Full Length Research Paper

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

Nontuthuko R. Ntuli¹, Alpheus M. Zobolo ¹*, Pangirayi B. Tongoona², Nokuthula W. Kunene²

¹Department of Botany, University of Zululand, Private Bag X1001, KwaDlangezwa, 3886, South Africa.
²Department of Agriculture, University of Zululand, Private Bag X1001, KwaDlangezwa, 3886, South Africa.
³African Centre of Crop Improvement, University of KwaZulu-Natal Pietermaritzburg, Private Bag X01, Scottsville, 3209, South Africa. *Corresponding author. E-mail: azobolo@pan.uzu.ac.za. Tel: +27 35 902 6109. Fax: +27 35 902 6491.

Accepted 9 October, 2013

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 (GST) 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 (IN = 0.9996) and the closest in the genetic distance (D = 0.0004). The unselfed population from Umkhanyakude (green) (CPSP) and unsselfed population from Umkhanyakude (yellow) (MNS) populations as well as CPSP and TNS populations were the lowest in genetic identity (IN = 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 cultivars 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 cultivated 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 is a-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 are indicative 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 fingerprinting 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 geographical origins clustered together with both sequence-related amplified polymorphism (SRAP) and AFLP markers (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 landraces of this country. However, according to Yuan et al. (2007) genetic diversity of plant germplasm is the important basis of conservation biology and genetic improvement. The aim of the present work was to analyze the polymorphism and genetic diversity among and within unselved 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 (Mseleli: 27°38’S 32°47’E) and Zululand (Umundu: 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

Abbreviations: CPSP, Unselfed population from Umkhanyakude (green); MNS, selfed population from Umkhanyakude (yellow); MS, selfed population from Umkhanyakude (yellow); TNS, unselfed population from uThungulu (yellow); TS, selfed population from uThungulu (yellow); ZNS, unselfed population from Zululand (yellow); ZS, selfed population from Zululand (yellow) district; RAPD, random amplified polymorphic DNA; AFLP, amplified fragment length polymorphism; SRAP, sequence-related amplified polymorphism; PCR, polymerase chain reactions; Ne, effective number of alleles; H, Nei’s gene diversity index; I, Shannon’s information index; ISSR, inter-simple sequence repeat.
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 Umkhanayakude, 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 Erkan 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 gently rubbed on the stigma lobes of the pistillate flower in the same plant (Thrall 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 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 was considered pure and were used for the polymerase chain reactions (PCR).

**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       | Umkhanayakude       | Unselfed         | Yellow/orange     |
| MS   | iThanga       | Umkhanayakude       | 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 | Umkhanayakude  | Unselfed         | Green             |

**RAPD amplification**

Approximately 50 ng of DNA was amplified through the PCR using 25 µl reactions under the following conditions: 1X of GoTaq<sup>®</sup> 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 cyces 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 1X 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 constitutive 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: Umkhanayakude unselfed (MNS); Umkhanayakude selfed (MS); Umkhanayakude 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), 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

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

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 $N_m = 0.5 (1-G_{ST})/G_{ST}$ (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   | Hs   | GsT | Nm   |
|-------------|--------------------|---------------------|-----|----|-----|-----|------|-----|------|-----|------|
| CB9         | GGTGACGCAG         | 100-1300            | 10  | 9  | 90  | 1.1216 | 0.1084 | 0.2201 | 0.1081 | 0.0027 | 183.3149 |
| CB12        | AGTGCAGGCC         | 100-1300            | 8   | 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        | GGCTGTTTCC         | 75-1400             | 12  | 11 | 92  | 1.2169 | 0.1783 | 0.3227 | 0.1772 | 0.0060 | 82.5414 |
| CB17        | GTAACAGGCC         | 100-1400            | 12  | 11 | 92  | 1.2167 | 0.1781 | 0.3225 | 0.1774 | 0.0039 | 127.1654 |
| CB19        | GGTCGTCCGT         | 75-1400             | 14  | 14 | 100 | 1.1540 | 0.1335 | 0.2585 | 0.1330 | 0.0035 | 143.1361 |
| CB21        | CAGCAGCTG         | 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        | AAGTGCAGACC        | 200-1300            | 10  | 10 | 100 | 1.1541 | 0.1335 | 0.2586 | 0.1329 | 0.0047 | 106.6980 |
| Total       | -                  | -                   | 100 | 94 | 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 index; I, Shannon’s information index; Hs, genetic diversity index within populations; GsT, 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 (GsT), 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 (Hs) varied from 0.1081 (CB9) to 0.1847 (CB23), with an average of 0.1499 ± 0.0007.

The genetic differentiation coefficient between populations (GsT) 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 Umkhandakude 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
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 districts.

### 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            | 0.0003 | 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.
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 unsselfed; MS, Umkhanyakude selfed; TNS, uThungulu unsselfed; NS, uThungulu selfed; ZNS, Zululand unsselfed; 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 the 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 lowest 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), because they were collected from distant geographical regions, where most of the farmers 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 conclusion is supported by the findings of Ferriol et al. (2004a,b,2004b) 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 accessions 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 researchers 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.

REFERENCES

Agbagwa IO, Ndukuw BC, Mensah SI (2007). Floral biology, breeding system, and pollination ecology of Cucurbita moschata (Duch. ex Lam) Duch. ex Poir. varieties (Cucurbitaceae) from parts of the Niger Delta, Nigeria. Turk J. Bot. 31: 451-458.

Amadou HI, Bebeli PJ, Kalsikesi PJ (2001). Genetic diversity of Bambara groundnut (Vigna subterranea L.) germplasm revealed by RAPD markers. Genome 44: 995-999.

Barboza N, Albertazzi FJ, Sibaja-Cordero JA, Mora-Umaña F, Astorga C, Ramirez P (2012). Analysis of genetic diversity of Cucurbita moschata (D.) germplasm accessions from Mesoamerica revealed by PCR SSCP and chloroplast sequence data. Sci. Hortic. 134: 60-71.

Barracosa P, Lima MB, Cravador A (2008). Analysis of genetic diversity in Portuguese Ceratonia silqua L. cultivars using RAPD and AFLP markers. Sci. Hortic. 118: 189-199.

Cardoso All (2004). Depression by inbreeding after four successive self-pollination squash generations. Sci. Agric. (Piracicaba, Braz.) 61(2): 224-227.

Cuevas-Marrero H, Wessel-Beaver L (2008). Morphological and RAPD marker evidence of gene flow in open-pollinated populations of Cucurbita moschata interplanted with C. argyrosperma. Cucurbitaceae 2008. Proceedings of the IX EUCARPIA Meeting on Genetics and Breeding of Cucurbitaceae (Pitrat M, ed), INRA, Avignon (France), May 21-24, 2008: pp. 347-352.

De Wolf H, Blust R, Backeljau T (2004). The use of RAPD in eco-ziology. Mutat. Res. 566: 249-262.

Decker-Walters DS, Walters TW, Poslusnzy U, Kevan PG (1990). Genealogy and gene flow among annual domesticated species of Cucurbita. Can. J. Bot. 68: 782-789.

Dey SS, Singh AK, Chandel D, Behera TK (2006). Genetic diversity of bitter gourd (Momordica charantia L.) genotypes revealed by RAPD markers and agronomic traits. Sci. Hortic. 109: 21-28.

Du X, Sun Y, Li X, Zhou J, Li X (2011). Genetic divergence among inbred lines in Cucurbita moschata from China. Sci. Hortic. 127: 207-213.

Ercan N, Kurum R (2003). Plant, flower, fruit and seed characteristics of five generation inbred summer squash lines (Cucurbita pepo L.). Pak. J. Bot. 35(2): 237-241.

Ferrari MJ, Du D, Winsor JA, Stephenson AG (2007). Inbreeding depression of plant quality reduces incidence of an insect-borne pathogen in a wild gourd. Int. J. Plant Sci. 168(5): 603-610.

Ferrari MJ, Stephenson AG, Mescher MC, De Moraes CM (2006). Inbreeding effects on blossom catolilies in Cucurbita pepo subsp. texana (Cucurbitaceae). Am. J. Bot. 93(12):1768-1774.

Ferriol M, Picó B, de Córdova PF, nuez F (2004b). Molecular diversity of a germplasm collection of squash (Cucurbita moschata) determined by SRAP and AFLP markers. Crop Sci. 44: 653-664.

Ferriol M, Picó B, Nuez F (2003). Genetic diversity of a germplasm collection of Cucurbita pepo using SRAP and AFLP markers. Theor. Appl. Genet. 107: 217-282.

Ferriol M, Picó B, Nuez F (2004a). Morphological and molecular diversity of a collection of Cucurbita maxima landraces. J. Amer. Soc. Hort. Sci. 129(1): 60-69.

Fike MS (2011). Pumpkin passion. The Canadian Organic Grower: 14-18.

Formisano G, Roig C, Esteras C, Ercolano MR, Nuez F, Monforte AJ, Picó MB (2012). Genetic diversity of Spanish Cucurbita pepo landraces: an unexploited resource for summer squash breeding. Genet. Resour. Crop Evol. 59: 1169-1184.

Ghobary HMM, Ibrahim KhY (2010). Improvement of summer squash through inbreeding and visual selection. J. Agric. Res. Kafer El Sheikh Univ. 36: 340-350.

Hadia HA, Abdel-Razzak HS, Hafez EE (2008). Assessment of genetic relationships among and within Cucurbita species using RAPD and ISSR markers. J. Appl. Sci. Res. 4(5): 515-525.

Hadrys H, Balick M, Schierwater B (1992). Application of random amplified polymorphic DNA (RAPD) in molecular ecology. Mol. Ecol. 1: 55-63.

Katherivan K, Vengedesan G, Singer S, Steinitz B, Paris HS, Gaba V (2006). Adventitious regeneration in vitro occurs across a wide spectrum of squash (Cucurbita pepo) genotypes. Plant Cell Tissue Organ Cult. 85: 285-295.

Khan ASMMR, Rabbani MG, Islam MS, Rashid MH, Alam AKMM (2009). Genetic diversity of inbred lines in a wild gourd (Trichosanthes dioica Roxb) revealed by random amplified polymorphic DNA (RAPD) markers. Thai J. Agric. Sci. 42(2): 61-69.

Modi AT (2004). Short-term preservation of maize landrace seed and taro propagules using indigenous storage methods. S. Afr. J. Bot. 70(1): 16-23.

Molebatsi LY, Siebert SJ, Cilliers SS, Lubbe CS, Davoren E (2010). The Tswana Tshimo: A homegarden system of useful plants with a particular layout and function. Afr. J. Agric. Res. 5(21): 2952-2963.

Montes-Hernandez S, Eguiarte LE (2002). Genetic structure and indirect estimates of genetic flow in three taxa of Cucurbita (Cucurbitaceae) in western Mexico. Am. J. Bot. 89(7): 1156-1163.

Morimoto Y, Maundu PK, Kawase M, Fujimaki H, Morishima H (2006). RAPD polymorphism of the white-flowered gourd (Lagenaria siceraria (Molina) Standl.) landraces and its wild relatives in Kenya. Genet. Resour. Crop Evol. 53: 963-974.

Mujaću C, Sehić J, Werlemark G, Garkava L, Ntuli et al. 20027

Nepi M, Pacini E (1993). Pollination, pollen viability and pistil receptivity in Cucurbita pepo. Ann. Bot. 72: 527-536.

Ntuli NR, Zobolo AM (2008). Effect of water stress on growth of col-
chicine induced polyploid *Coccinia palmata* and *Lagenaria sphaerica* plants. Afr. J. Biotechnol. 7(20): 3648-3652.

Paris HS (2000). *Quiscence Intense* (*qi*): A gene that affects young but not mature fruit colour intensity in *Cucurbita pepo*. J. Hered. 91(4): 333-339.

Paris HS (2009). Genes for “reverse” fruit striping in squash (*Cucurbita pepo*). J. Hered. 100(3): 371-379.

Spencer LJ, Snow AA (2001). Fecundity of transgenic wild-crop hybrids of *Cucurbita pepo* (Cucurbitaceae): implications for crop-to-wild gene flow. Heredity 86: 694-702.

Thralls E, Treadwell D (2008). Home vegetable garden techniques: hand pollination of squash and corn in small gardens. The Institute of Food and Agricultural Sciences (IFAS), University of Florida.

Torquebiau E, Dosso M, Nakaggwa F, Philippon O (2010). How do farmers shape their landscape: A case-study in KwaZulu-Natal, South Africa. ISDA June 2010, Montpellier, France: 1-15.

Tsivelikas AL, Koutita O, Anastasiadou A, Skaracis GN, Traka-Mavrona E, Koutsika-Sotiriou M (2009). Description and analysis of genetic diversity among squash accessions. Braz. Arch. Biol. Technol. 52(2): 271-283.

Wessel-Beaver L (2000). *Cucurbita argyrosperma* sets fruits in fields where *C. moschata* is the only pollen source. Rep. Cucurbit Genet. Coop. 23: 62-63.

Winsor JA, Peretz S, Stephenson AG (2000). Pollen competition in a natural population of *Cucurbita foetidissima* (Cucurbitaceae). Am. J. Bot. 87(4): 527-532.

Wu J, Chang Z, Wu Q, Zhan H, Xie S (2011). Molecular diversity of Chinese *Cucurbita moschata* germplasm collections detected by AFLP markers. Sci. Hortic. 128: 7-13.

Yeh FC, Yang R-C, Boyle T (1999). POGENE Version 1.31. Microsoft Windows-based Freeware for Population Genetic Analysis. Department of Renewable Resources, University of Alberta, Edmonton.

Yıldız M, Ekbic E, Keles D, Sensoy S, Abak K (2011). Use of ISSR, SRAP, and RAPD markers to assess genetic diversity in Turkish melons. Sci. Hortic. 130: 349-353.

Yuan Z, Yin Y, Qu J, Zhu L, Li Y (2007). Population genetic diversity in Chinese pomegranate (*Punica granatum* L.) cultivars revealed by fluorescent-AFLP markers. J. Genet. Genomics 34(12): 1061-1071.