GENETIC VARIATION OF *WARBURGIA UGANDENSIS* IN KENYA AND IMPLICATIONS FOR ITS CULTIVATION

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Abstract

Warburgia ugandensis is one of the ten species identified as high priority medicinal plants in Kenya for detailed study. Although locally common in some areas, the populations of this species have been wiped out in many areas due to the use of its bark by traditional healers for medicinal purposes against malaria and other ailments as well as skin cream. It has been commercialised in Kenya although it has shown variation in active ingredients associated with its medicinal properties. It grows over a wide altitudinal range, from high altitude to the lowlands. The question addressed in the research is whether this is just phenotypic variation due to site differences or genetic variation. Preliminary results showed genetic variation, meaning that it is important to promote the best material from the available wild resources to facilitate optimal, cost-effective use in cultivation and protection of the important wild populations. It will be mainly cultivated for incorporation into farming systems as well as in natural forests. The impact of the cultivation will be monitored both on the farms and in the wild. Other current studies include germination and rooting ability through tissue culture.

1. Background

Kenya is rich in biological diversity. The country harbours over 6000 species of higher plants including 2000 tree and shrub species. Most of the fauna species are associated with forest and woody vegetation. Trees of medicinal value are found mainly within the natural forests but most of these forests are being degraded by anthropogenic activities. This has called for an increased awareness of conservation of these indigenous species both *in-situ* and *ex-situ*.

Threats to sustainable use of medicinal plants in Kenya include high population growth rate, competing land uses, environmental degradation, loss of indigenous knowledge, increased commercialisation of traditional medicine, increased demand in the local and international markets due to low prices of medicinal plants, high poverty levels and unemployment rate.

Many people in Kenya prefer traditional medicine to modern medicines attributed to good accessibility, affordability, local knowledge and expertise among local communities. Traditional medicinal plant species in Kenya were ranked according to utility value and sustainable use. The top ten priority species were as follows (Kariuki & Simiyu, 2005): *Prunus africana, Warburgia ugandensis, Ceasalpinia volkensii, Fagaropsis hidebrandtii, Securidaca longipendunculata, Zanthoxylum gilletti, Zanthoxylum usambarense, Strychnos henningsii, Zanha africana* and *Zanthoxylum chalybeum*. *Warburgia ugandensis* was rated as second highest priority medicinal plant species in Kenya.

Warburgia ugandensis (included within *W. salutaris* by Palgrave, 2002) is an important antimicrobial medicinal species. It is endemic to East Africa and widely distributed in lower rain forest and drier highland forest areas at altitudes between 1 000 m to 2 000 m (Maundu & Tengnas, 2005). The populations are mainly found on the western and south western parts of Kenya. Besides the medicinal value, *W. ugandensis* is a fairly slow growing tree species used as firewood, timber, fodder, tool handles, food seasoning, mulch for soil conservation, ornamental, shade and resin (Maundu & Tengnas, 2005). Although a highly valuable species, it is being destroyed due to the high demand for the medicine extracted from bark, roots, young twigs, leaves and fruit for use by the traditional healers.

This tree species has a high pharmaceutical value both for humans and livestock, exhibiting a broad spectrum antimicrobial activity (Olila *et al.*, 2001) with sequiterpene dialdehyde, warburganal (Haraguchi, 1998), muzigadial and polygodial (Taniguchi and Kubo, 1993). For instance in Kenya, as a painkiller and antimicrobial remedy, *W. ugandensis* has been used to treat malaria, chest pains, toothache and manufacture of some skin creams in humans (Traditional healers, personal communication). In animals a cytotoxic sesquiterpine, characterized as muzigadial, has been isolated from *W. ugandensis* against trypanosomiasis (Olila *et al.*, 2001) and it has been used widely to treat parasitic diseases (Kioy *et al*., 1990).

To enhance biodiversity conservation, a deliberate effort has been geared towards conserving and sustainable use of *W. ugandensis* both *in-situ* and *ex-situ* in Kenya. In South Africa, specifically Kwa-Zulu Natal, the closely related species, *W. salutaris*, has been documented as scarce due to sourcing of the

material from the wild population for traditional medicine (Cunningham, 1990). *W. salutaris* has been proclaimed a protected tree species in South Africa in terms of the National Forests Act (Act 84 of 1998).

The use of *W. ugandensis* has already reached commercialisation scale in Kenya. There is therefore a need for an intensive cultivation programme to conserve it. Due to its medicinal importance, more people are growing them on their farms and it takes about 18 to 45 days to germinate and about 3 to 4 months for seedlings to be ready for planting in the field. Propagation through tissue culture of the species has been successfully done at the Kenya Forestry Research Institute (KEFRI) to support rapid multiplication of planting material. Through tissue culture, one explant is likely to produce over 100 plantlets in four months (Ms Wahu, KEFRI, personal communication, May 2006). Although propagation of the species is on the rise, there was a need to rightly advise the stakeholders on which provenance would be effective in both active ingredients and site conditions.

This species, commonly used by the traditional practitioners in Kenya, has gained a lot of popularity. Some provenances are considered more effective than other provenances (Traditional practitioner, personal communication). Similar observations of popularity of some provenances have been highlighted in South Africa (Geldenhuys & Mitchell, 2006). The preference of some provenances over others by the end users could be attributed to factors such as the environmental or genetic variation. Further research to be done on *W. ugandensis* will be of great value if the underlying genetic make-up of this species is known.

Randomly Amplified Polymorphic DNAs (RAPDs) were used for analysis of the genetic variation and successfully mapped other indigenous wood carving populations such as *Olea europaea*, *Brachylaena huillensis*, and *Dalbergia melanoxylon* (Machua & Muturi, 2005). RAPDs will be used to determine the genetic diversity of *Warburgia ugandensis* because primer sequences of the sample need not be known. This will then help farmers or other stakeholders to use the best seed source for optimal gain from *W. ugandensis* in both the conservation areas and on farms.

The purpose of this paper is to give a short description of the sampling sites and methods, and preliminary results, for the study of the genetic variation of *W. ugandensis*.

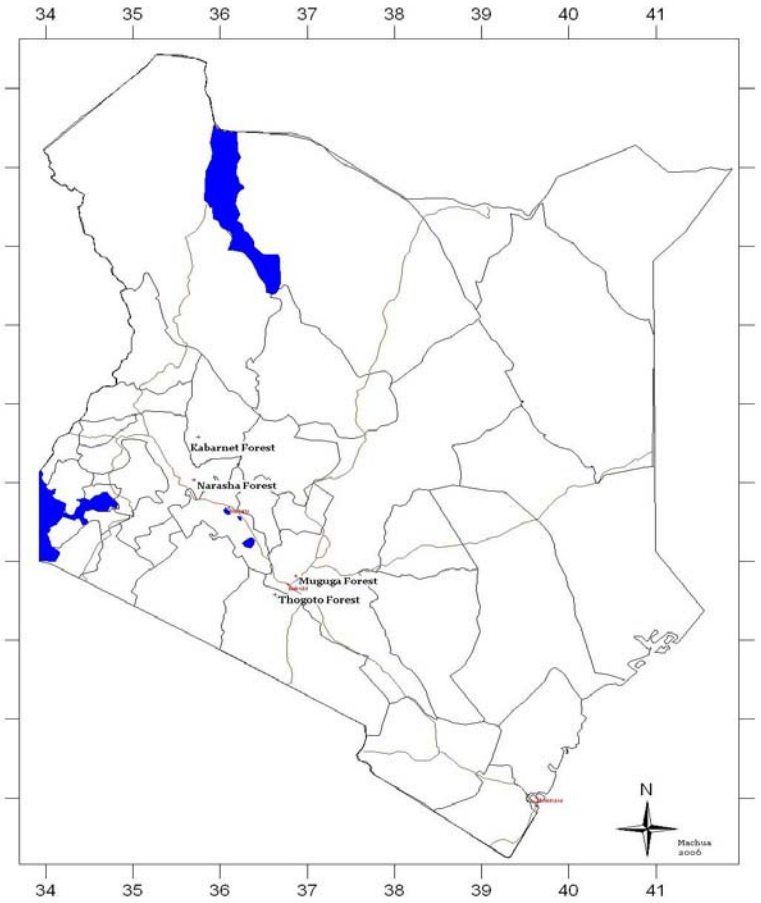

Figure 1. Map of Kenya showing the sites where *W. ugandensis* was collected for this study.

2. Materials and methods

Ten young leaf samples of *W. ugandensis* were collected from each of five sites in Kenya for mapping the various genotypes available (Figure 1). These sites were Kibwezi, Thogoto forest, Muguga forest, Kabarnet forest and Narasha forest in the Eldama-ravine. Kibwezi is located at an altitude of 910 m with a bi-modal rainfall of less than 600 mm per annum. The mean height of trees from which samples were taken was about 12 m. Thogoto forest is situated at an altitude of 1935 m and the mean height of the sample trees were 14.4 m. Muguga is located at an altitude of 2000 m and the samples were collected from trees of mean height 20.3 m. Kabarnet forest occurs at an altitude of 2178 m and the samples were collected from trees with mean height of 14.4 m. Eldama-ravine is situated at an altitude of 2100 m and the samples collected were from trees of mean height 12.2 m.

Kibwezi is located at a lower altitude compared to Muguga, Thogoto, Kabarnet and Eldama-ravine and there have been variations in the concentrations of the curative component from these sites. This has been attributed to the climatic conditions that in dry areas, the curative component concentrations are much higher than in the wet or higher altitudes and so the traditional practitioners prefer using medicine from these drier areas.

Kabarnet forest, Narasha forest, Muguga forest and Thogoto forests are close to human habitats and this has accelerated over-exploitation of the medicinal *W. ugandensis*. Kibwezi has *W. ugandensis* but the populations are far less compared to those of the highlands, which could be attributed to preference for the concentrations of the active ingredients for curative purposes by the traditional practitioners.

Morphological data were collected alongside the leaf samples for all the populations for height and diameter at breast height. DNA was extracted for all the samples using Sodium Dodecyl Sulphate (SDS) method of extraction. DNA quantification was done for all the samples to get the required 5 mg for the amplification process. Pure samples of DNA were isolated for amplification using Randomly Amplified Polymorphic DNAs (RAPDs) primers of known sequences.

3. Results

Preliminary results of the gel profiles for *W. ugandensis* populations showed polymorphism for some samples for the five populations (Figure 2). There were variations both within the highland provenances and between the highland and lowland areas. From the morphological data, traditional practitioners' knowledge and the molecular work, it could be concluded that *W. ugandensis* is affected by both the genotype and environment, or interaction between the two, although more studies are being done on the interaction of the two factors.

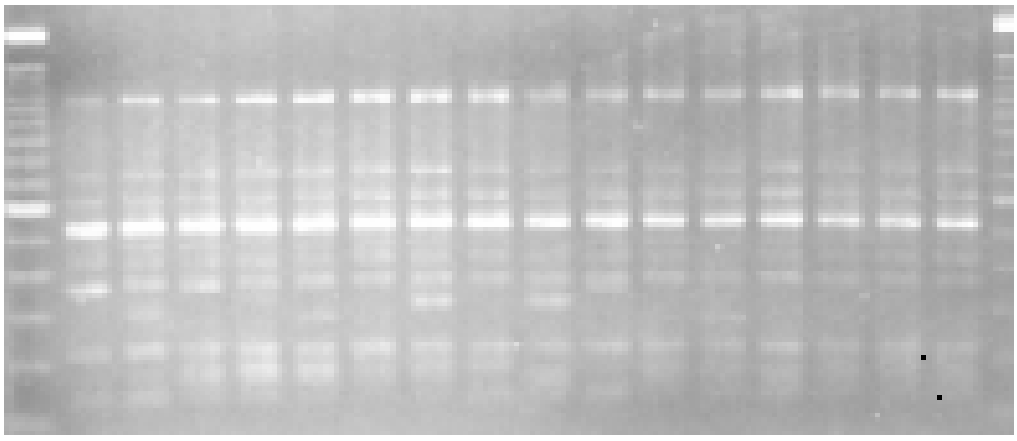

Figure 2. Gel profiles for *Warburgia ugandensis* for samples from Kabarnet, Muguga, Thogoto, Eldama-ravine and Kibwezi

Primer six of known sequence was used for the first and second columns for two samples from Ngong forest (Figure 3). There was a clear difference in the bands on the profile showing population differences, i.e. polymorphism (Figure 3). The third and fourth columns represent another primer for the same samples and the bands between the two columns still showed variations within the Ngong population. For the eighth column, the same primer was used for two samples but only one sample showed bands showing a difference in the same population of Ngong. The last column to the right shows the marker onto which the size of the bands can be estimated.

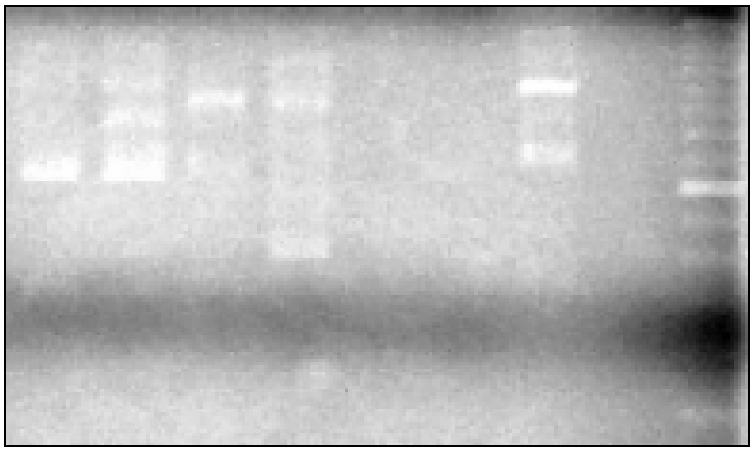

Figure 3. Gel profiles for two *Warburgia ugandensis* samples from Ngong forest showing clear within population genetic differences.

Other profiles not shown in this report revealed polymorphism for the five populations although some primers showed a consistent pattern on the profiles meaning some had similar bands at given loci.

These are preliminary results, and more conclusive results and discussion will follow once the study has been completed.

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