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Full Length Research Paper

Assessment of genetic variation of selected spiderplant (*Cleome gynandra* L.) morphotypes from western Kenya

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In the genetic variation study of the 4 spiderplant morphotypes, 10 out of the 12 primers screened generated 31 polymorphic bands. Nei's coefficient of gene differentiation showed that the morphotypes were differentiated. Nei's genetic identity value calculated from the RAPD data ranged from 0.3 to 0.6 indicating the degree of genetic identity between the morphotypes. Dendrograms constructed from a matrix calculated on the basis of UPGMA clustering algorithm revealed that the 4 morphotypes formed 3 groups.

Key words: *Cleome gynandra*, genetic variation, morphotypes, RAPD-PCR, spiderplant, western Kenya.

INTRODUCTION

Spiderplant is an herbaceous leafy vegetable, indigenous to many parts of sub-saharan Africa. A number of attempts have been made to establish the chromosome counts, resulting in variable diploid counts of 2n = 18, 20, 30, 32, 34, 36 (Schippers, 2000; Mnzava and Chigumira, 2004), with Schippers (2000) reporting 2n = 20 as being most frequent. Polyploidy has also been observed in the species (Chweya and Mnzava, 1997). It is reported to occur all over Kenya, especially as weed of cultivation and disturbed areas, from 0-2400 m (Maundu et al., 1999b).

While systematic characterization and evaluation of spiderplant has not been done in Kenya, some studies in Kenya and Zimbabwe indicate significant variations in many characteristics among spiderplant populations (Chweya, 1990; Kemei et al., 1995; Mnzava and Chigumira, 2004). Little information exists to what extent these differences are due to environmental factors such as climate, soil fertility and stress conditions and genetic factors (Mnzava and Chigumira, 2004). The magnitude of these variations within the indigenous germplasm pool found in Kenya is also not known. Farmers frequently grow landraces of spiderplant and make a few selections out of them. Lanteri et al. (2003) defines a landrace as a population of plants growing in a specific geographical environment, whose individuals share a common gene pool. From the study carried out in Kenya by Maundu et al. (1999a), the occurrence of different morphotypes of spiderplant has been indicated but it is not clear to what extent they are genetically different. Compared with exotic vegetable species, there is need to understand the growth patterns and characteristics of spiderplant for further studies, e.g. breeding and nutritional studies.

Advances in molecular genetics have made available a range of new techniques that can be used in the conservation of plant genetic resources and crop improvement (Ayad et al., 1997). Although morphological characterization is recommended as a first step for measuring crop diversity (Watson and Eyzaguirre, 2002), morphological characters are known to be susceptible to environmental influences (Berinyuy et al., 2002). Molecular techniques can provide genomic information and estimates of genetic variability and molecular markers, which are free of environmental influence, are therefore useful in crop improvement (Bernatzky and Tanksley, 1986a,b).

Selection of parental material meant for the improvement of spiderplant as a vegetable should ideally be based on genetic information that is reliable and consistent. Molecular markers are now widely used to estimate

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genetic diversity, assess genetic relationships, map genes underlying important agronomic traits in crop species and conduct linkage analyses of quantitative and qualitative traits (Grattapagalia and Sederroff, 1994; Brummer et al., 1995; Vroh et al., 2006). Markers provide descriptors complementary to conventional morphological traits, particularly when used to assess genetic diversity at both inter- and intra- specific levels (Prince et al., 1995; Rodriguez et al., 1999; Tams et al., 2005).

There are several techniques currently available to detect molecular variability within and among species. The most frequently employed include random amplification of polymorphic DNA fragments with PCR (RAPD-PCR), restriction fragment length polymorphism (RFLP), amplified fragment length polymorphism (AFLP) and sequence-tagged microsatellites (STMS) (Weining and Langridge, 1991; Vos et al., 1995; Watson and Eyzaguirre, 2002). RAPD and AFLP techniques have been extensively used in assessing genetic variation and taxonomic relationships of both wild and cultivated species (Gaudel et al., 2000; Mateu-André and Segarra-Moragues, 2000; Lanteri et al., 2001; Müller-Schärer and Fischer, 2001). The RAPD marker analysis (Williams et al., 1990), based on a polymerase chain reaction (PCR) with arbitrary primers is not influenced by the environment, although its repeatability has some limitations. It has been used effectively for analyzing genetic diversity in various plant species, including widely grown cereal, oilseed and root crops as well as several fruit, vegetable and ornamental species (Kelly and George, 1998). It can discriminate between individuals, varieties or biotypes of the same species. Lee et al. (1996b), for example, could readily discriminate over 95% of a collection of 50 oilseed rape cultivars with one primer and a combination of 2 or more primers achieved complete discrimination. The technique will detect polymorphism throughout the genome, with primer amplifying sequences into several bands, each of which originating from a different locus.

The objective was to study the genetic variation of the 4 spiderplant morphotype selections from western Kenya at the molecular level.

MATERIALS AND METHODS

Plant materials

The seeds of the 4 morphotypes used in this experiment were collected from small-scale farmers in Kakamega District and from wildly growing plants within Chepkoilel Campus, Moi University in Uasin Gishu District, both in western Kenya. The morphotypes for the study on morphological characterization were identified and selected from plants which were raised in a plastic greenhouse at the Department of Seed, Crop and Horticultural Sciences in Moi University. From this study, seeds of each morphotype (GG- green stem/green petiole type of plants; GP- green stem/purple petiole type of plants; PG- purple stem/green petiole type of plants and PP- purple stem/purple petiole type of plants) were saved and stored in hermatically-sealed aluminium foil packets at room temperature (about 20^0C) until the time to execute the RAPD-PCR experiment.

Plants for the experiment were raised in a plastic greenhouse at the Department of Seed, Crop and Horticultural Sciences, Moi University. Seeds of each morphotype were densely sown in separate plastic flowerpots filled with soil collected within Chepkoilel Campus. Thinning was done 15 days after seedling emergence, to leave 10 seedlings per pot. RAPD-PCR analyses were done in the molecular biology laboratory at the Department of Botany, Tea Research Foundation of Kenya, Kericho, Kenya.

RAPD-PCR experiment

A number of methods of DNA extraction were evaluated. These were the mini cetyl trimethyl ammonium (CTAB) method in which the extraction buffer was CTAB while the extraction agent was mercaptoethanol; mini CTAB method with extraction buffer as CTAB and extraction agent as dithiothreitol (DTT); mini sodium dodecyl sulphate (SDS) method with extraction buffer being SDS and extraction agent as mercaptoethanol and mini SDS method in which the extraction buffer was SDS and extraction agent was DTT. The running of the gels for the extracted DNA samples was done in a mini-gel tank with 2 Lambda DNA standards (100 and 250 ng/µL) included into the wells of the gels to help estimate the concentration of the DNA in the samples. The samples were quantified by viewing them under UV light in the dark room, in which SDS-DTT method was indicated as the best because it had most DNAs comparable to 250 ng/µL. The most successful method in our case is described while the others are listed in Table 1.

Total genomic DNA was extracted from healthy young leaves of a 3 week old plants of each spiderplant morphotype, by using the modified 'sodium dodecyl sulphate (SDS) Mini-preparation' method (Edwards et al., 1991; Wachira, 1996). Samples of approximately 0.6 g of fresh 5 - 6 foliate leaves were frozen in liquid nitrogen, ground to a fine powder and added to 400 µL extraction buffer [200 mM tris (hydroxymethyl) aminomethane-HCl of pH 7.5; 25 mM ethylenediamine tetra acetic acid (EDTA) of pH 8.0; 250 mM NaCl; 0.5% SDS; 0.1% dithiothreitol (DTT)] and a pinch (about 10 - 20 mg) of polyvinyl poly-pyrrolidone (PVPP). The PVPP was added to prevent browning of the material due to the presence of polyphenols. Another 400 µL extraction buffer was added, the homogenate vortexed and centrifuged (Microcentrifuge, Hermle, Model Z 252 M) at 7000 rpm for 4 min to pellet the plant debris. The supernatant was poured off into a clean centrifuge tube of 1.5 mL. The nucleic acid was precipitated from the aqueous phase by adding 600 µL cold isopropanol and gently shakened. The DNA pellet was recovered by centrifugation at 7000 rpm for 10 min, the pellets rinsed with 1000 µL cold 70% ethanol and centrifuged for a further 2 min. The alcohol was removed and the remaining pellet was air-dried for about 1 h. The pellet was resuspended in 500 µL sterile distilled water (SDW), dissolved in 1 µL RNase solution (100 µg/mL in SDW) and incubated in a hot water bath overnight at 55^0C for complete digestion of RNA.

200 µL of chloroform/isoamyl (CIA) was added to an equal volume of unpurified DNA sample, inverted gently and the mixture centrifuged at 7000 rpm for 20 min. The aqueous phase was then transferred into a new tube and to the old tube, 80 µL of 1 M NaCl-TE was added, after which the tube was centrifuged at 7000 rpm for 20 min. The resultant aqueous phase was again transferred into the new tube, which was then centrifuged at 7000 rpm for 15 min to mix the 2 phases. The supernatant was transferred to a clean tube and 300 µL of isopropanol was added and gently mixed, then the tube was centrifuged at 5000 rpm for 15 s. The supernatant was removed and 500 µL ice-cold 70% ethanol was added to sterilize, before centrifuging at 5000 rpm for 5 min. The supernatant was removed and the tube centrifuged at 5000 rpm for 1 min; then the pellet that remained was air-dried for 10 min. The pellet was later resuspended in 200 µL SDW and incubated in a hot water bath

Table1. Other DNA extraction methods that were evaluated.

Method	Extraction buffer	Extraction agent	Remarks
1. Mini - Cetyl trimethyl ammonium bromide (CTAB)–mercaptoethanol [2 x CTAB + Mercaptoethanol]	CTAB	Mercaptoethanol	2nd best compared to the method adopted for the study
2. Mini – CTAB - Dithiothreitol (DTT) [2 x CTAB + DTT]	CTAB	DTT	3rd best compared to the method adopted for the study
3. Mini - Sodium Dodecyl Sulphate (SDS)–mercaptoethanol [SDS + Mercaptoethanol]	SDS	Mercaptoethanol	3th best compared to the method adopted for the study

overnight at 55^0C.

The purified DNA was quantified by the agarose electrophoresis method described by Manniatis et al. (1982). Mini-agarose gel of 1.5% was melted in a microwave for 2 min, poured in a mini-gel tank and 5 µL gel loading dye [50% of glycerol; 250 mM EDTA of pH 8.0; 0.01% bromophenol blue] added to help make up the volume for quantification. When the gel had solidified, 1 x TBE buffer [89 mM Tris-HCl of pH 8.3; 89 mM boric acid; 2.5 mM EDTA] was added to fill the tank. 5 µL each of the purified DNA samples were pippeted into the wells of the gel, together with 2 lambda DNA standards (100 ng/µL and 250 ng/µL). The gel was run at 40 V for 40 min. After electrophoresis the gel was placed in a tray and stained using 5 µL ethidium bromide (EtBr), with gentle agitation on a shaker for 2 h. The gels were then viewed in a dark room under UV light (312 nm) on a transilluminator. The concentrations of the samples were determined by comparing band sizes and staining intensities of the test DNA samples with those of the standard Lambda DNA. Most band sizes and intensities for other methods (Table 1) were not as good as the SDS-DTT method used in this study.

The samples of the 4 spiderplant morphotypes were evaluated for polymorphism using 10-base RAPD primers purchased from Operon Technologies, Alameda Calif., USA and ABgene, Epsom, UK. Based on results of a previous study in tea (*Camellia sinensis*) (Wachira et al., 1995), a total of 12 different primers were selected and screened (OPD-18, OPW-04, OPW-06, OPW-11, AB4-13, OPW-18, OPU-15, OPV-15, OPW-03, OPU-10, OPU-20 and OPD-05). PCR conditions for RAPD reaction were modified from the method by Williams et al. (1990) as follows: 0.5 µL DNA sample was added to 8.5 µL master mix containing 5.9 µL SDW, 1 µL reaction buffer [750 mM Tris-HCl of pH 8.8; 200 mM $(NH_4)_2SO_4$; 0.1% (v/v) Tween 20®], 1 µL 25 mM $MgCl_2$; 0.5 µL 200 mM template DNA (dNTPs) [dATP, dCTP, dGTP and dTTP]; 1 µL 20 mM 10-base primer, 0.1 µL unit Taq DNA polymerase; to make a final volume of 10 µL in a PCR tube. The above PCR samples were then placed in a Techne thermal cycler, Model PHC-3. The samples were subjected to 93^0C for 5 min (hot start step), 40 cycles of: 93^0C for 1 min (denaturation step), 42^0C for 1.5 min (primer-template annealing step), 72^0C for 1 min (polymerisation step) and final extension step of 10 min at 72^0C. The amplification products were stored at 4^0C before analyzing by gel electrophoresis.

After amplification in the thermocycler, 10 µL of the loading dye [50% of glycerol; 250 mM EDTA of pH 8.0; 0.01% bromophenol blue] was mixed with 5 µL of the PCR products. The mixture was then loaded onto a 1.5% mini-agarose gel together with a Lambda DNA EcoR I/Hind III digest. The gel was then electrophoresed in 1 x TBE running buffer [89 mM Tris-HCl of pH 8.3; 89 mM boric acid; 2.5 mM EDTA] at a constant voltage of 150 for 180 min. After electrophoresis, the gel was stained in EtBr for 30 min before rinsing and viewing in a dark room under ultraviolet light of 312 nm on a transilluminator. The PCR bands were photographed using a Nikon camera, model FM 2 (Nikon Corporation, Japan). The exposed films were then processed in a photographic studio and pictures of DNA bands produced.

Data analysis

For each morphotype, the DNA bands were scored by visual inspection. Each PCR band was assumed to represent a single locus and data were scored as presence (1) or absence (0) of specific RAPD-bands. Different bands produced with each primer were numbered sequentially. Bands with a clear medium or strong signal were taken into account. Bands at comparable places on the gel but with different intensities were not distinguished from each other when morphotypes were compared.

The resulting data were analysed using the POPGENE 3.2 (Yeh et al., 1999) software programme. Diversity estimates for the morphotypes were calculated as (i) percentage polymorphic bands (PB), where PB = X/M x 100, X is the number of polymorphic bands in the sample and M the total number of bands and (ii) genetic diversity (H), calculated by Nei's (1978) genetic diversity. Genetic diversity was calculated for each locus (including monomorphic and polymorphic loci) by $H = 1 - \sum f_i^2$. For species values $(H_s)f_1$ is the mean frequency of the *i*th allele (*fi*) pooled across all populations. For population values $(H_p)f_i$ is the frequency of the *i*th alleles in each population.

To analyze the differentiation between morphotypes by statistical procedure, the Gst statistic, was used (Gst = Ht - Hs/Ht), where, Gst is the coefficient of gene differentiation, Ht is the total genetic diversity and Hs is the mean heterozygosity within populations. Gst was estimated using POPGENE 3.2

Pairwise comparisons of genetic similarities among the morphotypes were computed with POPGENE 3.2., according to Nei's (1978) unbiased, standard genetic identity, where I = 0.0 shows no common alleles and I = 1.0 indicates equal gene frequencies.

The genetic relationships between the 4 morphotypes studied were inferred from a cluster analysis procedure, the Neighbour-Joining using the unweighted pair group method with arithmetic average (UPGMA) algorithm based on the standard genetic distance of Nei for the various loci [D = -ln (I)], where I is the genetic identity for multiple loci (Nei, 1978). The result was used to create a dendrogram. Cluster analysis was performed using POPGENE programme. D = 0 indicates no genetic differences, while D = 1 means distantly genetically related.

RESULTS

Genetic diversity

Out of the 12 RAPD primers screened for their capacity to differentiate among the 4 spiderplant morphotypes from western Kenya, 10 (83.3%) amplified polymorphic products and generated 148 bands (14.8 bands for each

Table 2. Polymorphic RAPD primers used and their characteristics.

Primer	Nucleotide sequence	GC content (%)	Total number of bands	Number of Polymorphic bands	Polymorphic bands (%)
OPD-18	5´GAGAGCCAAC3´	60	10	3	30.0
OPW-04	CAGAAGCGGA	60	8	1	12.5
AB4-13	GTCAGAGTCC	60	15	2	13.3
OPW-18	TTCAGGGCAC	60	19	1	5.3
OPU-15	ACGGGCCAGT	70	10	1	10.0
OPV-15	CAGTGCCGGT	70	20	2	10.0
OPW-03	GTCCGGAGTG	70	22	6	27.3
OPU-10	ACCTCGGCAC	70	13	2	15.4
OPU-20	ACAGCCCCCA	70	16	6	37.5
OPD-05	TGAGCGGACA	60	15	7	46.7
Total			148	31	20.95

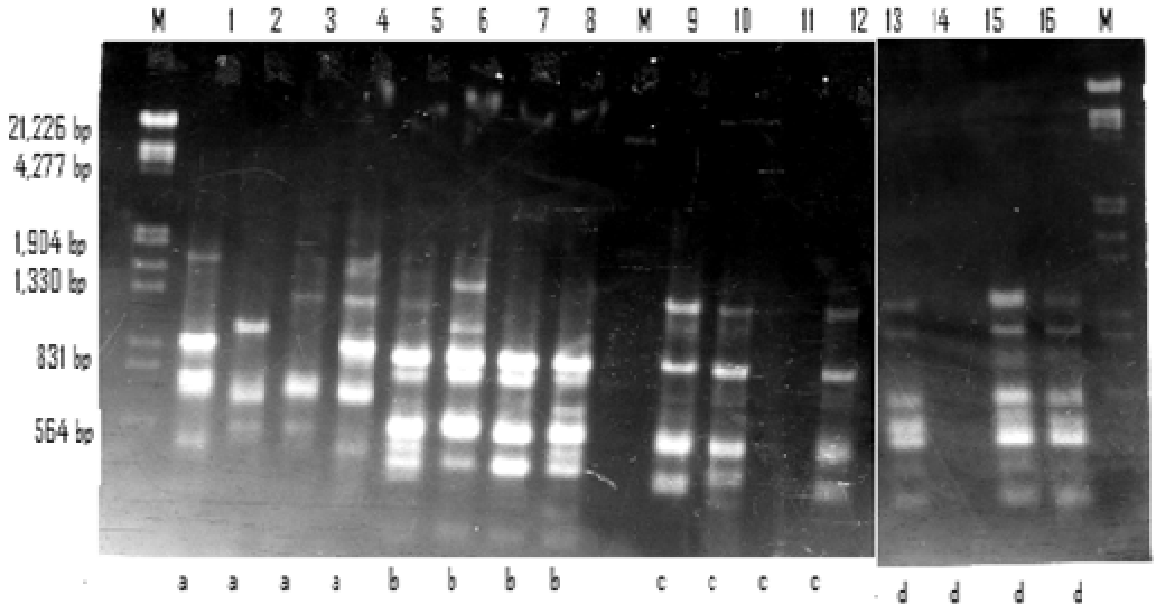

Figure 1. Subset of RAPD amplification products generated from 4 spiderplant (*C. gynandra*) morphotypes from western Kenya using primers a: OPD-05 (lanes 1 - 4), b: OPU-20 (lanes 5 - 8), c: OPU-10 (lanes 9 - 12) and d: OPV-15 (lanes 13 - 16). The order of morphotypes in a, b, c and d is PP-purple stem/purple petiole type of plants; PG- purple stem/green petiole type of plants; GP-green stem/purple petiole type of plants and GG-green stem/green petiole type of plants. M is a Lambda DNA EcoR I/Hind III. The approximate molecular weight (bp) is shown on the left. Arrows indicate examples of polymorphism.

primer), of which 31 (20.95%) were polymorphic (Table 2), with an average of 3.1 polymorphic loci/RAPD primer. The other 2 primers showed no polymorphism. The highest number of bands was generated by primers OPW-03 (22 bands) and OPV-15 (20 bands), the highest number of polymorphic bands was generated by primers OPD-05 (7 bands) and OPW-03 and OPU-20 (6 bands each) (Table 2). The product sizes ranged from 564 - 3530 bp; with primer OPU-15 having the longest size. Figure 1 shows a subset of RAPD amplification products generated from the 4 spiderplant (*C. gynandra*) morphotypes from western Kenya.

The mean number of alleles per locus (N) was 1.97, while the mean Nei's genetic diversity index (H) was 0.41 (data not shown). The results above revealed the RAPD variation between morphotypes.

Genetic differentiation

RAPD markers gave a high coefficient of gene differentiation (Gst) of 1 (data not shown). The 4 morphotypes

Table 3. RAPD matrix of Nei's (1978) unbiased measure of genetic identity (I).

Morphotypes	GG	GP	PG	PP
GG		0.6	0.3	0.6
GP			0.4	0.5
PG				0.3
PP				

Table 4. RAPD matrix of Nei's (1978) unbiased measure of genetic distance (D).

Morphotypes	GG	GP	PG	PP
GG		0.5	1.0	0.5
GP			0.9	0.8
PG				1.0
PP				

were well differentiated.

Nei's genetic identity (I)

The lowest genetic identity value of 0.3 was estimated for morphotypes GG and PG and PG and PP respectively, thus they shared the lowest number of common alleles and differed the most (Table 3). Morphotypes GG and GP and GG and PP had respectively the highest genetic identity values (0.6) and more common alleles (Table 3). Morphotypes GP and PG and GP and PP had intermediate genetic identity values, respectively. The analysis shows genetic diversity for the used primer set and may well give a general picture of the morphotype diversity. The genetic identity complements the genetic distance (Table 4). Therefore relationship between morphotypes represented by genetic distance is similar to that of genetic identity.

Nei's genetic distance (D) and relationships between morphotypes

Nei's genetic distance (D) is shown in Table 4. As with the analysis for the genetic identity, the lowest genetic distance of 1.0 was estimated for morphotypes GG and PG and PG and PP respectively indicating that they were the most distantly related. Similarly, morphotypes GG and GP and GG and PP had respectively the smallest genetic distance value (0.5); on the other hand, GP and PG and GP and PP had intermediate genetic distances, respectively.

Figure 2 depicts a dendrogram in the relationships between the morphotypes, based on distance coefficients. Three clusters are distinguished showing genetic variations among the morphotypes.

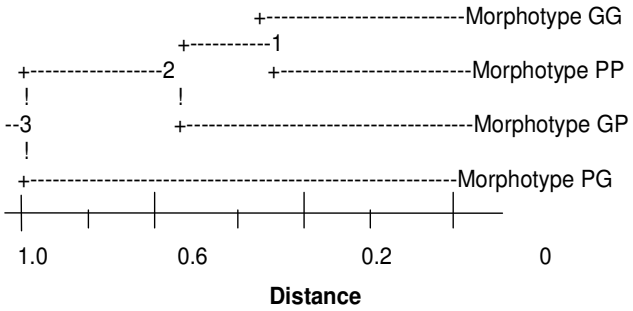

Figure 2. Neighbour-joining dendrogram based on Nei's (1978) genetic distances based on RAPD-PCR results for 4 spiderplant morphotypes from western Kenya.

DISCUSSION

The research was conducted to genetic assess variations in 4 spiderplant morphotypes from western Kenya, using RAPD-PCR technology. In earlier studies (Lee et al., 1996b; Kelly and George, 1998; Gaudel et al., 2000; Mateu-André and Segarra-Moragues, 2000; Lanteri et al. 2001; Müller-Schärer and Fischer, 2001), the extensive and effective use of RAPD analysis in genetic diversity studies has been reported for both wild and cultivated plant species that include cereal, oilseed, root, fruit, vegetable and ornamental crops. To the best of our knowledge the present study is the first molecular assessment of genetic variability between morphotypes of spiderplant from western Kenya.

The DNA amplification and fingerprinting (DAF) technique detected molecular polymorphism among the *Cleome gynandra* morphotypes indicating that the RAPD technique can also be used to examine the extent of genetic diversity in this indigenous vegetable. The average number of polymorphic bands per primer was 3.1 (Table 2), which is comparable with results of Lanteri et al. (2003) for a landrace of *Capsicum annuum*, where the 18 primers gave an average number of polymorphic bands/primer of 3.3. They found 18 out of 59 RAPD primers to produce polymorphic bands. The RAPD primers in the present study were also used in tea research and is concluded that they were useful in genetic variation studies of both species. Large number of polymorphisms can be detected by adding primers. It is realized that differences in both coding and non coding regions of the genome can be revealed, including sequence differences that may exist between genes coding for the same protein. In tea, RAPDs have been used to establish differences between samples from Kenya, Korea and China (Muzin, 1994; Wachira et al., 1995; Liang et al., 1998). However, as stated by Weeden et al. (1989), a complication in using the RAPD technique for diversity studies is the continuum of band intensities observed:

some RAPD products show intense, well resolved bands, while other bands are faint or fuzzy and are difficult to score (Figure 1). RAPDs are dominant markers, making them unable to distinguish heterozygotes from homozygotes (Williams et al., 1990; Miklas et al., 2006).

Variation occurring between morphotypes was at Gst = 1 (data not shown), adequate for complete differentiation between the 4 spiderplant morphotypes. A most interesting observation is that the selected 4 “morphotypes”, with selection based on colour only, show clear differences in RAPDs bands.

The diversity in spiderplant could probably be explained for by it being semi-cultivated in Kenya without much selection by farmers, thus allowing maintenance of variation. The genetic diversity in Africa of Ethiopian mustard (*Brassica carinata*) based on molecular markers is much less than that of brown mustard (*Brassica juncea*) (Mnzava and Schippers, 2004). The 2 species belong to the family Brassicaceae, are closely relatives of *Cleome* (Rodman et al., 1996, 1998). The cultivation of Ethiopian mustard started in Ethiopia about 4000 BC, while brown mustard is a later introduction from Asia and Europe (many landraces of Ethiopian mustard exist both as oilseed and leafy vegetable, with differences in earliness, plant structure, leaf size, shape and structure, seed yield and glucosinolate and erucic acid levels) (Mnzava and Schippers, 2004). The narrower genetic base of Ethiopian mustard compared to brown mustard could possibly be explained by its long cultivation and with selection for desirable characteristics by African farmers.

In the present study, a dendrogram was generated based on RAPD bands as characters. The morphotypes were grouped into 3. Morphotypes GG and PP formed the first close group, followed by the addition of GP in group 2. Morphotype PG was at distance from groups 1 and 2.

Comparing the molecular dendrogram with the morphological one in an earlier study, it shows the clusters differ, not surprisingly, as they are different criteria for grouping. Since genes and environments are responsible for the various expressions of characters in plants, gene mutation could bring about a change in characters and can also lead to the development of new plant types (Dutta, 1979). This may have happened in the 4 morphotypes, which differ genetically. As pointed out by McClintock (1984), the genome is dynamic and can modify in response to environmental stresses. Even elite crop gene pools possess inherent mechanisms for new genetic variability, named genome’s plasticity (Rasmusson and Phillips, 1997).

Flavonoids, including anthocyanins are considered survival compounds of flowering plants. These were found in the stems and petioles of 3 of the spiderplant morphotypes studied (Beggs and Wellmann, 1994). Anthocyanins are credited with many functions in plants, which include preinfection mechanisms of disease resistance; increase in osmotic pressure of cell sap, thus enhancing absorption of water by root hairs from the soil and water movement within the plant, which are all important for plant growth, further, assistance in respiratory and photosynthetic processes as well as protection of chlorophyll from being decomposed in strong light (Hill et al., 1981; Kochhar and Krishnamoorthy, 1992). While external factors such as strong light, low temperature, drought, low soil nitrogen content and low oxygen supply tend to favour the synthesis of anthocyanins, genetic factors are essential in their formation (Kochhar and Krishnamoorthy, 1992; Beggs and Wellmann, 1994). It is likely that the many roles cited for anthocyanin apply to spiderplant, also providing for its wide distribution in various agro-ecological zones in Kenya.

The 4 morphotypes are clearly distinct, exhibiting morphological and nucleotide sequence (Tables 3 and 4, Figure 2).

Conclusions

The genetic identification of a cultivated crop variety is of particular importance for the stabilization of its commercial value and for the promotion of its status. To date, there has been no systematic molecular description of spiderplant resources in western Kenya. Although a more comprehensive collection is needed to elucidate genetic variation within *C. gynandra* indigenous to western Kenya, the present study demonstrates that genetic variation exists within western Kenya’s *C. gynandra* germplasm, which is useful information for germplasm conservation and genetic improvement of the species. The results provide support for the utility of RAPD-PCR markers for assessing genetic diversity in spiderplant germplasm collections in the country. Other authors have effectively utilized such markers in genetic variability studies of various plant species. In addition to using morphological traits to distinguish plant types the RAPD technology is of great benefit since it is capable of generating large numbers of polymorphisms with different primers, while at the same time detecting differences in coding and non-coding regions of the genome.

Eventually, an extensive study, covering the entire Kenyan spiderplant germplasm collection by molecular markers, will reveal the total genetic diversity available in this vegetable crop species. For purposes of conservation, seed of many populations should be collected and samples of individuals analysed for estimating genetic differentiation.

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