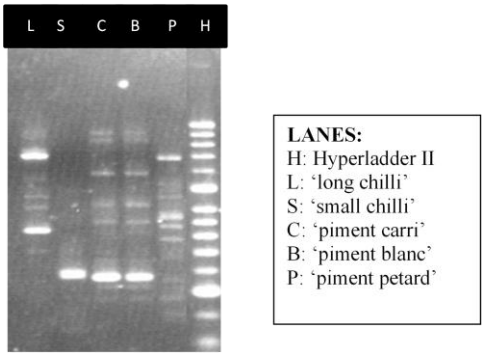

Fig. 7: Amplification products from all five varieties with primer OPD13

clustered together in the dendogram (Fig. 11), showing that they are closely related due to sharing of bands in their DNA profile. A 500 bp marker was shared between 'Piment carri' and 'Piment blanc' with primer OPD13 as shown in Fig. 7. A 800 bp marker was present in 'Piment carri' and 'Piment blanc' with primer OPB11 as seen in Fig. 8. A 300 bp marker was present in 'Piment carri' and 'Piment blanc' with primer OPL05 (Fig. 9).

Dissimilarity values (Table 3) ranged from 0.444 to 0.994. The dendogram had a high goodness of fit and was robust as indicated by the cophenetic ratio of 0.9658 and was hence reliable for the analysis of the genetic relatedness among the chilli varieties.

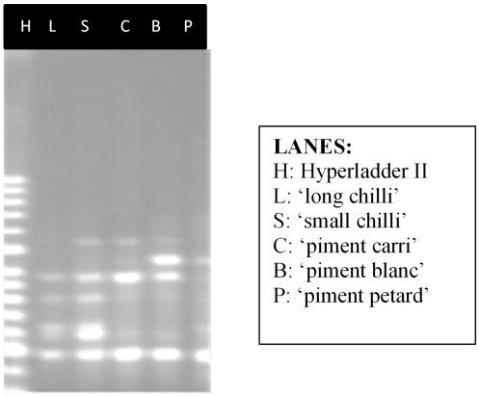

Fig. 8: Amplification products from all five chilli varieties with OPB11

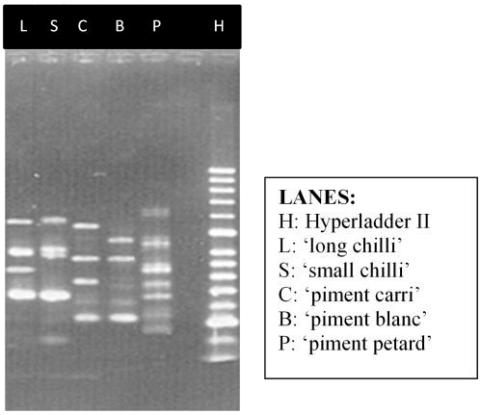

Fig. 9: Amplification products from all five chilli varieties with OPL05

Morphological markers used for comparison of the chilli varieties include parameters such as plant height, plant canopy width, leaf color, leaf size, leaf shape, fruit color, fruit size and fruit shape. Other parameters, which could have been used to increase the efficiency in assessing the morphological markers, are plant growth habit, length of leaf blade, type of flower, shape of fruit apex, stalk length and thickness and time of beginning of flowering. A striking phenotypic difference that helps to differentiate between the five chilli varieties is the fruit shape. Each variety of chilli has its own characteristic fruit shape. The 'Long chilli' fruit is the longest compared to 'Small chilli', 'Piment carri', 'Piment blanc' and 'Piment petard' and it has a narrow triangular shape. Both 'Small chilli' and 'Piment carri' have an elongated fruit shape but 'Piment petard' is pointed at the end. 'Piment carri' can be easily distinguished as it has a rectangular shape. 'Piment blanc' has a triangular shape. All five varieties differ in fruit length. When ripen, only 'piment blanc' is reddish pink in color, while the other four varieties are red.

At the vegetative stage, it is quite difficult to identify the different varieties using parameters such as plant height,

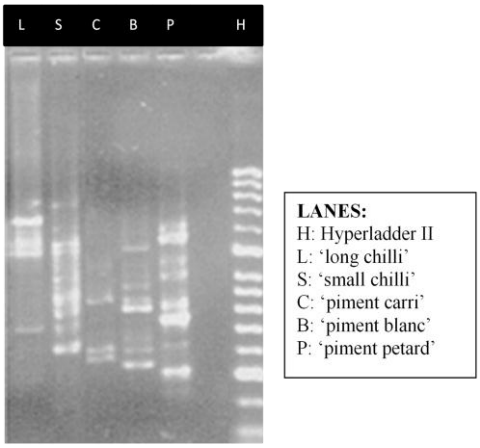

Fig. 10: Amplification products from all five chilli varieties with OPC03

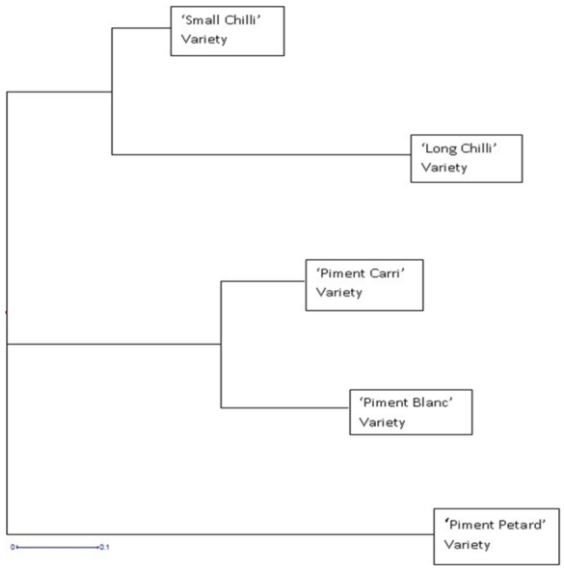

Fig. 11: Dendogram generated using UPGMA cluster analysis showing the relationships and diversity among the chilli varieties

plant canopy width, leaf shape, and leaf size. Therefore, molecular techniques can be used to provide a precise and rapid identification of the different varieties. Morphological characterization is also important as it gives an overall idea on the different chilli varieties thus allowing the collection of the plant material for molecular analysis.

Young, green, and healthy leaves were chosen for DNA extraction in this study due to their low levels of polyphenols, tannins, and polysaccharides with maturity. The development and use of molecular markers for the detection and exploitation of DNA polymorphism is one of the most important developments in the field of molecular genetics (Semagn *et al.*, 2006). Many reports have shown the value of molecular markers like RAPD to study genetic diversity of *Capsicum*. RAPD markers can give robust classification criteria that could be useful in species separation (Sitthiwong *et al.*, 2005). Small differences in amplification efficiencies can give significant differences in the overall product pattern and yield (Bassam *et al.*, 1992).

The 'Piment carri' and 'Piment blanc' variety unexpectedly clustered together despite the large morphological differences like fruit shape, fruit size, fruit color (young and ripen), and leaf color. Very few morphological characters are shared between the two varieties mainly as far as fruit size and fruit color are concerned however, there is evidence for genetic similarity between these two varieties using the RAPD technique.

Surprisingly, the 'Long chilli' and 'Small chilli' variety clustered together despite the major phenotypic differences like plant height, plant canopy width, leaf length, width, color, and shape, fruit size and fruit shape. Very few morphological character such as fruit color (young and ripen) are shared between the two varieties compared to all the other varieties. A 500 bp marker was present in 'Long chilli' and 'Small chilli' with primer OPL05. Though these two species are morphologically dissimilar, the RAPD technique showed that there is homology at the genomic level.

'Piment petard' is a variety which is closer to 'Small chilli' on the basis of morphological traits such as plant height, plant canopy width, leaf color and width, and fruit color (young and ripen). As reflected in the dendogram, 'Piment petard' is seen to be the closest relative of 'Small chilli' and 'Long chilli' than to 'Piment carri' and 'Piment blanc'.

The Jaccard's similarity analysis depicted a good degree of genotypic diversity existing in the chilli genotypes studied. The minimum and maximum similarity values were 0.006 and 0.556. The dendogram reflect a good genetic analysis, which is based on amplification signals from RAPDs proving that it is a good marker to evaluate the genetic relationships among chilli accessions as previously reported (Prince *et al.*, 1995; Wang *et al.*, 1996; Makari *et al.*, 2009; Akbar *et al.*, 2010).

The RAPD method is very useful and plays an important role in securing plant variety rights as the need to protect propriety germplasm is increasing in Mauritius. These findings can pave the way to the use of other non-dominant markers to eventually facilitate marker validation for agronomically important characters, genome mapping and recombination breeding program for the development of new cultivars using the elite local chilli cultivars.

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