AGRONOMIC EVALUATION AND MOLECULAR GENOTYPING OF SELECTED SOYBEAN (*GLYCINE MAX* (L) MERRIL) VARIETIES GROWN IN DIFFERENT ENVIRONMENTAL REGIONS OF EAST AFRICA

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DECLARATION

This thesis is my original work and has not been presented for a degree in any other University.

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DEDICATION

To my loving and caring parents.

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Prior appreciation goes to the Almighty God for everything He has done in my life this far.

Many thanks go to my family for their pieces of advice, prayers and financial support. My parents Mr. Moses Okwany Agina (father), Mrs. Caroline Okwany (mother) and loving brother Mr. Joshua Caleb Okwany. They have been by my side all the times.

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ABSTRACT

Soybean (*Glycine max*. (L)Merril) is one of the most popular pulses in the world. It has been known to man for a long time since its first record goes as far as 2500 BC in China and Manchuria. *Glycine max* belongs to the subgenus *soja,* which also contains *G. soja* and *G. gracilis*. Soybean is grown primarily for the production of seed, has a multitude of uses in the food and industrial sectors, and represents one of the major sources of edible vegetable oil and of proteins for livestock feed use. Genes greatly determine morphological characteristics of living organisms. Environmental conditions may also determine these characteristics, some of which are important in agronomy. This study aimed at investigating the relationship that exists between agronomic and molecular characteristics of selected soybean cultivars grown in different regions of East Africa. The results will be used to advice both the farmers and researchers on the cultivar they cultivate/study and their relationship to agronomic value respectively. This information will also be used in breeding programmes. In this research, different soybean cultivars from different regions of East Africa (Kenya, Uganda and Tanzania) were grown in different blocks within one region (Mbita point in Kenya). Agronomic characteristics were observed and recorded from germination to post harvest time. For molecular analysis, genomic DNA was extracted according to Phenol Chloroform method. This was followed with PCR process using custom ordered pair of primers that corresponded to the flanking ends of the targeted gene fragment (5S ribosomal genes). Restriction Fragment Length Polymorphism (RFLP) was then performed on the isolated PCR fragments by digesting DNA using various restriction enzymes determined by computer based simulation of already sequenced genes of known *Glycine* species. Gene clean was performed on PCR materials. The elute was confirmed on agarose gel then sent for automated sequencing ABI prism (AppliedBiosystems) at (ILRI). The resulting gene sequences were compared with gene sequences of known *Glycine* species using various enzymes in computer based simulations. This enabled get a marker (*Sac* II) for the 5S ribosomal genes in *Glycine max.* The gene sequences were then subjected to gene blast using MEGA 4 and resulted with a phylogenetic tree for the East African soybean. The studied cultivars were found to be closely related to *Glycine max.* RFLP results were compared with agronomic performance. There was significant difference in plant height ($F= 106.131$, $df = 12$, $p<0.05$); number of branches ($F=25.227$, $df =12$, $p<0.05$); weight of the whole plant ($F = 6.634$, $df = 12$, $p<0.05$); total number of seeds per plant from the various cultivars ($F = 13.330$, $df = 12$, $p<0.05$); weights of total seeds per plant from various cultivars ($F = 5.124$, $df =12$, $p<0.05$). Significance differences observed in various parameters analysed in this study were not reflected in the cultivars' genetic characteristics. The marker (*sac* II) identified in this research will be useful in further research in soybeans. The best performing cultivars (TGX-1876-2E, Namsoy 4M, Nam II and Nyala) will also be recommended for soybean farmers around Lake Victoria region. These results will also assist plant breeders to produce hybrids from the best performing cultivars.

ACRONYMS AND ABBREVIATIONS

ABI	Applied Biosystems
ANOVA	Analysis of variance
DM	Dry Matter
DNA	Deoxyribonucleic acid
ICIPE	International Center of Insect Physiology and Ecology
ILRI	Institute of Livestock Research Infections
LAI	Leaf Area Index
MAAL	Monosomic Alien Addition in Lines
MEGA	Molecular Evolutionary Genetics Analysis
PAGE	Polyacrylamide Gel Electrophoresis
PCR	Polymerase Chain Reaction
RFLP	Restriction Fragment Length Polymorphism
SFP	Seed-filling Period
SNP	Single Nucleotide Polymorphism
SSR	Simple Sequence Repeat
TBA	Tris Borate EDTA
T.E	Tris-HCl EDTA
UV	Ultra violet

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CHAPTER ONE

INTRODUCTION

1.1 Study Background

Soybean (*Glycine max*. (L)Merril) is one of the most popular pulses in the world. It has been known to man for a long time since its first record goes as far as 2500 BC in China and Manchuria (Morse, 1950). It is generally agreed that soybean probably originated in the Northern and Central regions of China (Gibson and Benson, 2002). According to Ngeze (1993), soybean originated from a wild plant known as glycine from the Manchuria region of China.

Glycine max belongs to the subgenus *soja*, which also contains *G. soja* and *G. gracilis*. *Glycine soja*, a wild species of soybean, grows in fields, hedgerows, roadsides and riverbanks in many Asian countries. Cytological, morphological and molecular evidence suggest that *G. soja* is the ancestor of *G. max. G. gracilis* is considered to be a weedy or semi-wild variant of *G. max*, with some phenotypic characteristics intermediate to those of *G. max* and *G. soja*. (Fekuda, 1993; Hymowitz, 1970).

Soybean was introduced in East Africa at the turn of twentieth century. It is believed that soybean varieties currently grown in the East African countries trace their roots to these early varieties. Although no literature exists showing the performance of these early foreign varieties, satisfactory productivity was noted in the Bukoba region of Kagera province during the second world war period (1939-1945).

In 1974 Harrison moved from Uganda to Kenya. He settled at Kisii, in Southwestern Kenya, just across Lake Victoria from Kampala. The Kenya Department of Agriculture had identified the area around Kisii to be the best in the country for growing soybeans. Starting in 1974, Harrison began to work with the Kenya government to promote the cultivation of soybeans in Kenya. Many of farmers he worked with were members of Adventist Church (William and Akiko, 2004).

Soybean is grown as a commercial crop in over 35 countries. The major producers of soybeans are the United States, China, Democratic People’s Republic of Korea, Argentina and Brazil. Currently the largest producer of soybeans in the world is North America whose production stands at 70.5 million metric tones per year followed by South America (44.8 million metric tones per year). Africa produces 0.6 million metric tones per year, while the rest of the world produces 28.0 million metric tones. Most of the soybean produced in Africa comes from Kenya, Nigeria, Zimbabwe, Egypt, South Africa, Zambia, Malawi and Uganda. The main soybean producing areas in Kenya are Rift Valley province (Nakuru, Nandi, Trans Nzoia, Koibatek, Narok, Trans Mara, Laikipia and Bomet districts); Eastern province (Meru, Embu, Mbere and Machakos districts); Western province (Kakamega, Busia, Bungoma, and Vihiga districts); Nyanza province (Rachuonyo, Homabay, Kisii, Nyamira and Siaya districts); and Central province (Kirinyaga, Muranga and Nyeri districts). Since 1993, the total production in Kenya is estimated to have increased from 1000 metric tones (MT) to 5000 MT per year (GTZ, 1998).

1.2 Economic importance of soybeans

Soybean is grown primarily for the production of seed, has a multitude uses in the food and industrial sectors, and represents one of the major sources of edible vegetable oil and of proteins for livestock feed use.

The mature soybean seed contains a higher food percentage than any other pulse. The seed has 13-25% oil, 30-50% protein and 14-24% carbohydrates. It also contains vitamins B1, B2 and B5 (Borget, 1992; Schery, 1972). The oil is used industrially in the manufacture of margarine and shortening, candles, soaps, vanishes, insecticides and many more products. Its seeds can be eaten green (boiled or baked); the seeds are also cooked and eaten with a cereal accompaniment. Another product is soy milk which is recommended for diabetics and infant formula. Soybean flour has been reported to reduce the level of blood cholesterol thereby reducing the risk of heart problems (Borget, 1992). Soybean alone or in combination with cereal forage such as oats and barley is also used as animal feed. Soybean has agronomic value such as soil conservation and as green manure. Due to their ability to fix nitrogen in the soil, soybean helps to improve productivity of other food and cash crops particularly in mixed crops, and rotational farming (Borget, 1992; Rodriguez-Kabana *et al*., 1990).

1.3 Problem statement

Most farmers and researchers in East Africa do not have sufficient information on agronomic nor genetic characteristics of the soybean cultivars that they have.

1.4 Justification

This study aimed at investigating the relationship that exists between agronomic and molecular characteristics of these soybean varieties. The results would be used to advice both the farmers and researchers on the cultivar they cultivate/study and their relationship to agronomic value respectively. This information can also be used in breeding programmes.

1.5 Hypotheses

i. Variation in agronomic performance in various soybean cultivars is represented in the cultivars' genetic make up.
ii. Most East African soybean cultivars are the same genotypes.
iii. Nyala (control) performs better than other cultivars agronomically.
iv. East African soybeans are the same genotype as their ancestral genotype.

1.6 Objectives

i. To evaluate the agronomic performance of the selected cultivars.
ii. To determine molecular characteristics of selected cultivars.
iii. Associate the molecular characteristics with morphological features of the soybean cultivars.
iv. To define the genotype of selected cultivars grown in East Africa.

CHAPTER TWO

LITERATURE REVIEW

2.1 Physiological and Agronomic characteristics of soybeans

The soybean plant is a branched, non frost tolerant, annual about one meter above ground level and two meters below ground level. Like other legumes, soybeans root develops nodules if the appropriate rate of strain of *Rhizobium* bacteria is present. The root system is extensive, with a tap root which may exceed 1.5 meters in length, giving rise to many lateral branches usually in the 1-30 centimeters horizon. However, there is considerable variation between cultivars in respect to growth, total amount, spread and degree of penetration of roots, factors which must be considered when choosing varieties to be grown in specific conditions. For instance, according to Mayaki *et al.*, (1976) on a dry matter basis at physiological maturity, 50 percent of dry root weight of rain grown soya was in the 0-15 centimeters horizon and 30 percent in the 15-90 centimeters horizon. Root development ceases at about the time seed development begins. This compares with 70 percent and 20 percent when irrigated. (Mayaki *et al*., 1976).

Stem is normally rounded, often hairy, varying in colour according to the variety, usually less than 75 centimeters in height, the lower internodes becoming woody with age. Plants are naturally much branched, although modern cultivars usually have less than six branches, and there are determinate and indeterminate types. Tall varieties which may reach up to 200 centimeters and recumbent types are generally grown for fodder or as smallholder crops, since they are unsuitable for mechanized production. Trials indicate that plants which achieve maximum height at flowering with subsequent minimum growth produce higher seed yield than plants which significantly increase their height after flowering (AVRDC, 1978).

Usually branches arise from the base of the stem and do not rebranch. Indeterminate types have the terminal growing point elongating until the plant matures and does not terminate with a cluster of flowers or pods while determinate types have stems terminating in a cluster of flower or pods. They have more branches and are shorter than indeterminate.

The soybean pods have generally 2-4 seeds with main colour being grey or black all of which may be modified in appearance by presence of the hairs on the surface. Seed development requires about 35 days after pollination. Dry matter accumulation varies from 60-90 pounds per day per acre. Commercial varieties grown for oil and protein have seeds almost spherical in shape, ranging in size from 10-20 grams per 100 seeds and often flattened. There have however, been reports of nitrogen fertilizers increasing the seed yields of soya and groundnuts. Some of these responses may be due to poor nodulation in the absence of an effective strain of *Rhizobium* or to high soil temperature depressing the rate of nitrogen fixation. At Ibadan Nigeria, it was found that at this season surface soil temperature exceed 40^{o}C and that temperature exceeding 36^{o}C significantly reduced germination rate and percentage emergence of soybean, but cow pea emerged equally well with or without shade. Solution culture studies have shown that the activities of monomeric aluminium species and calcium account for reduction in growth of tap root of soybeans. Furric and humic compounds commonly found in acid soils can alleviate aluminium toxicity. In acid soils with no aluminium toxicity it may be calcium deficiency which restricts plant growth while it appears that soybeans are generally quite sensitive to soil acidity, reasonable grain yield of 2-2.5 t h^{-1} and quite satisfactory nodulation are sometimes obtained with tolerant varieties on soils with pH values in the range of 4.1-4.6 and aluminium saturation in the range of 36-55% as found in Guyana, Nigeria, Bromfield and Puerto Rico (Alva *et al.*, 1986; Bruce *et al.*, 1988; Wahab, 1979; Olsen *et al.*, 1975; Bromfield and Ayanaba, 1980; Le mare, 1959).

Research on the association between dry matter (DM) accumulation and soybean yield have also reported contradictory results. In early research, no association between DM accumulation and yield were found (Weber *et al.*, 1966). These researchers used different planting patterns and populations to increase DM accumulation. The treatments they imposed affected DM accumulation predominantly during the vegetative period. A more recent study of four Japanese soybean cultivars reported that difference in DM accumulation between old and modern genotypes was most apparent after the beginning of the seed-filling period (SFP) (Shiraiwa and Hashikawa, 1995). The contradictory finding in earlier and more recent studies may be evidence of a temporal relationship between DM accumulation and yield improvement. A number of researchers have attempted to identify critical periods for soybean yield determination (Egli, 1988; Board and Harville, 1993; Hayati *et al.*, 1995; Board *et al.*, 1996). Investigations on the vegetative period have reported no evidence to link either DM accumulation during the vegetative period or the duration of this period with yield (Weber *et al.*, 1966; Egli, 1993). While many researchers have found a correlation between the duration of the SFP and seed yield (Hanway and Weber, 1971; Gay *et al.*, 1980; Smith and Nelson, 1986), the contribution of the duration of the SFP to soybean yield has been contested (Egli *et al.*, 1984). However, assimilate supply after the beginning of the SFP (R4) may affect yield. Board *et al*. (1996) found that total DM and leaf area index (LAI) at R5 were positively correlated with seed yield. Furthermore, it has been reported that increasing assimilate supply after beginning pod development (by either shade removal or CO_2 enrichment) increased seed yield (Hardman and Brun, 1971; Hayati *et al*., 1995). Therefore, the association between total DM and seed yield may be apparent only if greater DM accumulation occurs after the beginning of the SFP.

Physiological maturity (maximum dry weight at a moisture content of 50-60%) coincides with the growth stage R7 of Fehr and Caviness i.e. when one pod on the meristem has reduced its mature colour. Delaying harvesting after physiological maturity by as little as two weeks increases seed discoloration and damage (Fehr and Caviness, 1977, 1979; Nangju, 1980; Te Krony, Egli and Henson, 1981).

In the tropics the growth duration of adapted soybean cultivars and landraces is commonly 90-110 days and up to 140 days (Osafo, 1977). This relatively short growth period is due primarily to sensitivity to short day length. Numerous studies have shown that the induction of flowering, the duration and extent of vegetative growth, the production of viable pollen, the length of flowering and pod-folling periods and maturity characteristics are all subject to modification by photoperiod. Time of flower initiation shows a complex day length temperature interaction and sensitivity to short days or high temperature. Temperature alone is a poor predictor of time of flowering or total growth duration (Criswell and Hume, 1972; Lawn and Byth, 1979; Major *et al.*, 1975). At relatively high latitudes within the tropics there is linear decline in number of days to flowering and total plant height with delay in sowing after midsummer. Flowers first appear on lower meristem nodes and form progressively towards the tip of the meristem and along auxiliary branches. There are determinate and indeterminate genotypes. The flowering period lasts 15 days in the determinate soybeans. Many flowers may abort, the most common stage of loss being the first week after flowering. Pod and seed development takes 30-70 days (Osafo, 1977).

2.2 Local and Global Cultivation and Utilization of soybeans

The new production areas of soybean are characterized by warmer and more humid conditions, which pose different production problems such as pod shattering, reduced seed viability, pest and diseases. Seed losses of 34-99% are often associated with pod shattering in susceptible varieties and delayed harvesting after maturity. Shortage of labour and harvesting materials can delay harvesting leading to seed yield loss. To overcome these there is need to develop varieties that are resistant to shattering, that can stand relatively longer periods in the field after dry maturity without shattering.

The cultivation and utilisation of soybean locally and at industrial level in Kenya has continued to grow and probably motivated by the search for alternative sources of proteins and cooking oil. Soybean grows between 0 and 2000m above sea level. The maturity period is longer at higher altitudes than at lower altitudes. It grows in a wide range of soil and climatic conditions; it requires deep well drained soils with soil pH of 5.8 to 6.8. vertisols (black cotton soils) can be used as long as germination is not impeded by soil capping. Soya is very sensitive to day length and varieties selected for certain areas may not do similarly elsewhere (GTZ, 1998).

In Kenya soybean germplasm evaluation started in 1993 with 96 local and imported soybean varieties, which were tested for yields and management requirements, seed colour and height. The imported varieties were from regions that are similar to Kenya in latitude (day length), soil age but not necessarily in altitude such as Nigeria, Zimbabwe, Zambia and Ecuador on the basis of high yields. Genotype by environment interactions caused by differential responses of genotypes in environmental changes is a factor that often complicates and confounds the selection of superior varieties (Nassiuma and Wasike, 2002). Cultivars crossover interactions and genotypic rank change across environments are of importance in plant breeding and may slow down the selection process (Abdalla *et al.,* 1997). The environment is defined as the sum total of the external conditions which influence growth and development of an organism (Allard, 1960). The different attributes of the environment include moisture supply, temperature and soil type among others (Yau *et al.,* 1991).

2.3 Soybean Breeding and Genetics

Soybean breeding programs in USA have successfully developed hundreds of improved cultivars through hybridization of elite cultivars and breeding lines that trace back to a small number of original plant introductions and selection. The narrowness of the North American soybean germplasm base has been well documented by pedigree analysis. In the analysis of pedigree of 258 North American cultivars were released between 1947 and 1988. Gizlice *et al.,* (1994) determined that only 35 ancestors contributed more than

95% of all alleles. An increase in the coefficient of percentage has been noted when ancestry of cultivars developed for the Southern and Northern growing regions of North America are examined separately, indicating an even greater restriction of genetic base of cultivars within these regions. The limited germplasm bases of North American soybean cultivars threaten the ability of breeders to sustain genetic improvement. It also increases vulnerability of the crop to the changes in pathogen and pest populations. Introgression of new genetic diversity through hybridization with introduced germplasm is one of the ways to increase genetic variation in breeding populations, the base upon which gain from selection depends. At present the use of exotic germplasm in soybean cultivar development generally has been limited to small number of introductions that have served as sources of genes for resistance to disease and insect pest and have contributed little overall genetic diversity (Brown-Guedira, *et al.,* 2000; Gizlice *et al.,* 1994).

According to Scott and Samuel (1970), the method of producing hybrid soybean seed on the commercial scale is yet to be defined. The problem is that soybean is a perfect flower. Both the staminate and the pistillate are present in the same flower. When pollen is shed it immediately drops on the stigma of the same flower. This often happens before the flower opens (blooms). The stigma is receptive to pollen approximately 24 hours before anthesis and remains receptive 48 hours after anthesis. The anthers mature in bud and directly pollinate the stigma in the same flower. As a result, soybeans exhibit a high percentage of self-fertilization and cross pollination is usually less than one percent (Caviness, 1966).

Brown-Guedira *et al.,* (2000) proposed that to utilize the introduced germplasm to increase productivity and to provide new resource of genetic variation for future gain, selection criteria for parental stock need to consider genetic diversity as well as agronomic value. Several diversity studies in soybean have been conducted using morphological characters, pedigree information, and biochemical variation. Although morphological and agronomic characters are useful in evaluating genetic diversity, collecting such data can be laborious and phenotypic values are often strongly influenced by the environment. The development of hybrids could result in the introgression of the novel traits into these related species and result in the related species becoming weedier, with potential for ecosystem disruption into related species. For a trait to become incorporated into a species genome, recurrent backcrossing of plants of that species by hybrid intermediaries, survival and fertility of the resulting offspring is necessary. The taxonomy of species within the genus Glycine has been confused, and it long remained a subject for argument, although recent revisions have removed many spurious taxa from the genus. As an example of taxonomic problems, when verdcourt (1996) was preparing a revised treatment of the genus Glycine for the flora of Tropical East Africa, he found the type specimen for the entire genus was infact a pueraria. At present, G. max (L) Merril, synonymous with G. hispida, Soya max, Dolichus Soja etc. is placed in the subgenus Soya (Moench) F.J. Herm. Together with G. soya sieb and Zucc. (also known as G. ussuriensis). Both have 2n=40 as have most members of the subgenus Glycine L. Verdc. The subgenus Bracteata which consisted of G. wightii and its various subspecies, has now been removed from the genus Glycine altogether, and a new designation has been proposed; Neonotonia weightii. (Lu 1966; Leppik, 1971; Broue, 1978; Hymowitz and Newal, 1981).

Interspecific hybridization within subgenus Glycine, or between the subgenus soja has been generally unsuccessful. Cytogenetic evidence suggest that *G. max* and its putative ancestor *G. soja* are the same species, but as a practical aid to plant breeders they have been maintained as separate by taxonomists, since the first is a wholly domesticated erect annual, the second is basically a wild annual twining vine. In common with other crop long domesticated and used intensively by man, modern soybean has lost much of its original genetic diversity through continuous selection for high yield and uniform maturity. Thus in spite of superficial differences between modern cultivars, they are genetically similar. The sub-sequential yield increase which has been achieved by some commercial cultivation of soybean began in the USA is unlikely to be maintained. A study by the University of Missouri showed that modern cultivars have 50% higher yields than varieties available in the 1930s (Anon, 1978). There would presently appear to be a yield barrier problem in soybean, and current research indicates that interactions between environment and plant growth could be as important in this respect as genetic factors. It could well be that selection for, or incorporation from other genotypes, or increased ability to assimilate or more efficient use applied nutrients would be valuable attributes. There would also appear to be a strong negative correlation between the two important factors of oil and protein content, which renders improvement of the two most difficult. Reported habitability of yield would appear to be low, averaging some 50%, while maturity, lodging, height and seed size varied between 70 and 90%, with protein and oil content around 75%. These relations imply that selection for yield is more difficult than for other attributes (Miller and Fehr, 1979). Soybean is considered to be a diploidized tetraploid with a putative diploid ancestor (n=11) that underwent an aneuploid loss to (n=10) followed by genome duplication (Shoemaker *et al.*, 1996).

2.4 Use of Molecular Techniques in soybean characterization

In Zou *et al.*, (2004), a selection of 96 soybeans by simple sequence repeat (SSR) markers was evaluated for cross-specific amplification and polymorphism in *G. tomentella.* Thirty-two SSR markers (33%) revealed specific alleles for *G. tomentella* PI 483218 (2n=78). In the same study these SSR markers were further examined with an amphidiploid line (2n=118) and Monosomic Alien Addition in Lines (MAALs), each with 2n=40 chromosomes from soybean and one from *G. tomentella*. The results show that the use of SSR markers is a rapid and reliable method of detecting *G. tomentella* chromosomes in MAALs. The subgenus *Soja*, to which *G. max* belongs, also includes *G. soja* Sieb. and Zucc. (2n=40) and *G. gracilis* skvortz. (2n=40), wild and semi-wild annual soybean relatives from Asia. *G. soja* (2n=40) is a wild viny annual with small and narrow trifoliate leaves, purple flowers and small round brown-black seeds. It grows wild in Korea, Taiwan, Japan, Yangtze Valley, N.E China and areas around the border of the former USSR. *G. gracilis*, an intermediate in form between *G. soja* and *G. max*, has been observed in North East China. Inter-specific, fertile hybrid between *G. max* and *G. soja* (Sieb and Zucc.) and between *G. max* and *G. gracilis* have been easily obtained. (Gizlice *et al.*, 1994; Ahmed *et al*., 1977; Hadley and Hymowitz, 1973; Broich, 1978; Karasawa, 1952; Skvortzow, 1927)).

Agronomic performance of exotic germplasm in the target environment may be taken into account in parental selection; but is not known what effect this has on the probability of obtaining allelic diversity. Morphological differences are usually determined by a small number of genes and may not be representative of genetic divergence in the entire genome. Geographical origin also may not be an adequate indicator of genetic diversity (Brown-Guedira, *et al*., 2000).

The coding portion of the nuclear ribosomal rDNA cistron (18S, 5.8S, and 26S rDNA) has been extensively characterized at the sequence level for several hundred flowering plants, mainly owing to interest in using these data for phylogenetic analyses. Sequences include those from small-subunit (18S) rDNA (Nickrent and Soltis, 1995; Soltis *et al*., 1997), large subunit (26S) rDNA (Bult *et al*., 1995; Kuzoff, *et al*., 1998), and a large number of internal transcribed spacer (ITS) and 5.8S rDNA sequences (reviewed in Baldwin *et al.*, 1995). The ITS is the most variable portion of the rDNA cistron and is sometimes referred to as the NTS (non transcribed spacer), a misnomer, since large portions of it are transcribed. In contrast to the conserved 18S and 26S rDNA, which have phylogenetic utility at deeper divergence levels, ITS sequences often contain sufficient variation to allow examination of genetic relationships between cultivated varieties (cultivars), populations, and individuals (Rogers and Bendich, 1987; Hemleben *et al*., 1988; Jorgansen and Cluster 1988; Schaal and Learn, 1988). The ITS rDNA varies widely in length among different plant groups, ranging in size from 0.1 to over 1.2 kb (Rogers and Bendich, 1987). Such length heterogeneity is a result of the presence of at least one and often many, tandem (direct) or dispersed subrepeat domains. The advent of direct sequencing of PCR product has precluded the need for cloning, thus greatly facilitating broad-scale sequencing studies of many genes; however, the length of plant ITS sequences and the lack of universally conserved sites among different species within ITSs may explain the relative paucity of these sequences. The development of DNA-based markers is important for selection and improvement of varieties and hybrids in plant breeding programs. (Gupta *et al*., 2001; Kota *et al*., 2003).

Single nucleotide polymorphisms (SNPs) including insertion/deletions (indels) can provide a rich source of useful molecular markers in genetic analysis. Because SNPs can be analyzed using high-throughput and cost effective systems, they are useful for construction of high-density genetic maps as well as for genetic association studies (Cho *et al.,* 1999, Picoult-Newberg *et al*., 1999; Nairz *et al.,* 2002; Rafalski 2002; Kota *et al*., 2003).

The relatively high level of linkage disequilibrium (LD) that would be anticipated in self-fertilizing plant species such as soybean may permit whole genome scans using SNPs for QTL discovery. In contrast, the lower levels of LD in out crossing species such as maize (*Zea mays* sp. *mays* L.) will require the use of the candidate gene approach to discover the specific gene(s) underlying phenotypic changes (Rafalski, 2002; Tenaillon *et al*., 2001; Zhu *et al.,* 2003).

Mutations in coding DNA sequences (cSNPs) may change amino acid sequences and affect gene function and could therefore be valuable as markers of agronomic traits. Expressed sequence tag (EST) data serve as a useful source of DNA sequences in which SNPs can be discovered. The Soybean EST Project database contained more than 342,000 publicly available ESTs from 84 libraries as of December 2004. This resource provides an excellent source for the development of gene-derived SNP markers (Collins *et al.*, 1998; Brookes 1999; Marth *et al.*, 1999; Picoult-Newberg *et al*., 1999).

Restriction Fragment Length Polymorphism (RFLP) can also be used as a genetic marker. Instead of examining some features of the phenotype; the method directly assesses the genotype, as revealed by the restriction map. Recombination frequency can be measured between a restriction marker and a visible phenotypic marker. A genetic map can include both genetic and phenotypic markers. Because restriction markers are not restricted to the genomic reprint changes that affect the phenotype, they provide the basis for an extremely powerful technique for identifying genetic loci at molecular level (Lewis, 2000).

Minisatellites are also useful for genetic mapping. These are sequences that are satellites consisting of tandem repeats of a short unit, consisting of from 5-50 bp repeats. The variability is seen when a population contains variations fragments of many different sizes that form the same genomic region. When individuals are examined, there is extensive polymorphism, and that many differences in alleles, can be found. The cause of variation here is that individual alleles have different numbers of the repeating unit. The cause of this variation is the genetic recombination between misaligned repeat units as for satellite DNA. The rate of genetic exchange at minisatellite sequences is high, approximately 10^{-4} per kb of DNA. This rate is approximately x10 greater than the rate of homologous recombination at meiosis that is in any random DNA sequence. So minisatellites are hotspots for meiotic recombination (Lewis, 2000).

Sometimes the presence of a minisatellite is correlated with a high rate of exchange during recombination events occurring in sister chromatids. Because of the variations of length with repeat unit it changes the length of the minisatellite, but has no effect on flanking phenotype makers, because these are non coding sequences of DNA (Lewis, 2000).

CHAPTER THREE

MATERIALS AND METHODS

3.1 Study materials and area

A collection of thirteen (13) soybean cultivars were used in this research. Both field and laboratory experiments were done. Farm experiment was performed at ICIPE, Mbita Point. The study area was as shown in fig 1.

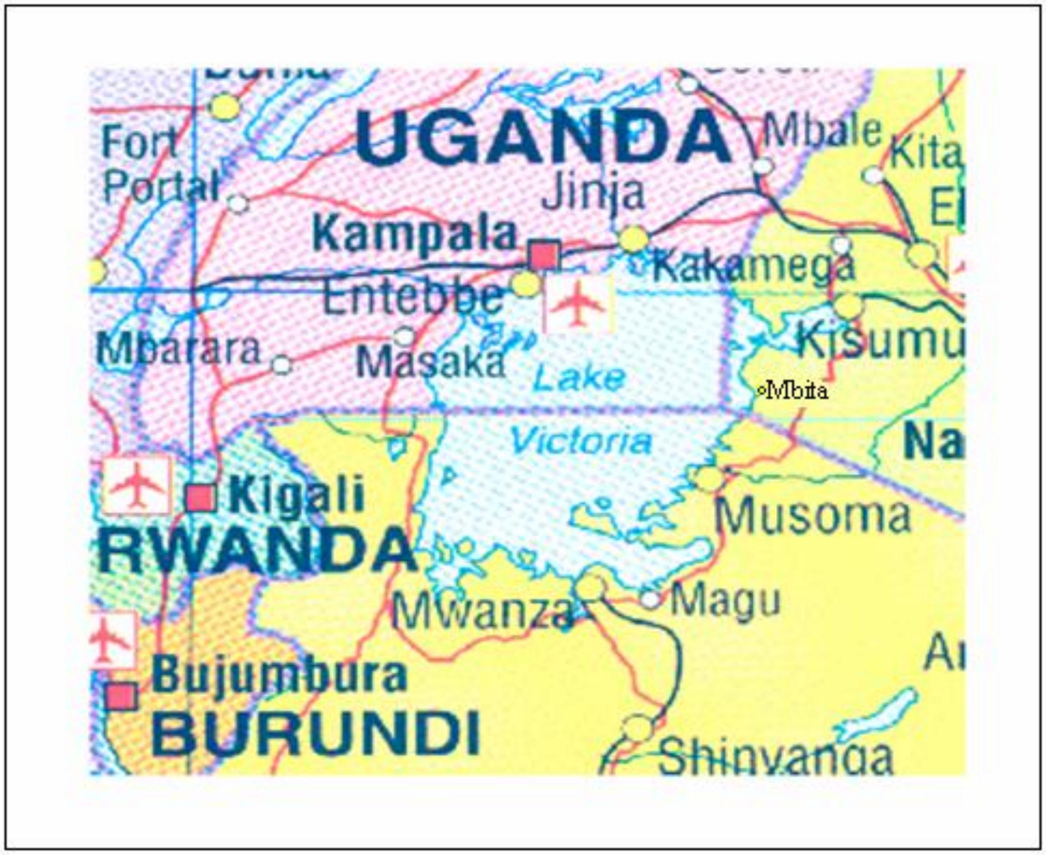


Figure 1 Map showing study area

3.2 Field Experiment

Performance of local cultivars and experimental cultivars was studied for all the three countries (Kenya, Uganda and Tanzania). There were three replications of experimental blocks each consisting of the standard variety (high yielding landrace) grown along the introduced cultivars.

Each block contained 5 cultivars (4 experimental and one control). Each cultivar was planted in three rows. The distance between each plant and the other was 5 centimeters, between one row and another was 50 centimeters. The distance separating one cultivar from the other was 1 meter.

In this experiment, cultivars were planted in blocks as follows: Kenyan block (SB 20, SB19, SB9, SB8, and Nyala); Tanzanian block (Mikumi, Mikese, Duicker, TGX 1876-2E, and Nyala); Ugandan block (Maksoy IN, Namsoy 4M, NAM I, NAM II and Nyala). Nyala was planted in all the blocks as a control. The experimental blocks are shown in figure 2.

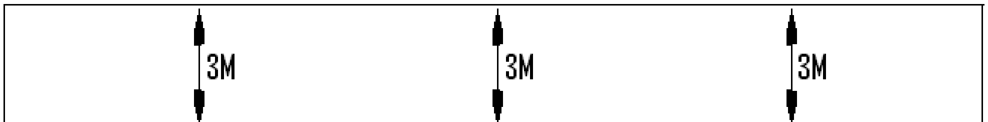


Figure 2 Experimental layout

Cultivars were harvested and labeled according to their regions and replications. This was followed by laboratory analysis, where individual measurements and weights of cultivars were taken. A meter rule was used to measure the length of the whole plant from the bottom most part of the shoot to the tallest branch. Individual plants were weighed carefully making sure they did not touch the ground. The number of branches emerging from each plant was counted. Seeds from individual plants were threshed, counted and weighed. Seeds from different replications but same cultivars were bulked in a hark bag. Biomass without seeds was determined by subtracting the total weight of seeds from the weight of the whole plant. 1000 kernel weight was determined by counting about 100 seeds and multiplying with a coefficient. All this data was recorded in tables and subjected to statistical analysis. The raw data from the tables were then fed into SPSS program for analysis. The parameter's means were compared by the use of ANOVA.

3.3 Laboratory (molecular) Experiment

3.3.1 Genomic DNA extraction

Approximately 1.0g young shoot of soybean was obtained from the set experimental blocks, taken to the laboratory, washed with distilled water and placed in sterile mortar and quickly ground into pulp using a pestle then immediately subjected to DNA extraction procedures. STE extraction buffer (200 µℓ) (1M Tris (pH 7.5), 5MNaCl, 0.5M EDTA, 10% SDS and 7µℓ of mecaptoethanol) was added to the ground leaf materials then transferred to eppendorf tubes. An aliquot of 500 µℓ of the Phenol: Chloroform (1:1) was added and vortex for 10 seconds, then left at room temperature for about 45 minutes. It was then centrifuged at 10000 rpm for 10 minutes. Supernatant (500 µℓ) was transferred into a fresh eppendorf tube and an equal volume of cold Chloroform: Isoamyl alcohol (24:1) added and mixed well by inversion to emulsify then centrifuged at 10000 rpm for 10 minutes. Chloroform: Isoamyl wash was repeated twice. Supernatant (500 µℓ) was transferred into a fresh eppendorf tube then an equal volume of chilled Isopropanol (propan-2-ol) was added, mixed and incubated at -20 °C for 15 minutes. It was then centrifuged at 14000 rpm for 5 minutes at room temperature to pellet the DNA and the pellets washed with 450 µℓ of 70% ethanol then centrifuging at 14000 rpm for 5 minutes. Alcohol was poured off and eppendorf tubes were inverted to air dry the DNA pellet. The pellet was then re-suspended in 100 µℓ T.E (10mM Tris-HCl, 1mM ETDA [pH 7.5]). The isolated genomic DNA was stored at -20°C. Quality of extracted DNA was determined by electroprhoresis on 1% agarose gel run for about 30 minutes at 90mA (150V), and then visualized under UV illumination. The image was captured with Polaroid film type 667.

3.3.2 PCR- polymerase Chain Reaction

PCR on the extracted DNA was done using custom ordered pair of primers (Syrib F 5'-GCGGAAGGATCATTGTCGATG-3' and Syrib R 5'-TGACCTGAGGTCTCGTTG-3') that correspond to the flanking ends of 5S ribosomal gene including the intergenic sequences of the soy gene. Amplification reaction was performed in a 25 µℓ volume, containing 14 µℓ double distilled water, 2.5 µℓ PCR buffer (pH 8.4), 2.0 µℓ $MgCl_2$, 2.0 µℓ of dNTP's, 1.0 µℓ of each primer, 2.0 µℓ of genomic DNA and 0.5 µℓ of Taq DNA polymerase. The temperature profile was as follows: initial denaturation at 94 °C for 7 minutes; 35 cycles of denaturation at 94°C for 1 minute, annealing at 53°C for 1 minute, and extension at 68°C for 1 minute, and final extension at 72°C for 7 minutes. PCR products were loaded into separate lanes of a 1% agarose gel. The gel ran for about 30 minutes at 90mA (150V), and then was visualized under UV illumination and photographed with Polaroid film type 667.

3.3.3 Restriction Fragment Length Polymorphisms (RFLPs) Analysis

Restriction endonuclease digestion of genomic DNA with Hae III, Hpa II, Mse I, Taq I, Sac II and Sma I enzymes was conducted according to manufacturer's instructions.

Digests were incubated for 3 hours using 2 to 5 units of enzymes per µg DNA. DNA fragments were separated according to size by electrophoresis in a 1% agarose gel using TAE running buffer and Polyacrylamide Gel Electrophoresis (PAGE) in TBE (90mM Tris, 90mM borate, 2mM EDTA) for approximately 30 minutes at 90mA (150V). The restriction pattern was visualized under UV illumination and photographed with Polaroid film type 667. 123 base pair ladder was used as molecular size marker.

3.3.4 Gene Clean

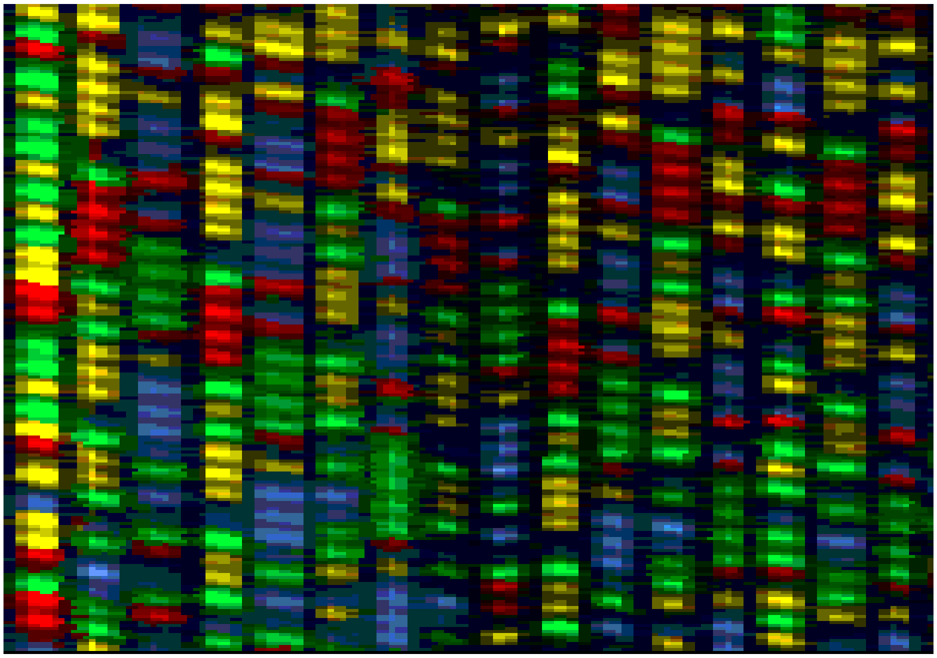
Target DNA bands were excised from agarose gel using straight-edge razor blade and placed in different well labeled microcentrifuge tubes. Three volumes (1.2 ml for 0.4 g slice) NaI stock solution was added. It was then incubated at 56°C in water bath for 15-30 minutes, mixing after 5 minutes until all agarose had melted. Glassmilk stock solution was vortexed to mix well then 20 µl glassmilk was added to the microcentrifuge tubes. The tubes containing the mixtures were incubated for 15 minutes on ice, mixing every 3 to 5 minutes to keep glassmilk in suspension. The tubes were then microcentrifuged for 5 seconds to pellet the glassmilk. Supernatant was removed and placed into new tubes. 500 µl (10-50 volumes) ice-cold New Wash was added to silica gel pellet and the pellets resuspended. The tubes then microfuged for 10 seconds, and the supernatant discarded. The wash was repeated two more times (3 washes total). After discarding supernatant from last wash, the pellets were spinned for 10 seconds; and the residual new wash removed with pipette. The pellets were then resuspended in 15 µl dH2O; incubated for 5 minutes at 45°C to 55°C in a water bath; microfuged for 30 seconds to pellet silica gel. DNA-containing supernatant was then transferred to new tubes. The tubes were then spinned for a few seconds to remove any residual glassmilk carried over with supernatant transfer. Supernatant was then transferred to new tubes.

3.3.5 Sequencing

Presence of DNA in eluate was confirmed on agarose gel (Plate 9) then sent for automated sequencing (ABI prism AppliedBiosystems) at (ILRI). Sequencing procedures utilizes fluorescent tagged NTP's in the sanger's dideoxy chain termination method. The PCR reaction consists of DNA template, primer, DNA polymerase, unlabeled dNTP's, fluorescently labeled ddNTP's, and appropriate buffer in ultra pure sterile water. All customer samples are run using the same standard linear PCR cycle on a GeneAmp 9600. During the PCR reaction, the double stranded DNA template is denatured one time, before the cycles start, for 2 min. at 96^{o}C. After this "hot start", the temperature cycle begins with denaturation at 96^{o}C for 10 sec., a ramp down to 50^{o}C for 15 sec. to allow the primers to anneal to the template's priming site, then a ramp up to 60^{o}C for 4 min. to allow extension of the primer by the polymerase. This temperature cycle is repeated for 25 cycles. The DNA polymerase used is "Amplitaq FS", a thermo stable modified form of Thermus aquaticus DNA polymerase. Amplitaq has no 3'-5' exonuclease activity, enabling rapid and efficient incorporation of dNTP's and the fluorescent ddNTP's.

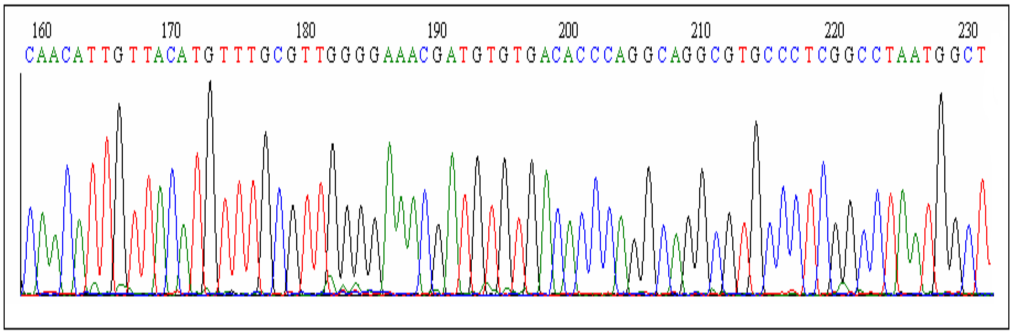
When the PCR is complete, the reaction mix contains a population of PCR fragments of different lengths, each terminating in a fluorescent-dye-containing dideoxynucleotide. Each of the four dideoxynucleotide bases contains a different fluorescent dye which emits a characteristic wavelength, thus the identity of the dye corresponds to the final base on that fragment. The entire reaction is purified, then run in a single lane on a polyacrylamide gel in an ABI 377 Sequencer, so that the fragments separate according to size (see illustration plate 1). As the fragments are electrophoresed, they run past a laser detector at the bottom of the gel, and the emission wavelength of each fragment is detected.

Plate 1. Fragments electrophoresed past a laser detector at the bottom of a gel



The data is compiled into a gel image, analyzed by ABI software and the resulting sequence is written into a text file and a chromatogram file as shown in plate 2 below.

Plate 2 A chromatogram file showing a gene sequence



3.3.6 BLAST identification of clones.

The identities of the generated nucleotide sequences were assigned in the NCBI's non-redundant nucleotide BLAST search (Altschul *et al*., 1997) and ClustalW alignment program in BioEdit suite.

3.3.7 Phylogenetic analysis

Phylogenetic relationships were inferred from the aligned nucleotide sequences by the neighbor-joining method implemented in the Phylip package (Felsentein, 1997) as implemented in the software package MEGA (Tamura *et al*., 2007). Consensus trees were bootstrapped for 100 replicates and rooted to the *Glycine soja* to determine divergence from the other taxa.

CHAPTER FOUR

RESULTS AND DISCUSSION

4.1. **Soil and weather conditions in Mbita region during vegetative period**.

Soybean grows between 0 and 2000M above sea level. It grows in a wide range of soil and climatic conditions. Soybean requires deep well drained soils with soil pH of 5.8 to 6.8. Vertisols (black cotton soils) can be used as long as germination is not impeded by soil capping (GTZ, 1998).

Most soils in Mbita region are black cotton loam with a pH varying between 5.5 and 7.0. they are moderately deep with a range of 30-35 centimeters. They are deficient in nitrogen (N), phosphorus (P) and potassium (K). However, our experiment was laid in an area that had been set aside free from cultivation for over two years. This was enough time for soils to replenish essential soil elements.

Daily temperatures during the vegetative period were sufficient having a mean of 24.3^{o}C and monthly distribution ranging between 23.5^{o}C (May) to 25.4^{o}C (March) as shown in table 1. Rainfall was distributed unevenly during the vegetative period. There was a mean of 256.5mm with monthly ranges between 147mm (June) and 378mm (April). The four months received a total of 1026mm which is far below the optimum soybean requirements.

Monthly distribution of temperature and rainfall (figure 3) shows that cultivars underwent crucial stages of plant development (flowering and pod-filling) when the temperatures were high but rainfall was low. This explains why most studied cultivars had few and light seeds.

Table 1. Temperature and rainfall distribution during the vegetative period.

Month	Average temperature (^oC)	Rainfall (mm)
March	25.4	277
April	24.3	378
May	23.5	224
June	23.9	147
Total	97.1	1026
Mean	24.3	256.5

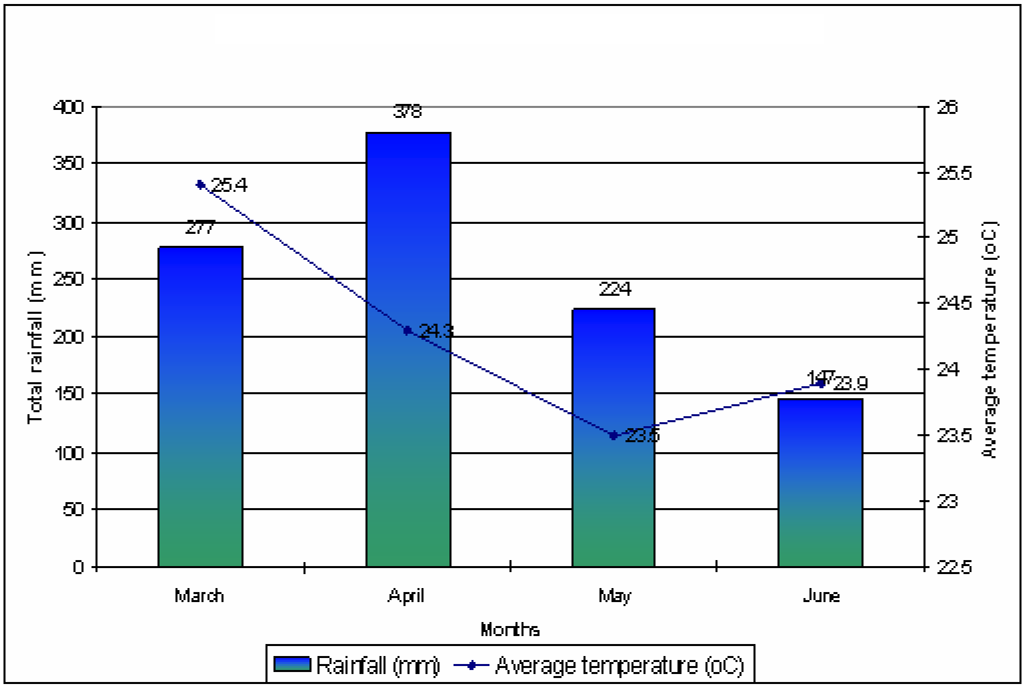


Fig 3. Interaction between temperature and rainfall during vegetative period.

4.2. Physiological characteristics of cultivars in the field experiment.

Soybean cultivars were sown on 17th March, 2006. Nam I took 3 days for 50% of seeds to germinate while Maksoy IN, Nam II, Mikumi, TGX-1876-2E and SB20 took 4 days. All the other cultivars took 5 days. It was noted that two Tanzanian cultivars (Bossier and TGX-1805-2E) failed to germinate so they were replaced with Mikumi and Mikese.

Numerous studies have shown that the induction of flowering, production of viable pollen, length of flowering and pod-filling periods are subject to manipulation by photoperiod. Time of flower initiation shows a complex day-length temperature interaction (Criswell and Hume, 1972; Jones and Laing, 1978; Lawn and Byth, 1979).

The studied cultivars differed in flowering duration ranging between 47 and 49 days. Physiological maturity (maximum dry weight at a moisture content of 50-60%) coincides with the growth stage R7 of Fehr and Caviness i.e when one pod on the meristem has reduced its mature colour (Fehr and Caviness, 1979). According to Stanton (1966) soy maturity periods are classified as early 85-95 days, medium 95-110 days and late 110-140 days. The studied cultivars had different maturity periods as follows: Nam I matured earliest at 104 days while Nam II matured latest at 136 days. Mikumi took 124 days to mature while Mikese took 135 days. The other cultivars took between 106 and 115 days to mature. These maturity periods fell within Osafo's (1977) proposed period of 90-140 days.

4.3. Agronomic performance of the studied cultivars.

Table 2 Significance differences in various parameters among the cultivars

	Cultivars													
Parameters	Nyala	SB8	SB9	SB19	SB20	Nam I	Nam II	Nam 4M	Maksoy 1N	Duicker	Mikese	Mikumi	Tgx-1876-2E	F Value
Plant height (cm)	38.77e	46.71g	41.49ef	36.16de	38.81e	41.96f	46.62g	45.93g	31.38d	27.66c	32.43d	34.39d	28.28c	106.131
Weight of whole plant (gms)	14.82b	12.71ab	14.72b	11.12a	12.46a	15.01b	16.28b	13.46b	13.04b	9.23a	12.08a	12.30a	16.09b	6.634
No. of branches per plant	4.52cd	3.30bc	3.17b	3.27b	3.70c	4.11c	3.83c	2.07a	3.93c	2.26a	2.49ab	3.94c	5.12d	25.227
Total No of seeds per plant	55.82b	43.3ab	52.14ab	41.26a	40.73a	45.89ab	40.9a	32.02a	49.31ab	34.93a	42.11a	47.83ab	70.82c	13.330
Weight of total seeds per plant	6.18ab	4.8a	5.38ab	4.75a	4.95a	4.88a	5.87ab	4.58a	5.16ab	3.9a	4.74a	5.05a	6.67b	5.124
1000 seed weight	20.16b	20.04b	19.4b	20.08b	20.39b	19.14ab	23.52c	22.72c	18.7a	19.9b	19.8b	19.56b	18.19a	38.946
Biomass without seeds	8.83ab	7.92ab	9.04ab	6.53a	7.48a	10.12b	10.40b	8.51ab	7.81ab	5.34a	7.54a	7.29a	9.42ab	8.140

NB: Mean values denoted by similar letters are not significantly different $P \leq 0.05$

Table 3. Average agronomic performance per cultivar

CULTIVAR	**N**	**Plant height (cm)**	**Weight of whole Plant (g)**	**No. of branches/plant**	**Total number of Seeds/ plant**	**Weight of total Seeds/ plant (g)**	**1000 seeds weight (g)**	**Biomass without seeds (g)**	**% of seed/whole wt. of plant**
Duicker	90	27.66±0.46	9.23±0.58	2.26±0.20	34.93±1.86	3.89±0.26	19.90±0.31	5.34±0.35	42
Tgx-1876-2E	90	28.28±0.44	16.09±0.86	5.12±0.16	70.82±3.89	6.67±0.40	18.19±0.16	9.42±0.48	41
Maksoy 1N	90	31.38±0.72	13.04±0.73	3.93±0.17	49.31±2.77	5.16±0.31	18.70±0.21	7.81±0.45	40
Mikese	90	32.43±0.46	12.08±0.69	2.49±0.20	42.11±2.56	4.74±0.31	19.80±0.26	7.54±0.45	39
Mikumi	90	34.39±0.55	12.30±0.64	3.94±0.14	47.83±2.74	5.05±0.30	19.56±0.25	7.28±0.39	41
SB 19	90	36.16±0.49	11.12±0.80	3.27±0.15	41.26±2.24	4.75±0.28	20.08±0.24	6.53±0.66	43
Nyala	90	38.77±0.97	14.82±0.87	4.52±0.15	55.82±3.05	6.18±0.38	20.16±0.25	8.83±0.54	42
SB 20	90	38.81±0.77	12.46±0.79	3.70±0.18	40.73±2.48	4.95±0.31	20.39±0.21	7.48±0.50	40
SB 9	90	41.49±0.60	14.72±0.86	3.17±0.20	52.14±3.05	5.38±0.33	19.40±0.17	9.04±0.51	37
Nam I	90	41.96±0.68	15.01±0.86	4.11±0.17	45.89±3.03	4.88±0.33	19.14±0.25	10.12±0.56	33
Nam 4M	90	45.93±0.66	13.46±0.81	2.07±0.20	32.02±2.13	4.57±0.32	22.72±0.26	8.51±0.46	34
Nam II	90	46.62±0.78	16.28±0.94	3.83±0.18	40.90±2.32	5.87±0.35	23.52±0.27	10.40±0.63	36
SB 8	90	46.71±0.61	12.71±0.68	3.30±0.17	43.30±2.60	4.79±0.30	20.04±0.19	7.92±0.40	38
Total Av.	***1170**	**37.74±0.26**	**13.33±0.22**	**3.52±0.05**	**45.93±0.80**	**5.15±0.09**	**20.12±0.08**	**8.17±0.14**	**39**

*** Total number of analysised plants**

There was a significant difference in plant height in the various cultivars (F= 106.131, df = 12, p<0.05) (see table 2). The mean height ranged among the cultivars from Duicker (27.66 ± 0.46cm) to SB 8 (46.71 ± 0.61cm) as shown in figure 4.

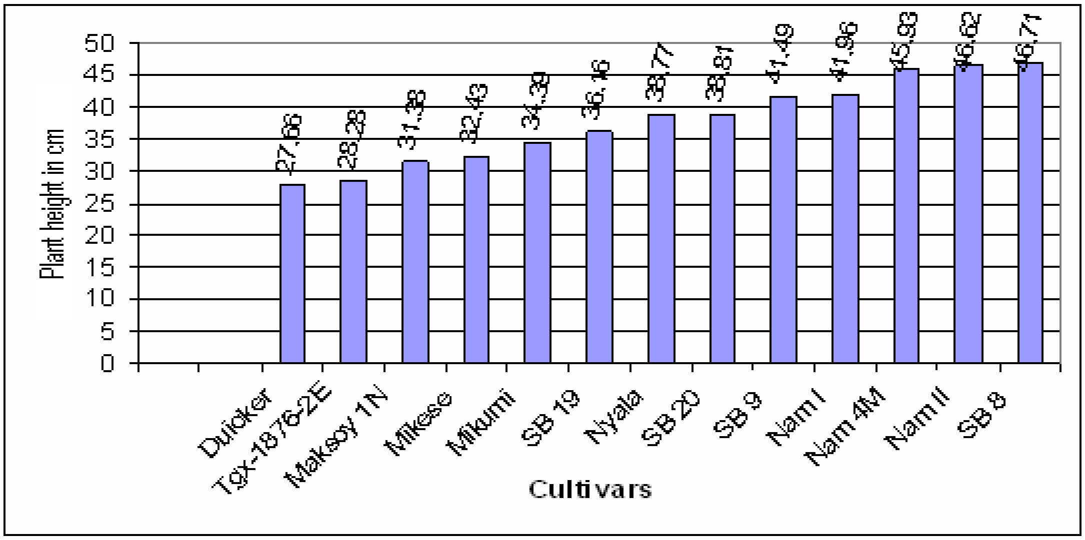


Fig 4. Average plant height in centimeters per cultivars

This height was within the recommended height according to AVDC (1978). The best height is usually less than 75 centimeters; these are within the acceptable range. However, tall varieties may reach up to 200 centimeters. They are generally grown for fodder or as small holder crops since they are unsuitable for mechanized production.

The number of branches produced among the studied cultivars significantly differed (F=25.227, df =12, p<0.05) (see table 2). All the studied cultivars had an average of 3.52 ± 0.1 branches per plant. Tgx-1876-2E had the highest average number of branches (5.12±0.16) while Namsoy 4M had the lowest average number of branches (2.07±0.20).

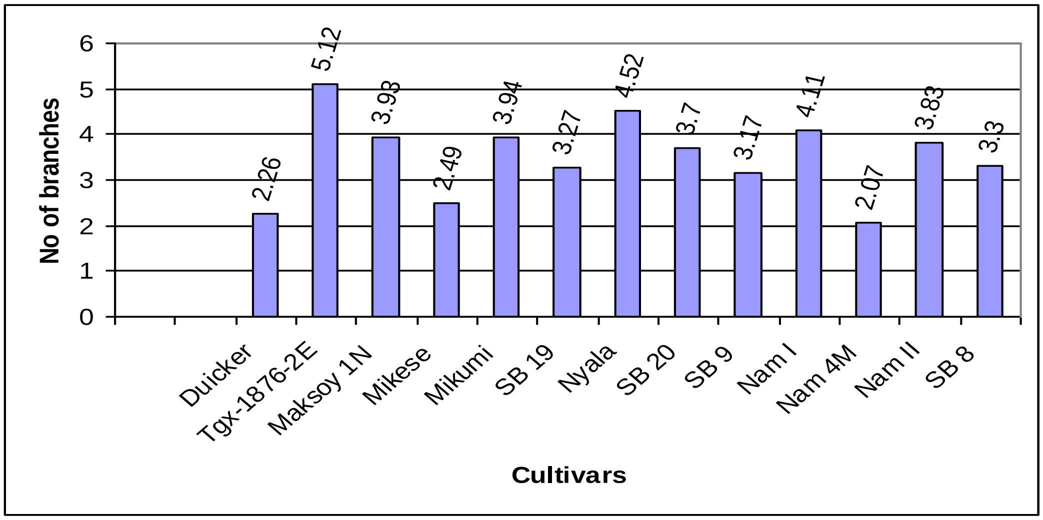


Fig. 5 Average number of branches per cultivar

It was noted that there was a positive correlation between the average height of plants and the number branches (r = 0.135).

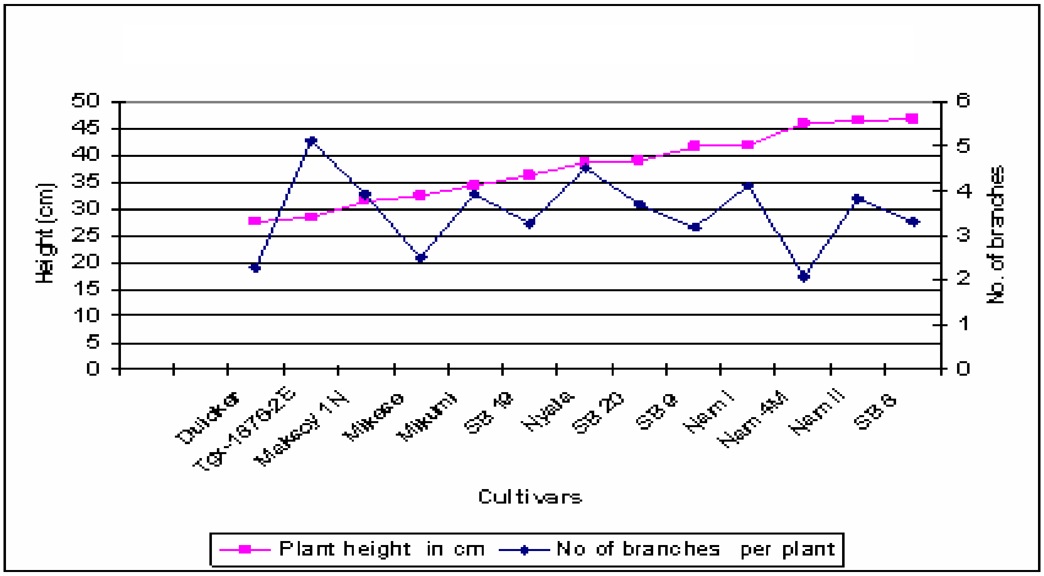


Fig. 6 Comparison of average plant height and average number of branches per cultivar.

Usually branches arise from the base of the stem and do not rebranch. Indeterminate types have the terminal growing point elongating until the plant matures and does not terminate with a cluster of flowers or pods while determinate types have stems terminating in a cluster of flower or pods. They have more branches and are shorter than indeterminate (Osafo 1977). According to these results, it was found out that highly branched cultivars were relatively short in height, had relatively high weight in grams, many seeds and therefore generally performed well agronomically. The relationship between number of branches and height is true as recorded by Osafo (1977) that the determinate types have more branches and are shorter than the indeterminate types.

All the studied cultivars had an average weight of whole plant of 13.33 ± 0.2 grams. There was a significant difference in weight of the whole plant (F = 6.634, df = 12, $p<0.05$) (see table 2). Nam II scoring the highest average value (16.28 ± 0.94) grams followed by Tgx-1876-E (16.09 ± 0.86) grams while Duicker was the least with 9.23 ± 0.58 grams.

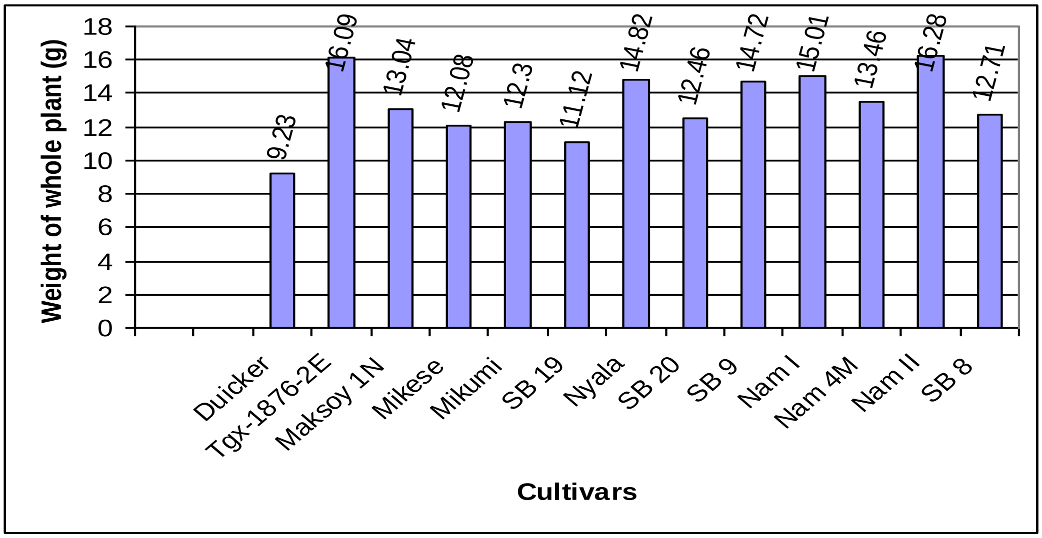


Fig. 7 Average weight of whole plant in grams.

There was a positive correlation between the plant height and weight of the whole plant (r = 0.289).

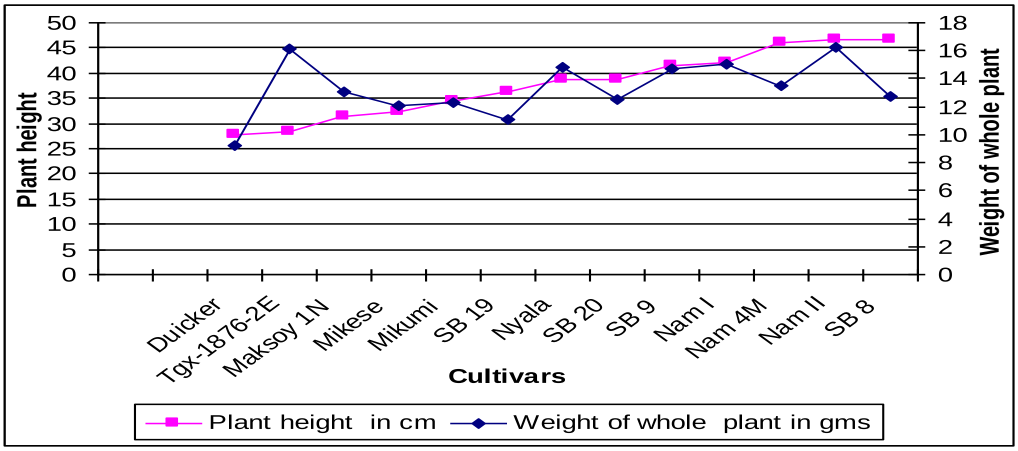


Fig. 8 Comparison of average plant height and average weight of whole plant per cultivar.

Most tall cultivars had more seeds per plant than shorter ones. The cultivars had an average number of seeds of 45.93 ± 0.8. It was noted that there was a significant difference in the total number of seeds per plant from the various cultivars (F = 13.330, df = 12, $p<0.05$) (see table 2). Tgx-1876-2E had the highest average number of seeds per plant (70.82 ± 3.89) while Namsoy 4M had the smallest average number of seeds per plant (32.02 ± 2.13) which could be due to the small number of branches in Namsoy 4M (average of 2.07±0.20). It was noted that Tgx-1876-2E had the highest number of branches (average of 5.12 ± 0.2), biggest number of seeds per plant, (average of 70.82 ± 3.9), heaviest weight of whole plant (16.09 ± 0.9) and highest number of seeds per plant (6.67 ± 0.4) but had low weight of 1000 kernels (18.19 ± 0.2) and short height (28.28 ± 0.4 cm). There was a positive correlation between the number of seeds per plant and number of branches (r =0.638).

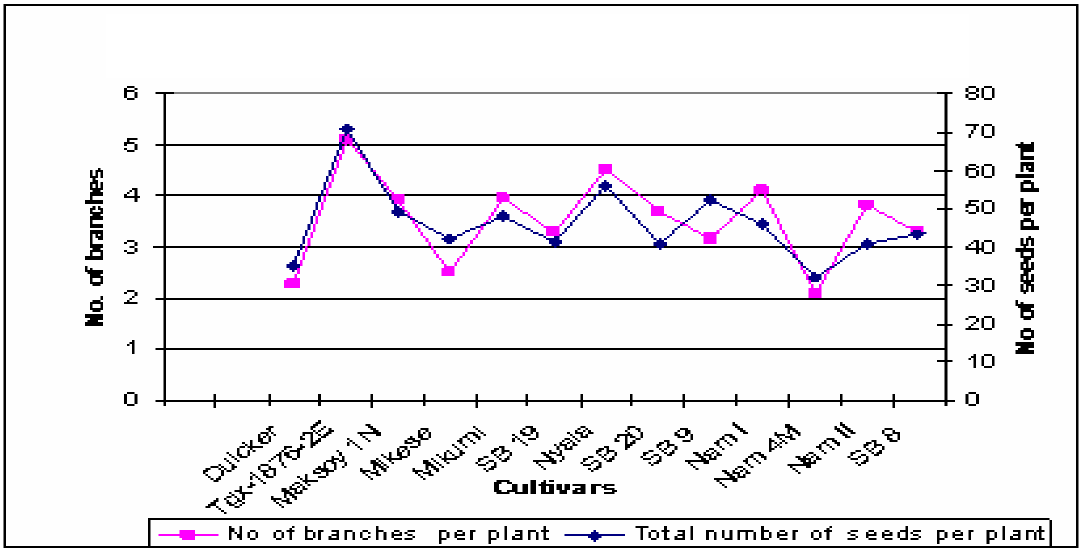


Fig. 9 Comparison of average number of branches and average number of seeds per plant.

Cultivars had an average weight of 5.15 ± 0.1 grams of seeds per plant. Duicker had the lowest weight of 3.89 ± 0.3 grams while TGX-1876-2E had the highest (6.67 ± 0.4 grams).

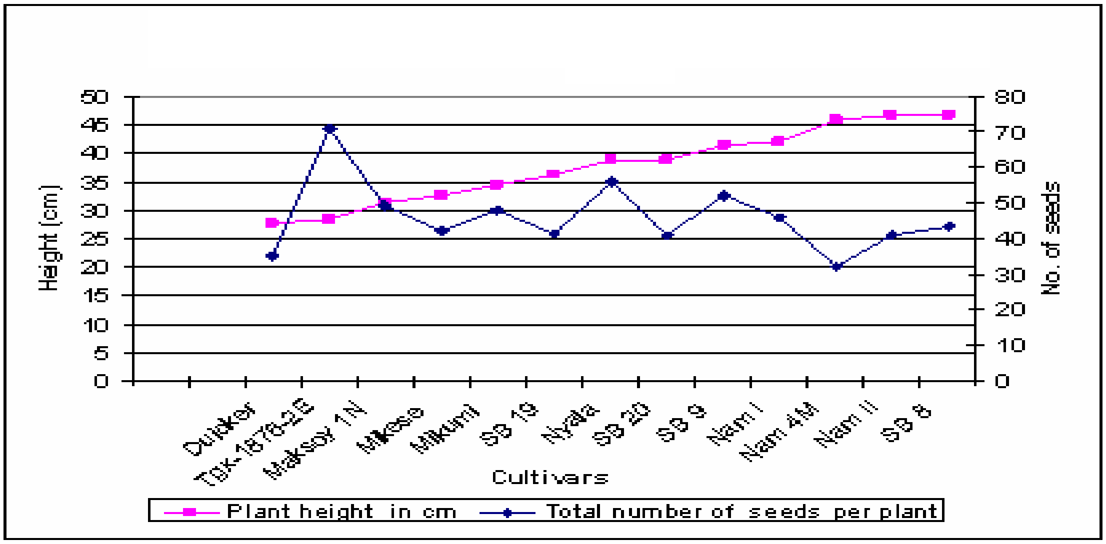


Fig. 10 Comparison between plant height and total number of seeds per plant.

There was a significant difference in the weights of total seeds per plant from various cultivars (F = 5.124, df =12, $p<0.05$) (see table 2). There was a positive correlation between plant height and the total weight of seeds per plant.

Cultivars had an average of 20.12 ± 0.1 grams of 1000 kernel weight. The lowest cultivars were TGX-1876-2E (18.19 ± 0.2 grams) and Maksoy IN (18.70 ± 0.2 grams). A significant difference was observed among all the cultivars (F = 38.946, df = 12, $p< 0.05$) (see table 2).

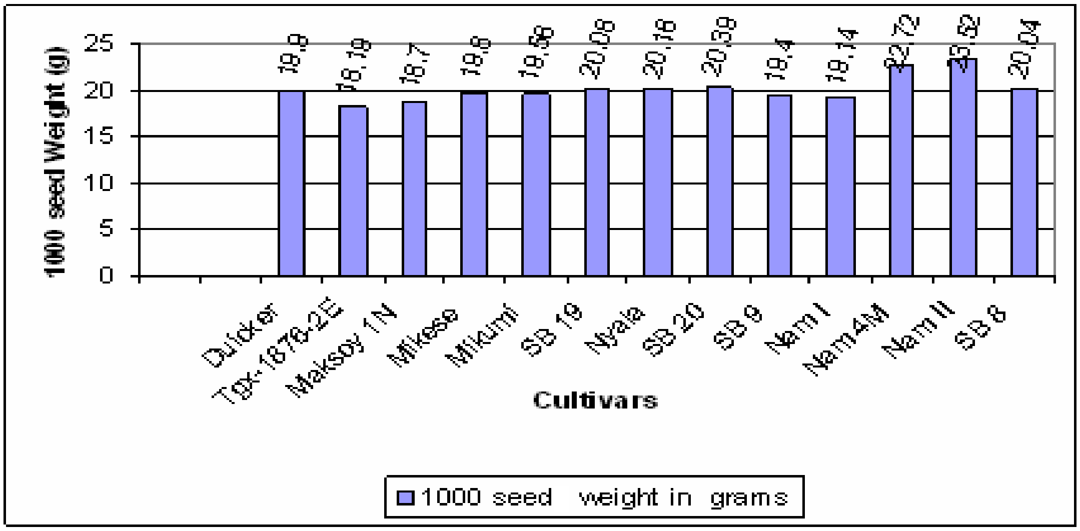


Fig. 11 Average of 1000 seed weight in grams per cultivar.

Comparison between weight of whole plant and 1000 kernel weight showed that there was a positive correlation among cultivars (r = 0.298)

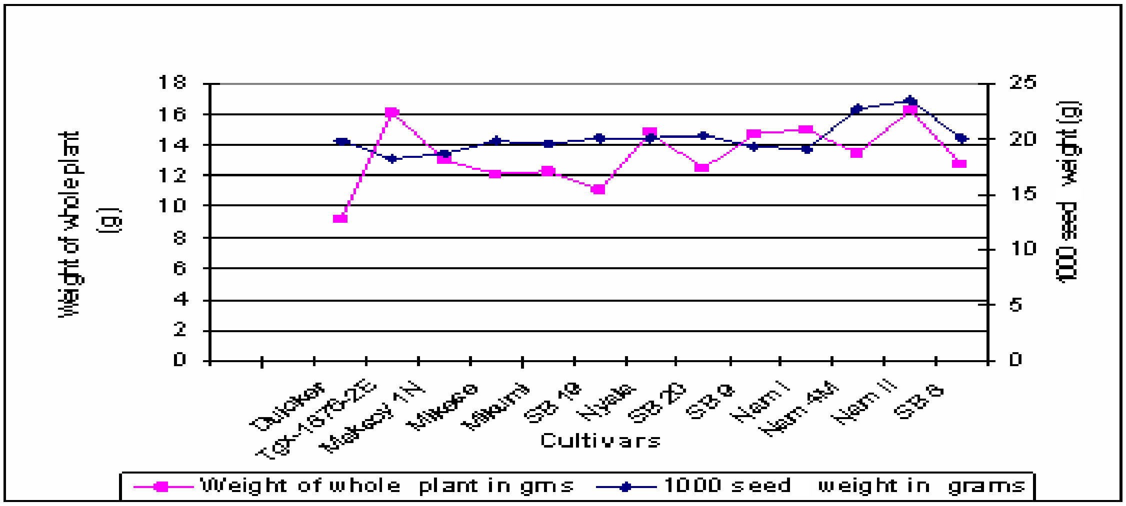


Fig. 12 Comparison between weight of whole plant and 1000 seed weight in grams

Studied cultivars had biomass without seed (total plant weight – total seeds per plant) of an average of 8.17 ± 0.1 grams. Duicker had the lowest biomass of 5.34 ± 0.4 grams while Nam II had the highest 10.40 ± 0.7 grams. There was a positive correlation between plant height and biomass without seeds among the studied cultivars (r = 0.431). Low biomass without seeds in Duicker may be attributed to its short height and few branches.

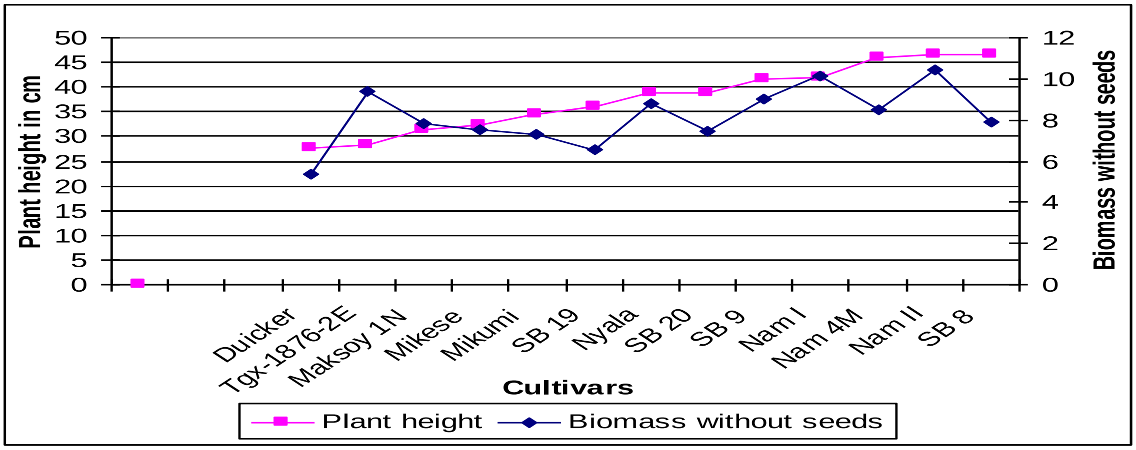


Fig. 13 Comparison between plant height and biomass without seed.

Generally, there was a positive correlation between the average plant height and the average number of seeds per plant (r = 0.161). Most cultivars must have formed less seeds due to the harsh weather conditions. The highest variance was observed in TGX-1876-2E.

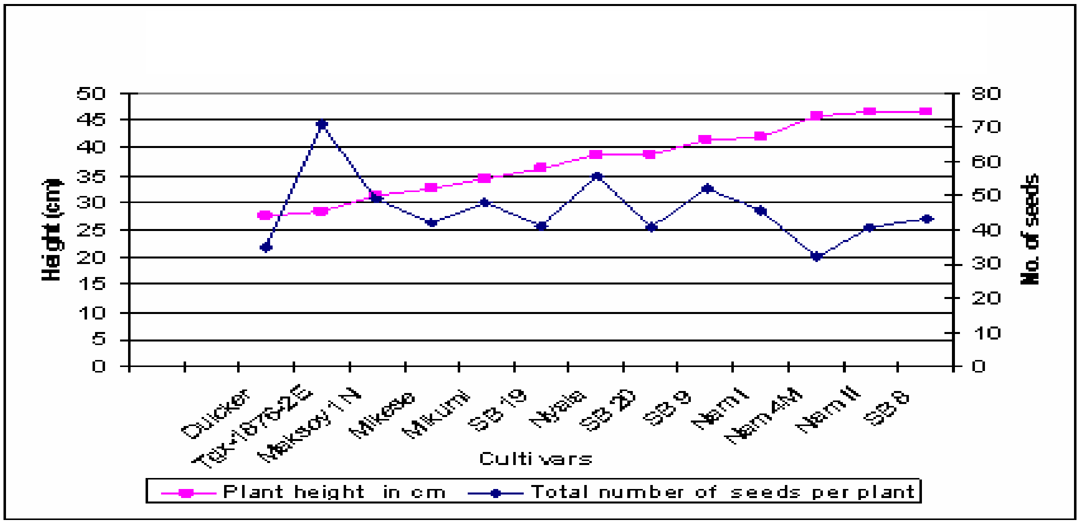


Fig. 14 Comparison of average plant height and average number of seeds per plant

Comparison between 1000 seed weight and biomass without seed showed that for most cultivars, the more the weight of 1000 seeds the higher the biomass without seed. This is attributed to the fact that seeds formed part of the total plant biomass. However, this was specific in each cultivar.

Results on the proportion of seed to the whole weight of plant showed that Nam I had the lowest (33%) while SB19 had the highest proportion (43%). Other cultivars with low percentages included Nam 4M (34%), Nam II (36%), SB9 (37%), SB8 (38%) and Mikese (39%). Cultivars with high percentages included Maksoy 1N (40%), TGX-1876-2E, Mikumi (41%), Duicker and Nyala (42%).

Number of branches and biomass without seed were positively correlated (r = 0.599).

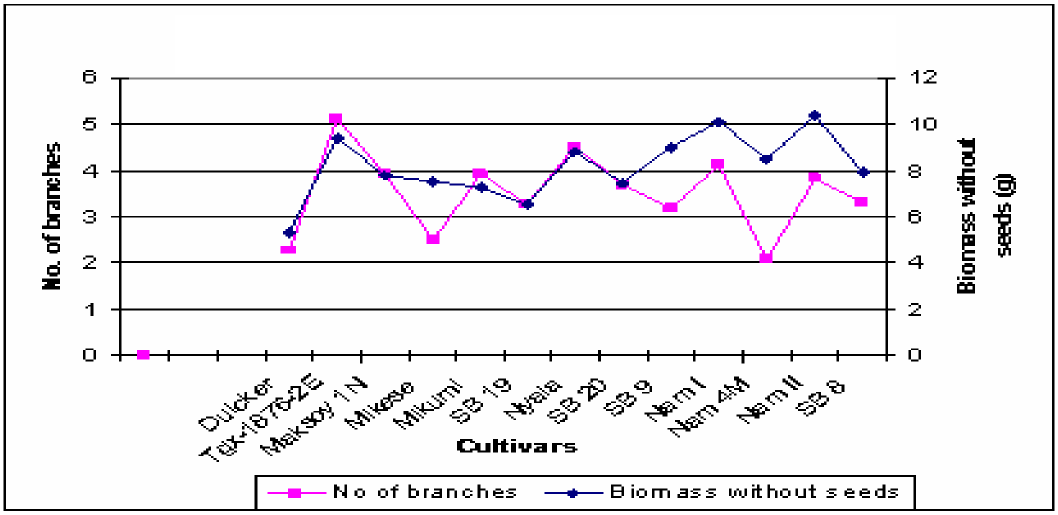


Fig. 15 Comparison of average number of branches and average biomass without seeds per cultivar

A few exceptions were observed in Mikese, Mikumi and SB9. This correlation can be attributed to the fact that branches form a substantial part of the total plant weight.

4.4 Performance of cultivars between East African countries.

Results on plant height showed that Kenyan cultivars had height ranging between 36.6 cm (SB19) and 46.71 cm (SB8); Ugandan cultivars ranged between 31.38 cm (Maksoy 1N) and 46.62 cm (Nam II) while Tanzanian cultivars ranged between 27.66 cm (Duicker) and 34.39cm (Mikumi). The mean of Ugandan cultivars was biggest 41.47 (37%) followed by Kenyan 40.39 (36%) then Tanzanian 30.69 (27%). There was significant difference in performance among cultivars per country (F = 231.565, df = 2, $p < 0.05$). However, the mean differences between the Kenyan and Ugandan cultivars were not significantly different $p \leq 0.05$

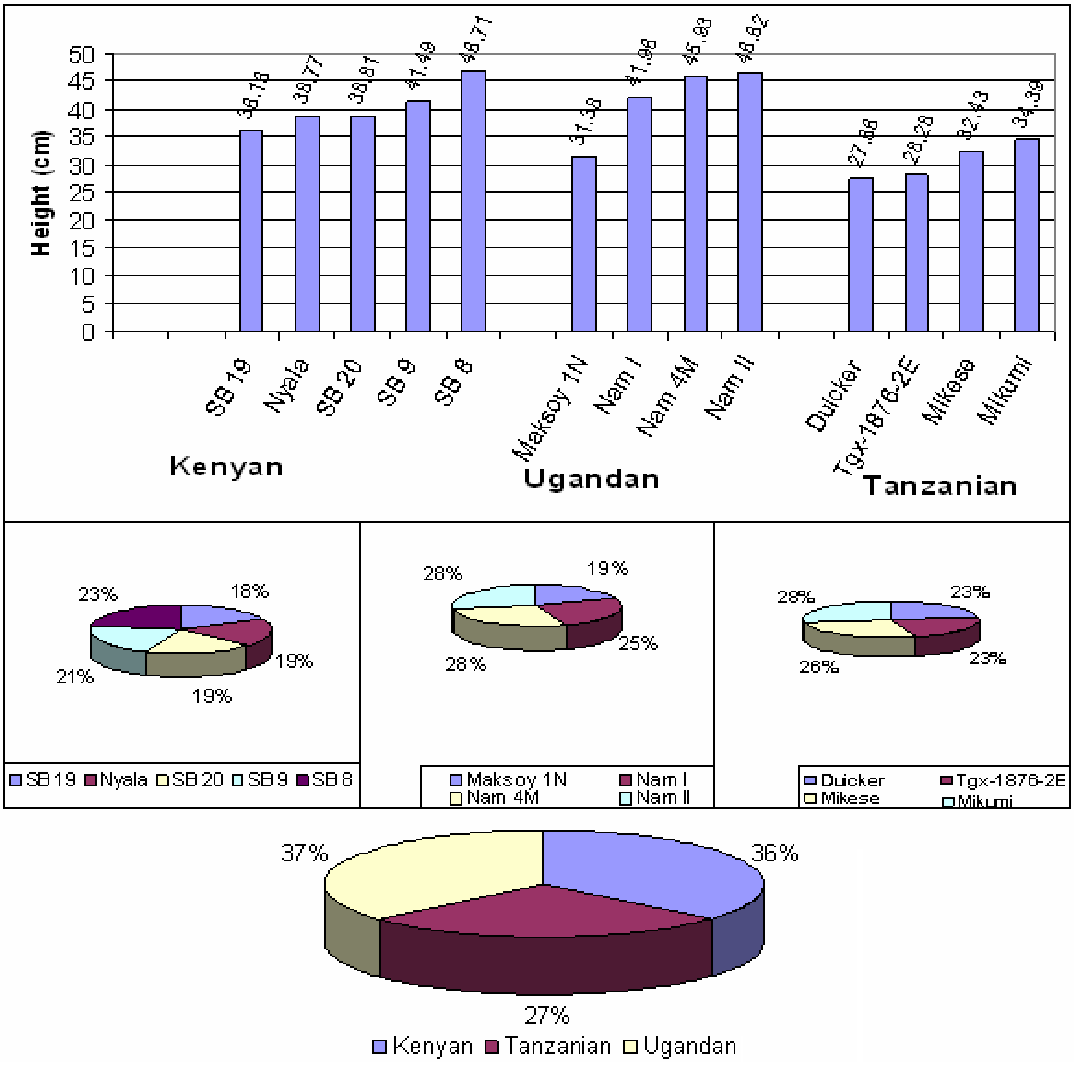


Fig.16 Plant height among East African cultivars.

Kenyan cultivars had weights of whole plant ranging from 11.12g (SB19) to 14.82g (Nyala); Ugandan ranged from 13.04g (Maksoy 1N) to 16.28g (Nam II) while Tanzanian ranged from 9.23g (Duicker) to 16.09g (TGX-1876-2E) as shown in figure 17 below.

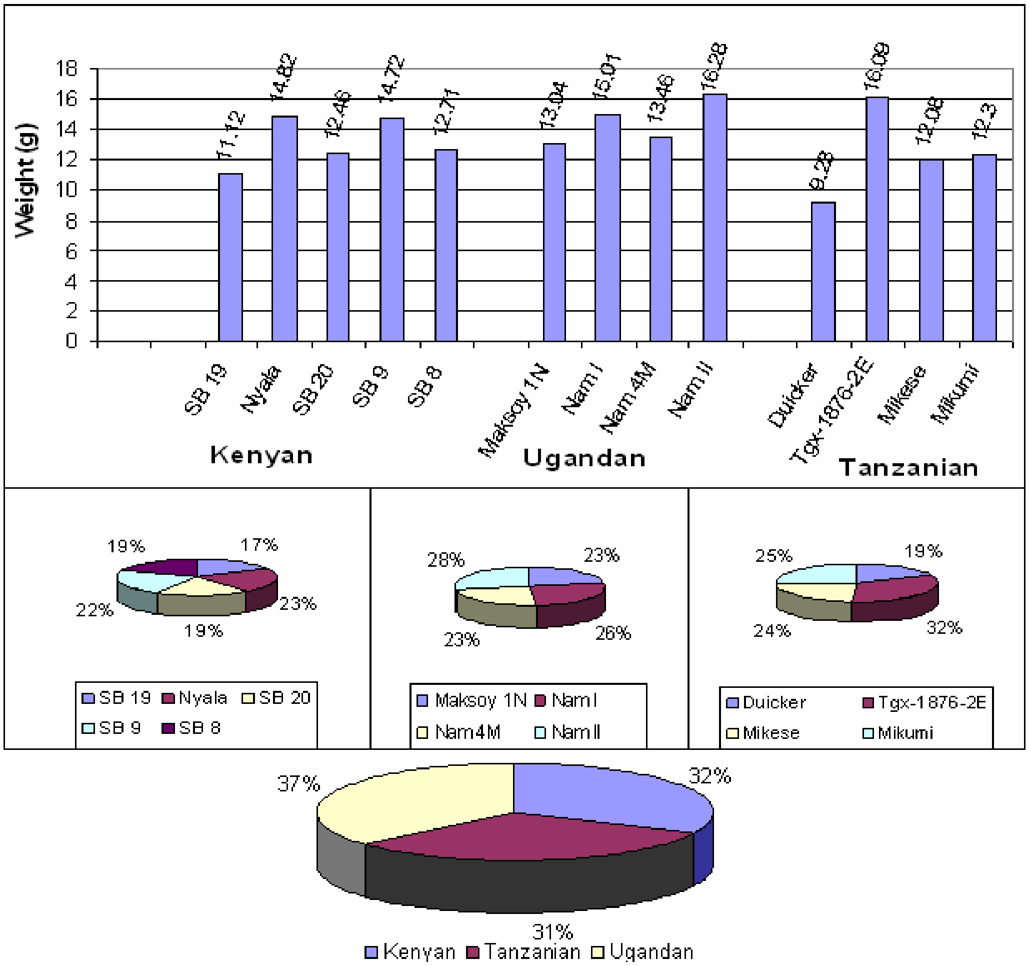


Fig.17 Weight of whole plant among East African cultivars.

Overall analysis showed that Ugandan cultivars had highest weight per plant with a mean on of 14.45 (37%), followed by Kenyan 13.17 (32%) and then Tanzanian 12.43 (31%). There was significant difference in performance among cultivars per country (F = 6.513, df = 2, $p< 0.05$). However, the mean difference between the Kenyan and Tanzanian cultivars was not significantly different $p\leq 0.05$.

It was observed that the average number of branches was distributed as follows: Kenyan ranged between 3.17 (SB9) to 4.52 (Nyala); Ugandan ranged between 2.07 (Nam 4M) to 4.11 (Nam I) and Tanzanian 2.26 (Duicker) to 5.12 (TGX-1876-2E). Figure 18. below shows this distribution.

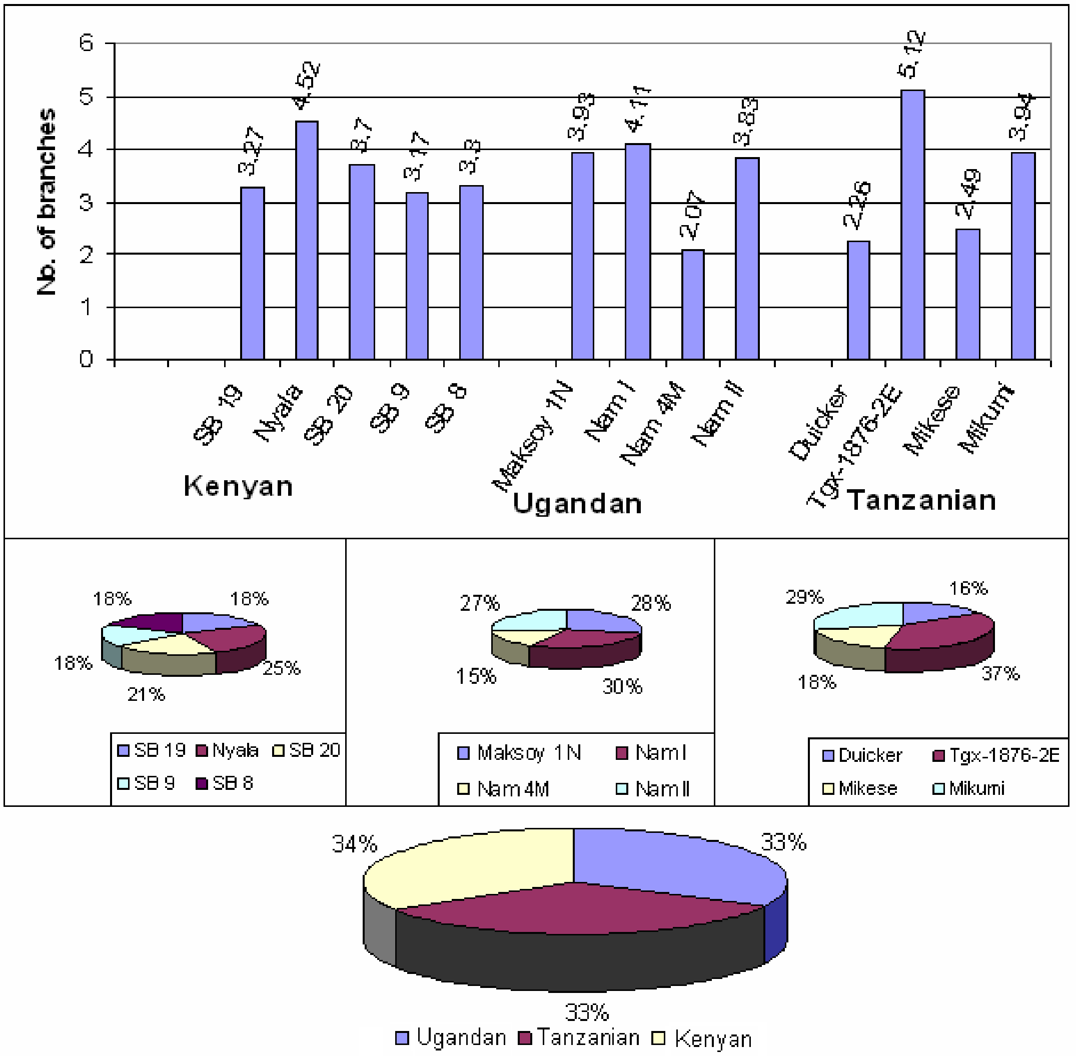


Fig. 18 Average number of branches per cultivar among East African cultivars

Overall analysis showed that Kenyan cultivars had a mean of 3.59 (34%) followed by Ugandan 3.48 (33%) and then Tanzanian cultivars 3.45 (33%). There was no significant difference in performance among cultivars per country (F = 0.620, df = 2, $p < 0.05$)

Kenyan cultivars had total number of seeds ranging from 40.73 (SB20) to 55.82 (Nyala); Ugandan ranged from 32.02 (Nam 4M) to 49.31 (Maksoy 1N) while Tanzanian ranged from 42.11 (Mikese) to 70.82 (TGX-1876-2E) as shown in figure 19.

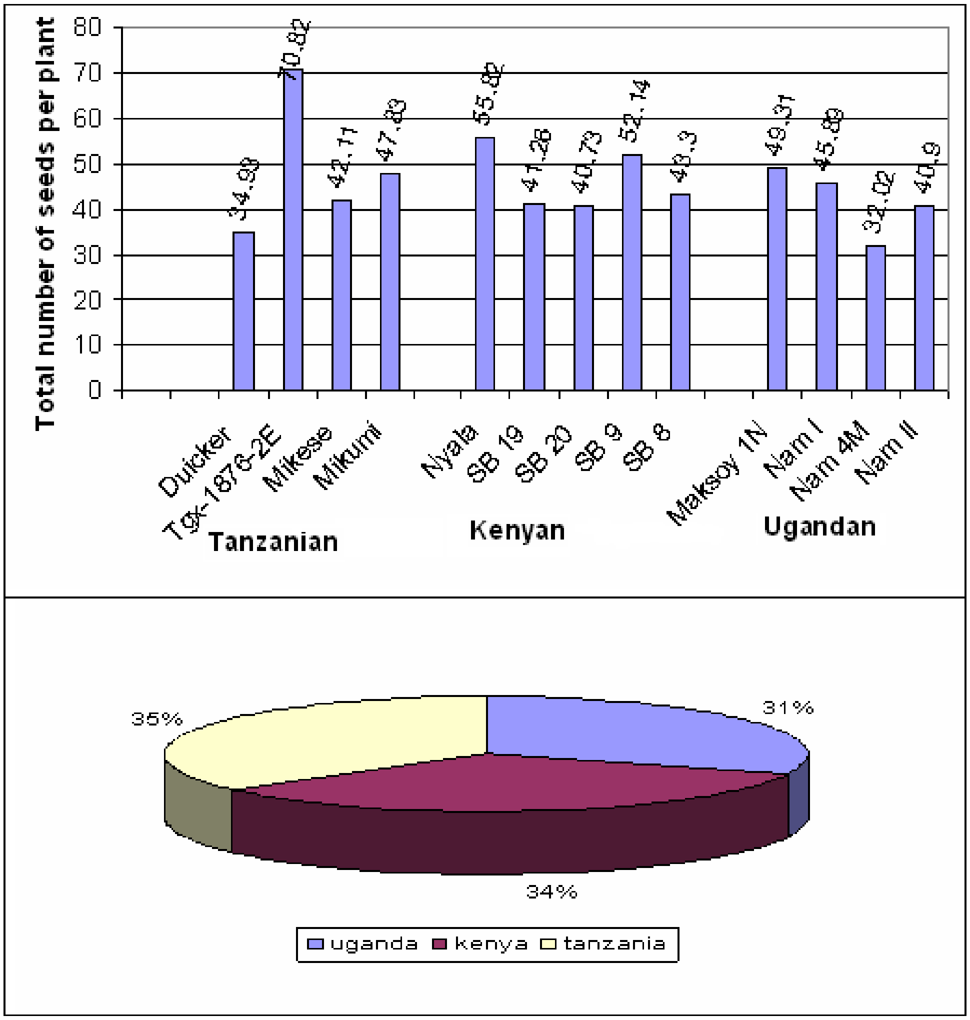


Fig.19 Average total number of seeds among East African cultivars.

Overall analysis showed that Tanzanian cultivars had a mean of 48.92 (35%) followed by Kenyan 46.65 (34%) and then Ugandan cultivars 42.03 (31%). There was a significant difference in performance among cultivars per country (F = 6.020, df = 2, $p < 0.05$). However, the mean difference between the Kenyan and Tanzanian cultivars was not significantly different $p \leq 0.05$.

Kenyan cultivars had average weight of total seeds per plant ranging from 6.18 (Nyala); 5.38 (SB9); 4.95 (SB20); 4.79 (SB8) to 4.75. Ugandan cultivars ranged from 5.87 (Nam II); 5.16 (Maksoy 1N); 4.88 (Nam I) to 4.57 (Nam 4M) while Tanzanian cultivars ranged from 6.67 (TGX-1876-2E); 5.05 (Mikumi); 4.74 (Mikese) to 3.89 (Duicker). These distribution were as shown in figure 20 below.

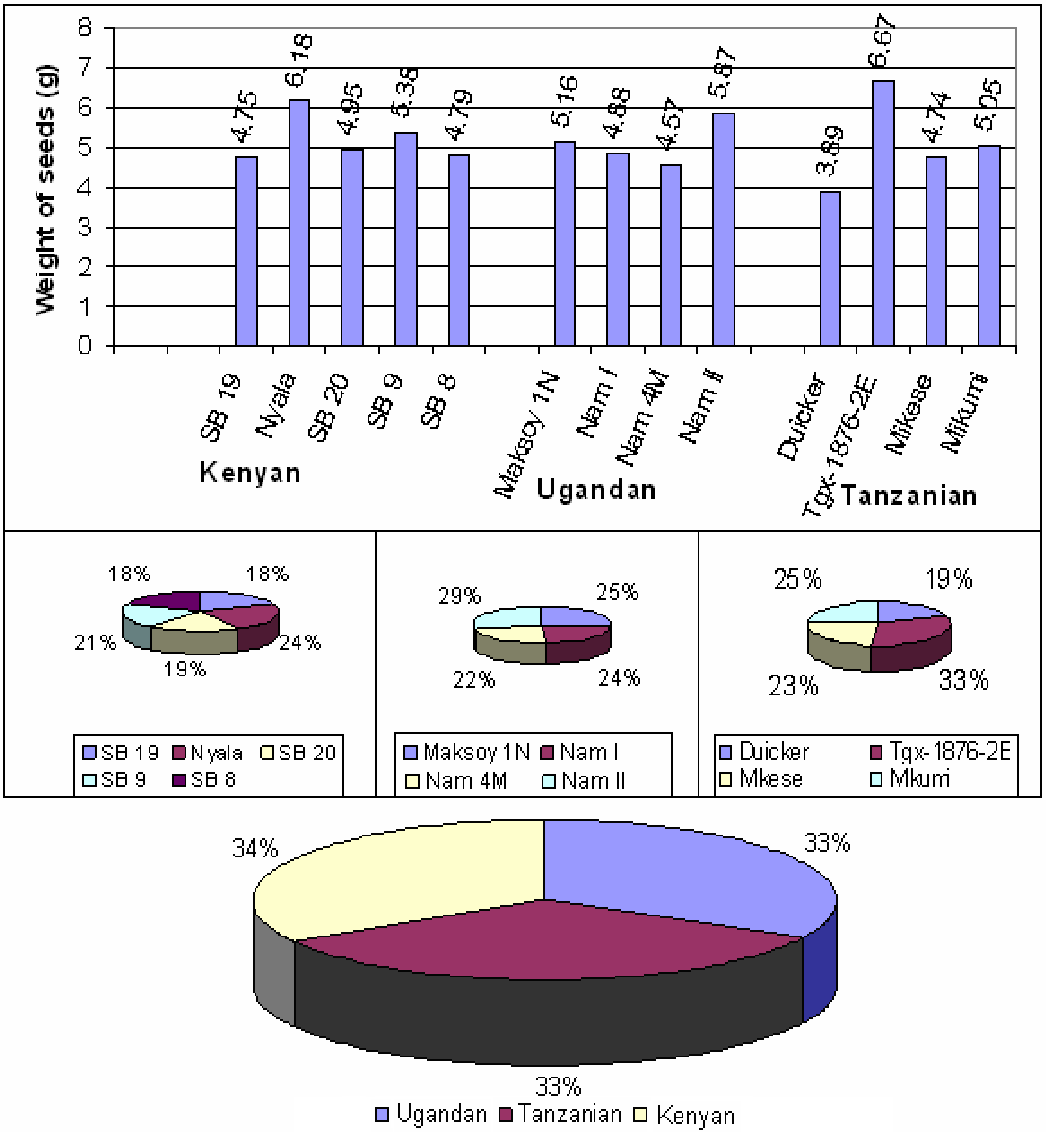


Fig.20 Average weight of total seeds per plant among East African countries

Kenyan cultivars contributed to a mean of 5.21 (34%), Ugandan 5.12 (33%) and Tanzanian 5.09 (33%) of the weight of total seeds per plant. There was no significant difference in performance among cultivars per country (F = 0.620, df = 2, $p< 0.05$).

Kenyan cultivars had an average 1000 seed weight ranging from 20.39 g (SB20); 20.16 g (Nyala); 20.08 g (SB19) to 19.40 g (SB9). Ugandan cultivars ranged from 23.52 g (Nam II); 22.72 g (Nam 4M); 19.14 g (Nam I) to 18.70 g (Maksoy 1N) while Tanzanian cultivars ranged from 19.9 g (Duicker); 19.8 g (Mikese); 19.56 g (Mikumi) to 18.19 g (TGX-1876-2E). These distributions were as shown in figure 21 below.

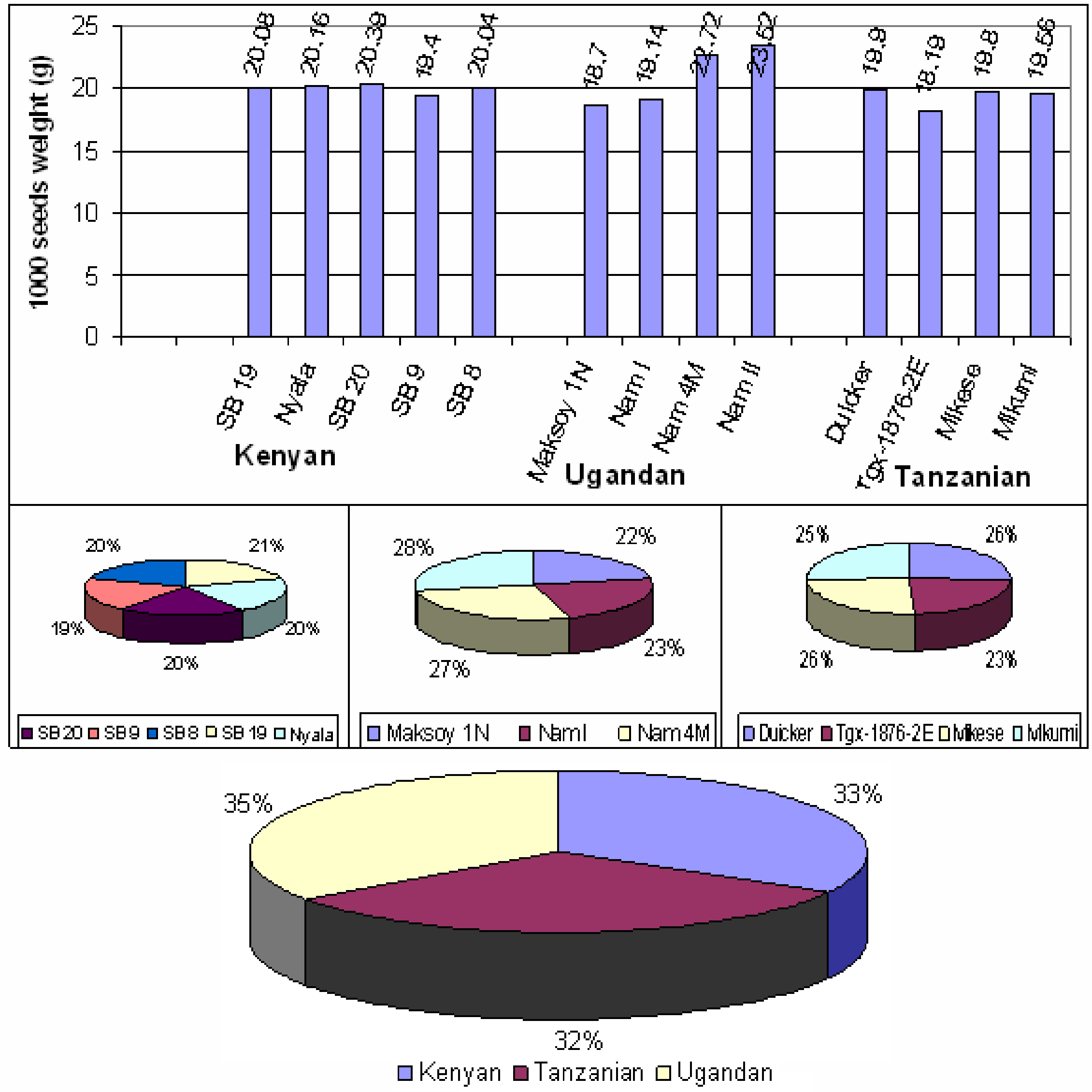


Fig. 21 Average 1000 seed weight among East African cultivars

An overall performance in 1000 seed weight showed that Ugandan cultivars scored the highest mean value 21.02 (35%), followed by Kenyan 20.01 (33%) then Tanzanian 19.36 (32%). There was a significant difference in performance among cultivars per country (F = 38.491, df = 2, $p < 0.05$).

Kenyan cultivars had average biomass without seeds per cultivar ranging from 9.04 g (SB9); 8.83 g (Nyala); 7.92 g (SB8); 7.48 g (SB20) to 6.53 g (SB19). Ugandan cultivars ranged from 10.4 g (Nam II); 10.12 g (Nam I); 8.51 g (Nam 4M) to 7.81 g (Maksoy 1N) while Tanzanian cultivars ranged from 9.42 g (TGX-1876-2E); 7.54 g (Mikese); 7.28 g (Mikumi); to 5.34 g (Duicker). These distributions were as shown in figure 22 below.

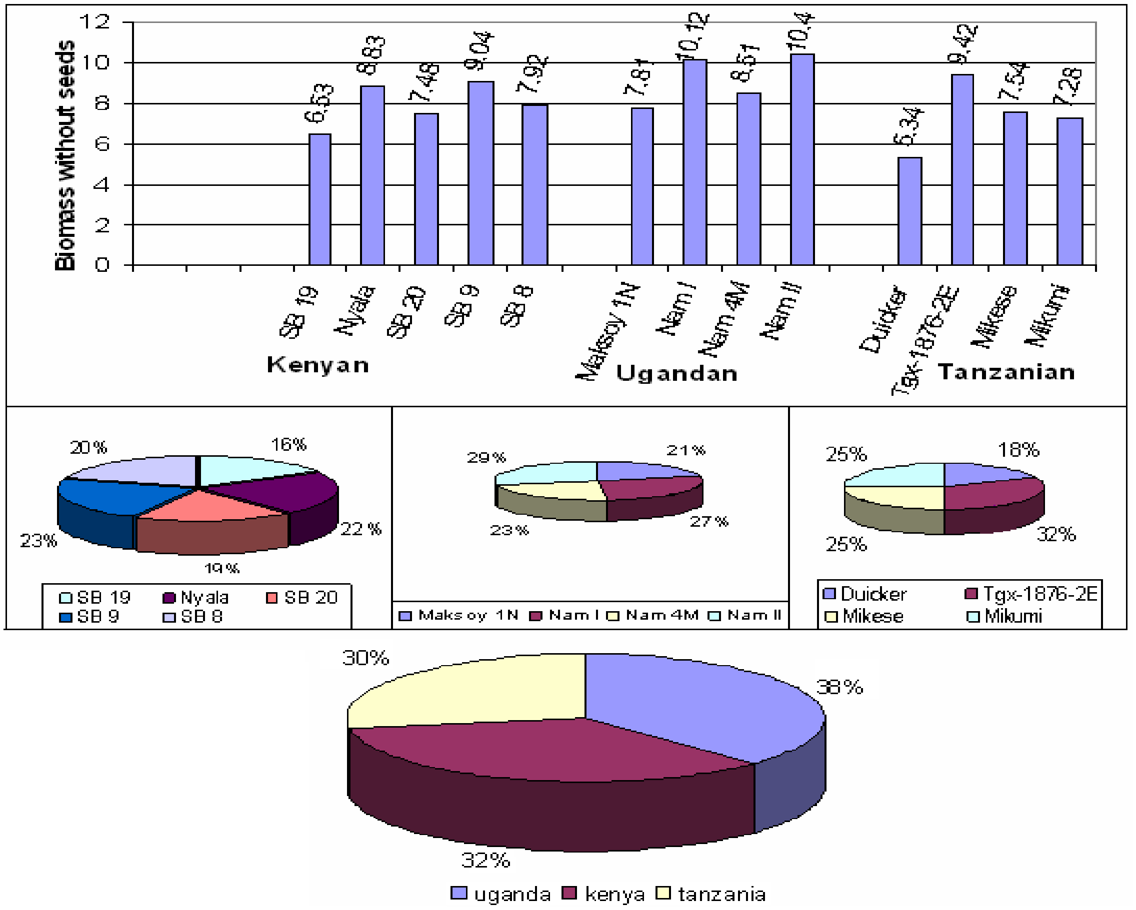
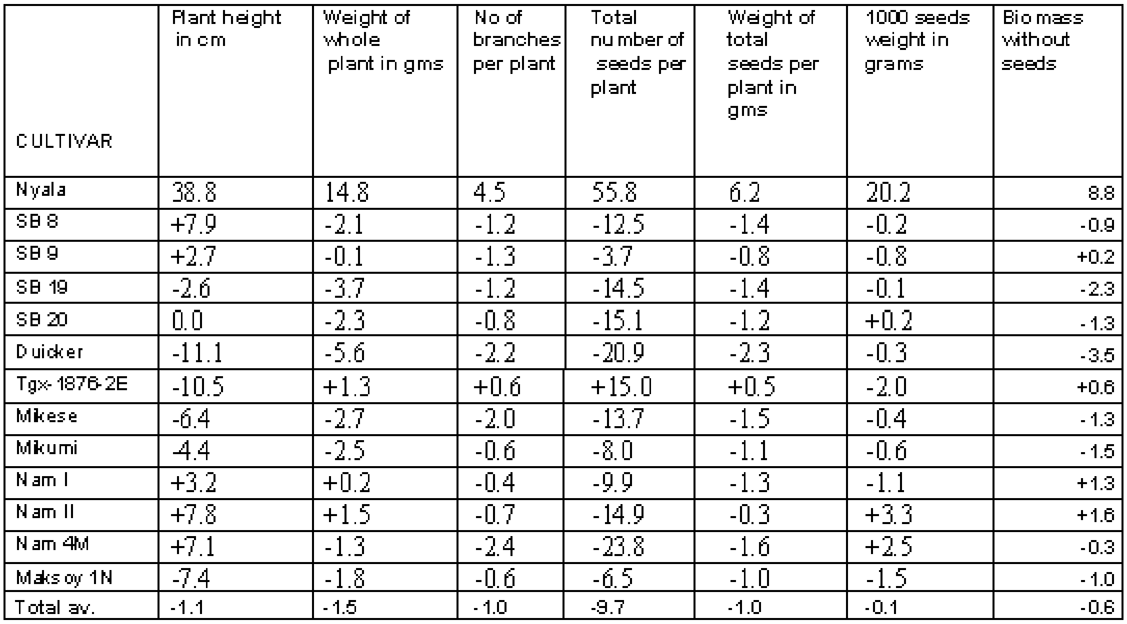


Fig. 22 Average biomass without seeds per cultivar among East African countries

An overall performance in average biomass without seeds per cultivar showed that Ugandan cultivars scored the highest mean value 9.21 (38%), followed by Kenyan 7.96 (32%) then Tanzanian 7.40 (30%). There was a significant difference in performance among cultivars per country (F = 13.303, df = 2, $p < 0.05$). However, the mean difference between the Kenyan and Tanzanian cultivars was not significantly different at $p \leq 0.05$.

Table 4. Comparative performance of cultivars in relation to the standard (Nyala)



CULTIVAR	Plant height in cm	Weight of whole plant in gms	No of branches per plant	Total number of seeds per plant	Weight of total seeds per plant in gms	1000 seeds weight in grams	Biomass without seeds
Nyala	38.8	14.8	4.5	55.8	6.2	20.2	8.8
SB 8	+7.9	-2.1	-1.2	-12.5	-1.4	-0.2	-0.9
SB 9	+2.7	-0.1	-1.3	-3.7	-0.8	-0.8	+0.2
SB 19	-2.6	-3.7	-1.2	-14.5	-1.4	-0.1	-2.3
SB 20	0.0	-2.3	-0.8	-15.1	-1.2	+0.2	-1.3
Duicker	-11.1	-5.6	-2.2	-20.9	-2.3	-0.3	-3.5
Tgx-1876-2E	-10.5	+1.3	+0.6	+15.0	+0.5	-2.0	+0.6
Mikese	-6.4	-2.7	-2.0	-13.7	-1.5	-0.4	-1.3
Mikumi	-4.4	-2.5	-0.6	-8.0	-1.1	-0.6	-1.5
Nam I	+3.2	+0.2	-0.4	-9.9	-1.3	-1.1	+1.3
Nam II	+7.8	+1.5	-0.7	-14.9	-0.3	+3.3	+1.6
Nam 4M	+7.1	-1.3	-2.4	-23.8	-1.6	+2.5	-0.3
Maksoy 1N	-7.4	-1.8	-0.6	-6.5	-1.0	-1.5	-1.0
Total av.	-1.1	-1.5	-1.0	-9.7	-1.0	-0.1	-0.6

Two Kenyan cultivars (SB8 and SB9) and three Ugandan cultivars (Nam I, Nam II and Nam 4M) had average heights greater than the standard. All Tanzanian cultivars were shorter than the standard.

Nyala had greater weight of whole plant than all cultivars except TGX-1876-2E, Nam I and Nam II.

Nyala had the highest number of branches, seeds per plant and weight of seeds per plant than all cultivars except TGX-1876-2E. the standard had less 1000 kernel weight than SB20, Nam II and Nam 4M.

4.5 Molecular results

PCR amplification of the 5S ribosomal gene from the cultivars yielded uniform PCR product of about 700 bps. as was expected based on the targeted DNA region amplified The patterns were uniform (plate 3).

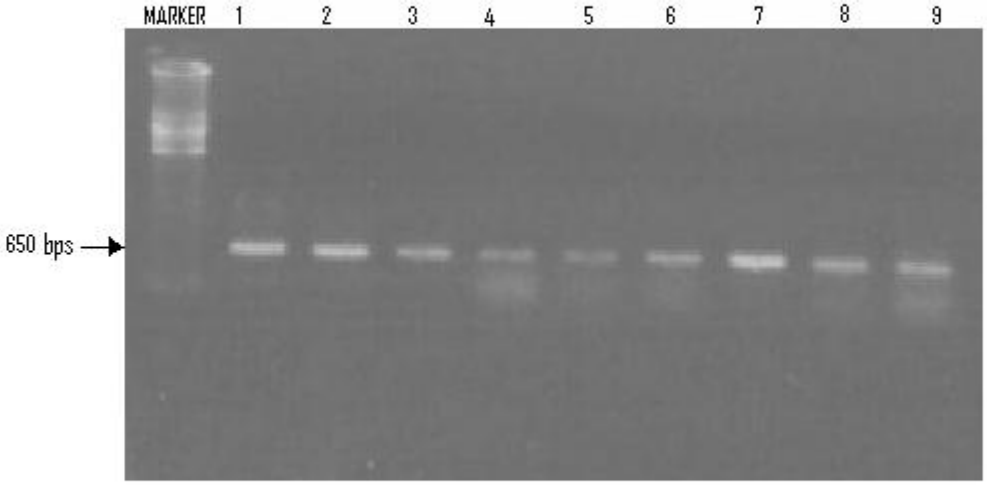


Plate 3. PCR product of the East African soybean *Glycine max* 5S rDNA gene on an agarose gel. The order of the cultivars from left to right is as follows; Nyala, Mikumi, Duicker, Mikese, SB20, Nam 4M, SB9, SB8 and Maksoy IN.

Gene clean

This was carried out to remove any impurity from the genomic material that may interfere with the restriction process and sequencing.

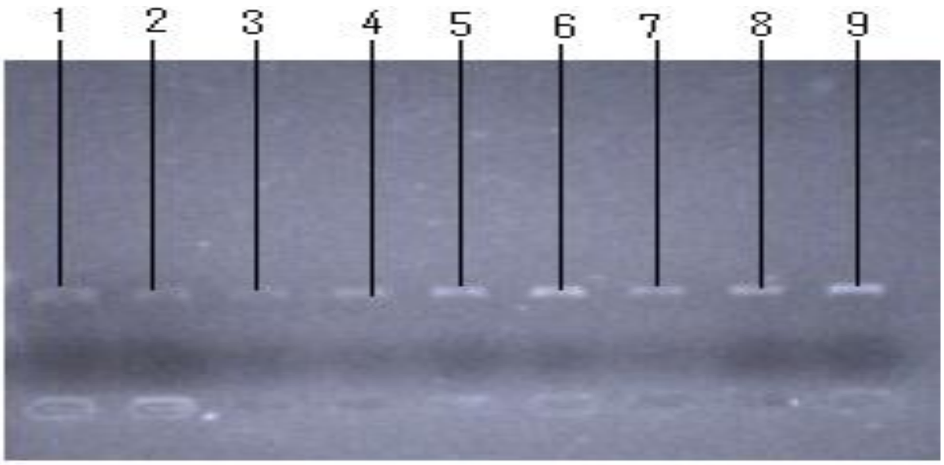


Plate 4. Gene Clean of the East African soybean *Glycine max* 5S rDNA gene on an agarose gel.

The order of the cultivars from 1-9 is as follows; Nyala, SB8, SB19, Mikumi, TGX-1876-2E, Duicker, Maksoy IN, Nam 4M and Nam I respectively.

Restriction Fragment Length Polymorphism

Digestion with restriction enzymes *Hae*III, *Hpa*II and *Mse*I gave similar patterns across the cultivars (plates 5-9). The choice of these enzymes had been determined by simulation restriction digests across the known Glycine spp (see **appendix 2**).

Digestion with restriction enzymes *Mse*I was done and electrophoresis done on agarose gel. The bands produced similar patterns. Digestion with enzyme *Hae*III also produced similar pattern in agarose gel (plate 5).

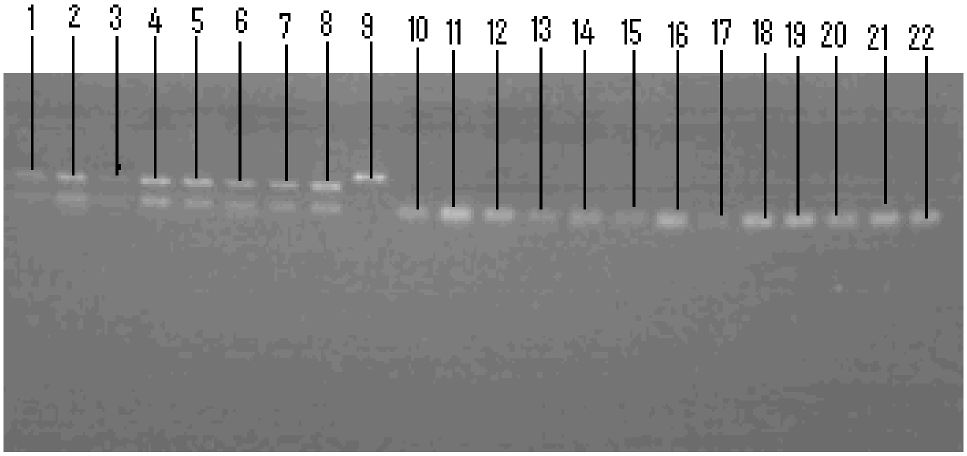


Plate 5. Restriction digest of 5S rDNA gene with enzyme *Mse*I. (Lanes 1-8) and *Hae*III (Lanes 10-22)

Lane 1,2,3,4,5,6,7 and 8 were Nyala, Mikumi, Maksoy 1N, SB20, Mikese, TGX-1876-2E and Nam II respectively. Lane 9 was uncut PCR product. This was loaded to act as a marker. Lines 10 to 22 were Nyala, SB8, SB9, SB19, SB20, Mikumi, Mikese, TGX-1876-2E, Duicker, Nam I, Nam II Nam 4M and Maksoy 1N in that order. These could not be analysed further thus the digests were electrophoresised on polyacrylamide gel for better analysis. PCR DNA was loaded on the far left. Lines 1 to 4 were Nyala, Mikumi, Maksoy 1N and SB20 in that order. The restricted bands move the same distance in the same pattern. There two band in the restriction with enzyme *Mse*I as was predicted from simulation restriction digests across the known *Glycine* spp (see **appendix 2**). Restriction digest with *Hae* III produced four bands as in the simulation restriction digest in **appendix 2**. The patterns were as shown below. The restriction enzyme did not discriminate between the local cultivars. This is because these cultivars are closely related. However, in distantly related cultivars, these enzymes are able to be markers.

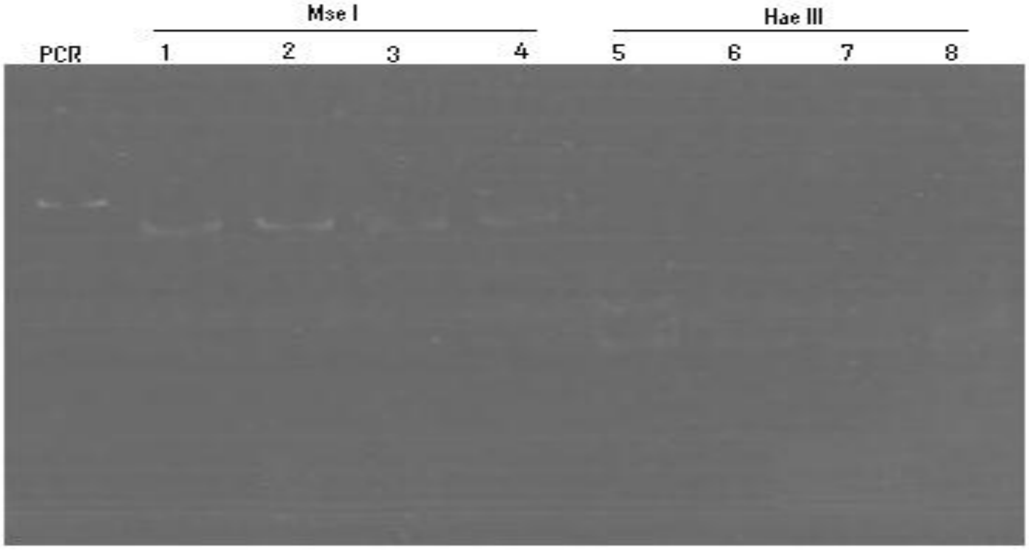


Plate 6. Restriction digests of 5S rDNA gene with enzyme *Mse*I. (Lanes 1,2,3 and 4);*Hae*III (Lanes 5,6,7 and 8)

Restriction digest with restriction enzymes *Hpa*II was done and electrophoresis done on agarose gel. The bands could not be easily analyzed since they were close to one another. Line 1 was PCR DNA product. Lines 2 to 14 were Nyala, SB8, SB9, SB19, SB20, Mikumi, Mikese, TGX-1876-2E, Duicker, Nam I, Nam II Nam 4M and Maksoy 1N in that order. All these produced bands with similar patterns.

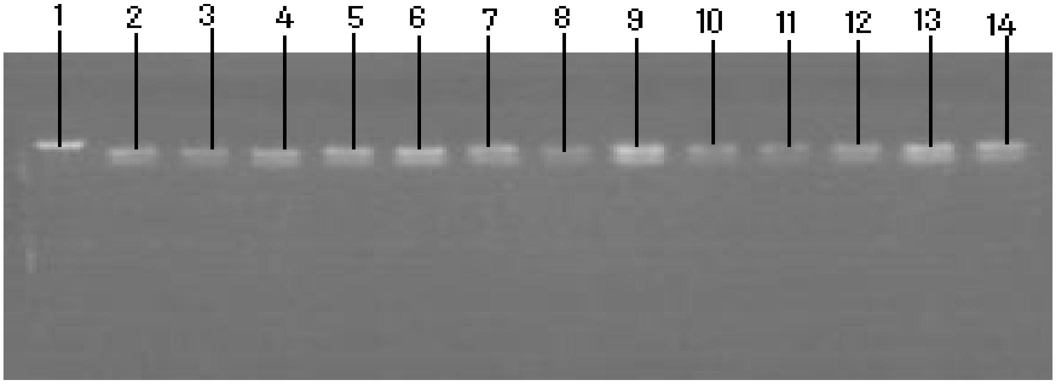


Plate 7. Restriction digests of 5S rDNA gene with enzyme *Hpa*II on Agarose gel.

When PAGE was done five bands were observed as predicted from simulation restriction digests across the known *Glycine* spp (see **appendix 2**).

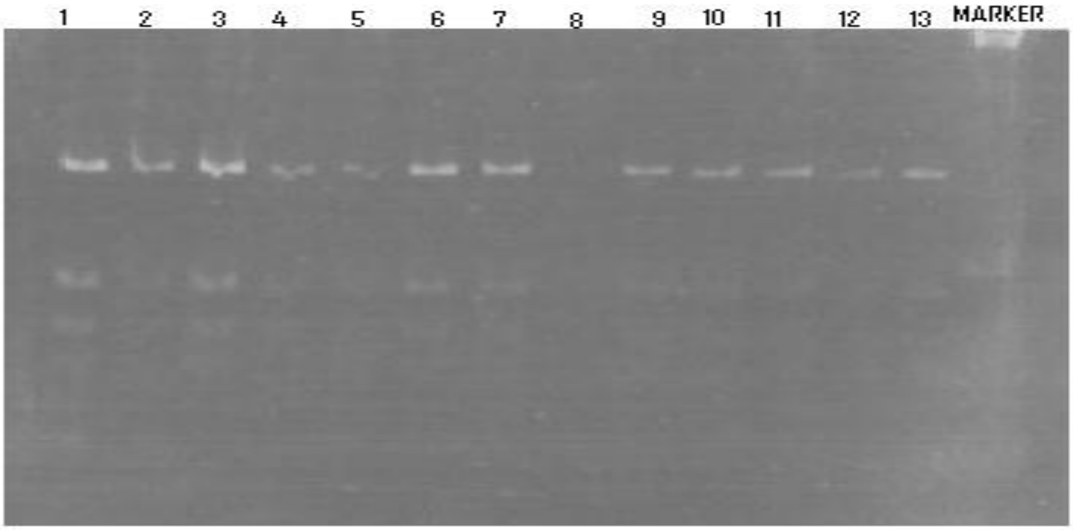


Plate 8. Restriction digests of 5S rDNA gene with enzyme *Hpa*II. Lanes 1-13 shows Nyala, SB8, SB9, SB19, SB20, Duicker, Mikese. TGX1876-2E, Maksoy IN, Nam I, Nam II and Nam 4M respectively.

However, digestion with restriction enzyme *Sac*II gave distinct restriction patterns. However, these mutations did not correspond to differences as shown by agronomic performance.

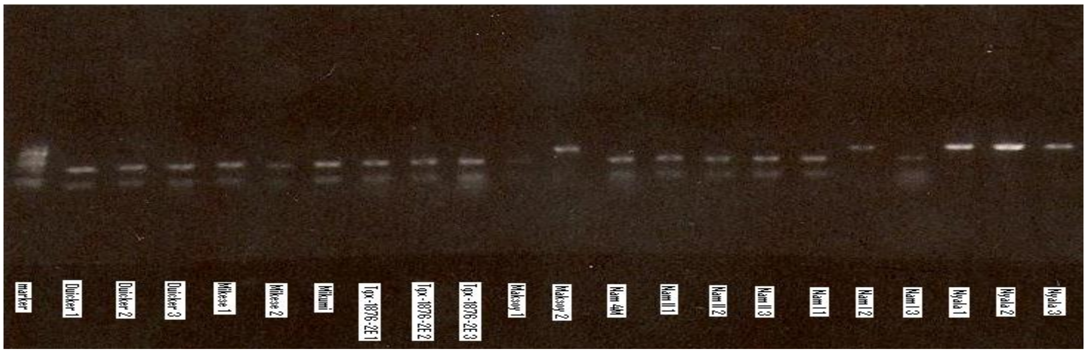
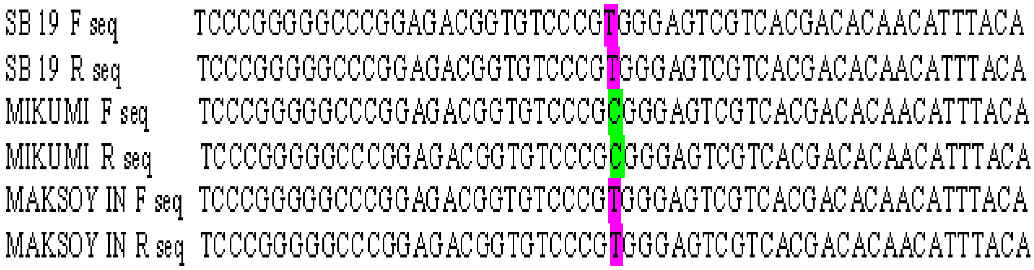


Plate 9. Restriction digests of 5S rDNA gene of the soybean cultivars using an enzyme *Sac* II on agarose gel.

4.6 Sequence analysis of 5S rDNA genes

Limited sample sequencing of the 5S gene fragment was carried out to determine possible sequence variations. When results were analyzed, a restriction marker site within the 5S ribosomal gene was determined (**figure 29**). This shows similar sequences for the East African soybean except for one region that appeared different in Mikumi. Inspite of limited number of cultivar sequences, this process enabled me to determine a marker for the East African soybean cultivars.



```
SB 19 F seq       TCCCGGGGGCCCGGAGACGGTGTCCCGTGGGAGTCGTCACGACACAACATTTACA
SB 19 R seq       TCCCGGGGGCCCGGAGACGGTGTCCCGTGGGAGTCGTCACGACACAACATTTACA
MIKUMI F seq      TCCCGGGGGCCCGGAGACGGTGTCCCGCGGGAGTCGTCACGACACAACATTTACA
MIKUMI R seq      TCCCGGGGGCCCGGAGACGGTGTCCCGCGGGAGTCGTCACGACACAACATTTACA
MAKSOY IN F seq   TCCCGGGGGCCCGGAGACGGTGTCCCGTGGGAGTCGTCACGACACAACATTTACA
MAKSOY IN R seq   TCCCGGGGGCCCGGAGACGGTGTCCCGTGGGAGTCGTCACGACACAACATTTACA
```

Plate 10. Section of gene sequences of one cultivar each from East African countries

Gene BLAST analysis

Blast analysis of the East African cultivars and different species of genus *Glycine* confirmed that all the cultivars studied belong to the genus *Glycine*. Maksoy 1N scored highly with various *Glycine* species (E= 0.00) (≥99%) as seen in appendix 7.4.

A phylogenetic tree was constructed based on 5S rDNA gene sequences of East African cultivars and different *Glycine* species. This phylogenetic analysis suggests that cultivar Mikumi is similar to *Glycine max* while SB 19 of Kenya and Maksoy of Uganda are the same species and also closely related to *Glycine max*

This study therefore managed to trace the East African soybeans origin in comparison with *Glycine* species globally. Further analysis is needed to reflect the data of specific region of the world

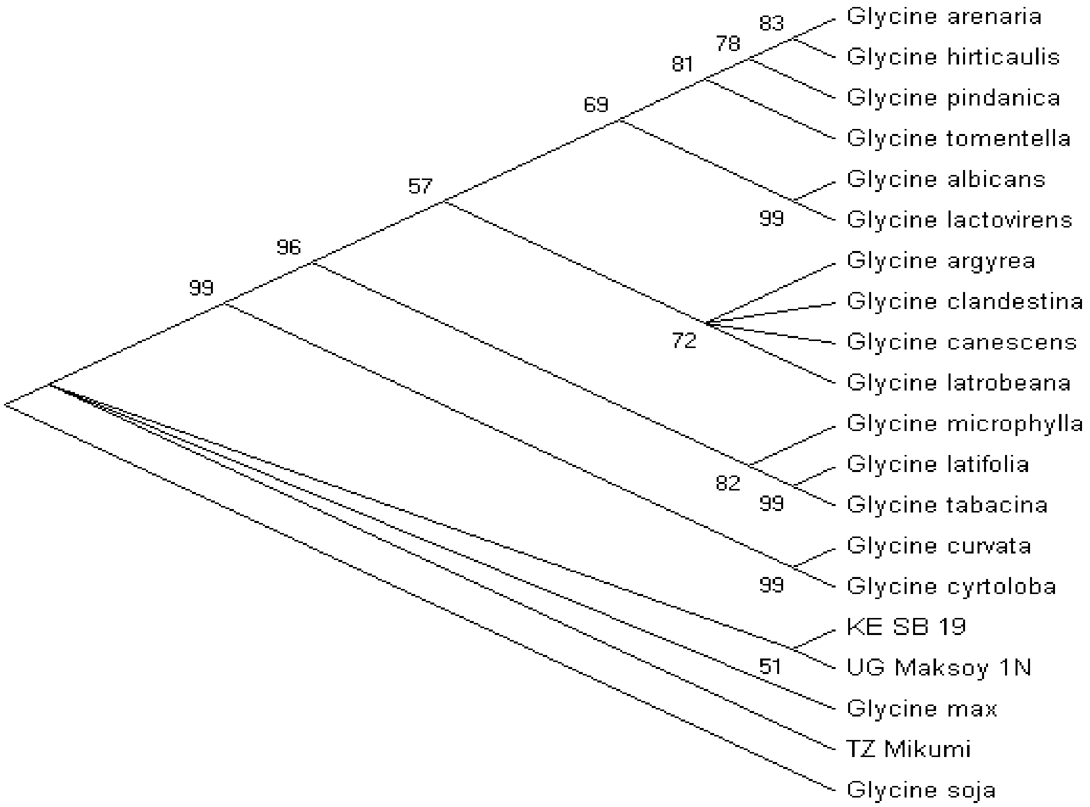


Fig. 23 Phylogenetic relationship between the East African cultivars and global *Glycine* species.

CHAPTER FIVE

CONCLUSION AND RECOMMENDATIONS

A general study of cultivars showed that they were of moderate heights. They were grown when there was water deficit but high temperatures which led to small seed sizes for most cultivars. They took the expected germination period and underwent all physiological processes. All cultivars had the recommended height of less than 75 centimeters for easy mechanization. Highly branched cultivars were relatively short in height, had relatively high weight, many seeds and therefore performed well agronomically. Most tall cultivars had relatively more seeds per plant than shorter ones with averages ranging between 32.02 and 70.82 seeds per plant. Cultivar TGX-1876-2E had the highest number of branches, biggest number of seeds per plant, heaviest weight of whole plant but had low weight of 1000 kernel and short height.

Generally most cultivars formed few and light seeds due to lack of enough rainfall. All studied cultivars had a good proportion of seed to whole plant ranging between 33% and 43%. This means that they are suitable for seed production.

Since branches form a substantial part of total plant weight, it was observed that the larger the number of branches the greater the biomass without seeds. Comparison of general agronomic performance between countries showed that Ugandan cultivars did well.

Molecular analysis showed that most restriction enzymes gave similar banding patterns on all studied cultivars indicating similar genotype. However, restriction with enzyme *Sac* II gave distinct restriction patterns. These mutations did not correspond to differences observed in agronomic performance. The observed differences reflect genotype-environment interaction, indicating genotype differences among the cultivars. This variation was within the ITS (non coding) region of 5S gene and therefore did not reflect a change in amino acid. A phylogenetic tree based on 5S rDNA gene sequences suggested that Mikumi is similar to *Glycine max*. SB19 and Maksoy are the same species and closely related to *Glycine max.* The study therefore managed to trace selected East African soybeans origin in comparison with *Glycine* species globally.

There is no link between agronomic performance and the genetic make up of the studied cultivars.

Recommendations

Cultivars TGX-1876-2E, Namsoy 4M, Nam II and Nyala should be recommended to farmers since they have many positive characteristics such as many seeds, high 1000 seed weight.

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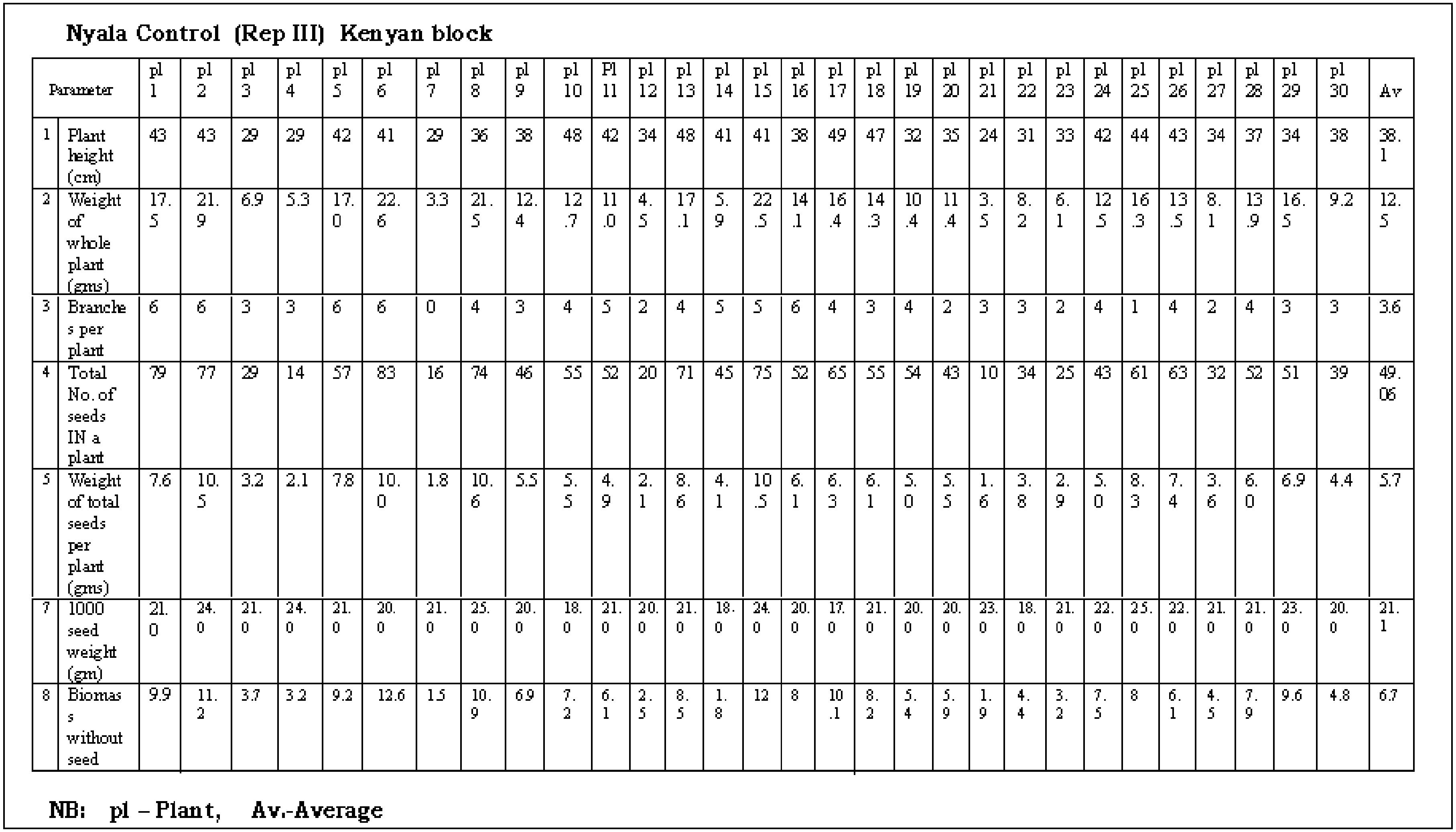
APPENDICES

7.1 Table of analysis



Nyala Control (Rep III) Kenyan block

Parameter		pl 1	pl 2	pl 3	pl 4	pl 5	pl 6	pl 7	pl 8	pl 9	pl 10	Pl 11	pl 12	pl 13	pl 14	pl 15	pl 16	pl 17	pl 18	pl 19	pl 20	pl 21	pl 22	pl 23	pl 24	pl 25	pl 26	pl 27	pl 28	pl 29	pl 30	Av
1	Plant height (cm)	43	43	29	29	42	41	29	36	38	48	42	34	48	41	41	38	49	47	32	35	24	31	33	42	44	43	34	37	34	38	38.1
2	Weight of whole plant (gms)	17.5	21.9	6.9	5.3	17.0	22.6	3.3	21.5	12.4	12.7	11.0	4.5	17.1	5.9	22.5	14.1	16.4	14.3	10.4	11.4	3.5	8.2	6.1	12.5	16.3	13.5	8.1	13.9	16.5	9.2	12.5
3	Branches per plant	6	6	3	3	6	6	0	4	3	4	5	2	4	5	5	6	4	3	4	2	3	3	2	4	1	4	2	4	3	3	3.6
4	Total No. of seeds IN a plant	79	77	29	14	57	83	16	74	46	55	52	20	71	45	75	52	65	55	54	43	10	34	25	43	61	63	32	52	51	39	49.06
5	Weight of total seeds per plant (gms)	7.6	10.5	3.2	2.1	7.8	10.0	1.8	10.6	5.5	5.5	4.9	2.1	8.6	4.1	10.5	6.1	6.3	6.1	5.0	5.5	1.6	3.8	2.9	5.0	8.3	7.4	3.6	6.0	6.9	4.4	5.7
7	1000 seed weight (gm)	21.0	24.0	21.0	24.0	21.0	20.0	21.0	25.0	20.0	18.0	21.0	20.0	21.0	18.0	24.0	20.0	17.0	21.0	20.0	20.0	23.0	18.0	21.0	22.0	25.0	22.0	21.0	21.0	23.0	20.0	21.1
8	Biomass without seed	9.9	11.2	3.7	3.2	9.2	12.6	1.5	10.9	6.9	7.2	6.1	2.5	8.5	1.8	12	8	10.1	8.2	5.4	5.9	1.9	4.4	3.2	7.5	8	6.1	4.5	7.9	9.6	4.8	6.7

NB: pl – Plant, Av.-Average

7.2 Simulation restrictions digest across known *Glycine* species.

BioEdit version 7.0.5.2 (6/5/05) Restriction Mapping Utility(c)1998, Tom Hallgi|1885372|gb|U60551.1|GMU60551 Glycine max small subunit ribosomal RNA gene, partial sequence, internal transcribed spacer 1, 5.8S ribosomal RNA gene and internal transcribed spacer 2, complete sequence, and large subunit ribosomal RNA gene, partial sequence Restriction Map

```
1/4/2008 10:56:53 AM

662 base pairs

Translations:  none

Restriction Enzyme Map:

1
TGCGGAAGGATCATTGTCGATGCCTCACAATCAGATTGACCCGCGAACTTGTTTATTCATCTACCGTCGGG
AGGGAGGGG   80

1
ACGCCTTCCTAGTAACAGCTACGGAGTGTTAGTCTAACTGGGCGCTTGAACAAATAAGTAGATGGCAGCCC
TCCCTCCCC   80

          AciI   MboI      TaqI                    AciI

                   DpnI                             BstUI

                    BstKTI

81
ATGACCACGGCGCCCCGTGCGCCCGGCCTCCTCGTCCTCGCGACAAACACAAACCCCGGCGCTTCGTGCGC
CAAGGAACT   160

81
TACTGGTGCCGCGGGGCACGCGGGCCGGAGGAGCAGGAGCGCTGTTTGTGTTTGGGGCCGCGAAGCACGCG
GTTCCTTGA   160

                 KasI            HinP1I                            NruI
HpaII        HinP1I

                   NarI             HhaI                          BstUI
HinP1I      HhaI

                   HinP1I                                         HpaII
HhaI

                    SfoI           HaeIII

                     HhaI

                      BbeI
```

```
161
CAAATCTGTTAAGTGCGACTCCCGGGGGCCCGGAGACGGTGTCCCGCGGGAGTCGTCACGACACAACATTT
ACATACAAT   240

161
GTTTAGACAATTCACGCTGAGGGCCCCCGGGCCTCTGCCACAGGGCGCCCTCAGCAGTGCTGTGTTGTAAA
TGTATGTTA   240

                 MseI         XmaI    HaeIII           AciI

                               HpaII    ApaI             BstUI

                                SmaI    HpaII            AciI

                                   PspOMI                SacII

241
GACTCTCGGCAACGGATATCTCGGCTCTTGCATCGATGAAGAACGTAGCGAAATGCGATACTTGGTGTGAA
TTGCAGAAT   320

241
CTGAGAGCCGTTGCCTATAGAGCCGAGAACGTAGCTACTTCTTGCATCGCTTTACGCTATGAACCACACTT
AACGTCTTA   320

                         EcoRV               HpyCH4V            HpyCH4IV
Tsp509I

                                          ClaI                        TaiI
HpyCH4V

                                          TaqI

321
CCCGTGAACCATCGAGTCTTTGAACGCAAGTTGCGCCCGAAGCCATTAGGCCGAGGGCACGCCTGCCTGGG
TGTCACACA   400

321
GGGCACTTGGTAGCTCAGAAACTTGCGTTCAACGCGGGCTTCGGTAATCCGGCTCCCGTGCGGACGGACCC
ACAGTGTGT   400

                    TaqI                  HinP1I              HaeIII

                                            HhaI

401
TCGTTTCCCCAACGCAAACATGTAACAATGTTGCTGCGCGGGGTGTATGCTGACCTCCCGCGAGCACCCGC
CTCGTGGTT   480

401
AGCAAAGGGGTTGCGTTTGTACATTGTTACAACGACGCGCCCCACATACGACTGGAGGGCGCTCGTGGGCG
GAGCACCAA   480

                          PciI                                      HinP1I
AciI       AciI

                           FatI                                       HhaI
BstUI        BssSI
```

```
                                 NlaIII           BstUI

                                              AciI

481
GGTTGAAATCTGGGTTCATGGCCGACTTCGCCGTGATAAAATGGTGGATGAGCCACGCTCGAGACCAATCA
CGTGCGAGC   560

481
CCAACTTTAGACCCAAGTACCGGCTGAAGCGGCACTATTTTACCACCTACTCGGTGCGAGCTCTGGTTAGT
GCACGCTCG   560

                        FatI                                       XhoI
HpyCH4IV

                             NlaIII
TaqI          PmlI

                              HaeIII
TaiI

561
CGGTCAGTTCTGGACCCATCGACGACCCTTTGCGTGCACGCACGCTCCCAACGAGACCTCAGGTCAGGCGG
GGCTACCCG   640

561
GCCAGTCAAGACCTGGGTAGCTGCTGGGAAACGCACGTGCGTGCGAGGGTTGCTCTGGAGTCCAGTCCGCC
CCGATGGGC   640

        HpaII                           TaqI                      ApaLI
AciI       AciI

                                            HpyCH4V

641     CTGAGTTTAAGCATATCAATAA   662

641     GACTCAAATTCGTATAGTTATT   662

               MseI
```

7.3 ANOVA TABLES

Plant height in cm

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	48072.631	12	4006.053	106.131	.000
Within Groups	43672.289	1157	37.746		
Total	91744.920	1169			

Plant height in cm

Tukey HSD

CULTIVAR	N	Subset for alpha = .05						
		1	2	3	4	5	6	7
Duicker	90	27.656						
Tgx-1876-2E	90	28.278						
Maksoy 1N	90		31.378					
Mikese	90		32.433					
Mikumi	90		34.389	34.389				
SB 19	90			36.156	36.156			
Nyala	90				38.767	38.767		
SB 20	90				38.811	38.811		
SB 9	90					41.489	41.489	
Nam I	90						41.956	
Nam 4M	90							45.933
Nam II	90							46.622
SB 8	90							46.711
Sig.		1.000	.055	.778	.161	.134	1.000	1.000

Means for groups in homogeneous subsets are displayed.
a Uses Harmonic Mean Sample Size = 90.000.

Weight of whole plant in gms

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	4398.823	12	366.569	6.634	.000
Within Groups	63933.297	1157	55.258		
Total	68332.120	1169			

Weight of whole plant in gms

Tukey HSD

CULTIVAR	N	Subset for alpha = .05				
		1	2	3	4	5
Duicker	90	9.230				
SB 19	90	11.120	11.120			
Mikese	90	12.083	12.083	12.083		
Mikumi	90	12.304	12.304	12.304		
SB 20	90	12.462	12.462	12.462	12.462	
SB 8	90	12.713	12.713	12.713	12.713	12.713
Maksoy 1N	90		13.040	13.040	13.040	13.040
Nam 4M	90		13.456	13.456	13.456	13.456
SB 9	90		14.722	14.722	14.722	14.722
Nyala	90			14.823	14.823	14.823
Nam I	90			15.010	15.010	15.010
Tgx-1876-2E	90				16.092	16.092
Nam II	90					16.283
Sig.		.084	.062	.287	.057	.067

Means for groups in homogeneous subsets are displayed.
a Uses Harmonic Mean Sample Size = 90.000.

Number of branches per plant

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	846.881	12	70.573	25.227	.000
Within Groups	3236.689	1157	2.797		
Total	4083.571	1169			

Number of branches per plant

Tukey HSD

CULTIVAR	N	Subset for alpha = .05					
		1	2	3	4	5	6
Nam 4M	90	2.067					
Duicker	90	2.256					
Mikese	90	2.489	2.489				
SB 9	90		3.167	3.167			
SB 19	90		3.267	3.267			
SB 8	90		3.300	3.300	3.300		
SB 20	90			3.700	3.700	3.700	
Nam II	90			3.827	3.827	3.827	
Maksoy 1N	90			3.933	3.933	3.933	
Mikumi	90			3.944	3.944	3.944	
Nam I	90				4.111	4.111	
Nyala	90					4.522	4.522
Tgx-1876-2E	90						5.122
Sig.		.897	.061	.090	.061	.053	.440

Means for groups in homogeneous subsets are displayed.
a Uses Harmonic Mean Sample Size = 90.000.

Total number of seeds per plant

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	106304.834	12	8858.736	13.330	.000
Within Groups	768910.278	1157	664.572		
Total	875215.112	1169			

Total number of seeds per plant

Tukey HSD

CULTIVAR	N	Subset for alpha = .05				
		1	2	3	4	5
Nam 4M	90	32.022				
Duicker	90	34.933	34.933			
SB 20	90	40.733	40.733	40.733		
Nam II	90	40.900	40.900	40.900		
SB 19	90	41.256	41.256	41.256		
Mikese	90	42.111	42.111	42.111		
SB 8	90	43.300	43.300	43.300	43.300	
Nam I	90		45.889	45.889	45.889	
Mikumi	90			47.833	47.833	
Maksoy 1N	90			49.311	49.311	
SB 9	90			52.144	52.144	
Nyala	90				55.822	
Tgx-1876-2E	90					70.822
Sig.		.147	.181	.135	.060	1.000

Means for groups in homogeneous subsets are displayed.
a Uses Harmonic Mean Sample Size = 90.000.

Weight of total seeds per plant in gms

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	578.315	12	48.193	5.124	.000
Within Groups	10882.631	1157	9.406		
Total	11460.946	1169			

Weight of total seeds per plant in gms

Tukey HSD

CULTIVAR	N	Subset for alpha = .05			
		1	2	3	4
Duicker	90	3.899			
Nam 4M	90	4.577	4.577		
Mikese	90	4.744	4.744	4.744	
SB 19	90	4.751	4.751	4.751	
SB 8	90	4.796	4.796	4.796	
Nam I	90	4.881	4.881	4.881	
SB 20	90	4.954	4.954	4.954	
Mikumi	90	5.053	5.053	5.053	
Maksoy 1N	90	5.156	5.156	5.156	5.156
SB 9	90	5.380	5.380	5.380	5.380
Nam II	90		5.874	5.874	5.874
Nyala	90			6.183	6.183
Tgx-1876-2E	90				6.672
Sig.		.064	.186	.083	.050

Means for groups in homogeneous subsets are displayed.
a Uses Harmonic Mean Sample Size = 90.000.

1000 seeds weight in grams

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	2350.144	12	195.845	38.946	.000
Within Groups	5818.133	1157	5.029		
Total	8168.277	1169			

1000 seeds weight in grams

Tukey HSD

CULTIVAR	N	Subset for alpha = .05				
		1	2	3	4	5
Tgx-1876-2E	90	18.189				
Maksoy 1N	90	18.700	18.700			
Nam I	90	19.144	19.144	19.144		
SB 9	90		19.400	19.400	19.400	
Mikumi	90		19.556	19.556	19.556	
Mikese	90		19.800	19.800	19.800	
Duicker	90			19.900	19.900	
SB 8	90			20.044	20.044	
SB 19	90			20.078	20.078	
Nyala	90			20.156	20.156	
SB 20	90				20.389	
Nam 4M	90					22.722
Nam II	90					23.522
Sig.		.177	.055	.117	.139	.449

Means for groups in homogeneous subsets are displayed.

a Uses Harmonic Mean Sample Size = 90.000.

Biomass without seeds

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	2180.081	12	181.673	8.140	.000
Within Groups	25821.587	1157	22.318		
Total	28001.668	1169			

Biomass without seeds

Tukey HSD

CULTIVAR	N	Subset for alpha = .05				
		1	2	3	4	5
Duicker	90	5.336				
SB 19	90	6.529	6.529			
Mikumi	90	7.288	7.288	7.288		
SB 20	90	7.479	7.479	7.479		
Mikese	90	7.541	7.541	7.541		
Maksoy 1N	90		7.813	7.813	7.813	
SB 8	90		7.919	7.919	7.919	
Nam 4M	90		8.506	8.506	8.506	8.506
Nyala	90		8.826	8.826	8.826	8.826
SB 9	90			9.043	9.043	9.043
Tgx-1876-2E	90			9.424	9.424	9.424
Nam I	90				10.120	10.120
Nam II	90					10.400
Sig.		.087	.060	.114	.057	.259

Means for groups in homogeneous subsets are displayed.
a Uses Harmonic Mean Sample Size = 90.000.

7.4 BLAST Analysis

Sequences producing significant alignments

Accession	Description	Max score	Total score	Query coverage	E value	Max ident	Links
EF517917.1	Glycine max 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence	1016	1016	100%	0.0	99%	
U60551.1	Glycine max small subunit ribosomal RNA gene, partial sequence, internal transcribed spacer 1, 5.8S ribosomal RNA gene and internal transcribed spacer 2, complete sequence, and large subunit ribosomal RNA gene, partial sequence	1016	1016	100%	0.0	99%	
L36612.1	Glycine max internal transcribed spacer DNA	1016	1016	100%	0.0	99%	
U60550.1	Glycine soja small subunit ribosomal RNA gene, partial sequence, internal transcribed spacer 1, 5.8S ribosomal RNA gene and internal transcribed spacer 2, complete sequence, and large subunit ribosomal RNA gene, partial sequence	1011	1011	100%	0.0	99%	
AF144652.1	Glycine max isolate 43Virginia internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene, complete sequence; and internal transcribed space 2, partial sequence	1005	1005	100%	0.0	99%	
AF144651.1	Glycine max isolate 38virginia-s internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene, complete sequence; and internal transcribed space 2, partial sequence	1005	1005	100%	0.0	99%	
AJ224109.1	Glycine soja DNA for internal transcribed spacer, isolate 043	998	998	100%	0.0	99%	
AJ009791.1	Glycine soja 5.8S rRNA gene and internal transcribed spacers, isolate 039, partial	981	981	100%	0.0	98%	
AJ009790.1	Glycine soja 5.8S rRNA gene and internal transcribed spacers, isolate 001	974	974	100%	0.0	98%	
AJ009787.1	Glycine max 5.8S rRNA gene and internal transcribed spacers, isolate 001, partial	957	957	100%	0.0	98%	
AF144653.1	Glycine soja isolate 39soja internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene, complete sequence; and internal transcribed space 2, partial sequence	955	955	99%	0.0	98%	
AJ011337.1	Glycine max internal transcribed spacers ITS1 and ITS2, isolate Williams	955	955	100%	0.0	98%	
AF144654.1	Glycine max isolate 48Hidatsa internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene, complete sequence; and internal transcribed space 2, partial sequence	942	942	99%	0.0	97%	
U60548.1	Glycine cyrtoloba small subunit ribosomal RNA gene, partial sequence, internal transcribed spacer 1, 5.8S ribosomal RNA gene and internal transcribed spacer 2, complete sequence, and large subunit ribosomal RNA gene, partial sequence	802	802	92%	0.0	94%	

Accession	Description	Max score	Total score	Query coverage	E value	Max ident	Links
AY433834.1	Glycine tomentella specimen-voucher CSIRO G1415 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 26S ribosomal RNA gene, partial sequence	798	798	99%	0.0	93%	
AY168308.1	Glycine tomentella isolate G1413-S3 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene and internal transcribed spacer 2, complete sequence; and 26S ribosomal RNA gene, partial sequence	798	798	99%	0.0	93%	
EF517918.1	Glycine microphylla voucher PGGC 1272 (CSIRO) 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence	795	795	100%	0.0	92%	
AY433934.1	Glycine stenophita specimen-voucher CSIRO G2223 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 26S ribosomal RNA gene, partial sequence	795	795	92%	0.0	94%	
AY168326.1	Glycine clandestina isolate G1575-DJ1 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene and internal transcribed spacer 2, complete sequence; and 26S ribosomal RNA gene, partial sequence	795	795	99%	0.0	92%	
U60537.1	Glycine microphylla small subunit ribosomal RNA gene, partial sequence, internal transcribed spacer 1, 5.8S ribosomal RNA gene and internal transcribed spacer 2, complete sequence, and large subunit ribosomal RNA gene, partial sequence	795	795	100%	0.0	92%	
AY433841.1	Glycine tomentella specimen-voucher CSIRO G1367 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene and internal transcribed spacer 2, complete sequence; and 26S ribosomal RNA gene, partial sequence	787	787	99%	0.0	92%	
AY433840.1	Glycine tomentella specimen-voucher CSIRO G1361 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene and internal transcribed spacer 2, complete sequence; and 26S ribosomal RNA gene, partial sequence	787	787	99%	0.0	92%	
AY433839.1	Glycine tomentella specimen-voucher CSIRO G1274 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene and internal transcribed spacer 2, complete sequence; and 26S ribosomal RNA gene, partial sequence	787	787	99%	0.0	92%	
AY433838.1	Glycine tomentella specimen-voucher CSIRO G1133 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene and internal transcribed spacer 2, complete sequence; and 26S ribosomal RNA gene, partial sequence	787	787	99%	0.0	92%	
AY433837.1	Glycine tomentella specimen-voucher CSIRO G1392 18S ribosomal RNA gene,	787	787	99%	0.0	92%	

Accession	Description	Max score	Total score	Query coverage	E value	Max ident	Links
	partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 26S ribosomal RNA gene, partial sequence						
AY433836.1	Glycine tomentella specimen-voucher CSIRO G1157 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 26S ribosomal RNA gene, partial sequence	787	787	99%	0.0	92%	
AY433835.1	Glycine tomentella specimen-voucher CSIRO G1316 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 26S ribosomal RNA gene, partial sequence	787	787	99%	0.0	92%	
AF187086.1	Myrospermum sousanum small subunit ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene and internal transcribed spacer 2, complete sequence; and large subunit ribosomal RNA gene, partial sequence	787	787	92%	0.0	94%	
AY168311.1	Glycine tomentella isolate G1763-HS7 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene and internal transcribed spacer 2, complete sequence; and 26S ribosomal RNA gene, partial sequence	787	787	99%	0.0	92%	
AY168310.1	Glycine tomentella isolate G1180-HV9 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene and internal transcribed spacer 2, complete sequence; and 26S ribosomal RNA gene, partial sequence	787	787	99%	0.0	92%	
AY168309.1	Glycine tomentella isolate G1288-HV1 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene and internal transcribed spacer 2, complete sequence; and 26S ribosomal RNA gene, partial sequence	787	787	99%	0.0	92%	
U60544.1	Glycine tomentella small subunit ribosomal RNA gene, partial sequence, internal transcribed spacer 1, 5.8S ribosomal RNA gene and internal transcribed spacer 2, complete sequence, and large subunit ribosomal RNA gene, partial sequence	787	787	99%	0.0	92%	
AY433882.1	Glycine clandestina specimen-voucher CSIRO G1123 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 26S ribosomal RNA gene, partial sequence	784	784	99%	0.0	92%	
AY433842.1	Glycine tomentella specimen-voucher CSIRO G1427 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene and internal transcribed spacer 2, complete sequence; and 26S ribosomal RNA gene, partial sequence	784	784	99%	0.0	92%	
U60547.1	Glycine curvata small subunit ribosomal RNA gene, partial sequence, internal	784	784	92%	0.0	94%	

Accession	Description	Max score	Total score	Query coverage	E value	Max ident	Links
	transcribed spacer 1, 5.8S ribosomal RNA gene and internal transcribed spacer 2, complete sequence, and large subunit ribosomal RNA gene, partial sequence						
U60534.1	Glycine clandestina small subunit ribosomal RNA gene, partial sequence, internal transcribed spacer 1, 5.8S ribosomal RNA gene and internal transcribed spacer 2, complete sequence, and large subunit ribosomal RNA gene, partial sequence	784	784	99%	0.0	92%	
AY433884.1	Glycine tomentella specimen-voucher CSIRO G2073 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 26S ribosomal RNA gene, partial sequence	782	782	100%	0.0	92%	
AY168322.1	Glycine tomentella isolate G1777-OF09 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene and internal transcribed spacer 2, complete sequence; and 26S ribosomal RNA gene, partial sequence	782	782	100%	0.0	92%	
AY433889.1	Glycine tomentella specimen-voucher CSIRO G1854 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 26S ribosomal RNA gene, partial sequence	780	780	99%	0.0	92%	
AY433888.1	Glycine tomentella specimen-voucher CSIRO G1811 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 26S ribosomal RNA gene, partial sequence	780	780	99%	0.0	92%	
AY433887.1	Glycine tomentella specimen-voucher CSIRO G1286 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 26S ribosomal RNA gene, partial sequence	780	780	99%	0.0	92%	
AY433886.1	Glycine tomentella specimen-voucher CSIRO G1410 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 26S ribosomal RNA gene, partial sequence	780	780	99%	0.0	92%	
AY433843.1	Glycine tomentella specimen-voucher CSIRO G1136 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene and internal transcribed spacer 2, complete sequence; and 26S ribosomal RNA gene, partial sequence	780	780	99%	0.0	92%	
AY168325.1	Glycine tomentella isolate G1393-E4 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene and internal transcribed spacer 2, complete sequence; and 26S ribosomal RNA gene, partial sequence	780	780	99%	0.0	92%	

Accession	Description	Max score	Total score	Query coverage	E value	Max ident	Links
AY168324.1	Glycine tomentella isolate G1188-E3 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene and internal transcribed spacer 2, complete sequence; and 26S ribosomal RNA gene, partial sequence	780	780	99%	0.0	92%	
AY168323.1	Glycine tomentella isolate G1134-ET3 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene and internal transcribed spacer 2, complete sequence; and 26S ribosomal RNA gene, partial sequence	780	780	99%	0.0	92%	
AF023446.1	Glycine tomentella small subunit ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene and internal transcribed spacer 2, complete sequence; and large subunit ribosomal RNA gene, partial sequence	780	780	99%	0.0	92%	
AY433881.1	Glycine clandestina specimen-voucher CSIRO G2160 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 26S ribosomal RNA gene, partial sequence	778	778	99%	0.0	92%	
AY433933.1	Glycine pindanica specimen-voucher CSIRO G2939 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 26S ribosomal RNA gene, partial sequence	776	776	99%	0.0	92%	
AY433912.1	Glycine tomentella specimen-voucher CSIRO G2583 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 26S ribosomal RNA gene, partial sequence	776	776	99%	0.0	92%	
AY433911.1	Glycine tomentella specimen-voucher CSIRO G1944 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 26S ribosomal RNA gene, partial sequence	776	776	99%	0.0	92%	
AY433910.1	Glycine tomentella specimen-voucher CSIRO G2051 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 26S ribosomal RNA gene, partial sequence	776	776	99%	0.0	92%	
AY433906.1	Glycine tomentella specimen-voucher CSIRO G1934 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 26S ribosomal RNA gene, partial sequence	776	776	99%	0.0	92%	
AY433904.1	Glycine tomentella specimen-voucher CSIRO G2401 18S ribosomal RNA gene,	776	776	99%	0.0	92%	

Accession	Description	Max score	Total score	Query coverage	E value	Max ident	Links
	partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 26S ribosomal RNA gene, partial sequence						
AY433903.1	Glycine tomentella specimen-voucher CSIRO G1303 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 26S ribosomal RNA gene, partial sequence	776	776	99%	0.0	92%	
AY433901.1	Glycine tomentella specimen-voucher CSIRO G3075 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 26S ribosomal RNA gene, partial sequence	776	776	99%	0.0	92%	
AF023447.1	Glycine tomentella small subunit ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene and internal transcribed spacer 2, complete sequence; and large subunit ribosomal RNA gene, partial sequence	776	776	99%	0.0	92%	
U60546.1	Glycine pindanica small subunit ribosomal RNA gene, partial sequence, internal transcribed spacer 1, 5.8S ribosomal RNA gene and internal transcribed spacer 2, complete sequence, and large subunit ribosomal RNA gene, partial sequence	776	776	99%	0.0	92%	
U60543.1	Glycine arenaria small subunit ribosomal RNA gene, partial sequence, internal transcribed spacer 1, 5.8S ribosomal RNA gene and internal transcribed spacer 2, complete sequence, and large subunit ribosomal RNA gene, partial sequence	776	776	99%	0.0	92%	
AY433885.1	Glycine tomentella specimen-voucher CSIRO G2321 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 26S ribosomal RNA gene, partial sequence	774	774	99%	0.0	92%	
U60539.1	Glycine tabacina small subunit ribosomal RNA gene, partial sequence, internal transcribed spacer 1, 5.8S ribosomal RNA gene and internal transcribed spacer 2, complete sequence, and large subunit ribosomal RNA gene, partial sequence	774	774	92%	0.0	94%	
AJ011340.1	Glycine dolichocarpa internal transcribed spacers ITS1 and ITS2, isolate 039	774	774	99%	0.0	92%	
AJ224110.1	Glycine dolichocarpa DNA for internal transcribed spacer, isolate 038	774	774	99%	0.0	92%	
AY433915.1	Glycine tomentella specimen-voucher CSIRO G2469 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 26S ribosomal RNA gene, partial sequence	773	773	99%	0.0	92%	
AY433856.1	Glycine tomentella specimen-voucher CSIRO G1427 18S ribosomal RNA gene,	773	773	100%	0.0	92%	

Accession	Description	Max score	Total score	Query coverage	E value	Max ident	Links
	partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 26S ribosomal RNA gene, partial sequence						
AY433855.1	Glycine tomentella specimen-voucher CSIRO G1367 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 26S ribosomal RNA gene, partial sequence	773	773	100%	0.0	92%	
AY433854.1	Glycine tomentella specimen-voucher CSIRO G1361 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 26S ribosomal RNA gene, partial sequence	773	773	100%	0.0	92%	
AY433853.1	Glycine tomentella specimen-voucher CSIRO G1274 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 26S ribosomal RNA gene, partial sequence	773	773	100%	0.0	92%	
AY433852.1	Glycine tomentella specimen-voucher CSIRO G1136 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 26S ribosomal RNA gene, partial sequence	773	773	100%	0.0	92%	
AY433851.1	Glycine tomentella specimen-voucher CSIRO G1136 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 26S ribosomal RNA gene, partial sequence	773	773	100%	0.0	92%	
AY168318.1	Glycine tomentella isolate G1180-SV12 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene and internal transcribed spacer 2, complete sequence; and 26S ribosomal RNA gene, partial sequence	773	773	100%	0.0	92%	
AY168317.1	Glycine tomentella isolate G1763-HR3 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene and internal transcribed spacer 2, complete sequence; and 26S ribosomal RNA gene, partial sequence	773	773	100%	0.0	92%	
AY168316.1	Glycine tomentella isolate G1288-C3 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene and internal transcribed spacer 2, complete sequence; and 26S ribosomal RNA gene, partial sequence	773	773	100%	0.0	92%	
AF467484.1	Ophrestia radicosa small subunit ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene and internal transcribed spacer 2,	773	773	100%	0.0	92%	

Accession	Description	Max score	Total score	Query coverage	E value	Max ident	Links
	complete sequence; and large subunit ribosomal RNA gene, partial sequence						
AY433932.1	Glycine hirticaulis specimen-voucher CSIRO G2591 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 26S ribosomal RNA gene, partial sequence	771	771	99%	0.0	92%	
AY433905.1	Glycine tomentella specimen-voucher CSIRO G1946 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 26S ribosomal RNA gene, partial sequence	771	771	99%	0.0	92%	
AY433902.1	Glycine tomentella specimen-voucher CSIRO G2576 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 26S ribosomal RNA gene, partial sequence	771	771	99%	0.0	92%	
AY433900.1	Glycine tomentella specimen-voucher CSIRO G3119 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 26S ribosomal RNA gene, partial sequence	771	771	99%	0.0	92%	
AY433899.1	Glycine tomentella specimen-voucher CSIRO G3101 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 26S ribosomal RNA gene, partial sequence	771	771	99%	0.0	92%	
AY433898.1	Glycine tomentella specimen-voucher CSIRO G1397 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 26S ribosomal RNA gene, partial sequence	771	771	99%	0.0	92%	
AY168315.1	Glycine tomentella isolate G1403-OF05 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene and internal transcribed spacer 2, complete sequence; and 26S ribosomal RNA gene, partial sequence	771	771	92%	0.0	94%	
U60535.1	Glycine argyrea small subunit ribosomal RNA gene, partial sequence, internal transcribed spacer 1, 5.8S ribosomal RNA gene and internal transcribed spacer 2, complete sequence, and large subunit ribosomal RNA gene, partial sequence	771	771	99%	0.0	92%	
AY168314.1	Glycine tomentella isolate G1739-CO3 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene, complete sequence; and internal transcribed spacer 2, partial sequence	769	769	97%	0.0	92%	

Accession	Description	Max score	Total score	Query coverage	E value	Max ident	Links
AY168313.1	Glycine tomentella isolate G1487-CO1 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene, complete sequence; and internal transcribed spacer 2, partial sequence	769	769	97%	0.0	92%	
AY168312.1	Glycine tomentella isolate G1969-CO5 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene, complete sequence; and internal transcribed spacer 2, partial sequence	769	769	97%	0.0	92%	
EF517915.1	Glycine canescens voucher PGGC 1120 (CSIRO) 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence	767	767	99%	0.0	91%	
AY433867.1	Glycine tomentella specimen-voucher CSIRO G2539 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 26S ribosomal RNA gene, partial sequence	767	767	100%	0.0	91%	
AY433866.1	Glycine tomentella specimen-voucher CSIRO G2100 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 26S ribosomal RNA gene, partial sequence	767	767	100%	0.0	91%	
AY433865.1	Glycine tomentella specimen-voucher CSIRO G2099 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 26S ribosomal RNA gene, partial sequence	767	767	100%	0.0	91%	
AY433864.1	Glycine tomentella specimen-voucher CSIRO G2059 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 26S ribosomal RNA gene, partial sequence	767	767	100%	0.0	91%	
AY433863.1	Glycine tomentella specimen-voucher CSIRO G1766 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 26S ribosomal RNA gene, partial sequence	767	767	100%	0.0	91%	
AY433862.1	Glycine tomentella specimen-voucher CSIRO G1930 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 26S ribosomal RNA gene, partial sequence	767	767	100%	0.0	91%	

Accession	**Description**	**Max score**	**Total score**	**Query coverage**	**E value**	**Max ident**	**Links**
AY433861.1	Glycine tomentella specimen-voucher CSIRO G1394 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 26S ribosomal RNA gene, partial sequence	767	767	100%	0.0	91%	
AY433848.1	Glycine tomentella specimen-voucher CSIRO G1820 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 26S ribosomal RNA gene, partial sequence	767	767	100%	0.0	91%	
AY433847.1	Glycine tomentella specimen-voucher CSIRO G1380 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 26S ribosomal RNA gene, partial sequence	767	767	100%	0.0	91%	
AY433846.1	Glycine tomentella specimen-voucher CSIRO G1366 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 26S ribosomal RNA gene, partial sequence	767	767	100%	0.0	91%	
AY433845.1	Glycine tomentella specimen-voucher CSIRO G1364 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 26S ribosomal RNA gene, partial sequence	767	767	100%	0.0	91%	
AJ009789.1	Glycine tabacina 5.8S rRNA gene and internal transcribed spacer, isolate 016, partial	767	767	99%	0.0	92%	
AY433893.1	Glycine tomentella specimen-voucher CSIRO G2058 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 26S ribosomal RNA gene, partial sequence	763	763	100%	0.0	91%	
AY433844.1	Glycine tomentella specimen-voucher CSIRO G1945 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene, complete sequence; and internal transcribed spacer 2, partial sequence	763	763	97%	0.0	92%	