



DOCTORAL THESIS No. 2011:21  
FACULTY OF FOREST SCIENCES

# Clonal Propagation of *Detarium microcarpum* and *Khaya senegalensis*

A Step toward Clonal Forestry in Burkina Faso

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Doctoral Thesis

Swedish University of Agricultural Sciences

Alnarp 2011

Acta Universitatis agriculturae Sueciae

2011:21

Cover: Top - Excavated root segment and rootlings of *Detarium microcarpum*  
Bottom - Seedlings, rooted stem cuttings and stecklings of *Khaya senegalensis*  
(Photo: C. Ky-Dembele)

ISSN 1652-6880

ISBN 978-91-576-7556-9

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Print: SLU Service/Repro, Alnarp 2011

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## A Step toward Clonal Forestry in Burkina Faso

### Abstract

The slow growth of seedlings and the impact of insect pests are major limitations to the use of indigenous species in plantations in Burkina Faso. Thus, the use of vegetative propagules and resistant clones may enhance the success of plantations. The objectives of this thesis were to develop efficient and simple clonal propagation methods for two indigenous species, *Detarium microcarpum* and *Khaya senegalensis*, and to compare the growth of sexual and asexual propagules.

Two clonal propagation methods were developed: root cuttings for *D. microcarpum* and stem cuttings for *K. senegalensis*. Root segment length and diameter were key factors that affect sprouting and rooting ability. Root segments of 20 cm length and 15–60 mm diameter were the most successful. Stockplant and auxin application influenced root formation by leafy stem cuttings of *K. senegalensis*. High proportions of cuttings taken from seedling have rooted, while cuttings obtained from older trees rooted poorly, highlighting maturation as critical factor. The rooting ability of cuttings from older trees was improved by pollarding and auxin application.

Comparison of sexual and asexual plantlets of *D. microcarpum* revealed that root suckers and seedling sprouts had a closer morphological resemblance. The well-established root system and the high carbohydrate concentrations in the roots of seedling sprouts may favor a growth comparable to that of root suckers. Seedlings and stecklings of *K. senegalensis* had similar growth patterns with respect to: the relative growth rates of stem length, leaf, stem, root and the total plant biomass; the biomass fraction to total plant biomass of leaf, stem and root; leaf area productivity; foliar carbon isotope ratio; and carbohydrate concentrations in roots. However, water stress was a major growth-limiting factor, resulting in a reduction in plant growth, biomass production, and carbohydrate concentration.

As these studies constitute a first step toward the effective use of clonal propagules of *D. microcarpum* and *K. senegalensis* to ensure successful plantation, more investigations examining the effects of donors, the application of plant growth regulators are required in order to optimize the techniques.

**Keywords:** Carbohydrates, carbon isotope ratio, root sucker, rootling, seedling, seedling sprout, steckling, vegetative propagation, water stress, West Africa.

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*To Lawadoun and Lawali*

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## List of Publications

This thesis is based on the work contained in the following original papers, which are referred to in the text by their respective Roman numerals (I-IV):

- I Ky-Dembele C., Tigabu M., Bayala J., Savadogo P., Boussim I.J., Odén P.C. 2010. Clonal propagation of *Detarium microcarpum* from root cuttings. *Silva Fennica*, 44(5), 775-786.
- II Ky-Dembele C., Tigabu M., Bayala J., Savadogo P., Boussim I.J., Odén P.C. 2010. Propagation of *Khaya senegalensis* by stem cuttings. (Submitted manuscript).
- III Ky-Dembele C., Tigabu M., Bayala J., Ouédraogo S.J., Odén P.C. 2008. Comparison between clonal and sexual plantlets of *Detarium microcarpum* Guill. & Perr., a savanna tree species in Burkina Faso. *African Journal of Ecology*, 46(4), 602-611.
- IV Ky-Dembele C., Bayala J., Savadogo P., Tigabu M., Odén P.C., Boussim I.J. 2010. Comparison of growth responses of *Khaya senegalensis* seedlings and stecklings to four irrigation regimes. *Silva Fennica*, 44(5), 787-798.

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# 1 Introduction

## 1.1 Background

Deforestation and land degradation are major concerns over large parts of sub-Saharan Africa. Some of the key factors affecting these phenomena are agricultural expansion, logging, firewood collection, charcoal production, overgrazing, and uncontrolled fires. Natural regeneration, enrichment planting, plantations, agroforestry, controlled burning, and soil and water conservation are some of the techniques used to mitigate the effects of deforestation and land degradation in sub-Saharan Africa (FAO, 2004). While natural regeneration is passive, resulting from the unassisted recruitment of seedlings produced by seed dispersed from existing mature trees (Dorrrough *et al.*, 2008), and the regrowth of coppices following logging, plantation establishment is an active process that involves planting seedlings or vegetative propagules either in single or mixed stands (FAO, 2004). Natural regeneration, which is cheaper and less labor-intensive, is however spatially and temporally unpredictable because trees may not establish where land managers want them and their densities may be greater or less than desired; this means there is considerable risk associated with any investment in natural regeneration (Vesk & Dorrrough, 2006; Dorrrough *et al.*, 2008). Plantation establishment, which is often promoted as it allows faster growth and has higher potential to produce biomass, can be expensive and labor-intensive (Kanowski, 1997; Dorrrough *et al.*, 2008).

In addition, in arid and semiarid areas such as African savannas, land managers responsible for afforestation or reforestation are faced with numerous and complex biological, environmental, and economic challenges because of the effects of the severe climate and the many disturbances such as fire, grazing, and excessive harvesting of trees and shrubs. These

environments are characterized by low variable rainfall, high incident radiation, high summer or year-round temperature and evaporation rates, low atmospheric humidity, and frequently strong winds, all leading to a short growing season. The severe climate also greatly affects soil formation, most of the processes involved being progressively slower or impeded with increasing aridity; as aridity decreases, soils become more developed, deeper, and more leached (Armitage, 1985; FAO, 1989; Malagnoux *et al.*, 2007). Therefore the methods that are appropriate for plant propagation and vegetation restoration depend on many interrelated variables including the propagation characteristics of a given species, site and environmental factors, economics and management goal considerations (Kanowski, 1997; Hartmann *et al.*, 2002; FAO, 2004; Scianna *et al.*, 2004).

## 1.2 General overview of plant propagation in forestry

Plant propagation, the intentional act of reproducing plants, has been defined as the science and art of multiplying plants and preserving their unique qualities by either sexual or asexual means (Hartmann *et al.*, 2002). Sexual reproduction involves meiotic cell division occurring in the gamete and forming seed tissues that ultimately produce progeny (seedlings) with a new or unique genotype relative to their male and female parents. Most woody plants are highly heterozygous so that the progeny of woody plants grown from seed tend to exhibit a relatively high level of genetic variation (Libby & Rauter, 1984; Hartmann *et al.*, 2002; Scianna *et al.*, 2004; Eriksson *et al.*, 2006). On the other hand, asexual propagation is the reproduction from the vegetative parts, such as stems, leaves, roots, tissues or organs, of the donor plant and involves mitotic cell division in which the chromosomes duplicate and divide to produce two nuclei which are genetically identical to the original (Hartmann *et al.*, 2002; Eriksson *et al.*, 2006). This can occur through the formation of adventitious roots and shoots or by combining vegetative tissues, as in grafting (Macdonald, 1990; Hartmann *et al.*, 2002).

Such clonal processes, in which the genotype of the parent plant is exactly duplicated, are possible because of two unique plant characteristics, *totipotency* and *dedifferentiation*. *Totipotency* is the ability of vegetative plant cells to carry all of the genetic information necessary to regenerate the original plant. *Dedifferentiation* is the ability of mature (differentiated) cells to return to a meristematic condition and produce a new growing point (Hartmann *et al.*, 2002; Scianna *et al.*, 2004). Clonal individuals are referred

to as stecklings (plantable rooted stem cuttings); rootlings (plantable individuals grown from root segments) or root suckers (individuals arising vertically from superficial lateral roots in field conditions).

The selection of a propagation method often depends on the reproductive characteristics of the species involved. Some woody species can be propagated readily from seed because they frequently produce abundant viable seed, while others possess one or more dormancy mechanisms which prevent seed germination until environmental conditions are favorable for germination, survival, establishment, and ultimately species perpetuation. Warm or cold stratification, mechanical or chemical seed coat scarification, or some combination of these or other treatments are usually needed before germination will occur. These conditions are fulfilled naturally by passage through the guts of animals, bush fires, micro-climatic conditions and soil processes (Baskin & Baskin, 2001; Hartmann *et al.*, 2002; Scianna *et al.*, 2004). Embryo culture can be used to break down the dormancy of some species (Rambabu *et al.*, 2006).

As with propagation by seed, some species are easily propagated by clonal methods while others are difficult to propagate. In some cases, vegetative propagation is difficult or there is a lack of technology to do so. Adventitious roots or shoots may be produced, but at a rate so low as to be impractical for wide scale applications. Some species can be propagated by cuttings but only at certain times of the year, or only from hardwood or softwood. Tissue culture provides an alternative approach to traditional cloning techniques but needs technical input and facilities that could increase plant production costs (Macdonald, 1990; Hartmann *et al.*, 2002).

There are numerous site and environmental conditions, such as climate (temperature, rainfall, relative humidity and wind), soil, topography, and biotic factors, that can influence the type of propagation system selected for producing plants. These conditions can directly influence propagation by affecting the production of seeds or by reducing plant vigor. Factors inhibiting the use of seeds include poor weather such as drought, since it affects production and timely collection, consumption by animals, attack by insects and diseases, and fire (FAO, 1989; Hartmann *et al.*, 2002; Scianna *et al.*, 2004). Site conditions may also favor one type of asexual propagation technique over another. For example, superficial soil or disturbances such as fire and logging are known to favor root suckering in some tree species (Tredici, 2001; Silla *et al.*, 2002). In most cases, propagation by seed is the most labor and cost effective method of reproducing plants if genetic

variability, such as germination requirements, can be managed within acceptable limits (Macdonald, 1990; Hartmann *et al.*, 2002). Although seed may be in abundant supply, viability may be low, available seeds may be expensive (Scianna *et al.*, 2004), seedling survival rate may be low and seedlings may grow slowly (Kaboré, 2005; Zida *et al.*, 2007). In such situations, when the conditions for collecting, sowing, and culturing seeds are inadequate, asexual propagation may be a viable production alternative (Libby & Rauter, 1984; Leakey, 1987).

### 1.3 Clonal propagation in forestry

#### 1.3.1 Methods and application of clonal propagation

There are many clonal propagation methods, including cuttings, grafting, budding, layering and tissue culture. However, grafting, cuttings and the recent technique of tissue culture are the main vegetative propagation methods that have been developed and are the most widely used in forestry (Zobel & Talbert, 1984; Libby, 1986).

Grafting has been undertaken from the earliest times and is still in use on a large scale to preserve and multiply desired genotypes (Zobel & Talbert, 1984). It is the most common technique employed to preserve trees in clone banks or for seed orchards in which the objective is large-scale seed production. It can be performed reliably with most species. It is especially important for the propagation of fruit trees where ontogenetic maturity has to be retained. However, in some species, grafting incompatibilities develop anywhere from a year or so to over a decade after grafting, resulting in failure of the graft union. The cost per graft is generally high, and the effects of the rootstocks are an additional variable (Libby, 1986). In contrast to grafting, stem cuttings can in some cases produce stecklings for costs similar to those of seedlings; in few cases for even less (Libby, 1986; Leakey, 1987). Low costs plus freedom from delayed mortality and from rootstock interaction mean that stecklings can be considered for many uses, from mass propagation for plantation establishment to research requiring high genetic control (Libby, 1986). However, unlike grafting, the rooting of stem cuttings is highly sensitive to the effects of maturation of the donor tree. In general, cuttings from juvenile donors root easily and subsequently grow like seedlings, while cuttings from mature donors root with difficulty, and these stecklings differ substantially from seedlings in many respects (Libby, 1986; Greenwood & Hutchison, 1993).

In forestry, propagation by root cuttings is a less widely utilized technique than propagation by stem cuttings. But, recent studies have demonstrated that root cuttings can be a useful and efficient method for cloning forest trees such as aspen (Hall *et al.*, 1989; Stenvall, 2006; Snedden *et al.*, 2010). The main advantages of root cuttings are that it requires limited propagation facilities, provides a relatively fast way to multiply clonal material, and may supplement other propagation techniques (Macdonald, 1990). The main limitation of clonal propagation by root cuttings is the occurrence of chimeras, in which the cells of the outer layer are of a different genetic make-up from those of the inner tissues; these will not regenerate true-to-type from root cuttings (Macdonald, 1990; Hartmann *et al.*, 2002). However, root suckering occurs naturally in some species such as poplars following disturbance of forest stands, promoting the colonization of new ground. Frequent fires and heavy logging are disturbances known to favor the spread of root suckering species (Jenik, 1994; Del Tredici, 2001).

Tissue culture is particularly useful as a very effective and rapid method to multiply clonal material for early release of a selected genotype; it is especially valuable for producing stockplants that are normally difficult or slow to propagate by conventional vegetative methods (Libby, 1986; Ahuja, 1993). The technique encompasses regeneration from shoot and root tips, callus tissue, leaves, seed embryos, anthers, and even a single cell; shoot tips are the most commonly cultured tissue for woody plant propagation (Macdonald, 1990). The most promising technique is somatic embryogenesis. However, genetic variation or somaclonal variation can occur in plant tissue cultures and hamper the production of true-to-type propagules from a selected genotype (Chen, 1993; Kleinschmit *et al.*, 1993). In addition, costs per plant are generally high but will probably come down, although intensive work will be needed before confidence in the performance of plantlings (plantable tissue culture plantlets) approaches that of stocklings and seedlings (Zobel & Talbert, 1984; Feyissa *et al.*, 2005).

In forestry, clonal propagation is used mainly for the preservation of genotypes in clone banks, for the multiplication of desired genotypes for special uses, such as in seed orchards or breeding orchards, for the evaluation of genotypes and their interactions with the environment through clonal testing, and for maximizing genetic gains in operational planting programs. These can be separated into two major groups of uses, research and operational production (Zobel & Talbert, 1984). Cloning of trees has been a useful tool in traditional tree improvement. The reasons for cloning and the ability to clone effectively vary among species. With a few minor exceptions

(mutation and maturation-related differences), all members of a clone are genetically identical. For this reason, clones are often used in forestry research to control or to assess genetic variability in experiments, since uncontrolled genetic variability can introduce unwanted biases or at least unnecessary variation in many kinds of experiments. By using identified clones, these problems can be eliminated.

Tree improvement programs also use clones to increase the multiplying power of selected parents. In addition, clones are used in silvicultural research to study questions such as the effects of spacing, fertilizers, and site changes. Such experiments become even more useful if some of the research clones can then be used in production plantations (Libby & Rauter, 1984; Zobel & Talbert, 1984; Libby, 1986). It is presumed that some of the limitations to domestication in Africa (i.e. long generation times, irregular flowering/fruitletting periods, as well as out-breeding) can be overcome through vegetative propagation (Leakey *et al.*, 1982a; Teklehaimanot, 2004).

Clonal forestry has been practiced the longest with sugi (*Cryptomeria japonica*), and over the greatest areas with poplars, willows, and eucalypts (Zobel & Talbert, 1984; Ohba, 1993; Zobel, 1993; Zsuffa *et al.*, 1993). In Japan, sugi has been propagated as rooted branch cuttings for production forestry since about 1400 (Ohba, 1993) and, for 3-4 centuries in Europe, Asia and the Middle East, poplars and willows have frequently been vegetatively propagated by planting unrooted cuttings (Zsuffa *et al.*, 1993). Currently, the largest operational clonal forestry programs are conducted with several species in the genus *Eucalyptus* (Zobel, 1993). As in many regions of the tropics, in Africa vegetative propagation has been used extensively in operational forest planting mainly with *Eucalyptus* and *Gmelina* (Leakey, 1987). For all the species involved, vegetative propagules are often found to be more effective in plantation establishment than seedlings (Ohba, 1993; Zobel, 1993; Zsuffa *et al.*, 1993).

However, aside from these few genera, vegetative propagation has not been used extensively in operational forest-planting programs (Leakey, 1987; Kleinschmit *et al.*, 1993; Libby & Ahuja, 1993a). However, now that most of the biological problems that prevented successful and efficient cloning of forest tree species have been solved, or are sufficiently well understood, clonal forestry is becoming an economic and realistic option and a viable alternative to conventional seedling-based forestry for many species and programs (Libby & Rauter, 1984; Kleinschmit *et al.*, 1993).

### 1.3.2 Advantages and limitations of clonal propagation

Clonal propagation has both pros and cons. It is viewed as a powerful means of exploiting genetic gains through capture of the two broad components of genetic variation, namely additive and non-additive. When seed regeneration is used, only the additive portion of the genetic variation can be manipulated, unless special approaches, such as controlled pollinations to mass-produce specific seed lots or two-clone orchards, are employed. In general, the use of vegetative propagation makes it possible to capture and transfer to the new tree all the genetic potential from the donor tree. For characteristics, such as volume growth, that have low narrow-sense heritability, it appears that it is possible to more than double short-term genetic gain in many species by using vegetative propagules rather than seed regeneration (Libby & Rauter, 1984; Zobel & Talbert, 1984; Kleinschmit *et al.*, 1993; Libby & Ahuja, 1993b). For example in Brazil, the use of *Eucalyptus* clones in plantations has improved the crop yield by up to 112% and productivity by 135% compared to seedling plantations (Zobel, 1993).

The ability to obtain a high degree of crop uniformity for many traits (size, form, growth requirements, stress tolerance and wood quality) is a major advantage of clonal forestry (Libby & Ahuja, 1993b). Uniformity is particularly important in reducing crop wastage and in ensuring that the final product is of high quality (Zobel & Talbert, 1984; Macdonald, 1990). The use of clonal propagules can also be very effective in the reduction of pests. After suitable testing, resistant clones can be deployed so that plantations can be maintained free from infection (Zobel, 1993). Another advantage of clonal forestry is the opportunity to match clones to sites and silvicultural treatments or to select clones that can tolerate specific environmental stresses (Libby & Rauter, 1984; Zobel, 1993).

The major problems in operational and research uses of vegetative propagules relate to the effect of the age and location of the propagule from the parent and its ability to grow as a tree. Both age and location differences are very important. With increasing age, there is a progressive loss of the capacity for vegetative propagation. Physiologically mature tissue has lower rooting ability, takes longer to initiate roots, and develops fewer roots than physiologically juvenile material. In addition, juvenile material tends to assume an orthotropic (upright or normal tree form) growth habit much more readily than mature material. Cyclophysis, defined as the process of maturation of the apical meristems, is related to age effects and topophysis,



the phenomenon that occurs when scions, buddings, and rooted cuttings maintain for some time a branch like growth habit (plagiotropic growth), is related to location or origin effects. A third cause of variation is periphysis, which refers to locations in different environments, such as shade and sun shoots on an individual tree. Although the concepts of cyclophysis and topophysis are widely recognized, their effects are not always understood (Zobel & Talbert, 1984; Bonga & von Aderkas, 1993; Kleinschmit *et al.*, 1993; Hartmann *et al.*, 2002; Leakey, 2004). Methods for developing or maintaining juvenility, such as hedging or successive grafting, are essential for further development of the operational planting of vegetative propagules. Rejuvenation of mature clones occurring during the tissue culture process could be of great value (Zobel & Talbert, 1984; Becwar, 1993; Bonga & von Aderkas, 1993).

The main criticism of clonal forestry is that it reduces biodiversity, restricting the genetic base of the forest which makes clonal plantations vulnerable to unexpected outbreaks of diseases and insects, and leads to plantation failure since a single pathogen could affect all trees equally. Thus, the idea of growing forest trees with relatively similar genotypes over large areas is a cause of concern among foresters and the public (Zobel & Talbert, 1984; Kleinschmit *et al.*, 1993; Lane, 2004). If the genetic base in forestry is narrowed, the likelihood of losses will increase. Although yields are enhanced, the forester must address the following question: how much risk of death or loss of yield can be tolerated for a given amount of additional product uniformity, volume or quality (Zobel & Talbert, 1984)?

However, it has become apparent that the use of diverse clones offers possibilities for greater genetic diversity in plantations, compared to natural regeneration or to planting seedlings from wild or orchard collections and thereby reduces the relative risk of loss in clonal plantations to below that of seedling plantations (Libby, 1982; Zobel & Talbert, 1984; Libby, 1986; Kleinschmit *et al.*, 1993). According to many authors, by maintaining a high genotypic diversity within a plantation when using a mosaic-like distribution or small planting blocks, it is possible to buffer the effects of any pest over the entire plantation (Kleinschmit *et al.*, 1993; Lane, 2004). In some regions, widespread intimately mixed plantations of many clones (WIMPs) may be favored whereas mosaics of monoclonal stands (MOMs) may be required in other regions (Zobel & Talbert, 1984; Libby, 1987; Kleinschmit *et al.*, 1993; Lindgren, 1993). A mixture of clones with seedlings or other species has been also suggested as a means of reducing risk

and increasing diversity (Lindgren, 1993). The benefits and risks associated with vegetative propagation in forestry have been reviewed by a number of authors, who also emphasize that the technology has the potential to create a revolution in forestry (Libby & Rauter, 1984; Kleinschmit *et al.*, 1993; Talbert *et al.*, 1993).

## 1.4 Relevance of clonal forestry in Burkina Faso

### 1.4.1 Current situation of forests in Burkina Faso

Burkina Faso (formerly Upper Volta) is a landlocked country of 273 600 km<sup>2</sup> and 15 234 000 inhabitants (FAO, 2010), located in the middle of West Africa. Forests in Burkina Faso have been established from the savanna woodlands that characterize the dominant vegetation structure. In 2010, the estimated forest area of Burkina Faso was about 5 649 000 ha accounting for 21% of the country's land area, while net annual forest lost was about 60 000 ha (1.03%) for the period 2005-2010 (FAO, 2010). The decline of the forests is expected to persist because of intense pressures as a result of grazing, expansion of crop lands, and collection of wood and other products (FAO, 2003b). Forest areas are categorized as 'classified' or protected (Fig.1) and 'non-classified' domains. The classified domain includes state forests (880 000 ha), national parks (390 000 ha) and wildlife reserves (2 545 000 ha). The non classified areas are where the human population freely conducts farming activities, livestock breeding and wood collection (Ouédraogo, 2001). Within the state forests, 600 000 ha are assigned to management mainly for fuelwood production (FAO, 2010), but only 29% of this area has been under effective management since 1986 (Kaboré, 2005).

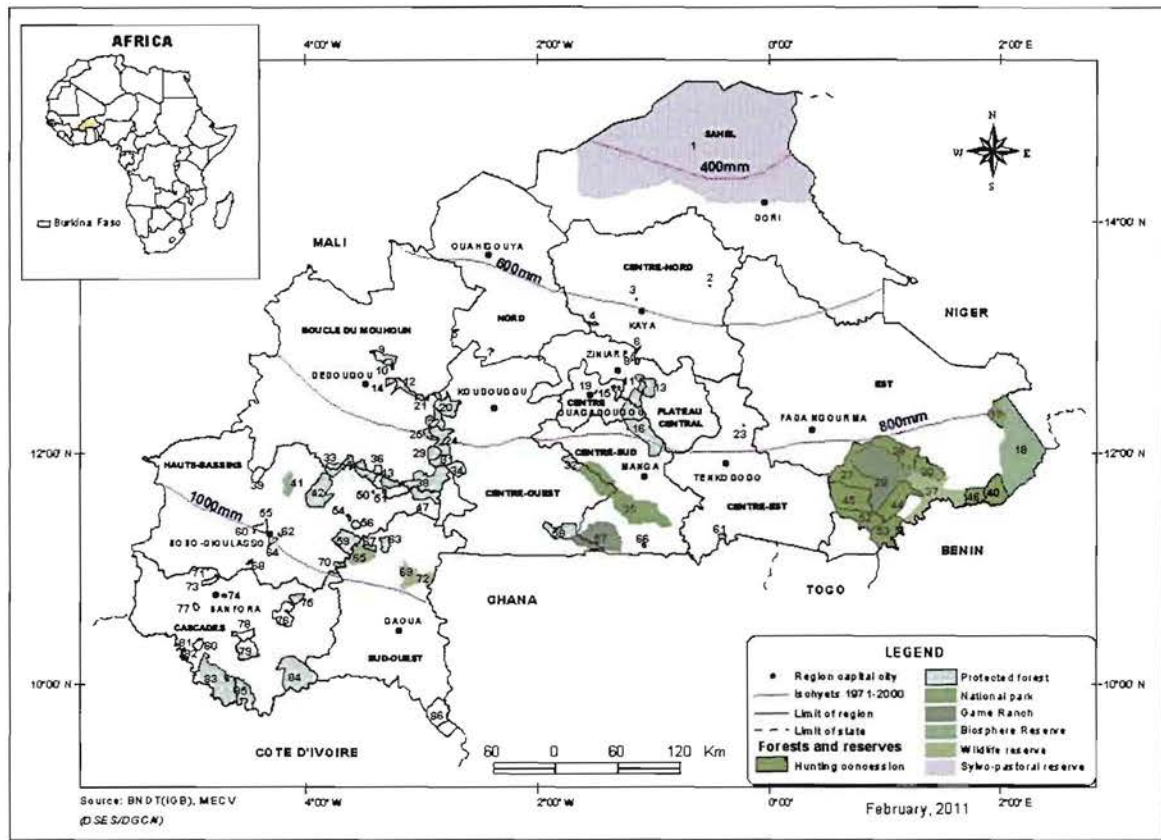


Figure 1. Forests and reserves in Burkina Faso (Ministry of Environment and Quality of Life).

The management regime entails annual early fire setting and selective tree cutting of 50% of the merchantable volume over a 15–20 year rotation. The harvested stands are mainly left to regenerate naturally by coppice growth and the establishment of seedlings. In some cases this is supplemented by direct seeding. However, studies have revealed that natural regeneration from seeds is inadequate and supplementary direct seeding has failed due to high mortality rates of both seeds and seedlings (Kaboré, 2005).

Forest plantations have started in 1970 and their total area amounts to about 109 000 ha, representing 2% of the total forest area in 2010 (FAO, 2010). Both farmers and the public sector plant forests for non industrial uses, primarily fuelwood and poles, but the survival and productivity of the trees are often low. For instance the estimated production yield for *Eucalyptus camaldulensis* is about 1.38 – 3.71 m<sup>3</sup> ha<sup>-1</sup> year<sup>-1</sup> (Ouédraogo, 2001). Eighty percent of the plantations are of exotic species (FAO, 2010) with *Eucalyptus camaldulensis* dominating followed by *Acacia nilotica* an indigenous tree species (Anonymous, 2006). It is partly because of the slow growth of the indigenous tree species that most forest plantations in Burkina Faso comprise exotic fast growing tree species. Moreover, while the current expansion of planted forests is about 6 000 ha per year (FAO, 2010), involving around 4 000 000 seedlings (Anonymous, 2006), it does not compensate for the annual natural forest loss of 60 000 ha to meet the growing needs for fuelwood, building materials, and other products required by the expanding population.

Trees outside forests form a major source of wood and wood products in all ecological zones in Burkina Faso. Such trees are mainly located in sacred forests, usually protected by customary edicts, and trees grown on farmland as part of various agroforestry practices. Usually small in their extent, pockets of woodland are sometimes found as dense stands constituting "sacred woods". They are dominated by *Anogeissus leiocarpa*, *Acacia pennata*, *Celtis integrifolia*, *Diospyros mespiliformis*, and *Pterocarpus erinaceus* (Fontès & Guinko, 1995). Farmers have, for many generations, maintained a traditional rational land use system, known as agroforestry parkland, characterized by the deliberate retention of trees in cultivated or recently fallowed lands (FAO, 2003a). Common tree species selected by farmers and maintained on their farmland include *Adansonia digitata*, *Borassus aethiopum*, *Faidherbia albida*, *Lannea microcarpa*, *Parkia biglobosa*, *Sclerocarya birrea*, *Tamarindus indica*, and *Vitellaria paradoxa* (Fontès & Guinko, 1995; Nikiema, 2005). Trees are an integral part of the system, providing a range of

products, and contributing to the maintenance of soil fertility (Bayala *et al.*, 2006; Bayala & Ouedraogo, 2008), water conservation and environmental protection. Most rural communities rely on trees outside forests as the main source of fuelwood, fodder, oil, poles, construction material, and shade for animals during the dry season (FAO, 2003b; Nikiema, 2005).

#### 1.4.2 Potential advantages of clonal forestry in Burkina Faso

In Burkina Faso, about 40% of the gross domestic product (GDP) comes from agricultural activities, employing 81% of the total population and including agriculture, livestock and forests (Anonymous, 2004). The contribution of forest products to the GDP in Burkina Faso was 11% in 1991 (FAO, 1995) while the value of fuel from the 7 333 000 m<sup>3</sup> of wood removed from the forests in 2005 was estimated to amount to 63 million US\$ (FAO, 2010). Forests offer a wide range of both material and intangible benefits, all of which have a value but only some of which are currently expressed in monetary terms (FAO, 1997; FAO, 2010). Forest and trees outside forests provide goods and services such as timber, fiber, fuelwood, food, fodder, gum, resins, medicines, environment stabilization, and biodiversity conservation (FAO, 2000). As in most African countries, wood is by far the most important source of energy, indeed 90% of all harvested wood is used for energy (FAO, 2007) satisfying 91% of the total energy consumption in Burkina Faso (Ouédraogo, 2001). In addition to providing goods and services, the forests, woodlands, and trees outside forests are integral to the vegetation structure of the landscape.

With the increasing population and the declining forest cover, the forestry sector in Burkina Faso will presumably face a more difficult situation of satisfying the growing, divergent, and conflicting demands of different sections of the population with respect to their needs for forest products and services. To cope with this, a great investment is needed to ensure long term sustainable management of the natural forests, plantations and agroforestry and to support higher production and productivity (FAO, 2003b). In such a situation, the use of vegetative propagules in research and operational forestry could be a viable option for delivering rapid short-term tree improvement to increase forest production. Vegetative propagation could be advantageous for selecting trees with resistance to disease and insect pests and for adaptations to adverse environments such as arid and semi-arid areas which are so dry and hot that they limit the vigorous growth of trees in Burkina Faso. One of the greatest benefits of vegetative propagation is the

speed with which the desired genetic qualities of selected trees can be utilized, making it unnecessary to wait for seed production before producing propagules for operational planting. As soon as plants have been proven to have good genotypes, they can be used directly in operational reforestation, for example enrichment planting or small scale plantations, by employing vegetative propagation.

In addition, planting vegetative propagules grown from genotypes that have performance traits such as a high growth rate or drought resistance and good wood quality might reduce the long juvenile growth period and improve the quality and quantity of forest products, especially from indigenous tree species. In agroforestry, it would be possible to select clones that take advantage of highly productive sites, or tolerate the problems associated with degraded sites, better than seedlings. The selection of particularly useful and contrasting clones could serve the multiplicity of agroforestry objectives such as production of wood, fruits, fodder, and medicine (Kleinschmit *et al.*, 1993).

Despite advances in cloning techniques and their practical applications in forestry elsewhere, they are still in their infancy in Burkina Faso. Detailed protocols for cloning the indigenous tree species of West Africa are not available, with the exception of a few species. Such techniques and species include stem cuttings from *Khaya ivorensis*, *K. anthotheca* (Tchoundjeu & Leakey, 1996; Opuni-Frimpong *et al.*, 2008), and *Parkia biglobosa* (Teklehaimanot *et al.*, 2000), grafting of *Vitellaria paradoxa* (Sanou *et al.*, 2004), and micro-cuttings and grafting of *K. senegalensis* (Danthu *et al.*, 2003; Ouédraogo, 2004). Thus, this project was initiated to develop appropriate clonal propagation techniques for two highly exploited species, *Detarium macrocarpum* Guill & Perr. and *Khaya senegalensis* A.Juss.

## 2 Objectives

The overall objective of the work underlying this thesis was to develop simple and efficient clonal propagation techniques for two commercially valuable tree species indigenous to Burkina Faso, namely *Detarium microcarpum* Guill & Perr. and *Khaya senegalensis* A.Juss. This represents a first step toward the use of clonal materials for improving the success of plantation establishment and production.

The specific objectives of this research were:

1. To determine factors which affect the success of clonal propagation by stem and root cuttings of *D. microcarpum* and *K. senegalensis* (I, II);
2. To examine differences in morphological traits and carbohydrate contents of clonal and sexual plantlets of *D. microcarpum* (III);
3. To compare growth, biomass allocation and intrinsic water use efficiency of seedlings and stecklings of *K. senegalensis* in response to varying irrigation regimes (IV).

## 3 Materials and methods

### 3.1 Description of *Detarium microcarpum* and *Khaya senegalensis*

Wood fuel is by far the most important output from the forests of Burkina Faso. Fuelwood accounts for 85-90% of all commercial wood products in the country, followed by raw material such as poles and then timber. The annual consumption of timber is about 25 000 m<sup>3</sup>, of which 93% is imported from neighboring countries (Ouédraogo, 2001). To avoid fuelwood deficits and reduce the country's dependence on imported wood as demand increases, forest policy emphasizes the management of natural forests and plantations to maximize fuelwood production and increase the national production of raw material and timber. However, slow growth and the impact of insect pests are major limitations to growing indigenous species in plantations, even though they are compatible with the environmental conditions and their products are well accepted in the country where they grow naturally. Therefore, it is important to improve the establishment and growth in plantations of indigenous tree species such as *D. microcarpum* and *K. senegalensis*, known for their high potential in terms of the production of both fuelwood and raw material.

#### 3.1.1 Botany, distribution and importance

*D. microcarpum* is a deciduous tree species that sprouts late in the dry season (Kouyaté & van Damme, 2006; Bastide & Ouédraogo, 2009). It belongs to the Family Leguminosae, the subdivision Caesalpinioideae and the Tribe Detarieae (Watson & Dallwitz, 1993). *D. microcarpum* is known locally in Burkina Faso by various common names including *Kagedga* (More), *Tama koumba* (Dioula) and *Koro* (Samo). *D. microcarpum* is distributed across semi-arid sub-Saharan Africa, from Senegal to Cameroon, extending east to the



Sudan (Fig. 2). It has an irregular distribution and can be locally very common (Kouyaté & van Damme, 2006; Vautier *et al.*, 2007). The genus *Detarium* comprises two other species, *D. senegalense* and *D. macrocarpum* (Kouyaté & van Damme, 2006).

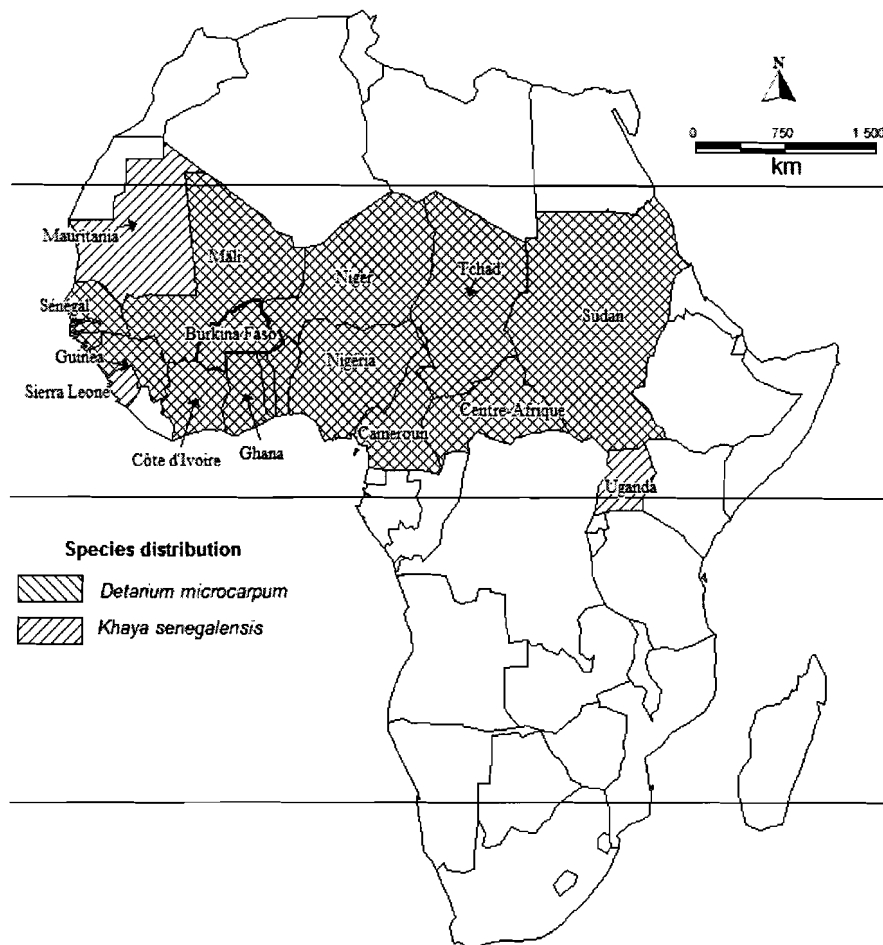


Figure 2. Spatial distribution of *Detarium microcarpum* and *Khaya senegalensis* in Africa, adapted from (Kouyaté & van Damme, 2006) and (Nikiema & Pasternak, 2008).

*D. microcarpum* is catalogued as a major African medicinal plant. The roots, stems, bark, leaves and fruits are all used to treat ailments such as tuberculosis, meningitis, itching and diarrhea. The isolation of terpenoids and anti-HIV flavans from *D. microcarpum* extracts has been reported (Abreu & Relva, 2002; Kouyaté & van Damme, 2006; Vautier *et al.*, 2007).



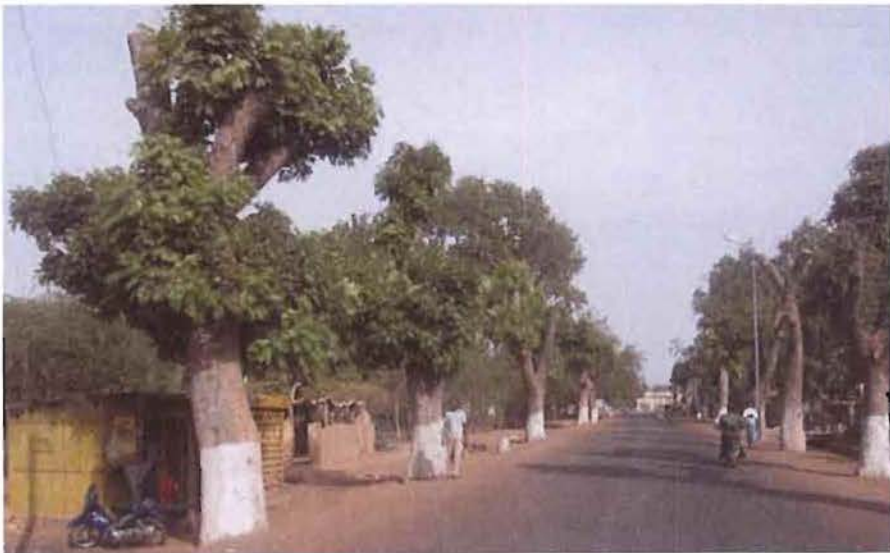
Mature trees of *Detarium microcarpum* in the Nazinon forest (C. Ky-Dembele)

The fruits are rich in vitamin C, potassium and calcium, and are widely eaten and marketed within the species' range in West Africa (Akpata & Miachi, 2001). The seed flour has been found to comprise 11% proteins, 36% lipids and 42% carbohydrates (Anhwange *et al.*, 2004). The species produces timber which can serve as a mahogany substitute. Its hard dark brown wood provides very good quality timber, which is used in carpentry and construction (Vautier *et al.*, 2007). It is also used for good quality charcoal and fuelwood delivering 19 684 kJ kg<sup>-1</sup> of calorific power (Kaboré, 2005). It is the most important commercial fuelwood species and is harvested preferentially from the state forests in Burkina Faso (Kaboré, 2005; Sawadogo, 2007).

*K. senegalensis* is an evergreen tree species of the family Meliaceae, the subfamily Swietenioideae, and the tribe Swietenieae (Styles & Vosa, 1971; de Bie *et al.*, 1998); it reaches a height of 15–20 m, a diameter of 1.5 m and has an 8–16 m clean bole (Joker & Gamene, 2003; Nikiema & Pasternak, 2008). The genus *Khaya* comprises four species in mainland Africa, *K. anthotheca*, *K. grandifoliola*, *K. ivorensis*, and *K. senegalensis*, as well as one or two species endemic to the Comoros and Madagascar, for example *K. madagascariensis* (Maroyi, 2008).

*K. senegalensis*, known as dry zone mahogany (English) or acajou cailcedrat (French), is a multipurpose tree across its natural range in Africa (Fig. 2), occurring in the Sudanian zone of 650-1300 (up to 1800) mm isohyets from Mauritania to northern Uganda (Nikiema & Pasternak, 2008). In Burkina Faso, the northern limit of the natural distribution of *K. senegalensis* is 13°55'N in the South Sahelian zone. The population density increases from North to South reaching 17 trees per hectare in various ecosystems such as river banks, fields, fallows and state protected woodlands. *K. senegalensis* is particularly valued for timber, fuelwood, medicinal purposes, and amenity (Joker & Gamene, 2003; Arnold, 2004; Nikiema & Pasternak, 2008).

*K. senegalensis* is one of the main timber wood species of dry areas of Africa, rated as one of the densest and hardest of the African mahogany woods (Normand & Sallenave, 1958). In some countries such as Mali and Burkina Faso, *K. senegalensis* wood may contribute up to 80% of all logs entering local sawmills (Nikiema & Pasternak, 2008). The wood, moderately resistant to fungi and termites, is valued for carpentry, high-class joinery, furniture, cabinet making, ship building and as a decorative veneer. Even though fuelwood from this species is in short supply because of difficulties with splitting it, the gross energy value of the wood is high: 19 990 kJ kg<sup>-1</sup> (Nikiema & Pasternak, 2008).



Pollarded roadside *Khaya senegalensis* trees in Ouagadougou (C. Ky-Dembele)

*K. senegalensis* has many medicinal uses. The bark is highly valued in traditional medicine as a treatment for fever caused by malaria, diarrhoea, dysentery, anaemia, etc. In traditional veterinary practice, the bark extract is used for treating internal ailments in cattle, camels, donkeys and horses (Joker & Gamene, 2003; Nikiema & Pasternak, 2008; ICRAF, 2010). Recently, the stem bark has been found to contain chemicals (limonoids) that have anti-proliferative activity against human cancer cell lines (Zhang *et al.*, 2007). In its natural range *K. senegalensis* provides fodder with high dry matter but relatively low crude protein content (Ouedraogo-Kone *et al.*, 2008). In West Africa, the species has become an important urban amenity tree, commonly planted as a roadside and ornamental shade tree and as an exotic in South Africa, Australia, Indonesia, etc. (Arnold, 2004; Nikiema & Pasternak, 2008).

### 3.1.2 Regeneration and the need for clonal propagation

*D. microcarpum* is capable of regenerating vigorously both sexually from seeds and vegetatively from lateral roots when the above-ground parts have been damaged, removed or killed by harvesting or fire (Ky-Dembele *et al.*, 2007). However, it has been noted that sexual reproduction rather than vegetative recruitment is responsible for most of the plants found in the Nazinon forest (Ky-Dembele *et al.*, 2007). Seeds of *D. microcarpum* have desiccation tolerance and exhibit orthodox storage behavior, remaining viable for at least 10 years with no dormancy mechanism (Zida *et al.*, 2005; Vautier *et al.*, 2007). Within the natural vegetation, in general, current year seedlings constitute the only true seedlings (individuals of seed origin that have never been affected by shoot dieback). For instance in the Nazinon forest, very few true seedlings (5%) have been found for *D. microcarpum* compared to seedling sprouts (71%) (individuals of seed origin that have been affected by shoot dieback, but resprouted from the root collar of the seedlings), coppices (20%) and root suckers (5%) (Ky-Dembele *et al.*, 2007). Regeneration by root suckers is the most important clonal reproduction mechanism of *D. microcarpum* following disturbance of forest stands in the savanna areas.

Seedling sprouting therefore remains the most important regeneration mechanism of *D. microcarpum* in the savanna woodlands because seedling shoots die back annually during the dry season for an unknown number of years before the sapling stage (Alexandre, 1992; Bationo *et al.*, 2001; Ky-Dembele *et al.*, 2007). In the Tiogo forest in Burkina Faso, Sawadogo

(personal communication) has observed seedling shoot die back in *D. microcarpum* over a period of 15 years since direct sowing. As for many species growing in African savannas, natural regeneration is significantly influenced by the physiological process of shoot die-back, known as the suffrutex phenomenon (Jackson, 1968; Alexandre, 1992; Menaut *et al.*, 1995; Mwitwa *et al.*, 2008). It is generally assumed that the purpose of this developmental habit is to reallocate all resources to the taproot, probably as a storage organ (Ky-Dembele *et al.*, 2008; Mwitwa *et al.*, 2008). It appears that a juvenile plant must resprout several times until at some stage it manages to escape the damage cause by drought, fire or herbivory to be able to grow to full maturity. When this occurs there is a sustained and rapid growth which produces a sapling that is strong enough to be able to withstand or recover from the subsequent effect of disturbances (Jackson, 1968; Bationo *et al.*, 2001). Whether this phenomenon is genetic or a combination of environmental and physiological factors is still unknown, although one study has indicated the existence of moderate heritability of shoot die-back in *Pterocarpus angolensis* in Southern Africa (Mwitwa *et al.*, 2008).

*D. microcarpum* is also known for its excellent potential for coppicing (Sawadogo *et al.*, 2002; Ky-Dembele *et al.*, 2007; Bastide & Ouédraogo, 2008). Therefore it is obvious that the persistence of *D. microcarpum* is related to its ability to resprout vigorously from the juvenile to the mature stages. Moreover, the high mortality rate of the seedlings combined with the slow growth accentuates the need for developing alternative propagation techniques (Kaboré, 2005; Zida *et al.*, 2008). Because they would establish quickly and could survive and grow satisfactorily, the use of good quality vegetative propagules in operational forestry would improve forest products derived from *D. microcarpum*.

Natural regeneration of *K. senegalensis* is poor (Joker & Gamene, 2003) as the seeds rapidly lose viability, over just two to three weeks under natural conditions (Opuni-Frimpong *et al.*, 2008). However, viability of the seeds can be retained when dried to below 5% moisture content and stored at a temperature of about 5°C (Danthu *et al.*, 1999; Gamene & Eriksen, 2005). *K. senegalensis* is listed as vulnerable on the International Union for Conservation of Nature (IUCN) 2010 red list of threatened species because of overexploitation for timber, fodder and medicine, habitat loss and degradation (Nikiema & Pasternak, 2008). In addition, efforts to restore the depleted mahogany resource base on plantations have been impeded by persistent attacks by the mahogany shoot borer *Hypsipyla robusta* (Danthu *et*

*al.*, 2003; Nikiema & Pasternak, 2008). Selection and propagation of genetically resistant individuals has been sought to ensure better establishment of plantations while conserving the germplasm (Newton *et al.*, 1993; Danthu *et al.*, 2003). However, to date, the species is still in the early stages of its domestication within its native area in West Africa.

## 3.2 Development of clonal propagation methods

### 3.2.1 Root cuttings of *Detarium microcarpum* (l)

Naturally regenerated mature trees of *D. microcarpum* were selected from the Nazinon forest, a tree and shrub savanna woodland located ca. 100 km south of Ouagadougou in Burkina Faso, and used as donors. Lateral roots were excavated, and a 1-1.5 m long section was removed from each tree. Root fragments were cut to the desired size. The distal end (toward the root tip) of each root segment was cut obliquely in order to differentiate it from the proximal end. The cuttings were treated with fungicide and planted in a sterilized mixture of soil, sand and cattle manure (1:1:1, v/v/v) in plastic containers, which were placed in a greenhouse at a humidity of 70-100% and a temperature of 22-37°C at the Department of Forest Production of the Environment and Agricultural Research Institute (INERA/DPF) in Ouagadougou. Cuttings were watered manually every second day.

Four series of experiments were performed to identify factors that influence the sprouting ability of root segments; the experiments were based on completely randomized designs with 10 replicates and three cuttings per experimental unit. In the first experiment, the effects of root segment length (5 and 10 cm) and diameter (11-20 mm and 21-40 mm) combined with propagation environment (inside a greenhouse with high humidity of 70-100% and temperatures in the range 22-37°C or outdoors in the shadow of a tree, where the humidity was low. i.e. 25-70%, and temperatures in the range 22-40°C) were tested. Cuttings were buried horizontally 1 cm below the surface of the growing medium. In the second experiment, the effects of root segment length were further tested, using 10 cm and 20 cm lengths, all with a diameter of 20-40 mm, in combination with vertical insertion modes (exposed versus buried). For the exposed insertion, the proximal ends of the segments were kept 2 cm above the surface of the medium while in the buried mode, the proximal ends were kept 1 cm below the surface of the growing medium. In the third experiment, we examined whether regeneration from root segments is dependent on distance from the root

collar of the mother tree: 0, 60 and 120 cm away. The root segments were buried vertically, with the proximal end 1 cm below the surface of the medium. Finally, we examined the effect of alignment of root segments (vertical versus horizontal) in combination with cutting length (10 cm and 20 cm). The root segments were buried 1 cm below the surface of the growing medium in plastic boxes (75 × 15 × 12 cm) for the horizontal alignment and in perforated black polythene bags (27 cm diameter × 40 cm height) for the vertical alignment treatments. A total of 21 sprouted root segments, 15 derived from 20 cm root segments and six from 10 cm root segments were planted and examined for root formation seven months after planting.

At the end of each experiment, all root segments were removed from the growing medium, washed and the number of sprouts taller than 0.5 cm and the number of new roots were recorded per cutting, the length of the longest sprout was also measured. The origins of the sprouts on each root segment (whether in the proximal, central or distal region of the segment) were also recorded. The sprouting efficiency was calculated as the percentage of sprouted cuttings from the total number of root segments planted in each experimental unit. For rootling establishment, the number of sprouts, the length and the basal diameter of the longest sprouts, were recorded and the biomass of the stems, leaves and roots were determined. The dry biomass of the stems, leaves and roots was determined after oven drying at 70 °C for 48 hours. The total biomass of the rootling was calculated by summing the stem, root and leaf biomass.

### 3.2.2 Stem cuttings of *Khaya senegalensis* (II)

Cuttings were collected from four stockplant types: 3-8 month-old seedlings, 5- and 15-year old planted trees and rejuvenated branches of pollarded old trees. Seedlings were raised from seeds purchased from the National Forest Seed Centre (CNSF) of Burkina Faso. The two mature stockplant donors (5- and 15-years old) were both from roadside plantation in Ouagadougou on “Avenue Charles de Gaulle” and “Avenue de la Jeunesse”, respectively. The rejuvenated stockplants were roadside trees planted about 100 years ago on “Rue Nongremason” in Ouagadougou and pollarded five or six months before cutting collection. Leafy shoots were harvested from the donors. The leaves were trimmed so that only two remained and these were cut to a length of 2-3 cm. Cuttings were 10 cm in length, unless otherwise stated. They were soaked in a fungicide solution for

10 min before planting in a rooting medium comprising a sterile sand and perlite (1:1 v/v) mixture, in plastic trays covered with transparent plastic sheets and kept under intermittent mist in a greenhouse at INERA/DPF. The experiments were run for eight weeks.

To test the effect of cutting length on rooting ability, leafy cuttings were collected from 5-month old seedlings and randomly allocated to each of four cutting lengths: 5, 10, 15 and 20 cm in a completely randomized design with five replications and six cuttings per replication. To examine the effect of leaf area on the rooting ability of cuttings, cuttings were collected from one-year old hedged seedlings and randomly allocated to each of four leaf area treatments, 0 cm<sup>2</sup> (leafless), 6-8 cm<sup>2</sup> (one leaf with one pair of cut leaflets), 12-16 cm<sup>2</sup> (two leaves with two pairs of cut leaflets), 22-28 cm<sup>2</sup> (two leaves with four pairs of cut leaflets), in a completely randomized design with five replications and six cuttings per replication. To investigate the effects of donor plant maturation and the application of Indole-3-butyric acid (IBA), a full factorial experiment with a split plot design involving 16 treatments was employed: stockplants (seedling shoots, resprouts of pollarded trees, 5-year and 15-year old tree crown sprouts) combined with IBA at four concentrations (0, 2500, 5000, 10000 ppm). The four stockplant types were randomly assigned to the main plots and the four IBA concentrations to the subplots. IBA was applied to the basal ends of cuttings for 5 seconds. Each treatment had seven replicates with six cuttings per replication.

To improve further the rooting ability of cuttings from resprouts of pollarded trees, a follow-up experiment was conducted using naphthalene acetic acid (NAA) alone or in combination with IBA. Cuttings were collected from two types of stockplants (4-month old seedling shoots and 6-month resprouts from pollarded trees) and two auxin treatments (NAA and NAA+IBA) at each of four concentrations (1000, 2000, 3000, 4000 ppm), arranged in a split-split plot design with five replications and six cuttings per replication. Stockplant donors were randomly assigned as the main plot factors; auxin treatments as sub-plot factors; and the four different concentrations as sub-sub-plot factors.

To examine the potential of smoke as an alternative to commercial auxins, the application of smoke solution was tested in two experiments. In the first experiment, the basal ends of cuttings collected from 5-month old seedlings were immersed in 5% or 10% smoke solution for 30, 60, 120 or 180 min; the results were compared with a water control in a split plot design. Smoke concentrations were tested at the main plot level while the



four immersion times were tested at the subplot level. Each treatment was replicated five times with six cuttings per replication. In the second experiment, 0, 20, 40, 60, 80 and 100% smoke solutions were tested in a completely randomized design. Five replicates of six cuttings each, collected from 8-month old single-shoot seedlings, were randomly assigned to each treatment. The bases of the cuttings were immersed in the smoke solutions for 60 min.

At the end of each experiment, the number of roots measuring at least 1 mm long was determined for each cutting, the length of the longest root measured and the secondary roots originating from the longest root counted. The percentage of rooted cuttings was determined as the proportion of the rooted cuttings from the total cuttings in each experimental unit.



Clonal propagation trays for stem cuttings of *Khaya senegalensis* placed on top of the staging and root cuttings of *Detarium microcarpum* on the middle shelf of the staging, inside a greenhouse at INERA/DPF (C. Ky-Dembele).

### 3.3 Comparison of sexual and clonal plantlets

#### 3.3.1 True seedlings, seedling sprouts and root suckers of *Detarium microcarpum* (III)

To examine differences in morphological traits and carbohydrate contents of clonal and sexual plantlets of *D. microcarpum*, 93 naturally regenerated plantlets (Fig. 3) were sampled from the Nazinon forest and categorized into two size classes corresponding to the two first development stages distinguished from previous studies related natural regeneration strategies of *D. microcarpum* in Burkina Faso (Bationo *et al.*, 2001). Class 1 was composed of individuals up to 50 cm tall while Class 2 consisted of individuals measuring 51 to 120 cm tall. The numbers of root suckers and seedling sprouts were 22 and 23, respectively in Class 1, and 26 and 22, respectively in Class 2. True seedlings (14 individuals) were raised in a greenhouse for 30 days.

From each plantlet, two leaves out of four along the stem were collected and placed under slight pressure for morphological data measurement. The rest of the leaves were collected and oven dried at 75°C for 3 days along with a portion of the main root collected from beneath the root collar, to be used for carbohydrate analysis. Morphological characters of each plantlet relating to canopy coverage, stem shape, root and leaf dimensions were measured. A total of 4272 leaflets from 573 leaves were measured. A total of 52 plantlets (19 root suckers, 19 seedling sprouts and 14 true seedlings) were selected for carbohydrate analyses. Leaves and roots were ground before taking samples for analysis. Soluble sugars (glucose, fructose and sucrose) were extracted with ethanol and starch was obtained after enzymatic digestion to a glucose equivalent. The concentrations of soluble sugars and starch were determined enzymatically using a Beckman DU 600 spectrophotometer. The sum of soluble sugars is, hereafter, referred to as total soluble sugars (TSS) and the sum of the soluble sugars and starch as total non-structural carbohydrates (TNC).

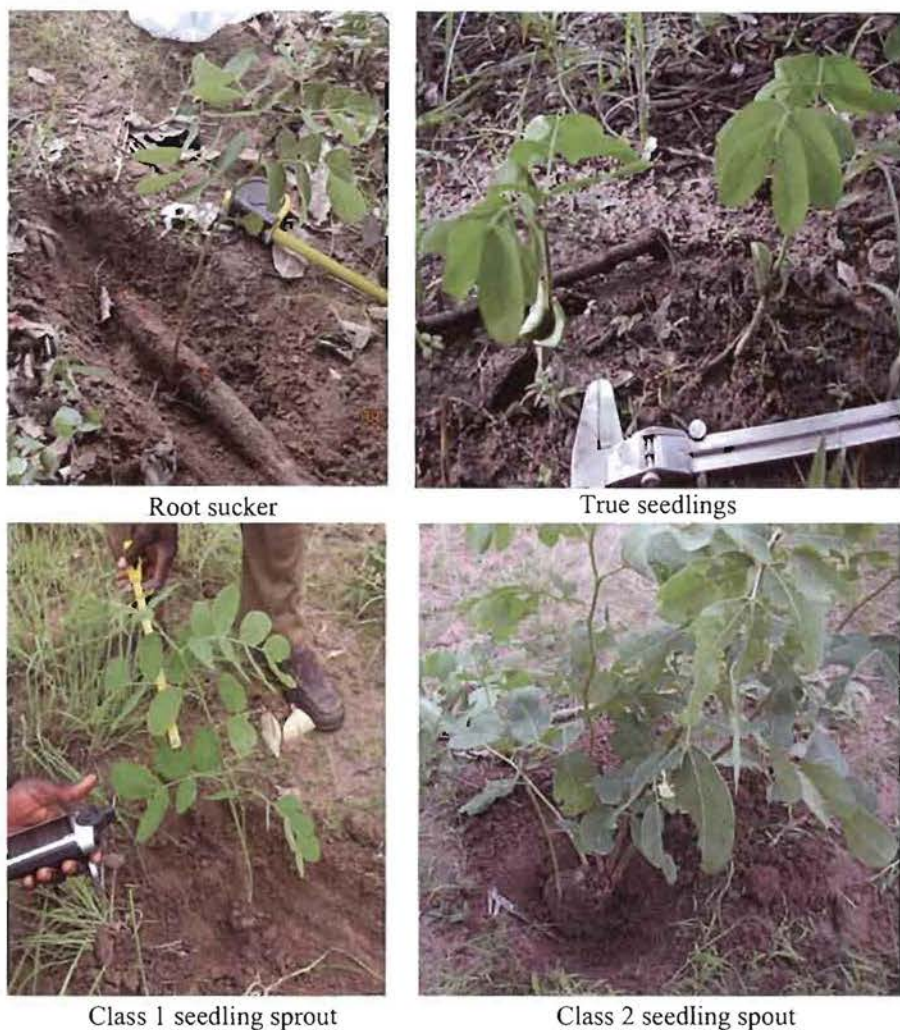


Figure 3. Illustrations of plantlet types of *Detarium microcarpum*: Class 1 (height = 50 cm), Class 2 (height > 50 cm) (C. Ky-Dembele).

### 3.3.2 Seedlings and stecklings of *Khaya senegalensis* (IV)

To determine the effects of irrigation regimes on the growth, biomass allocation and foliar carbon isotope ratio ( $^{13}\text{C}$ ) of seedlings and stecklings of *K. senegalensis*, an experiment was performed outdoors at INERA/DPF in Ouagadougou. Seedlings and stecklings originated from a common seed source purchased from CNSF in Burkina Faso. Rooted cuttings were obtained from 3-month old seedlings and grown on for four months. The stecklings (54) along with 8-month old seedlings (54) were replanted into

perforated 6-L plastic buckets, placed in full sun and grown on for 12 weeks. Six individuals of each propagule type were randomly selected for an initial harvest and the data were used to achieve the irrigation treatments and to assess growth rate from initial to final harvest. The remaining 48 seedlings and 48 stecklings were used in a completely randomized block design experiment with two factors, propagule type (seedlings and stecklings) and irrigation regime (25, 50, 75 and 100% field capacity), with four blocks and three plants per experimental unit. Field capacity was estimated by measuring the amount of water held in the soil of 12 control pots which had been fully wetted, covered and weighed after two days of drainage. The pots were weighed every 72 hours and watered according to the appropriate irrigation regime by supplementing the soil water content with a percentage (25, 50, 75 or 100) of the field capacity adjusted for the plant biomass estimated from regressions established on the basis of the initial harvest.

At both harvests, initial and final, the stem length and basal diameter of all plants were recorded. Harvested plants were separated into leaves, stems and roots. The total area of fresh leaves was measured. The biomass of the stems, leaves and roots was determined after drying at 70°C for 48 hours. The relative growth rate (RGR) from initial to final harvest was calculated according to Hunt (1982):  $RGR_A = (\ln A_f - \ln A_i) / (t_f - t_i)$ . Where  $A_f$  denotes the measured trait at final ( $f$ ) harvest and  $A_i$  denotes it at the initial ( $i$ ) harvest calculated as the mean of the six plants per propagule type for the destructive variables; ( $t$ ) is the time in weeks at final ( $f$ ) and initial ( $i$ ) harvest. Leaf area productivity, specific leaf area, leaf area ratio, leaf biomass ratio, stem biomass ratio, root biomass ratio, and root to stem ratio were calculated using data collected at the final harvest and taken as additional variables to the RGR. Samples of seedlings and stecklings subjected to 50 and 100 % field capacity watering regimes were analyzed to determine the carbon isotope ratios in the leaves using a mass spectrometer in the Radio Carbon Dating Laboratory at the University of Helsinki, Finland and carbohydrate concentrations (glucose, fructose, sucrose and starch) in the roots, at Eurofins Food and Agro Sweden in Lidköping. For carbohydrate analysis, the root samples were pooled in two groups, (blocks 1 + 2) and (blocks 3 + 4) to obtain the minimum amount required of the sample.

### 3.4 Data analysis

For all studies, data were checked for normality and when it was possible, Johnson-transformation was done for variables that did not fulfill the requirement of normal distribution. The equal variance requirement was observed from the residual plots obtained from Minitab. In study I, the two-sample T-Test procedure in Minitab 15 (Minitab Inc., State College, PA, USA) was used for data relating to rootling establishment and GLM procedure of Statistical Analysis System (SAS Institute Inc., 2002-2008) was performed for the other variables. In study II, because of the great number of null values in the percentage of rooted cuttings, transformation was not successful for normal distribution requirement. The GLM (SAS) procedure was used for completely randomised and split-plot design experiments while the Mixed (SAS) procedure was used for the split-split-plot design. Means that exhibited significant differences ( $p < 0.05$ ) were further compared using Tukey's multiple comparison test. In both study I and II means that exhibited significant differences ( $p < 0.05$ ) were further compared using Tukey's HSD multiple comparison test.

In study III, the GLM procedure and two-sample T-Test were performed to determine differences among plantlet origins with Minitab 14 (Copyright: 1972 - 2003 Minitab Inc.). Significant differences, when  $p < 0.05$ , were further tested using Bonferroni's test. Linear discriminant analysis was performed to classify plantlets according to their origin using Minitab for single variable and the software R (R Development Core Team 2006) for multiple variables. In study IV, two way-analysis of variance (ANOVA) was performed in order to compare propagule types (seedlings and stecklings), irrigation regimes (25, 50, 75 and 100% field capacity) and the interactions between these two factors using GLM (SAS) procedure. Significant differences, when  $p < 0.05$ , were further tested using Tukey's HSD multiple comparison test.

## 4 Results and discussion

### 4.1 Factors affecting the propagation of *Detarium microcarpum* from root cuttings

The results obtained from the series of experiments clearly demonstrate that *D. microcarpum* can be regenerated from root segments collected from mature field-grown trees. The segments exhibited a relatively good capacity to produce new shoots and roots; success was mainly affected by the diameter ( $p < 0.05$ ) and the length ( $p < 0.000$ ) of root segments (Table 1). Sprouting in *D. microcarpum* was possible from 10 and 20 cm long root segments with a diameter of 15-60 mm, while 5 cm long cuttings were unsuitable due to their poor sprouting ability. Rootling assessment indicated that sprouted root segments of both 10 cm and 20 cm were able to produce new roots from the initial root segments. However, rootlings derived from 20 cm root segments produced a greater biomass of new roots ( $0.62 \pm 0.08$  g) than those from 10 cm root segments ( $0.34 \pm 0.09$  g). This is in agreement with a number of previous studies, in which a similar range of lengths or diameters has resulted in successful sprouting of *Faidherbia albida* (Harivel *et al.*, 2006), *Spathodea campanulata* (Meunier *et al.*, 2008), *Maeria crassifolia* (Houmey *et al.*, 2007), *Prunus avium* (Ghani & Cahalan, 1991) and *Malus domestica* (Robinson & Schwabe, 1977a).

It is also well known that root thickness has a clear effect on survival, shoot production and vigor when propagating woody species from root segments. This may be related to greater assimilate reserves available for regeneration (Robinson & Schwabe, 1977a; Lawes & Sim, 1980). In particular, carbohydrates have been considered to be key determinants of good shoot regeneration from root segments (Lawes & Sim, 1980). Very thin root segments may lack sufficient nutritional reserves for bud and shoot

growth (Eliasson, 1971a; Robinson & Schwabe, 1977b; Stenvall *et al.*, 2009). On the other hand, thick roots may regenerate slowly because the tissue may be too mature and inactive (Stenvall *et al.*, 2006). Thus, there is an optimum diameter that results in successful regeneration of root segments; in our case 21-60 mm seems promising.

Table 1. *Effects of environment, root segment length and diameter on sprouting efficiency, the number of sprouts, and the diameter and length of the longest sprouts per sprouted root segment of Detarium microcarpum.*

Factors		Sprouting (%)	No. sprouts	Diameter (mm)	length (cm)
Environment	Greenhouse	16±4a	1.8±0.2a	2.5±0.2a	7.44±1.56a
	Outdoor	11±4a	1.6±0.4a	3.6±0.3b	10.56±2.13a
Cutting length	5 cm	1±1a	1.0±0.0a	2.1±0.0a	7.00±- a
	10 cm	26±5b	1.8±0.2a	2.9±0.2a	8.50±1.34a
Cutting diameter	11-20 mm	8±3a	1.3±0.2a	2.5±0.3a	7.29±1.59a
	21-40 mm	19±5b	2.0±0.2a	3.0±0.2b	8.94±1.73a

Values (Mean ± SE) followed by the same letter for a given factor are not significantly different at the 5% level according to Turkey's multiple comparison test.

The slower regeneration of new roots compared to shoot regeneration is in accordance with all previous studies consulted, in which rooting time is often longer than sprouting time (Hartmann *et al.*, 2002; Stenvall *et al.*, 2005). As suggested by these authors, this feature may indicate that the sprouting process promotes initiation of adventitious rooting because the carbohydrate supply from the leaves might be able to support root elongation (Eliasson, 1968). Sprouting efficiency did not vary significantly between different distances from the root collar, even though cuttings taken from near the root collar of the mother tree exhibited the best sprouting efficiency (40%) compared to the middle part, 60 cm (27%) and the distal part, 120 cm (20%) away from the root collar.

Moreover, root segments of *D. microcarpum* showed strong polarity, with most of the shoots developing toward the proximal ends. This was expected because of hormonal control, a mechanism which interacts with carbohydrate supply for bud initiation and subsequent growth from root segments of woody plant species (Eliasson, 1971b; Schier & Campbell, 1976; Robinson & Schwabe, 1977a; Ede *et al.*, 1997). According to these authors, the polarity is due to the transport of auxin, a shoot suppression hormone that is acropetal in roots, away from the proximal end toward the root tip. In attached roots, auxin from the aboveground part of the tree

would normally prevent bud initiation, but when this supply ceases upon detachment of the root, depletion of auxin will allow preferential bud initiation to occur at the proximal end.

## 4.2 Factors affecting the propagation of *Khaya senegalensis* by stem cuttings

The most critical factor affecting vegetative propagation of *K. senegalensis* by stem cuttings was found to be the age of the stockplant ( $p < 0.0001$ ). Cuttings taken from 3- and 5-month old seedlings rooted well and produced more roots than cuttings obtained from older trees (Fig. 4). The rooting ability of cuttings from older donors was improved by crown pollarding and auxin application (16%) compared to cuttings from unpruned 5-year old trees (5%). Cuttings from 15-year old stockplants did not root at all.

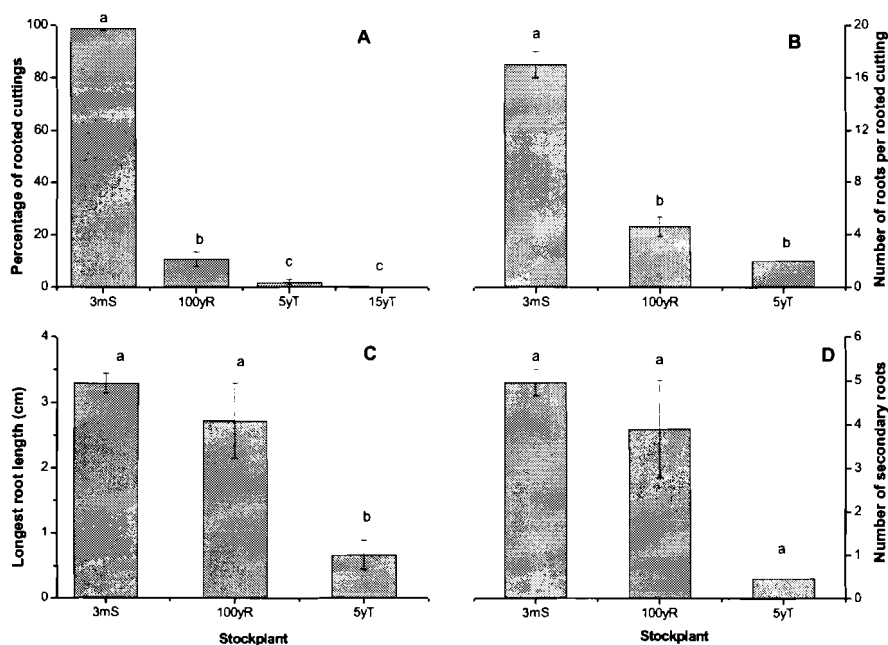


Figure 4. Main effect of stockplant donor type on the percentage of rooted cuttings (A), the number of roots per rooted cutting (B), the length of the longest root (C), and the number of secondary roots (D) of *Khaya senegalensis*. 3mS, 3-month-old seedling shoots; 100yR, respouts of 100-year-old pollarded trees; 5yT, crown sprouts of 5-year-old trees; 15yT, crown sprouts of 15-year-old trees. Bars represent standard errors of means. Means followed by the same letter(s) are not significantly different at the 5% level according to Tukey's multiple comparison test.



These results are consistent with many previous studies, which have shown that cuttings derived from juvenile stockplants are easier to root than those derived from mature stockplants (Browne *et al.*, 1997; Berhe & Negash, 1998; Bhardwaj & Mishra, 2005; Amri *et al.*, 2010) and that shoots originating from juvenile zones of a mature tree exhibit juvenile characteristics (Bonga & von Aderkas, 1993; Hartmann *et al.*, 2002; Bhardwaj & Mishra, 2005; Amri *et al.*, 2010). The superior rooting ability of cuttings from seedlings over those from trees has been attributed to the effect of changes in the woody plant developmental process that occur with increasing age; these are known as maturation or ontogenetic aging. Serial grafting or rooting of cuttings, annual hedging, crown-pruning and *in vitro* serial subcultures have been used to reduce the effects of aging (Greenwood & Hutchison, 1993; Hartmann *et al.*, 2002). The rooting ability of juvenile cuttings may be ascribed to optimum levels of sugars, the total carbohydrate content and low nitrogen levels (Bhardwaj & Mishra, 2005), while the reduction in rooting potential of cuttings from the stem of mature donors might be due to a decrease in the content of endogenous auxins or an accumulation of inhibitory substances (Hartmann *et al.*, 2002).

Depending on the maturation of the stockplant from which cuttings had been taken, three major effects of auxin on the rooting ability of cuttings were noted: effects on root formation ( $p < 0.05$ ), the number of roots per rooted cutting ( $p < 0.01$ ) and root length ( $p < 0.001$ ). The effectiveness of applied auxin in inducing rooting and in increasing the total number of roots increased with stockplant maturation. For cuttings derived from seedlings, auxin application did not influence root induction; the most significant effect of auxin application was on the number of roots per rooted cutting. Overall root number increased by up to 216% in cuttings treated with 10000 ppm IBA compared to the control. For cuttings taken from resprouts of pollarded trees, the application of high doses of auxin increased root length and the number of secondary roots. Similar effects have been reported for African mahoganies and other African woody species (Badji *et al.*, 1991; Tchoundjeu & Leakey, 1996; Teklehaimanot *et al.*, 1996; Tchoundjeu *et al.*, 2002; Opuni-Frimpong *et al.*, 2008). In the present investigation there was no advantage of applying smoke solution compared to the control. However, lower doses of smoke solution (5-10%) were associated with more root induction and a greater number of roots than higher doses. Whether this was related to the age of the seedlings or to the smoke effect requires further investigation.

Cutting length did not significantly affect any of the traits evaluated in contrast to leaf area which significantly affected the percentage of rooted cuttings (Fig. 5). Successful rooting was restricted to leafy stem cuttings ( $p=0.004$ ) of *K. senegalensis*. This is a common response in tropical trees (Leakey, 2004). The inability of leafless cuttings to root has been associated with the rapid depletion of carbohydrates in stem tissues; in contrast, the concentrations in leafy cuttings tend to increase (Leakey *et al.*, 1982b). This suggests that rooting is dependent on carbohydrates formed and utilized after cuttings have been excised from the donor plant (Leakey & Coutts, 1989). The lack of any pronounced relationship between cutting length and rooting ability may be related to the large size of the cuttings used in the present study.

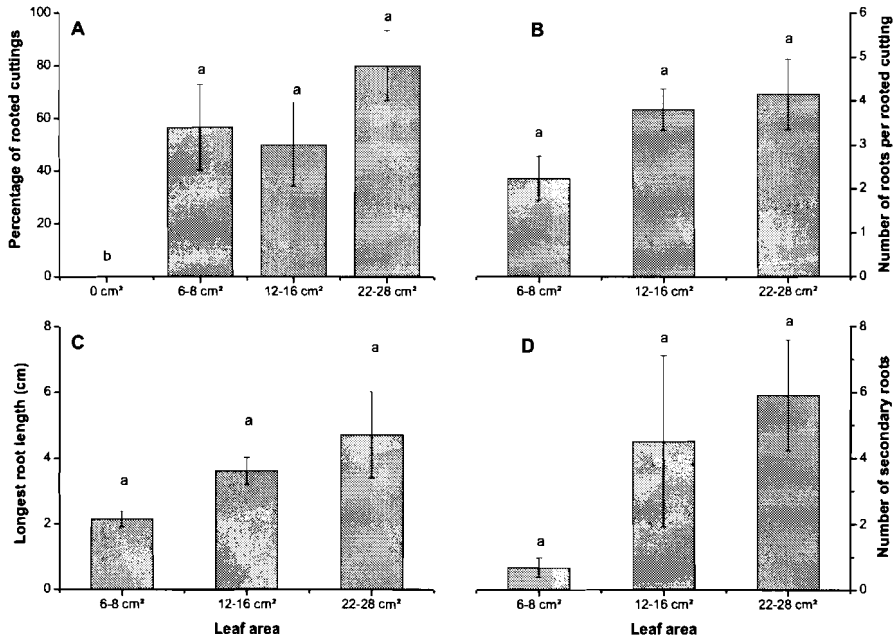


Figure 5. Effect of leaf area on the percentage of rooted cuttings (A), the number of roots per rooted cutting (B), the length of the longest root (C), and the number of secondary roots (D) of *Khaya senegalensis*. Bars represent standard errors of means. Means followed by the same letter(s) are not significantly different at the 5% level according to Tukey's multiple comparison test.

### 4.3 Comparison of true seedlings, seedling sprouts and root suckers of *Detarium microcarpum*

Root suckers had the highest values ( $p < 0.001$ ) for almost all traits relating to the stem, canopy and leaves for individuals shorter than 50 cm (Class 1), followed by seedling sprouts and then true seedlings. These results are in agreement with the opinion that root suckers grow faster than sexually reproduced seedlings (Silla *et al.*, 2002; Homma *et al.*, 2003). Similar results have been reported by Hoffmann (1998) and Kennard *et al.* (2002), who found that height, crown area, stem diameter or number of stems of root suckers were significantly greater than those of plantlets originating from seeds. Initial growth might be relatively more important for root suckers, so that Class 1 root suckers were very difficult to find during the fieldwork, as noted by Homma *et al.* (2003), who reported the rarity of intermediate sized suckers with a height growth ranging from 12 to 40 cm per year.

However for individuals taller than 50 cm (Class 2), root suckers exhibited higher values than seedling sprouts with respect only to stem length ( $p = 0.041$ ) and root diameter ( $p = 0.019$ ). The variables leaflet length and width exhibited significantly higher values ( $p < 0.000$ ) for the seedling sprouts in both Class 1 and Class 2. This might be an advantage for increased production of photosynthate resulting from increased leaf surface area, a feature that appears more important for the juvenile stage of sexually produced seedlings than clonal plants. The root size appeared to be important for differentiating classes of seedling sprouts in contrast to root suckers, for which the analysis revealed no difference between Classes 1 and 2. This variable was significantly higher for Class 2 in comparison with Class 1 individuals (4.1 cm and 1.9 cm mean diameter, respectively). This supports previous results highlighting the importance of root growth to ensure seedling survival and growth within disturbed biomes such as savannas (Cruz *et al.*, 2002; Luoga *et al.*, 2004). The sprouting abilities of plants have been linked to higher levels of resources, particularly starch, in plant tissues (Iwassa & Kubo, 1997; Bell & Ojeda, 1999). For instance Bell & Ojeda (1999) found that *Erica* seeder species had consistently lower amounts of root starch than resprouters. The present study also revealed high variability in starch and TNC concentrations among regeneration mechanisms of individuals of *D. microcarpum* growing under the same environmental conditions. For small individuals, starch and TNC concentrations in root samples of seedling sprouts were higher ( $p < 0.001$ ) than corresponding samples from root suckers and true seedlings (Table 2).

Table 2. Carbohydrate concentration ( $\text{mg g}^{-1}$  biomass) in leaf and root samples for individuals up to 50 cm tall (Class 1) and 51-120 cm tall (Class 2). For a given tissue, leaves or root, values (Mean  $\pm$  SE) followed by the same letter within a column are not significantly different at the 5% level using Bonferroni's test for class 1 and the 2-sample *t*-test for class 2.

Tissue	Origin	Glucose	Fructose	Sucrose	TSS	Starch	TNC
A) Class 1							
Leaves	Root sucker	18.5 $\pm$ 3.3a	21.8 $\pm$ 3.4a	32.0 $\pm$ 3.8a	72.3 $\pm$ 9.9a	2.3 $\pm$ 0.4c	74.6 $\pm$ 9.8c
	Seedling sprout	26.8 $\pm$ 5.7a	31.5 $\pm$ 6.0a	41.6 $\pm$ 7.0a	99.9 $\pm$ 18.6a	1.3 $\pm$ 0.3c	101.2 $\pm$ 18.7c
	True seedling	11.9 $\pm$ 2.3a	13.3 $\pm$ 2.4a	44.4 $\pm$ 3.8a	69.5 $\pm$ 7.5a	18.2 $\pm$ 4.8c	87.7 $\pm$ 8.7c
Root	Root sucker	19.0 $\pm$ 6.0a	19.9 $\pm$ 6.6a	47.4 $\pm$ 12.5a	86.3 $\pm$ 22.1a	85.0 $\pm$ 10.8b	171.3 $\pm$ 24.7b
	Seedling sprout	14.1 $\pm$ 4.9a	17.9 $\pm$ 5.5a	39.0 $\pm$ 8.7a	71.0 $\pm$ 14.8a	224.7 $\pm$ 36.2a	295.7 $\pm$ 27.6a
	True seedling	17.1 $\pm$ 2.1a	21.1 $\pm$ 2.4a	65.3 $\pm$ 5.8a	103.4 $\pm$ 8.1a	22.3 $\pm$ 5.2c	125.7 $\pm$ 11.7bc
B) Class 2							
Leaves	Root sucker	13.5 $\pm$ 3.4a	16.8 $\pm$ 4.0a	29.5 $\pm$ 4.3a	59.8 $\pm$ 10.7a	2.7 $\pm$ 1.3a	62.4 $\pm$ 10.8a
	Seedling sprout	12.1 $\pm$ 2.3a	15.3 $\pm$ 2.8a	25.3 $\pm$ 3.5a	52.7 $\pm$ 8.1a	1.8 $\pm$ 0.4a	54.5 $\pm$ 8.3a
Root	Root sucker	08.9 $\pm$ 2.1b	12.5 $\pm$ 2.8b	39.8 $\pm$ 11.1a	61.1 $\pm$ 13.6b	126.9 $\pm$ 30.7a	188.0 $\pm$ 28.0a
	Seedling sprout	29.9 $\pm$ 6.5a	32.3 $\pm$ 7.2a	82.7 $\pm$ 21.0a	145.0 $\pm$ 29.5a	144.1 $\pm$ 31.3a	289.1 $\pm$ 43.3a

SE: standard error; TSS: total soluble sugars; TNC: total non-structural carbohydrates.

However, within Class 2, glucose ( $p=0.007$ ), fructose ( $p=0.021$ ) and TSS ( $p=0.020$ ) concentrations were higher in root samples of seedling sprouts than in those of root suckers (Table 2). Starch conversion into sugars might explain the high level of soluble sugars in roots of Class 2-seedling sprouts. For Class 1 individuals, the depletion of starch could be limited as a result of shoot die back during the dry season, in contrast to Class 2 plants, in which shoot die back may be less pronounced. Larger individuals, which are more resistant with thicker bark preventing disturbance damage (Wilson & Witkowski, 2003), may also need more resources for maintaining live shoots. This is in accordance with Kozłowski (1992), who reported that starch-sugar conversions are common in woody plants and that starch is transformed to sugars whenever sugar levels are low, and Latt *et al.* (2000) who noted that plants which were repeatedly cut maintain a high proportion of carbohydrate reserves as readily transportable and usable sugars. It is also known that allocation of resources to belowground stores reduces growth by decreasing construction of resource gaining organs (leaves and root tips), thereby reducing the potential for further growth (McPherson & Williams, 1998).

The success of differentiating seedling sprouts from root suckers using morphological characters and carbohydrate concentrations in leaves and roots was limited (Fig. 6). Except for true seedlings, none of the morphological variables resulted in more than 63% accuracy in distinguishing seedling sprouts from root suckers. This highlights the difficulty of distinguishing between plants derived from root suckers and those of seed origin using morphological observations, as also reported by Bellefontaine *et al.* (1997) and Sawadogo *et al.* (2002). Out of 11 characters evaluated, only stem length, internode number and root diameter discriminated the regeneration mechanisms, having 70%, 72% and 71% accuracies, respectively. Of the leaf characters examined, the most important variable was rachis length, which correctly classified 65% of Class 1 seedlings into three groups of plantlets. Among Class 2 individuals, 52% was correctly classified using leaflet length. The classification accuracy varied between 30% and 73% for seedling sprouts, indicating high variability in growth behavior of seedling sprouts. Carbohydrate concentrations in roots seemed more important for classifying plantlets according to their origin, and the maximum classification accuracy was about 80%. The variables that individually provided the best discrimination accuracy between plantlet groups were starch (82%) for Class 1 individuals and glucose (83%) for Class 2 individuals.

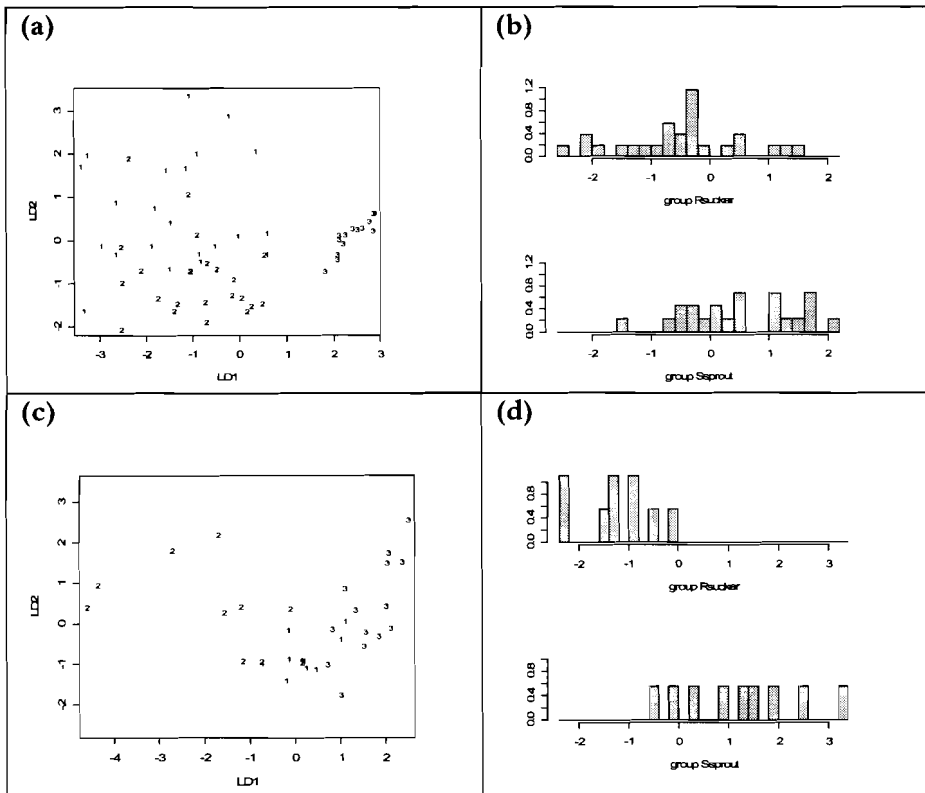


Figure 6. Linear discriminant analysis. The classification of root sucker (1, Rsucker), seedling sprout (2, Ssprout) and true seedling (3) based on morphological characters (a,b; height, stem length, stem diameter and canopy widths) and carbohydrate contents (c,d; glucose, fructose, sucrose and starch from root) for plants up to 50 cm tall, Class 1 (a,c) and plants of 51-120cm tall, Class 2 (b,d) from the Nazinon forest, Burkina Faso.

The resemblance in morphological characters between seedling sprouts and root suckers, especially for Class 2 individuals, could be explained by a relative similarity in their growth performance. The rate of growth may not differ much between seedling sprouts and root suckers because of their well-established root system and the high carbohydrate reserves. It has been reported that a vigorous resprouting response would be favored by a greater allocation to storage in the root (Cruz *et al.*, 2002) since a larger root system would offer more surface area for water and nutrient uptake (Kennard *et al.*, 2002). While seedling sprouts and root suckers can draw up reserves using preexisting root systems, true seedlings must produce both above and belowground tissues, thus slowing their growth. Moreover, non destructive methods such as molecular markers might be useful for segregating seedling sprouts from root suckers, because they can measure the genomic response

to adaptation or selection in a given environment (Hoffmann & Willi, 2008; Srivastava & Mishra, 2009). The presence of different alleles due to any segregation at the genetic markers could be indicative of the difference between root suckers and seedling sprouts.

#### 4.4 Comparison of seedlings and stecklings of *Khaya senegalensis*

The overall results showed large and significant differences between plants grown under different irrigation regimes, but only small differences between seedlings and stecklings of *K. senegalensis*. The two types of propagule originated from two different modes of propagation, sexual from seeds and asexual from rooted cuttings.

Except for the relative growth rate (RGR) of the stem basal diameter ( $p=0.016$ ), the specific leaf area ( $p=0.005$ ) and total non-structural carbohydrate (TNC) contents in roots ( $p=0.047$ ), plant responses related to growth, biomass production, biomass fractions,  $^{13}\text{C}$ , soluble sugars, and starch contents did not differ significantly between seedlings and stecklings. Seedlings had higher stem basal diameter RGR, a greater specific leaf area, and a greater TNC (Table 3) than stecklings. The comparable mean RGRs between stecklings and seedlings indicate that these two types of propagule exhibit a similar growth pattern during the early growth phase.

Table 3. The effects of *Khaya senegalensis* propagule type (seedling and steckling) and irrigation regime (50 and 100% field capacity) on carbohydrate concentration in roots ( $\text{mg g}^{-1}$  biomass) in Ouagadougou, Burkina Faso. Values (Mean  $\pm$  SE) followed by the same letter for a given factor are not significantly different at the 5% level according to Turkey's multiple comparison test.

Factors		Glucose	Fructose	Sucrose	TSS	Starch	TNC
Propagule	Seedling	2.0 $\pm$ 0.7 <sup>a</sup>	3.0 $\pm$ 1.0 <sup>a</sup>	35.8 $\pm$ 9.4 <sup>a</sup>	40.7 $\pm$ 10.4 <sup>a</sup>	125.0 $\pm$ 14.1 <sup>a</sup>	165.7 $\pm$ 14.3 <sup>a</sup>
	Steckling	1.4 $\pm$ 0.5 <sup>a</sup>	2.1 $\pm$ 0.7 <sup>a</sup>	31.0 $\pm$ 5.8 <sup>a</sup>	34.4 $\pm$ 6.8 <sup>a</sup>	85.8 $\pm$ 26.8 <sup>a</sup>	120.2 $\pm$ 33.4 <sup>b</sup>
Irrigation	50%	0.8 $\pm$ 0.1 <sup>b</sup>	1.2 $\pm$ 0.2 <sup>b</sup>	21.8 $\pm$ 1.1 <sup>b</sup>	23.7 $\pm$ 1.0 <sup>b</sup>	82.0 $\pm$ 25.0 <sup>a</sup>	105.7 $\pm$ 25.8 <sup>b</sup>
	100%	2.6 $\pm$ 0.5 <sup>a</sup>	3.8 $\pm$ 0.6 <sup>a</sup>	45.0 $\pm$ 5.8 <sup>a</sup>	51.4 $\pm$ 5.7 <sup>a</sup>	128.8 $\pm$ 13.6 <sup>a</sup>	180.1 $\pm$ 09.0 <sup>a</sup>

TSS: total soluble sugars; TNC: total non-structural carbohydrates.

Differences between seedlings and stecklings are diverse; there are differences between tree species, and sometimes within the same species or from nursery to field plantations (Frampton Jr & Foster, 1993; Russell, 1993; Hennon *et al.*, 2009). While some studies, usually of field plantations, have shown that seedlings grow faster, others have reported growth equal to or slower than that of stecklings. Our findings are consistent with the results

obtained frequently for *Pinus radiata* (Fielding, 1970; Talbert *et al.*, 1993), *Chamaecyparis nootkatensis* (Karlsson & Russell, 1990) and *Pinus taeda* (Frampton *et al.*, 2000). It has been reported that, generally, growth of stecklings of radiata pine is similar to that of seedlings when cuttings are taken from juvenile sources (Fielding, 1970; Talbert *et al.*, 1993). This contrasts with the results obtained with *Faidherbia albida* (Ouédraogo, 1993), *Olea europaea* (Negash, 2003) and *Fraxinus angustifolia* (Cicek *et al.*, 2006), where stecklings have been found to exhibit better growth than seedlings. However, according to these studies, more variations could be expected within clones or between stecklings of differing origins than in seedlings, because the growth of stecklings is influenced by their genetic potential, the maturity of the donor plant, the morphology of the regenerated root system, the vigor of the propagules and the elapsed time after planting.

The higher specific leaf area of seedlings compared to stecklings may have been due to the reduction in the leaf area and density of stecklings as shown in leaf area ratio and leaf area productivity. The higher RGR of stem diameter for seedlings might be due to a growth variation between seedling stems derived from hypocotyls and the shoots of the rooted stem cuttings, because, in some species such as *Colophospermum mopane* (Johnson *et al.*, 1996), hypocotyl tissues are able to adjust their osmotic potential in response to varying external water potentials. This feature may not be maintained for a prolonged growth period. Since the root is known as the main storage organ of TNC for savanna tree species (Bond & Midgley, 2001; Hoffmann *et al.*, 2004), a greater content of TNC in seedlings may provide larger reserves for seedlings than stecklings and consequently improve their survival because a vigorous resprouting response would be favored by a greater allocation to storage (Cruz *et al.*, 2002; Myers & Kitajima, 2007).

In contrast to propagule type, water stress had significant effects on plant growth during the ten-week period of the experiment. Significant differences were detected between the well watered (75 and 100% field capacity) plants and those with a limited water supply (25 and 50% field capacity) in terms of their relative growth rate, biomass allocation, soluble sugars, and TNC. The response of the two propagule types to water stress was a decline in growth and biomass production (Fig. 7), a decrease in carbohydrate contents (Table 3) and an increase in the stem and root biomass fraction and carbon isotope ratio ( $^{13}\text{C}$ ). Similar results have been reported in several previous studies (Roupsard *et al.*, 1998; Gindaba *et al.*, 2005; Karacic & Weih, 2006; Regier *et al.*, 2009; Sanon, 2009; Niinemets, 2010; Yang & Miao, 2010).



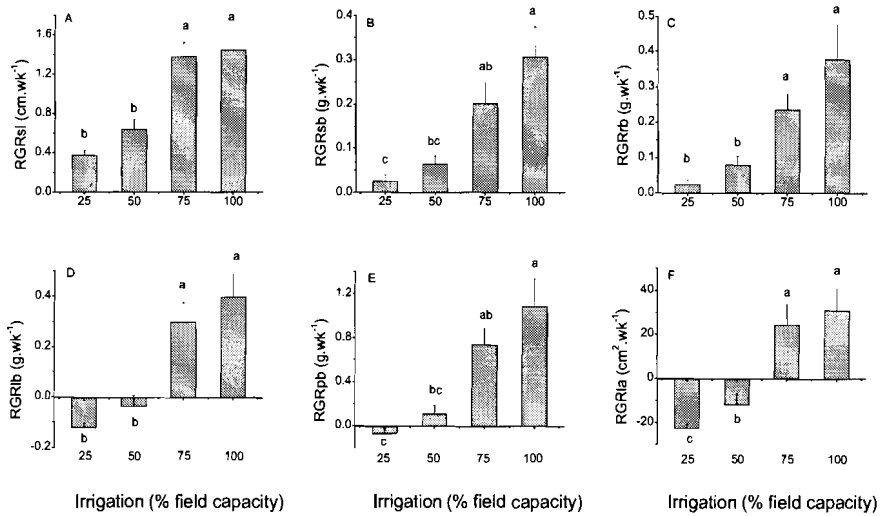


Figure 7. The effect of irrigation regimes (25, 50, 75 and 100% field capacity) on the relative growth rate (RGR) of stem length (A), stem biomass (B), root biomass (C), leaf biomass (D), plant biomass (E) and leaf area (F) of seedlings and stecklings of *Khaya senegalensis* in Ouagadougou, Burkina Faso. Bars represent standard errors of means. Different letter(s) indicate significant differences at the 5% level according to Tukey's multiple comparison test.

It is well established that plants respond to a reduced water supply by structural or physiological acclimation or both. When severely water stressed, plants minimize water loss by reducing their total leaf area, shedding the lower leaves and reducing the formation of new leaves. Consequently, this reduction in leaf area diminishes the total photosynthetic output which in turn results in decreased growth; usually this is consistent, as in our study, with a positive correlation between plant biomass and leaf area (Farquhar *et al.*, 1989; Chapin III, 1991; Hall *et al.*, 1994). Water limitation has different effects on carbohydrate contents in tree species but it is recognized that, in general, the concentrations of non-structural carbohydrates in young plants decrease in stressed conditions (Regier *et al.*, 2009; Niinemets, 2010). This is because, as with most stress factors, water limitation results in reduction in plant assimilation rates, thus reducing the newly assimilated carbon pool in leaves and further translocation to growing and storage organs (Niinemets, 2010) and activating utilization of carbohydrate reserves (Kozłowski, 1992; Sudachkova *et al.*, 2009). In addition, an increase in root biomass ratio could be a better strategy for maintaining growth under water-limited conditions, as this can increase water and nutrient absorption, returning carbon and nutrient contents to more favorable levels for storage in order to support rapid growth when

conditions do become favorable (Chapin III *et al.*, 1987; Kozłowski & Pallardy, 2002).

The interaction effect between propagule types and irrigation regimes was significant for five parameters: leaf area ratio ( $p=0.031$ ), leaf area productivity ( $p=0.037$ ), root to stem ratio ( $p=0.035$ ),  $^{13}\text{C}$  ( $p=0.037$ ), and TNC ( $p=0.042$ ). However, the observed variations were more obvious between stressed and well watered conditions for stecklings than for seedlings (Fig. 8), indicating that the variation in growth and WUE between seedlings and stecklings would be more noticeable in stressed conditions.

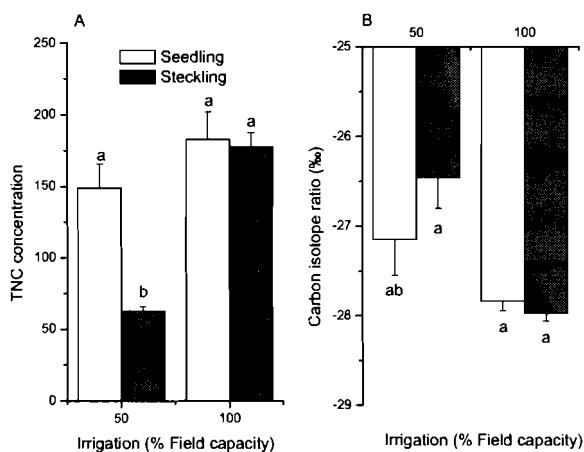


Figure 8. The effects of the interaction (propagule type  $\times$  irrigation regime) on the total non-structural carbohydrate (TNC) concentration in roots (A) and carbon isotope ratio ( $^{13}\text{C}$ ) in leaves (B) of seedlings and stecklings of *Khaya senegalensis* in Ouagadougou, Burkina Faso. Bars represent standard errors of means. Different letter(s) indicate significant differences at the 5% level according to Tukey's multiple comparison test.

According to the relationship found between  $^{13}\text{C}$  and the intrinsic WUE (Hall *et al.*, 1994; Devitt *et al.*, 1997), stecklings exhibiting a similar  $^{13}\text{C}$  could be expected to have a WUE similar to that of seedlings. However, because of a greater amount of TNC in the roots under water-stressed conditions, seedlings may exhibit better recovery than stecklings. Thus the development of stecklings' root systems should be examined in further experiments aiming to compare the two propagule types.

## 5 Concluding remarks and perspectives

Clonal propagation can be advantageous for multiplying plants and creating successful forest plantations provided that efficient vegetative propagation methods exist and that the growth of asexual propagules is comparable to or greater than that of sexual propagules. The findings obtained from the studies reported in this thesis indicate that: (a) lateral roots from field-grown mature trees can be used for clonal propagation of *D. microcarpum* in a nursery but cutting length and diameter are both important factors that affect the sprouting and rooting ability of root segments; and (b) *K. senegalensis* can be propagated vegetatively from leafy stem cuttings derived from seedlings. As the investigations presented here represent a first step toward effective clonal propagation of *D. microcarpum* and *K. senegalensis*, which might lead to successful plantation establishment in Sahelian and Sudanian Africa, further work is required to optimize the techniques. Future studies should focus on various factors relating to the effects of age of donor plants, the application of shoot and root inducing hormones or fertilizers, and the season of collection for root segments on shoot and new root formation in *D. microcarpum*. For *K. senegalensis*, studies are required to determine the optimum concentration of auxins for enhancing the rooting of leafy stem cuttings. A greater number of individuals of diverse environments should be selected in future work for good representativeness. Tissue culture techniques may well be further explored for mass propagation of both *D. microcarpum* and *K. senegalensis*.

The comparisons of sexual and clonal propagules illustrate that root suckers and seedling sprouts of *D. microcarpum* within natural forest stands have a close morphological resemblance, especially for individuals taller than 50 cm, and that the growth of stecklings from *K. senegalensis* juvenile donors follows a similar trend to that of its seedlings under both well watered and water-stressed conditions. Water-stress was found to be an important factor

limiting the establishment and the growth of the two types of propagules. Limited water supply, under 25 and 50% field capacity conditions, produced stress in all plants: they exhibited a reduction in plant growth, biomass production, and soluble sugars and an increase in the root biomass fraction, water use efficiency, and TNC. This highlights the need to select genotypes for drought-tolerance in addition to mahogany shoot borer-resistance in order to ensure the success of *K. senegalensis* plantation establishment for timber production in its native areas in Africa. For *D. microcarpum* future work is needed to examine the growth of rootlings compared to that of seedlings or seedling sprouts with respect to their growth response to water limitation and the shoot die back phenomenon. In addition, molecular markers as a non destructive method might be advantageously used for the discrimination of seedling sprouts from root suckers of *D. microcarpum*.

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## Acknowledgements

This work would not be possible without the support of a great number of people and institutions. First of all I express my gratitude to my main supervisor, Prof. Per-Christer Odén, for his kindness, patience, constant encouragement and support throughout the study period. I am very grateful to my deputy supervisors, Jules Bayala, Mulualet Tigabu, Patrice Savadogo and Prof. Joseph Boussim for their support, guidance and constructive criticism when reviewing the protocols, the draft manuscripts.

Special thanks to those who helped me during the fieldwork: S. Compaoré, D.N. Compaoré, T. Larba, A. Waneweogo, K. Ouédraogo, F. Nandweogo, T.A. Rosalie Dembélé, A. Sanou, S. Diasso<sup>1-2</sup>, N. Koné, A. Samne, C. Garba, R. Ouédraogo, M. Meda, O. Ky, D. Kibora, H. Yonly, Z. Sanon, P. Bazié, H. Bazié, and anyone else who assisted me in this study. Thanks to Jan-Erik for help with analyzing the data. Thanks to the Nazinon forest management staff for permission to work in the forest, to the Ouagadougou City Hall administrator and Issa Sawadogo for permission to work on roadside plantations and Sees-editing Ltd for linguistic improvements.

The Swedish International Development Cooperation Agency (Sida) and Swedish Institute (SI) are gratefully acknowledged for their kind financial support and many cultural activities which allowed me to discover and learn about Sweden. I am grateful to SLU (Swedish University of Agricultural Sciences), GENFYS (Department of Forest Genetics and Plant physiology) and ESS (Southern Swedish Forest Research Centre) for assistance and support, to the authorities of CNRST (Centre National de Recherche Scientifique et Technologique), INERA (Institut de l'Environnement et de Recherches Agricoles) and DPF (Département Productions Forestières) for logistical support and permission to pursue a PhD education, and to the regional office of CIFOR (Centre for International Forestry Research) in Ouagadougou, for their kind support.



Many thanks to all my colleagues and friends, K. Sanon, M. Belem, M.C. Millogo, A. Da, B. Bastide, M. Somé, L. Sawadogo, D. Tiveau, N. Lamien, O. Dibloni, A.A. Maiga, M. Dianda, B. Diallo, T. Guissou, A.B. Bationo, J.M. Ouadba, J.F. Pallo, M. Ouédraogo, M. Traoré, S. Ganaba, A. Salawu, S. Soulama, N. Belé, B. Ouattara, D. Dianou, H. Sawadogo, and R. Sanou for continuous encouragement and support. Thanks to B. Wetian, J.N. Poda, S.J. Ouédraogo and E. Bonkougou for advice and encouragement. I also thank those who helped me to get started, P. Ouédraogo, Prof. S. Guinko, L. Thiombiano, M. Compaoré, F. Lompo, P. Sérémé and M. Sedogo for their support.

I am thankful to my African, Asian and Latin America friends in Sweden, L. Dapi Nzefa, O. Sanon, S. Traoré, D. Zida, S. Paré, Z. Gnankamari, K. Ouattara, H. Sidibé, B. Fidèle, D. Dayamba, I. Ouédraogo, H.N. Bouda, I.P. Saptoka, S. Xengvue, H. Priyadi, A. Daneshvar, A. Uasuf, and G. Shavez for good companionship. Thank you, P. Coulibaly-Lingani for companionship and joint “Gympa” training to keep body and mind healthy. I am also grateful to C. Soma, M. Djembré, T. Ouédraogo, S. Ilboudo, and M. Bouda for their friendship, encouragement and support.

I am deeply grateful to all the lovely friends I made in Sweden, Kajsa, Mats, Yvonne, Carina, Amanda, Mother, Muster, Milla, Alex, Felix, Edith, Agneta, Susanna, Katerine, Margareta, Hans, Thomas, Ann-Sofie, Simone and Gladys, who made my stay in the cold weather truly worthwhile. I will miss you all!

I am most grateful to my great family, father, mother, uncles, aunts, brothers, sisters, nieces, nephews and friends from households Dembélé, Ky, Paré, Toé, Toni, Zan and Zerbo for their continuous encouragement and support. Special thanks to Lawadoun Marina, my lovely daughter who patiently endured this lengthy study period.

Let us finally give thanks to the Lord God for all his blessings!

## French summary (Résumé)

Au Burkina Faso, l'utilisation des espèces ligneuses locales dans les reboisements est limitée en raison de la croissance lente et l'impact d'insectes tels que la chenille foreuse des pousses de l'acajou. De ce fait, l'utilisation de clones résistants ou à croissance rapide pourrait favoriser l'installation et la productivité des plantations. Ainsi, les objectifs de cette thèse étaient de mettre au point des méthodes de multiplication végétative simples et efficaces pour deux espèces locales importantes pour la production de bois de feu et de bois d'œuvre, *Detarium microcarpum* et *Khaya senegalensis* et d'examiner la croissance des plants d'origine sexuée et asexuée.

Deux méthodes de propagation ont été développées: le bouturage de racines pour *D. microcarpum* et le bouturage de tiges pour *K. senegalensis*. La longueur et le diamètre du segment de racine ont été deux facteurs importants qui ont affecté la capacité de bourgeonnement et d'enracinement. Les segments de racines, mesurant 20 cm de long et 15-60 mm de diamètre, ont été les plus performants. L'âge des plants mères et l'application d'auxine ont affecté l'enracinement des boutures de tiges de *K. senegalensis*. Les boutures prélevées sur des plantules ont été plus efficaces avec une proportion élevée (95-100%) de boutures enracinées que celles prélevées sur des plants plus âgés (0-5%). Ceci met en évidence le fait que le phénomène de maturation serait un facteur limitant dans le clonage de *K. senegalensis*. La capacité d'enracinement des boutures prélevées sur des arbres plus âgés a été améliorée (10-16%) par l'écimage et l'utilisation des auxines.

La comparaison des plantules sexuées et asexuées de *D. microcarpum* a révélé une ressemblance morphologique des drageons et des rejets de semis. Le système racinaire bien établi et les fortes concentrations de glucides dans les racines des rejets pourraient favoriser une croissance comparable à celle des drageons. Les semis et les boutures de *K. senegalensis* ont eu une croissance similaire concernant les taux de croissance relative pour la hauteur des tiges, la biomasse produite des feuilles, des tiges, des racines, la biomasse totale des plants, le ratio de la biomasse de feuilles, tiges, et racines par rapport à la biomasse totale, le ratio de la biomasse de racines en rapport avec celle de la tige, la productivité de la surface foliaire, le ratio isotopique de carbone ( $^{13}\text{C}$ ) et la concentration en glucides des racines. Toutefois, le stress hydrique (25 et 50% de la capacité au champ) a été un facteur important qui a limité la croissance des plants à travers une réduction significative de leur croissance, la biomasse produite et la concentration de glucides.

Comme ces travaux représentent une première étape en vue de la multiplication clonale effective de *D. microcarpum* et *K. senegalensis* qui pourrait améliorer l'établissement et la productivité des plantations au Burkina Faso, d'autres études concernant les effets des plants mères, l'application des hormones de croissance et les types de plantules sont nécessaires pour optimiser les procédés.

Mots-clés: Afrique de l'Ouest, bouture de racine, drageon, glucides, multiplication végétative, ratio isotopique du carbone, rejet de semis, semis, stress hydrique.



Clonal Propagation of *Detarium  
microcarpum* and *Khaya senegalensis*  
A Step toward Clonal Forestry in Burkina Faso

Catherine Ky-Dembele

Akademisk avhandling som för vinnande av skoglig doktorexamen kommer att offentlig försvaras i Crafoordsalen, Alnarp, fredagen den **01 april 2011** klockan 14.00.

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Distribution:  
SLU, Southern Swedish Forest Research Centre,  
P.O. Box 49, SE-230 53 Alnarp, Sweden

Alnarp 2011  
ISSN 1652-6880  
ISBN 978-91-576-7556-9





## Clonal Propagation of *Detarium microcarpum* from Root Cuttings

Catherine Ky-Dembele, Mulualet Tigabu, Jules Bayala, Patrice Savadogo, Issaka Joseph Boussim and Per Christer Odén

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**Ky-Dembele, C., Tigabu, M., Bayala, J., Savadogo, P., Boussim, I.J. & Odén, P.C.** 2010. Clonal propagation of *Detarium microcarpum* from root cuttings. *Silva Fennica* 44(5): 775–786.

*Detarium microcarpum* is a valuable tree species for fuelwood, timber, food and medicine in sub-Saharan Africa. However, its population is dwindling due to overexploitation, its seedlings' low survival rate and slow growth. Vegetative propagation might enhance both survival and growth, but to date a successful clonal method does not exist for *D. microcarpum*. We conducted four experiments to examine the effects of propagation environment (high versus low humidity), cutting length and diameter, alignment of root segments (horizontal versus vertical) and distance from the root collar of donors on the regeneration ability of root segments collected from field-grown *D. microcarpum* trees in Burkina Faso. The size of root segments significantly affected their regeneration ability, while alignment had no effect. Sprouting was possible from 10 and 20-cm long segments of 15–60 mm diameter with 7–43% sprouting efficiency and multiple shoots while 5 cm long segments were unsuitable with 0–3% sprouting efficiency. Cuttings maintained at low humidity produced larger diameter sprouts than those in greenhouse. All cuttings showed strong polarity with most of the shoots developing at the proximal end. Rootlings from 20 cm root segments produced more new roots ( $0.62 \pm 0.08$  g) than those from 10 cm segments ( $0.34 \pm 0.09$  g), but they were similar for sprout and leaf growth. We conclude that lateral roots of field-grown mature trees can be used to produce rootlings in a nursery. Since this study is the first attempt to propagate *D. microcarpum* from root cuttings, further investigations are required to optimize the technique.

**Keywords** Burkina Faso, sprouting efficiency, rootling, vegetative propagation

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**Received** 11 June 2010 **Revised** 27 October 2010 **Accepted** 17 November 2010

**Available at** <http://www.metla.fi/silvafennica/full/sf44/sf445775.pdf>

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## 1 Introduction

*Detarium microcarpum* Guill. & Perr. is a deciduous tree of the family *Leguminosae*, subfamily *Ceasalpinioideae* (Watson and Dallwitz 1993, Vautier et al. 2007). It is found in semi-arid sub-Saharan Africa from Senegal to Cameroon, extending east to the Sudan. It has an irregular distribution, but it can be locally very common. Typically, it is found in high rainfall savanna areas, dry forests and fallow lands on sandy or iron rich hard soils as well as scattered trees on farms. It also occurs in dry savanna as a more stunted tree with smaller fruits (Vautier et al. 2007) reaching ca. 10 m high and with a dense rounded crown; in wet areas it can grow up to 25 m tall.

The fruits that are drupe-like, circular and disc-shaped, containing fibers are edible and rich in vitamin C, potassium and calcium. The seeds, singly embedded within the hard fruits are used to thicken soups (Akpata and Miachi 2001). *D. microcarpum* is classified as a major African medicinal plant. The roots, stems, bark, leaves and fruits are all used to treat ailments such as tuberculosis, meningitis, itching, syphilis and diarrhea (Arbonnier 2000, Abreu and Relva 2002, Kouyaté and van Damme 2006, Vautier et al. 2007). Isolation of terpenoids and anti-HIV flavans from *D. microcarpum* extracts have been reported (Abreu and Relva 2002). In Burkina Faso, *D. microcarpum* is also known as the most important commercial fuelwood species harvested from the State forests (Kaboré 2005, Sawadogo 2007). Its hard dark brown wood provides very high quality fuelwood (19 684 kJ/kg) and charcoal (Kaboré 2005) and good quality timber that is used in carpentry and construction (Vautier et al. 2007).

In Burkina Faso, current forest management regimes are based on coppicing and natural regeneration supplemented by direct seeding; these approaches are often unsuccessful. This may threaten the sustainability of the most commonly cut tree species, such as *D. microcarpum*, *Vitellaria paradoxa* C.F.Gaertn., *Terminalia* spp. and *Crossopteryx febrifuga* Benth. (Kaboré 2005, Sawadogo 2007). Savadogo et al. (2007) have, for example, classified *D. microcarpum* and *V. paradoxa* as being vulnerable species in Tiogo forest, one of the State forests of Burkina Faso, due to overexploitation and diminishing populations.

Natural regeneration of *D. microcarpum* is often established as a mixture of seedlings and suckers (Bationo et al. 2001, Ky-Dembele et al. 2007), but seedlings have a low survival rate (Bationo et al. 2001, Kaboré 2005) and slow growth (Bationo et al. 2001, Ky-Dembele et al. 2008). Seedling shoot die back for several unknown number of years is one of the reasons for the slow progression of seedlings towards an advanced stage of regeneration (Bationo et al. 2001, Bastide and Ouédraogo 2008, Ky-Dembele et al. 2008). Selection and vegetative propagation of superior families could be a way to improve the regeneration of *D. microcarpum* and to increase the quality and quantity of forest products derived from this species. However, no successful clonal propagation method is currently available.

We, therefore, examined the potential of using root segments for the clonal propagation of *D. microcarpum*. Stem cuttings seem to be difficult to root irrespective of whether they are derived from the shoots of mature trees, coppices or 3-month-old seedlings (Ky-Dembele, personal observation), but the species' ability to sucker naturally suggests that there is potential for propagating it from root cuttings. Root cuttings has been defined as a propagation technique in which plant roots are severed into individual pieces, segments or cuttings, each of which is capable of developing adventitious buds and roots and, therefore, of regenerating into complete plants (Macdonald 1990). The plants thus formed are known as 'rootlings' (Hall et al. 1989, Snedden et al. 2010). The method has been used for propagating some forest trees species, such as poplars (Eliasson 1961, Hall et al. 1989, Stenvall et al. 2004, Snedden et al. 2010), with varying success. An understanding of the main factors affecting the regeneration vigor of root segments, such as cutting size (length, diameter), the original location of the segment in the root system and growing conditions are essential (Hartmann et al. 2002). Hence, the present study was intended to determine the effects of root segment length and diameter, propagation environment, alignment and insertion mode and the distance from the root collar on the regeneration ability of root segments collected from mature plants of *D. microcarpum*. As the method is inexpensive and does not require special equipment (Hall et al. 1989, Meunier et

al. 2008), root cuttings could be a cost-effective method of cloning *D. microcarpum*.

## 2 Materials and Methods

### 2.1 Stockplants and Cuttings Preparation

Naturally regenerated mature trees of *D. microcarpum* from the Nazinon forest were used; this is a tree and shrub savanna woodland located ca. 100 km south of Ouagadougou (11°30′–11°51′N and 1°27′–1°50′W) in Burkina Faso. The donor trees involved in the study were, on average, 6.3 ± 0.2 m tall, had an average diameter at breast height (DBH) of 12.3 ± 0.6 cm and an average canopy width of 4.1 ± 0.2 m. Lateral roots were excavated, and a 1–1.5 m long section was removed from each tree. Root fragments were cut to the desired size, placed in water to avoid dehydration and then taken to the laboratory of the Forest Productions Department of the Environment and Agricultural Research Institute (INERA/DPF). The distal end (towards the root tip) of each root segment was cut obliquely in order to differentiate it from the proximal end. In the laboratory, cuttings were soaked in a solution of Ivory 80 WP, a fungicide containing 80% Mancozeb for 10 min. The cuttings were planted in a sterilized mixture of soil, sand and cattle manure (1:1:1, v/v/v) in plastic containers. The mixture had a sandy-clay-silt texture (Bayala et al. 2009). The containers were covered with transparent plastic sheets and placed in a greenhouse at a humidity of 70–100% and a temperature of 22–37 °C, unless otherwise stated. Cuttings were watered manually every second day.

### 2.2 Experimental Designs

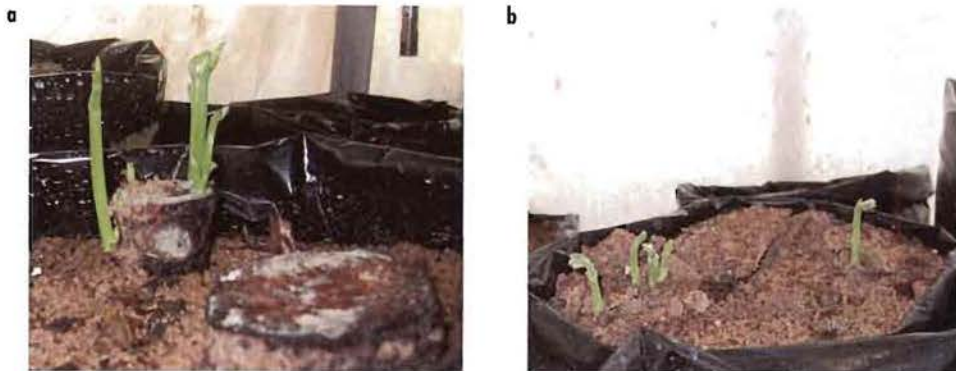
Four series of experiments were performed to identify factors that influence the sprouting ability of root segments. In the first experiment, the effects of root segment length (5 and 10 cm), diameter (11–20 mm and 21–40 mm) and propagation environment (inside a greenhouse with high humidity of 70–100% and a temperature in the range 22–37 °C or outdoors in the shadow of a tree, where the humidity was low. i.e. 25–70%,

and the temperature in the range 22–40 °C) were tested in a completely randomized factorial design with 10 replicates and three cuttings per experimental unit. The tree which was used for shade was a mature *Sclerocarya birrea* Hochst., measuring 13.5 m tall, 55.4 cm DBH, 15.2 m across the canopy from east to west and 8.5 m from north to south. From March 26 to 27, 2009, a total of 240 root segments were prepared from eleven lateral roots (cut to 5 or 10 cm) collected from eleven mature trees. Root segments were grouped into four categories: 5 cm long, 11–20 mm diameter; 5 cm long, 21–40 mm diameter; 10 cm long, 11–20 mm diameter; and 10 cm long, 21–40 mm diameter. Three segments were taken randomly from each set and assigned to the corresponding experimental unit. They were then buried horizontally, 1 cm below the surface of the growing medium in a plastic box (75 × 15 × 12 cm). The experiment lasted for eight weeks, starting on March 26, 2009.

In the second experiment, the effects of root segment length was further tested, using 10 cm and 20 cm lengths, all with a diameter of 20–40 mm, in combination with vertical insertion modes (exposed versus buried) in a completely randomized factorial design involving four treatments and 10 replicates, with three root segments per replicate. In the exposed insertion mode, the proximal ends of the segments were kept 2 cm above the surface of the medium while in the buried insertion mode, the proximal ends were kept 1 cm below the surface of the growing medium (Fig. 1). A total of 120 root segments were collected from twenty mature trees, four trees at a time (to set up two replicates), from May 29 to June 2, 2009. Each day segments were grouped into two sets (10 and 20 cm). Three segments were taken randomly from each set and planted in a perforated black polythene bag (27 cm diameter × 40 cm height). The experiment ran for ten weeks starting on May 29, 2009.

In the third experiment, we examined whether regeneration from root segments is dependent on distance from the root collar of the mother tree; we used a completely randomized design with 10 replicates. One lateral root (1.5 m long) was excavated from each of 30 mature trees, six trees at a time (to compose two replicates) between October 29 and November 2, 2009. Twenty-centimeter





**Fig. 1.** Vertical insertion modes used for clonal propagation of *Detarium microcarpum* from root cuttings: proximal end exposed (a) or buried 1 cm below the surface of the medium (b) in Ouagadougou, Burkina Faso.

root segments were cut at three distances from the root collar, 0, 60 and 120 cm; these are referred to as treatments, and pooled in three sets. Three cuttings were taken randomly from each set and assigned to the corresponding experimental unit. The root segments were 15–60 mm in diameter. The root segments were buried vertically, with the proximal end 1 cm below the surface of the medium, which had been placed in perforated black polythene bags (27 cm diameter  $\times$  40 cm height). The experiment ran for eleven weeks.

Finally, we examined the effects of alignment of root segments (vertical and horizontal) in combination with cutting length (10 cm and 20 cm) in a completely randomized factorial experiment with 10 replicates and three cuttings per experimental unit. Root segments (10 cm and 20 cm) of 20–40 mm diameter were obtained from the same lateral roots collected for the third experiment. One set of root segments (10 cm and 20 cm) was taken 20–50 cm from the root collar and the other set 80–110 cm from the root collar. To avoid a potential confounding effect of distance from the root collar, root segments from both sets were equally distributed between treatments. The root segments were buried 1 cm below the surface of the growing medium in plastic boxes (75  $\times$  15  $\times$  12 cm) for the horizontal alignment and in perforated black polythene bags (27 cm diameter  $\times$  40 cm height) for the vertical alignment. The experiment ran for eleven weeks in a greenhouse from October 29, 2009.

In order to assess rootling establishment further, a total of 21 sprouted root segments, 15 from 20 cm root segments and six from 10 cm root segments were replanted in perforated black polythene bags (27 cm diameter  $\times$  40 cm height) filled with the same mixture as described previously, containing soil, sand and manure. On August 14, 2010, seven months after planting, 21 rootlings (the surviving sprouted root segments) were examined.

### 2.3 Data Recording and Analysis

At the end of each experiment, all root segments were removed from the growing medium, washed, the number of sprouts taller than 0.5 cm and the number of new roots were recorded per cutting and the length of the longest sprout was measured. The origins of the sprouts on each segment (whether in the proximal, central or distal region of the segment) were also recorded. The sprouting efficiency was calculated as the percentage of sprouted cuttings to the total number of root segments planted in each experimental unit. For rootling establishment, the number of sprouts, the length and the basal diameter of the longest sprouts were recorded. Because the new roots were fine, embedded in the soil and therefore difficult to count, the root systems were gently washed manually over a 0.5 mm sieve to separate roots, excluding the initial root segments. The dry

biomass of the stems, leaves and roots was determined after oven drying at 70 °C for 48 hours. The total biomass of the rootling was calculated by summing the stem, root and leaf biomass.

Data were checked for normality and analyzed using the GLM procedure of Statistical Analysis System (SAS Institute Inc. 2002–2008). The two-sample T-Test procedure in Minitab 15 (Minitab Inc., State College, PA, USA) was used for data relating to rootling establishment. The dependent variables were mean sprouting percentage (sprouting efficiency), mean number of sprouts per sprouted segment, mean length and basal diameter of the longest sprout per sprouted segment, number of sprouts per rootling, length of the rootling's longest sprout, new root biomass, and total rootling biomass. Significant differences at  $P < 0.05$  were further tested using Tukey's HSD multiple comparison test.

### 3 Results

#### 3.1 Effects of Size of Root Segments and Propagation Environment

New shoots started to appear above the surface of the growing medium from the fifth week after the root segments were planted; this was the case in both the high-humidity environment of the greenhouse and the dry outdoor environment. Propagation environment only significantly influenced the diameter of the longest sprout ( $P = 0.0056$ ). The length of the root segments significantly affected sprouting efficiency ( $P < 0.0001$ ), while the diameter

of the root segments influenced both sprouting efficiency ( $P = 0.0318$ ) and diameter of the longest sprout ( $P = 0.0271$ ). Cuttings grown outdoors had a larger collar diameter than those grown in the greenhouse, while longer root segments (10 cm) exhibited a higher sprouting efficiency than shorter ones (5 cm). Root segments with a larger diameter (21–40 mm) produced the largest, most vigorous sprouts (Table 1). There were no interaction effects on any of the parameters assessed. Shoot formation occurred most frequently at the proximal ends of cuttings (62%) compared to the distal end (20%) and the middle section (18%). No segment produced new roots from either the proximal or distal end in eight weeks of observation.

#### 3.2 Effects of Root Segment Length and Vertical Insertion Mode

The length of root segment affected sprouting efficiency ( $P = 0.0002$ ) and the length of the longest sprout produced per sprouted segment ( $P = 0.0306$ ), while the mode of insertion had a significant effect on the diameter of sprouts ( $P = 0.0088$ ). No interaction effect was observed for any of the parameters assessed. On average, 20 cm long root segments sprouted five times more efficiently than 10 cm long segments. Buried root segments produced larger sprouts than unburied root segments. Neither the length of the root segments nor their insertion mode significantly influenced the number of sprouts per sprouted root segment, but multiple shoots were produced in many cases (Table 2). There was a pronounced polarity along the root segments, with the major

**Table 1.** Effects of environment, root segment length and diameter on sprouting efficiency, the number of sprouts, and the diameter and length of the longest sprouts per sprouted root segment of *Detarium microcarpum* in Ouagadougou, Burkina Faso.

Factors		Sprouting (%)	No. sprouts	Diameter (mm)	Length (cm)
Environment	Greenhouse	16 ± 4a	1.8 ± 0.2a	2.5 ± 0.2a	7.44 ± 1.56a
	Outdoor	11 ± 4a	1.6 ± 0.4a	3.6 ± 0.3b	10.56 ± 2.13a
Cutting length	5 cm	1 ± 1a	1.0 ± 0.0a	2.1 ± 0.0a	7.00 ± -a
	10 cm	26 ± 5b	1.8 ± 0.2a	2.9 ± 0.2a	8.50 ± 1.34a
Cutting diameter	11–20 mm	8 ± 3a	1.3 ± 0.2a	2.5 ± 0.3a	7.29 ± 1.59a
	21–40 mm	19 ± 5b	2.0 ± 0.2a	3.0 ± 0.2b	8.94 ± 1.73a

Values (Mean ± SE) followed by the same letter are not significantly different at the 5% level according to Tukey's multiple comparison test.

**Table 2.** Effects of root segment length and vertical insertion mode on sprouting efficiency, the number of sprouts, and the diameter and length of the longest sprouts per sprouted root segment of *Detarium microcarpum* in Ouagadougou, Burkina Faso.

Factors		Sprouting (%)	No. sprouts	Diameter (mm)	Length (cm)
Length	10 cm	7 ± 4a	2.5 ± 0.5a	3.0 ± 0.9a	10.70 ± 3.22b
	20 cm	33 ± 5b	4.7 ± 1.0b	4.0 ± 0.4b	5.24 ± 0.80a
Insertion	Buried	22 ± 6a	4.4 ± 1.1a	5.1 ± 0.4b	7.42 ± 0.92a
	Exposed	18 ± 5a	4.3 ± 1.5a	2.7 ± 0.4a	4.92 ± 1.49a

Values (Mean ± SE) followed by the same letter are not significantly different at the 5% level according to Tukey's multiple comparison test.

**Table 3.** Effects of distance from the root collar of the donor tree on sprouting efficiency, the number of sprouts, and the diameter and length of the longest sprouts per sprouted root segment of *Detarium microcarpum* in Ouagadougou, Burkina Faso.

Distance	Sprouting (%)	No. sprouts	Diameter (mm)	Length (cm)
0 cm	40 ± 12a	3.3 ± 0.6a	3.6 ± 0.4a	10.60 ± 2.23a
60 cm	27 ± 11a	3.4 ± 0.5a	4.2 ± 0.7a	11.84 ± 3.81a
120 cm	20 ± 0.9a	3.5 ± 0.9a	3.4 ± 0.5a	6.99 ± 1.56a

Values (Mean ± SE) followed by the same letter are not significantly different at the 5% level according to Tukey's multiple comparison test.

**Table 4.** Effects of root segment length and alignment on sprouting efficiency, the number of sprouts, and the diameter and length of the longest sprouts per sprouted root segment of *Detarium microcarpum* in Ouagadougou, Burkina Faso.

Factors		Sprouting (%)	No. sprouts	Diameter (mm)	Length (cm)
Length	10 cm	12 ± 4a	1.8 ± 0.4a	3.6 ± 0.6a	7.10 ± 2.17a
	20 cm	25 ± 5a	3.0 ± 0.6a	3.8 ± 0.2a	8.88 ± 1.90a
Alignment	Horizontal	15 ± 4a	1.9 ± 0.4a	3.7 ± 0.4a	6.33 ± 1.48a
	Vertical	22 ± 6a	3.3 ± 0.8a	3.7 ± 0.2a	10.24 ± 2.38a

Values (Mean ± SE) followed by the same letter are not significantly different at the 5% level according to Tukey's multiple comparison test.

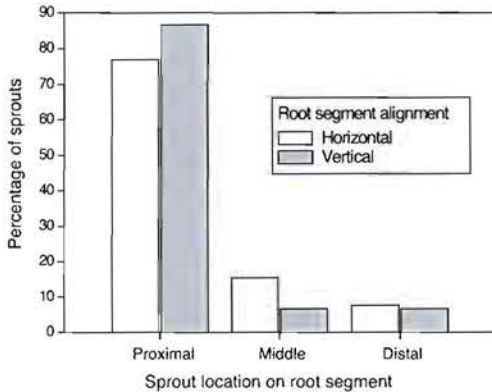
ity of shoots arising from the proximal end (88%) compared to the distal end (4%) and the middle part of the root segments (8%). No more new roots were produced within ten weeks.

### 3.3 Effects of Distance From Tree Root Collar, Root Segment Length and Alignment

There was no clear effect of distance from the root collar of the mother tree on the regeneration of root

segments, but sprouting efficiency varied from 20% to 40% and there was multiple shoot production (Table 3). Most of the shoots were produced from the proximal end (85%), followed by the middle part of the root segments (15%), but no shoots originated from the distal end of the cuttings. None of the cuttings produced new roots.

The alignment of root segments and their length did not significantly influence the sprouting efficiency, the number of sprouts per sprouted segment or the diameter and length of the longest sprout per sprouted segment, even though the



**Fig. 2.** Percentage of sprouts produced at different locations, the proximal ends (towards the mother tree root collar), the middle and the distal end (towards the root tip), on root segments of *Detarium microcarpum* aligned horizontally and vertically (experiment 4) in Ouagadougou, Burkina Faso.

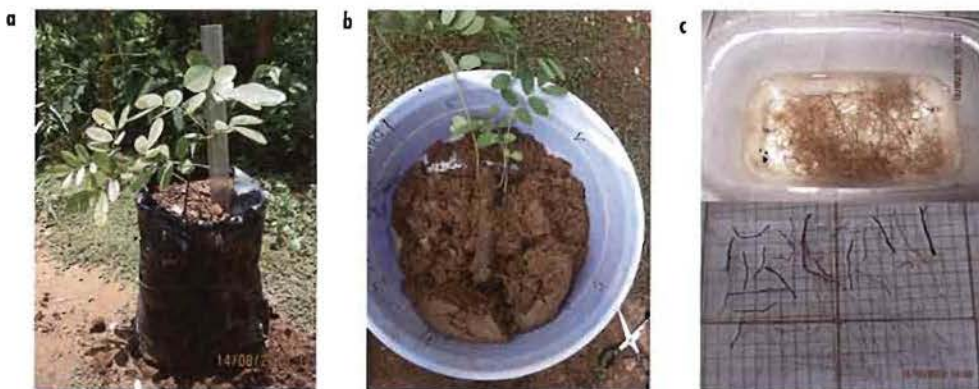
overall mean tended to be higher for root segments that were 20 cm long (Table 4). However, the polarity was greater for the vertical alignment than the horizontal (Fig. 2). Root segments aligned vertically produced 87% of their sprouts along the proximal third part compared to 77% for the horizontally aligned cuttings.

All sprouted root segments, when replanted, produced new roots (0.05 g–1.08 g) originating

from the original root segments (Fig. 3). There was a significant difference in mean new root biomass ( $p=0.044$ ) between the two root segment lengths, 10 and 20 cm. Rootlings derived from 20 cm root segments produced a greater biomass of new roots ( $0.62\pm 0.08$  g) than 10 cm root segments ( $0.34\pm 0.09$  g). Root segment length did not influence the other parameters used to assess rootling establishment even though the mean values tended to be higher for those derived from 20 cm root segments. The mean values regarding these parameters for rootlings derived from 10 cm and 20 cm long segments, respectively, were:  $1.5\pm 0.3$  and  $1.7\pm 0.2$  for sprout number;  $16.5\pm 3.8$  cm and  $19.0\pm 2.1$  cm for sprout length; and  $0.98\pm 0.20$  g and  $1.7\pm 0.30$  g for the total rootling biomass.

## 4 Discussion

The results from the present study clearly demonstrate that *D. microcarpum* can be regenerated from root segments collected from mature, field-grown trees. The segments exhibited a relatively good capacity to produce new shoots and roots. The average sprouting efficiency obtained in our study appears to be lower than that reported for *Ficus* spp. (Danthu et al. 2002), *Maerua crassifolia* Forssk. (Houmey et al. 2007) and *Spathodea campanulata* P.Beauv. (Meunier et al. 2008), but



**Fig. 3.** *Detarium microcarpum* rootling (a) obtained from a vertically planted root segment (b) and regenerated new roots (c) in Ouagadougou, Burkina Faso.

though cuttings taken from near the root collar of the mother tree exhibited the best sprouting efficiency (40%) compared to the middle part, 60 cm (27%) and the distal part, 120 cm (20%) away from the root collar. These results are in accordance with previous studies of aspen (Starr 1971) and *Paulownia tomentosa* (Thunb.) Steud. (Ede et al. 1997), where no clear differences were found between the number of shoots and roots produced near the main tree and those produced at the extremity. Our data, however, contrast with results presented by Houmey et al. (2007) who found the middle part of the root better at producing shoots than the proximal or distal parts. As thickness and location are related to each other, the location of the root segment is often significantly correlated with cutting regeneration ability (Ede et al. 1997); thicker root segments originating near the root collar usually produce more shoots, faster and more efficiently than thinner cuttings from the distal parts of the root system.

Assuming that the type of containers, the boxes and perforated polythene bags, did not affect the sprouting capacity of the root segments, they should not have confounded the comparison between horizontal and vertical alignments. Therefore, we consider that sprouting efficiency did not differ between horizontally and vertically aligned roots or between the exposed and the buried cuttings. This is in agreement with Brouard et al. (2005) but not with Ghani and Cahalan (1991), who found that exposing the proximal end of *P. avium* roots increased the number of successfully regenerated segments. However, vertical insertion, where the proximal end was buried 1 cm below the surface of the medium, was preferable, because root segments in this alignment are better anchored than the horizontally aligned ones, which are unstable and prone to lodging. In addition, the buried cuttings produced sprouts with a larger basal diameter than the exposed ones, thus providing support for a better shoot growth and a higher survival rate.

Root segments of *D. microcarpum* showed strong polarity, with most of the shoots developing towards the proximal end. This was expected because of hormonal control, a mechanism which interacts with carbohydrate supply for bud initiation and subsequent growth from root segments of woody plant species (Eliasson 1971b, Schier and

Campbell 1976, Robinson and Schwabe 1977a, Ede et al. 1997). According to these authors, the polarity is due to the transport of auxin, a shoot suppression hormone that is acropetal in roots, away from the proximal end towards the root tip. In attached roots, auxin from the aerial part of the tree would normally prevent bud initiation, but when this supply ceases upon detachment of the root, depletion of auxin will allow preferential bud initiation to occur at the proximal end, a phenomenon regarded as an extension of apical dominance.

Rootling assessment showed that both sprouted root segments of 10 cm and 20 cm were able to produce new roots from the initial root segments. However, the regeneration of new roots was a slower process compared to shoot regeneration. This is in accordance with all previous studies consulted, in which rooting time is often longer than sprouting time (Hartmann et al. 2002, Stenvall et al. 2005). As suggested by these authors, this feature may indicate that the sprouting process promotes initiation of adventitious rooting because the carbohydrate supply from the leaves may support root elongation (Eliasson 1968). However compared to aspen, which has a rooting time of less than a month (Stenvall et al. 2005), *D. microcarpum* roots slowly, requiring more than two months. The optimal time needed for root formation ought to be determined in relation to other factors, such as root length, hormone application and season of collection. The development of new roots directly from the original root segments instead of the base of the new shoot has been revealed as a feature common in poplars but not aspen (Schier and Campbell 1976), and may suggest that such new roots originate from latent lateral root initials on the original root segments (Hartmann et al. 2002). Moreover, because the longest root segments that we investigated (20 cm) produced more new roots than the 10 cm root segments, these may be better for *D. microcarpum* propagation from root cuttings.

In conclusion, the findings from our study indicate that lateral roots from field-grown mature trees can be used for clonal propagation of *D. microcarpum* in a nursery. Cutting length and diameter are both important factors that affect the sprouting and rooting ability of root segments. Root segments, measuring 20 cm long and 15–60

mm in diameter, were the most successful in terms of sprouting efficiency and new roots production, whether planted horizontally or vertically and with the proximal end exposed or buried. Because this study is the first attempt to clonally propagate *D. microcarpum* from root cuttings, further work is required to optimize the technique. In particular, the effects of age of donor plants, application of shoot and root inducing hormones, and season of collection of root segments on shoot as well as new root formation need to be studied.

## Acknowledgements

Funding for this study was provided by the Swedish International Development Cooperation Agency (Sida). Special thanks are due to Séni Compaoré, David Noaga Compaoré, Augustin Waneweogo, Karim Ouédraogo, François Nandweogo, T.A. Rosalie Dembele, and Abou Sanou for their help with collecting cuttings and establishing the trials, Apollinaire Samne for driving the team to the field and the Nazinon forest management staff for permission to work in the forest. We are grateful to anonymous reviewers for useful comments on the manuscript and to Sees-editing Ltd for linguistic improvements.

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# Propagation of *Khaya senegalensis* by stem cuttings

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## Abstract

*Khaya senegalensis* is a multipurpose African timber species. The development of clonal propagation could improve plantation establishment, which is currently impeded by mahogany shoot borer. To examine its potential for clonal propagation, the effects of cutting length, leaf area, stockplant maturation, auxin and smoke solution treatments were investigated. The length of cuttings had no significant effect on their rooting ability when taken from five-month old seedlings. Leafy cuttings rooted well (50-80%) compared to leafless cuttings (0%). Cuttings taken from seedlings rooted well (95-100%), but cuttings obtained from older trees rooted poorly (0-5%). The rooting ability of cuttings from older trees was improved (10-16%) by pollarding. Auxin application enhanced root length and the number of roots while smoke solution did not improve cuttings' rooting ability. These results indicate that juvenile *K. senegalensis* is amenable to clonal propagation, but further work is required to improve the rooting of cuttings from mature trees.

**Keywords:** Burkina Faso, cloning, rooting, Senegal mahogany, stockplant

# 1 Introduction

*Khaya senegalensis* A.Juss. (Meliaceae), commonly known as Senegal mahogany in English and acajou caïcedrat in French, is an evergreen tree that typically grows to a height of 15–20 m (up to 35 m on fertile soils) and has a diameter at breast height of 1.5 m, with a clean bole of 8–16 m. Its natural distribution extends from Mauritania and Senegal east to northern Uganda, within the rainfall range 650–1300 mm (and even up to 1800 mm) [1]. In Burkina Faso, the northern limit of the natural distribution of *K. senegalensis* is 13°55'N within the South-Sahelian zone. It occurs in various habitat types, such as on river banks, and in fields, fallows and protected woodlands, and its population density increases from North to South, reaching up to 17 trees per hectare [2].

*K. senegalensis* is a multipurpose tree with a variety of economic and environmental values [1]. It is one of the major timber species in West Africa owing to its hard and fungus- and termite-resistant red wood; it is highly valued for carpentry, joinery, furniture, ship building, and as a decorative veneer. The bark is used in traditional medicine to treat malaria, diarrhoea, dysentery, anaemia etc. Recently, the stem bark has been found to contain chemicals (limonoids) that exhibit anti-proliferative activity against human cancer cell lines [3]. It is also a good source of fodder for cattle, because of its high dry matter but relatively low crude protein content [4], it is also a source of edible and cosmetic oils [1]. In West Africa, the species has become an important urban amenity tree, commonly planted as a roadside or ornamental shade tree. It is also increasingly planted in other countries such as South Africa, Egypt, Australia, Sri Lanka, China, Indonesia, Malaysia and Vietnam for both amenity and timber production [5].

Natural regeneration of *K. senegalensis* is poor [1] as its seeds lose viability after only two or three weeks under natural conditions [6]. The viability of the seeds can be prolonged by drying to moisture content below 5% and storing them at a low temperature of around 5°C [7, 8]. *K. senegalensis* is classified as vulnerable on the IUCN 2010 red list of threatened species (International Union for Conservation of Nature) because of overexploitation for timber, fodder and medicine, and as a result of habitat

loss and degradation [1]. Efforts to restore the depleted mahogany resource base on plantations have been thwarted by persistent attacks of the mahogany shoot borer *Hypsipyla robusta*, which kills the main stem of young trees, causing excessive branching and contributing to mortality and poor quality timber production [1, 9].

Several methods have been recommended to contain this insect pest, including mixed planting with resistant species [10, 11], overhead shading of saplings, removal of lateral shoots, and chemical treatments [1, 6, 9]. Selection of genetically resistant individuals and cloning them would ensure better establishment of plantations while conserving the germplasm from genetic erosion [11].

Vegetative propagation using leafy stem cuttings has been successful in African mahoganies such as *K. ivorensis*, *K. anthotheca*, *Lovoa trichilioides* [6, 12] and *K. senegalensis* [9, 13, 14]. Chip budding, cleft grafting and side-veneer grafting have been successfully used for *K. senegalensis* seedling grafting in Burkina Faso [14]. Micro-cutting has also been successfully applied to micrografted plants with scions collected from 6-year-old trees of *K. senegalensis* [9]. As in many other tropical tree species [15], the varying results from these studies still leave some scope to improve the techniques. In this study, the objective was to determine the effects of cutting length, leaf area, stockplant maturation, auxin application, and aqueous smoke solution on the rooting ability of stem cuttings of *K. senegalensis*.

## 2 Materials and methods

### 2.1 Sources and preparation of cuttings

Cuttings were collected from four different types of stockplants: 3–8 month-old seedlings, 5- and 15-year old planted trees and rejuvenated branches of pollarded old trees. Seedlings were raised from seeds purchased from the National Seed Centre (CNSF) collected in 2008 from naturally regenerated tree stands in Tiakaré village within Gourma Province, Burkina Faso. The seedlings were grown in perforated black polythene bags (7 cm diameter × 25 cm tall), filled with a mixture of sand, soil and manure (2:2:1 v/v/v), outdoors in the nursery of the Forest Productions Department, Environmental and Agricultural Research Institute (INERA) in Ouagadougou, Burkina Faso. Because of an important concern regarding

the use of materials of known age, the two mature stockplant donors were both street trees in Ouagadougou: one set of donors from “Avenue Charles de Gaulle” and the other from “Avenue de la Jeunesse”, planted in 1994 and 2004, respectively. The rejuvenated stockplants were street trees planted about 100 years ago on “Rue Nongremason” in Ouagadougou and pollarded five or six months before cutting collection.

Leafy shoots were harvested from the seedlings and from the basal branches of trees between 06:00 - 08:00 h, placed in water to prevent drying before being taken to the laboratory. Leaves were trimmed so that only two leaflets of two opposite leaves remained on the cuttings collected from mature stockplants, and two simple leaves remained on the cuttings from seedlings, unless otherwise stated. The retained leaves and leaflets were cut to a length of 2-3 cm. Cuttings were 10 cm in length, unless otherwise stated.

The cuttings were kept under intermittent mist on a propagation bed maintained at 70%-100% relative humidity measured with a GP1 data logger in a greenhouse (3 × 15 m) located at INERA, Ouagadougou, Burkina Faso. The mean minimum and maximum temperatures in the greenhouse during the whole study period were 22°C (night) and 37°C (day), respectively. The mean quantum flux density was 450  $\mu\text{mol m}^{-2} \text{s}^{-2}$  during daytime, monitored using a Photosynthetically Active Radiation (PAR) light sensor with readout on a LI-19 unit. To minimise fungal attacks, cuttings were soaked in a solution of Ivory 80 WP, a fungicide containing 80% Mancozeb, for 10 min before planting. The cuttings were then planted at a depth of 2-3 cm in a rooting medium consisting of a mixture of sterile sand and perlite (1:1 v/v) in plastic propagation trays covered with transparent plastic sheets. The cuttings were regularly watered manually to avoid desiccation. The experiments ran for eight weeks.

## 2.2 Experimental designs

### 2.2.1 *Effects of cutting length and leaf area*

To test the effect of cutting length on rooting ability, leafy cuttings were collected from 5-month old seedlings and randomly allocated to each of four cutting lengths: 5, 10, 15 and 20 cm in a completely randomised design with five replications and six cuttings per replication of each of the four treatments. In total 120 cuttings were used, collected from the shoots of 120 seedlings.

To examine the effect of leaf area on the rooting ability of cuttings, 10 cm long cuttings were collected from one-year old hedged seedlings and randomly allocated to each of four leaf area treatments, 0 cm<sup>2</sup> (leafless), 6–8 cm<sup>2</sup> (one leaf with one pair of cut leaflets), 12–16 cm<sup>2</sup> (two leaves with two pairs of cut leaflets), 22–28 cm<sup>2</sup> (two leaves with four pairs of cut leaflets) in a completely randomised design with five replications and six cuttings per replication. Two shoots were cut from each of 60 one-year old hedged seedlings and mixed together before random allocation to the treatments.

### *2.2.2 Effects of stockplant maturation and auxin treatment*

To investigate the effect of donor plant maturation and Indole-3-butyric acid (IBA), cuttings were collected from four types of stockplants (3-month old seedlings shoots, 5-month old young sprouts from pollarded old trees, and crown sprouts from 5- and 15-year old trees). The young sprouts of the pollarded trees are henceforth referred to as “resprouts”. A full factorial experiment with a split plot design involving 16 treatments was employed: stockplants (seedling shoots, resprouts of pollarded trees, 5-year and 15-year old tree crown sprouts) × IBA (0, 2500, 5000, 10000 ppm). Demineralised water was used for dissolving the auxin and served as a control treatment. The four stockplant types were randomly assigned to the main plots, while the four IBA concentrations were tested at the subplot level. Each treatment had seven replicates with six cuttings per replication; thus the total number of cuttings used was 672. The basal ends of the cuttings were dipped in the IBA solutions for 5 seconds. In total, 168 single-shoot seedlings were used for cutting collection while eight trees represented each of the three categories of tree (pollarded, 5- and 15-year old); these were used equally for cutting collection and IBA treatment allocation.

To improve further the rooting ability of cuttings from resprouts of pollarded trees, a follow-up experiment was conducted using naphthalene acetic acid (NAA) alone and in combination with IBA. Resprouts and cuttings from seedling donors were compared. Cuttings were collected from two types of stockplants (4-month old seedling shoots and 6-month resprouts from pollarded trees) and two auxin treatments (NAA and NAA+IBA) at each of four concentrations (1000, 2000, 3000, 4000 ppm), arranged in a split-split plot design with five replications and six cuttings per replication of the 16 treatments. Stockplant donors were randomly assigned as the main plot factors; auxin treatments as sub-plot factors; and the four different concentrations as sub-sub-plot factors. The basal ends of the cuttings were dipped in the auxin solutions for 5 seconds. Overall, 30 shoots were cut from each of eight pollarded trees, which were different from



those sampled for the previous experiment; 240 single-shoot seedlings were used for cutting collection.

### *2.2.3. Effects of smoke solution*

This experiment examined the potential of smoke as an alternative to commercial auxins. Smoke solution was produced according to the method described by Dayamba et al. [16], it involved burning a mixture of dry wood and herbs and allowing the smoke to bubble into a plastic bottle of water for about 10 h. The stock solution (100%) was then diluted to 80%, 60%, 40%, 20%, 10% and 5% concentrations of smoke solution (v/v) while de-mineralized water served as a control (0%). Two experiments were performed.

In the first experiment, the basal ends of cuttings collected from 5-month old seedlings were immersed in 5% and 10% smoke solution for 30, 60, 120, 180 min; the results were compared with a water control in a split plot design. Smoke concentrations were tested at the main plot level while the four immersion times were tested at the subplot level. Each treatment was replicated five times with six cuttings per replication. In total, 240 cuttings were used, collected from 240 single-shoot seedlings.

In the second experiment, 0, 20, 40, 60, 80 and 100% smoke solutions were tested in a completely randomised design. Five replicates of six cuttings collected from 8-month old single-shoot seedlings were randomly assigned to each treatment. The bases of the cuttings were immersed in the smoke solutions for 60 min.

## **2.3 Data collection and analysis**

After eight weeks, the root systems were gently washed and the number of roots measuring at least 1 mm long was determined for each cutting. The length of the longest root was measured and the secondary roots originating from the longest root were counted. The percentage of rooted cuttings was determined, i.e. rooted cuttings as a proportion of planted cuttings in each experimental unit. Data were checked for normality and subjected to analysis of variance using Statistical Analysis System software (SAS Institute Inc., 2002-2008). As a large number of cuttings failed to produce roots, transformation (SQRT, arcsine, and Johnson) of the variable percentage of rooted cuttings was not successful for normal distribution requirement.

Johnson-transformed data were used for other variables that did not fulfil the requirement of normal distribution. The equal variance requirement was observed from the residual plots obtained from Minitab 15 (Minitab Inc., State College, PA, USA). The dependent variables were mean percentage of rooted cuttings, mean number of roots per rooted cutting, mean number of secondary roots per rooted cutting and mean length of the longest root per rooted cutting. The GLM procedure was used for completely randomised and split-plot design experiments while the Mixed procedure was used for the split-split-plot design. Means that exhibited significant differences ( $p < 0.05$ ) were further compared using Tukey's multiple comparison test.

### 3 Results

#### 3.1 Effects of cutting length and leaf area

Cutting length did not significantly affect ( $p > 0.05$ ) any of the traits evaluated (Table 1). However, leaf area significantly ( $F_{(3, 16)}=6.53$ ;  $p=0.004$ ) affected the percentage of rooted cuttings. No leafless cuttings rooted and mortality was 93% within eight weeks (Fig. 1). No significant differences were found for any of the parameters evaluated for leafy cuttings with different leaf areas even though cuttings with a leaf area of 22–28 cm<sup>2</sup> rooted better (80%) than cuttings with leaf areas of 6–8 cm<sup>2</sup> (57%) and 12–16 cm<sup>2</sup> (50%).

Table 1. *Effects of cutting length on the percentage of rooted cuttings, the number of roots, the length of the longest root, and the number of secondary roots per rooted cutting of Khaya senegalensis. Means  $\pm$  SE (standard error) followed by the same letter(s) are not significantly different at the 5% level according to Tukey's multiple comparison test.*

Length	Rooting percentage	No. roots / rooted cutting	Longest root length (cm)	No. secondary roots
5 cm	53 $\pm$ 16a	1.8 $\pm$ 0.3a	5.62 $\pm$ 1.38a	9.5 $\pm$ 4.1a
10 cm	47 $\pm$ 12a	2.0 $\pm$ 0.3a	4.10 $\pm$ 1.38a	3.9 $\pm$ 2.3a
15 cm	43 $\pm$ 16a	1.8 $\pm$ 0.2a	4.30 $\pm$ 0.32a	3.0 $\pm$ 1.7a
20 cm	63 $\pm$ 3a	3.0 $\pm$ 0.6a	5.16 $\pm$ 1.05a	7.7 $\pm$ 2.0a

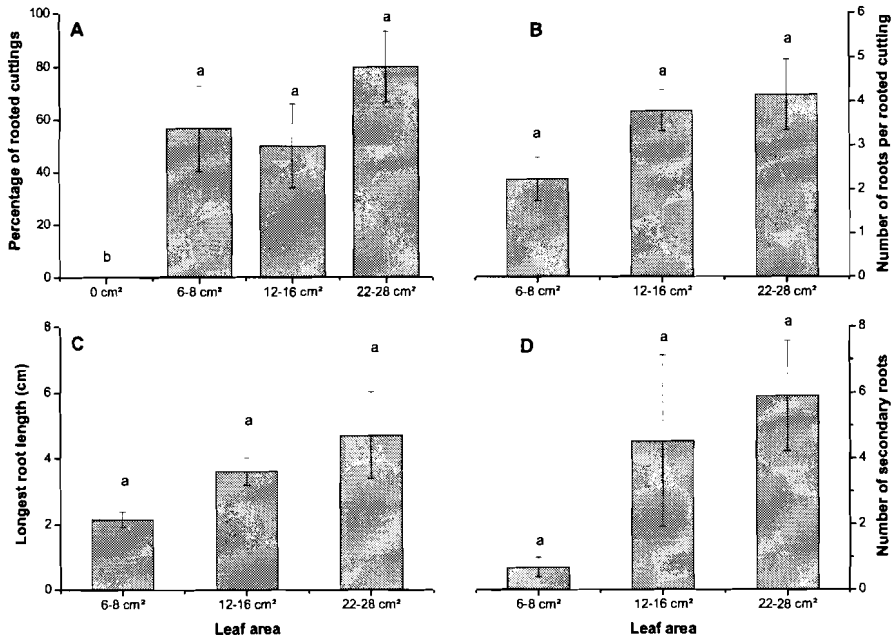


Figure 1. Effect of leaf area on the percentage of rooted cuttings (A), the number of roots per rooted cutting (B), the length of the longest root (C), and the number of secondary roots (D) of *Khaya senegalensis*. Means followed by the same letter(s) are not significantly different at the 5% level according to Tukey's multiple comparison test.

### 3.2 Effect of stockplant maturation and auxin

Rooting responses of cuttings varied significantly with stockplant types or donor plant maturation and IBA concentrations (Table 2). Cuttings taken from seedlings rooted significantly better (99%) than cuttings from resprouts of old pollarded trees (11%) and crown shoots of old trees (2%) (Fig. 2) and produced more roots per rooted cutting. The IBA treatment significantly affected the percentage of rooted cuttings, the number of roots per rooted cutting, and the root length. Mean root length did not differ between cuttings from seedlings and resprouts (3.3 cm versus 2.7 cm), but was significantly lower (0.7 cm) for cuttings taken from 5-year old trees (Fig. 2). Considering all stockplants together, IBA application did not improve the percentage of rooting, but the number of roots per rooted cutting increased considerably with greater IBA concentrations: up to 216% (Table 3). The number of secondary roots was unaffected by IBA application (Table 2). Significant interactions were identified between stockplant donor and IBA with respect to root length and the number of secondary roots per rooted cutting, with longer roots from cuttings originating from pollarded mature tree resprouts treated with high concentrations of IBA (Table 4, Fig. 3).

Table 2. Analysis of variance for the effect of stockplant maturation and IBA treatment on the percentage of rooted cutting (peRC), the number of root per rooted cutting (nbRC), the length of cutting longest root (leRC) and the number of secondary root (nbSR) of *Khaya senegalensis*.

Source	DF	peRC		DF	nbRC		leRC (cm)		nbSR	
		F	P		F	P	F	P	F	P
Rep (R)	6	0.85	0.550	6	0.94	0.543	0.33	0.896	1.12	0.466
Stoc (S)	3	334.93	<0.001	2	39.95	0.002	1.84	0.246	3.85	0.081
Error:										
MS(R×S)	18			5						
Rep×Stoc	18	6.35	<0.001	5	2.39	0.071	9.3	<0.001	3.01	0.032
IBA	3	2.75	0.049	3	5.04	0.008	8.88	0.005	2.83	0.062
Stoc×IBA	9	0.68	0.728	3	1.81	0.174	8.06	0.008	3.73	0.026
MS(Error)	72			22						
Total	111			42						

DF, degrees of freedom; Rep, replicate; stoc, stockplant; IBA, Indole 3-butyric acid.

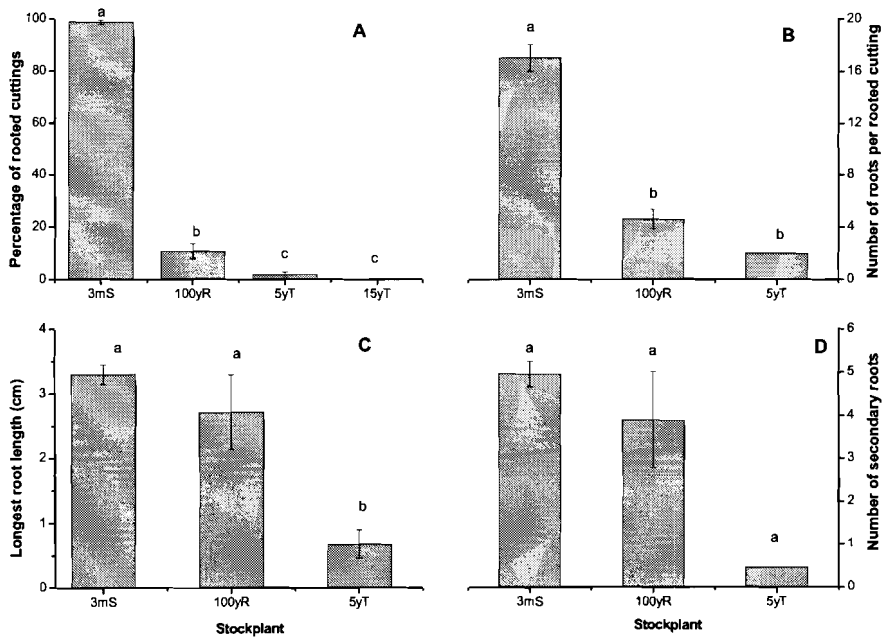


Figure 2. Main effect of stockplant donor on the percentage of rooted cuttings (A), the number of roots per rooted cutting (B), the length of the longest root (C), and the number of secondary roots (D) of *Khaya senegalensis*. 3mS, 3-month-old seedling shoots; 100yR, respouts of 100-year-old pollarded trees; 5yT, crown sprouts of 5-year-old trees; 15yT, crown sprouts of 15-year-old trees. Means followed by the same letter(s) are not significantly different at the 5% level according to Tukey's multiple comparison test.

Table 3. Main effects of Indole-3-butyric acid (IBA) on the percentage of rooted cuttings, the number of roots, the length of the longest root, and the number of secondary roots per rooted cutting of *Khaya senegalensis*. Means  $\pm$  SE (standard error) followed by the same letter(s) are not significantly different at the 5% level according to Tukey's multiple comparison test.

IBA	Rooting percentage	No. roots / rooted cutting	Longest root length (cm)	No. secondary roots
0 ppm	28 $\pm$ 8ab	7.9 $\pm$ 1.4a	2.59 $\pm$ 0.45ab	3.5 $\pm$ 0.9a
2500 ppm	30 $\pm$ 8a	10.0 $\pm$ 2.1ab	2.53 $\pm$ 0.35b	3.8 $\pm$ 0.8a
5000 ppm	28 $\pm$ 8ab	12.0 $\pm$ 2.1b	3.51 $\pm$ 0.50a	4.6 $\pm$ 0.9a
10000 ppm	26 $\pm$ 8b	17.1 $\pm$ 3.1c	3.27 $\pm$ 0.31ab	5.2 $\pm$ 1.0a

Table 4. Interactions between stockplant donors and Indole-3-butyric acid (IBA) treatments with respect to the length of the longest root and the number of secondary roots per rooted cutting of *Khaya senegalensis*. Means  $\pm$  SE (standard error) followed by the same letter(s) are not significantly different at the 5% level according to Tukey's multiple comparison test.

Stockplant	IBA (ppm)	Longest root length (cm)	No. secondary roots
Seedlings	0	3.30 $\pm$ 0.34a	4.9 $\pm$ 0.7ab
	2500	3.30 $\pm$ 0.22a	5.2 $\pm$ 0.6ab
	5000	3.48 $\pm$ 0.39a	5.2 $\pm$ 0.8ab
	10000	3.10 $\pm$ 0.24ab	4.2 $\pm$ 0.6ab
5-month resprouts*	0	1.15 $\pm$ 0.55bc	0.3 $\pm$ 0.3b
	2500	2.08 $\pm$ 0.70abc	3.1 $\pm$ 1.8ab
	5000	3.56 $\pm$ 1.33a	3.5 $\pm$ 2.0ab
	10000	3.88 $\pm$ 1.37a	8.8 $\pm$ 3.8a
5-year old trees	0	0.50 $\pm$ 0.00c	00b
	2500	0.75 $\pm$ 0.35c	00b
	5000	-	-
	10000	-	-

\* young sprouts of 100-year old pollarded trees

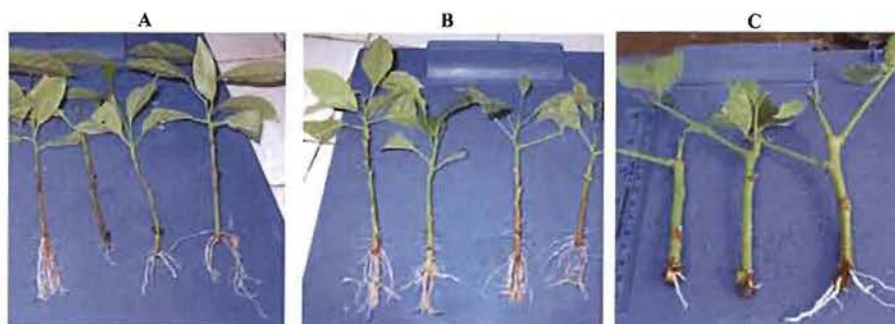


Figure 3. Roots of cuttings exposed to different concentrations of Indole Butyric Acid: 0 ppm (A) 10000 ppm (B) from 3-month seedlings and 2500 ppm (C) from young sprouts of 100-year-old pollarded trees.

When cuttings were treated with NAA, significant differences were observed among stockplant types; this was true for NAA alone or in combination with IBA. Cuttings from seedling donors produced a significantly higher percentage of rooted cuttings [ $F_{(1, 60)}=479.03$ ;  $p<0.001$ ], a greater number of roots per rooted cutting [ $F_{(1, 33)}=18.79$ ;  $p=0.001$ ], longer roots [ $F_{(1, 33)}=12.15$ ;  $p=0.001$ ] and a greater number of secondary roots per rooted cutting [ $F_{(1, 33)}=10.09$ ;  $p=0.003$ ] than cuttings from resprouts (Table 5). Cuttings treated with NAA alone had significantly [ $F_{(1, 33)}=6.4$ ;  $p=0.0163$ ] longer roots than cuttings treated with a combination NAA and IBA (Table 5), although the latter treatment tended to increase the number of roots per rooted cutting ( $F_{(1, 33)}=4.05$ ;  $p=0.052$ ). The interaction between stockplant and auxin significantly [ $F_{(1, 60)}=8.56$ ;  $p=0.005$ ] influenced the percentage of rooted cuttings. For cuttings taken from resprouts of pollarded mature trees, application of NAA alone improved rooting percentage (12%) compared to cuttings treated with a combination of NAA and IBA (0%). No significant differences were found between auxin treatments in cuttings from seedling stockplants ( $79 \pm 5\%$  versus  $88 \pm 3\%$  for NAA and NAA+IBA treatments, respectively).

Table 5. Main effects of stockplant maturation, Naphthalene acetic acid (NAA) and NAA in combination with Indole butyric acid (NAA + IBA) on the percentage of rooted cuttings, the number of roots, the length of the longest root, and the number of secondary roots per rooted cutting of *Khaya senegalensis*. Means  $\pm$  SE (standard error) followed by the same letter(s) are not significantly different at the 5% level according to Tukey's multiple comparison test.

Main effects		Rooting (%)	No. roots / rooted cutting	Longest root length (cm)	No. secondary roots
Stockplant:	Seedlings	84 $\pm$ 3a	9.4 $\pm$ 0.6a	2.25 $\pm$ 0.12a	1.5 $\pm$ 0.2a
	Resprouts	6 $\pm$ 2b	2.7 $\pm$ 0.5b	1.31 $\pm$ 0.33b	0.3 $\pm$ 0.3b
Auxin	NAA	45 $\pm$ 6a	6.7 $\pm$ 0.8a	2.18 $\pm$ 0.19a	1.3 $\pm$ 0.2a
	NAA+IBA	44 $\pm$ 7a	10.3 $\pm$ 0.8a	1.92 $\pm$ 0.13b	1.1 $\pm$ 0.3a
Concentration	1000 ppm	42 $\pm$ 10a	7.1 $\pm$ 1.0a	2.23 $\pm$ 0.28a	1.8 $\pm$ 0.5a
	2000 ppm	48 $\pm$ 10a	9.2 $\pm$ 1.2a	2.11 $\pm$ 0.27a	0.7 $\pm$ 0.2a
	3000 ppm	42 $\pm$ 9a	9.0 $\pm$ 1.2a	2.20 $\pm$ 0.28a	1.4 $\pm$ 0.4a
	4000 ppm	48 $\pm$ 10a	7.3 $\pm$ 1.4a	1.80 $\pm$ 0.20a	1.2 $\pm$ 0.3a

### 3.3 Effect of smoke solution

A low concentration (5%) smoke solution significantly inhibited the rooting of cuttings ( $F_{(2, 8)} = 11.59$ ,  $p = 0.004$ ) and the number of roots per rooted cutting, compared to the control ( $F_{(2, 8)} = 11.07$ ,  $p = 0.005$ ). Time of exposure to smoke solution did not significantly affect the rooting ability of cuttings (Table 6). We did not observe significant interactions between the concentrations of the smoke solution and exposure time. Further exposure of cuttings to low and high smoke solution concentrations had no positive effect on the rooting ability of cuttings (Table 7).

Table 6. Main effects of low concentrations of smoke solution and immersion time on the percentage of rooted cuttings, the number of roots, the length of the longest root, and the number of secondary roots per rooted cutting of *Khaya senegalensis*. Means  $\pm$  SE (standard error) followed by the same letter(s) are not significantly different at the 5% level according to Tukey's multiple comparison test.

Main effects		Rooting Percentage	No. roots / rooted cutting	Longest root length (cm)	No. secondary roots
Smoke solution	0%	100 $\pm$ 0a	10 $\pm$ 0.3a	2.92 $\pm$ 0.17a	4 $\pm$ 0.4a
	5%	88 $\pm$ 3b	7 $\pm$ 0.4b	3.28 $\pm$ 0.20a	6 $\pm$ 0.8a
	10%	93 $\pm$ 3ab	7 $\pm$ 0.3b	3.31 $\pm$ 0.28a	5 $\pm$ 0.8a
Immersion time	30 min:	92 $\pm$ 3a	8 $\pm$ 0.4a	3.01 $\pm$ 0.25a	5 $\pm$ 1a
	60 min	93 $\pm$ 3a	8 $\pm$ 0.6a	3.10 $\pm$ 0.24a	5 $\pm$ 1a
	120 min	97 $\pm$ 2a	8 $\pm$ 0.5a	3.22 $\pm$ 0.26a	5 $\pm$ 1a
	180 min	91 $\pm$ 4a	8 $\pm$ 0.6a	3.34 $\pm$ 0.26a	6 $\pm$ 1a

Table 7. Rooting ability (percentage of rooted cuttings, number of roots, length of the longest root and number of secondary roots per rooted cutting) of *Khaya senegalensis* cuttings in response to exposure to various concentrations of smoke solution for one hour. Means  $\pm$  SE (standard error) followed by the same letter(s) are not significantly different at the 5% level according to Tukey's multiple comparison test.

Smoke solution (%)	Rooting percentage	No. roots / rooted cutting	Longest root length (cm)	No. secondary roots
0	47 $\pm$ 6a	2.7 $\pm$ 0.4a	6.44 $\pm$ 0.75a	16.2 $\pm$ 3.8a
20	27 $\pm$ 17a	2.3 $\pm$ 1.3a	7.92 $\pm$ 0.66a	8.1 $\pm$ 3.3a
40	60 $\pm$ 16a	2.0 $\pm$ 0.4a	4.32 $\pm$ 0.40a	4.1 $\pm$ 1.1a
60	53 $\pm$ 14 a	2.7 $\pm$ 0.6a	6.10 $\pm$ 0.59a	6.9 $\pm$ 2.9a
80	67 $\pm$ 9a	2.2 $\pm$ 0.3a	3.95 $\pm$ 0.90a	7.5 $\pm$ 2.6a
100	53 $\pm$ 12a	2.4 $\pm$ 0.4a	3.99 $\pm$ 1.45a	6.7 $\pm$ 2.6a

## 4 Discussion

### 4.1 Effects of leaf area and cutting length

Successful rooting was restricted to leafy stem cuttings of *K. senegalensis*. This is a common response in tropical trees [15]. The inability of leafless cuttings to root has been associated with the rapid depletion of carbohydrates in stem tissues; in contrast, the concentrations in leafy cuttings tend to increase [17]. This suggests that rooting is dependent on carbohydrates formed and utilised after cuttings have been excised from the donor plant [18]. In addition to providing a source of carbohydrates, the leaf also influences the water status of the cutting. The trimming of leaves minimises water loss via transpiration while allowing sufficient photosynthesis to occur during propagation to enable root development [18, 19]. Our findings are consistent with studies of *K. ivorensis* [6, 12], *K. anthotheca* [6], *Acacia senegal* [20], *Prunus africana* [21], *Triplochiton scleroxylon* [17], and *Allanblackia floribunda* [22]. However, in contrast to these previous studies, no optimum leaf area was found in *K. senegalensis* although cuttings with a retained leaf area of 22–28 cm<sup>2</sup> seemed to root better than the others. The lack of significant differences may be due to the available carbohydrate reserves in the larger cuttings used in the present study.

Positive relationships between rooting and cutting length have been reported for a number of tropical tree species, including *Triplochiton scleroxylon* [23], *K. ivorensis* [12] and *Eucalyptus* spp. [24]. Such relationships between cutting length and rooting ability are thought to reflect the importance of carbohydrate reserves stored in the stem, which support adventitious root development [23]. The lack of any pronounced relationship between cutting length and rooting ability may be related to the much larger cuttings used in the present study. Although similar results have been recorded for *Triplochiton scleroxylon* [25] and *Allanblackia floribunda* [26], the data highlight the importance of post-severance over pre-severance carbohydrate production [18] in the rooting ability of cuttings, suggesting that leaf area might be more important than cutting length. For example, in *K. ivorensis* cutting length affected rooting ability only for cuttings with a large leaf area (100 cm<sup>2</sup>) in contrast to small (10 cm<sup>2</sup>) and medium (50 cm<sup>2</sup>) leaf areas [12].



## 4.2 Effects of stockplant maturation and auxins

The most critical factor affecting vegetative propagation of *K. senegalensis* by stem cuttings was found to be the age of the stockplant. Cuttings taken from 3- and 5-month old seedlings rooted well and produced more roots than cuttings obtained from older trees. The rooting ability (% rooting and root number) of older *K. senegalensis* cuttings was marginally improved by pollarding crown branches and by auxin application (16%) compared to cuttings from unpruned 5-year old trees (5%). Cuttings from 15-year old stockplants did not root at all.

These results are consistent with many previous studies showing that cuttings derived from juvenile stockplants are easier to root than those derived from mature stockplants [27-30] and that shoots originating from juvenile zones of the mature tree exhibit juvenile characteristics [29-32]. The superior rooting ability of cuttings from seedlings over that of trees has been attributed to the effect of changes in the woody plant developmental process that occur with increasing age; these are known as maturation or ontogenetic aging. Ontogenetic aging is often found to be most advanced in the upper parts of a tree and least advanced near the base of the trunk, with intermediate conditions between. As tree species possess meristems that are normally perennially dormant and mature more slowly than active ones, these meristems often produce vigorous sprouts (e.g. stump sprouts) after the release of dormancy [31-33], but typically these are still difficult to root. The rooting ability of juvenile cuttings may be ascribed to optimum levels of sugars and the total carbohydrate content and low nitrogen levels [30], while the reduction in rooting potential of cuttings from the stem of mature donors might be due to a decrease in the content of endogenous auxins or an accumulation of inhibitory substances [31].

Serial grafting or rooting of cuttings, annual hedging, crown-pruning and *in vitro* serial subcultures have been used to reduce the effects of aging. For instance, micrografting on *in vitro* seedlings of *K. senegalensis* facilitated rooting of microcuttings from 6-year-old trees [9]; annual pruning increased and then maintained the elevated percentage of cuttings that rooted from Douglas fir for a period of up to 14-years [34]; and hedging and repeated cutting enabled clonal propagation of Sitka spruce for up to 18 years [35]. Crown pollarding offers the opportunity to select *K. senegalensis* trees with demonstrated resistance or tolerance to mahogany shoot borer attacks. However more work is needed to enhance the rooting ability of cuttings from pollarded mature trees from more diverse environments to get a good

representation, because the present results are based on street trees, which might not be representatives of trees growing in natural stands.

Depending on the maturation of the stockplant from which cuttings had been taken, three major effects of auxin on the rooting ability of cuttings were noted: effects on root formation, the number of roots per rooted cutting and root length. The effectiveness of applied auxin in inducing rooting and in increasing the total number of roots increased with stockplant maturation. For cuttings derived from seedlings, auxin application did not influence root induction; the most significant effect of auxin application was on the number of roots per rooted cutting. Overall root number increased by up to 216% in cuttings treated with 10000 ppm IBA compared to the control. For cuttings taken from resprouts of pollarded trees, the application of high doses of auxin increased root length and the number of secondary roots. Good root systems are essential for growth and the survival of stecklings because they increase the volume of soil that can be accessed as a source of water and nutrients. Similar effects have been reported for African mahoganies and other African woody species [6, 12, 20, 21, 36].

#### 4.3 Effects of smoke solution

It has been reported that aqueous smoke extract stimulates root initiation and development as well as the growth of primary root sections of *Lycopersicon esculentum*, depending on the dilution of the smoke extracts and immersion time [37]. The active compound in the smoke (butenolide) has been shown to exhibit cytokinin- and auxin-like effects, stimulating cell division in soybean callus and rooting in mung bean [38]. However, in the present investigation there was no comparative advantage of applying smoke solution over the control. This discrepancy might be related to the concentration of the initial stock solution and the species-specific nature of the response. But lower doses of smoke solution (5-10%) were associated with more root induction and a greater number of roots than higher doses. Whether this was related to the age of the seedlings or to the smoke effect requires further investigation. However, the response to low doses of smoke solution indicates that it would be appropriate to explore further its application alone or in combination with other plant growth regulators to enhance the rooting ability of cuttings from juveniles as well as mature donors.

## 5 Conclusions

These results indicate clearly that *K. senegalensis* can be propagated vegetatively from leafy stem cuttings derived from seedlings; success was, however, very limited with cuttings from older plants. With the increasing demand for high quality timber and raw materials for pharmaceuticals, tree improvement programmes could benefit from our findings, contributing to rapid and mass propagation of *K. senegalensis*. The selection of mahogany shoot borer resistant genotypes within the seedling population could be carried out in order to enhance the establishment of plantations of *K. senegalensis* within its native range in West Africa.

## Acknowledgments

Thanks to Swedish International Development Cooperation Agency (Sida) for financial support. Special thanks to François Nandweogo, Karim Ouédraogo, Augustin Waneweogo, Rosalie T. A. Dembele, Abou Sanou and Zezouma Sanon for their help in establishing and evaluating the trials; to Modeste Meda and Djibril Dayamba for providing the smoke solution; to Ouagadougou City Hall administrator and Issa Sawadogo for permission to work on the planted roadside trees; and to Sees-editing Ltd for linguistic improvements.

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# Comparison between clonal and sexual plantlets of *Detarium microcarpum* Guill. & Perr., a savanna tree species in Burkina Faso

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## Abstract

An understanding of the proportion of true seedlings, seedling sprouts and root suckers in the forest is essential for directing the genetic composition of the future crop. We conducted a study to determine the difference between these plantlets of *Detarium microcarpum* based on morphological characters and carbohydrate contents in leaves and roots. For individuals  $\leq 50$  cm in height, root suckers had the highest values for height, stem length, internode number, root diameter, rachis length and leaflet number. The concentrations of starch and total nonstructural carbohydrates in the roots of seedling sprouts were superior. Plantlets did not differ in the concentration of leaf carbohydrates. For individuals  $> 50$  cm in height, root suckers had larger values for stem length, root diameter, leaflet length and width. Roots of seedling sprouts showed higher concentrations of soluble sugars and total soluble sugars. True seedlings were distinguished from seedling sprouts and root suckers using all morphological traits except collar diameter and leaflet number. Root suckers and seedling sprouts showed a closer morphological resemblance; thus resulted in slightly more than 50% discrimination success. In conclusion, discrimination between seedling sprouts and root suckers appeared to be more difficult than between true seedlings and clonal plantlets.

**Key words:** carbohydrates, discrimination, morphology, root sucker, seedling

## Résumé

Une bonne compréhension de la proportion de vrais semis, de rejets et de drageons en forêt est essentielle pour orienter la composition génétique du peuplement forestier. Nous avons réalisé une étude pour déterminer la différence entre les plantules de *Detarium microcarpum* en nous basant sur les caractéristiques morphologiques et la concentration en hydrate de carbone des feuilles et des racines. Parmi les individus  $\leq 50$  cm de haut, les drageons avaient les valeurs les plus élevées pour la hauteur, la longueur des tiges, le nombre des entrenœuds, le diamètre des racines, la longueur du rachis et le nombre de folioles. La concentration d'amidon et le total des hydrates de carbone non structuraux étaient supérieurs dans les racines des rejets. La concentration des hydrates de carbone dans les feuilles des plantules ne différait pas. Chez les individus  $> 50$  cm de haut, les dragons présentaient des valeurs supérieures pour la longueur de la tige, le diamètre des racines, la longueur et la largeur des folioles. Les racines des drageons présentaient une plus forte concentration de sucres solubles et du total des sucres solubles. Les semis ont pu être distingués des rejets et des drageons au moyen de toutes leurs caractéristiques morphologiques sauf le diamètre du collet et le nombre de folioles. Les drageons et les rejets montraient une ressemblance morphologique plus grande et n'ont donc permis qu'une discrimination réussie à un peu plus de 50% seulement. En conclusion, la distinction entre rejets et drageons apparaît plus difficile à effectuer qu'entre semis et plantules clonées.

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## Introduction

Most savanna species regenerate both sexually and asexually following disturbances, such as fire and selective

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logging (Bellefontaine, 1997; Bationo, Ouédraogo & Guinko, 2001; Kennedy & Potgieter, 2003; Luoga, Witkowski & Balkwill, 2004). In dry environments, such as tropical dry forests and savanna woodlands with 500–1000 mm rainfall and experiencing prolonged drought for more than 3 months per year (FAO, 2001), regeneration by root suckers is the most important clonal reproduction mechanism that occurs following disturbance of forest stands (Kozłowski, Kramer & Pallardy, 1991; Homann, 1998; Kennedy & Potgieter, 2003). Seedling resprouting is also identified as the dominant regeneration mechanism following selective cutting of Sudanian savanna woodland (Ky-Dembele *et al.*, 2007). An understanding of the relative proportion of individuals originating from seeds or vegetative buds as well as their growth and development is very essential for directing the genetic composition of the future crop while enhancing wood production.

Generally, the shoots of root suckers are known to grow faster than newly established seedlings because of the well-established root system with stored reserves (Homma *et al.*, 2003). However, sprouting especially in the case of root suckering and layering is known to increase the number of clonal individuals and reduce the genetic diversity within a population (Tredici, 2001; Eckert, 2002). In addition, suckers grow rapidly and then stop growing when carbohydrate reserves are depleted (Tew, 1970). Even though root suckers are larger than the corresponding true seedlings and have lower mortality rates (Homann, 1998; Silla *et al.*, 2002), the difference in size is not high enough to distinguish root suckers from seed origin seedlings for all woody species within different environments. For instance, root suckers of *Fitzroya cupressoides* can easily be distinguished visually from seed origin seedlings in Chile (Silla *et al.*, 2002) whereas in Burkina Faso, the only method used so far to discriminate them remains the excavation of the root system. Similar difficulties to distinguish between root suckers and seedlings originating from seeds have been reported by Bellefontaine (1997) and Sawadogo, Nygard & Pallo (2002).

Therefore, the aim of this study was to examine the difference between true seedlings, seedling sprouts and root suckers of *Detarium microcarpum* Guill. & Perr. using morphological characters, soluble sugars and starch contents as discriminating variables. *Detarium microcarpum* is a model tree species for such a study because it regenerates vigorously both sexually from seeds and vegetatively from lateral root buds once the above-ground parts have been damaged, removed or killed by harvesting or fire (Bationo

*et al.*, 2001; Sawadogo *et al.*, 2002; Ky-Dembele *et al.*, 2007). *Detarium microcarpum* is the most important commercial fuel wood species harvested from Burkina State Forests (Sawadogo *et al.*, 2002; Kabore, 2005). It is deciduous and belongs to the Caesalpinaceae family. The tree is between 8 and 10 m high and commonly grows in West African savanna (Arbonnier, 2000).

## Materials and methods

### Definitions

In this study, the term true seedling refers to a plantlet of seed origin (Homann, 1998) that had never been affected by shoot dieback. A plantlet of seed origin with progressively downward taproot (Barsoum, 2002) and affected by shoot dieback is categorized as seedling sprouts. Root suckers are shoots arising from superficial lateral root (Barsoum, 2002). All individual plantlets with height up to 120 cm are considered as seedlings.

### Plant sampling

Ninety-three naturally regenerated plantlets of *D. microcarpum* were sampled from 26 to 28 of July 2003 in the Nazinon forest, a tree and shrub savanna in Burkina Faso, located *c.* 100 km south of Ouagadougou (11°30'–11°51'N and 1°27'–1°50'W). These seedlings were categorized into two size classes: class 1 composed of individuals with height up to 50 cm and class 2 consisted of individuals with height ranging from 51 to 120 cm. The numbers of root suckers and seedling sprouts were 22 and 23, respectively in class 1, and 26 and 22, respectively, in class 2. True seedlings (fourteen individuals) were raised in a greenhouse for 30 days. From each plantlet, two leaves out of four leaves along the stem were collected and placed under slight press. In the case of branched plantlets, the same scheme was followed for one out of two branches. The rest of the leaves were collected and preserved in paper bags while a portion of the main root under the root collar was collected from each plantlet with a 4-mm diameter auger for carbohydrate analyses.

### Morphological characters measurement

Morphological characters of each plantlet related to canopy coverage, stem shape, root and leaf dimensions were measured (Table 1). These morphological characters were

**Table 1** Description of seedling morphological characters used as discriminating variables in the study

Characters	Description
Canopy 1	Highest width of the canopy
Canopy 2	Width perpendicular to canopy 1
Seedling height	From soil surface to terminal bud, vertically
Seedling length	The stem length
Collar diameter	Diameter at transition zone between stem and root aboveground
Internode number	The number of portion of a stem between the level of insertion of two successive leaves or branches
Root diameter for root sucker	Mean of diameter of the root at each part, toward the mother tree and the opposite site
Root diameter for seedling sprouts and true seedling	Tap root diameter, under the transition zone between stem and root belowground
Rachis length	Strig or the axis of the compound leaf
Leaflet number	Number of leaflet on the rachis
Leaflet length	Length of the leaflet axis
Leaflet width	Width at the widest point on the leaflet

chosen in accordance with some botanical studies (Barnes *et al.*, 2000; Turner *et al.*, 2001). A total of 4272 leaflets from 573 leaves were measured.

#### Carbohydrate analyses

A total of 52 plantlets (nineteen root suckers, nineteen seedling sprouts and fourteen true seedlings) were selected for carbohydrate concentration analyses at the Swedish University of Agricultural Sciences – Seed Laboratory. Collected leaf and root samples were oven dried at 75°C for 3 days and ground altogether before taking samples for the analyses. As ethanol/water has proved to be a good general-purpose extraction for monosaccharides, ethanol buffers were used for sugars extraction (Chaplin, 1994). Soluble sugars (glucose, fructose and sucrose) were extracted from 5 to 100 mg of dried and ground plant material twice with 0.5 ml of 80% (v/v) ethanol and once with 1 ml of 50% (v/v) ethanol at 80°C. The insoluble material including starch was incubated for 18–20 h at 50°C with amyloglucosidase as enzyme to convert starch into glucose. The starch as glucose equivalent and the concentration of soluble sugars were determined enzymatically using a Beckman DU 600 spectrophotometer

(Boehringer, Mannheim, Germany). The absorbance was read at 340 nm and results expressed in mg g<sup>-1</sup> dry weight. Carbohydrates measured after ethanol extraction were referred to as soluble sugars, carbohydrates measured after enzymatic digestion were referred to as starch, the sum of soluble sugars was referred to as total soluble sugars (TSS) and the sum of the soluble sugars and starch was referred to as total nonstructural carbohydrates (TNC).

#### Data analyses

The mean values of all morphological character and carbohydrate data were computed for the three types of plantlet: true seedling, seedling sprout and root sucker. Analysis of variance and two-sample *t*-test were performed to determine differences among plantlet origins with Minitab 14 (Minitab Inc., State College, PA, USA). As true seedlings were all <50 cm in height, two regeneration mechanisms, root suckers and seedling sprouts, were considered in the analysis to compare class 1 and 2 individuals. Linear discriminant analysis was performed to classify plantlets according to their origin using Minitab for single variable and the software R (R Development Core Team) for multiple variables. All statistical tests were considered significant at  $P \leq 0.05$ .

## Results

#### General patterns of morphological characters

Significant morphological differences among true seedlings, seedling sprouts and root suckers were noted for all morphological characters (Table 2). The analysis of variance revealed three different groups ( $P < 0.001$ ) within class 1 individuals where root suckers had the highest values followed by seedling sprouts and then true seedlings for six traits (height, stem length, internode number, root diameter, rachis length and leaflet number). Root suckers did not differ significantly from seedling sprouts in canopy widths whereas both plantlet types differed significantly from true seedlings with respect to this variable. For class 2 individuals, plantlets differed significantly with respect to four traits out of eleven; namely stem length ( $P = 0.041$ ), root diameter ( $P = 0.019$ ), leaflet length ( $P = 0.000$ ) and leaflet width ( $P = 0.000$ ). Root suckers had the highest values for stem length and root diameter whereas they displayed the lowest values for leaflet length and width.

**Table 2** Morphological characters of seedlings with height up to 50 cm (class 1) and with height within 51–120 cm (class 2) from three regeneration mechanisms (root sucker, seedling sprout and true seedling)

Character	Class 1		Class 2	
	Origin	Mean ± SE	Origin	Mean ± SE
Height (cm)	Root sucker	34.5 ± 2.1a	Root sucker	73.5 ± 3.5a
	Seedling sprout	27.4 ± 2.1b	Seedling sprout	69.4 ± 2.6a
	True seedling	6.7 ± 0.4c		
Stem length (cm)	Root sucker	52.9 ± 4.8a	Root sucker	86.6 ± 3.6a
	Seedling sprout	31.8 ± 2.8b	Seedling sprout	76.8 ± 2.8b
	True seedling	7.0 ± 0.4c		
Collar diameter (cm)	Root sucker	0.7 ± 0.1a	Root sucker	1.2 ± 0.1a
	Seedling sprout	0.5 ± 0.0b	Seedling sprout	1.1 ± 0.1a
	True seedling	0.4 ± 0.0b		
Canopy1 (cm)	Root sucker	34.3 ± 3.2a	Root sucker	46.4 ± 2.7a
	Seedling sprout	28.0 ± 1.5a	Seedling sprout	48.0 ± 2.3a
	True seedling	9.0 ± 0.6b		
Canopy2 (cm)	Root sucker	17.3 ± 1.4a	Root sucker	28.8 ± 3.0a
	Seedling sprout	16.6 ± 1.1a	Seedling sprout	28.8 ± 2.7a
	True seedling	5.5 ± 0.4b		
Internode number	Root sucker	8.9 ± 1.1a	Root sucker	14.4 ± 0.9a
	Seedling sprout	4.6 ± 0.5b	Seedling sprout	13.0 ± 0.8a
	True seedling	1.4 ± 0.1c		
Root diameter (cm)	Root sucker	6.8 ± 0.6a	Root sucker	5.6 ± 0.5a
	Seedling sprout	1.9 ± 0.3b	Seedling sprout	4.1 ± 0.3b
	True seedling	0.2 ± 0.0c		
Rachis length (cm)	Root sucker	9.8 ± 0.1a	Root sucker	10.7 ± 0.1a
	Seedling sprout	7.7 ± 0.1b	Seedling sprout	10.9 ± 0.1a
	True seedling	2.6 ± 0.1c		
Leaflet number	Root sucker	6.9 ± 0.0a	Root sucker	7.2 ± 0.0a
	Seedling sprout	5.8 ± 0.1b	Seedling sprout	7.2 ± 0.0a
	True seedling	5.4 ± 0.1c		
Leaflet length (cm)	Root sucker	5.7 ± 0.0b	Root sucker	6.2 ± 0.0b
	Seedling sprout	6.2 ± 0.1a	Seedling sprout	6.8 ± 0.0a
	True seedling	2.5 ± 0.1c		
Leaflet width (cm)	Root sucker	3.3 ± 0.0b	Root sucker	3.5 ± 0.0b
	Seedling sprout	3.6 ± 0.0a	Seedling sprout	3.8 ± 0.0a
	True seedling	1.2 ± 0.0c		

Mean values followed by the same letter for each character are not significantly different at 5% level using Bonferroni's for class 1 and 2-sample *t*-test for class 2.

Considering all plantlets together, class 1 and 2 individuals differed significantly in all eleven morphological traits ( $P < 0.0001$ ) with class 1 displaying the lowest values except root diameter ( $P = 0.137$ ) that did not differ between the two classes.

#### Carbohydrate contents

Mean concentrations of glucose, fructose, sucrose, TSS, starch and TNC in leaves and root of true seedlings, root suckers and seedling sprouts are shown in Table 3. Roots

were the dominant organ for carbohydrate storage in all plantlets from three modes of regeneration. The analysis of variance indicated that only starch ( $P < 0.001$ ) and TNC ( $P < 0.001$ ) concentrations were significantly different among true seedlings, seedling sprouts and root suckers in class 1. Starch and TNC concentrations in the roots of seedling sprouts were higher than in the roots of true seedlings and root suckers, whereas no differences were found in concentration of carbohydrates in leaves. For class 2 individuals, carbohydrate in leaves did not show a significant difference between seedling sprouts and root

**Table 3** Carbohydrate concentration (mg g<sup>-1</sup> dry weight) in leaf and root samples for individuals with height up to 50 cm (class 1) and with height within 51–120 cm (class 2)

Tissue	Origin	Glucose	Fructose	Sucrose	TSS	Starch	TNC
<b>Class 1</b>							
Leaves	Root sucker	18.5 ± 3.3a	21.8 ± 3.4a	32.0 ± 3.8a	72.3 ± 9.9a	2.3 ± 0.4c	74.6 ± 9.8c
	Seedling sprout	26.8 ± 5.7a	31.5 ± 6.0a	41.6 ± 7.0a	99.9 ± 18.6a	1.3 ± 0.3c	101.2 ± 18.7c
	True seedling	11.9 ± 2.3a	13.3 ± 2.4a	44.4 ± 3.8a	69.5 ± 7.5a	18.2 ± 4.8c	87.7 ± 8.7c
Root	Root sucker	19.0 ± 6.0a	19.9 ± 6.6a	47.4 ± 12.5a	86.3 ± 22.1a	85.0 ± 10.8b	171.3 ± 24.7b
	Seedling sprout	14.1 ± 4.9a	17.9 ± 5.5a	39.0 ± 8.7a	71.0 ± 14.8a	224.7 ± 36.2a	295.7 ± 27.6a
	True seedling	17.1 ± 2.1a	21.1 ± 2.4a	65.3 ± 5.8a	103.4 ± 8.1a	22.3 ± 5.2c	125.7 ± 11.7bc
<b>Class 2</b>							
Leaves	Root sucker	13.5 ± 3.4a	16.8 ± 4.0a	29.5 ± 4.3a	59.8 ± 10.7a	2.7 ± 1.3a	62.4 ± 10.8a
	Seedling sprout	12.1 ± 2.3a	15.3 ± 2.8a	25.3 ± 3.5a	52.7 ± 8.1a	1.8 ± 0.4a	54.5 ± 8.3a
Root	Root sucker	08.9 ± 2.1b	12.5 ± 2.8b	39.8 ± 11.1a	61.1 ± 13.6b	126.9 ± 30.7a	188.0 ± 28.0a
	Seedling sprout	29.9 ± 6.5a	32.3 ± 7.2a	82.7 ± 21.0a	145.0 ± 29.5a	144.1 ± 31.3a	289.1 ± 43.3a

Values are given as mean ± SE.

TSS, total soluble sugars; TNC, total nonstructural carbohydrate.

For class 1, mean values followed by the same letter within a column are not significantly different at 5% level using Bonferroni's. For class 2, mean values followed by the same letter for a given tissue, leaves or root are not significantly different at 5% level using 2-sample *t*-test.

suckers while glucose, fructose and TSS concentrations in roots were significantly higher for seedling sprouts than for root suckers. The concentrations of glucose ( $P = 0.034$ ), fructose ( $P = 0.030$ ) and TNC ( $P = 0.042$ ) in leaves of seedling sprouts were higher in class 1 than in class 2. The TSS concentration in roots of seedling sprouts was significantly higher ( $P = 0.033$ ) for class 2 individuals than for class 1 individuals. Carbohydrate concentration in leaves and roots of root suckers did not vary significantly between classes.

#### Classification of plantlets according to their origin

Linear discriminant analysis carried out on morphological characters showed that true seedlings could be fully distinguished with nearly all morphological traits except collar diameter and leaflet number (Table 4, Fig. 1a). Most morphological traits of true seedlings were smaller in magnitude compared with root suckers and seedling sprouts. Root suckers and seedling sprouts within class 1 were correctly classified with a maximum of 73% accuracy. Stem length, internode number and root diameter accounted for 70% correct classification of true seedlings, root suckers and seedling sprouts. Within class 2, the best classifying single variable between seedling sprouts and root suckers was canopy1 with 63% correct classification accuracy (Table 4). The combination of height, stem length, stem diameter, canopy widths and internode

number resulted in 76% and 63% classification accuracy for class 1 and 2 individuals, respectively (Table 4, Fig. 1a,b).

Based on the carbohydrate concentration in leaves, 71% of true seedlings were correctly classified by glucose and fructose concentrations while the combination of all sugars (glucose, fructose, sucrose and starch) resulted in 86% correct classification of true seedlings (Table 5). Root suckers and seedling sprouts in class 1 were discriminated with 70% and with 56% in class 2 using carbohydrate concentrations in leaves as discriminating variables. Carbohydrate moieties either in leaves or roots could not correctly separate true seedlings, root suckers and seedling sprout within class 1 (Table 5, Fig. 1c). However, root glucose alone resulted in 100% classification of root suckers in class 2. The maximum proportion of correctly classified seedling sprouts from class 2 was 67% using glucose and the combination of soluble sugars and starch as discriminating variables (Table 5, Fig. 1d).

## Discussion

#### Morphological characters and carbohydrate contents

Root suckers had the highest values for almost all traits of stem, canopy and leaf for individuals with shoot height <50 cm (class 1), followed by seedling sprouts and then true seedlings (Table 2). These results are in agreement

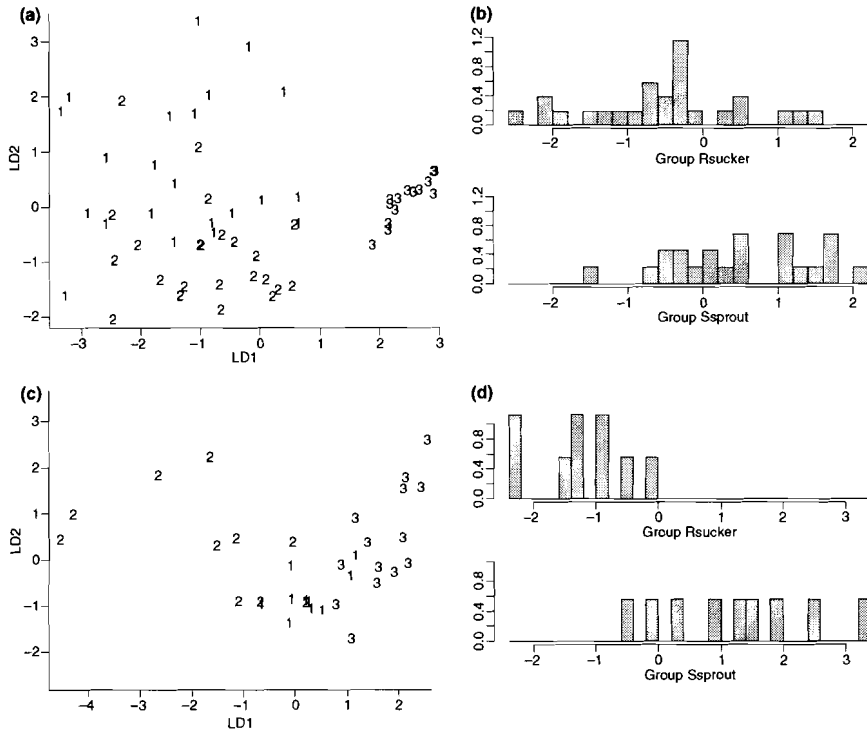
**Table 4** Proportion of correctly classified seedlings per regeneration mechanism (root sucker, seedling sprout and true seedling) by means of morphological character variables using linear discriminant analysis for plants up to 50 cm height (class 1) and plants of 51–120 cm height (class 2)

Variable	Root sucker	Seedling sprout	True seedling	All origins
<b>Class 1</b>				
Height	0.64	0.52	1	0.68
Stem length	0.68	0.52	1	0.70
Collar diameter	0.73	0.30	0.57	0.53
Canopy1	0.55	0.52	1	0.64
Canopy2	0.46	0.35	1	0.54
Internode number	0.55	0.73	1	0.72
Root diameter	0.73	0.52	1	0.71
Height, stem length, stem diameter, canopy1, canopy2, internode number	0.55	0.83	1	0.76
Rachis length	0.66	0.51	1	0.65
Leaflet number	0.62	0.50	0.50	0.57
Leaflet length	0.46	0.58	1	0.55
Leaflet width	0.53	0.56	1	0.59
Rachis length, leaflet length, leaflet width, leaflet number	0.73	0.72	1	0.75
Variable	Root sucker	Seedling sprout	All origins	
<b>Class 2</b>				
Height	0.50	0.50	0.50	
Stem length	0.62	0.55	0.58	
Collar diameter	0.42	0.64	0.52	
Canopy1	0.73	0.50	0.63	
Canopy2	0.35	0.64	0.48	
Internode	0.54	0.50	0.52	
Root diameter	0.50	0.73	0.60	
Height, stem length, stem diameter, canopy1, canopy2, internode number	0.65	0.59	0.63	
Rachis length	0.53	0.51	0.52	
Leaflet number	0.58	0.43	0.52	
Leaflet length	0.60	0.54	0.57	
Leaflet width	0.55	0.55	0.55	
Rachis length, leaflet length, leaflet width, leaflet number	0.62	0.57	0.60	

with the opinion that root suckers grow faster than sexually reproduced seedlings (Silla *et al.*, 2002; Homma *et al.*, 2003). Similar results were reported by Homann (1998) and Kennard *et al.* (2002) who found that height, crown area, stem diameter or number of stems of root suckers were significantly greater than seed origin seedlings. The initial growth might be relatively more important for root suckers so that class 1 root suckers were even more difficult to find during the fieldwork in agreement with Homma *et al.* (2003) that had reported the rarity of middle sized suckers with a height growth ranging from 12 to 40 cm year<sup>-1</sup>. However, within individuals higher than 50 cm (class 2), root suckers showed higher values than

seedling sprouts only in stem length and root diameter (Table 2). The leaflet length and width were the variables with significantly higher values in seedling sprouts within both class 1 and 2. This might be an advantage for increased production of photosynthate because of increased leaf surface area that appears more essential for the juvenile stage of sexually reproduced seedlings than clonal plants.

The root size appeared to be important to differentiate classes of seedling sprouts in contrast to root suckers where the analysis did not show any difference between class 1 and 2. The success and growth of resprouting individuals from seed origin might be correlated with tap root size.



**Fig 1** Linear discriminant analysis. The classification of root sucker (1, Rsucker), seedling sprout (2, Ssprout) and true seedling (3) based on morphological characters (a, b; height, stem length, stem diameter and canopy widths) and carbohydrate contents (c, d; glucose, fructose, sucrose and starch from root) for plants up to 50 cm height, class 1 (a, c) and plants of 51–120 cm height, class 2 (b, d)

This variable was significantly higher for class 2 in comparison with class 1 individuals (4.1 cm and 1.9 cm of mean diameter, respectively). This supports previous results on the importance of root growth to insure seedling survival and growth within disturbed biomes such as savannas (Cruz *et al.*, 2002; Luoga *et al.*, 2004). However, seedling-resprouting success could also depend on the size of above-ground part as reported by Sakai & Sakai (1998) who found that *Euptelea polyandra* could not sprout sufficiently without a considerably large volume of above-ground parts. Sprouting abilities of plants have been ascribed to higher levels of resources, particularly starch, in plant tissues (Iwassa & Kubo, 1997; Bell & Ojeda, 1999). For instance, Bell & Ojeda (1999) found that *Erica* seeder species had consistently lower amounts of root starch than resprouters. The present study also showed high variability

in starch and TNC concentrations among regeneration mechanisms of individuals of the same species, *D. microcarpum*, within the same environmental conditions.

For small size individuals (class 1), starch and TNC concentrations in root samples of seedling sprouts were higher than corresponding samples of root suckers and true seedlings (Table 3). However, in class 2, starch and TNC did not differ significantly between plantlet types probably because of high variability in the data. Glucose, sucrose, fructose and TSS concentrations were higher in root samples of seedling sprouts than in those of root suckers within class 2 (Table 3). Starch conversion into sugars might explain the high level of soluble sugars in roots of class 2-seedling sprouts. For class 1 individuals, the depletion of starch might be minor as a result of shoots die back during the dry season in contrast to class 2 in

**Table 5** Proportion of correctly classified seedlings per regeneration mechanism (root sucker, seedling sprout and true seedling) by means of carbohydrate concentrations in leaves and root as variables for plants up to 50 cm height (class 1) and plants of 51–120 cm height (class 2)

Class	Variable	Carbohydrate in leaves				Carbohydrate in root			
		Root sucker	Seedling sprout	True seedling	All origins	Root sucker	Seedling sprout	True seedling	All origins
1	Glucose (Gl)	0.00	0.50	0.71	0.44	0.40	0.60	0.21	0.38
	Fructose (Fr)	0.30	0.50	0.71	0.53	0.10	0.60	0.50	0.41
	Sucrose (Su)	0.60	0.20	0.36	0.38	0.20	0.60	0.79	0.56
	TSS	0.20	0.50	0.60	0.40	0.00	0.70	0.64	0.47
	Starch (St)	0.70	0.70	0.43	0.59	0.80	0.70	0.93	0.82
	TNC	0.50	0.50	0.20	0.40	0.20	0.70	0.79	0.59
2	Gl+Fr+Su+St	0.60	0.60	0.86	0.71	0.60	0.60	0.78	0.68
	Glucose	0.44	0.56		0.50	1	0.67		0.83
	Fructose	0.44	0.56		0.50	0.78	0.56		0.67
	Sucrose	0.56	0.44		0.50	0.78	0.44		0.61
	TSS	0.56	0.56		0.56	0.89	0.56		0.72
	Starch	0.33	0.56		0.44	0.78	0.56		0.67
	TNC	0.56	0.56		0.56	0.78	0.56		0.67
	Gl+Fr+Su+St	0	0.22		0.11	0.78	0.67		0.72

which shoot die back might be less pronounced. Because larger individuals, which are more resistant with thicker bark preventing disturbance damage (Wilson & Witkowski, 2003), may need also more resources for maintaining their shoot alive. This is in accordance with Kozłowski (1992) who reported that starch–sugar conversions are common in woody plants and that starch is transformed to sugars whenever sugar levels are low. The difference in carbohydrate contents between class 1 and 2 individuals was more perceptible for seedling sprouts than root suckers. In leaves of seedling sprouts, glucose, fructose and TNC were higher for class 1 individuals while TSS concentrations were higher in roots of class 2 individuals. Plants that were repeatedly cut maintain a higher proportion of carbohydrate reserves as readily transportable and usable sugars (Latt, Nair & Kang, 2000). It is also known that allocation of resources to belowground stores reduces growth by detracting from the construction of resource gaining organs (roots and leaves), thereby reducing the potential for further growth (Mcpherson & Williams, 1998).

Root suckers and seed origin individuals on one hand, class 1 and 2 seedling sprouts on the other hand might have different features regarding carbohydrate allocation. According to Iwassa & Kubo (1997), the storage is used as a shock-absorber of disturbance and that the plant chooses the pattern of growth, reproduction, storage and recovery after disturbances by reallocation of stored material to maximize the total lifetime reproductive success. It seems likely that root suckers allocate fewer resources to the

belowground parts than seedling sprouts but, more studies would be needed to understand better the difference in resources allocation of true seedlings, seedling sprouts and root suckers of *D. microcarpum*. The concentrations of carbohydrate found in true seedlings were not significantly different from that of root suckers. As noted by Kabeya & Sakai (2003), the carbohydrates in seedlings root at that early stage might be translocated from the cotyledons and are considered as stored reserve than resources used for immediate growth. These stored reserves in roots could support successful resprouting and enhance seedling adaptation to environmental stresses and disturbances, the main causes of seedling shoot dieback (Bond & Midgley, 2001; Kabeya *et al.*, 2003).

#### *Discrimination of plantlet according to origin*

The success of differentiating seedling sprouts from root suckers using morphological characters as well as carbohydrate concentrations in leaves and roots is limited (Fig. 1). Except for true seedlings, none of the morphological variables resulted in more than 63% accuracy in distinguishing seedling sprouts from root suckers. This indicates the difficulty to distinguish root suckers from seed origin individuals based on morphological observations, as also reported by Bellefontaine (1997) and Sawadogo *et al.* (2002). Out of eleven characters evaluated, only stem length, internode number and root diameter discriminated the three regeneration mechanisms with 70%, 72% and



71% accuracies respectively. Within leaf characters, the most important variable was rachis length, which correctly classified 65% of class 1 seedlings into three groups of plantlets. Among class 2 individuals, 52% was correctly classified using leaflet length. The classification accuracy varied between 30% and 73% for seedling sprouts, indicating high variability in growth behaviour of seedling sprouts (Fig. 1a,b). Carbohydrate concentrations in roots seemed more important for classifying plantlets according to origin even though the maximum classification accuracy was about 80% (Table 5, Fig. 1c,d). The variables that individually provided the best discrimination accuracy between plantlet groups were starch (82%) for class 1 individuals and glucose (83%) for class 2 individuals.

The resemblance in morphological characters between seedling sprouts and root suckers, especially within class 2 individuals, could be explained by a relative similarity in their growth performance. The rate of growth may not differ much between seedling sprouts and root suckers because of their well-established root system and the high concentration of carbohydrate reserve. It has been reported that a vigorous resprouting response would be favoured by a greater allocation to storage in the root (Cruz *et al.*, 2002) as a larger root system of sprouts would offer more surface area for water and nutrient uptake (Kennard *et al.*, 2002). While seedling sprouts and root suckers can draw up reserves in pre-existing root systems, true seedlings must produce both above and belowground tissues. Seedling sprouts and root suckers have a rapid growth because they benefit from their well-established root system contrary to true seedlings. However, there is a carbon cost of maintaining an extensive root system with only sprouting photosynthetic tissue (Miller & Kauman, 1998). Seedlings with balanced root/shoot ratios such as root suckers may be able to allocate more resources to aboveground tissue than seedling sprouts can, which have to export more carbon to the belowground to support larger root systems (Miller & Kauman, 1998). Nonetheless, future work to elucidate survival and growth patterns of root suckers compared with seedling sprouts or true seedlings should be attempted to evaluate the morphological growth rhythm according to the root size and the seasonal variations in carbohydrate of each regeneration mechanism.

In conclusion, the present results illustrate that true seedlings ( $\leq 50$  cm) can be distinguished from seedling sprouts and root suckers using shoot morphological traits except root collar diameter and leaflet number. Starch

concentration in roots and the sum of glucose, fructose, sucrose and starch in leaves resulted in higher classification rate of true seedlings. Root suckers and seedling sprouts have a closer morphological resemblance; especially for individuals with height more than 50 cm, thus resulted in slightly more than 50% discrimination success. The well-established root system and the high carbohydrate concentrations of seedling sprouts might favour a growth performance comparable to that of root suckers. Thus, future work is needed to evaluate the growth of root suckers compared with seedling sprouts regarding age, root size, above ground morphological characters and carbohydrate allocation to roots and shoots.

### Acknowledgements

The study was financially supported by Swedish International Development Agency (Sida) and Centre National de la Recherche Scientifique et Technologique (CNRST), Burkina Faso. Special thanks to Daniel Kibora who measured the leaflet length and width, to Didier Zida and Niyaidouba Lamien for valuable discussions, to Margareta Söderström for laboratory assistance.

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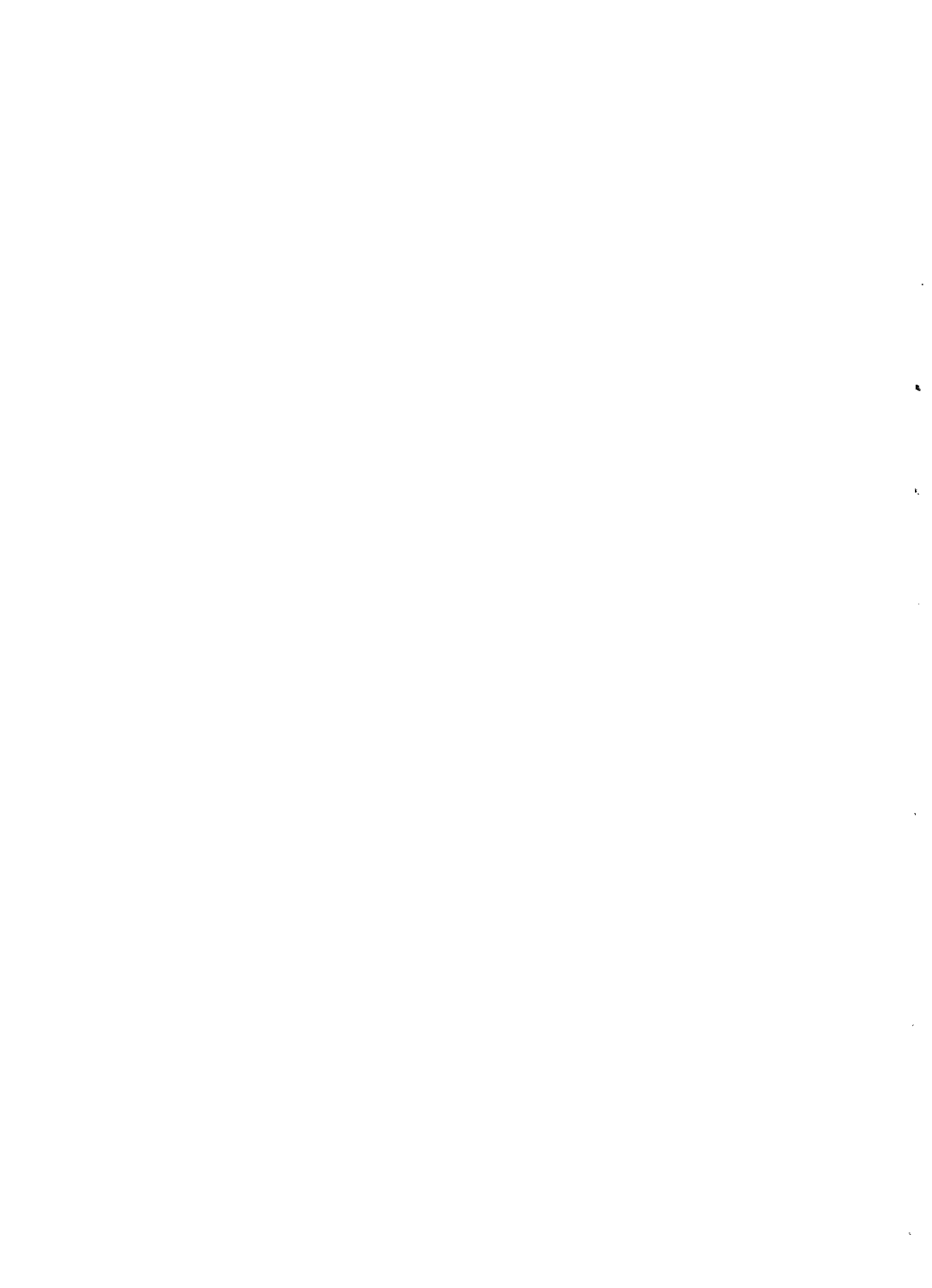
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(Manuscript accepted 16 November 2007)

doi: 10.1111/j.1365-2028.2007.00912.x





## Comparison of Growth Responses of *Khaya senegalensis* Seedlings and Stecklings to Four Irrigation Regimes

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Ky-Dembele, C., Bayala, J., Savadogo, P., Tigabu, M., Odén, P.C. & Boussim, I.J. 2010. Comparison of growth responses of *Khaya senegalensis* seedlings and stecklings to four irrigation regimes. *Silva Fennica* 44(5): 787–798.

*Khaya senegalensis* is an important tree species for timber production, native to West Africa, but mahogany shoot borer attacks prevent successful plantations. This research was aimed at comparing the growth of two propagule types, seedlings and stecklings, of *Khaya senegalensis* subjected to four irrigation regimes, 25, 50, 75 and 100% field capacity in Burkina Faso. The relative growth rate, biomass allocation and intrinsic water use efficiency of the propagules were assessed in a full-factorial pot experiment in block design. Except the relative growth rate of stem basal diameter and specific leaf area, for which mean values were significantly higher for seedlings than stecklings, the two propagule types had similar growth patterns regarding relative growth rates of stem length, leaf, stem, root and the total plant biomass. There was no significant difference between propagule types concerning biomass fraction to total plant biomass of leaf, stem and root, root to stem ratio, leaf area productivity and carbon isotope ratio ( $\delta^{13}\text{C}$ ). However, the irrigation regimes significantly affected all parameters. In contrast to 75 and 100% field capacity irrigation regimes, the low water supply of 25 and 50% field capacity resulted in plant stress, which was evident from the significant reduction in plant growth and biomass production and an increase in the root biomass to total plant biomass ratio and  $\delta^{13}\text{C}$ . It can be concluded that seedlings and stecklings have comparable growth patterns, while water stress is a major growth-limiting factor highlighting the need for selecting drought and borer resistant genotypes for successful plantations.

**Keywords** rooted cuttings, water stress, Senegal mahogany

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**Received** 6 September 2010 **Revised** 10 November 2010 **Accepted** 19 November 2010

**Available at** <http://www.metla.fi/silvafennica/full/sf44/sf445787.pdf>

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# 1 Introduction

*Khaya senegalensis* A.Juss. (Meliaceae), also known as acajou caïcedrat (French) or Senegal mahogany (English), is the most suitable indigenous tree species for timber production in Burkina Faso. Growing up to 35 m in height and 1.5 m in diameter on fertile soil, with an 8–16 m clean bole, its wood is hard, dense and red, resistant to fungi and termites. It is valued for carpentry, joinery, furniture making, cabinet work, ship building and in the production of decorative veneers (Nikiema and Pasternak 2008). But its natural regeneration is poor, and mahogany shoot borer *Hypsipyla robusta* (Moore) attacks prevent the success of plantations within the native area in West Africa (Newton et al. 1993, Nikiema and Pasternak 2008). Cloning resistant individuals to *Hypsipyla* has been sought as a solution for enhancing plantation establishment and production (Newton et al. 1993, Danthu et al. 2003).

Accordingly, our preliminary investigations have indicated that juvenile *K. senegalensis* plants are amenable to clonal propagation, allowing screening from seedling populations and multiplication of eventual resistant genotypes by stem cuttings. However, in savanna areas which are subjected to seasonal drought, such as Burkina Faso, water stress is known to be a key factor limiting plant growth, survival and productivity (Wilson and Witkowski 1998), and it often adversely affects forest plantations and agroforestry practices. It is considered to be a major cause of failure during re-establishment while also affecting seedling ability to use water efficiently, crucial to their post-planting survival (Margolis and Brand 1990, Sun et al. 1996). In addition, survival and initial growth of seedlings may be associated with one or more other factors, such as the mode of propagation, plant quality and age, silvicultural practices, browsing, fire and other disturbances (Zida et al. 2008, Bayala et al. 2009). Since the climate predictions for African savanna areas suggest an increase in the severity of droughts (Sheffield and Wood 2008), the ability to adapt to drought ought to be an important consideration when selecting genotypes to plant.

Because of problems associated with vegetative propagation such as cyclophysis or topophysis, field testing has been necessary to evaluate prop-

agule types and to quantify differences between vegetative propagules and seedlings (Frampton Jr and Foster 1993). As for a short term evaluation, pot trials have been a useful tool for clone testing prior to extensive field evaluation (Weih and Nordh 2002), a pot experiment was initiated to compare the growth pattern of seedlings and stecklings (plantable rooted cuttings) of *K. senegalensis* submitted to water stress by means of relative growth rate (RGR) and intrinsic water-use efficiency (WUE) based on carbon isotope ratio ( $\delta^{13}\text{C}$ ) in leaves. Stable carbon isotope ratio is a measure of the heavy isotope ( $^{13}\text{C}$ ) to the light isotope ( $^{12}\text{C}$ ) ratio (Lajtha and Michener 1994).

Relative growth rates are frequently used to compare the growth of seedlings that differ in initial size in order to eliminate any growth differences related to size and to determine which seedlings are inherently more efficient (Hunt 1982). Seedlings and stecklings of *K. senegalensis* may vary in production of biomass, resource allocation and adaptations to drought. Water use efficiency is a functional characteristic which is related to plant growth and performance under drought conditions. It is defined as the amount of carbon biomass produced per unit water transpired by the crop and corresponds to the transpiration efficiency in  $\text{C}_3$  plants (Farquhar et al. 1989). In theory, increasing WUE could affect plant growth. Moreover, measurement of WUE has been simplified by the discovery of a strong correlation between WUE and stable carbon isotope discrimination in  $\text{C}_3$  plants (Farquhar et al. 1989, Devitt et al. 1997). Commonly used for screening cultivars of dryland crops and rangeland grass species (Lajtha and Michener 1994), carbon isotope discrimination is becoming a valuable tool in tree breeding (Brendel et al. 2002, Raddad and Luukkanen 2006). The selection of genotypes with high  $\delta^{13}\text{C}$  and, therefore, high WUE, would have the potential to increase growth of total tree biomass in arid environments, such as the Sudanian zones of Burkina Faso (Farquhar et al. 1982, Hall et al. 1994, Sun et al. 1996). Thus, in order to generate information that could be used for the selection of improved plant materials suitable for propagation and successful plantations in Burkina Faso, an experiment was conducted to determine the effect of four irrigation regimes on growth, biomass allocation, foliar  $\delta^{13}\text{C}$  of two

propagule types, stecklings and seedlings, of *K. senegalensis*.

## 2 Materials and Methods

### 2.1 Plant Material and Experimental Design

The experiment was performed outdoors at the Forest Productions Department (DPF) of the Environmental and Agricultural Research Institute (INERA) in Ouagadougou, Burkina Faso. Seedlings and stecklings originated from a common seed source purchased from the National Seed Centre (CNSF) in Burkina Faso. Seeds were collected in 2008 from Tiakaré village (11°11'N–1°12'W) in Nahouri province, Burkina Faso. The seedlings were grown first in black perforated polythene bags (7 cm diameter × 25 cm height), which were filled with a mixture of sand, arable soil and manure (2:2:1 v/v/v), in the nursery at DPF. Ten-centimeter-long cuttings were collected from 3-month old seedlings and rooted in a 1:1 (v:v) perlite/sand medium in a mist greenhouse for two months. Rooted cuttings or stecklings were planted in plastic bags (20 cm diameter × 30 cm height), filled as previously described. After sprouting and then growing for about four months, 54 healthy stecklings, all approximately the same size, were chosen and replanted into 6-L plastic buckets filled with the arable soil, sand and manure mix. Concomitantly, five-month old seedlings (54 in total) which had previously been grown in plastic bags (7 cm diameter × 25 cm height) were transferred into 6-L plastic buckets. The bottom of each bucket has been manually perforated by means of five holes. Both, propagule types, seedlings and stecklings originating from seeds and rooted cuttings, respectively, were then placed in full sun and grown for 12 weeks, from September 28, 2009. The buckets were placed flat on the ground. All plants were watered once a day until October 12, when six individuals of each propagule type were selected at random for the initial harvest, which data were used in the assessment of growth rate. At this time, the mean length of stems was 21.6±0.6 cm (seedlings) and 17.7±0.7 cm (stecklings). The remaining 48 seedlings and 48 stecklings were used in a

completely randomized block design experiment with two factors, propagule type (seedlings and stecklings) and irrigation regime (25, 50, 75 and 100% field capacity). Three plants were randomly assigned accordingly to each of the eight experimental treatment units and arranged randomly in each of the four blocks (3 plants × 2 propagule types × 4 irrigation regimes × 4 blocks).

Field capacity was estimated by measuring the amount of water held in the soil of 12 control pots, which had been fully wetted, covered and weighed after 2 days of drainage. From October 13 until December 20, 2009, the pots were weighed every 72 hours and watered according to the appropriate irrigation regime by supplementing the soil's water content with a percentage (25, 50, 75 or 100%) of the field capacity adjusted for the plant biomass. Plant diameters were measured; biomass was, however, estimated from regressions of the basal diameters and fresh biomass of seedlings and stecklings determined at initial harvest and these data were used for two consecutive irrigation periods.

### 2.2 Harvest Procedure and Carbon Isotope Analysis

The initial harvest was carried out on October 13, 2009 and involved 6 seedlings and 6 stecklings; the second was on 22 December, 10 weeks after the treatments began. At both harvests, the stem length and basal diameter of all plants were recorded. Harvested plants were separated into leaves, stems and roots. The root systems were gently washed with tap water. The total leaf area of fresh leaves was measured with a laser area meter (CI-202, CID Inc., USA). The dry biomass of the stems, leaves and roots was determined after drying at 70 °C for 48 hours. The total dry biomass of the plant was calculated by summing the stem, root and leaf dry biomass. The dry biomass is henceforward referred as biomass.

As chemical analyses are expensive, only foliar samples of seedlings and stecklings subjected to watering regimes 50 and 100 % field capacity were analyzed, to determine their carbon isotope ratios, using a mass spectrometer in the Radio Carbon Dating Laboratory at the University of Helsinki, Finland. The carbon isotope ratio of the

sample ( $\delta^{13}\text{C}_{\text{sample}}$ ) was expressed as

$$\delta^{13}\text{C}_{\text{sample}} (\text{‰}) = [(R_{\text{sample}}/R_{\text{PDB}}) - 1] \times 1000 \quad (1)$$

where  $R_{\text{sample}}$  is the carbon isotope molar abundance ratio  $^{13}\text{C}/^{12}\text{C}$  of the sample and  $R_{\text{PDB}}$  is the Pee Dee Belemnite standard for carbon, the usual standard to which all measurements are referred (Lajtha and Michener 1994, Raddad and Luukkanen 2006).

### 2.3 Data Analysis

In order to compensate for differences in initial plant development, a functional growth analysis approach was used to compare plant growth between the two harvests (Hunt 1982). The relative growth rate (RGR) was calculated for stem, leaf, root, total plant biomass, leaf area, stem length and diameter. The RGR from initial to final harvest was calculated according to Hunt (1982):

$$\text{RGR}_A = (\ln A_F - \ln A_I) / (t_F - t_I) \quad (2)$$

$A_F$  denotes the measured trait at final ( $F$ ) harvest and  $A_I$  denotes it at the initial ( $I$ ) harvest calculated as the mean of the six plants per plant type for the destructive variables; ( $t$ ) is the time in weeks at final ( $F$ ) and initial ( $I$ ) harvest. Thus in the following text, the RGR of the stem, leaf, root and whole plant biomass, leaf area, stem length and diameter are referred to as RGRsb, RGRlb, RGRrb, RGRpb, RGRla, RGRsl and RGRsd, respectively. Leaf area productivity (plant biomass growth rate per unit of leaf area, LAP), specific leaf area (leaf area per unit of leaf biomass, SLA), leaf area ratio (leaf area per unit of plant biomass, LAR), leaf biomass ratio (leaf biomass per unit of plant biomass, LBR), stem biomass ratio (stem biomass per unit of plant biomass, SBR), root biomass ratio (root biomass per unit of plant biomass, RBR), and root to stem ratio (root biomass per unit stem biomass, RSR) were calculated using data collected at the final harvest and taken as additional variables to the RGR.

For all variables, two way-analysis of variance (ANOVA) was performed in order to compare propagule types (seedlings and stecklings),

irrigation regimes (25, 50, 75 and 100% field capacity) and the interactions between these two factors. Data were checked for normality and analyzed using the GLM procedure of the Statistical Analysis System (SAS Institute Inc., 2002–2008). Johnson transformed data were used for variables (RGRlb, RGRrb, RGRpb, RGRsl, RGRsd, SLA, LAP, and  $\delta^{13}\text{C}$ ) that did not fulfill the requirement for normality. Significant differences, when  $p < 0.05$ , were tested further using Tukey's HSD multiple comparison test.

## 3 Results

### 3.1 Plant Growth and Biomass Allocation

Except for the growth rate of the stem basal diameter and the specific leaf area, plant responses related to growth, biomass production, biomass fractions and  $\delta^{13}\text{C}$  did not differ significantly between seedlings and stecklings (Tables 1–2). Seedlings had higher stem basal diameter RGRs and a greater specific leaf area than stecklings (Table 3). With respect to the other variables associated with RGR and biomass allocation, seedlings were found to be similar to stecklings. The overall mean biomass fractions for seedlings and stecklings, respectively, were  $0.32 \pm 0.01$  and  $0.31 \pm 0.02$  for stem biomass ratio,  $0.34 \pm 0.01$  and  $0.32 \pm 0.02$  for root biomass ratio,  $1.06 \pm 0.07$  and  $1.08 \pm 0.05$  for root to stem ratio,  $0.34 \pm 0.02$  and  $0.37 \pm 0.03$  for leaf biomass ratio,  $34.5 \pm 3.0$  and  $31.5 \pm 4.4 \text{ cm}^2 \text{ g}^{-1}$  for leaf area ratio, and  $3.5 \pm 0.5$  and  $4.8 \pm 0.9 \text{ mg cm}^{-2} \text{ wk}^{-1}$  for leaf area productivity.

Conversely, irrigation regimes significantly affected all the variables relating to growth, biomass production and allocation except root to stem ratio (Tables 1–2). Investigation of the plant growth responses to irrigation treatments showed two distinct groups; the group supplied with high amounts of water (those plants subjected to 75 and 100% field capacity irrigation regimes) and the group supplied with less water (plants subjected to 25 and 50% field capacity irrigation regimes). Where a high water supply was maintained, higher relative growth rates of leaf and root biomass resulted, as well as higher growth



**Table 1.** ANOVA F-values for the effects of *Khaya senegalensis* propagule types (seedling and steckling) and irrigation regimes (25, 50, 75 and 100% field capacity) on the relative growth rate of plant, stem, root and leaf biomass, leaf area, stem length and stem basal diameter in Ouagadougou, Burkina Faso.

Source of variation	df	Relative growth rate of						
		Plant biomass	Stem biomass	Root biomass	Leaf biomass	Leaf area	Stem length	Stem diameter
Propagule type	1	0.44 <sup>ns</sup>	2.21 <sup>ns</sup>	0.05 <sup>ns</sup>	4.15 <sup>ns</sup>	0.17 <sup>ns</sup>	0.44 <sup>ns</sup>	6.91*
Irrigation	3	17.13 <sup>***</sup>	11.99 <sup>***</sup>	10.71 <sup>***</sup>	24.83 <sup>***</sup>	36.83 <sup>***</sup>	14.14 <sup>***</sup>	16.25 <sup>***</sup>
Propagule × Irrigation	3	1.15 <sup>ns</sup>	0.69 <sup>ns</sup>	1.63 <sup>ns</sup>	1.58 <sup>ns</sup>	2.55 <sup>ns</sup>	1.21 <sup>ns</sup>	0.33 <sup>ns</sup>

\*p<0.05, \*\*p<0.01, \*\*\*p<0.001, <sup>ns</sup> (not statistically significant, p>0.05).

**Table 2.** ANOVA F-values for the effects of *Khaya senegalensis* propagule types (seedling and steckling) and irrigation regimes (25, 50, 75 and 100% field capacity) on stem biomass ratio (SBR), leaf biomass ratio (LBR), root biomass ratio (RBR), root to stem ratio (RSR) leaf area ratio (LAR), specific leaf area (SLA), leaf area productivity (LAP) and foliar carbon isotope ratio ( $\delta^{13}\text{C}$ ) in Ouagadougou, Burkina Faso.

Source of variation	df	SBR	LBR	RBR	RSR	LAR	SLA	LAP	$\delta^{13}\text{C}$
Propagule type	1	0.22 <sup>ns</sup>	0.96 <sup>ns</sup>	0.81 <sup>ns</sup>	0.09 <sup>ns</sup>	1.00 <sup>ns</sup>	9.99 <sup>**</sup>	0.56 <sup>ns</sup>	2.48 <sup>ns</sup>
Irrigation	3(1)	12.32 <sup>***</sup>	12.69 <sup>***</sup>	3.48*	1.01 <sup>ns</sup>	20.62 <sup>***</sup>	5.46 <sup>**</sup>	17.02 <sup>***</sup>	30.30 <sup>***</sup>
Propagule × Irrigation	3(1)	1.87 <sup>ns</sup>	1.14 <sup>ns</sup>	0.26 <sup>ns</sup>	3.46*	3.60*	2.00 <sup>ns</sup>	3.39*	4.89*

\*p<0.05, \*\*p<0.01, \*\*\*p<0.001, <sup>ns</sup> (not statistically significant, p>0.05). (1) df for  $\delta^{13}\text{C}$

**Table 3.** The effects of *Khaya senegalensis* propagule types (seedling and steckling) and irrigation regimes (25, 50, 75 and 100% field capacity) on the relative growth rate of stem basal diameter RGRsd ( $\text{wk}^{-1}$ ), specific leaf area, SLA ( $\text{cm}^2 \text{g}^{-1}$ ) and foliar carbon isotope ratio,  $\delta^{13}\text{C}$  (‰) in Ouagadougou, Burkina Faso.

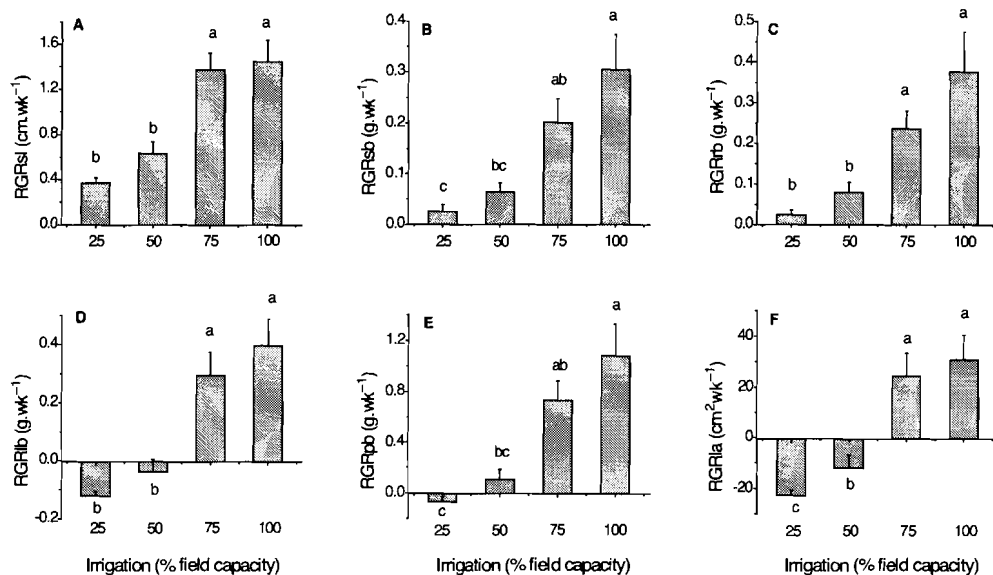
		RGRsd	SLA	$\delta^{13}\text{C}$
Propagule type	Seedling	0.052 ± 0.007 <sup>a</sup>	101.5 ± 7.6 <sup>a</sup>	-27.49 ± 0.23 <sup>a</sup>
	Steckling	0.040 ± 0.007 <sup>b</sup>	80.6 ± 6.4 <sup>b</sup>	-27.21 ± 0.33 <sup>a</sup>
Irrigation	25%	0.018 ± 0.004 <sup>b</sup>	67.9 ± 10.8 <sup>b</sup>	
	50%	0.031 ± 0.005 <sup>b</sup>	94.3 ± 15.4 <sup>ab</sup>	-26.80 ± 0.28 <sup>a</sup>
	75%	0.066 ± 0.007 <sup>a</sup>	104.1 ± 4.5 <sup>a</sup>	
	100%	0.070 ± 0.004 <sup>a</sup>	97.7 ± 1.3 <sup>a</sup>	-27.90 ± 0.07 <sup>b</sup>

Within the same column mean ± SE followed by different letters indicates significant differences at the 5% level according to Tukey's multiple comparison test.

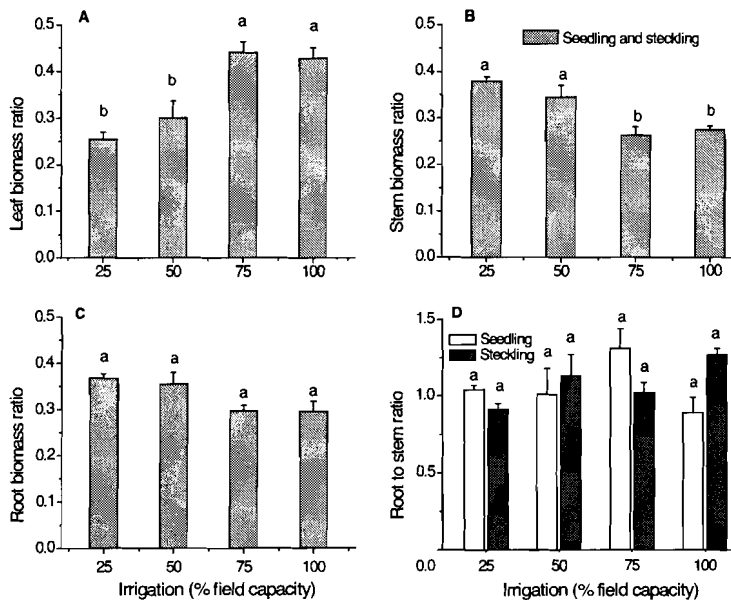
rates of stem length, stem basal diameter and leaf area, than when the water supply was low (Fig. 1, Table 3). The low water supply induced negative values for the RGR of leaf biomass (Fig. 1D) and leaf area (Fig. 1F). Reduction in leaf area was even more significant in plants supplied with a 25% than a 50% field capacity watering regime (Fig. 1F). Consequently, the leaf, root and stem

biomass fractions were significantly affected by the low water supply, resulting with a decrease in the leaf biomass fraction and increases in the stem and root biomass fractions (Fig. 2).

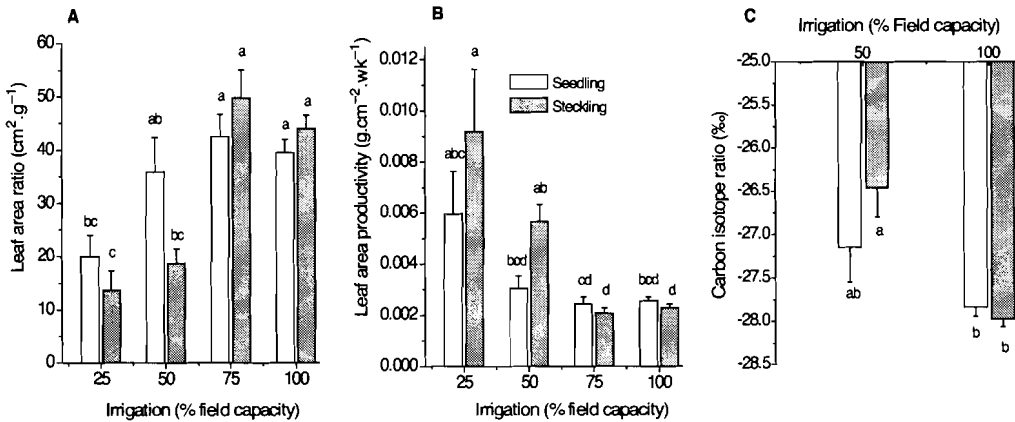
The effect of interactions between propagule types and irrigation regimes was significant for three parameters: root to stem ratio, leaf area ratio and leaf area productivity (Table 2). The group



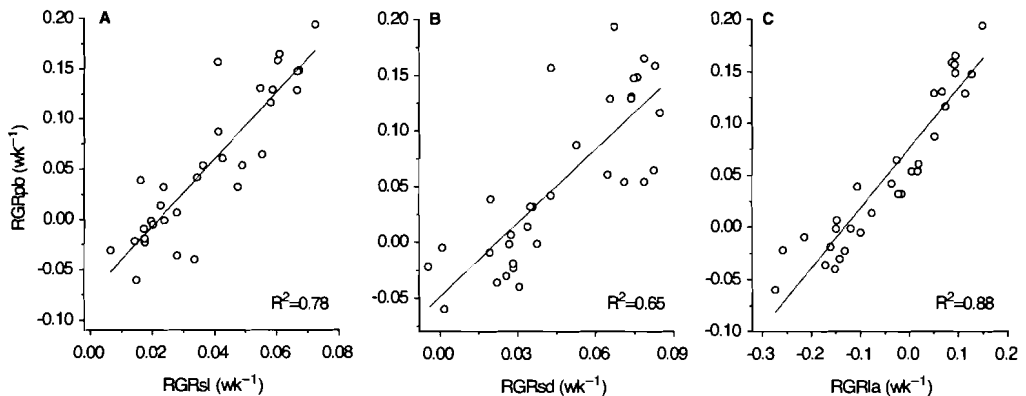
**Fig. 1.** The effect of irrigation regimes (25, 50, 75, 100% field capacity) on the relative growth rate of stem length (A), stem biomass (B), root biomass (C), leaf biomass (D), plant biomass (E) and leaf area (F) of seedlings and stecklings of *Khaya senegalensis* in Ouagadougou, Burkina Faso. Bars represent standard errors of means. Different letters indicate significant differences at the 5% level according to Tukey's multiple comparison test.



**Fig. 2.** The effect of irrigation regimes (25, 50, 75, 100% field capacity) on leaf biomass ratio (A), stem biomass ratio (B), root biomass ratio (C), and root to stem ratio (D) of seedlings and stecklings of *Khaya senegalensis* in Ouagadougou, Burkina Faso. Bars represent standard errors of means. Different letters indicate significant differences at the 5% level according to Tukey's multiple comparison test.



**Fig. 3.** The effect of propagule types of *Khaya senegalensis* (seedling and steckling) and irrigation regimes (25, 50, 75 and 100% field capacity) on leaf area ratio (A), leaf area productivity (B) and foliar carbon isotope ratio (C) in Ouagadougou, Burkina Faso. Bars represent standard errors of means. Different letters indicate significant differences at the 5% level according to Tukey's multiple comparison test.



**Fig. 4.** Relationships between relative growth rate of total plant biomass (RGRpb) and the relative growth rates of stem length (RGRsl), stem basal diameter (RGRsd) and leaf area (RGRla) of *Khaya senegalensis* seedlings and stecklings after 10 weeks of growth under four irrigation regimes, 25, 50, 75 and 100% field capacity in Ouagadougou, Burkina Faso.

with a low water supply (25–50% field capacity) had lower leaf area ratios and higher leaf area productivity than the group which received a high water supply (Fig 2D, Fig 3AB). However, there was no clear difference between seedlings and stecklings with respect to these variables when any of the four irrigation regimes were considered alone, even though seedlings had higher leaf area ratios and lower leaf area productivity than stecklings when receiving a low water supply.

Furthermore, the RGRs of stem length, stem basal diameter and leaf area were found to be significantly correlated ( $p < 0.0001$ ) with the RGR of plant biomass, indicating a high degree of association between the biomass of the whole plant and these three non-destructive variables (Fig. 4).

### 3.2 Carbon Isotope Ratio ( $\delta^{13}\text{C}$ )

Mean foliar  $\delta^{13}\text{C}$  values ranged from  $-29.2$  to  $-25.3$  ‰. They were affected significantly by the irrigation regimes and the interaction (propagule types  $\times$  irrigation regimes) but similar between propagule types (Table 2). Mean values were significantly increased by the low water supply of 50% field capacity with  $-26.8$  ‰ compared to  $-27.9$  ‰ for 100% field capacity watering regime. This increase was clearly distinguished for stecklings in contrast to seedlings which had similar  $\delta^{13}\text{C}$  mean value for the two water supply conditions, 50 and 100% field capacity (Fig. 3C).

## 4 Discussion

### 4.1 Main Effect of Propagule Types

The overall pattern in the results showed large and significant differences between plants grown under different irrigation regimes, but only a small difference between seedlings and stecklings of *K. senegalensis*. Stecklings and seedlings had comparable mean RGRs for stem length, leaf, root, stem and total plant biomass, biomass allocation and intrinsic water use efficiency, indicating that these two types of propagule had a similar growth pattern during the early growth phase. Differences between seedlings and stecklings are diverse and

differ between tree species, and sometimes within the same species or from nursery to field plantations (Frampton Jr and Foster 1993, Russell 1993, Hennon et al. 2009). While some studies, usually of field plantations, have shown that seedlings grow faster, others have reported growth equal to or slower than that of stecklings. For example, in a nursery trial, yellow-cedar stecklings grew taller and had a greater root collar diameter than seedlings, but both were found to grow to similar sizes in a field comparison trial in British Columbia (Russell 1993); conversely, in Alaskan field experiments, stecklings were reported to be smaller in size than seedlings (Hennon et al. 2009). Our findings are consistent with the results obtained frequently for radiata pine (Fielding 1970, Talbert et al. 1993), yellow cypress (Karls-son and Russell 1990) and loblolly pine (Frampton et al. 2000). It was reported that generally, growth in stecklings of radiata pine was similar to that of seedlings when cuttings were taken from juvenile trees which were less than 10 years old (Talbert et al. 1993). It has also been found that the method of propagation itself (seeds or cuttings) had no strong influence on the growth rate of radiata pine when stecklings were propagated from juvenile plant material in Australia (Fielding 1970). This contrasts with the results obtained for *Faidherbia albida* (Delile) A. Chev. (Ouédraogo 1993), African wild olive (Negash 2003) and narrow-leaved ash (Cicek et al. 2006), where stecklings showed better growth than seedlings; conversely, in field tests on farms, white spruce seedlings exhibited higher relative growth rates than stecklings (Beaulieu and Bernier-Cardou 2006). However, according to these previous studies, more variations could be expected within clones or between stecklings of differing origin than in seedlings, because the growth of stecklings is influenced by their genetic potential, the maturity of the donor plant, the morphology of the regenerated root system, the vigor of the propagules and the elapsed time after planting.

The higher specific leaf area of seedlings compared to stecklings may have been due to reduction in the leaf area and density of stecklings as shown in leaf area ratio and leaf area productivity (Fig 3). Indeed, variations in specific leaf area have frequently been assumed to explain most of the interspecific variation in the RGR of seedlings

or clones (Poorter and Remkes 1990, Cornelissen et al. 2003, Karacic and Weih 2006). Since these previous investigations have shown that seedlings of fast-growing species or clones showed higher specific leaf area, seedlings would be expected to grow faster than stecklings. Unexpectedly, in the present study the pattern of RGR was similar for seedlings and stecklings, except for stem collar diameter. The higher RGR of stem diameter for seedlings might be due to a growth variation between seedling stems derived from hypocotyls and the shoots of the rooted stem cuttings, because hypocotyl tissues are able to adjust the osmotic potential in response to varying external water potentials in some species such as *Colophospermum mopane* (Kirk ex Benth.) (Johnson et al. 1996). This feature might not be maintained for a prolonged growth period.

#### 4.2 Main Effect of Irrigation Regimes

In contrast to propagule type, water stress had a significant effect on plant growth during the ten-week period of the experiment. Significant differences were detected between the well watered (75 and 100% field capacity) plants and those with a low water supply (25 and 50% field capacity) in terms of their relative growth rate and biomass allocation. The response of the two propagule types to water stress was a decline in growth and biomass production and an increase in the root biomass fraction and intrinsic WUE. The RGR of the stem, root, leaf, total plant biomass, leaf area, stem length and diameter, and the leaf biomass ratio were significantly decreased, while stem biomass ratio, root biomass ratio and  $\delta^{13}\text{C}$  increased significantly. Similar results have been reported in several previous studies (Roupsard et al. 1998, Gindaba et al. 2005, Karacic and Weih 2006, Sanon 2009, Yang and Miao 2010).

It is well established that plants respond to a reduced water supply with either structural or physiological acclimation or both. When severely water stressed, plants minimize water loss by reducing their total leaf area, shedding the lower leaves and reducing the formation of new leaves. Consequently, this reduction in leaf area diminishes the total photosynthetic output which in turn results in a decreased growth rate; usually this is

consistent with, as in our study, a positive correlation between plant biomass and leaf area (Farquhar et al. 1989, Chapin III 1991, Hall et al. 1994, Kozłowski and Pallardy 2002). It has also been stated that drought induces a reduction in leaf growth through a hormonal signal from the roots, as water stress causes a decrease in cytokinin transport from roots to shoots and/or an increase in leaf abscisic acid content; these changes in hormone balance would result in changes in cell wall extensibility and, therefore, in growth (Chapin III 1991). Alternatively, an increase in root biomass ratio could be a better strategy for maintaining growth under water-limiting conditions, as this can increase water and nutrient absorption, returning carbon and nutrient contents to more favorable levels for storage in order to support rapid growth when conditions do become favorable (Chapin III et al. 1987, Kozłowski and Pallardy 2002).

#### 4.3 Interaction Effect of Propagule Types and Irrigation

The interaction effect between propagule types and irrigation regimes was significant for four parameters: root to stem ratio, leaf area ratio, leaf area productivity and  $\delta^{13}\text{C}$ . However, the variations observed were more obvious between stressed and well watered conditions for stecklings than for seedlings, indicating that stecklings may respond faster to water stress than seedlings and that the variation in growth and WUE would be more noticeable in stressed conditions. According to the relationship found between  $\delta^{13}\text{C}$  and the intrinsic WUE (Hall et al. 1994, Devitt et al. 1997), stecklings exhibiting a similar  $\delta^{13}\text{C}$  could be expected to have a similar WUE to seedlings. But, before drawing any conclusions or making recommendations for practical applications, more investigations over a longer drought period are needed in order to determine variation in growth, biomass allocation,  $\delta^{13}\text{C}$ , plant survival and capacity to recover. Moreover, the RGR of total plant biomass is associated with indirect growth parameters, such as leaf area, stem length and diameter, suggesting that these could be reliable parameters, measurable without damaging plants, for assessing seedling and steckling growth in a nursery environment.

As a whole growth patterns of seedlings and stecklings are comparable. The growth of rooted cuttings from juvenile donors follows a similar trend to that of seedlings under both well watered and water stressed conditions. However, water stress was found to be an important factor limiting the establishment and growth of both types of propagule. A low water supply, resulting in 25 and 50% field capacity, produced stress in all plants, as they exhibited a reduction of plant growth and biomass production, and an increase in the root biomass fraction and WUE. This highlights the need to select genotypes for drought-tolerance in addition to mahogany shoot borer-resistance in order to ensure the success of *K. senegalensis* plantation establishment for timber production in its native areas in Africa.

## Acknowledgements

Financial support was provided by the Swedish International Development Cooperation Agency (Sida). We would like to thank: H.N. Bouda and P. Bazié for helpful discussions; S. Diasso, H. Yonly, N. Koné, T. A. R. Dembele, A. Wanewéogo, K. Ouédraogo, F. Nandwéogo, A. Sanou and Z. Sanon for laboratory and field assistance; F. Bognounou for providing the laser area meter; Eloni Sonninen for carrying out the  $\delta^{13}\text{C}$  analyses; and anonymous reviewers for constructive comments.

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*Total of 40 references*



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Two clonal propagation methods were developed: root cuttings for *Detarium microcarpum* and stem cuttings for *Khaya senegalensis*. Root segment length and diameter affected its sprouting and new root formation ability while stockplant age and auxin influenced rooting of leafy stem cuttings. Comparison of sexual and asexual plantlets of *D. microcarpum* revealed that root suckers and seedling sprouts had a closer morphological resemblance. Seedlings and stecklings of *K. senegalensis* had similar growth patterns but water stress was a major growth-limiting factor.

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ISSN 1652-6880

ISBN 978-91-576-7556-9

