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

A Step toward Clonal Forestry in Burkina Faso

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

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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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Contents

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 (Dorrough *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 & Dorrough, 2006; Dorrough *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; Dorrough *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 produced 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 stecklings 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/fruiting 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^2 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).

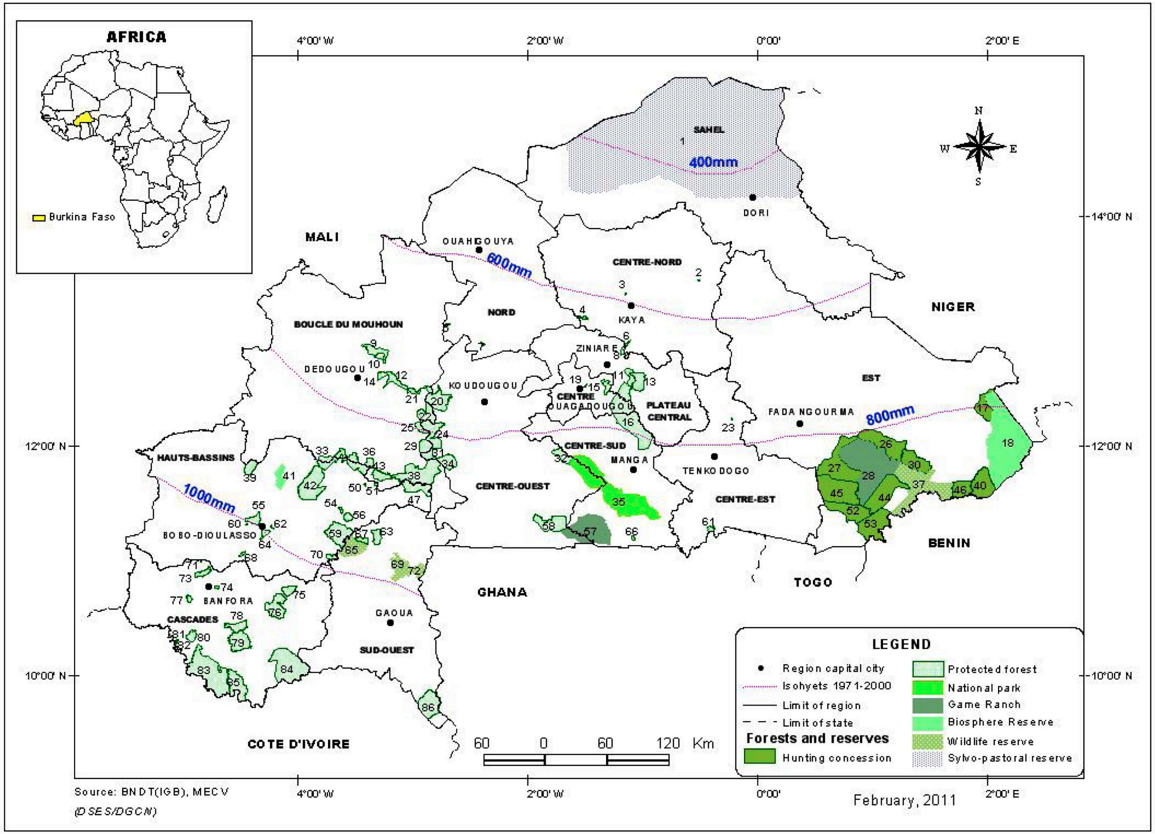

Figure 1. Forests and reserves in Burkina Faso (Ministry of Environment and Quality of Life).

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 $m^3 ha^{-1} year^{-1}$ (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^3 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^3, 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).

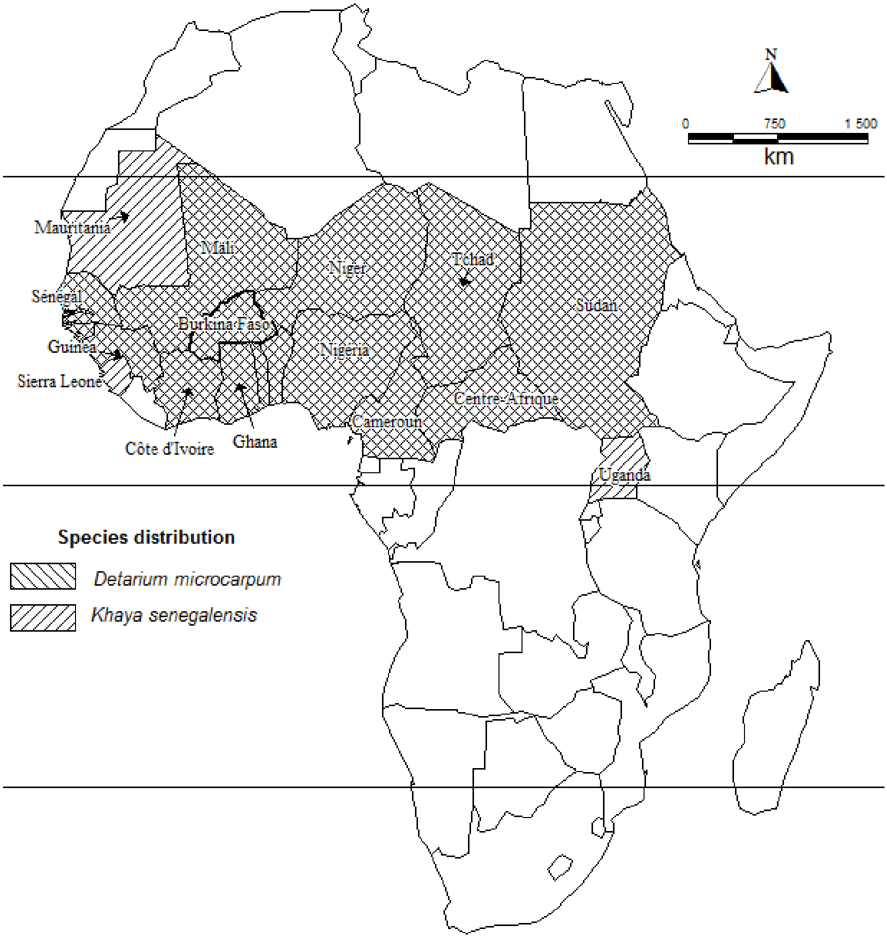

Figure 2. Spatial distribution of *Detarium microcarpum* and *Khaya senegalensis* in Africa, adapted from (Kouyaté & van Damme, 2006) and (Nikiema & Pasternak, 2008).

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^{-1} 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^{-1} (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. seneglensis* 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* (I)

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^2 (leafless), 6-8 cm^2 (one leaf with one pair of cut leaflets), 12-16 cm^2 (two leaves with two pairs of cut leaflets), 22-28 cm^2 (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}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 ± 0.08 g) than those from 10 cm root segments (0.34 ± 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), *Maerua 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.

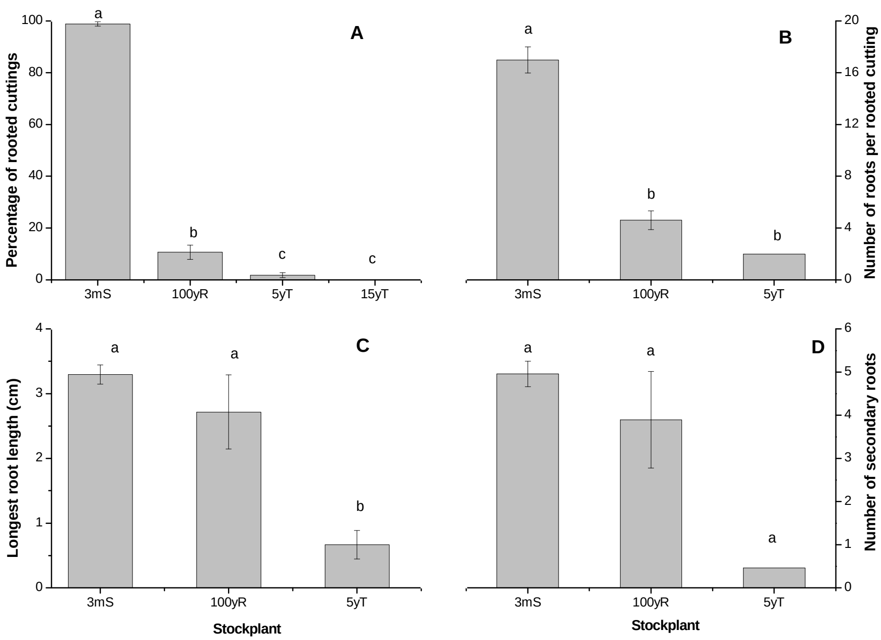

Figure 4. Main effect of stockplant donor type on the percentage of rooted cuttings (A), the number of roots per rooted cutting (B), the length of the longest root (C), and the number of secondary roots (D) of *Khaya senegalensis*. 3mS, 3-month-old seedling shoots; 100yR, resprouts of 100-year-old pollarded 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.

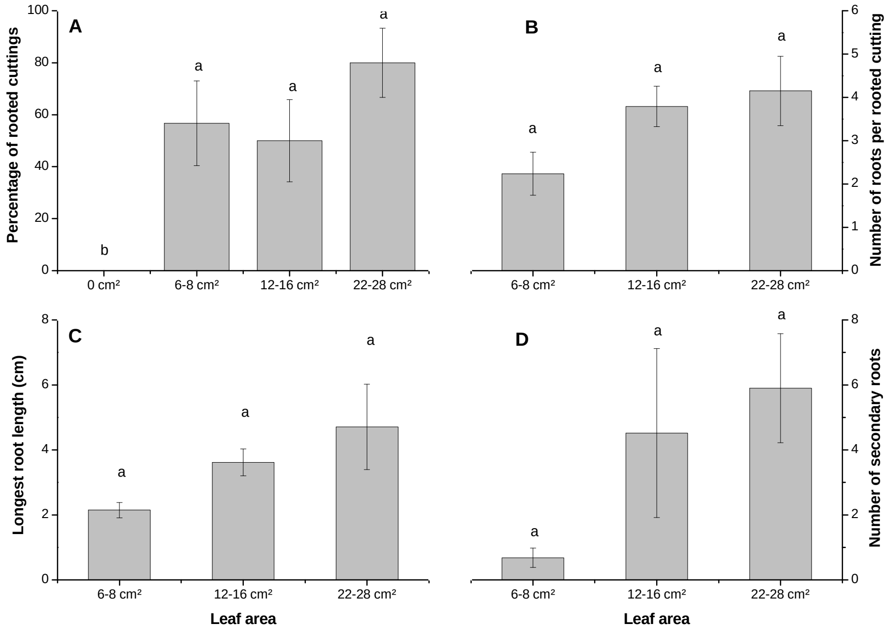

Figure 5. Effect of leaf area on the percentage of rooted cuttings (A), the number of roots per rooted cutting (B), the length of the longest root (C), and the number of secondary roots (D) of *Khaya senegalensis*. Bars represent standard errors of means. Means followed by the same letter(s) are not 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 (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 ± 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±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							
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

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 Kozlowski (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.

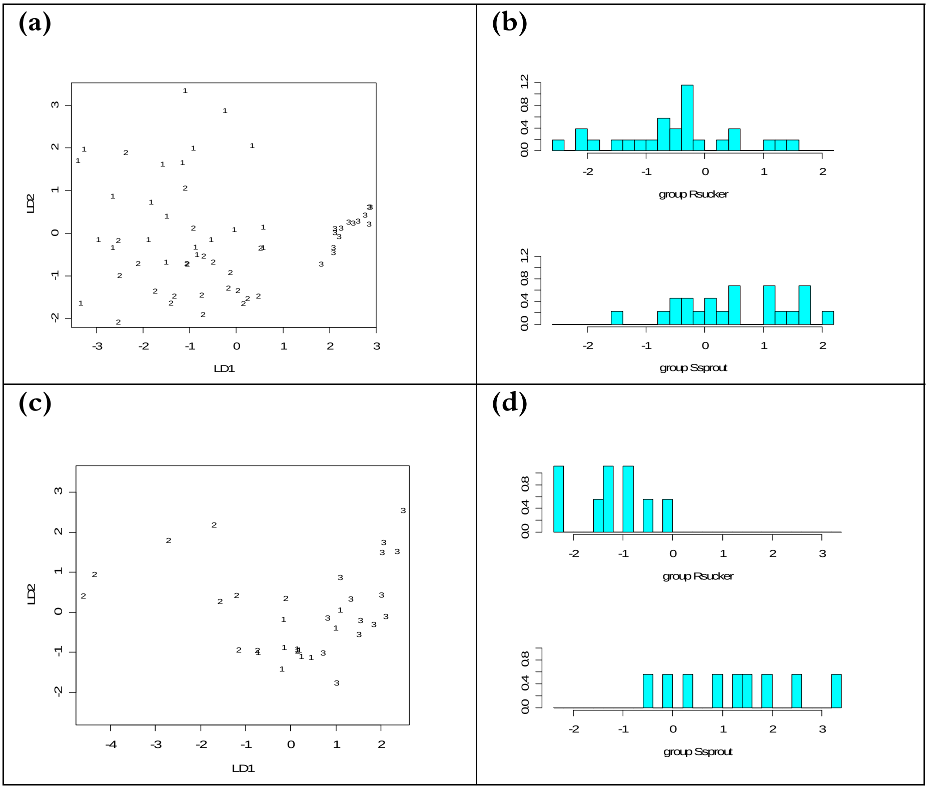

Figure 6. Linear discriminant analysis. The classification of root sucker (1, Rsucker), seedling sprout (2, Ssprout) and true seedling (3) based on morphological characters (a,b; height, stem length, stem diameter and canopy widths) and carbohydrate contents (c,d; glucose, fructose, sucrose and starch 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}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 (mg g^{-1} biomass) in Ouagadougou, Burkina Faso. 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.*

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

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}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).

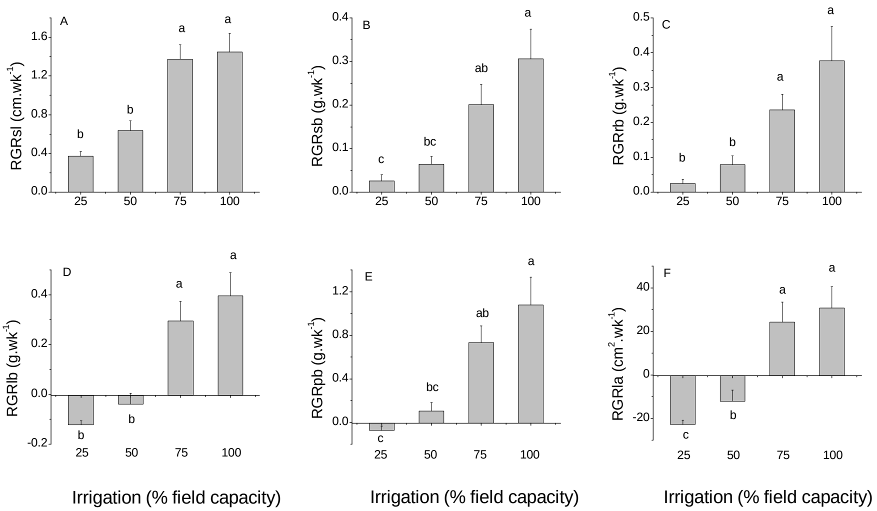

Figure 7. The effect of irrigation regimes (25, 50, 75 and 100% field capacity) on the relative growth rate (RGR) of stem length (A), stem biomass (B), root biomass (C), leaf biomass (D), plant biomass (E) and leaf area (F) of seedlings and stecklings of *Khaya senegalensis* in Ouagadougou, Burkina Faso. 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 (Kozlowski, 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; Kozlowski & 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}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.

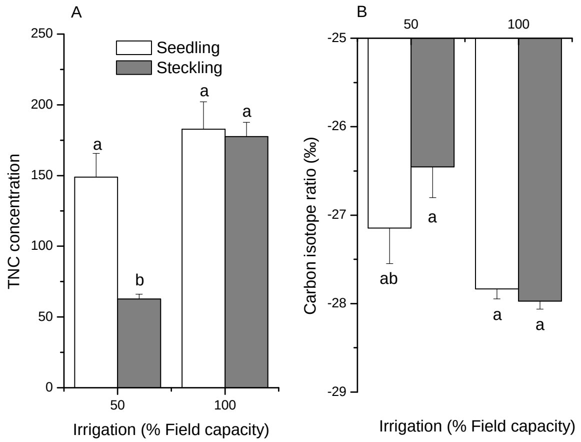

Figure 8. The effects of the interaction (propagule type × irrigation regime) on the total non-structural carbohydrate (TNC) concentration in roots (A) and carbon isotope ratio (^{13}C) in leaves (B) of seedlings and stecklings of *Khaya senegalensis* in Ouagadougou, Burkina Faso. Bars represent standard 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}C and the intrinsic WUE (Hall *et al.*, 1994; Devitt *et al.*, 1997), stecklings exhibiting a similar ^{13}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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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 développés: 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}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.