Tree Genetics & Genomes (2016) 12: 66
DOI 10.1007/s11295-016-1013-z

ORIGINAL ARTICLE

Genetic diversity of the African poplar (*Populus ilicifolia*) populations in Kenya

Sammy Muraguri Mutegi[1] · Alice Muchugi[2] · Sammy Carsan[2] · Robert Kariba[2] · Ramni Jamnadass[2] · Phanuel Oballa[3] · Amy M. Brunner[4] · Steven Runo[1]

Received: 20 November 2015 / Revised: 29 April 2016 / Accepted: 9 May 2016 / Published online: 9 June 2016

Abstract We evaluated the genetic diversity of the African poplar (*Populus ilicifolia*) populations found in Kenya compared with reference samples of five poplar species from North America and one species introduced in Kenya from India (KEFRI-Kenya). Amplified fragment length polymorphism (AFLP) was used with the objective of providing important information for breeding and in situ/ex situ conservation of this species. Samples collected from three locations along the species' natural range (Athi, Ewaso Nyiro, and Tana rivers) were compared with four samples of locally planted *Populus deltoides* stand introduced from India and ten reference samples from North America. Six AFLP primer combinations produced 521 clear bands for analysis. The percentage polymorphic loci were lowest in Tana (20.4 %) and highest in Athi (40.6 %). The average heterozygosity across the studied populations was between 0.07 and 0.3. AMOVA revealed more genetic variation partitioning within population (87 %; $P < 0.01$) than among populations (13 %; $P < 0.01$) suggesting significant genetic variation between populations. Further, UPGMA delineation showed two clusters of the Tana, Athi, and Ewaso Nyiro populations clustered together compared to the North America and India/KEFRI reference samples. Moreover, the study showed that the Athi population is more diverse than those of Tana and Ewaso Nyiro and may be important for conservation, domestication, and improvement studies. The genetic differentiation ($F_{ST} = 0.134$) among Kenyan *P. ilicifolia* populations suggests limited possibility of gene flow between these populations.

Keywords *Populus ilicifolia* · Kenyan bioenergy species · AFLP · Genetic variation · Kenyan riparian habitat

Communicated by P. Ingvarsson

Electronic supplementary material The online version of this article (doi:10.1007/s11295-016-1013-z) contains supplementary material, which is available to authorized users.

✉ Sammy Muraguri Mutegi
Sammy.muraguri88@gmail.com

[1] Department of Biochemistry and Biotechnology, Kenyatta University, P.O. Box 43844-00100, Nairobi, Kenya

[2] World Agroforestry Centre (ICRAF), P.O. Box 30677-00100, Nairobi, Kenya

[3] Kenya Forestry Research Institute (KEFRI), P.O. Box 20412-00200, Nairobi, Kenya

[4] Department of Forest Resources and Environmental Conservation, Virginia Tech, Blacksburg, VA 24061, USA

Introduction

Populus ilicifolia (Engl.) Rouleau, locally known as the African poplar, is a riparian species endemic to Kenya and Tanzania (Maundu and Tengnas, 2005; WCMC, 1998). It is the southernmost member of this genus in the world occurring naturally along the valleys of the River Tana, Athi, and Ewaso Nyiro in Kenya (Oballa, 1996; WCMC, 1998). The distribution range of this species is in the arid and semi-arid region of the country where annual rainfall is between 200–800 mm. It is deciduous, shedding off some of its leaves during seed maturation period mainly in the rainy season a phenomenon called reverse phenology. In Kenya, it is commonly referred to as the Tana poplar and is widely used by communities living close to its natural habitats for fodder, timber, posts, beehives and dugout canoes given its easy-to-work wood properties (Battiscombe, 1926). Its potential is however poorly known unlike in the temperate regions where poplar has had great environmental and economic contributions in both plantation and agroforestry settings (Oballa, 1996). Given the bioenergy

constraints in most African countries often compounded by fluctuation in oil prices, opportunities to develop *P. ilicifolia* for sustainable production of bioenergy could emerge as witnessed for other poplar species in the temperate regions (United States Department of Energy, 2006).

P. ilicifolia reproduce both asexually and sexually which may affect the genetic diversity dynamics especially in natural conditions. Adaptation to changing environments and occupation of new habitats is influenced by these two reproduction mechanisms (Eriksson, 1993). Pollen and seed dispersal in *P. ilicifolia* is important in maintaining genetic variability. Pollination occurs by wind, and in 3–6 weeks, seeds are formed. Seeds surrounded at the base by silky hair are often produced in large quantities and dispersed by wind and water (Barat-Segretain, 1996). Vegetative reproduction, on the other hand, is uncommon but can occur through cuttings and root sucker shoots which is essential in reestablishment of population after major disturbances (Eriksson, 1993). Within its natural range, the species is observed to regenerate poorly.

Studies conducted on propagation techniques have showed that with some improvement in growth conditions the species can be raised successfully both from seeds and cuttings (Oballa, 1996). Mature seeds collected and sown within 1–2 days germinated well under hot and humid conditions. Cuttings rooting success of up to 60 % has been reported under hot glasshouse conditions. Efforts on matching sites for plantation establishment can therefore help to conserve the species and increase its economic use (Oballa, 1996).

P. ilicifolia is currently listed as one of the endangered trees in Kenya by the Kenya Wildlife Services and as a vulnerable species in the IUCN red list (IUCN, 2016). Reduction of the species' population is attributed to habitat degradation due to cultivation along river valleys and protected areas, heavy floods, and large herbivores such as elephants that uproot mature and young trees. Prevailing threats on the species' degradation therefore demand concerted efforts in the species conservation. Knowledge of the existing genetic diversity within and among the species populations is not available to inform national restoration strategies for the species. Further, the genetic relatedness with its temperate relatives has not been investigated previously to support future breeding programs on the species.

A lot of work has nonetheless been carried out for the temperate *Populus* genus with the entire genome of *Populus trichocarpa* being sequenced (Tuskan et al. 2006). Knowledge on poplar tree improvement from America, Europe, and Asia can therefore help inform breeding strategies for the African poplar. Improvements that will lead to the cultivation of the species as an agroforestry tree are important especially if materials can provide good returns in a short rotation as demanded by communities depending on trees as a source of bioenergy. This study has contributed to this goal by assessing the levels of heterozygosity (gene diversity) of *P. ilicifolia* populations found in the Athi, Tana, and Ewaso Nyiro rivers in Kenya. It was hypothesized that there is no significant genetic variation within and among *P. ilicifolia* populations sampled in the Athi, Tana and Ewaso Nyiro rivers in Kenya, and there is no significant genetic relatedness between the Kenyan *P. ilicifolia* and the temperate *Populus* species from North America and some of India's material introduced in Kenya at KEFRI.

Methodology

A reconnaissance survey was carried out in 2012 to establish the status of *P. ilicifolia* populations along the river valleys (of Athi, Ewaso Nyiro and Tana) and their tributaries. There was a notable reduction in the number of trees in most of the locations compared to the 1996 and 2005 surveys (Fig. 1a, b). Due to the reduced population sizes and flooded rivers, only 30 tree clusters were sampled for the current study. At least six leaves per tree in the accessible tree clusters per location were collected maintaining a distance of approximately 100 m apart to reduce chances of collecting asexually reproduced clones of a single tree. The leaves were placed in labeled snap-top bag containing silica gel to dry the leaf samples. Two samples each of *Populus tremuloides*, *Populus deltoides*, *Populus fremontii*, *Populus*

Fig. 1 **a** *P. ilicifolia* trees along the Ewaso Nyiro River, Kenya in 2005. **b** Root suckers from remnants of the same population during the 2012 survey (Credit: P. Oballa). Indiscriminate logging and flooding effects has reduced the size of this natural population with no mature tree on sight

Table 1 Pre-selective amplification primers

Primer name	Sequence
EcoRI + 1-A	5′GACTGCGTAACCAATTC + A-3′
MseI + 1-C	5′GATGAGTCCTGAGTAA + C-3′

tremula, and *Populus balsamifera* were obtained from North America and four samples of *P. deltoides* introduced from India and planted at the Kenya Forestry Research Institute (KEFRI) in 2005 were included in the study as reference samples.

DNA was extracted using the CTAB method according to Doyle and Doyle (1987) and amplified fragment length polymorphism (Vos et al. 1995) analysis carried with modification as in the AFLP™ Plant Mapping Protocol of Applied Bio systems. Frequent cutter *Msel* and rare cutter *EcoR1* endonucleases from New England Biolabs were used for restriction of genomic DNA. To minimize artifacts, two amplification processes were involved, pre-selective and selective amplification. PCR pre-selective amplification was performed on the samples in which the threshold of selection was low since only a single nucleotide was added onto the primer sequence (Table 1). In the subsequent selective amplification, six AFLP primer combinations were used for this study (Table 2). Amplicon fragment sizes were resolved on capillary electrophoresis (ABI 3730 DNA analyzer). The ABI Genetic Analyzer recorded the fluorescence intensity as a function of time and wavelength from the regions on a CCD camera that correspond to different detection wavelength ranges. These signals were transmitted to a computer that has data collection software version 4.1.

Data was analyzed using GeneMapper software to display the fragment sizes as electrophoregrams and binary data. Genetic variation was computed to show genetic partitioning within and among Kenya's population (AMOVA; Excoffier et al., 1992) as well as genetic differentiation among population (F_{ST}) using GenALEx 6.5 software (Peakall and Smouse, 2009). Heterozygosity and the percentage of polymorphic loci in *P. ilicifolia* populations and reference species were computed at 99 % confidence interval using tool for population genetics analysis (TFPGA). Finally, genetic distances and relationships between sample populations including those of North America and India were done using principle coordinate analysis (PCoA) and dendograms using TFPGA software (Miller, 1997).

Table 2 Six primer sets that showed polymorphism used in the AFLP analysis

Name	Sequence
EcoRI + 3-ACA	5′GACTGCGTAACCAATTC + ACA 3′
MseI + 3-CTC	5′GATGAGTCCTGAGTAA + CTC 3′
EcoRI + 3-ACT	5′GACTGCGTAACCAATTC + ACT 3′
MseI + 3-CAA	5′GATGAGTCCTGAGTAA + CAA 3′
EcoRI + 3-ACT	5′GACTGCGTAACCAATTC + ACT 3′
MseI + 3-CAT	5′GATGAGTCCTGAGTAA + CAT 3′
EcoRI + 3-AAG	5′GACTGCGTAACCAATTC + AAG 3′
MseI + 3-CTT	5′GATGAGTCCTGAGTAA + CTT 3′
EcoRI + 3-AGC	5′GACTGCGTAACCAATTC + AGC 3′
MseI + 3-CAC	5′GATGAGTCCTGAGTAA + CAC 3′
EcoRI + 3-AGG	5′GACTGCGTAACCAATTC + AGG 3′
MseI + 3-CAT	5′GATGAGTCCTGAGTAA + CAT 3′

Results

Diversity estimates

Allele frequencies generated by AFLP markers were used to estimate the gene diversity (H) and percentage of polymorphic loci within populations (Table 3). The Tana population had the lowest gene diversity value ($H=0.079$) while Athi had the highest gene diversity $H=0.135$ and a higher heterozygosity (H) value than all the temperate species except *Populus fremontii* (North America) and *P. deltoides* introduced at KEFRI from India.

Genetic structure and differentiation

Results from analysis of molecular variance (AMOVA) showed that most of the genetic variation (87 %) was within the natural *P. ilicifolia* populations found in Kenya even though there was genetic differentiation among populations ($F_{ST}=0.134$) (Table 4).

Table 3 Mean diversity estimates (H) for *P. ilicifolia* populations and reference samples generated from 521 AFLP markers for 40 individuals sampled from Kenya and North America. Percentage polymorphic loci (% loci) and sample size (N) are also shown

Species (populations)	Sample size (N)	% polymorphic loci	Mean heterozygosity (*H*)
P. ilicifolia (Athi)	10	41.7	0.135
P. ilicifolia (Tana)	10	20.4	0.079
P. ilicifolia (Ewaso Nyiro)	6	23.8	0.091
P. deltoides (India/KEFRI)	4	53.0	0.24
P. tremuloides (N. America)	2	13.4	0.074
P. deltoides (N. America)	2	12.9	0.071
P. tremula (N. America)	2	15.7	0.087
P. fremontii (N. America)	2	33.8	0.186
P. balsamifera (N. America)	2	10.0	0.055

Table 4 Summary of analysis of molecular variance (AMOVA)

Source of variation	df	SS	MSD	% variation	F_{ST}	*P* value
P. ilicifolia populations						
Among populations	2	118.2	59.1	13	0.134	<0.01
Within populations	18	527.8	29.3	87		

df, SS, MSD, % variation, F_{ST}, and *P* values are shown

df degrees of freedom, *MSD* mean of square deviation, F_{ST} genetic differentiation among populations

Cluster analysis

Results from PCoA analysis showed that the first two principle axes accounted for 45.6 and 11.5 % variation, respectively (Fig. 2). One cluster was made of reference samples from the North America and India/KEFRI samples while the second cluster comprised of the Tana, Athi, and Ewaso Nyiro populations which were the Kenyan natural populations. Individuals that were close together were interpreted to have similar genetic characteristics (belonging to closely related species) while those apart were interpreted to be different or distantly related species.

Genetic relatedness between the populations (species) and individuals, Nei's unbiased genetic distances shown in Table 5 were used to draw dendograms. The smallest genetic distance was between the Athi and Tana populations (0.034) while the greatest genetic distance (0.79) was between the Tana and North American *P. deltoides* species (Table 5). Two main clusters were formed considering genetic distances (Fig. 3). One cluster consisted of the Tana, Athi, and Ewaso Nyiro populations, while the other comprised of the reference samples from the North America and India/KEFRI samples. Moreover, individuals from the same natural populations are shown to be regularly clustered in one group even though, curiously, three individuals from the Athi population appeared to cluster separately from the rest of the population (Fig. 4).

Discussion

Although a large sample size is desirable in genetic analysis studies (Bashalkhanov et al. 2009), use of a small sample size is common (Breinholt et al. 2009). To compensate for this, analysis of average heterozygosity and genetic distance of a given species can use a small sample size so long as a large number of loci are studied (Nei, 1978). In this study, 521 loci were used for the genetic studies.

Genetic diversity estimates assessed here showed significant genetic diversity for the different populations studied. The Kenyan natural populations had a mean heterozygosity (*H*) of between $H=0.07$ and $H=0.14$. Similar results were obtained by Chen and Peng (2010) on the genetic diversity of *Populus cathayana* analyzed using AFLP markers. Castiglione et al. (2010) studies on genetic diversity and introgression of *Populus alba* also reported gene diversity values that concur with these results. The Athi population was the most diverse among the Kenyan natural poplar, an indication that it could be the center of origin for the Kenyan *P. ilicifolia* populations thus spreading through wind dispersal to the Tana and Ewaso Nyiro rivers. This is in agreement with Oballa (1996) findings. Lower heterozygosity was recorded for the Tana ($H=0.079$) and Ewaso Nyiro ($H=0.091$) populations suggesting chances of genetic drift in these populations. This is attributed to low population densities of tropical tree species due to ecological and anthropogenic factors which result to a decrease in gene diversity (Dusan, 1992). Additionally, founder effects could have also caused the low gene diversity (Muchugi et al. 2012) in the Ewaso Nyiro and Tana populations. Such effects may have led to subsequent loss of unexploited genetic potential (Kotzé

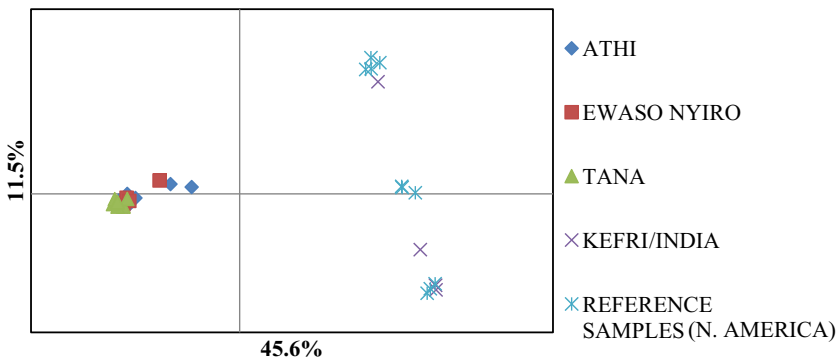

Fig. 2 Principle coordinate analysis of the *P. ilicifolia* populations and reference samples from N. America and India/KEFRI based on 521 AFLP loci

Table 5 Nei's unbiased genetic distance matrix among *P. ilicifolia* populations and reference samples based on 521 AFLP loci

	1	2	3	4	5	6	7	8	9
1	0								
2	0.037	0							
3	0.034	0.035	0						
4	0.667	0.699	0.724	0					
5	0.586	0.594	0.635	0.320	0				
6	0.753	0.746	0.788	0.124	0.454	0			
7	0.629	0.637	0.678	0.310	0.147	0.485	0		
8	0.693	0.722	0.757	0.107	0.399	0.108	0.3943	0	
9	0.655	0.705	0.716	0.294	0.410	0.396	0.4251	0.146	0

Key: (1) *P. ilicifolia* (Athi), (2) *P. ilicifolia* (Ewaso Nyiro), (3) *P. ilicifolia* (Tana), (4) *P. deltoides* (India/KEFRI), (5) *P. tremuloides* (North America), (6) *P. deltoides* (North America), (7) *P. tremula* (North America), (8) *P. fremontii* (North America), (9) *P. balsamifera* (North America)

and Muller, 1994) and probably resulted in erosion of genetic diversity in these populations.

The current study has shown that *P. fremontii* from North America had the highest gene diversity value ($H=0.186$) compared to the rest of the species studied from there. It was also more diverse than all *P. ilicifolia* populations from Kenya except for the Athi population. The high diversity value recorded for outgroup samples from India/KEFRI ($H=0.24$) suggested that the material could be of diverse origin. The possibility of hybridization of *P. fremontii* and *P. deltoides* from India/KEFRI with the local *P. ilicifolia* for genetic improvement could be explored especially for those populations that appear to possess low gene diversity values.

Molecular genetic studies on poplar species in the temperate regions report high levels of genetic diversity within populations (Stevens et al. 1999, Imbert and Lefèvre 2003), an important factor in conservation and breeding (Munthali et al. 2012). The level of genetic variation of (13 %) among populations and high variation within populations (87 %) reported here, could imply that many alleles are common among the populations with few rare alleles. High similarity of alleles may deduce sharing of common ancestral alleles and the low frequency allele as a subsequent result of evolution processes (Esselman et al., 2000).

The breeding system in trees is nonetheless one factor that influences the genetic variation within and among populations (Loveless and Hamrick, 1984). Forest trees have more genetic diversity within populations than among populations as they are outcrossing (Hamrick and Godt, 1996). *P. ilicifolia* as an outcrossing tree species (Nilsson et al. 1991) exhibit similar partitioning within population and among populations (Table 4). The genetic differentiation among the Kenyan populations suggested high genetic differentiation among populations implying limited gene flow between these populations in the form of seed dispersal and pollination.

The population disturbance caused by flooding and cultivation might have led to decimation of the riverine populations studied, which may have contributed to high genetic structuring among populations. Conservation strategies of *P. ilicifolia* in Kenya should therefore consider the three populations studied here in order to capture a wide genetic diversity. Studies of genetic differentiation are therefore important in determining which population should be conserved and how genetic mixing between populations should be carried out in introduction programs.

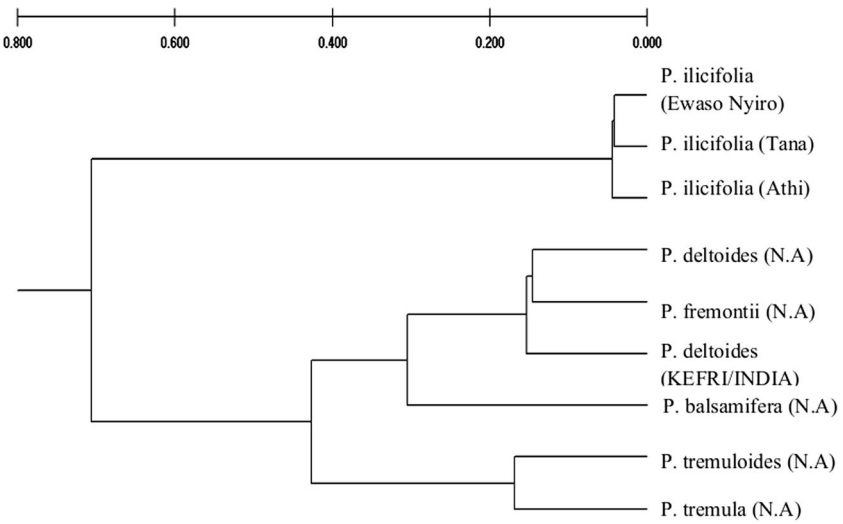

Fig. 3 UPGMA dendogram showing relationship among the *P. ilicifolia* populations and Reference species from North America and India/KEFRI based on 521 AFLP loci

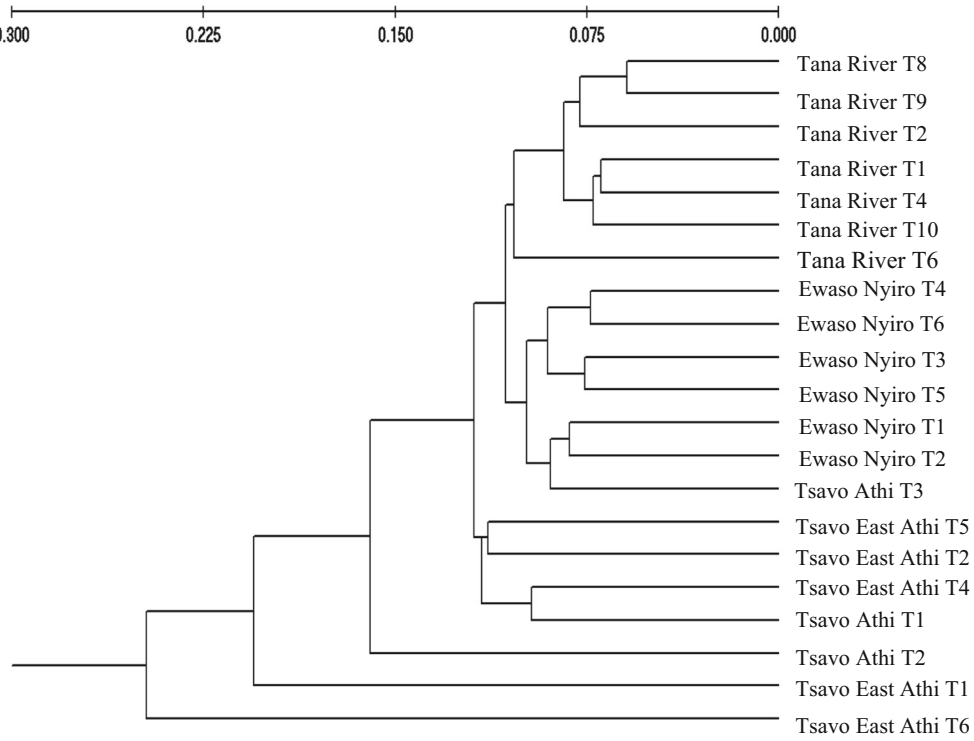

Fig. 4 UPGMA dendogram showing relationship among individual trees in the three Kenyan natural *P. ilicifolia* populations based on 521 AFLP loci

The resultant dendogram from UPGMA cluster analysis grouped the Kenyan natural *P. ilicifolia* populations in the same cluster. The other cluster was composed of the North America *Populus* species and India/KEFRI samples. This clustering could be attributed to the fact that the KEFRI samples were composed of temperate *P. deltoides* introduced in Kenya (KEFRI) in 2005 from India. Results suggest that the *P. ilicifolia* and temperate species from the North America and India/KEFRI materials were distantly related as confirmed by the genetic distances. Dendogram drawn only for individuals from the Kenyan natural *P. ilicifolia* showed three distinct clusters corresponding to the three natural populations. Three individuals from the Athi population appeared to cluster individually, an indication of diversity in this particular population. Such individuals can be included in breeding programs and ex situ collections to capture the diversity in this species.

Conclusion

This study has established low levels of polymorphic loci (bands) and low heterozygosity in some *P. ilicifolia* populations which suggests that the species contains little variation. National conservation strategies could therefore focus on in situ conservation in protected areas so as to maintain the evolutionary processes that shape the species diversity in its original habitat. Where this is not possible due to species destruction by flooding and destruction by herbivores ex situ as well as circu situ conservation should be considered to maintain diversity. Furthermore, seed collection from many randomly sampled trees is encouraged in order to capture the large diversity witnessed within populations. Finally, the possibility of hybridizing *P. ilicifolia* with the temperate poplar species such as *P. fremontii and P. deltoides* should be considered so as to avail quality planting material for cultivation as an agroforestry or plantation tree with additional benefits of bioenergy production.

Acknowledgments This research was made possible through Forests Trees and Agroforestry (FTA) funding availed to the World Agroforestry Centre (ICRAF).

Colleagues from the Virginia Polytechnic Institute and State University are thanked for their collaboration and providing reference samples used for the analysis.

The authors extend gratitude to the anonymous reviewers for their constructive comments to refine the manuscript.

References

Barat-Segretain MH (1996) Strategies of reproduction, dispersion, and competition in river plants. Veg 123:13–37

Bashalkhanov S, Pandey M, Rajora P (2009) A simple method for estimating genetic diversity in large populations from finite sample sizes. Genetics 10:1471–2156

Battiscombe E (1926) A descriptive catalogue of some of the common of some of the common trees and wood plants of Kenya colony, Nairobi, Kenya

Breinholt W, Renee V, Olga R, Catherine L (2009) Population genetic structure of an endangered Utah endemic, *Astragalus ampullarioides* (*fabaceae)*. Am J Bot 3:661–667

Castiglione S, Cicatelli A, Lupi R, Patrignani G, Fossati T, Brundu G, Sabatti M, Van Loo M, Lexer C (2010) Genetic structure and introgression in riparian populations of *Populus alba* L. Plant Biosyst 144(3):656–668

Chen K, Peng Y (2010) AFLP analysis of genetic diversity in *Populus cathayana* Rehd originating from South Eastern Qinghai-Tibetan plateau of China. Pak J Bot 42(1):117–12

Doyle JJ, Doyle JL (1987) A rapid DNA isolation procedure for small quantities of fresh leaf tissue. Phytochem Bull 19:11–15

Dusan G (1992) Effects of origin on the genetic diversity of Norway spruce (*Picea abies* Karst) populations. Forest Ecol Manag 54: 215–223

Eriksson O (1993) Dynamics of genets in clonal plants. Trends Ecol Evolut 8:313–316

Esselman EJ, Crawford DJ, Soren B, Stuessy TF, Anderson GJ, Silva MO (2000) RAPD marker diversity within and divergence among species of Dendroseris (Asteraceae: Lactuceae). Am J Bot 4:591–596

Excoffier L, Smouse P, Quattro M (1992) Analysis of molecular variance inferred from metric distance among DNA haplotypes: application to human mitochondria DNA restriction data. Genetics 131:479–491

Hamrick JL, Godt MJW (1996) Effects of life history traits on genetic diversity in plants. Philos T Roy Soc B 351:1291–1298

Imbert E, Lefèvre F (2003) Dispersal and gene flow of *Populus nigra* (Salicaceae) along a dynamic river system. J Ecol 91:447–456

International Union for Conservation of Nature (2016). The IUCN red list of threatened species: *Populus ilicifolia* (Tana River Poplar). http://dx.doi.org/10.2305/IUCN.UK.1998.RLTS.T32882A9731782.en

Kotzé A, Muller GH (1994) Genetic relationship in South African cattle breeds. In: Proceedings of the 5th world congress on genetics applied to livestock production, 21st edn. University of Guelph, Guelph, Canada, pp 413–416

Loveless MD, Hamrick JL (1984) Ecological determinants of genetic structure in plant populations. Annu Rer Ecol Evol S 15:65–95

Maundu P, Tengnas B (2005) Useful trees and shrubs for Kenya. Technical Handbook No.35. World Agroforestry Centre, Nairobi, Kenya

Miller MP (1997) Tools for population genetic analysis TFPGA, 1.3: a Windows program for analysis of allozymes and molecular population genetics data. Distributed by the author. Northern Arizona University. http://www.ccg.unam.mx/~vinuesa/tlem09/docs/TFPGADOC.PDF

Muchugi A, Kindt R, Muluvi GM, Muge E, Kipruto H, Jamnadass RH (2012) Genetic variation of Kenyan populations of *Warburgia ugandensis*, an important East African highlands medicinal tree species. J Life Sci 4:97–105

Munthali CR, Chirwa PW, Changadeya WJ, Akinnifesi FK (2012) Genetic differentiation and diversity of *Adansonia digitata* L. (baobab) in Malawi using microsatellite markers. Agroforest Syst 86:419–431

Nei M (1978) Estimation of average heterozygosity and genetic distance from a small number of individuals. Genetics 89:583–590

Nilsson C, Gardjell M, Greelson G (1991) Importance of hydrochory in structuring plant communities along rivers. Can J Botany 69:2631–2633

Oballa PO (1996) Status of *Populus ilicifolia* in Kenya. Occasional Paper No. 3. A report on conservation initiatives. Kenya Forestry Research Institute, Nairobi

Peakall R, Smouse P (2009) GENALEX6.5. Gene analyses in excel. Population genetic software for teaching and research. Mol Ecol Notes 6:288–295

Stevens MT, Turner MG, Tuskan GA, Romme WH, Gunter L, Waller DM (1999) Genetic variation in postfire aspen seedling in Yellowstone National Park. Mol Ecol 8:1769–1780

Tuskan GA, DiFazio S, Jansson S, Grigoriev I, Hellsten U, Putnam N, Ralph S et al (2006) The genome of black cottonwood, *Populus trichocarpa* (Torr. & Gray). Science 313:1596–1604

United States Department of Energy (2006) Breaking the biological barriers to cellulosic ethanol: a joint research agenda.U.S. Department of Energy Office of Science and Office of Energy Efficiency and Renewable Energy. DOE/SC–0095. http://www.doegenomestolife.org/biofuels/

Vos P, Hogers R, Bleeker M, Reijans M, Van De Lee T, Hornes M, Frijters A, Pot J, Peleman J, Kuiper M, Zabeau M (1995) AFLP: a new technique for DNA fingerprinting. Nucleic Acids Res 23:4407–4414

World Conservation Monitoring Centre (1998) Populus ilicifolia. In: IUCN 2012. IUCN Red List of Threatened Species. Version 2012.1. <www.iucnredlist.org>

Data archiving statement

Raw data generated by the AFLP markers in this study was in the form of binary data, that is, presence of allele denoted by 1 and absence of allele denoted by 0. 521. Polymorphic markers were generated by six AFLP primer combinations against each sample (ESM 1).