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DETERMINATION OF EXTENT OF GENETIC VARIATION IN *GOMPHOCARPUS* GERMPLASM USING SSR MARKERS

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ABSTRACT: *In Kenya two common species of Asclepias; Gomphocarpus physocarpa and Gomphocarpus fruticosus are commercially cultivated as cut flowers for their inflated green bolls. As a comparison, Gomphocarpus physocarpa has larger and more rounded ornamental seed bolls than Gomphocarpus fruticosus whose inflated seed bolls have sharp pointed end and are covered in short, stout hairs. These two species may be easily confused from each other. Unimproved commercial lines grown in Kenya are a mix of the two cultivars as the two species can easily hybridize. A study was carried out whose objective was to determine the extent of genetic diversity of Gomphocarpus in Kenya. The Gomphocarpus samples used in the study were collected both from the wild and commercial farms. Fourteen Gomphocarpus samples were collected from various areas in Kenya. All the SSR fragments obtained from gel electrophoresis in this experiment were easily recognizable under UV light. All the fourteen Gomphocarpus samples collected from different locations had SSR 650 bp. The SSR primers were monomorphic across all the Gomphocarpus samples indicating that there was no genetic variation among the Gomphocarpus collections used.*

KEYWORDS*: Asclepias spp., DNA markers, genetic variation*

INTRODUCTION

The term *Asclepias* is used to refer to milkweed species grown in American continent and other Western worlds, while *Gomphocarpus* refers to *Asclepias* species found in Africa and Arabian continents (Hodkiss, 2009). The genus *Gomphocarpus* comprises 25 – 32 species that occur in Africa and Arabian Peninsula. Ten other species occur in southern Africa. Morphologically, *Gomphocarpus* plants demonstrate an erect growth habit with multiple stems of 1 – 3m in height. The leaves are entire and arranged in pairs and opposite each other along the stem. The leaves are also dull green in colour. Flowers are borne in simple 6 – 10 flowered umbels, each flower being suspended on a pedicel. Flowers are often brightly coloured, with a characteristic five-fold symmetry. The pollen are found in pollinia or 'pollen sacs' rather than being in individual grains or tetrads typically for most plants (Parsons and Cuthbertson, 1992). When the follicles ripen, they split open and the seeds attached to the floss are blown away by wind (Ramanujan - Krishna, 2008).

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In Kenya two common species; *Gomphocarpus physocarpa* and *Gomphocarpus fruticosus* are commercially cultivated as a cut flower for its inflated green bolls. As a comparison, *Gomphocarpus physocarpa* has larger and more rounded ornamental seed bolls than *Gomphocarpus fruticosus* whose inflated seed bolls have sharp pointed end and are covered in short, stout hairs. These two species may be easily confused from each other. However, unimproved commercial lines grown in Kenya are a mix of the two cultivars as the two species can easily hybridize.

Genetic diversity refers to any variation in the nucleotides, genes, chromosomes, or whole genome of organisms. The genome is the entire complement of DNA within the cells or organelles of the organism (Andayani *et al.*, 2001). Genetic diversity at its most elementary level is represented by differences in the sequences of nucleotides (adenine, cytosine, guanine, and thymine) that form the DNA within the cells of the organism (Broyles, 2002).

Genetic diversity helps in species adaptation to ever changing environments. Where more variations are displayed, it is more likely that a proportion of the individuals in that population will possess different combination of sets of alleles that are suited most for the environment. Such individuals are bound to survive and give off springs bearing the set of adaptative allele. The population will survive many generations due to success of such individuals to prevailing environmental conditions (Broyles, 2002).

Thus, genetic diversity is the corner-stone for any genetic improvement in a population. Information regarding the genetic diversity of any germplasm is crucial to guide in the development of an efficient plant breeding programme and maintenance of genetic diversity in a given gene pool. In research, morphological, biochemical and DNA-based markers are used to estimate the genetic diversity in a population. However, morphological markers are greatly influenced by prevailing environmental conditions (CIAT, 1993) and not suitable for perennial crops that take long time to mature before one can generate sufficient data. DNA-based markers offer consistent results irrespective of cropping conditions, type and age of sampled tissue (Sakiyama, 2000). Microsatellite markers exhibit high degree of polymorphism. These have been developed to characterize and study the population genetic structure in *Asclepias exaltata* and *Asclepias syriaca* (Broyles *et al.*, 1994). These types of markers have been recommended for assessing population genetic structure and interspecific gene flow in these and other *Asclepias species* (Broyles, 2002).

Morphological characterization of *Gomphocarpus* has not revealed any genetic divergence in the two species (Watako *et. al*., 2014). The main objective of this study was to determine the extent of genetic diversity of *Gomphocarpus* in Kenya using SSR markers. The *Gomphocarpus* samples used in the study were collected both from the wild and commercial farms.

MATERIALS AND METHODS

Sample collection

Fourteen *Gomphocarpus* samples were collected from various areas in Kenya. Five of these samples were plant collections from Thika, Chumvi, Narok, Juja farm and Molo which were

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relatively taller with broad leaves. Sample 6 was a plant obtained in the wild among Juja farm plants possessing unique features different from the rest in that population, including narrower leaves and shorter stems; sample 7 was a collection from Siaya having narrow leaves and shorter stems. On the other hand, samples 9, 10 and 11 were short variety collections from Machakos whereas samples 8, 12, 13 and 14 were tall *Gomphocarpus* collections from Kitengela, Machakos, Makueni and Laikipia respectively (Table 1).

Table 1: *Gomphocarpus* sample collections and sites.

Gomphocarpus collection	Areas of collections	Characteristics
Samples 1	Thika	Broad leaves
Samples 2	Chumvi	Broad leaves
Samples 3	Narok	Broad leaves
Samples 4	Juja farm	Broad leaves
Samples 5	Molo	Broad leaves
Samples 6	Juja farm	Narrow leaves
Samples 7	Siaya	Narrow leaves
Samples 8	Kitengela	Broad leaves
Samples 9	Machakos	Narrow leaves
Samples 10	Machakos	Narrow leaves
Samples 11	Machakos	Narrow leaves
Samples 12	Machakos	Broad leaves
Samples 13	Makueni	Broad leaves
Samples 14	Laikipia	Broad leaves

All the leaves used in DNA extraction were collected from young and tender tissues especially from stem apex. These leaf tissues were put in polythene paper bags, labeled then kept in cool dry ice boxes in preparation for DNA extraction.

DNA extraction from *Gomphocarpus* collections

All equipments used in this experiment were first sterilized by autoclaving. Approximately 3 g of fresh *Gomphocarpus* leaves were crushed in liquid nitrogen to a finer powder using a mortar and pestle. The samples were then placed in eppendorf tubes. While working with this fresh tissue, it was necessary to ensure that the material did not thaw before it was added to the isolation buffer otherwise cellular enzymes might rapidly degrade the DNA. An isolation buffer (2% CTAB, 1% PVP, 1.4 M NaCl, 20 mM EDTA, and 100 mM Tris-HCl) was prewarmed at 60˚C for about 30 minutes; 2 % of β-mercaptoethanol was then added to this isolation buffer just before adding the isolation buffer mixture to each sample. To each of the fourteen tissue samples in eppendorf tubes, 600 µl of isolation buffer was added under a fume cupboard and vortexed gently. The samples were then incubated for 5 minutes at 15˚C in an incubator with mixing after every 10 minutes. This was followed by an addition of 400 µl of chloroform iso amyl alcohol to each sample eppendorf tube (Chloroform to iso amyl alcohol in 24:1 ratio). The samples were vortexed gently then inverted severally to avoid DNA shearing. The samples were then centrifuged for 15 minutes at 13,000rpm, the aqueous (upper) phase or the supernatant then extracted thrice with fresh chloroform–iso amyl alcohol. The supernatant from each of the

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fourteen samples was then transferred into a fresh clean eppendorf tube into which 500 µl of iso propanol and 20 µl NaCl (5M) were then added. The samples were then placed at -20˚C for 4 hours then centrifuged for DNA to precipitate. The supernatant was then poured out to remain with the pellets. These pellets were then washed with 1000µl of 70% ethanol and then gently agitated for a few minutes and DNA collection done by centrifugation. Residual CTAB was removed by this step. The tubes were inverted and drained on a paper towel for about 20 minutes.

At this stage, 60 µl of TE buffer (10 mM Tris-HCl, 1 mM EDTA, pH=8.0) was added to each sample in order to dissolve the dry pellets; 1-2µl of RNAseA (10mg/ml) were added to each sample, incubated at 37˚C for 2 hours. The DNA was then stored at -20˚C.

DNA quantity and quality determination

Agarose gel electrophoresis and spectrophotometry were used to determine the quantity and quality of DNA; 1g of agarose powder was dissolved in 100ml Tris Borate EDTA (0.5 x TBE x 45 mm Tris borate, 1mm EDTA , PH=8.0) buffer (1% w/v) in a microwave oven and then boiled at 1 minute 30 seconds. After cooling to about 50˚C, 5 µl ethidium bromide was added to the resulting mixture. As the gel was cooling, the gel trough was prepared by sealing the open ends of the clean glass with a tape. Plastic strips were also tightened on the open edges to prevent gel escape. A comb was set vertically in the gel tray ensuring the lower tips of the comb were slightly above the gel tray floor.

The warm agarose solution was then poured gently into the gel tray to form the wells. The gel was allowed to cool for 40 minutes before removing the autoclaved tape and the comb. The gel was then immersed in the electrophoresis tank containing 1X TBE buffer. At this stage, 5µl of each DNA sample mixed with 3µl of loading solution was loaded to the wells of the gel. DNA molecular weight marker or ladder was run alongside one lane of the gel. The gel ran at a constant voltage of 120 volts until the bromo-phenol blue migrated almost to the end of the gel. The gel was then removed from the trough, placed in a UV trans-illuminator and photographed. The DNA concentration was calculated in the sample using the formula 1.0 OD_{260} = 50 µg/ml (under standard conditions, that is a 1-cm light path).

Quantification of DNA in solution was done by measuring the absorbance of light (260 nm) in a spectrophotometer(Eppendors Biophotometer, Germany); 2µl of each sample was added to 98µl TE, mixed well and OD_{260} and OD_{280} read to determine the purity of the DNA (CYMMT, 2005). Following UV quantification, each DNA sample concentration was adjusted at 5ng/µl with TE and stored at 4˚C (CYMMT, 2005).

Polymerase chain reaction (PCR) using SSR primers

The microsatellites used to amplify the DNA from *Gomphocarpus* samples were selected from a set of primers earlier used in amplification of *Asclepias exaltata* and *Asclepias syriaca.* As such, these primers were recommended for use in any other *Asclepias species* (Agrawal and Fishbein, 2008). The PCR amplifications were carried out on a Gene-Amp PCR system 9700 (Applied Biosystems). The DNA sample volume used was 1µl per reaction tube with a concentration of 5ng/µl genomic DNA, 10 X PCR buffers (with 5/µl Taq. Polymerase), and 0.5 µM each of

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forward and reverse primers (Table 2 and 3). The final volume used for the reaction was 20 µl. The prepared samples containing genomic DNA in microtubes were then placed in the thermal cycler. The thermal cycler was programmed at 95˚C initial denaturization for 5 minutes, 30 cycles of 94˚C denaturization for 30 seconds; the annealing temperature was 57˚C for 1 minute, 72˚C elongation temperature for 30 seconds and 72˚C in 10 minutes being the elongation temperature and a final temperature was set at 4˚C until the samples were used.

Table 2: PCR optimization conditions of the SSR primers

	1st PCR Trial	**2nd PCR Trial**	**Final Optimized PCR conditions**	**Final concentration of various components**
PCR buffer with $MgCl_2$ (10X)	3 µl	3 µl	1.5 µl	1x
Forward primer	1 µl	2 µl	0.5 µl	20pM
Reverse primer	1 µl	2 µl	0.5 µl	20pM
Taq DNA polymerase (5/µl)	0.3 µl	0.3 µl	0.2 µl	2 µl/ml
Sterile water	14.2 µl	12.2 µl	16.3 µl	10 µl
Template DNA (5ng)	0.5 µl	0.5 µl	1 µl	2ng/ml
	20 µl	**20 µl**	**20 µl**	

Table 3: *Gomphocarpus* microsatellite primers used in the project

Total volume

Primer set number	**Locus**	**Primer sequence (50 – 30)**	**Repeat mortif**
1	Asyr-A106 FJ478394	F: TTTCTTCATTTGTCAGCTCAAC R: GGAGTCGTCAATATAGACACAC	$(GA)_{13}$
2	Asyr-B102 FJ478397	F: TTGGAAGAAGGAAGGAATACTGAG R: CACCAACAAGTAATGACATGAG	$(AAC)_7$

Source: (Agrawal and Fishbein, 2008)

Agarose Gel Electrophoresis of PCR products

Agarose powder was dissolved in 100 ml of TBX solution to prepare 3% agarose gel solution; 3µl of sample loading buffer (bromo-phenol blue) was added to 5µl of each PCR product by pipetting then mixing prior to loading the mixture in the preformed sample wells on the gel. The

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samples were then run alongside 5µl of 1kb DNA ladder at 100 volts for 45 minutes. At the end of the 45th minute, the gel was observed under UV light and photographed to visualize the amplified SSR fragments.

RESULTS

All the SSR fragments obtained from gel electrophoresis in this experiment were easily recognizable under UV light. All the fourteen *Gomphocarpus* samples collected from different locations (Table 1) had SSR 650 bp. The SSR primers were monomorphic across all the *Gomphocarpus* collection samples used in this study (Figure 1). The SSR primer sets used in this study did not show any genetic variation among the *Gomphocarpus* collections used.

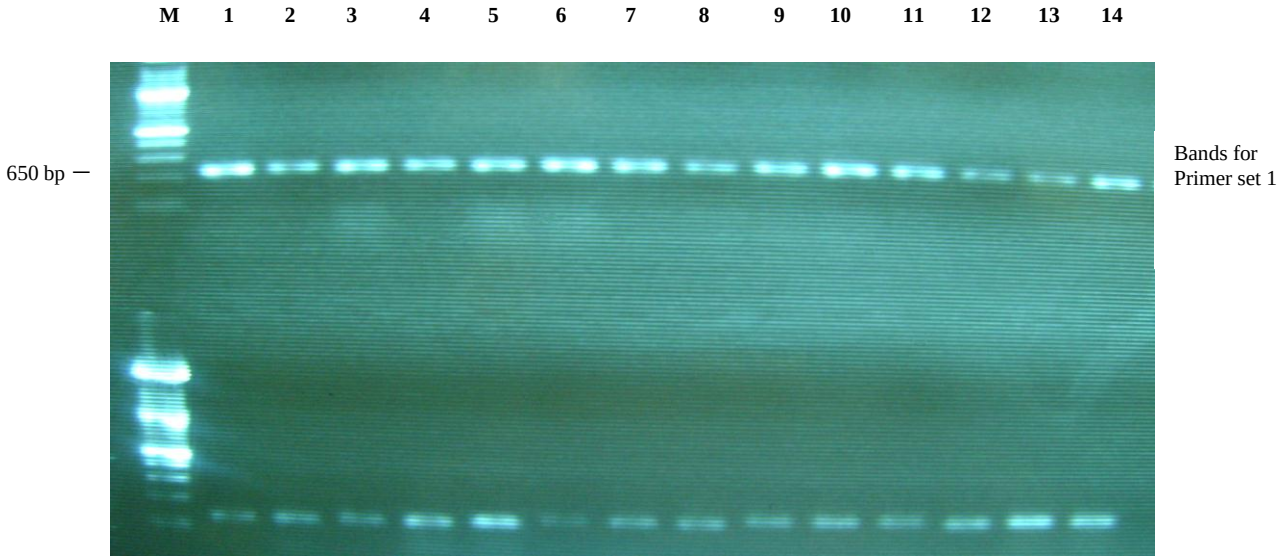

Figure 1: Comparison of SSR marker banding patterns obtained from a set of 14 *Gomphocarpus* collections from various sites in Kenya. Lane M is the molecular weight marker. The size of DNA was 650 bp.

DISCUSSION

The analysis of genetic diversity and relatedness between or within different populations, species, and individuals is a central task for many disciplines of biological science. Such assessment is of paramount importance for germplasm conservation and breeding purposes (Sakiyama, 2000).

The application of SSR markers in genetic fingerprinting of germplasms which are morphologically similar or indistinguishable, as in the case of *Gomphocarpus*, has been established as a reliable, efficient and very informative technique (Irwin *et al*., 1998). The microsatellite markers have shown high degrees of genetic polymorphism in many crops including *Cucumis melon* L. (Danin-Poleg *et al*., 2001), cassava cultivars (Moyib *et al*., 2007) and sweet potato (Karuri *et al*., 2009). However, the results obtained in the current study reveal

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that SSR markers were highly monomorphic in *Gomphocarpus* germplasm (Figure 1). That means no variation was detected in *Gomphocarpus* characterization using SSR markers.
The current study is a pioneer work in the genetic characterization of Kenyan *Gomphocarpus* germplasm using microsatellite or SSR markers. The study used one set of Primers (microsatellites), the SSR markers, which had been used before in a similar project in genetic characterization of *Asclepias syriaca* and *Asclepias exaltata*. As such, the primers were recommended useful for assessing population genetic structure and interspecific gene flow in any other species of *Asclepias* such as *Gomphocarpus* species or the African *Asclepias* (Broyles *et al*., 2002).

The results of *Gomphocarpus* germplasm characterization using the selected SSR genetic marker suggests that the populations of *Gomphocarpus* used in the study are considerably homogenous. Both the domesticated and wild *Gomphocarpus* are very similar implying low genetic diversity in the *Gomphocarpus* germplasm.

Information regarding the genetic relationships of germplasm is critical in addressing breeding programmes and germplasm resource management (Rolda´n-Ruiz *et al*., 2001). Results of genetic diversity using SSR markers in this study as well as morphological diversity analysis (Watako *et. al*., 2014) demonstrated the absence of variability among evaluated *Gomphocarpus* cultivars. With the results obtained, it is difficult to select diverse parents for breeding programmes and in maintaining genetic variation in *Gomphocarpus* germplasm. It might not be easy also to utilize the genetic potential of these genotypes in improvement of identified phenotypes for adaptation in changing environments. As a conclusion therefore it is essential to come up with strategies that will create variations in *Gomphocarpus* germplasm in order to determine the potential for improvement on the various characteristics.

REFERENCES

Agrawal AA and Fishbein M (2008). Phylogenetic escalation and decline of plant defense strategies. *Proceedings of the National Academy of Sciences* 105:10057–10060.

Andayani N, Morales JC, Forstner MRJ, Supriatna J, Melnick DJ (2001). Genetic variability in mtDNA of the silvery gibbon: implications for the conservation of a critically endangered species. *Conservation Biology*, 15(3), 1545-1548.

Broyles SB (2002). Hybrid bridges to gene flow: a case study in milkweeds (*Asclepias*). *Evolution* 56:1943–1953.

Broyles SB, Schnabel A and Wyatt R (1994). Evidence of long-distance pollen dispersal in milkweeds (*Asclepias exaltata*). *Evolution* 48**:**1032–1040.

Danin-Poleg, Y, Reis N, Baudraci S, Arnas S, Pitrat M, Staub J E, Oliver M, Arus P, Devicente CM, Katzir N (2001). Simple sequence repeats in cucumis mapping and map margin. *Genetics* 43:963-974.

Hodkiss RJ (2009). *Asclepias* – International Asclepiad Society Journal; *The succulent plant* 106: 24-58

International Center for Tropical Agriculture (CIAT). (1993). Biotechnology Research Unit. *Annual report*, Cali, Colombia.

ISSN 2054-6319 (Print), ISSN 2054-6327(online)

International Maize and Wheat Improvement Center (CIMMYT). (2005). Laboratory Protocols: CIMMYT Applied Molecular Genetics Laboratory. 3rd Edition. Mexico, D.F: CIMMYT.

Irwin SV, Kaufusi P, Banks K, de la Pena R and Cho.J.J (1998). Molecular characterization of taro (*Colocasia esculenta*) using RAPD markers. *Euphytica* 99, 183-189.

Parsons WT and Cuthberton EG (1992). Noxious weeds of Australia(Inkata Press, Melbourne / Sydney), pp. 179 – 182.

Moyib OK, Odunola OA and Dixon AGO (2007). SSR markers reveal genetic variation between improved cassava cultivars and landraces within a collection of Nigerian cassava germplasm. *African Journal of Biotechnology*. 6(23): 2666-2674.

Ramanujan – Krishna. (2008). Discoveries: Milhweed evolves to shrug off predation. Northern Woodlands; Centre for Northern Woodland Education, 15(4), pp. 56.

Rolda´n-Ruiz I, van Eeuwijk FA, Gilliland TJ, Dubreuil P, Dillmann C, Lallemand J, De Loose M and Baril CP (2001). A comparative study of molecular and morphological methods of describing relationships between perennial rye grass (*Lolium perenne* L.) varieties. *Theoretical and Applied Genetics* 103: 1138-1150.

Sakiyama NS (2000). DNA markers for coffee tree breeding. In: Sera, T. Soccol, C. R. Pandy, A. and Roussos, S. (Eds) Coffee biotechnology and quality. Kluwer Academic Publishers, Dordrecht pp. 179-185.

Watako AO, Saggafu SM and Mamati GE (2014). Morphological characterization of two endemic species of *Gomphocarpus* (Mobydick) in Kenya. J. agri. Sci. Technol. ISSN 1561 – 7625: 3 – 12.

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