Journal of Horticulture and Forestry

Volume 6 Number 11 December 2014
ISSN 2006-9782

Academic Journals

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Table of Contents: Volume 6 Number 11 December 2014

ARTICLES

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Vol. 6(11), pp. 99-106, December, 2014
DOI: 10.5897/JHF2014.0365
Article Number: F9DF58549453
ISSN 2006-9782 ©2014

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Journal of Horticulture and Forestry

Full Length Research Paper

Prediction of *Osyris lanceolata* (Hochst. & Steud.) site suitability using indicator plant species and edaphic factors in humid highland and dry lowland forests in Kenya

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Received 8 August, 2014, Accepted 20 November, 2014

Osyris lanceolata (African Sandalwood) belongs to the family _Santalaceae_ that hosts some of the most valuable species for perfumery oil extraction. In India and Australia, _Santalum album_ and _Santalum spicatum_ are well developed for perfumery oil extraction through establishment of commercial plantations. In Africa, _O. lanceolata_ has attracted significant attention as potential perfumery oils extraction species. However, African Sandalwood exploitation is through unsustainable smuggling from natural forests and woodlands. Since sustainable production of _O. lanceolata_ oils is only feasible through establishment of commercial plantations, there is need to understand ecological requirements of the species before the remaining natural stands disappear. The aim of this study was to determine plant species and edaphic factors that can predict African Sandalwood site suitability for domestication programs. Sample plots with and without _O. lanceolata_ were selected from natural stands in a humid highland forest and a dry lowland forest, vegetation sampled using nested-intensity plots and soils sampled in the plots simultaneously. Vegetation data was recorded according to species abundance. Soil samples were analyzed for nutrients, texture and moisture retention. Canonical Correspondence Analysis using CANOCO software was used to determine species association and relationship between species to soil variables. In the highland forest, _O. lanceolata_ clustered with _Rhus natalensis_ and six other species, and was correlated to soil nitrogen, moisture and clay. In lowland forest, _O. lanceolata_ clustered with _R. natalensis_ and _Hypoestes forskahlii_ but did not correlate with any of the soil variables. The clustering of African Sandalwood with _R. natalensis_ in both forest types suggests strong predictive capacity of _R. natalensis_ for _O. lanceolata_ site suitability in humid and dry areas. Inconsistence of _O. lanceolata_ relationship with soil variables in the two study sites provides opportunity for further studies in different soil types.

Key words: CANOCO, domestication, edaphic, hemi-parasites, species association, African Sandalwood.

INTRODUCTION

Osyris lanceolata (African Sandalwood) is an evergreen hemi-parasite that belongs to the family *Santalaceae* (Maundu and Tengnas, 2005, Irving and Cameron, 2009). The family hosts culturally and commercially

important species that have long been used for herbal medicine, religion and perfumery oil industry (Tshisikhawe et al., 2012, Subasinghe, 2013). Species such as *Santalum album* and *Santalum spicatum* have long been exploited for perfumery oil and are now more developed commercially with plantations of *S. album* showing an increasing trend in Australia, China, India, Fiji and Sri Lanka (Subasinghe, 2013). In recent past, trade in African Sandalwood oil has also increased because of ready markets in Asia and Europe (CITES Cop 16). However, trade in African Sandalwood is unsustainable because materials are smuggled from natural stands and without clear domestication programs (Mukonyi et al., 2011). Moreover, exploitation of the species for herbal medicine has also increased (Tshisikhawe, 2012) leading to its decline in natural stands (Githae et al., 2011). Arising from this concern, African Sandalwood is now listed as threatened species under USF and WS (2013). Since sustainable production of *O. lanceolata* oils is only feasible through establishment of commercial plantations, there is need to identify predictive abiotic and biotic variables for its occurrence before the remaining natural stands disappear.

African Sandalwood has wide ecological distribution in Africa (Beentje, 1994, Mwang'ingo et al., 2003, Tshisikhawe et al., 2012, International Plant Names Index website (www.ipni.org/). The species can parasitize over 300 species of plants from herbaceous weeds, grass, multi-stem shrubs and trees. Usually, it is found in association with various hosts such as *Dodonea viscosa, Tecomaria capensis, Catha edulis, Apodytes dimidiata, Brachytegia spiciforms, Rhus natalensis* and *Casuarina equisetifolia* (Mwang'ingo et al., 2010). In Kenya, the species grows naturally in both humid highland and dry lowland forests (Maundu and Tengnas, 2005) that differ in altitude, vegetation types, soils and climatic variables (Sombroek et al., 1980). However, the effect of abiotic and biotic variables diversity on African Sandalwood distribution is not well studied, thus limiting site suitability prediction capacity for *O. lanceolata* domestication. The objectives of this study were therefore to determine plant species that associate strongly with *O. lanceolata* in humid highland and dry lowland forests and to determine soil variables that may influence the occurrence of the species in natural stands.

MATERIALS AND METHODS

Study sites

The study sites were Gachuthi humid highland forest and Kibwezi dry lowland forest (Figure 1). Gachuthi forest occurs in agro-climatic zone III (Sombroek et al., 1980), at an altitude range of 2040 to 2200 m above sea level with temperatures ranging from 12 to 25°C and mean annual rainfall range of 990 to 1500 mm. The soils in this forest are nitosols that are derived from volcanic rocks (Okalebo et al., 2002). The characteristics of these soils include high clay content (more than 35%), good moisture-storage capacity, good aeration, and high organic matter content. Cation exchange capacity and the percentage base saturation range from low to high. The soils are acidic (pH < 5.5) due to the leaching of soluble bases (Okalebo et al., 2002). The natural vegetation of Gachuthi forest is dominated by *Calodendrum capense, Ehretia cymosa, Maytenus undata, Teclea simplicifolia,Vangueria madagascariences, Warburgia ugandensis* and *Zanthoxylum usambarense*.

Kibwezi forest lies in a semi-arid region (agro climatic zone V) in south eastern Kenya (Figure 1) within an attitude range of 900 to 1015 m above sea level with a temperature range of 19 to 30°C and mean annual rainfall ranges between 250 and 350 mm (Sombroek et al., 1980). The soils in this forest are classified as sandy loams, gravely volcanic and clayey (Okalebo et al., 2002). Acacia commiphora woodland is the dominant vegetation type. Dominant trees include *Acacia xanthophloea*, *Acacia tortilis, Adansonia digitata*, *Balanites aegyptiaca* and *Commiphora* species.

Vegetation data collection and soil sampling

A reconnaissance visit in both forests was undertaken where *O. lanceolata* was found to be more abundant at the edges than deep in the forest and a sampling framework was designed. The forest edges were found to be fairly heterogeneous over short distances. Subsequently, transects measuring 600 m were laid using a linear tape measure. Modified nested-intensity plots (Barnett and Stohlgren, 2003) were then laid along each transects. To avoid spatial autocorrelation (Tiegs et al., 2005; de Knegt et al., 2010), a distance of ≥ 50 m was adopted between any two plots. In total, 24 plots were sampled in each site. In Gachuthi forest, 7 plots randomly fell in plots with *O. lanceolata* and 17 in plots without *O. lanceolata*. In Kibwezi forest, 18 plots were with *O. lanceolata* and 6 plots without *O. lanceolata.*

A modified nested intensity plot consisted of a main plot "A" measuring 5 by 20 m, a middle sub-plot "B" measuring 2 by 5 m and four sub-plots "C" of 1 by 1 m (Figure 2). Normally, the 1 by 1 m sub-plots are located near the corners of the main plot but their location was modified in this study to be close to *O. lanceolata* trees located at the middle of the main plot (Figure 2). Vegetation data was captured in terms of trees from the main Plot A, shrubs from Sub-plot B and herbaceous species and grass in Sub-plot C. The species were identified in the field, using published keys (Beentje, 1994). If the species could not be identified, its vernacular name was used and a specimen collected for identification at the national herbarium. Species found in each of the three sub-plots were tabulated in appropriate tables and their frequencies recorded for further analysis.

Soil samples were collected under *O. lanceolata* trees and the main plot measuring at depths of 0 to 25 cm and 25 to 50 cm using a soil auger, bulked, homogenized according to their different depths and stored in polythene bags. Soil samples were taken to Kenya Forest Research Institute (KEFRI) soil laboratory for analysis. The analysis included soil moisture, texture, pH and electro conductivity, nitrogen, phosphorous and potassium. Soil moisture and texture was determined using improved hydrometer method for soil particle size analyses, pH and Electro Conductivity(E.C.) values were determined with glass electrode, pH meter Model 691 and E.C. meter Model TOA Cm-20s (Lawal

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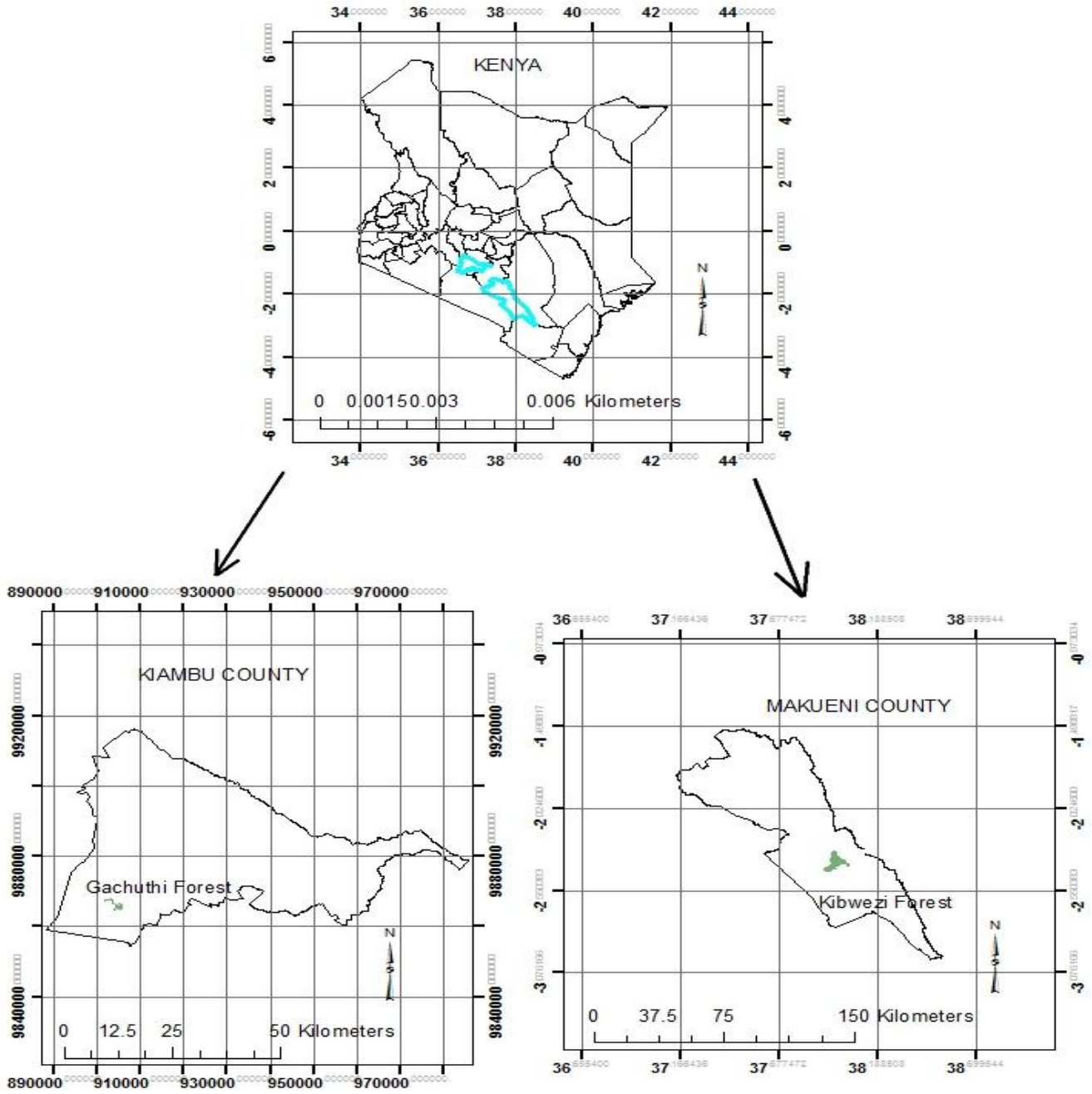

Figure 1. Geographical location of Gachuthi humid highland and Kibwezi dry lowland forests in Kenya.

and Girei, 2013). Total nitrogen was determined using Kjeldahl method with Skalar Block Digester System, Model SA 5640 as described by Okalebo et al. (2002). Available phosphorus was analyzed using UV spectrophotometer method (Olsen et al., 1982) with UV Spectronic Model 21-Milton Roy Co. Potassium was determined specto-photometically (Okalebo et al., 2002) using flame photometer, Model Corning M 410.

Data analysis

Species frequency data was combined into a single MS Excel© spreadsheet and used as species data. Soil nutrients, texture and moisture content data was then saved as a single MS Excel© spreadsheet and used as environmental data. The two data sets were used in Canonical Correspondence Analysis using CANOCO version 4.15 (Ter Braak, 1997) that relates species to measured environmental variables (Palmer, 1993). This relationship is shown graphically in biplots where lengths of the arrows reveal the relative influence of a measured variable to a species. In our case, plant species associations (clustering) was established from the species data and relationship between species and measured soil variables determined by using soil data as the environmental variable data in the analysis.

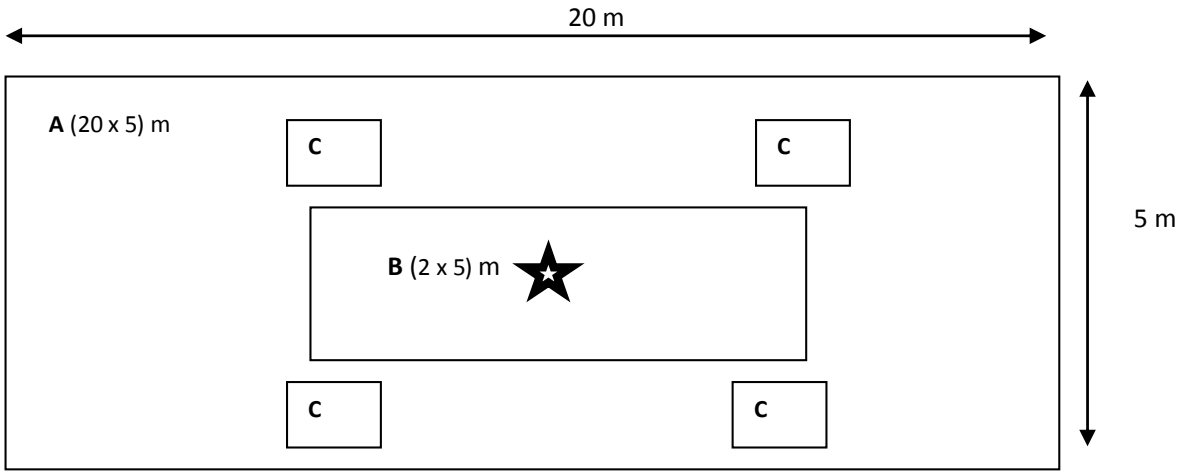

Figure 2. A modified nested-intensity sample plot used for field vegetation data collection. The star indicates approximate location of Osyris trees in the sample plots.

RESULTS AND DISCUSSION

Comparison of species occurrence in plots with and without *O. lanceolata* in Gachuthi and Kibwezi forests

In Gachuthi forest, 16 herbaceous species were found in plots with *O. lanceolata* and 24 herbaceous species found in plots with no *O. lanceolata* (Table 1). In Kibwezi forest, 11 herbaceous species were found in plots with *O. lanceolata* and 6 herbaceous species found in plots without *O. lanceolata* (Table 1). In Gachuthi, there were 3 grass species in plots with *O. lanceolata* and 5 grass species in plots without *O. lanceolata* (Table 1 In Kibwezi, there were 2 species of grass found co-occurring with *O. lanceolata* and only 1 grass species was found in plots without *O. lanceolata* only (Table 1). Twenty-two shrubs were found in plots with *O. lanceolata* and Twenty-one shrubs found in plots without *O. lanceolata* in Gachuthi (Table 2). This was in contrast with 14 and 11 shrubs found in plots with and without *O. lanceolata* in Kibwezi respectively (Table 2). Fourteen and seventeen tree species were found in plots with and without *O. lanceolata* in Gachuthi forest, respectively as compared to 21 tree species in plots with *O. lanceolata* and 7 tree species in plots without *O. lanceolata* in Kibwezi forest. In total, there were 55 species in plots with *O. lanceolata* as compared with 67 species without *O. lanceolata* in Gachuthi forest. This was in contrast to 48 species in plots with *O. lanceolata* and 25 species in plots without *O. lanceolata* in Kibwezi. Results of the study reveal inconsistence of trends in species co-occurrence with *O. lanceolata* between the two sites. The higher number of species found in highland humid forest is consistent with high species diversity of such forests when compared to lowland dry forests as influenced by variation in altitude, rainfall, temperature and soils (Sombroek et al., 1980). Also, the species found in *O. lanceolata* plots are among those reported in related studies (Mwang'ingo et al., 2010; Githae et al., 2011).

Abiotic and biotic factors associated with occurrence of *O. lanceolata* in Gachuthi and Kibwezi forests

Although, *O. lanceolata* was found co-existing with many species in both sites (Tables 1 and 2), CCA biplots (Figure 3a and b) revealed that the species could only cluster with a few species in each of the two sites. This suggests some of the species that coexisted may have little or no functional associational roles. Our findings are not surprising since studies on host preference of *O. lanceolata* have demonstrated that the species has a wide range of hosts but a few are more effective in its establishment and early growth (Mwang'ingo et al., 2005; Kamondo et al., 2007). The clustering of *O. lanceolata* with *R. natalensis* in both sites is consistent with the coexistence of both species in natural environments (Githae et al., 2011; Teklehaimanot et al., 2012) and effectiveness of *R. natalensis* as host species for *O. lanceolata* (Mwang'ingo et al., 2005; Kamondo et al., 2007). Therefore, we opine that *R. natalensis* is a good tree indicator for *O. lanceolata* site suitability. Since *O. lanceolata* also coexists with numerous herbaceous and grass species (Githae et al., 2011; Teklehaimanot et al., 2012), the functional association of the species with *Glycine wightii, Gutenbergia condifolia* and *Microglossa pyrifolia* at Gachuthi forest and *Hypoestes forskahlii* at Kibwezi is subject of further studies to provide a more effective stratification of *O. lanceolata* hosts among trees, shrubs and herbaceous species.

Relationship between species with soil nutrients (N, P, K), clay sand, silt, moisture, pH and EC revealed a contrasting trend between sites. In Gachuthi forest, *O. lanceolata* occurrence was correlated to nitrogen, clay

Table 1. Herbaceous (H) and grass (G) species found in plots with *O. lanceolata* (With Osyris) and those without Osyris (No Osyris) in Gachuthi and Kibwezi Forests. Species occurrence is denoted by √ whereas species absence is denoted by ×.

Plant species	Plant form	Gachuthi Forest		Kibwezi Forest	
		Osyris	No Osyris	Osyris	No Osyris
Abutilon mauritianum	H	√	√	√	×
Achyranthes aspera	H	√	√	√	×
Ageratum conyzoides	H	√	√	×	×
Asparagus racemosus	H	√	√	√	√
Barlelia acanthoides	H	×	×	√	√
Bidens pilosa	H	√	√	×	×
Chenopodium pumilio	H	×	√	×	×
Chloris sp	G	√	×	×	×
Cissus quadrangularis	H	×	×	√	√
Clematis brachiata	H	×	√	×	×
Commelina benghalensis	H	√	√	×	×
Conyza sumatrensis	H	√	√	×	×
Cyathula sp	H	×	√	×	×
Cynodon dactylon	G	×	√	√	√
Cyperus sp	G	×	×	√	×
Cyphostemma maranguense	H	×	√	×	×
Digitaria abyssinica	G	×	√	×	×
Duosperma kilimandscharicum	H	×	×	√	×
Fuarstia Africana	H	√	√	×	×
Galinsoga parviflora	H	√	√	×	×
Glycine wightii	H	√	√	×	×
Gutenbergia condifolia	H	√	√	×	×
Hyparrhenia rufa	G	√	√	×	×
Hypoestes forskahlii	H	√	√	√	√
Ipomea wightii	H	×	√	√	×
Justicia diclipteroides	H	×	×	√	×
Ocimum gratissimum	H	√	√	×	×
Oplismenus hirtellus	G	√	√	×	×
Oxalis obliquifolia	H	×	√	×	×
Pennisetum clandestinum	G	×	√	×	×
Periploca linearifolia	H	×	√	×	×
Seddera hirsute	H	×	×	√	×
Setaria verticillata	H	√	√	×	×
Sida tenuicarpa	H	×	×	×	√
Solanum incanum	H	√	√	√	√
Zehneria scabra	H	√	√	×	×
Total		19	29	13	7

and moisture in contrast to lack of such relationship in Kibwezi forest. The natural distribution of *O. lanceolata* in Kenya (Maundu and Tengnas, 2005; Githae et al., 2011; Mukonyi et al., 2011) and the soil maps of the range (Sombroek et al., 1980) revealed a great soil diversity in the range. Since our study was only restricted to two sites with two soil types, further studies with more representative soil types may be required to elucidate on edaphic factors that may influence *O. lanceolata* distribution.

Conclusion

In Gachuthi, Osyris clustered with *R. natalensis* and six other species whereas in Kibwezi, it clustered with *R. natalensis* and *H. forskahlii*. Therefore, *O. lanceolata* site suitability for domestication can be predicted using *R. natalensis*. CCA biplots showed clearly that *O. lanceolata* in Gachuthi forest positively correlated to soil nitrogen, moisture and clay whereas in Kibwezi forest; the species did not have a relationship with any of the soil variables.

Table 2. Shrub (S) and tree (T) species found in plots with *O. lanceolata* (With Osyris) and those without Osyris (No Osyris) in Gachuthi and Kibwezi Forests. Species occurrence is denoted by √ whereas species absence is denoted by ×.

Plant species	Plant form	Gachuthi Forest		Kibwezi Forest	
		Osyris	No Osyris	Osyris	No Osyris
Acacia brevispica	S	×	×	√	×
Acacia mearnsii	T	×	√	×	×
Acacia robusta	T	×	×	√	×
Adenium spp	S	×	×	×	√
Antidesma venosum	T	×	×	√	×
Aspilia mossambicensis	S	√	√	√	√
Balanites maughamii	T	×	×	√	×
Calodendrum capense	T	√	√	×	×
Cassipourea malosana	T	√	√	×	×
Celtis Africana	T	×	√	×	×
Clausena anisata	T	√	√	×	×
Clutia abyssinica	S	√	×	×	×
Combretum sp	T	×	×	×	√
Combretum sp	T	×	×	×	√
Commiphora baluensis	T	×	×	√	×
Commiphora eminii	S	×	×	√	×
Commiphora spp	T	×	×	√	×
Crotalaria mauensis	S	√	√	×	×
Croton dichogamus	S	×	×	√	×
Croton megalocarpus	T	×	×	√	×
Cussonia hostii	T	×	×	√	×
Diospyros consolatae	T	×	×	√	√
Dodonaea viscose	S	×	×	×	√
Dombeya burgessiae	S	√	×	×	×
Dombeya kirkii	S	×	×	√	√
Ehretia cymosa	T	×	√	×	×
Elaeodendron buchananii	T	√	√	×	×
Erythrococca bongensis	S	√	√	×	×
Euclea divinorum	T	√	√	√	×
Euphorbia candelabrum	T	×	×	√	×
Euphorbia scheffleri	S	×	×	√	×
Fagaropsis angolensis	T	√	×	×	×
Ficus vasta	T	×	×	√	√
Grewia similis	S	√	×	×	×
Grewia spp	S	×	×	√	×
Haplocoelum foliolosum	T	×	×	√	×
Helichrysum sp.	S	√	√	×	×
Heteromorpha trifoliate	T	×	×	√	×
Hibiscus diversifolius	S	√	√	×	×
Hibiscus fuscus	S	×	×	√	√
Hymenodictyon parvifolium	T	×	×	√	×
Indigofera swaziensis	S	×	√	√	√
Juniperus procera	T	×	√	×	×
Lantana trifolia	S	√	√	×	×
Leucas grandis	S	√	√	×	×
Leucas spp	S	×	×	√	√
Lippia javanica	S	√	√	×	×
Maerua oblongifolia	S	×	×	√	√
Maytenus senegalensis	S	×	×	√	×

Table 2. Contd.

Maytenus undata	S	×	√	×	×
Microglossa pyrifolia	S	√	√	×	×
Mystroxylon aethiopicum	S	√	√	×	×
Mystrxylon aethiopicum	T	×	×	√	×
Nuxia congesta	T	√	√	×	×
Ochna ovate	T	×	×	√	×
Olea europaea ssp. Africana	T	√	√	√	√
Pappea capense	T	×	×	√	√
Pittosporum viridiflorum	T	√	√	×	√
Plectrunthus barbatus	S	×	×	×	√
Pterolobium stellatum	S	√	√	×	×
Pterolobium stellatum	S	√	√	×	×
Rhus natalensis	S	√	√	√	√
Ritchiea albersii	T	×	√	×	×
Schrebera alata	T	√	√	×	×
Scutia myrtina	S	√	√	×	×
Steganoteenia oraliacea	T	×	×	√	×
Syphorstermma viminale	S	×	×	√	√
Teclea simplicifolia	T	√	√	√	×
Trimeria grandifolia	S	√	√	×	×
Triumfetta tomentosa	S	√	√	×	×
Turraea abyssinica	T	√	√	√	×
Vangueria madagascariensis	S	√	√	×	×
Vernonia brachycalyx	S	√	√	×	×
Vernonia lasiopus	S	√	√	×	×
Warburgia ugandensis	T	√	×	×	×
Zanthoxylum usambarense	T	√	√	×	×
Total		36	38	35	18

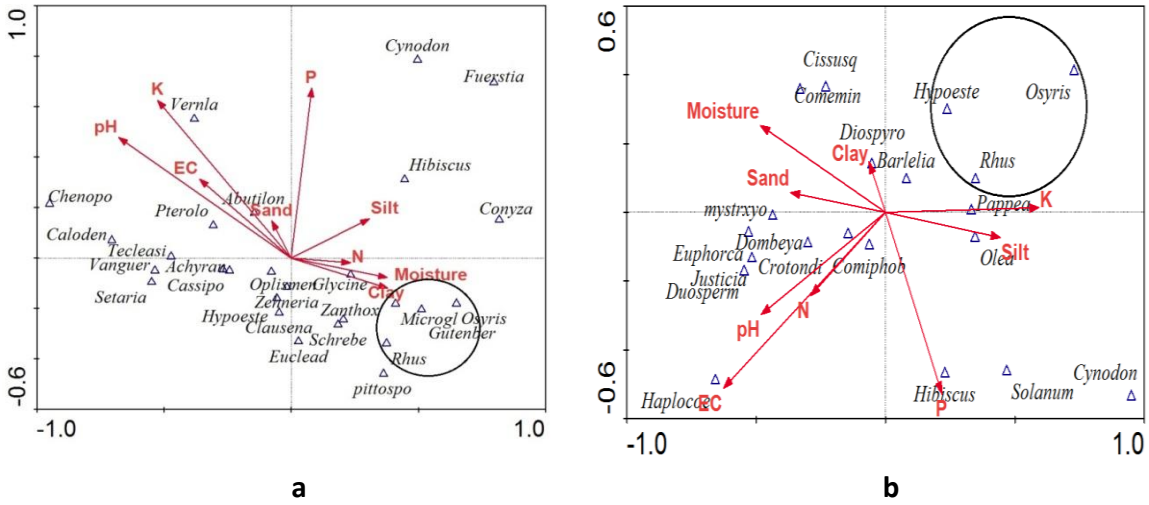

Figure 3a. CCA biplot of first and second axes showing species association and relationship between species with soil variable at Gachuthi humid highland forest. The first two axes explain 53.3% of species-soil variables relations. The circle highlights species that clustered with *Osyris lanceolata.*Species are abbreviated by the first 8 letters of their genus name shown in Tables 1 and 2**.** CCA biplot of first and second axes showing species association and relationship between soil variable at Kibwezi dry lowland forest. The first two axes explain 51.6% of species-soil variables relations. The circle highlights species that clustered with *Osyris lanceolata.* Species are abbreviated by the first 8 letters of their genus name shown in Tables 1 and 2.

Due to the limited number of sites used in the current study, we recommend further studies on relationship between soil variables and *O. lanceolata* occurrence in natural ecosystems.

Conflict of Interest

The authors have not declared any conflict of interest.

ACKNOWLEDGEMENTS

This study was funded by KEFRI through Drylands Forestry Research program in support of Mary Gathara's MSc study and we are grateful for the financial support. We appreciate the assistance extended by Mr. Eliud Macharia and Ms Margaret Nduta in field data collection and Mr. Shadrack Odhiambo in soil analysis. We are also grateful to Mr. Bernand Kamondo for assisting us in editing the manuscript before submission.

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Vol. 6(11), pp. 107-111, December, 2014
DOI: 10.5897/JHF2014.0366
Article Number:63999F749454
ISSN 2006-9782 ©2014

http://www.academicjournals.org/JHF

Journal of Horticulture and Forestry

Full Length Research Paper

The relation of endogenous abscisic acid and indole acetic acid on vigor of some selected dwarf mahaleb (*Prunus mahaleb* L.) genotypes

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Received 10 August, 2014; Accepted 6 November, 2014

Vigor reduction of sweet cherry varieties by dwarfing rootstock is well known, but the mechanism by which a rootstock induces dwarfing is not well understood. Plant hormones have been associated with dwarfing mechanism. This study was conducted, with the main purpose of determining the correlation between of endogenous abscisic acid (ABA) and indole acetic acid (IAA) and vigor of 10 selected dwarf mahaleb (*Prunus mahaleb* L.) genotypes. Endogenous IAA and ABA levels of selected dwarf genotypes were evaluated, which indicated significant differences for most traits. The IAA level in the shoot bark was the highest in H24 and lowest in M96 genotypes, while ABA level in the shoot bark was highest in M96 and lowest in H14. The mean ABA content decreased as the invigoration capacity of the genotype increased. ABA generally is regarded as an inhibitor of elongation. In our experiment, the endogenous ABA content was negatively correlated with shoot growth. ABA: IAA ratio in shoot bark decreased with increasing genotype vigor. ABA: IAA ratio of the dwarfing genotype M96 was about 1.118. Correlation coefficient showed a significant correlation between crown width, trunk diameter, crown volume, total IAA, total ABA, IAA: ABA and tree vigor. The concentration of ABA in shoot bark showed a good relationship with vigor, thus the content of this phytohormone in shoot bark could be a useful marker of dwarfing character in mahaleb genotype selections.

Key words: Mahaleb, Dwarf rootstock, indole acetic acid (IAA), abscisic acid (ABA).

INTRODUCTION

Vigor reduction of cherry varieties by dwarfing rootstocks is well known, but the mechanism for the dwarfing is not. There is limited data that deals directly with the mechanism of dwarfing by rootstocks or interstocks. Control of vigor is a characteristic being sought in every rootstock-breeding program (Ganji and Khalighi, 2006).

A long series of studies reported possible mechanisms related to the production and translocation of hormones dwarfin in the plant. However, exactly how hormones act in the g process is not well understood (Tréfois and Brunner, 1982). Several mechanisms have been proposed for a range of woody species. Hormonal, anatomical and nutritional mechanisms have been postulated (Lliso et al., 2004). The role of the rootstock in the dwarfing of grafted apple trees, suggested that a dwarfing mechanism is triggered by plant growth

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regulators (Noda et al., 2000).

It has also been reported that in some cases dwarfing might be caused by partial incompatibility between the scion and the rootstock, which may alter the transport of minerals and hormones (Webster et al., 2004). Tworkoski et al. (2007) reported that abscisic acid (ABA) concentrations did not differ, although they tended to be higher in the shoot tips of trees grown on M.9 and M.7 than on seedling rootstocks. The higher ABA in the shoot tips of trees grown on dwarfing rootstock may be attributed to higher ABA production in the rootstock, since ABA can move acropetally from roots via xylem (Ganji and Khalighi, 2006). In apple, higher ABA levels may have increased phloem differentiation, and the resulting high bark-to wood ratio could have reduced xylem conductivity in dwarfing rootstocks (Tworkoski and Miller, 2007b; Tworkoski et al., 2006). Brestic et al. (2011) reported that rootstock genotypes markedly influence phytohormonal composition of the scion part in fruit trees. Shoot bark of dwarfing rootstocks (M.27 and M.9) exhibited significantly higher ABA content than more vigorous rootstocks MM106 and MM111 (Kamboj, 1996). Comparing ABA-like activity in the shoot apices of normal and dwarf mutants of 'Cortland' and 'Golden Delicious' apples, reported higher concentrations of ABA in both dwarf mutants, the results were consistent during rapid shoot elongation, terminal bud formation and cessation of cambial growth stages. ABA has been found to decrease the rates of elongation of maize coleoptiles and also inhibited growth induced by auxin (Jindal et al., 1974), possibly through an effect on the translocation of auxin (Blažková et al., 2010).

Auxin generally is considered as a plant growth promoter. Auxin concentrations tended to be lowest in scions on M.7, and cytokinin concentrations highest in scions on M.9 rootstocks. Auxins produced and translocated from the apical meristem can inhibit subtending laterals and contribute to apical dominance in numerous species, including apple. Dwarfing apple rootstocks reduced the formation of nodes during shoot growth (Seleznyova et al., 2003), and the cumulative effect of such reduction over time can have a dramatic effect on branch development. Furthermore, dwarfing rootstocks and inter-stems reduced the number of extension shoots and promoted the formation of floral shoots (Seleznyova et al., 2008).

Since the reported possible mechanisms of dwarf genotypes mahaleb, so, this study was carried out in order to find out a relation between vigor, indole acetic acid (IAA) and ABA content in some dwarf selected mahaleb genotypes.

MATERIALS AND METHODS

Plant material

The research was carried out at the farm of Khorasan Razavi Agricultural and Natural Resources Center (Mashhad, Iran). Ten IAA included M96 (very dwarf), M188, M136, M6, M165, M266 (dwarf), M103, M82 (semi dwarf) and H14, H24 (standard) were used as experimental materials. Six shoots of each mahaleb genotypes were collected for IAA and ABA analysis and plants vigor (height, crown width, trunk diameter, and crown volume) was measured on 1-year-old rootstock plants in the nursery. The shoots were harvested and immediately brought to the laboratory in polythene bags. They were selected for bark removal. The bark (containing cortex, epidemis and layer of cambium cells) was removed from these internodes with a sharp blade, immediately frozen in liquid nitrogen and stored at -20°C to a wait analysis using MSTAT software. Bark samples from two shoots were pooled to give one replicate and there were, therefore, three replicates for each genotype.

Chemical reagents

The chemical reagents used were of analytical grade obtained from Merck Co.

Extraction and purification of IAA and ABA

Extraction, purification and quantitative determination of free and bound IAA, and ABA were done, with minor modifications, according to the methods of Rastegar et al. (2011). Spectrophotometric techniques were used to determine the amounts of IAA and ABA. One gram of fresh weight of each sample was taken and combined with 60 ml of methanol: chloroform: 2N ammonium hydroxide (12:5:3 v/v/v). Each combined extract (60 ml) was kept in a bottle at -20°C in a deep freezer for further analysis. IAA and ABA extraction assays were done according to the schematic diagram. Combined extract was treated with 25 ml of distilled water. The chloroform phase was discarded. The water-methanol phase was evaporated. The water phase was adjusted to the extract pH value of 2.5 or 7 or 11 with 1 N HCl or 1 N NaOH, respectively and 15 ml ethyl acetate was added at each of three steps. This procedure provided the isolation of free-form of IAA and ABA from the extraction solvent. After an incubation period of 1 h at 70°C, the same procedure was used for the isolation of the bound-form of IAA and ABA from the extraction solvent. Evaporation of ethyl acetate was performed at 45°C using a roteo evaporator system (BüchiInstruments). Thin-layer chromatography (TLC) was done using silica gel GF254 (Merck Chemicals, Germany) according to the method of Rastegar et al. (2011). TLC-separated IAA and ABA were isolated from the glass plaques according to the standard synthetic IAA and ABA Rf values. IAA and ABA were dissolved with 2 ml of methanol for filtration and separation from silica using cotton-glass filled transferring pipettes. Spectrophotometric assay was done at 280 nm for IAA and 263 nm for ABA and for all standard synthetic IAA and ABA and isolated samples.

All experiments were repeated three times. Total IAA and ABA was then obtained as the sum of free and bound IAA and ABA. The amounts of IAA and ABA in mahaleb samples were expressed as standard synthetic IAA and ABA equivalent.

Statistical analysis

Statistical analysis was performed using SPSS for windows statistical software (SPSS Inc., USA) for ± standard error and mean of each value.

Table 1. Vegetative characteristics of to selected dwarf *Prunus mahaleb* genotypes.

Genotype	Height (cm)	Crown width (cm)	Trunk diameter (mm)	Crown volume (m^3)
M96	100.0	87.5	35.4	0.3
M188	125.0	95.0	33.9	0.5
M136	170.0	95.0	53.2	0.7
M6	155.0	103.0	42.4	1.3
M165	115.0	90.0	33.4	0.5
M266	145.0	100.0	28.6	0.5
M103	115.0	105.0	38.3	0.6
M82	210.0	170.0	71.8	3.2
H14	280.0	235.0	84.4	7.8
H24	260.0	145.0	83.5	2.7

Table 2. Comparison of ABA and IAA total concentration than bound and free concentration in selected dwarf *Prunus mahaleb* genotypes.

Genotype	ABA (µg/ml)			IAA (µg/ml)		
	Total	Bound	Free	Total	Bound	Free
M96	118.9	31.67	84.26	106.4	41.71	64.69
M188	117.8	25.32	92.55	181.9	54.22	127.7
M136	113.6	34.34	79.31	187.6	78.46	109.1
M6	120.3	47.3	73.03	197.2	78.72	118.5
M165	90.51	23.6	69.92	266.3	97.32	169
M266	101.1	26.45	74.6	271.1	62.92	208.2
M103	132.4	35.71	96.7	303.3	80.86	222.4
M82	92.85	34.41	58.44	402.4	161.1	241.3
H14	46.16	18.09	28.07	527.4	153.1	347.2
H24	60.17	23.58	36.59	528.5	177.8	350.7
LSD $p < 0.01$	7.968	6.185	6.455	17.74	7.653	20.91
LSD $p < 0.05$	5.547	4.305	4.493	12.35	5.327	14.55

RESULTS AND DISCUSSION

Results showed vegetative of traits that the height, crown width, trunk diameter, crown volume, significant correlation with tree height and the factors controlling seedling size Mahaleb (Table 1). From among the vigor parameter height, width and crown volume of plants had significantly differed. H14 populations had the highest height, width trunk diameter, crown volume and crown volume, respectively (Table 1). This trial demonstrated a positive correlation between tree vigor, crown volume and crown width. However, our conclusion needs to be proved in further trials set on grafted rootstocks with commercial cherry cultivars (This experiment measured tree single, and need not analysis).

The amounts of IAA and ABA in the mahaleb genotypes samples are given in Table 2. The highest total IAA level was 527.40 and 528.50 µg/ml in standard genotypes H14 and H24, while the lowest was 106.40 µg/ml recorded in very dwarf genotypes M96 at 280 nm.

The mean IAA increased slightly with increasing genotype vigor. This result suggests that the vigor genotypes capacity of mahaleb results from the contribution of total IAA compounds. We found that shoot bark of invigorating genotypes had higher levels of diffusible IAA than comparable samples from dwarfing genotypes. So there was a direct relationship between vigor and total IAA level.

Differences in ABA concentrations in shoot bark were highly significant ($p < 0.001$) (Table 2). Mean ABA content decreased as the invigoration capacity of the genotype increased. In our experiment, the endogenous ABA content was negatively correlated with shoot growth. M103 (Semi dwarf) genotype showed significantly greater mean ABA concentration than H14 (standard) genotype. We also concluded that differences in the quantity of total ABA could be responsible for differences in vigor. The present study showed that total ABA content could be considered as a good screening method for helping to predict plant vigor. Interstocks do not affect the vigor of

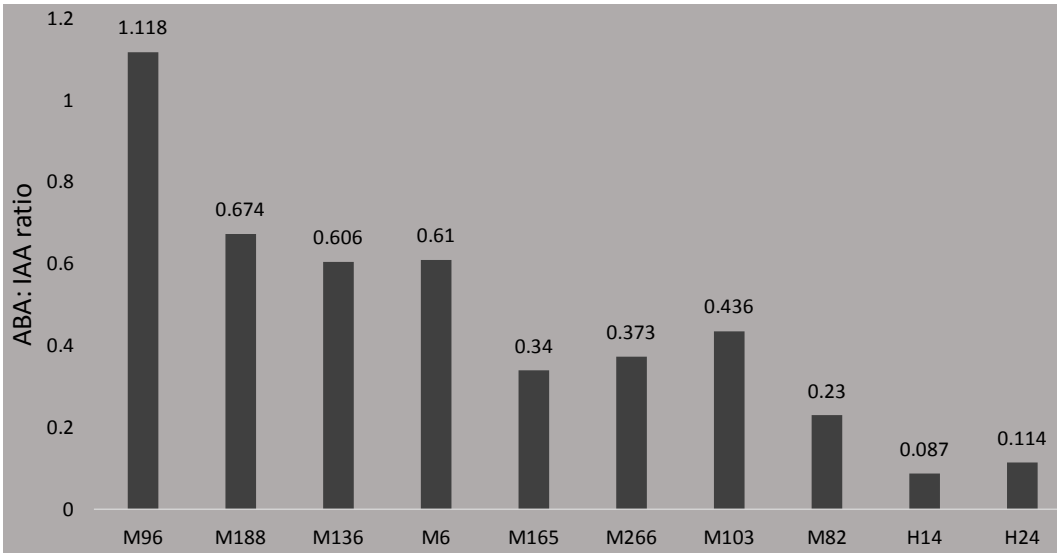

Figure 1. Comparison of ABA: IAA ratio for selected dwarf *Prunus mahaleb* genotypes.

Table 3. Correlation coefficient between some traits of different selected dwarf *Prunus mahaleb* genotypes.

S/No	Traits	1	2	3	4	5	6	7
1	Height	1						
2	Crown width	0.875**	1					
3	Trunk diameter	0.906**	0.906**	1				
4	Crown volume	0.878**	0.973**	0.929**	1			
5	Total IAA	0.761*	0.855**	0.742**	0.811**	1		
6	Total ABA	-0.757**	-0.862**	-0.761**	-0.878**	-0.855**	1	
7	IAA: ABA	-0.740**	-0.712**	-0.609**	-0.696**	-0.853**	0.761**	1

shoot growth in sweet cherry and were unable to find any relationship between the ABA content of bark tissues and the dwarfing effect of cherry rootstocks, suggesting that the results reported here may not extend to cherry (Blažková et al., 2010; Gyeviki et al., 2008). The greater concentrations of ABA found in shoot bark tissues from dwarfing rootstocks confirm earlier observations on ABA-like activity in dwarf-compared to invigorating rootstocks (Lliso et al., 2004). For example, the higher ABA content in the shoot tips of trees grown on dwarfing rootstock may be attributed to higher ABA production in the rootstock, since ABA can move acropetally from roots via xylem (Davies et al., 2005).

The ratio decreased with increasing rootstock vigor. The ranges of variation for vigor and ABA: IAA ratio were 1.118 in very dwarf mahaleb genotypes (M96) and 0.087 for standard mahaleb genotypes (H14), respectively (Figure.1).

The correlation coefficients among characteristics are mostly height, with the exception of those between tree vigor and total IAA (0.761), total ABA (-0.757) IAA: ABA (-0.740) (Table 3). The results showed that the ratio of IAA and ABA can be used as a feature in the selection procedure being considered as dwarf rootstocks.

Ganji and Khalighi (2006) reported that among the vigor parameters there was a positive correlation between tree vigor, height and crown volume. Jacyna (2004) showed that natural growth habit of pear cultivars, rootstock, and the interactions between them had no significant effect on tree height or diameter.

There was a significant positive correlation between total number of shoots and tree vigor. Growth rate of trees with spreading growth habits was much greater early in the growing season than trees with upright growth habits (Seleznyova et al., 2008; Tworkoski et al., 2007a; Lanauskas et al., 2014). Hooijdonk et al. (2011) reported that mean rates of IAA diffusion from the apex of the primary shoot declined during seasonal growth. Hence, a putative relationship existed whereby higher mean rates of IAA diffusion from the shoot apex, that with results are consistent with these researchers (Berestic et al., 2011; Tworkoski et al., 2006; Xiao et al., 2013). The concentration of auxin in the shoot bark of dwarfing and semi dwarfing rootstocks is similar. Although greater

auxin-like activity was identified in invigorating than in their dwarf mutants, it was quantified using bioassay and the results obtained may have been influenced by compounds in addition to free IAA (Jindal et al., 1974). A positive correlation between the endogenous IAA levels and of the above-ground parts of the three different rootstocks, indicate that endogenous IAA may have promoted the shoot growth. High concentrations of endogenous IAA in roots would inhibit root elongation. IAA in vigorous rootstock roots may be converted to esters or other conjugates and transported basipetally. Moreover, IAA metabolism may be more active in roots of vigorous than dwarf rootstocks. Sweet cherry rootstocks exhibited differences in their IAA oxidase activities 1 day after initial treatment. Mazzard, the most vigorous rootstock, had the lowest enzyme activity (Chong and Andrew, 2006).

Dwarfing rootstocks are known to have higher bark to wood ratios, suggesting that, although no differences in IAA levels were noted between rootstocks, higher ABA levels might have had overriding effects on vascular differentiation. Currently, the bark-to-wood ratios of roots are being used as markers for the early selection of dwarfing rootstocks in rootstock breeding programmes (Tréfois and Brunner, 1982; Jasyna et al. 2004). ABA and auxins may also affect the rootstock itself. The ratio of ABA: IAA was lower in vigorous rootstocks than in dwarf rootstocks. Maiden pear (does not specify any particular species) trees usually produce more lateral shoots when propagated on vigorous rootstocks, though this is not the case with some apple, sweet cherry and pear cultivars. The concentration of ABA in rootstock shoot bark shows a good relationship with rootstock vigor thus the content of this phytohormone in shoot bark, particularly early in the season, can be a useful marker of the dwarfing character in apple rootstock selections.

Conflict of Interest

The author(s) have not declared any conflict of interest.

ACKNOWLEDGEMENTS

This research was supported by Seed and Plant Improvement Research Institute. The authors express appreciation to Mrs. Sh. Akhavan and Mrs. S .Bina for lab analysis and M. Farokh and A. Eravani for technical assistance.

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