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Genetic diversity in *Cucurbita pepo* landraces from northern KwaZulu-Natal, South Africa, revealed by random amplified polymorphic DNA (RAPD) markers

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Genetic variation in seven selfed and unselfed *Cucurbita pepo* landraces from districts in KwaZulu-Natal Province was investigated using the random amplified polymorphic DNA (RAPD). Out of 36 primers screened nine were selected, which gave 100 clear and bright fragments, out of which 94 (94%) fragments were considered polymorphic. The sizes of bands ranged from 75 to 1800 bp. The number of bands per primer ranged between nine and 14. The genetic differentiation coefficient between populations (G_{ST}) varied between 0.0022 and 0.0100, while the gene flow ranged between 49.4545 and 223.7226. The effective number of alleles, Nei's gene diversity index and Shannon's information index were the highest in the selfed population from Zululand (yellow) district (ZS) population (Ne= 1.2046; H=0.1677; I=0.3060) and the lowest in unselfed population from uThungulu (yellow) (TNS) population (Ne=1.1512; H=0.1301; I=0.2518). The production of specific RAPD markers by different primers indicated gene diversity between: Selfed and unselfed populations from the same geographic origin; populations with yellow/orange mature fruit from a population with green mature fruit; and also among different populations in general. The selfed population from uThungulu (yellow) (TS) and TNS populations, both from uThungulu district, were the highest in genetic identity (I_N = 0.9996) and the closest in the genetic distance (D = 0.0004). The unselfed population from Umkhanyakude (green) (CPSP) and unselfed population from Umkhanyakude (yellow (MNS) populations as well as CPSP and TNS populations were the lowest in genetic identity (I_N = 0.9985) and the furthest in genetic distance (D = 0.0015). The dendrogram mainly grouped the populations according to their mature fruit colour, and then according to their geographical origin. All genetic parameters indicated that there was plentiful genetic diversity in *C. pepo* landraces of northern KwaZulu-Natal, South Africa.

Key words: *Cucurbita pepo* landraces, genetic variation, self-pollination, random amplified polymorphic DNA (RAPD) markers, northern KwaZulu-Natal.

INTRODUCTION

Cucurbita pepo is one of the most nutritionally and economically important species in the genus *Cucurbita* L. of Cucurbitaceae family that is cultivated worldwide and is of American origin (Tsivelikas et al., 2009; Ghobary and Ibrahim, 2010). Leaves of other wild Cucurbitaceae species indigenous to southern Africa, such as *Coccinia palmata* and *Lagenaria sphaerica* are cooked as leafy vegetables (Ntuli and Zobolo, 2008). *C. pepo* is a highly

polymorphic vegetable species, both in vegetative and reproductive characteristics, with a wide range of genetic variation occurring within it (Kathiravan et al., 2006; Formisano et al., 2012). Hadia et al. (2008) reported 84 and 87% polymorphism in *Cucurbita maxima* and *Cucurbita moschata*, respectively.

In traditional agriculture, genetic diversity is created by a diverse array of local varieties called landraces, which are well-adapted to local environmental conditions and inputs (Modi, 2004; Mujaju et al., 2010). Maintenance of landraces through *in situ* conservation is a preferred option for traditional small scale farmers (Modi, 2004). For example, part of the genetic variability of the first American summer squash cultigens remains intact in diverse landraces that are still cultivated for self-consumption and sale in local markets (Formisano et al., 2012).

In South Africa and other countries, communities and small scale farmers grow pumpkins as intercrop stands, whether with other plants, or with wild as well as culti-vated forms of other cucurbits (Mujaju et al., 2010; Molebatsi et al., 2010; Torquebiau et al., 2010). This intercropping practice enhances the geneflow among the cucurbit species due to the random bee pollination (Cuevas-Marrero and Wessel-Beaver, 2008; Mujaju et al., 2010). Gene exchange among plant populations loca-ted in distant geographical areas can be influenced by occasional introduction of seeds and seedlings as well as informal seed exchanges among farmers (Yuan et al., 2007; Du et al., 2011; Barboza et al., 2012).

Self-pollination increases plant mean homozygosity, which is not the natural genetic state of cross-pollinated species and reduces the proportion of heterozygosity in the population thus reducing the vigor of plants (Ercan and Kurum, 2003; Cardoso, 2004). Traits studies by Ghobary and Ibrahim (2010) in selfed *C. pepo* showed that the phenotypic expression of these traits were indi-cative of their genetic behaviour. Among the different types of molecular markers available, random amplified polymorphic DNA (RAPD) are useful for the assessment of genetic diversity because of their simplicity, and are fast and easy to perform and comparatively cheaper than other markers and require no prior knowledge of DNA sequences (Dey et al., 2006; Hadia et al., 2008; Khan et al., 2009). The potential applications of RAPD finger-printing in molecular biology include: Determination of taxonomic identities, detection of interspecific gene flow, assessment of kinship relationships, analysis of mixed genome samples and production of specific probes and

and gene mutation (Hadrys et al., 1992; De Wolf et al., 2004). RAPD markers have been used extensively to analyze genetic diversity in cucurbits (Ferriol et al., 2003; 2004a; 2004b; Dey et al., 2006; Morimoto et al., 2006; Hadia et al., 2008; Khan et al., 2009; Tsivelikas et al., 2009; Du et al., 2011).

Some RAPD primers produce bands that are uniquely amplified in single accessions (Barracosa et al., 2008), thus ensuring diversity among investigated accessions. In the dendrogram, accessions from different localities can either group themselves according to their agro-climatic regions of origin or not. With amplified fragment length polymorphism (AFLP), the *C. maxima* accessions from America and Spain were clearly grouped according to geographic origin (Ferriol et al., 2004a), whereas the Spanish *C. moschata* accessions from different geogra-phical origins clustered together with both sequence-related amplified polymorphism (SRAP) and AFLP mar-kers (Ferriol et al., 2004b).

Although *Cucurbita* species are valued as traditional leafy vegetables in South Africa, no molecular work has analyzed genetic diversity on different cultivated land-races of this country. However, according to Yuan et al. (2007) genetic diversity of plant germplasm is the impor-tant basis of conservation biology and genetic improve-ment. The aim of the present work was to analyze the polymorphism and genetic diversity among and within unselfed and selfed *C. pepo* landraces from Umkhanyakude, uThungulu and Zululand districts of northern KwaZulu-Natal, South Africa using the RAPD markers.

MATERIALS AND METHODS

Plant material

The seeds of *C. pepo* landraces collected from uThungulu (Nkandla: 28°37'S 31°25'E), Umkhanyakude (Mseleni: 27°38'S 32°47'E) and Zululand (Ulundi: 28°32'S 31°47'E) districts were grown at the Experiment Station in the Botany Department, University of Zululand (Empangeni: 28°51'S 31°50'E), and were used as the source of plant material. Seven accessions (Table 1) constituted two sets of plants that were used to harvest leaf material for DNA extraction: One set (four accessions) was from the seeds that were directly from the communities of three districts, and another set (three accessions) was from the seeds that were initially from the communities of these districts but the fruits were self-pollinated to fix the traits in an accession, where the natural pollinators (bees) were suspected to mix some pollen as the communities are practicing intercropping with other Cucurbitaceae

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Table 1. Description of *Cucurbita pepo* landraces identified in three districts of northern KwaZulu-Natal.

Code	Landrace name	Location (district)	Unselfed/ selfed	Mature fruit colour
MNS	iThanga	Umkhanyakude	Unselfed	Yellow/orange
MS	iThanga	Umkhanyakude	Selfed	Yellow/orange
TNS	iThanga	uThungulu	Unselfed	Yellow/orange
TS	iThanga	uThungulu	Selfed	Yellow/orange
ZNS	iThanga; iPhuzi	Zululand	Unselfed	Yellow/orange
ZS	iThanga; iPhuzi	Zululand	Selfed	Yellow/orange
CPSP	iNhlwathi emnyama	Umkhanyakude	Unselfed	Green

species in their fields. Young (folded to semi-folded) leaves were picked, freeze-dried and stored at 4°C for future use.

Self pollination procedure

Three *C. pepo* accessions from Umkhanyakude, Uthungulu and Zululand districts were grown in different areas that were about 2 km away from each other to prevent the incidence of pollen transference among plants from different districts. Both pistillate and staminate flowers that were to be selfed the following morning were covered with a light fine-porous cloth (curtain fabric), mimicking a cheesecloth bag (Winsor et al., 2000), in the afternoon prior to flower anthesis. These were noticed by the appearance of a slight touch of yellow or orange at the apex of the corolla tube or rather when the yellow/orange colour of the petals (corolla) was clearly seen or intensified from the outside according to the procedure of Ercan and Kurum (2003). At flower anthesis, soon after dehiscence of pollen sacs (pollen anthesis), self pollination was initiated from 04h00 until about 08h30 in the morning, when the viability and germination potential of pollen grains was still high (Nepi and Pacini, 1993; Agbagwa et al., 2007) and both male and female flowers were wide open. During selfing, the staminate flowers were picked and had their corolla tubes removed to expose the pollen-laden stamens and the pollen was gentle rubbed on the stigma lobes of the pistillate flower in the same plant (Thralls and Treadwell, 2008; Fike, 2011). One male flower was used for each female recipient (Spencer and Snow, 2001) due to high levels of irregularities in anthesis of both staminate and pistillate flowers of one plant. To prevent uncontrolled bee pollination, after self-pollination, the pistillate flowers were re-covered for the whole day, and the cover was removed the following day, since the female flowers are receptive on the ovules for only one day of flower anthesis (Nepi and Pacini, 1993; Agbagwa et al., 2007).

DNA extraction protocol

The DNA from the freeze-dried, ground *C. pepo* leaves was extracted according to the manufacturer's instructions using commercially available DNeasy Plant Mini Kit (Qiagen, Valencia, CA, USA).

Amount and purity of DNA

The yield of DNA in ng/µl was measured using the Nano Drop ND-1000 spectrophotometer (software ND-1000 V3.5.1; USA). The DNA purity was calculated at 260/280 nm wavelengths, where the DNA with an absorbance ranging between 1.7 and 1.9 were considered pure and were used for the polymerase chain reactions (PCR).

RAPD amplification

Approximately 50 ng of DNA was amplified through the PCR using 25 µl reactions under the following conditions: 1X of GoTaq® Green Master Mix, 2X (Promega Corporation); 0.4 µM random 10-mer oligonucleotide primer (Inqaba Biotechnical Industries (Pty) Ltd), and Nuclease-Free Water (Promega Corporation).

Amplifications were performed in a MJ Mini Personal Thermal Cycler (from BIO-RAD, Sweden) programmed for an initial denaturation at 95°C for 5 min followed by 40 cylces of 95°C for 30 s, 36°C for 30 s, and 72°C for 1 min, and final extension of 72°C for 4 min. Amplified products were separated in 1% agarose in 1x Tris-Borate-EDTA (TBE) buffer with 125 ng ethidium bromide per liter, using gel electrophoresis run at 70V for 1 h. The nucleic acid markers 100 bp (Promega Corporation) and 1 kb (Fermentas, Inqaba Biotechnical Industries (Pty) Ltd) were used to compare the amplification product sizes.

Of 36 primers tested, nine primers producing distinct polymorphic bands were selected for further analysis (Table 2). Each primer producing constituent amplification of well defined, brightly staining bands were used in further amplification of DNA from all individuals. Amplification was repeated to ensure reproducibility of scored products. RAPD markers were scored for presence or absence, and each marker was identified by primer and marker size.

The polymorphism rates of RAPD primers were evaluated using seven *C. pepo* landrace populations: Umkhanyakude unselfed (MNS); Umkhanyakude selfed (MS); Umkhanyakude green ripe fruits (CPSP); uThungulu unselfed (TNS); uThungulu selfed (TS); Zululand unselfed (ZNS); and Zululand selfed (ZS).

Data analysis

The data for RAPD was analysed using the Population Genetic Analysis (POPGENE version 1.31) (Yeh et al., 1999). The following genetic diversity parameters were determined: 1) The number of polymorphic bands (A) and the percentage of polymorphic bands (P); 2) the effective number of alleles per loci (Ne); 3) gene diversity (H) and Shannon's information index (I); 4) Nei's genetic distances (D) and genetic identity (I_N), which were evaluated using the cluster analysis that was performed with the unweighted pair group method of arithmetic average (UPGMA); and 5) the coefficient gene differentiation among the populations within species, which was determined using Nei's gene diversity method. The formula was

$$G_{ST} = D_{ST}/H_T, \quad H_T = H_S + D_{ST}$$

Where, H_T is the total gene diversity, H_S is the gene diversity within the population, and D_{ST} is the gene diversity between populations. The gene flow was determined as $Nm = 0.5 (1-G_{ST})/G_{ST}$ (Yuan et al., 2007).

Table 2. Sequence, produced band size range and polymorphism of different RAPD primers, as well as genetic variability within seven *Cucurbita pepo* populations.

RAPD Primer	Sequence (5' – 3')	Band size range (bp)	N	A	P	Ne	H	I	H_S	G_{ST}	Nm
CB9	GGTGACGCAG	100-1300	10	9	90	1.1216	0.1084	0.2201	0.1081	0.0027	183.3149
CB12	AGTCGACGCC	100-1300	9	8	89	1.1650	0.1417	0.2707	0.1412	0.0031	160.5899
CB13	ACGCATCGGA	100-1100	10	10	100	1.1539	0.1334	0.2584	0.1331	0.0022	223.7226
CB15	GGCTGGTTCC	75-1400	12	11	92	1.2169	0.1783	0.3227	0.1772	0.0060	82.5414
CB17	GTAACCAGCC	100-1400	12	11	92	1.2167	0.1781	0.3225	0.1774	0.0039	127.1654
CB19	GGTGCTCCGT	75-1400	14	14	100	1.1540	0.1335	0.2585	0.1330	0.0035	143.1361
CB21	CAGCACTGAC	100-1800	12	12	100	1.1937	0.1623	0.3004	0.1611	0.0070	70.7242
CB23	CTGGGCACGA	200-1400	11	9	82	1.2293	0.1865	0.3340	0.1847	0.0100	49.4545
CB27	AAGTGCGACC	200-1300	10	10	100	1.1541	0.1335	0.2586	0.1329	0.0047	106.6980
Total	-	-	100	94	94						
Average	-	-	11.11	10.44	94	1.1784	0.1506	0.2829	0.1499	0.0051	97.7840

CB, Cucurbita; N, total number of bands; A, number of polymorphic bands; P, percentage of polymorphism; Ne, effective number of alleles; H, Nei's gene diversity; I, Shannon's information index; H_S, genetic diversity index within populations; G_{ST}, genetic differentiation coefficient between populations; Nm, gene flow.

RESULTS

Polymorphism of RAPD amplified bands by different primers

The analysis of seven *C. pepo* populations with nine RAPD primers identified a total of 100 reproducible fragments (Table 2). Among them, 94 were polymorphic (94%), ranging in size from 75 to 1800 bp. Between nine and 14 fragments were amplified per primer, with an average of 11.11 fragments. Maximum number (14) of polymorphic fragments was obtained with the primer CB19. The number of polymorphic fragments for each primer varied from eight and 14, with an average of 10.44 fragments.

Population genetic diversity, differentiation (G_{ST}), and gene flow (Nm)

The effective number of alleles (Ne) estimated the reciprocal of homozygosity as ranging from 1.1216 (CB9) to 1.2293 (CB23), with an average of 1.1784 ± 0.0370. The Nei's gene diversity index (H) varied from 0.1084 (CB9) to 0.1865 (CB23), with an average of 0.1506 ± 0.0267. The Shannon's information index (I) ranged from 0.2201 (CB9) to 0.3340 (CB23), with an average of 0.2829 ± 0.0386. The genetic diversity index within populations (H_S) varied from 0.1081 (CB9) to 0.1847 (CB23), with an average of 0.1499 ± 0.0007.

The genetic differentiation coefficient between populations (G_{ST}) ranged between 0.0022 (CB13) and 0.0100 (CB23), with an average of 0.0051, which showed that the genetic variation between populations accounted between 0.22 and 1.00%, with an average of 0.51% of the total variation. The gene flow (Nm) ranged between 49.4545 (CB23) and 223.7226 (CB13), with an average of 97.7840, according to the genetic differentiation coefficient between populations, which indicated that there was a high exchange between *C. pepo* populations.

The effective number of alleles (Ne), Nei's gene diversity index (H) and Shannon's information index (I) were conducted to further understand the genetic diversity among the selfed and unselfed populations of *C. pepo* originating from three different districts (Table 2). The effective number of alleles, Nei's gene diversity index and Shannon's information index were the highest in ZS population (Ne= 1.2046; H=0.1677; I=0.3060) and the lowest in TNS population (Ne=1.1512; H=0.1301; I = 0.2518) (Table 3). Comparisons between selfed and unselfed populations within a district revealed that selfed populations of uThungulu and Zululand districts had higher effective number of alleles, Nei's gene diversity index and Shannon's information index than their analogous unselfed populations, while the opposite was evident in populations from Umkhanyakude district.

Specific RAPD marker production per primer per landrace(s)

Specific RAPD markers for CPSP population only were produced by: primers CB9 and CB12 (700 bp); primers CB13, CB19 and CB21 (1000 bp); and primer CB27 (1100 bp). Also primers CB15 and CB17 produced exclusive markers 100 and 500 bp, respectively, in all populations except CPSP population. The CPSP population had fruits that did not change their colour to orange or yellow at maturity.

Primer CB9 also showed the effect of selfing by

Table 3. Genetic variation among *Cucurbita pepo* populations.

Population	Effective number of alleles (Ne)	Nei's gene diversity (H)	Shannon's information index (I)
MNS	1.2009 ± 0.0773	0.1643 ± 0.0523	0.3003 ± 0.0733
MS	1.1651 ± 0.0661	0.1393 ± 0.0469	0.2645 ± 0.0681
TNS	1.1512 ± 0.0482	0.1301 ± 0.0349	0.2518 ± 0.0512
TS	1.1724 ± 0.0409	0.1461 ± 0.0302	0.2760 ± 0.0449
ZNS	1.1688 ± 0.0566	0.1427 ± 0.0391	0.2704 ± 0.0556
ZS	1.2046 ± 0.0655	0.1677 ± 0.0438	0.3060 ± 0.0613
CPSP	1.1901 ± 0.0440	0.1587 ± 0.0318	0.2941 ± 0.0467

Values are Mean ± standard deviation. MNS, Umkhanyakude unselfed; MS, Umkhanyakude selfed; TNS, uThungulu unselfed; NS, uThungulu selfed; ZNS, Zululand unselfed; ZS, Zululand selfed; CPSP, *C. pepo* species with dark green mature fruits.

Table 4. Nei's original measure of genetic identity and genetic distance among seven *C. pepo* populations.

Population ID	MNS	MS	TNS	TS	ZNS	ZS	CPSP
MNS	****	0.9995	0.9988	0.9992	0.9986	0.9989	0.9985
MS	0.0005	****	0.9988	0.9991	0.9987	0.9991	0.9992
TNS	0.0012	0.0012	****	0.9996	0.9994	0.9990	0.9985
TS	0.0008	0.0009	0.0004	****	0.9994	0.9990	0.9993
ZNS	0.0014	0.0013	0.0006	0.0006	****	0.9990	0.9988
ZS	0.0011	0.0009	0.0010	0.0010	0.0010	****	0.9989
CPSP	0.0015	0.0008	0.0015	0.0007	0.0012	0.0011	****

Nei's genetic identity (above diagonal) and genetic distance (below diagonal). MNS, Umkhanyakude unselfed; MS, Umkhanyakude selfed; TNS, uThungulu unselfed; NS, uThungulu selfed; ZNS, Zululand unselfed; ZS, Zululand selfed; CPSP, *C. pepo* species with dark green mature fruits.

identifying a unique band of 400 bp in unselfed populations from all districts including CPSP population, while primer CB21 identified marker 900 bp for only unselfed populations from all districts excluding CPSP population. Also CB15 produced specific band (200 bp) for only selfed populations from all districts, but also including the CPSP population. Primer CB9 produced unique band 600 bp in MS and ZS populations only, which were both selfed but from different districts. Primer CB23 produced a specific band 1000 bp in MNS and MS populations, both from one eco-geographic region, Umkhanyakude district.

The following specific RAPD markers amplified bands only in each of the following populations: MNS population [CB9 (800 bp), CB12 (900 bp), CB27 (800 bp)]; TNS population [CB23 (900 bp), CB27 (1000 bp)]; MS population [CB9 (1200 bp)] and TS population [CB13 (900 bp)].

Genetic identity and genetic distance between *Cucurbita pepo* populations

To further elucidate the gene differentiation among *C. pepo* populations, Nei's original measure of genetic identity (I_N) and genetic distance (D) was calculated (Table 4). The genetic identity ranged from 0.9985 to 0.9996, while the genetic distance varied from 0.0004 to 0.0015. The TS and TNS populations were the highest in genetic identity (I_N = 0.9996) and the closest in the genetic distance (D = 0.0004). The CPSP and MNS populations as well as CPSP and TNS populations were the lowest in genetic identity (I_N = 0.9985) and the furthest in genetic distance (D = 0.0015).

The phylogenetic relationship between populations was further illustrated by a dendrogram (Figure 1) using the UPGMA algorithm based on Nei's genetic distance (1972). The dendrogram grouped the populations into two main clusters, where cluster two had CPSP population (landrace with dark green and light green variegation at maturity) which was distant from a group with all other six populations. As with the results obtained in cluster one, which had two sub-clusters, a clear grouping according to geographical origin was observed. Sub-cluster one grouped the populations from Umkhanyakude district (MNS and MS). Sub-cluster two included populations from Uthungulu (TNS and TS) and Zululand (ZNS and ZS) districts, where populations from the former district formed a cluster and then assembled with ZNS and ZS
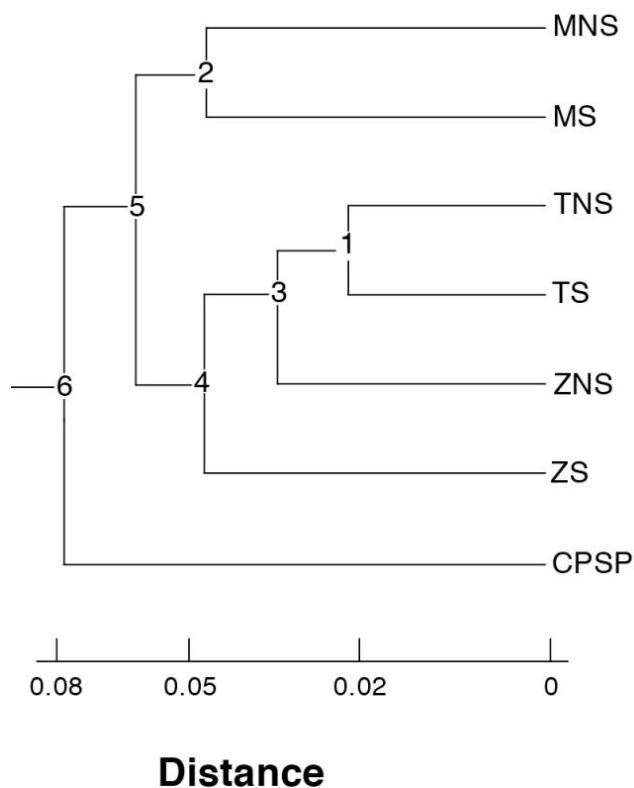
Figure 1. Unweighted pair group method of arithmetic mean (UPGMA) dendrogram based on Nei's (1972) genetic distance, summarizing data on differentiation in seven populations of *C. pepo* eco-geographical populations. MNS, Umkhanyakude unselfed; MS, Umkhanyakude selfed; TNS, uThungulu unselfed; NS, uThungulu selfed; ZNS, Zululand unselfed; ZS, Zululand selfed; CPSP, *C. pepo* species with dark green mature fruits.

populations, both from Zululand district.

DISCUSSION

Polymorphism in *C. pepo* populations

The level of polymorphism among the *C. pepo* population was relatively high, ranging between 82 and 100% with an average of 94% (Table 2). This high level of RAPD markers polymorphism in *C. pepo* genotypes is in accordance with the results of Kathiravan et al. (2006) Hadia et al. (2008) and Tsivelikas et al. (2009), who reported that *C. pepo* is a highly polymorphic species. In an earlier study, Hadia et al. (2008) reported 89% polymorphism among *C. pepo* genotypes.

Population genetic structure and geographic diversity of northern KwaZulu-Natal *C. pepo* landraces

The average value of G_{ST} was 0.0051, based on the RAPD markers for *C. pepo* in northern KwaZulu-Natal

(Table 2), indicating that the gene differentiation was higher within population (99.49%) than between the populations (0.51%). Gene differentiation and gene flow are important indices to evaluate the population genetic structure. The gene differentiation coefficients of *C. pepo* landraces, mainly from Spain, were 0.25 and 0.18, when analysed with SRAP and AFLP, respectively (Ferriol et al., 2003). Further, the gene differentiation coefficients of Spanish *C. moschata* landraces as determined by AFLP and SRAP analyses were 0.28 and 0.17, respectively (Ferriol et al., 2004b).

Based on the RAPD analysis of *C. pepo* landraces of northern KwaZulu-Natal, the average value of the gene flow was 97.7840 (Table 2), which according to Han et al. (2007) was overwhelming the effect of genetic drift because its value was above four. According to Yuan et al. (2007) and Mujaju et al. (2010), the movement of genes within and between populations, the gene flow, is negatively correlated with the gene differentiation, and is transferred by pollen and seed between populations for seed plants.

Also, the population genetic structure is mainly affected by a long distance diffusion of pollen and diffusion capability of pollen offspring owing to inbreeding and outcrossing propagation (Yuan et al., 2007). However, *C. pepo* distributed in different districts of KwaZulu-Natal with long geographical distance, had a very low possibility of the pollen spread by insects (particularly bees) between populations. Several authors have reported the evidence of gene flow and hybridization between several interplanted *Cucurbita* species (Decker-Walters et al., 1990; Wessel-Beaver, 2000; Montes-Hernandez and Eguiarte, 2002; Cuevas-Marrero and Wessel-Beaver, 2008).

The possible gene flow among *Cucurbita* species was also possible for long distances' pollen transfer by bees ranging between 800 and 1300 m (Montes-Hernandez and Eguiarte, 2002; Spencer and Snow, 2001). Therefore the main way of gene exchanges can be occasional introduction of seeds as enhanced by seed exchanges between farmers of different districts in KwaZulu-Natal (Montes-Hernandez and Eguiarte, 2002; Ferriol et al., 2004a; 2004b; Yuan et al., 2007; Mujaju et al., 2010; Du et al., 2011; Barboza et al., 2012).

The effective number of alleles (Ne=1.1784), Nei's gene diversity index (H=0.1506), Shannon's information index (I=0.2829), and genetic diversity index within populations (H_S=0.1499), also indicate molecular genetic diversity for the *C. pepo* populations studied herein (Table 2). The gene diversity obtained with RAPD markers among different *C. pepo* landraces from northern KwaZulu-Natal was less than that obtained with AFLP markers among different *C. moschata* accessions (Wu et al., 2011); with RAPD among *Trichosanthes dioica* accessions (Khan et al., 2009); and also with inter-simple sequence repeat (ISSR), SRAP and RAPD markers among Turkish and Foreign *Cucumis melo* genotypes (Yildiz et al., 2011). The production of a specific RAPD marker by primer CB23 (1000 bp)

in both MNS and MS populations from Umkhanyakude district indicated the effect of eco-geographic differences in gene diversity of plants, where landraces from the same geographic area are closely related (Ferriol et al., 2004a,b; Du et al., 2011).

Genetic variation between unselfed and selfed *C. pepo* populations

The higher effective number of alleles, which estimates the reciprocal of homozygosity, in selfed populations than unselfed populations from both uThungulu and Zululand districts (Table 3), showed the effect of self-pollination by enhancing homozygosity while reducing the heterozygosity in plant genomes, concurring with the reports by Ercan and Kurum (2003), Cardoso (2004) and Ferrari et al. (2006; 2007). However the higher Nei's gene diversity index and Shannon's information index in these selfed populations (Table 3) disagreed with these reports, but on the contrary the selfed population from Umkhanyakude district, showed this increase in homozygosity with selfing. This greater genetic variability among these selfed populations, particularly from uThungulu and Zululand districts, can increase their effectiveness of selection and the amount of genetic improvement in a breeding program as suggested by Ghobary and Ibrahim (2010).

The production of specific RAPD markers for either selfed or unselfed populations only, also confirmed that selfing had changed the genetic state of these *C. pepo* landraces from different districts of northern KwaZulu-Natal as reported earlier in other *Cucurbita* species (Ercan and Kurum, 2003; Cardoso, 2004; Ferrari et al., 2006; 2007).

Specific RAPD marker per *C. pepo* landrace(s)

The production of unique bands only in MNS population [CB9 (800 bp), CB12 (900 bp), CB27 (800 bp)]; TNS population [CB23 (900 bp), CB27 (1000 bp)]; MS population [CB9 (1200 bp)] and TS population [CB13 (900 bp)], indicated the genotype variation among *C. pepo* landraces in northern KwaZulu-Natal. Hadia et al. (2008) identified the specific RAPD markers that showed genotypes variation among *C. maxima*, *C. moschata* and *C. pepo* species, as well as those showing differences within their populations. Again, Barracosa et al. (2008) in their study of *Ceratonia siliqua*, made use of unique RAPD markers that were cultivar-specific to differentiate the Portuguese cultivars.

Six primers (CB9; CB12; CB13; CB19; CB21 and CB27) produced unique bands for CPSP population only, while two primers (CB15 and CB17) produced unique bands for all other six populations except CPSP. The absence and presence of these unique bands in CPSP populations only indicated that these primers were probably marking the genes or loci that affect fruit colour development or change at maturity among these landraces, where the

CPSP maintains its green fruit variegation at maturity. Paris (2000) reported 11 loci that have been identified as affecting developmental fruit colour in *C. pepo*, and of these, three genes of major effect – D, I-1, and I-2 – account for a considerable portion of the genetic variation in intensity of fruit colouration that is observed in this species. The developmental fruit colouration from light green fruits, several days past anthesis, except for some darkening of the main capillary veins, becoming blackish-green past anthesis, and then turn intense orange on ripening (Paris, 2000; 2009), as observed in other landraces, was conferred by genotype D/D I-1/I-1 L-2/L-2 (Paris, 2000). However, fruits of L-1/ — L-2/ — plants are intense green throughout development, as the case with the CPSP landrace, where, in contrast to plants homozygous recessive for either or both I genes, the fruits of L-1/— L-2/ — plants retain their black-green colour through maturity, not turning orange or yellow when ripe (Paris, 2000).

Genetic identity and genetic distance of *Cucurbita pepo* between populations

The range of genetic identity from 0.9985 to 0.9996 and genetic distance range from 0.0004 to 0.0015 indicated the presence of variability among the seven populations of *C. pepo* in northern KwaZulu-Natal (Table 4). The TS and TNS populations were the highest in genetic identity (I_N = 0.9996) and the closest in the genetic distance (D = 0.0004), probably because they originate from the same district, but CPSP and TNS populations were the lowest in genetic identity (I_N = 0.9985) and the furthest in genetic distance (D = 0.0015), because they were collected from distant geographical regions, where most of the farmers have maintained the productions of these landraces for many years. The same was reported among *Punica granatum* cultivars (Yuan et al., 2007), *T. dioica* accessions (Khan et al., 2009) and *C. moschata* accessions (Du et al., 2011; Barboza et al., 2012). However CPSP and MNS populations, both from Umkhanyakude district, were also the lowest in genetic identity (I_N = 0.9985) and the furthest in genetic distance (D = 0.0015), possibly because CPSP populations do not change fruit colour to orange or yellow at maturity whereas MNS population do change. Therefore the differences in genes that are responsible for colour formation at maturity as explained by Paris (2000; 2009) might have influenced this low genetic identity and wide genetic distance between these populations.

A clear grouping, first according to fruit colour change at maturity, and secondly according to geographical origin, was obtained (Figure 1). The separation of CPSP population from a group of six populations that changed in their fruit colour at maturity may support the wide genetic variation in these landraces with reference to their fruit colour formation as recorded by Paris (2000; 2009). A separate sub-cluster of Umkhanyakude district populations

(MNS and MS) from those of uThungulu (TNS and TS) and Zululand (ZNS and ZS) districts showed that these populations are grouped according to their agro-ecological regions. This concurs with the findings of Ferriol et al. (2004a,b;) in their study of *C. maxima* and *C. moschata*, respectively, who obtained the results with both SRAP and AFLP markers, where accessions were grouping according to their geographical origin. Also, Amadou et al. (2001) in their genetic diver-sity analysis of *Vigna subterranea* using RAPD markers, found the highest similarity on accessions that were originating from the same country. Further, Tsivelikas et al. (2009) report the genetic diversity analysis of *C. moschata* landraces using RAPD markers where acces-sions were grouped according to the agro-climatic regions and not according to the morphological traits.

Further, the grouping of population from Uthungulu district with those from Zululand district was probably due to existence of seed exchanges among farmers of these districts. The same was reported earlier by other resear-chers in cucurbits (Montes-Hernandez and Eguiarte, 2002; Ferriol et al., 2004a; 2004b; Barboza et al., 2012) and other species (Yuan et al., 2007).

Conclusion

The RAPD analysis discovered sufficient variations among the *C. pepo* landraces. This marker revealed the genetic diversity in landraces with differences in fruit colour change at maturity at a higher extent, and then landraces with different geographical origin. It further confirmed the effect of selfing on the change of plant's genetic make-up.

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