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Full Length Research Paper

Status of macadamia production in Kenya and the potential of biotechnology in enhancing its genetic improvement

L. N. Gitonga[1,3]*, A. W. T. Muigai[2], E. M. Kahangi[3], K. Ngamau[3] and S. T. Gichuki[4]

[1]Kenya Agricultural Research Institute, National Horticultural Research Center, P. O. Box 01000 - 220, Thika.
[2]Department of Botany, Jomo Kenyatta University of Agriculture and Technology, P. O. Box 00200-62000, Nairobi.
[3]Department of Horticulture, Jomo Kenyatta University of Agriculture and Technology, P. O. Box 00200-62000, Nairobi.
[4]Kenya Agricultural Research Institute, Biotechnology Center, P. O. Box 00200-57811, Nairobi.

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Macadamia (*Macadamia* spp.) is considered the world's finest dessert nut because of its delicate taste and numerous health benefits. It is grown in Kenya both as a cash crop and foreign exchange earner with Kenya producing about 10% of the world's total production. Macadamia has great potential for poverty reduction due to the high value of its products and its low requirement for external inputs. Although the crop has been grown in the country for over 5 decades, the growth of the industry is not commensurate with the demand and market potential that exists. Some of the challenges facing the macadamia industry in Kenya include lack of cultivars adapted to various agro ecological zones, inadequate planting materials of high quality, high cost of the available good quality planting materials and pests and diseases that affect nuts thus lowering post harvest quality. This paper discusses the potential of agricultural biotechnology relevant to genetic improvement of macadamia to compliment other efforts for its improved productivity and value.

Key words: Macadamia, dessert nut, biotechnology, genetic improvement.

INTRODUCTION

Macadamia (family *Proteaceae*) is an ever-green tree growing up to 20 m (Duke, 1983). 2 species *Macadamia integrifolia* Maiden and Betche (smooth-shelled), and *Macadamia tetraphylla* L.A.S. Johnson (rough- shelled) are cultivated for their edible nuts (McHargue, 1996). The mature fruit consists of a cream to white seed (kernel, nut) enclosed in a hard brownish seed coat (shell) which is then enclosed in a green or grayish green pericarp (husk) (Bittenbender and Hirae, 1990; Yokoyama et al., 1990) . The kernel can be eaten raw or fried (Duke, 1983) or as an ingredient into various confectionary products (Yokoyama et al., 1990; Sato and Waithaka, 1996). The oil extracted from macadamia is similar in composition to olive oil and is made up of 58.2% monounsaturated fatty acids (Cavaletto, 1980; Macfalane and Harris, 1981). It is considered a healthy food product as it contains no cholesterol, thus maintains blood cholesterol levels in check (Onsongo, 2003). The seed cake that remains after oil extraction is used as a constituent of livestock feed (Woodroof, 1967) . Rumsey (1927) also recommended the tree for timber and also as an ornamental.

Macadamia is mainly grown in Australia, Hawaii, South Africa, Kenya, Guatemala, Malawi, Brazil, Zimbabwe and Costa Rica in order of level of production (Table 1) (Wilkie, 2008). Other countries that cultivate the crop on a small scale include New Zealand, Mexico, Jamaica, Fiji, Argentina, Venezuela and Tanzania (Wasilwa et al., 2003.

Introduction in Kenya

Macadamia was introduced from New South Wales in Australia in to Kenya in 1946. 6 *M. tetraphylla* seeds were planted at Kalamaini estate in Thika district of central pro-

*Corresponding author. E-mail: lucygitonga2000@yahoo.com. Tel.: 254-67-24332.

Table 1. Macadamia production (Tons in-shell) in major macadamia growing countries in the years 2006 and 2007.

Country	Tons in-shell	
	2006	2007
Australia	42000	39700
Hawaii	22000	20000
S. Africa	17230	19230
Kenya	11400	11100
Guatemala	7000	8300
Malawi	4230	7110
Brazil	3125	3750
Zimbabwe	800	770
Costa Rica	500	500
Others	2200	2500
Total	108285	110460

Adapted from Wilkie (2008).

vince (Harris, 2004). More seeds of *M. integrifolia*, *M. tetraphylla* and hybrids of the 2 were introduced in 1964 from Australia, Hawaii and California. In 1968, grafted seedlings were produced using scion material of superior *M. integrifolia* varieties which were imported from Hawaii. The grafted Hawaiian varieties were planted in different agro-ecological zones. These 3 sources were used by the Harries family (Bob Harris Ltd, (BHL)) to propagate and supply macadamia seedlings to farmers in central, eastern, rift and coast provinces, as an alternative cash crop to tea and coffee (Harries, 2004). By 1974, BHL had already supplied 800,000 ungrafted seedlings to farmers in central and eastern provinces (Waithaka, 2001; Harris, 2004). However, when the trees begun to bear, no marketing infrastructure had been organized and this discouraged farmers and some started cutting down the trees. Since most of the trees were seed-propagated, there was wide variation in yield and quality of nuts. Most trees produced 5 - 10 kg/tree/season and kernels had less than 70% oil content which was considered low quality (Ondabu et al., 1996). Varieties adapted to various agro-ecological zones were also lacking. As a consequence, farmers were discouraged and some uprooted their trees.

History of Macadamia improvement in Kenya and current status

Improvement through selection breeding

Macadamia varietal development through selection breeding has extensively been done in other countries such as Hawaii (Bittenbender and Hirae, 1990), California (McHargue, 1996), Australia (Hardner et al., 2001) and South Africa (ARC, 2000) leading to varieties adaptable to various AEZ. Introduction of such varieties in Kenya would broaden the genetic base. However, they would need to be evaluated for adaptability to Kenyan conditions. Hence, selection of varieties from already adapted germplasm in Kenya was necessary. Between 1971 and 1973, a feasibility study funded by the food and agriculture organization (FAO) was carried out by the Kenya government to determine the potential of revitalizing the macadamia industry and providing farmers with high quality planting materials (Hamilton, 1971; Waithaka, 2001). Results of the feasibility study indicated that macadamia had high potential as a cash crop and as foreign exchange earner for Kenya (Kiuru et al., 2004). The government of Kenya requested the government of Japan for financial support and technical expertise to assist Kenyan counterparts in rehabilitation of the macadamia industry. Hence, in 1977 agronomic surveys of macadamia trees planted in the later part of 1960s revealed 300 'superior' trees in terms of yield and quality of nuts. Out of these, scions were obtained from 30 most promising trees in farmers' fields and grafted clones were planted at the National Horticultural Research Centre (NHRC, Thika) of the Kenya Agricultural Research Institute, for detailed observations on yield, nut and kernel characteristics (Wasilwa et al., 2003).

Nut quality has been accepted as the first criterion for the selection of improved macadamia cultivars (Nissen and Williams, 1980). First grade kernels contain over 72% oil. Oil content is determined by specific gravity using dried kernels of below 2% moisture content. First grade kernels readily float in tap water. Kernels that contain 72% or below sink in tap water but float or sink in 1.025 specific gravity brine solution and are 2^{nd} or 3^{rd} grade respectively. Such nuts are usually immature and harder and they become over brown when roasted (Yokoyama et al., 1990). Kernel recovery ratio, expressed as a percentage of recovered kernels' weight to the total in-shell nut weight, should be over 32% while the weight of kernel should range between 1.55-3.14 g. First grade kernel ratio expressed as a percentage of nuts that float in tap water to the total weight of recovered kernels should be over 90% (Nissen and Williams, 1980). Yield, expressed as the number of kilograms per tree per year should be over 50 kg if the tree is growing under highly suitable conditions or 40 kg under moderate conditions (Ondabu et al., 1996). Based on the factors mentioned above, 7 promising cultivars were selected from the 30 trees with a yield potential of between 50- 80 kg/tree/year, a 10 fold increase from average yield of 5 – 10 kg obtain-ed previously by farmers. Nut and kernel characteristics were also improved to ideal ranges. These varieties were recommended for commercial planting in the late 1980s (Table 2).

Several other varieties including EMB-2, MRG- 2, MRG-25, TTW-2, Hawaiian varieties; HAES 246, HAES 294, HAES 333, HAES 508, HAES 660, HAES 741, HAES 788 (*M. integrifolia*), KMB-4, KMB-25, KMB-9,

Table 2. Yield, nut and kernel weights of the 7 varieties recommended for commercial planting in Kenya in the late 1980s. Data on yield was obtained from original mother trees in farmers' fields where they were best adapted while data on nut and kernel characteristics was taken from grafted clones at NHRC.

Variety	Species	Yield (in shell) at 15 years (Kg/tree)	Kernel recovery ratio (%)	Kernel weight (g)	First grade kernel ratio (%)
KRG-15	*M. integrifolia*	80.0	39.3	2.53	91.99
EMB-1	*M. integrifolia*	70.0	33.5	1.99	95.50
MRG-20	*M. integrifolia*	55.0	32.6	2.24	95.77
KMB-3	(*M. integrifolia* x *M. tetraphylla*) hybrid	60.0	34.8	1.90	94.30
KRG-1	*M. integrifolia*	50.0	29.6	2.20	72.70
KRG-3	*M. integrifolia*	50.0	31.7	2.30	90.00
KRG-4	*M. integrifolia*	65.0	32.8	2.10	56.70

Source: Hirama et al. (1987).

Table 3. Yield, Nut and Kernel weights of additional 10 varieties recommended for commercial planting in the late 1990s. Data on yield was obtained from original mother trees in farmers' fields where they were best adapted while data on nut and kernel characteristics was taken from grafted clones at NHRC.

Variety	species	Yield (in shell) at 15 years (Kg/tree)	Kernel recovery ratio (%)	Kernel weight (g)	First grade kernel ratio (%)
EMB-2	*M. integrifolia*	40 - 60	34.63	2.38	94.95
EMB-H	Hybrid	40 - 60	36.0	1.96	99.67
KMB-4	Hybrid	40 - 60	36.24	2.88	96.30
KMB-25	Hybrid	40 - 60	36.00	3.15	87.17
MRG-2	Hybrid	50 - 60	38.66	3.91	93.92
MRG-25	Hybrid	40 - 60	37.22	2.51	97.25
MRU-23	Hybrid	40 - 60	35.02	2.67	81.50
MRU-24	Hybrid	40 - 60	35.28	2.17	97.00
MRU-25	Hybrid	40 - 60	33.45	2.12	81.00
TTW-2	*M. integrifolia*	40 - 60	41.33	2.06	91.67

Modified from Tominaga and Nyaga, (1997)

EMB-H, MRU-23, MRU- 24, MRU-25, MRG-1, MRG-2 and MRG-8 (*M. integrifolia* x *M. tetraphylla* hybrids) were planted in trials at different agro-ecological zones for continued observations on yield potential, tree and nut characteristics (Wasilwa et al., 2003). Subsequently, some addtional varieties, mostly natural (*M. integrifolia* x *M. tetraphylla*) hybrids were tentatively recommended for commercial planting in the late 1990s. These varieties have yield potential of 40 - 60 kg/tree/year, high kernel recovery ratio of between 33.45 - 41.33%, high kernel weight of between 1.96 - 3.15 g and over 80% first grade kernel ratio (Table 3) (Tominaga and Nyaga, 1997; Wasilwa et al., 2003)

Development of new varieties through cross breeding

Further agronomic observations revealed that some macadamia cultivars were adapted to different agro-ecological zones (AEZ) and were recommended accordingly (Table 4). Only 4 varieties KRG-15, EMB-1, MRG-20 were permanently recommendation for 3 agro- ecological zones, sunflower -maize zone, marginal coffee and main coffee zones. To date, only one variety (KMB-3) adapted to high altitudes of 1750 - 1870 m above sea level (coffee tea zone), was identified and recommended (Tominaga and Nyaga, 1997). Hence, cross breeding work between 4 selected clones (EMB-1, KRG-3, MRG-20 and KMB-3) and 2 Hawaiian varieties (HEAS 508 and HAES 333) and further cross-pollination with clones MRG-1, MRG-2, MRG-8, KMB-9 and KRG-4 (Table 5) was initiated in 1991 with an aim of combining adaptability to high altitude and other nut quality characteristics. The progenies were planted at an altitude of 1850 m above sea level (Kiuru et al., 2004) for evaluation.

Multiplication of selected superior clones through vegetative propagation

Macadamia is preferentially (> 75%) out crossing (Sedg-

Table 4. Macadamia varieties recommended for 4 agro-ecological zones of Kenya.

Agro-ecological zone	Altitude (meters)	Mean Temperature (^{o}C)	Rainfall in mm	Permanent recommendations	Tentative recommendations
Sunflower - Maize Zone	1280 - 1400 and below	19.5 - 20.7 and higher	800 - 900 and less	KRG-15, EMB-1 MRG-20	
Marginal Coffee Zone	1400 -1550	19.0 - 20.1	900 - 1200	KRG-15, EMB-1, MRG-20	KMB-4, MRG-25, TTW-2 (T/Taveta)
Main Coffee Zone	1550 - 1750	18.5 - 20.0	1200 - 1400	KRG-15, EMB-1, MRG-20, KMB-3	KMB-4, EMB-2, EMB-H, MRG-25 and TTW-2 (T/Taveta)
Coffee - Tea Zone	1750 - 1870	17.5 - 19.0	1400 - 1600	KMB-3	EMB-1, MRG-20, EMB-H, MRU-24 and MRU-25

Modified from Ondabu et al., (1996); Tominaga and Nyaga (1997); Wasilwa et al. (1999)

Table 5. Macadamia crosses performed in Kenya in 1991.

Cross	Number of crossed seed nuts	Cross	Number of crossed seed nuts
KMB-3 X KMB-9	474	MRG-20 X EMB-1	8
KMB-3 X KRG-3	33	MRG-20 X KRG-3	13
KMB-3 X EMB-1	3	KRG-3 X EMB-1	32
KMB-3 X MRG-20	26	EMB-1 X KRG-3	2
KMB-9 X KMB-3	27	EMB-1 X KRG-3	3
KRG- 3 X KMB-3	6	EMB-1 X MRG-20	2
MRG-20 X KMB-3	7		
Total			636

Source: JICA (1991)

ley et al., 1990) . Production of true-to-type planting material is therefore by grafting scions from selected parents on to rootstocks raised from seed (Stephenson, 1983). In Kenya, grafting methods were evaluated between 1985 and 1997. 3 grafting methods namely (a) Top wedge (90 - 100% successful takes), (b) splice and (c) side wedge (70 - 100%) were fine-tuned and used for mass propagation of planting material of selected superior material. Using these methods seedlings can be produced within 1.5 - 2 years (Nyakundi and Gitonga, 1993; Gitonga et al., 2002). Grafting reduces the juvenile period before bearing from 7 - 10 years to 3 - 4 years. Further, grafting also contributed to dwarfing of trees from over 15 m to manageable heights of less than 10 m. Average yields were increased from between 5 and10 kg to between 50 and 80 kg/tree/season while nut quality was improved by increasing nut oil content to over 72% (Onadabu et al., 1996) through propagation and distribution of superior cultivars. Selection of superior clones and development of vegetative propagation techniques and other agrono-mic packages has resulted into economic gain and importance of macadamia in Kenya. Currently, macadamia is a growing agro-processing industry that targets niche markets in Europe and the Orient (Rotich, 2004) . The area under macadamia has increased from 469 ha in 1989 to an estimated 8000 ha in 2003 (Onsongo, 2003; Kiuru et al., 2004).

Macadamia has since become the most important nut crop in Kenya, with an annual production of about 10,000 metric tons. Being a low-input crop, macadamia is grown by over 100,000 small-scale farmers for income and livelihood (Waithaka, 2001). Further, macadamia can be grown as an intercrop with other cash and food crops (Onsongo, 2003; CABI, 2005). Small-scale farmers pro-

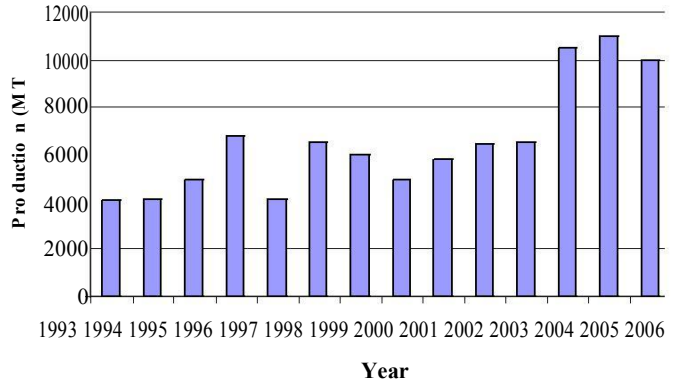

Figure 1. Macadamia nut production (in-shell MT) in Kenya from 1993-2006.
Source: USDA, 2008.

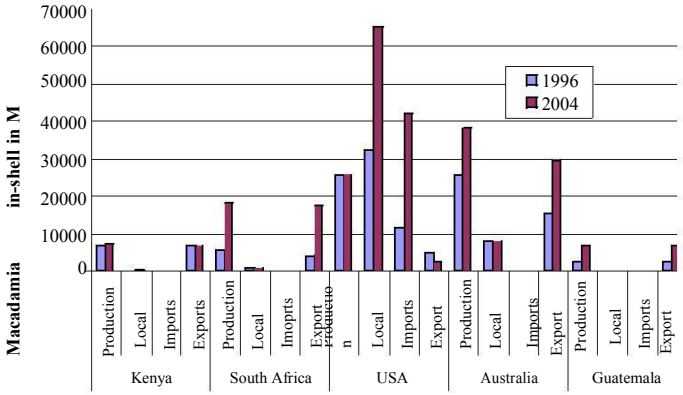

Figure 2. Macadamia in-shell basis - production, consumption, exports, and imports statistics for the years 1996 and 2004.
Source: USDA, 2008

duce about 70% of total production while 30% is produced by about 500 large-scale growers with at least 1000 trees each (Muhara, 2004). Kenya exports 93% of its total production to Japan, Hong Kong, Germany, USA, Canada and Switzerland (processed) and China, East Asia and India (unprocessed) while domestic market consumption is estimated at 50 tons of kernel annually (WHT and U.S.E.O, 2004; Rotich, 2004). The Kenya nut company (KNC), the major processor of macadamia nuts has branded its domestic products 'Nutfields' which include roasted nuts, chocolates and cookies sold in major supermarkets, hotels and airlines. International products are branded 'Out of Africa' (Onsongo, 2003).

Steady growth in production (Figure 1) and export of macadamia has been realized since the early 1990's. An increase from 4000 tons in 1993 to 7,300 tons in the year 2004 in export was registered (CABI, 2005). In 2003, the world production of processed kernel was 23,130 tons with Kenya producing 1520 tons (7%) from 1600 bearing trees (Onsongo, 2003; Lee, 2004).

However, the growth rate of the sub-sector is below the country's potential and is relatively slow when compared to the growth rate of competing countries such as South Africa to which Kenya has lost some of its market share (CABI, 2005) . While the production and exports from Kenya barely changed between 1996 and 2004, those of South Africa tripled (Figure 2). Within the same period, USA doubled its local consumption and increased its imports more than 4 times without changing its production levels thus offering huge market potential for Kenyan macadamia.

Several factors have been cited as responsible for the slow production growth of which include among others, inadequate cultivars adapted to various agro ecological zones, inadequate planting materials of high quality, high cost of the available good quality planting materials and pests and diseases that affect nuts thus lowering post-harvest quality.

Prospects of biotechnology applications for Macadamia improvement

Macadamia breeders must explore as much genetic diversity as possible from which to select and recombine favorable traits through cross-breeding (McHargue, 1996) so as to develop varieties that are adapted to Kenyan conditions and those that can compete in the world market (Rotich, 2004). However, the genetic diversity of macadamia germplasm in Kenya is not known and this slows breeding efforts.

Currently, the genetic improvement of macadamia is based on introduction and selection from existing germplasm. Germplasm characterization is based on agro-morphological traits. This process has been slow and since 1977 only 4 varieties have been permanently recommended for commercial planting (Ondabu et al., 1996). Moreover, morphological traits alone represent only a small portion of the plant genome and are influenced by environmental factors, there by limiting their utility in describing the potentially complex genetic structures that may exist between and within species (Avise, 1994).

Application of DNA markers for macadamia germplasm characterization

Genetic markers are simply heritable characters with multiple states at each character. Typically, in a diploid genus such as *Macadamia* (2n = 28) (Hardner et al., 2005), each individual can have 1 or 2 different states (alleles) per character (locus). All genetic markers reflect differences in DNA sequences (Sunnucks, 2000). Basic DNA profiling techniques (DNA markers) include restriction fragment length polymorphisms (RFLPs), random amplified polymorphic DNA (RAPDs), amplified fragment length polymorphisms (AFLPs) and microsatellites. These techniques are continually being modified to im-prove resolution and information content. With the inven-

tion of the polymerase chain reaction (PCR) technique, DNA profiling took huge strides in both discriminating power and the ability to recover information from very small (or degraded) starting samples. With sequencing technologies associated with automated and/or semi automated large-scale screening systems, DNA-based polymorphisms are now the markers of choice for molecular-based surveys of genetic variation (Hannotte and Jianlin, 2006).

Among the DNA–based markers, microsatellites (Goldstein et al., 1995) are well known for genetic diversity studies due to their potentially high information content (Ferreira, 2006), the results obtained are reproducible, the data can be scored and analyzed using standardized methods (Lanteri and Barcaccia, 2006). Characterization of diversity of macadamia germplasm using molecular tools has been previously applied in other countries. As a tool towards quantitative breeding of macadamia in Hawaii, Vithanage et al. (1998) reported on the use of isozyme analysis to assess the genetic relatedness but found it limiting since several isozyme alleles were shared by most of the commercial cultivars. They developed random amplified polymorphic DNA (RAPD) and sequence tagged sites (STSs) . Results showed that these markers followed Mendelian inheritance and these markers have been used to assay 76 individuals. Peace et al. (2002) used radio-labeled DNA amplification fingerprinting (RAF) technique for Macadamia chromosome mapping. They discovered 19 new RAF co-dominant markers which were used together with dominant markers to charac-terize 30 cultivars representative of the Hawaiian Maca-damia industry. These markers were also used to assign cultivars to germplasm groups that reflected species status and breeding origins. Recently, Schmidt et al. (2006) isolated 33 microsatellite markers from *M. integrifolia* that were used to genotype 43 commercial cultivars of Australia generating an average polymorphic information content of 0.480. These 33 microsatellite loci represent a significant tool for genome mapping and population genetic studies and can therefore be immediately applied for genetic diversity studies of macadamia selections in Kenya.

Other approaches for molecular characterization with increased robustness include single nucleotide polymorphisms (SNPs), allele mining and diversity array technology (DArT) . SNPs are DNA sequence variations that occur when a single nucleotide (A, T, C, or G) in the genome sequence is altered. For example the change of the DNA sequence AAGGCTAA to ATGGCTAA (Kahl et al., 2005). For a variation to be considered a SNP, it must occur in at least 1% of the population (allele frequency ≥1%) (Rapley and Habron, 2004).Since SNPs are located throughout the genome, whole genome scans may help identify regions associated with agronomically important traits and hence can be applied for high-throughput marker-assisted breeding, expressed sequence tags (EST) mapping and the construction of genetic linkage maps (Rafalski, 2002).

SNPs have been identified and utilized several crops including barley (Soleimani et al., 2003) and *Solanum caripense*, a wild relative of potato and tomato (Nakitandwe et al., 2007) . In nut crops, SNPs have been detected in coconut and are being used for studying genetic population structure (Mauro-Hennera et al., 2006) while in almond (Shu-Biao et al., 2008) validated 100 SNPs based on the predicted SNP information derived from the almond and peach EST database. Detection and validation of SNPs in macadamia would greatly enhance diversity analysis and genome mapping.

Allele mining exploits the DNA sequence of one genotype to isolate useful alleles from related genotypes (Lathar et al., 2004) . This can be done using DNA chip technology whereby the basic DNA sequence of a gene is spotted on a chip in the form of large series of sequence-overlapping probes consisting of 15 - 20 bases. Each base position in a fluorescently labelled sample is then interrogated for the presence of point mutations by monitoring hybridization signals with the spotted probes. Allele mining therefore helps to detect new point mutations, in relatively large DNA fragments. Once allelic variants of interest have been identified, the approach can be optimized by focusing on target sets of polymorphisms, for example by using SNP detection methods (Lathar et al., 2004; Upadhyaya et al., 2006). This method can therefore be applied to differentiate between genotypes or to detect new evolving macadamia varieties.

DArT was developed to provide a practical and cost effective whole genome fingerprinting tool with high through put (Jaccoud et al., 2001). The technology has the ability to generate reproducible molecular markers with no prior DNA sequence information (Kilian et al., 2005). Development of DArT starts with assembling a pool of DNA samples that encompass the diversity of the species (Wenzl et al., 2004). This may include the primary gene pool of a crop species, 2 parents of a cross (if the goal is creation of a genetic linkage map), or secondary gene pools. The DNA mixture representing the gene pool is then processed to produce a defined fraction of genomic fragments referred to as ‘representation’ which are then used to create a library in *Escherichia coli* (Kilian et al., 2005). DArT operates on the principle that the genomic 'representation' contains 2 types of fragments. Constant fragments, found in any 'representation' prepared from a DNA sample from an individual belonging to a given spe-cies and variable (polymorphic) fragments (molecular markers), only found in some but not all of the 'representations'. The variable fragments, referred to as the DArT markers are informative because they reflect sequence variation that determines the fraction of the original DNA sample that is included in the 'representation' (Kilian et al., 2005). The presence or absence of DArT markers in a genomic 'representation' is assayed by hybridising the 'representation' to the library of that species (Wenzl et al., 2004). Application of DArT for germplasm characteriza-

tion has been applied in a number of crops. Xia et al. (2004) developed DArT for cassava and typed a group of 38 accessions. The average call rate was 98.1% with scoring reproducibility of 99.8%. The DArT markers displayed fairly high polymorphism information content (PIC) values and revealed genetic relationships among the samples consistent with the information available on the accessions. Yang et al. (2006) developed DArT for pigeon pea and its wild relatives and typed 96 accessions representing nearly 20 species of *Cajanus*. A total of nearly 700 markers were identified with the average call rate and scoring reproducibility of 96.0 and 99.7% respectively. DArT markers revealed genetic relationships among the accessions consistent with the available information and systematic classification. Since DArT has high throughput due to a high level of multiplexing and requires no prior sequence information (Kilian et al., 2005), it can immediately be applied for diversity studies in macadamia and identification of superior varieties.

Marker assisted selection for enhanced breeding of macadamia

Considerable developments in biotechnology have led plant breeders to develop more efficient selection systems to replace traditional phenotypic-pedigree-based selection systems (Ribaut and Hoisington, 1998) . Marker assisted selection (MAS) is the indirect selection process where a trait of interest such as disease resistance, abiotic stress tolerance, and/or quality is selected based not on the trait itself but rather on a marker linked to it. The marker may be morphological, biochemical or one based on DNA/RNA variation (Semagn et al., 2006a). For example if MAS is being used to select individuals with disease, the level of disease is not quantified but rather a marker allele which is linked with disease is used to determine disease presence (Mingyao et al., 2005). Once genetic linkage has been identified for a disease, the next step is often association analysis, in which the markers within the linkage region are genotyped and tested for association with the disease (Mingyao et al., 2005). A review on the opportunities and constraints for marker assisted selection in macadamia breeding was done by Hardner et al. (2005). They suggested that detection of associations between marker-Quantitative trait loci (QTL's) using multiple families linked through pedigree was the most attractive strategy. Large scale association studies can be done using high throughput inexpensive genome-wide markers such as DArT (Baundouin et al., 2006) or SNP typing with DNA chips (Twyman, 2003; Ganal et al., 2009). DNA chips contain thousands of short DNA sequences immobilized at different positions and are used to discriminate between alternative bases at the site of a SNP. 2 chip-based typing methods are widely used. One method relies on allele-specific hybridization. Short DNA sequences on the chip represent all possible variations at a polymorphic site and a labeled DNA will only hybridize if there is an exact match. The base is identified by the location of the fluorescent signal (Ganal et al., 2009).

In the second method, the oligonucleotide on the chip may stop one base before the variable site and typing relies on allele- specific primer extension. A DNA sample stuck onto the chip is used as a template for DNA synthesis, with the immobilized oligonucleotide as a primer. The four nucleotides, containing different fluorescent labels, are added along with DNA polymerase. The incorporated base, which is inserted opposite to the polymorphic site on the template, is identified by the nature of its fluorescent signal and the variation of the added nucleotide is identified by mass spectrometry (Tang et al., 1999). A recent advance for the detection of SNPs known as the high resolution melting curve (HRM) has been developed. The technique measures temperature induced strand separation of short PCR amplicons and is able to detect variation as small as one base difference between samples. It has been applied to the analysis of almond SNP discovery and genotyping (Shu-Biao et al., 2008).

MAS will be useful in macadamia where selected characters like yield are expressed late in plant development due to long juvenile period. Selection of genotypes for such traits will not wait until fruiting time. Selection for high nut yield and quality of macadamia from an array of cross-bred population would also benefit from MAS such that useful crosses can be selected and advanced early enough. MAS can also be used to select for disease and pest resistance. However, limited molecular mapping work on macadamia has been reported. The first mole-cular linkage map of macadamia (*M. integrifolia* and *M. tetraphylla*) based on 56 F_1 progeny of cultivars ÁKeauhou' and ÁA16' was reported by Peace et al. (2003). The map comprised of 24 linkage groups with 265 frame-work markers: 259 markers from randomly amplified DNA fingerprinting (RAF), 5 random amplified polymorphic DNA (RAPD) and one sequence-tagged microsatellite site (STMS). This molecular study is the most compre-hensive examination to date of genetic loci of macadamia and is a major step towards developing marker-assisted selection for this crop. DArT is a powerful tool towards achieving faster marker assisted selection and con-struction of genetic linkage maps. Semagn et al. (2006b) constructed a genetic linkage map of 93 doubled-haploid lines derived from a cross between *Triticum aestivum* L. and a Norwegian spring wheat breeding line, NK93604, using DArT amplified fragment length polymorphism (AFLP), and simple sequence repeat (SSR) markers. The map has been successfully used to identify novel QTLs for resistance to *Fusarium* head blight and powdery mil-dew. In barley, Wenzl et al. (2006) developed a high den-sity consensus map of barley that resulted in identifica-tion of 14 ± 9 DArT loci within 5 cm on either side of SSR, RFLP or STS loci previously identified as linked to agri-cultural traits. Validation of DArTs for whole genome pro-filing was initiated in coconut and over 350 markers were

isolated from 120 lines from CIRAD and SriLanka (Wongtiem et al., 2005). It is proposed that, isolation of DArTs in macadamia be done to speed up genome mapping for agronomically important traits since a single DArT assay can simultaneously type hundreds to thousands of SNPs and insertion/deletion polymorphisms spread across the genome (Wenzyl et al., 2006).

Application of DNA markers for macadamia germplasm conservation

Most of the macadamia germplasm that is included in breeding programs is conserved on-farm. The existence of clonally propagated crops conserved on-farm is endangered by several factors including the introduction of alternative improved varieties (de Vicente et al., 2006). With multiplication of the few selected varieties of macadamia farmers have continued to cut or uproot original plantings and replacing them with the new cultivars. A recent survey (Gitonga et al., 2008a), revealed that out of 11 breeding selections 4 had already been cut down by the farmers despite a formal agreement with KARI to preserve the trees on-farm. 2 other trees had also died from natural causes between the years 2006 and 2007. Hence, there is risk of losing valuable germplasm leading to genetic erosion.

For *ex situ* conservation, genetic markers may contribute to the sampling, management and development of “core” collections as well as utilization of genetic diversity. For *in situ* and on-farm conservation strategies, genetic markers might help in recognizing the most representative populations within the gene pool (Lanteri and Barcaccia, 2006; de Vicente et al., 2006). Several criteria have been suggested for sampling the maximum possible genetic variation including, the number of alleles per locus, evaluated as those that are common in one to several populations but not in the species as a whole and those that are identified as unique (private) alleles (Lanteri and Barcaccia, 2006). Hence, information derived from genetic markers offers a good basis for better conservation decision making (de Vicente et al., 2006).

Genetic improvement of macadamia through tissue culture techniques

Mass multiplication of superior varieties

The conventional method of propagating macadamia in Kenya by grafting is constrained by requirement for long nursery period of 18-24 months (Nyakundi and Gitonga, 1993; Gitonga et al, 2002). Other methods of propagation have been found successful on macadamia including chip budding (Leigh, 1973), air layering (Kadman, 1982) and cuttings (Gitonga et al., 1997) but with little comer-cial viability.

Tissue culture techniques allow propagation of plant material with high multiplication rates on sterile artificial nutrient medium in an aseptic environment (George, 1993), a term usually referred to as cloning (Pfeiffer, 2003). Mass multiplication of clonal plant material through tissue culture techniques has now been widely used in nut crops such as cashew nut (Thimmappaiah et al., 2007), chestnut (Vieitez et al., 2007) and walnuts (Leal et al., 2007). Mulwa and Bhalla (2000) reported successful regeneration and rooting of shoots from nodal segments of *M. tetraphylla*. Regeneration of shoots from somatic embryos of *M. tetraphylla* has also been reported (Mulwa and Bhalla, 2006). Gitonga et al. (2004, 2008b) reported successful shoot regeneration from *M. integrifolia* , but with low multiplication rates and rooting problems. With the development of a viable tissue culture system for *M. integrifolia* and (*M. integrifolia* x *M. tetraphylla*) hybrids which form the main commercial cultivars in Kenya superior varieties can more efficiently be multiplied and distributed. Plantlets can also be enriched *in vitro* using endophytes to induce resistance of the resultant macadammia trees to disease phytotoxins and other biotic stresses (Saikkonen et al., 2004). *In vitro* enrichment with endophytes has been reported in *Theobroma cacao* (Arnold et al., 2003) and banana (Kavino et al., 2007).

Development of new varieties through somaclonal variation and genetic transformation

Somaclonal variation is the term used to describe the variation seen in plants that have been produced by tissue culture (Skirvin, 1993). The variations can be phenotypic or genotypic, caused by changes in chromosome numbers (polyploidy and aneuploidy), chromosome structure (translocations, deletions, insertions and duplications) and DNA sequence (base mutations) (Peschke and Phillips, 1992) . Hence, somaclonal variation leads to the creation of additional genetic variability. Somaclonal mutants can be enriched during *in vitro* culture to induce resistance to disease phytotoxins, tolerance to environmental or chemical stress (Bajaj, 1990; Haines, 1993). An example of such enrichment is with carrot somaclones resistant to *Alternaria dauci* (Dugdale et al., 2000) and *Pythium violae* (Cooper et al., 2006).

Plant genetic transformation involves the stable introduction of foreign DNA sequences, usually into the genome of the target plant. Specific new or modified genetic traits are added to existing plant varieties (Gayser and Fraley, 1989). Transgenic plants are then produced by regenerating shoots and roots from cells containing the foreign DNA through tissue culture techniques (McClean, 1998). Though macadamia is affected by relatively few pathogens that include *Phytophthora* spp., *Pythium* spp., *Botrytis* spp., *Rhizoctonia* spp., and *Pestalotia* spp. (Kiuru et al., 2004), the crop is highly affected by other stresses such iron chlorosis which affects both young and old plantations and can cause up to 50% losses (Wallace, 1957; Handreck, 1992) . Such stresses can benefit from somaclonal variation or genetic transformation by deve-

loping resistant varieties. Pena and Séguin (2001) reported on the successful incorporation of transgenes for shortening the juvenile phase into forest trees and this can be of great benefit to macadamia.

In vitro conservation of macadamia germplasm

Macadamia is currently conserved on-farm and in field gene banks. Field gene banks are costly to maintain and they require considerable inputs in the form of land, labour, management and material inputs (Uyoh et al., 2003) and this also limits the extent to which replication of accessions can be done and how much diversity can be maintained.

Tissue culture techniques (*in vitro* conservation techniques) offer 2 efficient complementary options to field gene bank *ex -situ* conservation. Slow growth storage (the medium-term conservation of stock cultures at few de-grees above zero, with or without the addition of compounds known to retard plant growth) and cryopreservation (freeze-preservation at ultra-low temperatures of 196°C in liquid nitrogen) (Benson, 1999). Cryopreservation is a sound alternative for long term conservation of plant genetic resources because at this temperature, all cellular divisions and metabolic processes are stopped (Panis and Lambardi, 2006) . In the recent years, the 'vitri-fication/one step freezing' technique has been continuously improved and applied to woody species after the invention of the Plant Vitrification Solution 2 (PVS2) (Sakai et al., 1990; Panis and Lambardi, 2006). However, for successful *in vitro* preservation an adequate protocol for micropropagation has to be worked out first for regeneration of the preserved tissues and hence this calls for concerted efforts to develop a tissue culture protocol for macadamia. *In vitro* cultures also simplify quarantine procedures for local and international exchange of germplasm since there is no risk of disease transfer, once the material is screened of known diseases while in the source country (Frison and Putter, 1993).

Conclusions

Macadamia genetic improvement in Kenya has been slow owing to the use of conventional breeding methods. It is clear that sustainable use and conservation of macadamia genetic resources would be enhanced greatly through biotechnology applications to complement conventional breeding and conservation efforts. However, use of biotechnology tools applications should be prioritized to yield maximum practical benefits in the shortest possible time. Use of molecular techniques for germplasm characterization and marker assisted selection should be a high priority.

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REFERENCES

Agricultural Research Council (ARC) (2000). ARC- Institute for Tropical and Subtropical crops, Pretoria, South Africa.

Arnold AE, Mejia LC, Kyllo D, Rojas E, Maynard Z, Robbins N, Herre EA (2003). Fungal endophytes limit pathogen damage in a tropical tree. Proceedings of the National Academy of Sciences of the United States of America. 100(26):15649-15654.

Avise JC (1994). Molecular markers, natural history and evolution. Chapman and Hall, Recent research and development in Forest Genetic Resources. Proceedings of the training workshop on the conservation and sustainable use of forest genetic resources in Eastern and Southern Africa. 6th -11th December 1999, Nairobi, Kenya.

Bajaj YPS (1990). Somaclonal variation - Origin, induction, cryopreservation and implication in plant breeding. In. Bajaj YPS (ed) Biotechnology in Agriculture and Forestry II – Somaclonal variation in crop improvement I. Springer-Verlag Berlin, Heidelberg, New York, Tokyo, pp. 1-48.

Baundouin L, Berger A, Lebrun P (2006). Assessing linkage disequilibrium in coconut. In: de Vicente MC, Glaszmann J-C (eds) Molecular markers for allele mining. Bioversity International, p. 36.

Benson EE (1999). Cryopreservation. In: Benson EE (ed) Plant conservation biotechnology. London, England, Taylor & Francis, pp. 83-95.

Bittenbender HC, Hirae HH (1990). Common problems of Macadamia Nut. In: Hawaii Research Extension Series 112. College of Tropical Agriculture and Human Resources, HITAHR, University of Hawaii.

CABI (2005). Report on the identification of income diversification options and development of small to medium enterprise for high quality coffee and macadamia in Kenya, CAB International Africa Regional Center, Nairobi, Kenya.

Cavaletto CG (1980). Macadamia nuts. In: Nagy SS, Shaw PE (eds) Tropical and subtropical Fruits, composition, properties and uses, AVI Publications Company pp. 542-559.

Cheplick GP, Faeth SH (2009). Ecology and Evolution of the Grass-Endophyte Symbiosis. Oxford University Press, Oxford.

Cooper C, Crowther T, Smith BM, Isaac S, Collin HA (2006). Assessment of the response of carrot somaclones to *Pythium violae*, causal agent of cavity spot. Pathology 55 (3) 427-432.

de Vicente MC, Guzman FA, Engels J, Rao VR (2006). Genetic characterization and its use in decision-making for the conservation of crop germplasm. In: Ruane J, Sonnino A (eds) The role of biotechnology in exploring and protecting agricultural genetic resources. FAO, Rome, Italy. pp. 129-138.

Dugdale LJ, Mortimer AM, Isaac S, Collin HA (2000). Disease response of carrot and carrot somaclones to *Alternaria dauci*. Plant Path. 49(1):57-67.

Duke JA (1983). Handbook of energy crops.

Ferreira ME (2006). Molecular analysis of gene banks for sustainable conservation and increased use of crop genetic resources. In: Ruane, J, Sonnino, A (eds) The role of biotechnology in exploring and protecting agricultural genetic resources FAO, Rome, Italy pp. 121-127.

Frison EA, Putter CAJ (eds) (1993). FAO/IBPGR Technical guidelines for the safe movement of coconut germplasm. Food and Agriculture Organization of the United Nations. Rome/International Board for Plant Genetic Resources, Rome.

Ganal MW, Altmann T, Röder MS (2009). SNP identification in crop plants. Current Opinion in Plant Biol. 12(2):211-217.

Gayser CS, Fraley RT (1989). Genetically engineered plants for crop improvement. Science 244: 1293-1299.

George PS, Ravishanker GA, Ventakaraman LV (1993). Clonal multiplication of *Gardenia jasminoides* Ellis through axillary bud culture. Plant Cell Rep. 13:59-62.

Gitonga LN, Kahangi EM, Muigai AWT, Ngamau K, Gichuki ST, Mutuma E, Cheluget W, Watiki BG (2008a). Farmers' knowledge on Macadamia genetic diversity in Kenya as a means for *in situ* conserveation. CATRINA 3(1): 55-60.

Gitonga LN, Kahangi EM, Gichuki ST, Ngamau K, Muigai AWT, Njeru ES, Njogu N, Wepukhulu S (2008b). Factors influencing the *in vitro* shoot regeneration in *Macadamia integrifolia*. Afr. J. Biotechnol. 7(22): 4202-4207.

Gitonga LN, Njeru ES, Kuria S, Muli S (2004). *In vitro* shoot proliferation of *Macadamia integrifolia*. Paper presented at the 9th KARI Scientific Conference. Nairobi.

Gitonga LN, Nyakundi W, Ruto ST, Watiki B, Balozi F, Takayama E (2002). Vegetative propagation of macadamia nut (*Macadamia integrifolia*, (*M. integrifolia* * *M. tetraphylla*) hybrids). In: Wesonga, JM, Losenge, T, Ndungu, CK, Obwara, FK, Agong, SG, Fricke, A, Hau, B, Stutzel, H (eds) Proceedings of the horticultural seminar on sustainable horticultural production in the tropics. 3rd-6th October 2001. JKUAT, Kenya.

Gitonga LN, Nyakund, W, Takayama E (1997). Effects of varietal differences on ability to root macadamia cuttings. Proceedings of the 1st National Horticultural seminar on sustainable horticultural production in the tropics. JKUAT, Kenya.

Goldstein DB, Linares AR, Cavalli-Sforza LL, Feldman MW (1995). An Evaluation of genetic distances for use with microsatellite loci. Genetics 139: 463-471.

Hamilton RA (1971). Macadamia nut growing, marketing, processing and research problems-Report to the Government of Kenya. FAO Rome No. TA 2996.

Hardner C, Peace C, Henshall J, Manners J (2005). Opportunities and constraints for marker-assisted selection in macadamia breeding. ISHS, Acta Hort. 694: 85-90.

Hardner C, Winks C, Stephenson R, Gallagher E (2001). Genetic parameters for nut and kernel traits in macadamia. Euphytica 117:151-61.

Haines RJ (1993). *In vitro* selection in breeding. In: Imrie BC, Hacker JB (eds) Focused plant improvement: towards responsible and sustainable agriculture pp. 210-219.

Handreck KA (1992). Iron-phosphorus interactions in the nutrition of seedling macadamia in organic potting media. Aust. J. Exp. Agri. 32(6): 773 - 779.

Hannotte O, Jianlin H (2006). Genetic characterization of livestock populations and its use in conservation decision-making. In: Ruane, J, Sonnino, A (eds) The role of biotechnology in exploring and protecting agricultural genetic resources. FAO, Rome, Italy pp. 89-96.

Harris M (2004). Involvement of the Harries family in the development of macadamia in Kenya, 1946-1974). Proceedings of the Macadamia Stakeholders meeting. 15th June, 2004. KARI HQTs. Nairobi, Kenya.

Hirama S, Ondabu N, Wasilwa L (1987). Observations of the differences on the characteristics of tree growth and bearing characteristics of five macadamia clones at different agro-ecology zones. Horticultural Development Project, Thika, Kenya.

Jaccoud D, Peng K, Feinstein D, Kilian K (2001). Diversity arrays: A solid state technology for sequence information independent genotyping. Nuc. Acid Res. 29: 25.

JICA (1991). Joint Review Report on the Horticultural Development Project in Kenya by Government of Japan, Japan International Cooperation Agency.

Kadman A (1982). Experiments with propagation of macadamia by air layering. Reprint from California Macadamia Society (CMS) Yearbook.

Kahl G, Mast A, Tooke N, Shen R, van den Boom D (2005). Single nucleotide polymorphisms. In: Meksem K, Kahl G (eds). The handbook of plant genome mapping: genetic and physical mapping. Wiley-VCH. pp. 75-104.

Kavino M, Harish, S, Kumar N, Saravanakumar D, Damodaran T, Soorianathasundaram K, Samiyappan R (2007). Rhizosphere and endophytic bacteria for induction of systemic resistance of banana plantlets against bunchy top virus. Soil biol. and Biochem. 39(5):1087-1098.

Kilian A, Huttner E, Wenzi P, Jaccoud D, Carling J, Caig V, Evers M, Heller-Uszynska, Uszynski G, Cayla C, Patarapuwadol S, Xia L, Yang S, Thompson B (2005). The fast and the cheap: SNP and DArTs-based whole genome profiling for crop improvement. In: Tuberosa R, Phillips RL, Gale M (eds). Proceedings of the international congress 'In the Wake of the Double Helix: From the Green Revolution to Gene Revolution, 27-42 May, 2003, Bologna, Italy pp. 443-461.

Kiuru P, Nyaga AN, Wasilwa L (2004). A Review of macadamia research in Kenya. Proceedings of the Macadamia Stakeholders meeting. KARI HQTs, Nairobi, Kenya.

Lanteri S, Barcaccia G (2006). Molecular marker based analysis for crop germplasm preservation. In: (eds) Ruane J, Sonnino A. The Role of Biotechnology in exploring and protecting agricultural genetic resources. FAO, Rome, Italy pp. 105-120.

Latha R, Rubia L, Bennett J, Swaminathan MS (2004). Allele mining for stress tolerance genes in Oryza species and related germplasm. Mol. Biotechnol. 27(2):101-108.

Leal DR, Sánchez-Olate M, Avilés F, Materan ME, Uribe M, Hasbún R, Rodríguez R (2007). Micropropagation of Juglans regia L. In: (Jain SM, Häggman H, (eds.) Protocols for micropropagation of woody trees and fruits. Springer, Heidelberg pp. 381-390.

Lee P (2004). Macadamia Working Group. Paper presented at the IX World Tree nut Convention, Las Vegas.

Leigh DS (1973). Notes on macadamia propagation in New South Wales. Reprint from California Macadamia Society Yearbook, 1973. http://www.coopersnuthouse.com/NotesOnMacadamiaPropagationInNewSouthWales20.ht.

Mauro-Herrera M, Meerow AW, Borrone JW, Kuhn DN, Schnell RJ (2006). Ten Informative markers developed from WRKY sequences in coconut (*Cocos nucifera*). Mol. Eco. Notes. 6(3):904-906.

McClean PE (1998). Analyzing plant gene expression with transgenic crops. Phillip McClean. http://www.ndsu.nodak.edu/instruct/mcclean/plsc731/transgenic/transgenic4.htm.

Macfalane N and Harris RV (1981). Macadamia nuts as an edible oil source. In: Pryde, EH, Princen, LH, MukNerjee, XD (ed) American Oil Chemiss Society.

McHargue LT (1996). Macadamia production in southern California. In. Janick J (ed), Progress in new crops. ASHS Press, Arlington, VA. pp. 458-462.

Mingyao L, Boehnke M, Abecasis GR (2005). Joint Modeling of Linkage and Association: Identifying SNPs Responsible for a Linkage Signal. Am. J. Hum. Genet. 76(6): 934–949.

Muhara J (2004). Role of private sector in processing and marketing of Macadamia in Kenya: Kenya Farm Nut experience. Proceedings of the Macadamia Stakeholders meeting. KARI HQTs, Nairobi, Kenya.

Mulwa RMS, Bhalla PL (2000). *In vitro* shoot multiplication of *Macadamia tetraphylla* L. Johnson. J. Hort. Sci. 75(1): 1-5.

Mulwa RMS and Bhalla PL (2006). *In vitro* plant regeneration from imamture cotyledon explants of macadamia (*Macadamia tetraphylla* L. Johnson). Plant Cell Rep. 25: 1281-1286.

Nakitandwe J, Trognitz F, Trognitz B (2007). Reliable allele detection using SNP-based PCR primers containing Locked Nucleic Acid: appli-cation in genetic mapping. Plant Methods 3:2.

Nissen RJ, Williams RR (1980). Assessment of Macadamia selections based on nut quality. Reprint from CMS Yearbook, 1980.

Nyakundi W, Gitonga L (1993). Macadamia Propagation handbook. JICA, Kenya.

Ondabu N, Nyaga AN, Tominaga K (1996). Macadamia clonal selection in Kenya. 5th Biennial KARI Scientific Conference, Nairobi, Kenya.

Onsongo M (2003). Kenya Trees Annual. USDA Global Agricultural Information Network.

Panis B, Lambardi M (2006). Status of cryopreservation technologies in plants (crops and forest trees). In: Ruane, J, Sonnino, A (eds) The role of biotechnology in exploring and protecting agricultural genetic resources. FAO, Rome, Italy pp. 61-78.

Peace C Vinathage V, Turnbull C, Carroll BJ (2002). Characterizing Macadamia germplasm with codominant radio-labeled DNA amplification fingerprinting (RAF) markers. International Symposium on Tropical and Subtropical fruits. Acta Hort. 575: 381-387.

Peace CP, Vithanage V, Turnbull CGN. Carroll BJ. (2003). A genetic map of macadamia based on randomly amplified DNA fingerprinting (RAF) markers. Euphytica, 134 (1): 17-26.

Pena L, Seguin A (2001). Recent advances in the genetic tra-nsformation of trees. Trends in Biotechnol. 19(12):500-506

Peschke V, Phillips RL (1992). Genetic implication of somaclonal va-riation in plants: Adv. Genet. 30: 42-75.

Pfeiffer TW (2003). From classical plant breeding to modern crop improvement. In. Chrispeels MJ, Sadava DE (eds). Plants, Genes and Crop Biotechnology. 2nd Edition. Jones and Bartlett Publishers, Inc. pp. 360-389.

Rafalski A (2002). Applications of single nucleotide polymorphisms in crop genetics. Current Opinion in Plant Biology 5(2):94-100.

Rapley R, Harbron S (Eds.) (2004). Molecular Analysis and Genome Discovery. Chichester. John Wiley & Sons Ltd.
Ribaut JM, Hoisington DA (1998). Marker assisted selection: New tools and Strategies. Trends Plant Sci. 3: 236-239.
Rotich M (2004). Development of macadamia industry: global markets of macadamia. Proceedings of the Macadamia Stakeholders meeting. KARI HQTs, Nairobi, Kenya.
Rumsey HJ (1927). Australian nuts and nut growing in Australia. Part 1. The Australian nut. Sidney.
Sakai A, Kobayashi S, Oiyama I (1990). Cryopreservation of nucellar cells of navel orange (Citrus sinensis Osb. var. brasiliencis Tanaka) by vitrification. Plant Cell Reports 9: 30-33
Sato Y, Waithaka JHG (1996). Integrating tree cash crops in agricultural production systems. Kenya.
Saikkonen K, Wäli P, Helander M, Faeth SH (2004).Evolution of endophyte-plant symbioses. Trends in Plant Science 9(6):275-280.
Schmidt AL, Scott L, Lowe AJ (2006). Isolation and characterization of microsatellite loci from Macadamia. Molecular Ecology Notes 6(4):1060-1063.
Sedgley M, Bell FDH, Bell D, Winks CW, Pattison SJ, Hancook TW (1990). Self-and cross compatibility of Macadamia cultivars. J. of Hort. Sci. 65: 205-218.
Semagn K, Bjørnstads , Ndjiondjop MN (2006a). Progress and prospects of marker assisted backcrossing as a tool in crop breeding programs. Afr. J. Biotechnol. 5:2588-2603.
Semagn K, Bjørnstad A, Skinnes H, Marøy AG, Tarkegne Y, William M (2006b) Distribution of DArT, AFLP, and SSR markers in a genetic linkage map of a doubled-haploid hexaploid wheat population. Genome 49(5): 545–555.
Shu-Biao-Wu SB, Wirthensohn MG, Hunt P, Gibson JP, Sedgley M (2008). High resolution melting analysis of almond SNPs derived from ESTs. Theor. Appl. Genet. 118:1-14.
Skrvin RM, Nortom M, McPheeters KD (1992). Somaclonal variation: Has it proved useful for plant improvement. Acta Hort. 336: 333-340.
Soleimanni VD, Baum BR, Johnson DA (2003). Efficient validation of Sngle Nucleotide Polymorphisms in plants by allele-specific PCR, with an example from barley. Plant Mol. Biol. Rep. 21: 281-288.
Stephenson RA (1983). Farm Characteristics and Management Practices in the Australian Macadamia Industry. Queensland Department of Primary Industries, Brisbane.
Sunnucks P (2000). Efficient genetic markers for population biology. Tree 15:199-203.
Tang K, Fu D-J, Julien D, Braun A, Cantor CR, Koster H (1999). Chip-based genotyping by mass spectrometry. Proceedings of the National Academy of Sciences of the United States of America 96(18):10016-10020.
Thimmappaiah, Shirly RA, Iyer RD (2007). Protocol for micropropagation of *Castanea sativa* Mill. In: (Jain SM, Häggman H, (eds.) Protocols for micropropagation of woody trees and fruits. Springer, Heidelberg pp. 313-320
Tominaga K, Nyaga AJN (1997). Breeding of Macadamia nuts. Evaluation report, 1994-1997.
Twyman R (2003). SNP typing with DNA chips. http://genome.wellcom.ac.uk/ doc_WTD021043.html.
United States Department of Agriculture (2008). Macadamia in-shell basis production by country in metric tons. http://www.indexmundi.com/agriculture/commodity=Macadamia&graph=production.
Upadhyaya HD, Furman BJ, Dwivedi SL, Udupa SM Gowda CLL, Baum M, Crouch JH, Buhariwalla HK, Singh S (2006). Development of a composite collection for mining germplasm possessing allelic variation for beneficial traits in chickpea. Plant Genet. Resour.: Characterization and Utilization, 4: 13-19.
Uyoh EA, Nkang AE and Eneobong EE (2003). Biotechnology, genetic conservation and sustainable use of bioresources. Afr. J. Biotechnol.2 (12): 704-709.
Vieitez AM, Sánchez MC, García-Nimo ML, Ballester A. (2007). Protocol for micropropagation of *Castanea sativa* Mill. In: (Jain SM, Häggman H, (eds.) Protocols for micropropagation of woody trees and fruits Springer, Heidelberg pp. 299-312.
Vithanage V, Hardner C, Aderson KL, Meyers N, McConachie C, Peace C (1998). Progress made with molecular markers for genetic improvement of macadamia. International Symposium on Biotechnology of Tropical and Subtropical Species, Part 2. Acta Horti. 461:199-207.
Waithaka JHG (2001). Sustainable commercial tree crop farming: A case for Macadamia nuts. Paper presented at the USAID African Sustainable Tree Crops Programme Conference, Nairobi, Kenya.
Wallace A (1957). Chlorosis in Macadamia. California Macadamia Society Year Book.
Wasilwa LA, Ondabu N, Watani G (2003). Adaptability of Kenyan macadamia selections and Hawaiian varieties in four agro ecological zones. A paper presented at the 1st KARI Adaptive Research Conference, Nairobi, Kenya.
Wasilwa LA, Watani GW, Ondabu N, Nyaga A, Muli H, Kagiri B, Kiiru S (1999). Performance of Macadamia varieties in three agro-ecology zones. Hortscience 35: 477.
Wenzl P, Li H, Carling J, Zhou M, Raman H, Paul E, Hearnden P, Maier C, Xia L, Caig V, Ovesná J, Cakir M, Poulsen D, Wang J, Raman R, Smith KP, Muehlbauer GJ, Chalmers KJ, Kleinhofs A, Huttner E, Kilian A (2006). A high-density consensus map of barley linking DArT markers to SSR, RFLP and STS loci and agricultural traits. BMC Genomics 7:206.
Wilkie JH (2008). Macadamia industry update. Paper presented at the 5th China International Foodstuff Exposition, Liuhua, China.
Wongtiem P, Perera C, Risterucci AG, Xia L, Patarapuwadol S, Carling J, Caig V, Evers M Uszynski G, Huttner E, Wenzi P, Fregene M, Vicente C, Glaszmann JC, Kilian A, (2005). Validation of Diverty Array Technology as a platform for whole-genome profiling in orphan crops. GCP meeting, Rome.
Woodroof JG (1967). Tree nuts, production, processing, products. AVI publications Company 1:313-337.
World Horticultural Trade & U.S. Export Opportunities (2004). Macadamia situation and outlook in selected countries.
Xia L, Peng K, Yang S, Wenzl P, de Vicente P, Fregene M, Kilian A (2005). DArT for high-throughput genotyping of cassava (*Manihot esculenta*) and its wild relatives. Theor. Appl. Genet. 110: 1092-1098.
Yang S, Pang W, Ash G, Harper J, Carling J, Wenzyl P, Huttner E, Kilian A (2006). Low level of genetic diversity in cultivated pigeon pea compared to its wild relatives is revealed by Diversity Arrays Technology (DArT) Theor. Appl. Genet. 113: 585-595.
Yokoyama KM, Wanitprapha K, Nakamoto ST and Bittenbender HC (1990). Macadamia Nut Economic Fact Sheet. 9. Dept Agric. Res. Econ., CTAHR, University of Hawaii.