Introgression of high protein and pest resistance genes from inter-specific hybrids of *Manihot esculenta* ssp *flabellifolia* into cassava *(Manihot esculenta* Crantz*)*

By

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Declaration

"I declare that the dissertation hereby submitted by me for the degree of Philosophiae Doctor in Agriculture at the University of the Free State is my own independent work and has not been previously submitted to another Faculty/University.

I furthermore cede copyright of the dissertation in favour of the University of the Free State"

Olalekan Abiodun **Akinbo**

10/10/08
Date

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11.2 Akinbo, O., M.T. Labuschagne and M. Fregene (2008). Quantitative trait loci (QTL) mapping of protein content in backcross derivatives of inter-specific hybrids from *M. esculenta* ssp *flabellifolia* and cassava (*Manihot esculenta* Crantz) (abstract/poster) Plant and Animal Genome XV. San Diego, California, USA. Pp150.

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Chapter 1

General introduction

Cassava is a perennial woody shrub, grown as an annual mainly for its starchy roots. It is a cheap source of carbohydrates for human populations in the humid tropics (Nweke *et al.*, 1994; Henry and Hershey, 2002; Hillocks, 2002; Onwueme, 2002). It is the staple food for over 500 million people in western and central Africa (Nweke, 1996; FAO, 1996; 2005; Egesi *et al*., 2007a) with an average consumption of approximately 500 cal/day (Iglesias *et al*., 1997). Originally domesticated in Brazil, cassava was carried to Africa and Asia by Portuguese traders from the Americas (Ross, 1975; Cock, 1982; 1985; Charrier and Lefevre, 1987). In 2006, annual world production was estimated at 208 million tons (FAO, 2006).

The largest producer of cassava world-wide is Nigeria, followed by Brazil, Thailand, Zaire, and Indonesia (Phillip *et al.*, 2005; FAO, 2006). Production in Africa and Asia continues to increase, while that in Latin America has remained relatively constant over the past 30 years. The total area harvested in 2005 was about 16 million hectares with 60%, 24% and 16% in Africa, Asia, and Latin America respectively (FAO, 2006). The storage roots are rich in carbohydrates (>85%) but poor in protein (2% - 3%, dry weight basis); the leaves are consumed as a green vegetable in many parts of Africa, providing protein, minerals and vitamins (Hahn, 1989). Due to its resilience to drought, cassava cultivation has expanded into marginal environments, particularly in regions with poor soils and lengthy dry seasons (El-Sharkawy, 1993; Aina *et al*., 2007a). It is used as a famine reserve crop in most parts of sub Saharan Africa (Charrier and Lefevre, 1987). Approximately 71% of world cassava production is utilised for human consumption, while the rest is for animal feed and industrial uses (Sarma and Kunchai, 1991; Ceballos *et al.*, 2008).

The potential to increase cassava yields through genetic improvement has been demonstrated successfully (Hahn *et al*., 1980b; IITA, 1982; 1990; Balyejusa Kizito *et al*., 2005; Okogbenin *et al*., 2007; Dixon *et al*., 2008). However, despite achievements in cassava improvement, many challenges remain. They include the low protein content of the major staple crop of some of the poorest populations in the world, presence of toxic cyanogenic glucosides in cassava, biotic stresses, and the need to tailor cassava to the myriads of agro-ecosystems where it is produced (Fregene *et al.,* 2007).

Low protein content in the roots of cassava has been a major factor for this unfavourable competition with other staples like potato, rice, soybean and cowpea in food and feed. Root protein content ranges between 2% - 3% (dry weight basis). In spite of this, the quality of this protein is fairly good, as is the proportion of amino acids. Methionine and lysine are, however, limiting amino acids in the root (Fregene *et al.,* 2006). If varieties can be developed with a higher quantity of protein and these amino acids, it would enhance the value of cassava as a food and/or feed. Only about 60% of the total nitrogen in cassava roots is derived from amino acids and about 1% of it is in the form of nitrates and hydrocyanic acid. The remaining 38% - 40% of the total nitrogen remains unidentified (Diasolua *et al*., 2002; 2003; Nassar, 2007).

Cassava protein is comparable to rice protein in digestibility. The crude protein content of roots appears to be relatively stable and constant with maturity of the plant. According to Close *et al*. (1953), the protein of processed cassava includes the highest percentage of glutamic acid and the lowest of methionine (1%). Sreermamurthy (1945) reported total absence of methionine whereas Osuntokun *et al.* (1968) reported that both cystine and cysteine are involved in cyanide detoxification. Cyanide is produced when the glycoside linamarine is hydrolysed by linamarinase (Ernesto *et al.,* 2002).

Several accessions of *Manihot esculenta* ssp *flabellifolia*, *M. esculenta* ssp *peruviana* and *M. tristis* collected in Brazil were found to have high protein content, between 10% - 18% (dry weight basis), in the storage roots (CIAT, 2003). Nassar (2000; 2007) reported that an inter-

specific hybrid had 10 times more lysine and three times more methionine than the common cassava cultivar. The genetic variability and quantity of the amino acid profile indicated the feasibility of selecting inter-specific hybrids that are rich in both crude protein and amino acids. The use of wild relatives in regular breeding programmes is complicated by the long reproductive breeding cycle of cassava, high genetic load that is released on backcrossing, and linkage drag associated with the use of wild relatives in crop improvement. A project was initiated at CIAT to accelerate the process of introgressing useful genes from wild relatives into cassava via a modified advanced back cross quantitative trait loci (ABC-QTL) (Tanksley and Nelson, 1996) breeding scheme.

Another nutritional handicap of cassava is the accumulation of cyanogenic glucosides in the roots. Cassava is well known for the presence of free and bound cyanogenic glucosides, linamarin and lotaustralin. They are converted to hydrogen cyanide (HCN) in the presence of linamarase, a naturally occurring enzyme in cassava. Linamarase acts on the glucosides when the cells are ruptured (Carlsson *et al.,* 1999; Ernesto *et al.,* 2002; Nassar *et al.,* 2008). All plant parts contain cyanogenic glucosides with the leaves having the highest concentrations. In the roots, the peel has a higher concentration than the flesh. In the past, cassava was categorised as either sweet or bitter, signifying the absence or presence of toxic levels of cyanogenic glucosides (Nassar and Marques, 2006). Sweet cultivars can produce as little as 20 mg of HCN per kilogram of fresh roots, while bitter ones may produce more than 50 times as much. The bitterness is identified through taste and smell. This is not a totally valid system, since sweetness is not absolutely correlated with HCN producing ability. In cases of human malnutrition, where the diet lacks protein and iodine, under-processed roots of high HCN cultivars may result in serious health problems (Phuc *et al.,* 2000; Nassar and Ortiz, 2007).

Cyanogens alone cannot be blamed for toxicity because other cyanogenic crops, such as sorghum and Lathyrus beans, which are widely used as food, cause few toxicity problems. But the protein contents of these two crops (11.0% - 18.7%, respectively) are higher. Many cassava products contain low amounts of cyanogens which can be efficiently eliminated by

the body if the protein intake is adequate (Bellotti and Riis, 1994; Dixon *et al*., 1994a; Siritunga *et al.*, 2004). However, the level of protein in cassava is far less than the levels found in rice, wheat, and tuber crops. If protein intake is more than adequate for both general metabolic requirements and cyanide elimination, toxic effects are lessened or even eliminated, even if cassava is improperly processed. Hence, the lack of protein in cassava roots is probably responsible for most non-fatal cases of cyanide poisoning associated with cassava (CGIAR, 1996; Siritunga *et al.*, 2004).

Biotic stress constitutes the principal production constraint in Africa and Latin America. Whiteflies in particular are considered one of cassava's major pests due to its role as vector for viruses that cause major diseases in cassava and due to direct damage. Host plant resistance to whiteflies is rare in cultivated crops but known in cassava (Bellotti and Arias, 2001). The largest complex of whiteflies on cassava is found in the Neotropics (Farias, 1994; Bellotti *et al*., 1999). The species *Bemisia tabaci* (Bellotti *et al*., 1999) is the vector of the most important production constraint in Africa, cassava mosaic disease (CMD). CMD is caused by several geminiviruses (Thresh *et al*., 1994; Wool *et al*., 1994; Akano *et al*., 2002; Ariyo *et al.*, 2002; Egesi *et al.*, 2007b) and causes yield losses of 20% - 100%.

Lawson (1988) noted that cassava genotypes find optimum physiological expression of their genetic potential within narrow ranges of biophysical conditions. Cock (1987) found that few cassava cultivars were stable over a wide range of ecological conditions. There exists growing consensus that stable productivity in cassava depends on a number of factors acting synergistically: abiotic factors (soils, temperature, photoperiod, and latitude), biotic elements (diseases, pests, and nematodes) and management practices (Allem and Hahn, 1991; Ariyo *et al.*, 2002; 2004). Given that cassava is produced principally by small holder farmers who rarely use inputs, there is a need to tailor cassava to production niches through breeding.

Cassava has a long growth cycle, low seed set, and is allogamous in nature, with a complex genetic structure. Cassava breeding is therefore considerably slowed down by the biology of the crop (Kawano *et al.*, 1998; Fregene *et al*., 2001a). Most agronomically important

characteristics such as yield and quality traits are inherited quantitatively (Zhuang *et al*., 1997). The joint influence of quantitative loci and the environment produces complex phenotypes (Geldermann, 1975). For most quantitative traits, little is known about the number, chromosomal position, action or individual and interactive effects of genes controlling their expression. If quantitative traits could be resolved into their individual genetic components, it might be possible to breed for these characters with the efficiency of dealing with single gene traits (Tanksley *et al*., 1989).

A major advance in unravelling the genetics of quantitative traits came with the discovery of DNA-based markers. Molecular markers have the potential of detecting higher levels of polymorphism, as genetic variation is surveyed directly at DNA level. Alleles of genes controlling virtually all traits can be tracked in segregating populations using genetically linked molecular markers, thereby dissecting genes controlling complex traits (Hayes *et al*., 1993). DNA markers have thus provided breeders with new tools to understand and more efficiently select for complex traits in breeding programmes (Akinbo *et al*., 2007; 2008).

Linkage maps have been constructed for many crops including potato (Bonierbale *et al*., 1988), barley (Bezant *et al*., 1996), sugarcane (Al-Janabi *et al*., 1993), and rice (Lin *et al*., 1996). Genetic maps have been published for relatively less researched crops which are, however, of great interest in the tropics such as plantain (Gawel and Jarret, 1991), groundnut (Kochert *et al*., 1991), cowpea (Fatokun *et al*., 1992; 1993) and cassava (Fregene *et al*., 1997; 2000; 2001b; Jorge *et al*., 2000; 2001; Mba *et al*., 2001; Akano *et al*., 2002; Okogbenin and Fregene, 2002; 2003; Okogbenin *et al*., 2006; Lokko *et al*., 2005; Akinbo *et al*., 2007; 2008).

These genetic maps provide opportunities for tagging genes and thereby improving the efficiency, precision and cost effectiveness in breeding traits of agronomic importance (Okogbenin *et al.,* 2008). The integration of these techniques into plant breeding promises to expedite the movement of genes among varieties, as well as the transfer of genes from wild progenitors. It will aid the analysis of complex polygenic characters as assemblages of single Mendelian factors (Villamon *et al.,* 2005).

The objectives of this study were to:

i Study the influence of the environment on the expression of protein content in the B_1P_2 population.

ii Study the influence of whitefly infestation on high protein clones.

iii Construct a linkage map of cassava using simple sequence repeat (SSR) markers in a backcross (B_1P_2) population derived from crossing an inter-specific hybrid of *M. esculenta* ssp *flabellifolia* with an elite cassava variety.

iv Identify QTL controlling protein content in the root, root yield, root quality traits, and pest resistance characters in first backcross derivatives of *M. esculenta* ssp *flabellifolia* using SSR markers from the linkage map and phenotypic measurements of the afore-mentioned traits.

v Determine the effects of each QTL in the B_1P_2 population.

Chapter 2

Literature review

2.1 The genus *Manihot* and cassava

Cassava is a member of the *Euphorbiaceae*, subfamily Crotonoideae, the tribe *Manihotae*, and the genus *Manihot.* The genus comprises of 98 species and is believed to have arisen and diversified recently. This argument is supported by the lack of variability in chromosome number, low levels of divergence in floral morphology (Rogers and Appan, 1973), DNA sequence data (Schaal *et al*., 1994), and by inter-fertility between morphologically divergent species in artificial crosses (Fregene *et al*., 1994; Roa *et al*., 1997). The species of the genus range from trees to shrubs and perennial herbaceous plants with a woody rootstock known for the production of latex and cyanogenic glucosides (Rogers and Fleming, 1973; Bailey, 1976; Fregene *et al.,* 2006). The species are grouped into 19 taxonomic sections (Rogers and Appan, 1973; Nassar, 2000).

Cassava is the only widely cultivated species of the genus *Manihot* and has been formally studied since 1886, when Alphonese de Candolle placed its geographic origin in the lowland tropical Americas (Smith, 1968). Following de Candolle, Vavilov (1951) considered north-eastern Brazil to be the most likely area of origin. Vavilov's consideration was based upon the fact that the largest numbers of cultivated variants are found in this area. Cassava shares the Brazilian-Paraguayan centre of origin with groundnuts, cacao, rubber, and other crops (Vavilov, 1992). Rogers (1963) identified two geographic centres of speciation: (i) the drier areas of western and southern Mexico and portions of Guatemala, and (ii) the dry north-eastern portions of Brazil. Nassar (1978a; b) identified four areas of diversity of the wild species: (i) central Brazil, (ii) north-eastern Brazil, (iii) south-western Mexico, and (4) western Mato Grosso (Brazil) and Bolivia.

Older domestication hypotheses of cassava have envisioned the crop to be a "compilospecies" derived from one or more species complexes, either in Mexico or Central America (Rogers, 1965; Rogers and Appan, 1973) or throughout the Neotropics (Rogers, 1963; Ugent *et al*., 1986; Sauer, 1993). But it is generally accepted now that cassava was domesticated from accessions of the wild *Manihot* species *M. esculenta* ssp *flabellifolia*, based on the close morphology and shared geographical distribution of both species in Brazil (Allem, 1987; 1994). Later studies based on DNA sequence and SSR marker data revealed that genetic variation found in cassava is a sub-set of that found in its putative progenitor (Olsen and Schaal 1999; 2001). This pattern of reduced genetic diversity with domestication seems to be the rule for crop-wild relative systems (Gepts, 1993; Tanksley and McCouch, 1997) and presumably reflects genetic drift over the course of domestication (Ladizinsky, 1985; Schaal and Olsen, 2000; Olsen and Schaal, 2001).

Although cassava is interfertile with subspecies *flabellifolia* (Roa *et al*., 1997), no evidence has been found to reflect introgression from the crop after domestication. Cassava was possibly domesticated in America between 5000 and 7000 BC (Lathrap, 1970). Sauer (1952) proposed the heart of domestication as north-western South America. Wild populations of *M. esculenta* occur primarily in west central Brazil and eastern Peru (Allem, 1994). All wild populations of this species are classified as *M. esculenta* ssp *flabellifolia* (Pohl) Ciferri (Roa *et al.*, 1997) or ssp *peruviana*. Collections of accessions of *M. esculenta* ssp *flabellifolia* have been conducted in Brazil and are kept *in vitro* or as seed at CIAT or Empresa Brasileira de Pesquisa Agropecuária (EMBRAPA).

Cassava was carried by the Arawak tribes of central Brazil to the Caribbean islands and central America in the 11th century (Brucher, 1989), and by the Portuguese to the west coast of Africa, via the Bight of Benin and the Congo River at the end of the 16th century (Jones, 1959). The crop reached the east coast of Africa via the islands of Reunion, Madagascar, and Zanzibar at the end of the 18th century (Barnes, 1975; Jennings, 1976) and arrived in India about 1800. The Spaniards took it to the Pacific, but it was not widely used as a food crop until the 1960s (Jennings, 1976). Gulick *et al*. (1983) have defined primary, secondary, and

tertiary levels of diversity for *M. esculenta* in modern times. Important secondary diversity lies in Africa outside the crop's centre of origin (Lefevre and Charrier, 1993; Fregene *et al.*, 2000; 2003; Hurtado *et al.*, 2008).

2.2 Broadening the genetic base of cassava using wild *Manihot* species

Wild *Manihot* species have been reported to be of potential benefit to cassava improvement (Rogers and Appan, 1973; Bryne, 1984; Asiedu *et al*., 1994; Nassar, 2000; Fregene *et al*., 2006). Evaluation of collections of *M. esculenta* ssp *flabellifolia* revealed resistance to important pests such as whiteflies, cassava green mites (CGM), and cassava mealybug (CM) (CIAT, 2003; 2005). High protein content has been identified in the roots of some accessions of the same species. Resistance to cassava mosaic disease (CMD) have been recovered in 4th backcross derivatives of *M. glaziovii* (Hahn, 1989) and delayed post harvest deterioration have been found in an accession of *M. walkerae* (Fregene *et al*., 2006).

Successful crosses have been made between *M. glaziovii* and *M. esculenta* and hybrids from these crosses produced viable seeds (Nichols, 1947; Jennings, 1957). *Manihot mellanobasis* x *M. esculenta* crosses have been successfully made (Jennings, 1959). For many years, the International Institute of Tropical Agriculture (IITA) carried out inter-specific hybridisation with *Manihot* species to investigate crossability barriers and other inter-genomic interactions among species (Hahn *et al*., 1990). Hybrids involved crosses between cassava clones and wild *Manihot* species such as *M. epruinosa, M. chloristicta, M. glaziovii, M. leptophylla*, and *M. brachyandra.* The most significant result was the isolation of polyploids from the early hybrids involving *M. glaziovii* and *M. epruinosa* as a result of production of unreduced gametes by one or both parents (Asiedu *et al.,* 1989*;* Hahn *et al.,* 1990).

2.3 Genetic variation of wild *Manihot* species for tuber formation and protein content

Thirty wild *Manihot* species collected from south and central America were examined for storage root formation and root protein content (Nassar, 1978a; b; 2000). It was reported that among these wild species, four species formed storage roots (*M. oligantha* ssp *nestili, M. tripartite, M. anomala* and *M. zehntneri).* Protein content of these species ranged from 3.06% - 7.10% on a dry weight basis. However, high percentages of protein occur in wild species, up to 18% dry weight basis (CIAT, 2003; Fregene *et al.*, 2006, 2007) and cultivated cassava as high as 7% or 8% (dry weight basis) in some cassava cultivars (Ceballos *et al.*, 2006).

According to Bolhuis (1953), cyanide storage in the root strongly influences the storage of protein. However, many reports state that crude protein content ranges from 2.2% in sweet to 2.7% in bitter cultivars (Anonymous, 1968; Rogers and Appan, 1973; Hajjar and Hodgkin, 2007). Nassar (2000) reported that since estimation of protein was based on total nitrogen, it must be viewed with caution, because it is not certain whether the breakdown products of cyanogenic glucosides enhance the total nitrogen content or not. Nartey (1968) showed that the hydrolytic products of glucosides are incorporated into amino acids for protein synthesis in cassava.

Two other wild Manihot species have been reported to have high protein content: *M. melanobasis* (Jennings, 1959) and *M. saxicola* (Lanjouw, 1939), but as there is no reference to their HCN content, it is not possible to say to what extent crude protein estimates were affected by hydrolytic products of glucosides. It seems logical to find wild cassava with high protein content, since selection for cultivation has aimed at increased tuber size without paying attention to protein content (Nassar, 2000). This could have led to the discard of protein-producing genes from cultivated varieties (Ceballos *et al*., 2006). More recently, several accessions of *M. esculenta* ssp *flabellifolia, M. esculenta* ssp *peruviana* and *M. tristis* collected in Brazil were found to have high protein content, between 10% -18% (dry weight basis) in storage roots (CIAT, 2003).

2.4 Characteristics of cassava

Manihot esculenta is a shruby perennial species that produces storage roots. Stems are either non-branching (slender and up to 4.5 m tall) or branched (from intermediate to highly branching patterns of no more than 1.5 m in height). Stems of the species are woody, usually with large pith and therefore brittle. The fully developed vegetative leaves have five to nine lobes, but the leaves found in association with the inflorescence are almost invariably reduced in number of lobes (most frequently three lobed but with occasional occurrences of an undivided simple leaf) (Rogers, 1965).

Pigmentation of the stems provides one of the most stable characteristics for differentiation of cultivars. One group of cultivars has light grey stems with a silvery aspect, due in part to the granular, waxy surface, whereas another group has varying amounts of anthocyanins, causing the stems to be yellow, orange, or brown. The application of a group of 53 morphological descriptors proposed by the International Plant Genetic Resources Institute (IPGRI) (Gulick *et al.,* 1983) has resulted in a non-anatomical model for characterisation of cassava genotypes.

Cassava is monoecious and predominantly out-crossing (Fregene *et al.*, 1997). Outcrossing is mediated by protogyny, and results in a high level of heterozygosity (Bryne, 1984; Hershey and Jennings, 1992). Cassava has few large basal pistillate and numerous smaller apical staminate flowers borne on the same inflorescence (Rogers, 1965). As flowering is always associated with branching, an early branching genotype may start flowering as early as three months after planting while non-branching types do not flower (Hahn *et al*., 1973; Conceicao, 1979). Based on the flowering habit, cassava varieties are classified as non-flowering, poor flowering, moderate flowering, profuse flowering with poor fruit setting and profuse flowering with high fruit setting (Indira *et al*., 1977).

Pistillate flowers have five petals and an ovary with three loci, each of which produces one seed (Rogers, 1965). Staminate flowers have ten stamens arranged in two rings of five and do

not initiate opening until after the last female flower of the inflorescence has bloomed (Rogers, 1965). One male flower produces about 1600 pollen grains of which only 50% are viable (Graner, 1942). *Manihot esculenta* is pollinated by insects (Rogers, 1965) but prolific production of readily disseminated pollen grains suggests that wind may be an important pollinating agent (Bueno, 1987). Profuse secretion of nectar attracts several insects, specifically bees, which are pollen disseminators. Although cassava is regarded as an allogamous species, considerable selfing may occur, especially in profusely flowering genotypes (Kawano *et al*., 1978). The fruit is a dehiscent capsule with three locules. Each locule contains a single carunculate seed. Most of the cultivars bear a relatively small number of fruits per plant as contrasted with the wild species (Rogers, 1965; Pujol *et al.,* 2005).

2.5 Importance of cassava

World cassava production grew at an annual rate of 2.2% from 1984 - 1994, the same rate as in the previous decade, reaching 164 million tonnes in 1997 (FAO and IFAD, 2000). Cassava production is expected to continue growing at almost the same rate, but this time because of yield increases (Rosegrant *et al.,* 2001; Phillip *et al.,* 2005).

World-wide, cassava has entered the modern market economy and there is growing demand for its use in processed food and feed products (Henry and Best, 1994; Jaramillo *et al*., 2005; Ceballos *et al.,* 2008). Owing to the diversity of its utilisation, adaptation and low input requirements, cassava often provides a valuable link for rural farmers to the market economy (Henry and Best, 1994). Their development is sensitive to both domestic and foreign trade policies and competes with alternative raw materials such as grains and sugarcane (Leihner, 1992; Henry and Gottret, 1995; Rosegrant, 2008).

Packaged cassava and cassava flour are gaining greater acceptance in some markets (Hershey and Henry, 1997). One of the potential outlets for cassava is the starch market. According to the International Starch Institute, cassava starch production has grown globally between 1980 and 1997, from 16 - 35 million tonnes (FAO and IFAD, 2000). Thailand and Indonesia are

the major suppliers of cassava to the world market, contributing some 80% and 10% total trade respectively, while the remainder is provided by small exporters in Africa, Asia, and Latin America (FAO and IFAD, 2000).

2.6 Cassava breeding

Cassava is one of the "orphan crops", so called because of limited investment in research, despite its importance as a major crop that feeds humankind (Fregene *et al.*, 2001a). Relatively little is known about its genetics. Given that in a cross fertilised species, inbreeding is deleterious (Kawano *et al*., 1978) and heterozygosity is largely essential for the maintenance of vigour, any breeding method should seek to maintain heterozygosity and take into account both additive and non-additive genetic variance (Bryne, 1984). The breeding process involves the choice of parental genotypes, sexual recombination and a multi-stage offspring selection that can last for 6 - 10 years aimed at genetic improvement of the crop by an accumulation of beneficial alleles and elimination of detrimental alleles (Kawano, 1998).

High frequencies of genes for specific desirable characteristics, including yield components, root quality, disease and pest resistance, tolerance to soil and climatic stresses, and stability of production across environments are progressively accumulated through recurrent selection (Hahn *et al*., 1980a; CIAT, 1981; 2002). Recurrent selection combined with a broad genetic base has been reported to be the most efficient procedure for improving cassava base populations (Hahn, 1978; CIAT, 1982; 2002; Bryne, 1984; Fregene *et al.,* 2007). For efficient recombination, good management of flowering is required (CIAT, 1981). Progenies resulting from each recombination cycle are evaluated and selections recombined again to form a new population. A conservative time-frame for developing an improved cultivar is between 8 - 10 years (Dixon *et al*., 2008).

Hybridisation in cassava is widely used in breeding programmes for the creation of genetic variability. Each hybrid seed is potentially a new cultivar (Bueno, 1987). Hybridisation involves hand and open pollination (Bryne, 1984). The success of this method depends

primarily on the choice of adequate parents and on the selection methods used (Kawano, 1980). This should be complemented by evaluation of the combining ability of the best genotypes (Hahn *et al*., 1979; Losado Valle, 1990; Morante *et al*., 2005).

A good selection site should include as many physical and biological constraints as possible, so that the final selection may have a chance of being widely adapted and adopted (Hahn *et al*., 1980a; Lozano *et al*., 1984; Egesi *et al.,* 2007a). In each selection site, the best genotypes with durable resistance or tolerance to most biotic and abiotic constraints are evaluated for several growing cycles and those which prove superior, are utilised in crosses (Hershey, 1984; Egesi *et al.,* 2007a). Often clones give variable results when grown in places other than the original selection sites, due to the strong genotype x environment (G X E) interaction found in cassava (Lozano *et al.*, 1980; Kawano, 1998; Aina *et al.*, 2007b; Egesi *et al.*, 2007a). Where sites have moderate to high stress conditions, sprouting could be low, with slow plant development and a delayed yield formation. Such a situation could impede efficient selection (Hershey, 1984).

Once agronomically acceptable gene-pools with adequate genetic bases are available for a target production area, additional desirable traits may be introduced by a modified backcrossing scheme, using different members of the adapted gene-pool as recurrent parents to avoid problems of inbreeding depression (Martin, 1976; Bueno, 1987). Significant progress has been made in breeding for pest and disease resistance, improved yield, and other agronomic and quality characteristics (Dixon *et al.*, 1995; Fokunang, 1995; Nukenine, 1995; Mahungu *et al*., 1996; Okogbenin *et al*., 2007; Dixon *et al*., 2008). Through the use of improved cultivars, cassava farmers in Africa, particularly in Nigeria, can obtain yields that are up to five times those of many CMD susceptible cultivars under severe disease pressure (IITA, 1990; Ogbe *et al*., 2006; Egesi *et al*., 2007b; Okogbenin *et al*., 2007). Root quality characteristics that are often considered in breeding schemes include cyanogenic potential (CNp), starch quality, protein content, and dry matter content (Mahungu, 1987; Ceballos *et al*., 2004; Jaramillo *et al*., 2005; Balyejusa Kizito *et al.,* 2007).

Attempts have been made to improve the protein content of cassava roots through conventional breeding methods involving hybridisation at inter-specific levels as well as by induced polyploidy and mutation (Mahungu, 1987; Asiedu *et al*., 1989; 1992). Screening of a large germplasm collection of about 1400 entries showed no significant variability in protein content (Hrishi and Jos, 1977). Jos *et al*. (1972) compared the protein content of the diploid and tetraploid plants and found that the average crude protein in the tetraploid was 42.3% higher than in the diploid. Chávez *et al*. (2005) reported that there was no correlation between dry matter content and protein content in the root but a weak positive correlation ($p = 0.14$) was observed between nitrogen and HCN contents in the roots.

Backcrossing, followed by selection (Hahn *et al.*, 1977; Albuquerque, 2007; Garzon *et al.*, 2008) has been used extensively to introduce new sources of pest or disease resistance from related *Manihot* species. Three backcrosses to cassava and further recombination was used to introgress cassava mosaic disease resistance from *M. glaziovii*. The backcross method has been the most common procedure used to incorporate CMD resistance into cultivated cassava (Singh and Hahn, 1982; Thresh and Cooter, 2005). Resistance to CMD is under quantitative genetic control (Doughty, 1958; Jennings, 1970; Hahn and Howland, 1972). The resistance appeared to be additive in nature with about 60% heritability (Hahn *et al*., 1977). Hahn *et al*. (1974) earlier reported that the resistance was recessive. Recently, dominant major genes involved in CMD resistance have been identified in landraces with high levels of resistance to the virus in Africa (Akano *et a1*., 2002).

Hahn *et al*. (1980b) noted a significant genotypic correlation between cassava bacterial blight (CBB) and CBD ($r = 0.90$), apparently due to introgression of blocks of genes from the wild relative.

Many scientists have implied that begomovirus resistance is largely unavailable for most susceptible crops, probably in reference to the difficulty in identifying ‘immune’ plant genotype (Morales, 2001). Despite the unavailability of immune cultivars observed for the majority of commercial crops affected by *Bemisia tabaci* that transmitted geminiviruses,

breeding for disease resistance has proven the most complementary and sustainable of the integrated whitefly control methods implemented to date (Morales, 2001; Bellotti and Arias, 2001). Undoubtedly, there are both direct and circumstantial evidence indicating the existence of adequate genetic variability in the primary and secondary gene pools of most cultivated species. This genetic variability can be exploited within and between cultivated species and their wild relatives (Debouck, 1991).

Root rot disease of cassava is an emerging problem in cassava growing regions of the world where cassava accounts for approximately one third of the total staple food production (FAO, 1993). The disease is caused by different root rot fungi, and has been reported to cause yield losses of up to 80% (Msikita *et al.,* 2005). As the rot pathogens affect the underground tuberous roots of cassava, the magnitude of the damage cannot be quantified until harvest (Onyeka *et al.,* 2005). The nature and effects of the disease are poorly understood by farmers and the disease remains a pressing concern in cassava growing regions (Onyeka *et al.,* 2005). Genetic improvement and search for varieties that are resistant to the various pests and diseases of cassava have formed the main focus of cassava research in the last decades (Ceballos *et al.,* 2004; Onyeka *et al.,* 2004; Bandyopadhy *et al.,* 2006). Prior to the mid-1980s, stories about wild genes preventing devastation by pests and diseases were dominated by a handful of crop success stories. The discovery and use of new resistance genes from the wild have steadily increased in crops (Hajjar and Hodgkin, 2007).

2.7 The cassava nuclear genome

The nuclear genome consists of the entire set of chromosomes bound by the nuclear membrane (Liu, 1998). It is distinct from the genomes of cytoplasmic organelles such as mitochondria and plastids (Vedel and Delseny, 1987). Genomes of different organisms vary in terms of total DNA content (genome size), ploidy level, chromosome number, and nature and number of functional genes (Flavell, 1995; Sigareva *et al.,* 2004). Flow cytometry measurements of nuclear DNA in cassava have revealed a diploid DNA content of 1.67 pg per cell nucleus (Awoleye *et al*., 1994; Woodward and Puonti-Kaerlas, 2004). This value

corresponds to 772 mega-base pairs in the haploid genome and places the cassava genome size at the lower end of the range for higher plants (Bennett *et al.,* 1992). The relatively small size of this genome favours the development of a saturated genetic map and molecular tags which would contribute to the understanding of the inheritance of many important quantitative traits (Fregene *et al*., 1997; Mba *et al.,* 2001; Okogbenin *et al.,* 2006).

2.8 Molecular genetic markers

Molecular genetic markers are defined as differences at the genotype (DNA) level and can be used to answer and explain questions of genetics (Paterson *et al.,* 1991; Okogbenin and Fregene, 2002; 2003; Lokko *et al.,* 2005). To be useful as a genetic marker, the marker locus has to show experimentally detectable variation among individuals (Castelblanco and Fregene, 2006; Sørensen *et al.,* 2008). The variation can be due to single nucleotide polymorphisms or deletions/insertions, or major chromosomal changes. Molecular genetic markers can be used to study the diversity of the observable variation at population or species level (Lee, 1995; Zhang *et al.,* 2008). Molecular genetic markers can be used to map genomes, identify regions of the genome controlling a trait, and follow a segment of interest of the genome in a plant breeding scheme (Berloo *et al.,* 2008; Somta *et al.,* 2008; Okogbenin *et al.,* 2008).

Until the advent of molecular markers, the markers used to develop maps in plants have been those affecting morphological traits (Liu, 1998). Although these morphological markers are of value, their usefulness in mapping studies (Ellis, 1994) is limited by their paucity and nature because they can be influenced by environmental factors. The number of useful morphological markers for quantitative traits was limited, because in most studies only a few markers were used, representing only a small fraction of the genome (Dettori *et al.,* 2001). However, maps based on morphological markers have been developed and a large number of morphological markers have been described for some crop species (Ellis, 1994; Tanksley, 1994; Fregene *et al.,* 2003; Yan *et al*., 2005).

The discovery that allelic forms of enzymes (isozymes or allozymes) can be separated on electrophoretic gels and detected with histochemical activity stains heralded the era of biochemical markers in genetic research (Smithies, 1955; Hunter and Markert, 1957; Xia *et al.,* 2005). Enzyme coding genes could be screened for polymorphism in natural populations and mapped genetically using electrophoretic techniques independent of any phenotypic change (Lewontin and Hubby, 1966). By the early 1980s, isozyme markers were being employed as a general tool for mapping polygenes. These studies met with considerable success compared to previous studies using morphological markers (Tanksley *et al*., 1982; Vallejos and Tanksley, 1983; Edwards *et al*., 1987; Weller *et al*., 1988; Yan *et al.,* 2005).

The genome coverage situation improved with isozyme markers, but the number of available enzyme activity stains limited the number of markers (Liu, 1998). Consequently, informative isozyme markers were not enough to cover an entire genome (Tanksley *et al*., 1982; Vallejos and Tanksley, 1983; Edwards *et al.,* 1987). However, the paucity of isozyme loci and the fact that they are subjected to post-translational modifications often restricted their utility (Staub *et al.,* 1996; Huamán *et al.,* 2000).

The next major advance in the utilisation of molecular markers occurred with the development of DNA-based genetic markers (Lee, 1995). Botstein *et al*. (1980) suggested that large numbers of genetic markers might be found by studying differences in the DNA molecule. In principle, visible markers and isozymes are as useful as DNA markers. In practice, however, much greater numbers of DNA markers can be readily found. Crop plants have about 10^8 to 10^9 nucleotides of DNA in total (Paterson *et al*., 1991; Okogbenin and Fregene, 2003). Even if a small percentage of these is different between two individuals, an enormous number of potential DNA markers result. In contrast, relatively few visible markers or isozymes tend to be polymorphic between two randomly chosen individuals (Stuber, 1994; Staub *et al*., 1996; Mba *et al.,* 2001).

The level of polymorphism maintained at any given locus in natural populations is determined by many factors which include population size, mating habits, selection, mutation

rate and migration (Tanksley, 1994). Two of these factors, viz relaxed selection pressure and higher mutation rates caused allelic variation to be higher at molecular level than at morphological marker level (Huamán *et al.,* 2000).

The availability of complete genome maps, facilitated by DNA markers, opened the opportunity for studying and detecting polygenes (Tanksley, 1994). Thus, the advent of molecular markers has allowed polygene mapping in virtually any segregating population e.g., F_2, F_3, backcross, and recombinant inbreds (Okogbenin *et al.,* 2006; Okogbenin *et al.,* 2008). Because molecular marker loci do not normally exhibit epistatic or pleiotropic effects, a virtually limitless number of segregating markers can be used in a single population for mapping polygenes across an entire genome (Tanksley, 1994).

DNA sequence variations can be monitored using several techniques. One technique monitors variation as changes in the length of DNA fragments produced by restriction endonucleases. This method has, therefore, been termed restriction fragment length polymorphisms (RFLPs) (Groodzicker *et al*., 1974; Botstein *et al*., 1980; Roa *et al.,* 1997). At present, many types of molecular markers with different useful properties have emerged and can be utilised for genetic analysis (Rafalski and Tingey, 1993; Mohan *et al*., 1997; Jorge *et al.,* 2000; Fregene *et al.,* 2000).

These markers provide an unlimited opportunity to obtain detailed information about genetic variation in the nuclear genome at DNA level. The dominant, epistatic, or heterotic interactions between alleles from one or more loci can be estimated (Fatokun *et al*., 1992; Stuber *et al*., 1992; Fregene *et al.,* 1997; Akano *et al.,* 2002; Okogbenin *et al.,* 2006). The shift from genetics based on the inference of genotype from phenotype, as pioneered by Mendel, to genetics based on the direct analysis of DNA sequence variation has been hailed as an important genetic paradigm shift. Genetic maps have been constructed in many crop plants using these markers on a single segregating population (Mohan *et al*., 1997; Fregene *et al*., 1997; 2000; 2001b; Jorge *et al.,* 2000; 2001; Mba *et al*., 2001; Akano *et al*., 2002; Lokko *et al*., 2005; Okogbenin *et al.,* 2006).

2.8.1 Restriction fragment length polymorphism (RFLP)

Among the various molecular markers developed, RFLP were the first to be used in human genome mapping (Botstein *et al*., 1980) and later adopted for plant genome mapping (Weber and Helentjaris, 1989). RFLP are co-dominant and can identify a unique locus (Tanksley *et al.*, 1989). This technique arose from the discovery of restriction enzymes and natural variation in DNA base sequences of organisms (Beckmann and Soller, 1986). Restriction enzymes bind specifically to and cut (or modify) double stranded DNA at short, specific sites within or adjacent to a particular sequence known as the recognition sequence (Botstein *et al*., 1980; Huang *et al.,* 1997; Pallotta *et al.,* 2000).

These enzymes have been classified into three groups, on the basis of their functions, as Type I, Type II, and Type III restriction enzymes. Recognition sites for various enzymes vary from four to eight base pairs in length. Base changes in DNA can alter the sequences that are recognised by restriction enzymes, abolishing sites or creating new sites for particular enzymes (Beckmann and Soller, 1983). This creates an enormous variation in eukaryotic cells. This variation has been exploited with the advent of restriction enzymes, which by nature of their recognition, binding and cleavage properties reduce large segments of DNA to a series of small fragments of distinct sizes (Kochert, 1990). The number of fragments produced reflects the distribution of restriction enzyme recognition sites in the DNA (Bostein *et al.,* 1980).

Digested fragments can be separated on a solid support such as agarose gels. A potential difference (voltage) applied across the gel results in different rates of movement of the DNA fragments depending on their sizes. Movement across the electric field is possible due to the negatively charged nature of DNA (negative charge on the phosphate backbone at normal pH). Separated DNA on the agarose gel is visualized by staining with a dye, ethidium bromide, which fluoresces in the presence of ultra-violet light. Restriction enzyme digests of relatively small genomes such as the chloroplast DNA (cp DNA) and mitochondrial DNA (mtDNA) genomes produces 40-60 fragments that can easily be seen on an agarose gel.

Restriction enzyme digests of nuclear DNA produce millions of discrete DNA fragments in a continuous range of sizes and when subjected to gel electrophoresis and ethidium bromide stains no distinct fragment, but a continuous smear, is visualised. RFLP of nuclear DNA cannot therefore be directly seen (Fregene, 1996).

Based on DNA to DNA hybridisation, a piece of radioactively or chemically labelled chromosomal DNA fragment (probe) is used to detect polymorphisms; by hybridisation to specific fragments in the separated digestion mixture that possess some nucleotide sequence homologous to the probe (Botstein *et al*., 1980). In practice, difficulties can arise if the probe used hybridises to repeated sequences at multiple locations on the genome (Fregene *et al.,* 1997; Jorge *et al.,* 2000; López *et al.,* 2003). Therefore, it is important to use probes that detect single polymorphic loci in different pedigrees (López *et al.,* 2003). Using RFLP markers, genetic maps have been developed in many plant species including rice (Causse *et al*., 1994; Huang *et al.,* 1997), and cassava (Fregene *et al.,* 1997; Jorge *et al.,* 2000; López *et al.,* 2003) amongst many others (Mohan *et al*., 1997; Stein *et al.,* 2007).

However, RFLP analysis is labour-intensive, needs big amounts of DNA, and is time consuming. The newer approaches based on polymerase chain reaction (PCR) are relatively simple. PCR is a DNA synthesis technique that amplifies specific regions of DNA that lie between two sites defined by the complementary sequences of two specific primers (Liu, 1998; Jorge *et al.,* 2000; López *et al.,* 2003; Stein *et al.,* 2007).

2.8.2 Random amplified polymorphic DNA (RAPD)

RAPD analysis, a PCR based molecular marker technique, was developed independently by Welsh and McClelland (1990) and Williams *et al*. (1990). Since then many new modifications of the PCR-based molecular marker techniques have been developed. RAPD markers are generated by PCR amplification of random genomic DNA segments with single synthetic decamer primers of arbitrary sequence (Williams *et al*., 1990; Jacobson and Hedrén, 2007). Amplified products are separated by electrophoresis on agarose or

polyacrylamide gels. Polymorphisms are detected as DNA fragments, which amplify in one individual but not the other i.e. present or absent. These changes most probably include single base substitutions as well as deletions or insertions that either changes the primer binding site's sequence or the size of the amplified DNA fragment (Williams *et al*., 1990; Yasukochi *et al.,* 2006; Lima *et al.,* 2007).

RAPD markers can identify large numbers of genetic polymorphisms between closely related taxa and a large set of primers can be screened within a short period. It requires the use of minimal amounts of DNA, thus allowing simple and rapid methods for genomic DNA isolation. The technique is simple and straightforward, requiring no isolation of cloned probes or preparation of hybridisation filters. The presence or absence of a band of a particular size generally distinguishes different alleles at the same locus. The band-present phenotype is dominant to the band-absent phenotype. The band present phenotype may represent a homozygous or heterozygous genotype for the locus in question (Jacobson and Hedrén, 2007). The band absent phenotype can only represent a homozygous genotype for the alternate allele. A disadvantage is that it cannot distinguish between heterozygotes and homozygotes (Obradovic *et al.,* 2008).

As the PCR amplification process is dependent upon many components and their interactions (Devos and Gale, 1992; Caetano-Anolles and Bassam, 1993; Wolf *et al*., 1993), it is important to specify a set of reaction conditions in order to obtain reproducible results for a given species. Sources of reliability lie in the purity of the template DNA, magnesium (Mg^{2+}) concentration, the choice of thermal stable DNA polymerase and thermal cycler used in PCR amplification. It also depends on the imprecise matches between short oligonucleotide primers (decamers) and the template DNA at low annealing temperatures ($35^{o}C$ to $40^{o}C$) during amplification conditions (Iqbal and Rayburn, 1994; Kelly, 1995; Qiu *et al*., 1995; Akinbo *et al.,* 2007; Sharma *et al.,* 2008).

Efforts to overcome problems of reproducibility with RAPD markers, especially between laboratories, led to the development of sequenced characterised amplified regions (SCAR)

(Kesseli *et al*., 1992) and allele specific associated primers (Weeden *et al*., 1992). Reproducibility is increased by sequencing the two ends of the RAPD fragment and synthesising two long primers (24 base pairs) homologous to each end. These two primers, which include the original decamer sequence, are used in the PCR protocol at an elevated annealing temperature (50^{o}C - 65^{o}C), and generally produce a single fragment (SCAR) of the same size as the previously sequenced RAPD fragment (Kesseli *et al*., 1992; Jacobson and Hedrén, 2007). Paran and Michelmore (1993) and Nair *et al*. (1995; 1996) were able to increase the reliability of RAPD markers by converting them to SCARs which could be used in a PCR reaction to amplify the RAPD fragments. SCARs have the advantage of being inherited in a co-dominant fashion in contrast to RAPD, which are inherited in a dominant manner (Mohan *et al.,* 1997; Okogbenin *et al.,* 2007).

2.8.3 Amplified fragment length polymorphism (AFLP)

Amplified fragment length polymorphism (AFLP) is based on the selective PCR amplification of restriction fragments from a total digest of genomic DNA (Vos *et al*., 1995). The genomic DNA is digested with two restriction enzymes, usually a rare cutter and a frequent cutter. Double stranded oligonucleotides, known as adapters, are ligated to the ends of the genomic DNA at the specific restriction sites. Adapters have a nucleotide overhang known as a "sticky end", complementary to that of the restriction site. Separate adapters are needed for each of the different restriction enzymes. The ligated DNA is then used as template for PCR reactions. The primers are specific to the combination of the adapter sequence and restricted site sequence (Huang *et al.,* 2007).

The AFLP method generates a large number of restriction fragments which is then selectively reduced by primers that have one or three selective nucleotides at the 3' prime end, facilitating the detection of polymorphisms. Choosing different base numbers and composition of nucleotides in primers can control the number of DNA fragments which are amplified. PCR products are separated on denaturing polyacrylamide gels. Caution is needed in scoring the AFLP gel because of the large number of bands. AFLP fragments are usually

scored as dominant markers, but occasionally polymorphisms can be distinguished as co-dominant markers (Liu, 1998). To do this, a mixture distribution model can be used to fit the band intensity for three possible genotypes such as in a diallel model. This approach is useful in saturation mapping and for discrimination between varieties (Brugmans, 2005; Gbadegesin *et al.,* 2007).

Lin *et al.* (1996) compared three different DNA mapping techniques i.e. RFLP, RAPD, and AFLP, for efficiency in detecting polymorphism in soybean and found AFLP to be the most efficient technique. High reproducibility, rapid generation, and high frequency of identifiable polymorphisms make AFLP analysis an attractive technique for identifying polymorphisms and for determining linkages by analysing individuals from a segregating population (Tamiru *et al.,* 2007). The time and cost efficiency, repeatability, and resolution of AFLP are superior or equal to other markers (RAPD, RFLP, and SSR), except that it primarily generates dominant rather than co-dominant markers. Because of their high repeatability and ease of use, AFLP markers have emerged as one of the major genetic markers with broad application (Mueller and Wolfenbarger, 1999; Edwards *et al.,* 2008).

2.8.4 Minisatellites (VNTR) and microsatellites (SSR)

PCR with specific primers can only reveal polymorphisms that lie in the amplified area between the primers. An alternative approach to increase the utility of PCR based markers is to produce primers that flank genomic regions more likely to show variability than a randomly selected sequence (Kochert, 1994). Such hypervariable regions consist of tandem repeated DNA sequences. Markers based on such sequences include minisatellites and microsatellites. Minisatellites are tandem repeats of sequences ranging from 9 - 100 bp in the genome. The number of repeats varies and is usually less than 1000 bp. Minisatellites are also referred to as variable number of tandem repeats (VNTR) and are detected mainly by hybridisation approaches (Mathema *et al.,* 2008). In hybridisation, genomic DNA can be digested using restriction sites flanking the tandem repeats. The cutting yields fragments containing cores of the repeats with a different number of repeats (length variation). The

polymorphic bands result from the variation in the number of the tandem repeats (Mathema *et al.,* 2008).

Microsatellites or simple sequence repeats (SSR) are tandem repeats of a much smaller size (2 - 8 bp) and are ubiquitous in eukaryotes (Gianfranceschi *et al.*, 1998). SSR polymorphism (SSRP) reflects polymorphism based on the number of repeat units (Litt and Luty, 1989; Weber and May, 1989; Arunachalam and Chandrashekaran, 1994). They are highly variable DNA sequences that can be used as informative markers for the genetic analysis of plants and animals. A genetic map with over 6000 SSR has been constructed in mouse (Dietrich *et al*., 1996). The number and composition of microsatellite repeats differ in plants and animals. The frequency of repeats longer than 20 bp has been estimated to occur every 33 kb in plants, unlike mammals where it has been found to occur every 6 kb (Wang *et al*., 1994). The more common form of repeats are simple di-nucleotide repeats such as (CA)n, (GT)n, (GA)n:(CT)n, (CG)n:(GC)n, and (AT)n:(TA)n, where n is the number of repeats. In humans, AC or TC is a common repeat unit, but in plants AT is more common, followed by AG or TC (Powell *et al*., 1996). In general, plants have about 10 times fewer SSRs than humans.

Microsatellites with tri- and tetra-nucleotide repeats are also found, but their frequencies are lower than the di-nucleotide repeats (Hearne *et al*., 1992). Searching through DNA sequence databases for sequences containing simple repeats may help identify microsatellites (Liu, 1998). For some species, such as human, mouse, *Arabidopsis* and rice, a large amount of DNA sequence data has already been accumulated. The discovery, inheritance, and variability of 14 GA repeats have been described for cassava (Chavarriaga-Aguirre *et al*., 1998). A subset of those SSR markers was used to evaluate the genetic diversity of the core collection of about 600 accessions of the cassava world germplasm bank at the International Centre of Tropical Agriculture (Chavarriaga-Aguirre *et al*., 1999). The development and characterisation of 172 SSR primers in cassava have been reported (Mba *et al*., 2001).

A nucleotide sequence flanking the repeats is used to design primers to amplify the different number of repeats in different varieties (Mba *et al*., 2001). This type of polymorphism is highly reproducible. These primers are useful for rapid and accurate detection of polymorphic loci and the information could be used for developing a high-density genetic map based on these sequence tags (Schmidt and Heslop-Harrison, 1996; Roder *et al.*, 1998; Okogbenin *et al.*, 2006). For most plant and animal species where no sequence data is available, a large effort using hybridisation and sequencing is needed to identify microsatellites suitable for use as genetic markers. Hybridisation using simple repeats as probes is used to screen genomic clones, and to identify a clone containing the sequenced microsatellites (Balyejusa Kizito *et al.*, 2007). The clone is then sequenced and primers designed from sequences flanking the repeats. Microsatellite markers have proven to be one of the most effective tools for genetic mapping, marker-assisted breeding and diversity studies. With new techniques for enriching and pre-screening libraries, it is now possible to produce a greater number of microsatellite markers (Edwards *et al*., 1996; Mba *et al.*, 2001; He *et al.*, 2003; Lopez *et al*., 2004; 2005; Okogbenin *et al.*, 2006; Hurtado *et al*., 2008).

2.8.5 Single strand conformation polymorphism (SSCP)

When the objective for using markers is the detection of mutations involving a single nucleotide change, then a method that can detect a single change in a nucleotide sequence such as single strand conformation polymorphism (SSCP) will be appropriate (Bertin *et al.*, 2005; Castelblanco and Fregene, 2006). SSCP is a technique that can detect polymorphism and DNA sequence alterations as small as a single nucleotide change (Orita *et al*., 1989). It is a powerful and rapid method but can only be used with relatively short DNA fragments. SSCP can identify the heterozygosity of the DNA fragment in DNAs of similar molecule weight. Electrophoretic mobility of single-stranded DNA in non-denaturing polyacrylamide gels depends on both size, and nucleotide composition. The SSCP methodology exploits the tendency of single stranded DNA to form intra molecular base pairs, resulting in a sequence dependent conformation with a specific mobility in acrylamide gels. Changes in DNA sequence, even in a single base pair, can cause alterations in the conformation and result in

changes in electrophoretic mobility. In practice, SSCP are principally detected by PCR to amplify a specific fragment, which is then run on a conformational gel (high resolution acrylamide) (Bertin *et al.*, 2005; Castelblanco and Fregene, 2006).

2.8.6 Sequence tagged sites (STS)

Sequence tagged sites (STS) were proposed by Olson *et al.* (1989) as chromosome landmarks in the human genome. A STS is a short, unique fragment of DNA whose sequence and position in the genome are known (~300 bp). Large DNA clones contain the same STS overlap, so STSs can be used in physical mapping to order large DNA fragments (Liu, 1998). If a polymorphism can be detected using a STS as probe, then anchor points between genetic and physical maps can be established (Weissenbach *et al*., 1992; Gyapay *et al*., 1994). Polymorphic STS markers are used for genomic analysis in plants (Mazur and Tingey, 1995; López *et al.*, 2004).

2.8.7 Expressed sequence tags (EST)

Expressed sequence tags (ESTs) are subsets of STSs derived from cDNA clones. ESTs can serve the same purpose as random STSs, with the advantage that ESTs are derived from expressed genes, i.e. from spliced mRNA which is usually free of introns as well as repetitive DNA (López *et al.*, 2004). ESTs have the advantages of representing functional genes and are therefore more useful as genetic markers than anonymous non-functional sequences (Castelblanco and Fregene, 2006; Sakurai *et al.*, 2007). In species having large genomes, cDNA sequencing to obtain ESTs are advantageous for genome analysis (Ji *et al.*, 2006; Sakurai *et al.*, 2007). In cassava, ESTs have been developed from transcript-derived fragments (TDFs), which are AFLP fragments of expressed mRNA populations. Suarez *et al*. (2000) obtained more than 500 TDFs by applying the cDNA-AFLP technique to mRNA from parents of a cassava genetic mapping population. Sequence alignment of the EST revealed mostly genes of unknown function (Coles *et al.*, 2005; Kim *et al.*, 2006). Generation

of EST as differentially expressed sequences, in time or between different varieties, is an important way of developing ESTs around specific traits for the candidate locus approach to mapping complex traits (Boventius and Weller, 1994; López *et al.,* 2004, 2005; Ayeh, 2008).

2.8.8 Single nucleotide polymorphisms (SNPs)

In the past, molecular markers were mainly based on genomic DNA. The DNA could belong to transcribed or non-transcribed regions of the genome (Gupta *et al.,* 2002; Gupta and Rustgi, 2004). However, there is a rapid accumulation of new markers, obtained from a large number of cDNA clones in a variety of plants and the accumulation of a large number of expressed tags in the public database. These markers are gradually gaining popularity compared to the older DNA based markers (Ayeh, 2008).

The discovery of new molecular markers such as single nucleotide polymorphism (SNP) techniques do not always need these (RFLP, RAPD, AFLP) electrophoresis based assays. SNPs are excellent markers for association mapping of genes controlling complex traits and provide the highest map resolution (Brookes, 1999; Bhattramakki *et al.,* 2002; Botstein and Risch, 2003). SNPs can thus be explained as any polymorphism between two genomes that is based on a single nucleotide exchange. In plants, studies on the occurrence and nature of SNPs are beginning to receive considerable attention (Ching *et al.,* 2002; Jander *et al.,* 2002; Zhu *et al.,* 2003; López *et al.,* 2005).

2.9 Linkage analysis and genetic map construction

A genetic map can be defined as a linear ordering of a group of genes or markers that closely represent the chromosome. Genetic maps are constructed by analysing the segregation of genes in simple pedigree progeny or offspring derived from crossing two parental organisms with contrasting genes (Balyejusa Kizito *et al.,* 2007). The construction of a genetic map requires: (i) a large number of genetic markers and a fairly sized segregating population, (ii)

a method of grouping markers into different linkage groups, and (iii) ordering of markers in the same linkage group to ascertain their relative position on the genetic map, via the estimation of multipoint recombination fractions among adjacent loci (Churchill and Doerge, 1994; Zhang *et al.,* 2008).

If two or more markers are located close together on a chromosome, their alleles are usually inherited together through meiosis. If they are further apart they may be separated during crossing over at meiosis and form new (non parental) combinations with other markers in a process called recombination. The genetic basis of linkage mapping is genetic recombination resulting from crossing over between homologous chromosomes during meiosis (Li *et al.,* 2008).

Genetic recombination is measured by the recombination fraction, which is the ratio of recombinant gametes to total gametes. Many statistical procedures have been used to detect linkage and to estimate recombination fraction at two point- or multipoint levels (Ott, 1991; Teng and Siegmund, 1997; Franke and Ziegler, 2005). These procedures are the fundamentals of linkage map construction. The recombination fraction is not additive along a chromosome and the departure from additivity increases with distance between loci (Fregene *et al.,* 1997). Additivity is based on the assumption that the average number of crossovers per chromatid occurring between two loci is directly proportional to the distance between the two loci. Estimates of the frequency of crossing over are most reliable when genes are closely linked, i.e. 1 to 10 map units (centiMorgans or cM). Because map distance is not additive, mapping functions were developed to correct for this effect (Rogers and Bernatchez, 2005). Mapping functions, such as Haldane's function, Kosambi's function and some other functions have been developed to make recombination additive (Haldane, 1919; Kosambi, 1944). These functions may apply to general or specific situations.

Sturtevant (1913) and Morgan (1928), in their early work on gene mapping in *Drosophila*, used the estimated recombination fraction as map distance i.e. map distance (m) is equal to recombination fraction (r). This is known as the Morgan's mapping function. When a small

genome segment is considered, the chance that double or multiple crossovers occur in the segment is low. The original derivation of Haldane's mapping function was purely mathematical. A more biologically based derivation of Haldane's mapping function has been developed. When the recombination fraction is small, map distances using Haldane's mapping function and recombination fraction are approximately equal. As the size of the segment increases, the expected number of double crossovers increases, and the map is adjusted for double crossovers through Haldane's mapping function. The recombination fraction approaches 0.5 and is independent of map distance when map distance is large. Genetic map distances, which are based on recombination, cannot be directly related to distances in nucleotide pairs on DNA (Zane *et al.*, 2002; Rogers and Bernatchez, 2005).

Haldane's mapping function works for situations with absence of crossover interference [where coefficient of coincidence (C) = 1]. However, experimental evidence has been found to support crossover interference and crossovers occur non-randomly in genomes (Muller, 1916). This has given rise to the development of the Kosambi mapping function (Kosambi, 1944) which assumes positive interference [i.e. fewer double recombinants compared to no interference (C <= 1)]. The rationale behind Kosambi's (1944) function is that the crossover interference depends on the size of a genome segment. The interference increases as the segment decreases (e.g. C $\rightarrow$1 when r $\rightarrow$ 0.0). The relationship between the size of the segment and the crossover interference is C = 2r. Other mapping functions have been reported such as by Carter and Falconer (1951), and Felsenstein (1979). The Carter and Falconer (1951) mapping function is commonly used when there is evidence of strong crossover interference ($C = 8r^3$). The central issue regarding the use of a particular mapping function in practice is the fitness of the mapping function to the observed data (Tuberosa *et al.*, 2003).

2.10 Genetic mapping of quantitative trait loci (QTL)

When dealing with a qualitative character, the relation between the phenotype and the genotype of the parents is easily recognised from simple numerical proportions observed in the segregating progeny (van Eck, 1995). Quantitative traits cannot be described in discrete phenotypic classes, but are described through the trait values of individuals, which are conceived as samples drawn from a continuous distribution (Falconer, 1989). The relationship between the phenotypic value and the genotype for most quantitative traits therefore remains obscure with common unanswered questions such as: how many genes influence the trait? How much does each gene contribute to the trait? Is there additive or non additive interaction between alleles at the same locus, or epistatic interaction between loci? (van Eck, 1995; Manly and Olson, 1999)

Loci affecting or controlling quantitative traits have commonly been referred to as QTL. Many genes important in plant breeding are QTL. Five concepts have been proposed regarding QTL (Lamkey and Lee, 1993). QTL are (1) major genes with pleiotropic effects on other traits (Barton, 1990); (2) fundamentally different from major genes in that QTL alleles are limited to small effects (Mather, 1941); (3) modifiers of major genes (Mukai and Cockerham, 1977); (4) loci with an allelic series with a range of effects leading to their recognition as macromutations (mutants) and micromutations (Allard, 1960; Thompson, 1975; Robertson, 1985) and (5) tightly linked multilocus clusters (Allard, 1960; 1988).

The procedures for finding and locating the QTL are collectively called QTL mapping. QTL mapping involves construction of genomic maps and searching for association between traits and polymorphic markers. QTL mapping is a combination of linkage mapping and traditional quantitative genetics. QTL mapping creates the possibility of modelling quantitative traits at the individual gene level (Ritter *et al.*, 2008).

A significant association between quantitative traits and markers may be evidence of a QTL near markers (Beavis, 1998). Simple t-test, simple linear regression, multiple linear

regression, non-linear regression and interval test approach have been proposed and used to map QTL (Weller *et al*., 1988; Lander and Botstein, 1989; Lande and Thompson, 1991; Knapp *et al*., 1992; Stuber *et al.*, 1992; Zeng, 1993; 1994). To carry out data analysis, different software packages are available such as: MAPMAKER (Lander *et al*., 1987), QTLSTART (Liu and Knapp, 1992), QTL Cartographer (Basten *et al*., 1995), PGRI (Liu and Lu, 1995), MAPQTL (Van Ooijen and Maliepaard, 1996), Map Manager QT (Manly and Cudmore, 1996) and QGENE (Tanksley and Nelson, 1996).

Commonly used approaches for QTL mapping, such as the single marker t-test, are single QTL models. The number of markers in the single QTL models can vary from one to a large number. However, only one or two markers are directly related to the putative QTL (Weller and Soller, 2004; Mackey and Powell, 2007). Single marker analysis is based on comparisons between marker genotypic means through a t-test, analysis of variance, a likelihood ratio test or a simple regression for a trait on a coded marker genotype. The single marker analysis is carried out by analysing one marker at a time (Thoday, 1961; Soller *et al*., 1976). The QTL is determined to be located near a marker if the phenotypic values for the trait are significantly different among the marker genotypes. QTL mapping has been recognised as a multiple test problem (Zeng, 1994). One of the QTL mapping strategies is to search the whole genome by hypothesis test for a single marker or a single genome position and then to build a multiple-QTL model based on the results from single QTL analysis. Searching the whole genome simultaneously is better than scanning individual points, if information content is adequate to do so (Melchinger *et al.*, 2007).

The single marker analysis has the disadvantages of low statistical power and confounding estimates of QTL effects (especially overestimation of tightly linked QTL and underestimation of loosely linked QTL) and locations. Lander and Botstein (1989) proposed the interval mapping approach to correct this problem. Interval mapping is based on the joint frequencies of a pair of adjacent markers and a putative QTL flanked by the two markers (Liu, 1998). Interval mapping using either the maximum likelihood or the regression approach is the most commonly used method. However, problems exist, such as: (1) the

number of QTL cannot be determined (2) the locations of QTL are sometimes not well resolved and (3) the statistical power is still relatively low. These problems are due to linked QTL, QTL interactions, and limited information in the model (Weller and Soller, 2004; Mackey and Powell, 2007).

In general, the interval mapping approach has a higher statistical power for detecting QTL than the single marker t-test when linkage map density is low i.e. recombination fraction is large between markers (Liu, 1998). However, the single marker t-test is as powerful, or more powerful than the interval mapping approach when the linkage map density is high (Liu and Lu, 1995). The assumption for simple interval mapping is that a single segregating QTL influences the trait. However, interval mapping is not independent for different segments if more than one QTL exists (Mackey and Powell, 2007).

Alternative approaches are needed for QTL mapping, based on multiple QTL models that include QTL interactions. One of such models is the composite interval mapping (CIM) which is a combination of simple interval mapping and multiple linear regressions (Zeng, 1993; 1994; Rao *et al.,* 2007). In practice, CIM can be implemented using an iterative expectation/conditional maximisation (ECM) algorithm (Meng and Rubin, 1993). The CIM can be implemented using the linear regression model for interval mapping as well as multiple linear model to control the residual genetic effects (Zeng, 1993; 1994). The original CIM using the ECM algorithm can be performed using the computer software package QTL Cartographer (Manly and Cudmore, 1996). For composite interval mapping using regression approaches, commercial software such as SAS can be used. There are more variables in the model than in simple interval mapping and single marker analysis making CIM more informative and efficient. One of the most important advantages of CIM is that markers can be used as boundaries to narrow down the most likely QTL position (Bogdan and Doerge, 2005; Li *et al.,* 2007).

With the emergence of genetic maps integrated across taxa, QTL mapping may expedite determination of order and biological function to genomic regions of crops (Lee, 1995). The

enthusiasm for QTL mapping for applied research is strongly associated with the expectation of using genetically linked DNA markers as the means of indirect selection for genomic regions affecting quantitative traits in plant breeding programmes. QTL mapping and DNA markers may provide insights into facets of quantitative patterns and other complex processes and phenomena that have been exploited but are not yet fully understood. QTL mapping is one of the most important activities connecting recent plant and animal genome research to plant and animal improvement, being a key element in the application of breeding and map based cloning to economically important genes (Li *et al.,* 2006).

One of the most rewarding aspects of QTL mapping is that putative QTL of large and small effects are detected with regularity. Simulation studies (van Ooijen, 1992; Carbonell *et al*., 1993; Beavis, 1994) and limited empirical investigations (Beavis, 1994) have, however, provided ample evidence for concern. With small plant sample sizes (< 100), the potential for erroneous detection (overestimating QTL effects and finding false or "ghost" QTL) and characterisation is substantial (Lee, 1995). This is due to multicollinearity i.e. unlinked markers associated with QTL due to chance, and limited numbers of recombinant classes for any region of the map, assuming that the trait and marker data are complete and correct (Li *et al.,* 2006; 2007).

Some studies (Stuber *et al.,* 1992; Beavis, 1994) revealed little agreement among mapping samples for location and effects of QTL, suggesting that sampling could considerably influence QTL estimation. However, QTL mapping for insect resistance (Lee, 1993), morphological traits in maize (Veldboom *et al*., 1994) and in maize x teosinte populations (Doebley and Stec, 1993) detected several QTL in similar genomic regions between populations involving the same parents. In addition, a summary of QTL detected in several mapping populations identified genomic regions common to several genetic backgrounds (Abler *et al*., 1991; Helentjaris, 1992).

A potential use of QTL mapping is to select parents that combine several traits and to improve methods for predicting the performance of progeny produced by individuals selected

in breeding programmes (Paterson *et al*., 1991; Desai *et al.,* 2008). Estimates of recombination and effects of QTL are inherently confounded (Edwards *et al*., 1987). Assessments that are more accurate would be obtained with independent samples of progeny from the same population (Lande and Thompson, 1991), as opposed to the typical practice of using the same sample for estimating gene location and effects. Therefore, many estimates of the genetic effects of QTL are probably biased. The type and degree of bias depends on the genetic and environmental designs and models used to detect and characterise the QTL. The direction and degree of bias are important because the estimates are used to forecast genetic gain and merit (Hoeschele and Van Raden, 1993), assess gene action for breeding programmes, investigate complex phenomena such as heterosis (Stuber, 1994) and to select targets for map-based cloning (Song *et al.,* 2007).

Most QTL mapping designs permit estimates of additive effects. Usually this is biased upward to a degree dependent on numerous variables (Carbonell *et al*., 1992; Darvasi *et al.*, 1993; Hoeschele and Van Raden, 1993; Beavis, 1994). The source of the bias includes deficiency of recombination gametes, G X E, and underestimation of epistasis (Tan and Mak, 1995; Melchinger *et al.,* 2007).

2.11 QTL mapping in heterozygous species

Genetic mapping in allogamous crops is complicated by the absence of complete homozygosity in the parents and subsequent mixture of genotypic classes in the progeny. The use of highly heterozygous parents affects QTL mapping by: (1) re-definining mating type at a locus level rather than all loci in parental cross; and (2) allowing the detection of multiple QTL alleles within a single outcrossed pedigree using separate maps for each parent (Williams, 1998). Pedigrees for QTL mapping, which do not use inbred lines are outcrossed pedigrees, although they vary in the degree of relatedness between parents of the segregating progeny population (Śliwka *et al.,* 2008).

Genetic load tends to be higher for outcrossing perennial plants than for other plants and animals, thereby rendering related matings, inbred lines, or near-isogenic lines unavailable (Williams and Savolaine, 1996). Outcrossing pedigrees have different mating types within a single parental cross such that there may be backcross-mating types at a marker locus and intercross mating types at other loci. This heterogeneity of mating types makes QTL detection more complex and less efficient compared to inbreds (Jakayar, 1970; Hill, 1975; Soller and Genizi, 1978; Beckmann and Soller, 1988; Knott, 1994; Śliwka *et al.*, 2008).

The marker genotypes in the F_1 progeny population of outbred pedigrees result from independent meioses and crossover in the maternal and paternal parents. Thus, individual maps are often constructed for each parent if progeny numbers are sufficiently large (Groover *et al*., 1994; Grattapaglia and Sederoff, 1994; Cervantes-Martinez and Brown, 2004). With co-dominant markers, the maternal map includes segregation data for the following: (1) maternal informative loci; (2) fully informative loci recoded to contain only maternal segregations (i.e. the paternal parent marker data are recoded to be homozygous); and (3) both informative loci, excluding linkages between pairs of both informative loci. The paternal map is constructed similarly (Balyejusa Kizito *et al.,* 2007).

Partitioning of data from both informative loci and recoding fully informative loci result in the statistical independence of the two parental maps, which are then joined into a consensus map. With dominant markers such as RAPDs, informative backcross marker configurations are searched *a posteriori* in an F_1 cross between two heterozygous parents (Cervantes-Martinez and Brown, 2004). If one parent is heterozygous and the other homozygous, the segregation pattern will be 1:1. Separate genetic maps are generated for each parent based on backcross configurations only (Grattapaglia and Sederoff, 1994; Grattapaglia *et al*., 1995). Separate male- and female-derived genetic linkage maps of cassava were constructed from an F_1 progeny from an intra-specific cross (Fregene *et al.*, 1997). It is expected that the molecular genetic map will provide a better definition of the structure of the cassava genome (Gomez *et al*., 1995; Mba *et al.,* 2001; Okogbenin *et al.,* 2006; Balyejusa Kizito *et al.,* 2007).

2.12 Application of molecular markers in plant breeding

Recent developments in molecular genetic techniques present the plant breeder with a new set of tools to approach intractable traditional plant breeding problems (Balyejusa Kizito *et al.,* 2007). Molecular genetic mapping, DNA sequencing, and gene function analysis, will make it possible to locate genes (Paterson *et al*., 1988; Lander and Botstein, 1989; Fregene *et al.,* 1997; Okogbenin *et al.,* 2006; Balyejusa Kizito *et al.,* 2007), understand their function, and use them in designing progenitors. All these new tools require increased knowledge about plant genomes and new techniques for obtaining, storing, and using this information (Fregene *et al.,* 2006; Balyejusa Kizito *et al.,* 2007).

The development of DNA marker technologies and mapping strategies has exerted a considerable impact on the genetic improvement of many crop species of importance to the developed world (Landry *et al*., 1992; Young, 1992; Foolad *et al*., 1995). Recently, mapping projects for the so called "orphan crops" (Persley, 1990) of particular interest to the tropics and subtropics have been initiated. They include cassava (Fregene *et al*., 1997; Lokko *et al.,* 2005; Okogbenin *et al.,* 2006), plantain (Gawel and Jarret, 1991), groundnut (Kochert *et al*., 1991) and cowpea (Fatokun *et al*., 1992; 1993; Menancio-Hautea *et al*., 1993; Menendez *et al*., 1997; Ogundiwin *et al.,* 2008).

Various aspects of DNA markers and crop improvement have been reviewed in detail since 1991 (Paterson *et al*., 1991; Beckmann and Osborn, 1992; Phillips and Vasil, 1994; Lee, 1995). Resolving complex traits into their single gene components will offer the possibility of treating these characters with the efficacy of single gene traits (Lander and Schork, 2006). Thoday (1961) pointed out that the study of quantitative variation is hampered because of the lack of complete genetic maps, a limitation, which has largely been overcome with the advent of DNA markers (Botstein *et al*., 1980). Higher density molecular maps make it possible to identify and measure the effects of genes underlying quantitative traits (Tanksley *et al*., 1989; Paterson *et al*., 1991; McCouch and Doerge, 1995).

QTL analysis provides a way of selectively manipulating individual genetic components of a complex trait. Cytogenetic markers have been used to locate QTL for several decades in crops such as maize and wheat. However, the advantages of DNA markers such as improved resolution, coverage, and co-dominance, make them a better way for characterisation of genomes. In marker-assisted selection (MAS) for breeding and genetics, 15-20 cM is a practical limit of resolution (Lee, 1995; Fregene *et al.,* 2007). Smaller regions (1-5 cM), is ultimately necessary for maximum efficiency according to simulated MAS (Gimelfarb and Lande, 1994). Molecular markers linked to quantitative traits have been reported for many crop species (Lee, 1995; Lin *et al*., 1996; Zhuang *et al*., 1997; Mohan *et al.,* 1997; Okogbenin and Fregene, 2002; 2003; Akinbo *et al.,* 2008; Okogbenin *et al.,* 2008).

2.13 Introgressing genes from exotic germplasm into cultivated gene pools

Not only do molecular markers provide an unprecedented glimpse into the quantity of genetic diversity; they also provide an opportunity to assess the potential of genes from exotic germplasm once they are in an elite line background (Lee, 1995; Okogbenin *et al.,* 2007). Analysis of advanced backcrosses involving wild relatives with DNA markers indicate that exotic donor parents contribute more genes with positive effects that could have been predicted from their phenotypes alone (Fregene *et al.,* 2006). This has been shown in maize (Lee *et al*., 1990), tomato (de Vincente and Tanksley, 1993; Eshed and Zamir, 1994), wheat (Rogowsky *et al*., 1991; Schwarzbacher *et al*., 1992) and cassava (Fregene *et al.*, 2006).

Exotic germplasm is an important source of major gene resistance to abiotic and biotic stress and some quality traits (Vaughan, 1989; Okogbenin *et al.,* 2007). Introgression of such genes is enhanced through MAS and via an efficient introgression of the genome region without excessive linkage drag (Lee, 1995; Fregene *et al.,* 2006). In contrast, the role of exotic germplasm in improving quantitative traits has been less prominent. With the advent of DNA markers it has been suggested that it may be possible to develop efficient strategies for rapidly identifying and incorporating favourable exotic alleles into elite backgrounds to

realise a net improvement in trait performance. This has been proved in several crops (Edwards, 1992; Gebhardt *et al.*, 2007).

DNA markers could increase the efficiency of germplasm conversion programmes such as those used for sorghum (Duncan *et al*., 1991). The goal of the conversion programmes for sorghum is to adapt tropical germplasm such that it may be grown and evaluated in temperate regions. Once adapted growth habit has been achieved, the merit of the exotic genes may be assessed in breeding programmes. Such conversion programmes might utilise DNA markers at several stages. Selection of exotic parents should promote maximum diversity while minimising duplications *(*Lee, 1995). DNA markers could assist with the selection of exotic parents for conversion. When segregating progeny are selected for backcrossing, markers could be used to identify progeny that carry the derived genome region with minimal amounts of the donor parent genome (Pereira and Lee, 1995; Balyejusa Kizito *et al.*, 2007). This identification would reduce the number of backcross generations and facilitate maximum recovery of exotic alleles. Thus, breeders would have more opportunities for assessing the merits of truly exotic alleles with unique and favourable effects (Lee, 1995; Fregene *et al.*, 2006).

Chapter 3

Generation and agronomic evaluation of F_1 inter-specific hybrids

3.1 Introduction

As the major staple food crop, cassava can serve as a cheap means of deploying adequate protein amongst the poor. But cassava roots are low in protein content (1% - 2% protein dry weight basis) probably because it was selected by early farmers principally for its starchy storage roots and not for protein content. High protein content has not been a breeding objective for the majority of cassava breeders. Deficiency in protein is one of the most important problems in pregnant and nursing mothers and growing children in populations where cassava is a staple food in the developing world. The earliest reports on breeding for increased protein content included the use of *M. melanobasis* and *M. tristis* in a backcross breeding programme in East Africa in the 1940s (Nichols, 1947; Bolhuis, 1953). More recently, crosses between cassava and *M. tristis* revealed root protein content of more than 8% in F_1 hybrids (Bolhuis, 1953; Asiedu *et al*., 1992). Unfortunately the high protein content was lost during backcrossing to recover the desired characteristics and high root yield of cassava (Asiedu *et al*., 1992).

Centro Internacional de Agricultura Tropical (CIAT) houses the world germplasm collection of cassava and its wild accessions (Hershey and Amaya, 1983). Ceballos *et al.* (2006) evaluated 149 clones for root protein in the cassava germplasm collection at CIAT with variation between 0.95% and 6.42% crude protein, which suggested that genetic variation exists within the material. The correlation between dry matter and protein content in the root is negative and the linear regression was relatively small from the study reported by Ceballos *et al.* (2006). A better source of genetic variability for the trait is in its wild relatives known to possess up to 15% protein content in their roots (Asiedu *et al.*, 1989; CIAT, 2002).

Recognising the usefulness of wild *Manihot* species, CIAT conducted several collection expeditions of close cassava relatives in the southern basin of Brazil as well as a study of genetic diversity to understand the structure of diversity for conservation purposes (Roa *et al.*, 1997). Many accessions of *M. esculenta* ssp *flabellifolia*, *M. esculenta* ssp *peruviana* and *M. tristis* species collected in 1995 and 1997 were found to have protein content of up to 15% and dry matter content of more than 50%. This was combined with good storage root formation. This is an important resource for improving protein content and dry matter yield in cassava. To increase protein content in the root of cassava, the high protein content accessions of *M. tristis*, *M. esculenta* ssp *flabellifolia*, and *M. esculenta* ssp *peruviana* were crossed extensively to elite cassava varieties (CIAT, 2002).

The objective of this study was to evaluate the F_1 inter-specific hybrids for protein content and to select the best ones for the generation of a backcross (B_1P_2) mapping population that can serve as basis for elucidating the genetics of high protein content as a means of increasing efficiency of breeding for high protein content in cassava.

3.2 Materials and methods

3.2.1 Location and climatic conditions

The field experiments were carried out in 2001 and 2003 at CIAT headquarters, in Palmira, Valle del Cauca Department, Colombia. The experimental site at Palmira (1000 m above sea level, latitude $3^0 31^1$N and longitude $76^0 21^1$W), has a soil texture described as mollisol (Lian and Cock, 1979a; b). Two rainfall peaks occur at this location in the intervals March to June and October to December. Long term total annual rainfall is about 1000 mm, although yearly variations are considerable. The water holding capacity of the soil is such that cassava rarely suffers from water stress at this site. Mean temperature is 25 ± 1^0C monthly. Solar radiation is normally between 12000 and 14500 g cal cm^{-2} mo^{-1} throughout the year.

3.2.2 Crosses made

A number of collection expeditions have been carried out by CIAT, to collect seeds of *M. esculenta* ssp *flabellifolia*, *M. esculenta* ssp *peruviana*, and *M. tristis* (Bonierbale *et al.*, 1995; Roa *et al.*, 1997). These collections were from the same populations used by Olsen and Schaal (1999) in their phylogeographic study of the origin of cassava. In May 2000, more than 2000 of these seeds were germinated in seedling nurseries and transferred to the field two months after planting. At nine months, three roots were "milked" from each of the genotypes and evaluated for crude protein, dry matter content, crude fibre, ash, amylose and storage root production (Table 3.1). Controlled pollinations were performed following the standard procedure described by Kawano (1980) between selected accessions of *M. esculenta* ssp *flabellifolia* (Appendix 1) and four of the current elite parents of the cassava gene pools adapted to the three major agro-ecologies where cassava is grown.

3.2.3 Field evaluation

In 2001, sexual seeds from the F_1 hybrids were planted in a seedling nursery and transferred to the field a month later. The distance between plants was 1.7 m by 0.8 m to increase the between plant competition and reduce within plant competition. Seven months after planting six woody stakes were harvested from each plant, along with their parents, and established in a replicated, by family, trial of six plant rows. Plants were hand harvested individually and yield and root quality traits were measured and averaged for each genotype. Harvest index was measured as the ratio between fresh root weight and the total fresh biomass. Dry matter content (DMC) in the roots was estimated using the specific gravity methodology (Kawano *et al.*, 1987). Approximately between 1 kg to 5 kg of roots were weighed in a hanging scale (W_A) and then the same sample was weighed with the roots submerged in water (W_W). Dry matter content was estimated using the following formula:

$$\% \text{ DMC} = 158.3 \text{ X} \left(\frac{W_a}{W_a - W_w} \right) - 142$$

For protein analyses, two roots per plant were sampled. Selected roots were peeled and washed slices were taken from the proximal, central, and distal sections of each root. Samples from roots of all plants of a genotype were mixed together and chopped into small pieces. Resulting chips were mixed well to obtain a uniform sample of the roots. A 100 g sample was then taken and dried in an oven with forced ventilation at 60^0C for 24 hours. Dried samples were ground in a mill with a stainless steel grinding tool.

All samples were analysed at the plant tissue analytical laboratory at CIAT. Nitrogen determination was based on a modification of the Kjeldahl method (Skalar, 1995). Root samples were digested with a mixture of sulphuric acid, selenium and salicylic acid. The salicylic acid forms a compound with the nitrates present to prevent loss of nitrate nitrogen. The digestion of the samples was initiated with hydrogen peroxide and at this step, the larger part of organic matter was oxidised. After decomposition of the excess of H_2O_2, the digestion was completed by concentrated sulphuric acid at elevated temperature (330^0C) with selenium as catalyst (Novozamsky *et al.*, 1983; Walinga *et al.*, 1989). Nitrogen was quantified colourimetrically on a segmented flow analyser. In the colouring process, salicylate, nitroprusside (catalyst) and active chlorine were added to form a green coloured complex with the ammonium ion. The absorption was measured at 660 nm (Krom, 1980; Searle, 1984).

Hock-Hin and Van-Den (1996) reported that, in the case of cassava roots, the conversion factor to estimate protein contents based on N concentrations should probably range between 4.75 and 5.87. Preliminary results suggested that the higher conversion factor (5.87) could better adjust to the measurements presented in this study. However, the average between these two figures was used instead as a conservative conversion factor between total nitrogen and protein content. The following year (2003), protein, dry matter, and fresh root yield data

was again evaluated at seven months after planting. Evaluation was by milking three roots per plant.

3.2.4 Data analyses

Data obtained was subjected to simple analysis, analysis of variance (ANOVA) and Principal Component Analysis (PCA). SAS (2002) was used for the Pearson correlation analysis. Principal Component Analysis (Iezzoni and Pritts, 1991) was used to investigate the source of phenotypic variation among genotypes. General combining ability estimates with an emphasis on high protein were used for selecting parents for generating larger families used in this study.

3.3 Results

A total of 744 genotypes from the crosses between wild relatives and cultivated cassava were evaluated for different yield quality traits. Maximum values were: for number of roots per plant 51, of which 32 were commercial roots, 0.94 for harvest index, 55.35% for dry matter content, 114 ton ha^{-1} for yield, 11.25% for protein content (dry weight basis) and 8 for root rot in the genotypes evaluated (Table 3.2). The standard deviation for roots per plant was 8.55, 4.71 for commercial roots, 0.12 for harvest index, 16.20 ton ha^{-1} for yield, 1.35% for protein content, and 0.71 for root rot.

Across genotypes, roots per plant ranged from 0.00 to 51.00, commercial roots from 0.00 to 32.00, percentage dry matter from 13.04 to 55.35, harvest index from 0.05 to 0.94, yield from 1.30 ton ha^{-1} to 114.00 ton ha^{-1}, percentage protein content from 2.87 to 11.25, and root rot from 0.00 to 8.00, which demonstrated the potential of using the wild progenitor of cassava to improve these traits.

Table 3.1: Simple statistics of characters of agronomic interest in accessions (273 genotypes) of the *M. esculenta* ssp *flabellifolia* in CIAT, Palmira in March, 2001

Variable	Minimum	Maximum	Average	Standard deviation
DMC[a]	17.87	91.67	49.73	17.11
PC[b]	0.58	14.59	6.32	2.91
CF[c]	2.14	58.17	21.31	14.82
Ash (%)	0.51	4.69	1.97	0.75
Amylose (%)	9.71	19.71	14.11	1.85

[a]Dry matter content (%); [b]Protein content (%); [c]Crude fibre (%)

Table 3.2: Simple statistics of agronomic variables on the F_1 (744 genotypes) inter-specific hybrids of cassava in CIAT, Palmira in May 2004

Variables	Minimum	Maximum	Average	Standard deviation
Rtplt[a]	0.00	51.00	9.83	8.55
ComRt[b]	0.00	32.00	8.58	4.71
HI[c]	0.05	0.94	0.39	0.12
DMC[d]	13.04	55.35	29.62	4.36
Yld[e]	1.30	114.00	21.60	16.20
PC[f]	2.87	11.25	5.39	1.35
Rtrot[g]	0.00	8.00	0.17	0.71

[a]Roots per plant; [b]Commercial roots; [c]Harvest index (0 - 1); [d]Dry matter content (%); [e]Yield (ton ha^{-1}); [f]Protein content (%); [g]Root rot (0 – 8)

A total of 15 families with genotypes having commercial sized roots were evaluated to determine their performance. All families had root numbers ranging from 3.89±5.16 to 31.77±15.41 (Table 3.3). Family CW 99 had the highest root number (31.77±15.41) and CW 151 had the lowest number (3.89±5.16). Commercial root size varied from 1.00±1.41 in CW 201 to 15.25±17.25 in CW 205. Harvest index ranged from 0.30±0.04 in CW 201 to 0.56±0.09 in CW 205, percentage dry matter varied from 25.19±6.07 in CW 179 to 33.34±3.69 in CW 168, yield from 6.5±0.35 ton ha^{-1} in CW 201 to 53.75±40.66 ton ha^{-1} in CW 205 and root rot from 0.00±0.00 in CW 136; CW151; CW 164; CW 165; CW 168; CW 187; CW 201; CW 205 respectively to 1.27±1.84 in CW 179. Protein content was not included in this family evaluation due to the prohibitive cost involved in the analysis for protein samples.

Principal component analysis showed that the first three PCs were important and explained 85.04% of the total variation in yield (Table 3.4). Variables in PC1 were positively correlated, indicating that all contributed to yield. PC1 had an eigenvalue of 3.23 and accounted for 53.91% of the variation. This represents an equivalent of at least four variables and indicated that root weight, roots per plant, commercial roots, and yield were important contributing variables. PC2 had an eigenvalue of 0.97, contributing 16.24% of the variation and had harvest index and dry matter content as the main contributing factors. PC3 had eigenvalues of 0.89, indicating that only a single variable (harvest index: 0.82) was contributing in this case.

Harvest index and dry matter content were important in at least two PCs while root weight, roots per plant, commercial root, and yield were important in one of the PCs (Table 3.4), indicating their relative importance to yield. In plotting the PC scores for individual traits in relation to the important PC axes, Figure 3.1 shows a clustering of root weight, commercial roots, roots per plant and yield, indicating that a relationship exists among these traits.

Table 3.3: Means and standard deviation of root quality characteristics of F_1 inter-specific hybrids of cassava evaluated in CIAT in May 2004

Families	Pedigree	Variables					
		Root No	ComRt[a]	HI[b]	DMC[c]	Yield	Rtrot[d]
CW 99	CW 30-29XOW 280–1	31.77 ± 15.41	12.68 ± 8.29	0.36 ± 0.10	29.31 ± 3.13	26.68 ± 14.13	0.28 ±0.74
CW 122	CW 30–73XOW 181–2	20.25 ± 12.24	8.30 ± 7.62	0.31 ± 0.13	31.45 ± 5.83	25.50 ± 16.96	0.00 ±0.00
CW 125	CW 30–73XOW 280–1	26.90 ± 13.16	11.10 ± 7.37	0.41 ± 0.15	30.74 ± 2.72	20.04 ± 13.78	0.10 ±0.32
CW 136	CW 30–87XOW 280–1	25.13 ± 15.01	9.04 ± 7.79	0.41 ± 0.09	30.86 ± 4.59	23.49 ± 14.88	0.00 ±0.00
CW 146	CW 47–3XOW 280–1	30.81 ± 19.57	14.19 ± 9.99	0.41 ± 0.11	30.97 ± 6.33	30.14 ± 21.99	0.29 ±0.68
CW 151	CW 48–1XOW 280–1	3.89 ± 5.16	5.00 ± 1.66	0.39 ± 0.14	29.13 ± 3.67	16.91 ± 17.12	0.00 ±0.00
CW 164	CW 56–5XOW 280–1	17.90 ± 13.27	7.38 ± 6.95	0.39 ± 0.12	29.28 ± 3.79	16.69 ± 13.39	0.00 ±0.00
CW 165	CW 56–5XOW 284–1	7.67 ± 7.51	3.67 ± 3.21	0.53 ± 0.11	27.69 ± 0.65	12.33 ± 11.93	0.00 ±0.00
CW 168	CW 60–7XOW 280–1	25.33 ± 17.36	12.00 ± 8.91	0.39 ± 0.12	33.34 ± 3.69	19.32 ± 13.63	0.00 ±0.00
CW 179	OW 132–2XMTAI-8	26.18 ± 17.24	6.00 ± 4.02	0.41 ± 0.06	25.19 ± 6.07	25.66 ± 17.89	1.27 ±1.84
CW 187	OW 181–2XCW 48–1	7.09 ± 6.05	4.00 ± 4.38	0.34 ± 0.13	27.44 ± 4.82	12.87 ± 7.58	0.00 ±0.00
CW 198	OW 230–3XCW 30–65	17.77 ± 11.69	6.86 ± 6.84	0.35 ± 0.09	29.41 ± 4.36	16.66 ± 12.56	0.09 ±0.29
CW 201	OW 230–3XCW 56–5	11.00 ± 4.24	1.00 ± 1.41	0.30 ± 0.04	26.09 ± 9.57	6.50 ± 0.35	0.00 ±0.00
CW 205	OW 231–3XMTAI-8	25.00 ± 20.49	15.25 ± 17.25	0.56 ± 0.09	25.32 ± 3.70	53.75 ± 40.66	0.00 ±0.00
CW 208	OW 280–1XMTAI-8	30.29 ± 18.71	10.83 ± 9.96	0.43 ± 0.13	29.81 ± 4.95	20.70 ± 16.17	0.02 ±104

[a]Commercial roots; [b]Harvest index (0 - 1); [c]Dry matter content (%); [d]Root rot

Root weight was highly significantly correlated with roots per plant and yield ($p \leq 0.0001$: Table 3.5). Yield was highly significantly correlated with root weight, roots per plant and harvest index ($p \leq 0.0001$; $p \leq 0.001$ respectively). Protein content was negatively correlated with dry matter content ($p \leq 0.01$) and positively correlated with roots per plant ($P \leq 0.01$).

The variation of protein content from the roots of the CW 198 family ranged from 3.68% to 11.20% crude protein. Table 3.6 shows the range of genetic variation in the protein content from the family CW 198. A best selection scheme according to the CIAT selection index procedure based on genetics, and interaction, was used, putting more emphasis on protein content in the root. In this early evaluation stage, eliminating inferior phenotypes (Kawano *et al.*, 1998) is more beneficial than selecting superior phenotypes.

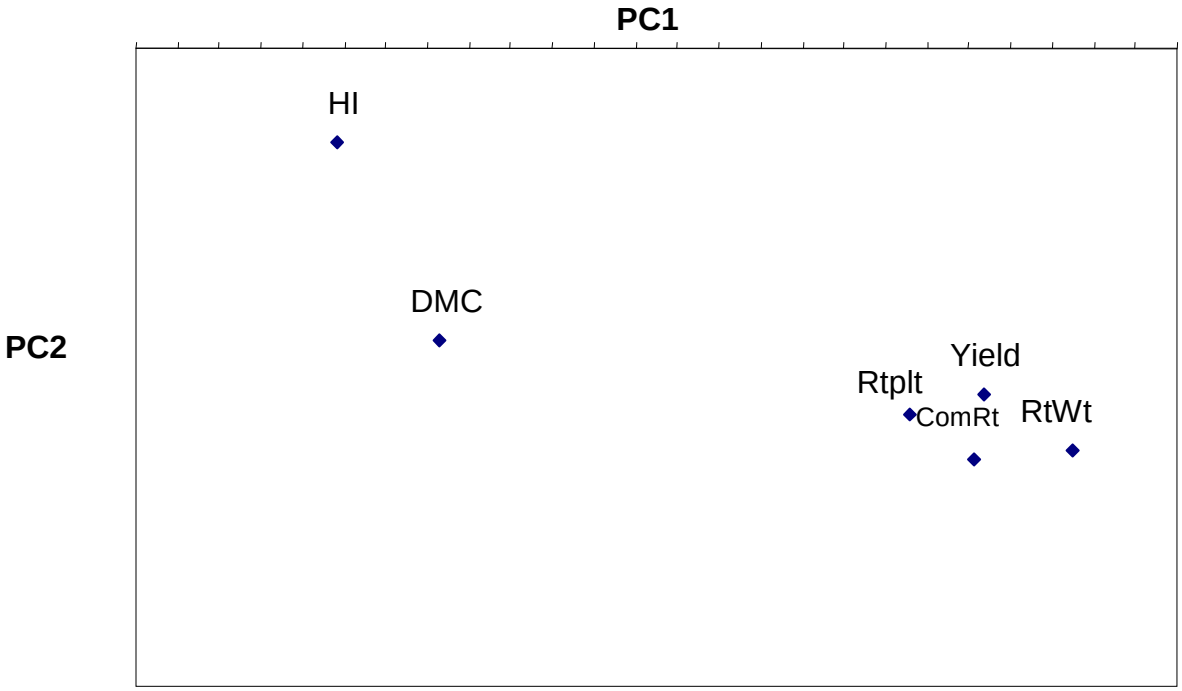
All of the above information was helpful in selecting a clone from a family with high protein content, average dry matter content, and high disease resistance to be used in the backcrossing of another generation with a cassava parent with high dry matter content, which is adapted to different agro-ecological zones.

Figure 3.2 shows the number of families that were analysed for crude protein content during the two planting seasons with the average percentage protein content and standard deviation. A total of 15 families were used with average protein content ranging from 5.01% to 8.02% and standard deviation ranging from 0.40 to 3.20. Dry matter content across the families ranged from 15.50% to 71.99%.

Table 3.4: Principal component coefficients of the various traits with principles of the various yield related traits evaluated on 774 genotypes in the F_1 population of inter-specific hybrids of cassava

Trait	PC1[a]	PC2	PC3
DMC[b]	0.15	0.81	-0.55
RtWt[c]	0.50	-0.14	-0.03
Rtplt[d]	0.43	-0.14	-0.13
ComRt[e]	0.49	-0.09	0.02
HI[f]	0.19	0.52	0.82
Yield (ton ha^{-1})	0.50	-0.10	-0.03
Eigenvalue	3.23	0.97	0.89
Percentage total variance	53.91	16.24	14.89
Cumulative	53.91	70.15	85.04

[a]Principal component; [b]Dry matter content (%); [c]Root weight (kg); [d]Roots per plant; [e]Commercial roots; [f]Harvest index (0 - 1)



HI = harvest index; DMC = dry matter content; Rtplt = roots per plant; RtWt = root weight; ComRt = commercial root

Figure 3.1: Plot of first and second principal components of various traits with principles of the various yield related traits evaluated in F_1 population of inter-specific hybrids of cassava

Table 3.5: Phenotypic correlation for selected F_1 (CW 198-11: 56 genotypes) for yield related traits and protein content recorded from inter-specific hybrids of cassava at harvest in CIAT Palmira, Colombia in May 2004

	Variables				
	DMC[a]	RtWt[b]	Rtplt[c]	HI[d]	Yield
RtWt	0.26ns				
Rtplt	0.03ns	0.59****			
HI	0.02ns	0.37ns	0.24ns		
Yield	0.12ns	0.83****	0.78****	0.43***	
PC[e]	-0.34**	0.01ns	0.33**	0.13ns	0.25ns

[a]Dry matter content (%); [b]Root weight (kg); [c]Roots per plant; [d]Harvest index (0 - 1); [e]Protein content (%);
** p≤0.01; *** p ≤0.001; **** p≤0.0001; ns=not significant

Table 3.6: Analysis of variance for protein content in roots from F_1 CW 198 inter-specific hybrids of cassava evaluated between 2002 and 2004 at CIAT, Palmira, Colombia

Sources of variation	Degrees of freedom	Sum of squares	Mean squares
Clone	11	2.19	0.199*
Error	10	0.46	0.045
Corrected	21	2.65	
Total			

R-square = 0.83; CV=8.81; Data were transformed by the √ (% protein) function, * p≤0.05

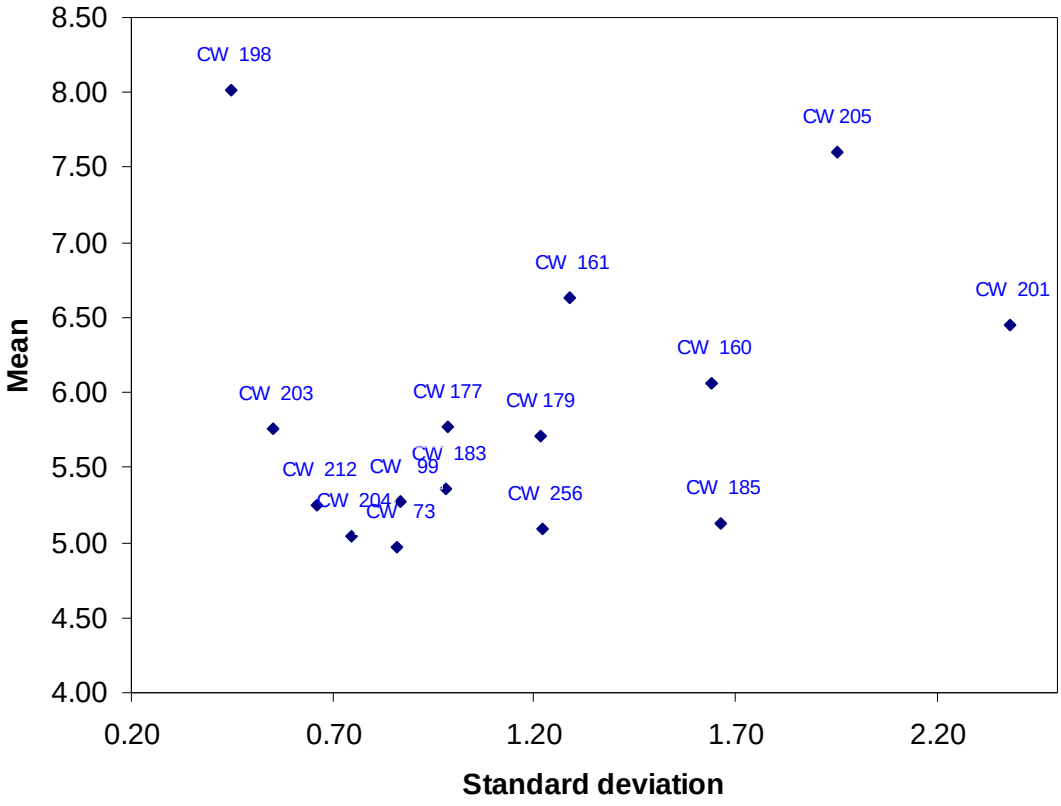


Figure 3.2: Plot of mean and standard deviation of percentage protein evaluated in the F_1 inter-specific hybrids of cassava

3.4 Discussion

The use of wild relatives for gene improvement is beneficial for modern agriculture, providing breeders with a broad pool of potentially useful genetic resources (Prescott-Allen and Prescott-Allen, 1986; 1988; Hajjar and Hodgkin, 2007). Wild relatives have been used to improve crops like sugar cane (Plucknett *et al.*, 1987), and tomatoes (Tanksley and McCouch, 1997). Results presented here revealed that protein genes in the root from the cassava progenitor have been introgressed into the F_1.

The overall dry matter content reported by Jaramillo *et al*. (2005), Ojulong (2006) and Ojulong *et al*. (2008a) for cassava were low compared to the highest value of 55.35% that was reported here for one of the F_1 families. Regarding protein content in the roots, Buitrago (1990) reported a mean crude content of 3.06%. However Chávez *et al*. (2005) reported clones of cassava with a higher protein content, ranging from 5.75% to 8.31% which are much higher than what was reported by Ceballos *et al*. (2006) from the evaluation of 149 clones for protein content that ranged from 0.95% to 6.42% but less than what was obtained from this study with the F_1 which had 11.25% protein content in the root.

Over 60% of the total families evaluated in this study had high resistance to root rot (*Botryodiplodia theobromae*), a disease that is prevalent in CIAT, Colombia where this evaluation was conducted. This showed that the gene that conferred this resistance from the wild relatives had been introgressed into the F_1. In a study conducted by Hajjar and Hodgkin (2007) they reported that wild relatives were a good source of resistance to diseases from where the resistance genes had been introgressed into cassava. But Onyeka *et al.* (2005) reported a high susceptibility of improved genotypes and African landraces of 83.6% and 83.1% respectively. Ojulong *et al*. (2008a) reported a high incidence of root rot ranging from a score of 0.1 ± 0.3 to 0.3 ± 0.4 on average per family of the cassava used in their experiments, which was high for cultivated cassava. However, for the inter-specific F_1 reported in this experiment, the root rot incidence was low.

The principal component analysis of yield quality traits revealed that PC1 explained 55.42% of the total variation, and PC2 and PC3 explained 26.70%. This represented an equivalent of six variables (dry matter content, root weight, commercial roots, roots per plant, harvest index and yield) that are important contributors.

The high positive correlation between yield, harvest index, root weight and roots per plant agrees with the results of other breeders (Kawano *et al*., 1998; Kawano, 2003; Okogbenin, 2004; Ojulong *et al*., 2008a). Contrary to the report of Ojulong *et al*. (2008a) that there is high correlation between yield and dry matter content, in this study, it was not the case.

Hopefully as introgression proceeds, there might be a significant correlation (Kawano *et al*., 1998). There was negative correlation ($p \leq 0.01$) between protein content and roots per plant and between dry matter content and protein, which is similar to the findings of Ceballos *et al*. (2006) suggesting that clones with high protein tend to have a lower level of dry matter content.

The analysis of variance results from these selected interspecific hybrids of the CW198 family provided strong evidence to support the genetic origin of protein content in the roots with sum of squares of 2.19 and coefficient of variance 8.8% which was greater than what was reported by Steel and Torrie (1960), Gomez and Gomez (1984), and Ceballos *et al*. (2006). The overall family means used against family standard deviation revealed accession CW 198 as having the highest protein content with the smallest standard deviation. This favoured selection of this family for further study and this family was used as a parent in the first backcross generation.

Results from this study are promising for the improvement of cassava root protein through introgression from its wild progenitor. Correlations among different traits suggested associations that can be used to facilitate cassava genetic improvement through traditional recurrent selection (Dudley, 1974) with emphasis on protein (CIAT, 2003).

Chapter 4

Embryo rescue establishment and micropropagation of a backcross family of cassava derived from a high protein inter-specific hybrid of *M. esculenta* ssp *flabellifolia*

4.1 Introduction

Manihot esculenta ssp *flabellifolia* is an important source of genetic variability for high protein content in roots for cassava breeding (CIAT, 2002). As demand for good cassava root quality is intensified, cassava breeders need to overcome the problem of poor germination rates and low multiplication rates which still cause bottlenecks in the population development of cassava (Jennings, 1963; Bryne, 1984; Ceballos *et al.,* 2004; Okogbenin *et al.,* 2008).

Improvement of germination rate holds the greatest promise for resolving some of the compelling problems of cassava population development. Germination of cassava seeds has been enhanced by various procedures including scarification, treatment by heat and/or acid, exposing seeds to red light, and more recently by embryo culture of mature and immature seeds (Nartey *et al.*, 1974; Kawano *et al*., 1978; Biggs *et al*., 1986; Roca *et al*., 1988; Ng, 1989; Fregene *et al*., 1999). Embryo culture provides a simple technique for breaking seed dormancy and ensuring a fairly uniform germination rate (Biggs *et al.*, 1986).

Producing breeding or mapping backcross derivatives from embryo rescue and hardening *in vitro* cassava plantlets in a greenhouse was found to be an effective way to ensure uniform germination and multiply healthy plants to establish good field experiments (Biggs *et al.*, 1986; Szabados *et al.,* 1987). The objective of this study was to use embryo rescue to generate sufficient planting material and reduce the time it will take for replicating trials of the protein mapping population.

4.2 Materials and methods

Selected F_1 inter-specific hybrids (the selection criteria used were mean percentage protein content and standard deviation of the F_1 family and individual hybrids as discussed in Chapter 3) were re-evaluated for protein content. Individuals with high protein content from families with low standard deviation and high average protein content were selected and used as parents for backcrossing. F_1 (CW 198 - 11) inter-specific hybrids were crossed to MTAI - 8, an elite cassava genotype from Thailand, that is an excellent parent.

Parents for crossing were planted in the crossing block at CIAT, Palmira in single rows of 1 m between plants and 2 m between rows, to facilitate movement during crosses. Genotypes were monitored daily for onset of flowering. At the onset of flowering plants were inspected every morning for flowers about to open, and such flowers were enclosed with transparent bags, to prevent contamination from stray pollen on opening. Pollen was collected in plastic bottles (perforated), from MTAI - 8 male parents. At around 11.00 am when flowers were open, the transparent bags were removed, and pollen from the MTAI - 8 parent dusted on the stigma of CW 198 - 11 (Figure 4.1). All non-mature flowers were removed from the inflorescence which was then tagged with a label containing the pedigree, number of female flowers pollinated, and date of pollination. The bag was removed to allow the fruit to develop freely. Four weeks after pollination, fruits were covered with bags made of gauze to collect the fruits that explode at maturity (Jennings and Iglesias, 2002). Seed was collected from the field after 60 days. They were cleaned, and viable seeds identified and germinated *in vitro*.

Seeds from the B_1P_2 (671) material were tested for viability by soaking in water. After the viability test, embryos excised from the 495 viable seeds were cultured *in vitro* using a 17N culture medium. The culture medium was supplemented with 0.01 mg l^{-1}NAA, 0.01 mg l^{-1} GA_3, 1.0 mg l^{-1} thiamine-HCl, 100 mg l^{-1} inositol, 2% sucrose, 0.7% agar (Sigma Co.) and 25 mg l^{-1} of a commercial fertilizer containing NPK (10:52:10). The medium had a pH of 5.7 to 5.8 (Roca, 1984).

Embryo culture was done at the tissue culture laboratory of cassava genetics of CIAT, Cali, Colombia in January 2005 as follows: mature seeds were treated with concentrated sulphuric acid for 50 minutes, washed thoroughly, and rinsed with water, before soaking in water for 30 minutes. Seeds were surface sterilised by immersion in 70% alcohol for 5 minutes followed by immersion in 5% sodium hypochlorite and Tween for 20 minutes, and rinsed three times with sterilised water.

Under aseptic conditions, seeds were split along the longitudinal axis and embryos removed by means of sterile forceps and a scalpel. Excised embryos were placed radicle down in the prepared 17N medium. Embryo cultures were incubated in darkness for three days to promote radicle growth and transferred to growth chambers with a 12 hour photoperiod. Plantlets remained in the growth chamber for six weeks before they were transferred to the greenhouse for intensive post flask management.

For the post flask management, plants were transferred from test tubes to a mixture of soil and sand in black polythene bags under protection from direct sun and insects. Optimal conditions were achieved using 7 cm to 10 cm polythene bags containing a sterilised mixture of three parts soil with one part of fine sand. In the greenhouse, to reduce the shock, plants were maintained in a humidity chamber created with a large transparent polythene bag for seven days with regular watering and addition of micro- and macro-nutrients at intervals. Spraying of the leaves against fungi was done intermittently for four weeks. After this time, plants were hardened and transplanted in the field in Corporación Colombiana de Investigación Agropecuaria (CORPOICA), Palmira in November 2005 and March 2006.

The B_1P_2 family was transplanted to the field in two batches in November 2005 and March 2006 to guarantee that sufficient plants from each genotype will be obtained from the hardening and field establishment process. This first round of field establishment was to generate the stakes for the first replicated trials. The field layout was 1.6 m x 0.8 m between and within rows for the first single row trial (SRT).

Plantlets were arranged genotype by genotype in the field. Plantlets were soaked in "Terravite" (fertilizer in solution) to increase recovery after hardening. In both fields, regular irrigation was done during the first three weeks, after which rainfall served as source of water supply. Hand weeding was done around each plantlet and herbicide was applied to control weeds. Immediately after planting, both foliar and soil nitrogen fertiliser was applied around plants to boost them after hardening.

The CIAT system was adopted to name the new genotypes from the tissue culture, with the first plantlet assuming number one and the rest subsequent numbers. Before harvesting, there was flooding in the first field (Figure 4.2) which made yield data recording impossible. In the second field, each genotype was harvested and harvestable biomass divided into storage roots, and vegetative biomass, comprising leaves and stems. Roots were weighed to obtain fresh root yield. Roots which would pass for sale in the local supermarkets were selected and counted to give number of commercial roots.

Harvest index was calculated by dividing fresh root yield by total biomass. Percentage dry matter content (DMC) of the roots was estimated using the standard CIAT procedure (Chapter 3: Kawano *et al*., 1987; Jaramillo *et al*., 2005). Dry yield was derived as a product of fresh root yield and dry matter content.

Data obtained was subjected to simple analysis (SAS, 2002). Whitefly infestation was scored on a scale of 1 to 5 (when the plant was clean, it was scored 1 and when it was heavily infested, it was scored 5).

Figure 4.1: The pollination processes of the B_1P_2 inter-specific hybrids of cassava for the establishment of the *in vitro* plantlets in the field

Figure 4.2: A flooded field of B_1P_2 interspecific hybrids of cassava germinated from the *in vitro* plantlets at CORPOICA, Palmira during the 2006 planting season

4.3 Results

A total of 671 seeds from the crosses between CW 198 - 11 and MTAI - 8 were produced, of which 495 (73.77%) passed the seed viability test (flotation in water). Embryonic axes of these seeds were excised and cultured. Three hundred and twenty-eight embryos (66.00%) germinated after four days. The B_1P_2 account of percentage germination of embryonic axes from the seeds is provided in Table 4.1a.

Due to heterozygosity of cassava, each seed is a different genotype. Depending upon the development of cultures, at least one single node cutting was obtained from each shoot, providing a multiplication rate of 1:4 after four weeks of culture. Growth and development rate of the plantlets was high (85.55%), the rate of establishment in the field was equally high (98.89%), which resulted in vigorous plants that produced sufficient material for replicated planting. The percentage survival and establishment is provided in Tables 4.1b-c.

The highest yield at CORPOICA (2007) was recorded in B_1P_2 - 11 (25.00 ton ha^{-1}), while the highest dry root yield was recorded from B_1P_2 - 189 (7.82 ton ha^{-1}, Table 4.2).

Harvest index estimates ranged from 0.07 (B_1P_2 - 89) to 0.87 (B_1P_2 - 220). Dry matter content (%) ranged from 17.22% in B_1P_2 - 292 to 70.28% in B_1P_2 - 317. The highest root weight was recorded for B_1P_2 - 11 (0.62 ton ha^{-1}). The average commercial root number was 0.91 with genotype B_1P_2 - 189 having the highest number of nine roots. There were a relatively high number of roots per plant, on average 3.90, with genotype B_1P_2 - 289 having the highest number of 10.

Table 4.3 shows that commercial roots were significantly correlated ($P \leq 0.0001$) with root weight, roots per plant, fresh root yield, and dry root yield but it had no association with harvest index. Root weight was significantly correlated ($P \leq 0.0001$) with fresh root yield and dry root yield. Roots per plant were highly correlated ($P \leq 0.0001$) with harvest index, fresh root yield, dry root yield but not significantly with dry matter content. Harvest index was

significantly correlated (P≤0.0001) with fresh root yield and dry root yield. Fresh root yield was significantly (P≤0.0001) correlated with dry root yield.

Table 4.1a: Seed generated from the crosses between CW 198 - 11 X MTAI - 8 and resulting plantlets from the B_1P_2 backcross population of cassava

Cross	Pedigree	Seed generated	Viable seeds	*In vitro* plants	% germination
B_1P_2	CW 198 - 11 X MTAI - 8	671	495	328	66.00

Table 4.1b: Resulting plantlets from the *in vitro* backcross population of cassava (*M. esculenta* Crantz) to the field phase

Cross	Pedigree	*In vitro* plantlets	Plantlets in the field	% survival
B_1P_2	CW 198 - 11 X MTAI - 8	2117	1811	85.55

Table 4.1c: Establishment of the backcross population of cassava (B_1P_2) from *in vitro* in the field

Cross	Pedigree	Plantlets in the field	Plants at harvest	% establishment
B_1P_2	CW 198 - 11 X MTAI - 8	1811	1791	98.89

Table 4.2: Simple statistics of agronomic variables evaluated in the B_1P_2 backcross population of cassava in Corpoica, Palmira March 2007

Variables	Minimum	Maximum	Average	Standard deviation
ComRt[a]	0.00	4.50	0.91	0.82
Rtwt[b]	0.04	0.62	0.21	0.09
Rtplt[c]	0.60	10.00	3.90	1.98
HI[d]	0.07	0.87	0.30	0.14
FRY[e]	0.39	25.00	6.70	4.89
DMC[f]	17.22	70.28	27.06	6.14
DRY[g]	0.07	7.82	1.84	1.44

[a]Commercial roots; [b]Root weight (kg); [c]Roots per plant; [d]Harvest index (0 - 1); [e]Fresh root yield (ton ha^{-1}); [f]Dry matter content (%); [g]Dry root yield (ton ha^{-1})

Table 4.3: Simple correlation coefficient matrix of yield components and incidence of whitefly symptoms for a cassava backcross population B_1P_2 evaluated in 2007 at Corpoica, Palmira, Colombia

	Wfly[a]	ComRt	Rtwt	Rtplt	HI	FRY	DMC
ComRt[b]	-0.04						
Rtwt[c]	0.00	0.63****					
Rtplt[d]	-0.05	0.66****	0.15ns				
HI[e]	-0.05	0.34ns	0.26ns	0.52****			
FRY[f]	-0.06	0.87****	0.67****	0.77****	0.49****		
DMC[g]	-0.17	0.23ns	0.17ns	0.28ns	0.13ns	0.28ns	
DRY[h]	-0.08	0.85****	0.65****	0.76****	0.46****	0.98****	0.40****

Whitefly (1-5); [b]Commercial roots; [c]Root weight (kg); [d]Roots per plant; [e]Harvest index (0-1); [f]Fresh root yield (ton ha^{-1}); [g]Dry matter content (%); [h]Dry root yield (ton ha^{-1}), ****$P \leq 0.0001$; ns=not significant

4.4 Discussion

In the “new biology” that has been flourishing for some time now, tissue culture is a basic technique for plant propagation and an excellent auxillary tool in the breeding of economically important plant species (Takeshita *et al.,* 1980). The preservation, conservation and distribution of germplasm are made possible using tissues cultured *in vitro* (Szabados *et al.,* 1987). The multiplication of inter-specific cassava hybrids through embryo axes can be considered a productive method of reducing seed dormancy, increasing germination rates, multiplication time, and reducing the breeding and selection cycle time, which is a major bottleneck in cassava breeding (Jain, 2006). Selection for uniform, healthy plants to be transferred from the *in vitro* phase to the field is possible at this stage, to give good planting material for replicated trials. Cassava plants can be regenerated via organogenesis from different types of explants which are immature zygotic embryo (Fregene *et al.,* 1999), cotyledons of somatic embryos (Li *et al.,* 1998), auxiliary buds and nodal explants (Konan e*t al.,* 1997), leaf explants (Mussio *et al.,* 1998), and through improvement of shoot organogenesis with silver nitrate (Zhang *et al.,* 2001).

The rate of establishment per genotype is usually high. For cultivated cassava, Fregene *et al*. (1999) demonstrated that, of 47 seeds germinated from mature fruits by culture, 91% germination was recorded after two days, but for inter-specific hybrids recording a 66% germination rate is a huge success, considering the level of seed inhibition from seed germination. In addition, Fregene *et al*. (1999) demonstrated that depending upon the development of cultures, at least one single node cutting was obtained from each shoot, providing a multiplication rate of 1:3 after four weeks which agreed with our finding of 1:4 ratios after four weeks.

The highest dry matter content recorded in this evaluation was higher (70.0%) than that reported by Rajendran and Hrishi (1982), of 66.4% and Magoon *et al*. (1973), of 47.2%. There was no association between fresh root yield and dry matter content, which Kawano *et al.* (1998) also observed at earlier stages of selection. They came to the conclusion that fresh

root yield and dry matter content can be handled largely as independent characters. This lack of association between fresh root yield and dry matter content is in agreement with findings reported by Ojulong (2006).

The usefulness of embryo culture, notably the rescue of inter-specific hybrids by culture of immature embryos (Raghavan, 1985; Mejia-Jimenaz *et al*., 1994) and mature embryos (Fregene *et al*., 1999) has been emphasised. It has been established from this study that this can be extended to the establishment of cassava populations, where low germination rate that prevents rapid establishment of a population trial, is a bottleneck in cassava breeding**.**

The use of tissue culture at this stage of the work has been tremendous helpfull in overcoming dormancy, which is common to wild relatives and its inter-specific hybrids that always result in non-uniform germination at the seedling stage and reduced the long germination period of the seeds. This is a novel contribution of this technique, which helped in the selection of good viable tissue culture materials that were sent to the field for the replication trial of the mapping population.

Chapter 5

Evaluation of protein content, post harvest physiological deterioration, and yield traits in a B_1P_2 family derived from an inter-specific hybrid with *M. esculenta* ssp *flabellifolia*

5.1 Introduction

Cassava has enormous potential to reduce hunger and malnutrition for millions of people that live on cassava as food security crop. Wild relatives of cassava have become a source of improving the crop by introgressing useful genes from it (Fregene *et al.,* 2007). One of the bottle necks of cassava is its low protein in the root, reduced shelf-life, and increment of the yield quality of the existing cassava germplasm (Fregene *et al.,* 2006).

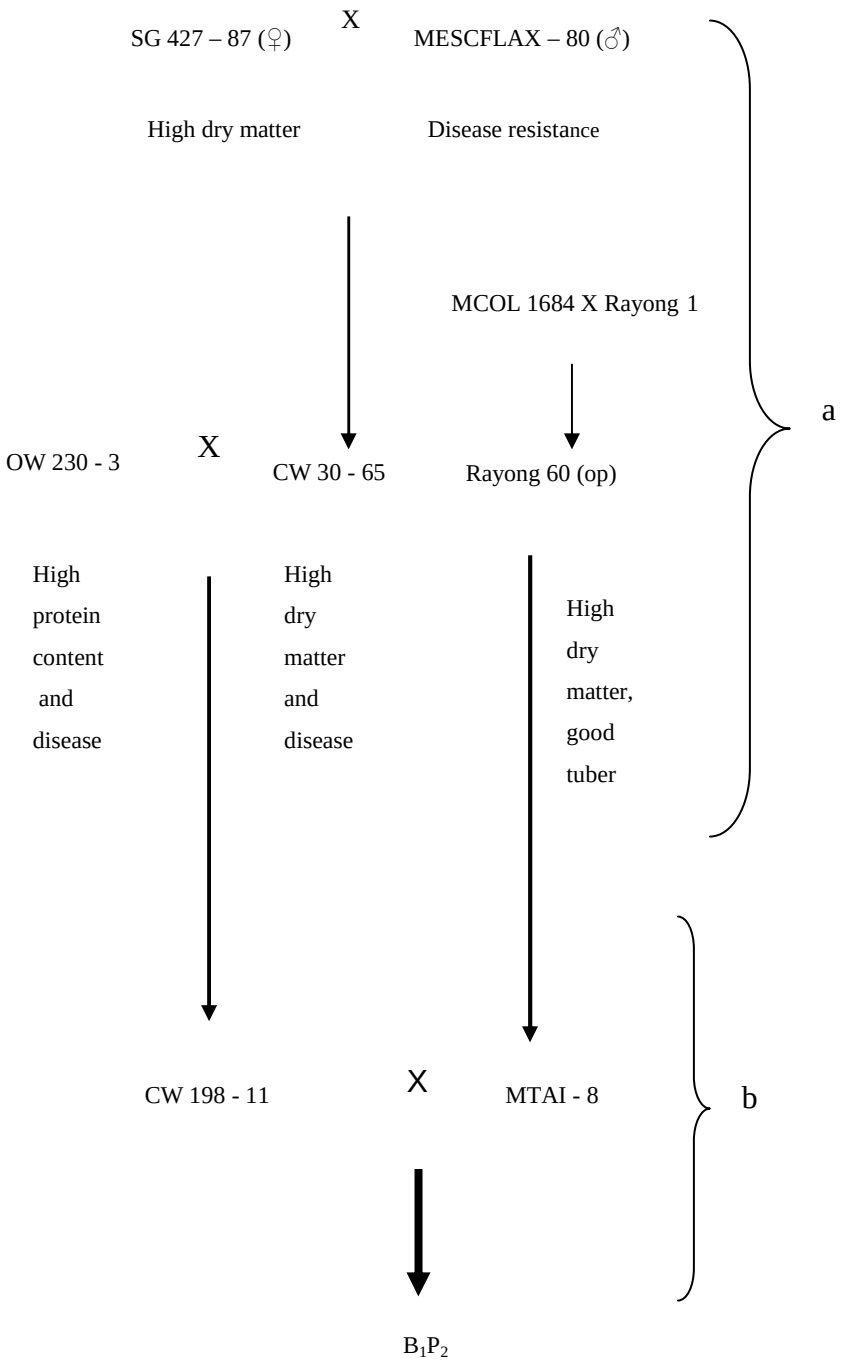
In general, breeding programmes seek to improve crop productivity, widen the genetic base, and maintain its adaptation to specific agro ecologies. The potential for genetic improvement of cassava has been demonstrated and progress made in increasing yield potential and stability (Ngoan *et al.,* 1995; Kawano, 1998). However, world mean yields for cassava are still far below the yield potential. Despite the progress already made by breeders, additional gains in productivity are demanded at a faster pace because of demographic pressures, changes in agricultural practices, biotic and abiotic stress, and consumer preferences. Other root quality traits relevant to different cassava breeding programmes world-wide are the cyanogenic potential in the root (Dixon *et al*., 1994a; Balyejusa Kizito *et al.,* 2007), early bulking capacity (Okogbenin and Fregene, 2002), post harvest physiological deterioration (PPD) (Sánchez *et al.,* 2005), and high protein content in the roots (Fregene *et al.,* 2006). Unfortunately, the genetic variability for the latter two traits is relatively small in *M. esculenta* and therefore, inter-specific crosses with other *Manihot* species are necessary to introgress useful alleles from them (Ceballos *et al*., 2004). Wild relatives of cassava are

known sources of resistance genes to virtually all cassava pests and diseases as well as PPD and high root protein content (CIAT, 2002).

Cassava cultivars are sometimes deficient in some economically important characters such as resistance to pests, diseases, and drought and have low protein content (Nassar and Dorea, 1982; Nassar and Grattapaglia, 1986) due to the bottle neck that occurred during domestication. Lost genes can be restored to the gene pool of the cultigen by inter-specific hybridisation with wild relatives which possess these genes (Nassar *et al.,* 1986). Wild species of cultivated crops have been frequently used as an important source of genetic diversity and have been employed effectively in a variety of breeding programmes (Hahn *et al*., 1990; Fregene *et al*., 1994; Tanksley and McCouch, 1997; Gupta and Sharma, 2007; Hajjar and Hodgkin, 2007; Okogbenin *et al.,* 2007). The objective of this study was to introgress genes from wild progenitors of cassava for increased root protein and dry matter content to commercial cassava.

5.2 Materials and methods

An inter-specific F_1 hybrid CW 198 - 11 was earlier developed at CIAT, Cali, Colombia (CIAT, 2002) by genetic crosses of OW 230 - 1 (FLA 441 - 5 with protein content of 10.45%) and CW 30 - 65, an inter-specific hybrid between an improved cassava variety SG 427 - 87 and an accession of *M. esculenta* ssp *flabellifolia*. The inter-specific cross was 'backcrossed', in the sense of another cross to cassava, to MTAI - 8 to generate a B_1P_2 family with 225 individuals. The wild maternal grand parent of B_1P_2 has, in addition to high protein content in the roots, high dry matter and resistance to various cassava diseases (African cassava mosaic disease, cassava bacterial blight, cassava anthracnose disease) and pests (hornworm, whiteflies). The male parent (MTAI - 8) is a successful elite Thailand cultivar with high dry matter content, good tuber formation, and cream coloured roots from the breeding programme at the Thailand Agricultural Research Centre. The pedigree of these parents is illustrated in Figure 5.1.



a = process of introgression of high protein content and disease resistance to F_1 progenies; b = process of backcrossing of the F_1 into cultivated cassava

Figure 5.1: Pedigree of the planting materials used for the B_1P_2 family

Embryo axes of sexual seeds from the B_1P_2 family were cultured *in vitro* and micro-propagated to produce six to eight plantlets per genotype. These were transferred to the screen house in 2005, and after 60 days planted in the field at CORPOICA field experiment station, Palmira, Colombia. At 10 months after planting, 1 - 2 roots were 'milked' from each genotype and used to evaluate protein content as described in Chapter 4. At 10 months after planting (MAP), matured stem cuttings from the plants harvested at CORPOICA were used to establish a preliminary yield trial experiment made up of 225 genotypes, in a complete block design with three replicates of 12 blocks, eight plants per row. The field trial was conducted in CIAT - Palmira at plot number P^2N in 2006, at Palmira in Valle del Cauca Department (elevation 965 m, 3^o49'N, 76^o36'W), located in the mid altitude tropics of Colombia. The site has bimodal rainfall, although there are yearly variations, with peaks usually between March - June and October - December. The soil in Palmira is a fertile alluvial clay loam. Meteorological data at the location during experimentation are presented in Table 5.1.

The total area of the trial was 5989 m^2, comprising of eight plants per genotype, with border plants on the edges. Planting was on ridges at a spacing of 0.7 m (within rows) x 1.4 m (between rows). The plants were not fertilised or sprayed with insecticide, but weeded when necessary. Yield and quality traits were evaluated on the seven middle plants and means were calculated.

Table 5.1: Meteorological data at Palmira in 2006 and 2007

	Palmira	
Climatic factors	2006	2007
Precipitation (mm)	104.50	82.85
Evaporation (mm)	135.73	135.08
Radiation (MJ m^{-2})	17.68	16.86
Maximum temperature (^{0}C)	30.14	30.23
Minimum temperature (^{0}C)	19.32	18.94
Mean relative humidity (%)	76.79	76.72
Mean wind velocity (m sec^{-1})	56.58	58.96

Harvesting was carried out at 10 MAP. The seven inside plants in the row were harvested and their storage roots weighed to determine yield. Samples of roots from several plants of a single genotype were taken for dry matter content determination. There are two methods used in measuring the dry matter content in CIAT breeding program, the first one, specific gravity method was used in the previous chapter and the drying sample method was use here for further analysis of the sample for protein. The results from the methods have been compared and no variation in the final results. Dry matter content (DMC) assessment was done by peeling of the back of the fresh tuber and oven drying at 60^{o}C for 48 hours after which the weight difference between the fresh weight and dry weight was measured and the percentage dry was calculated. Percentage dry matter content was determined using the formula:

$$\%DMC = \frac{\text{Weight of the oven dried sample}}{\text{Weight of the fresh sample}} \quad X \quad 100$$

The dry root yield was calculated as follows: %DMC x fresh root yield. Harvested plants were assessed for number of storage roots per plant. The aerial part (stems and leaves) of the plants were weighed to determine fresh shoot weight. Harvest index was computed as the ratio of root yield to the total harvested biomass per genotype on fresh basis.

Two root quality traits, protein content and post harvest deterioration were analysed: For protein analyses, samples used to determine DMC were used and the same procedure as described in Chapter 3 was followed. Protein analysis of the roots was estimated using the Kjeldahl method (Skalar, 1995) as it was described in Chapter 3.

Primary deterioration of cassava results from an endogenous physiological process, independent of the presence of pathogens, and has been described as a wound response cascade gone awry (Wheatley, 1982). Three to seven roots were randomly picked per genotype and used to determine PPD. Evaluation for PPD was done at seven days after harvest. Immediately after harvest, 10cm - 15 cm sections were taken from each randomly picked root (Marriot *et al*., 1978; 1979). The distal or tail end of the cut roots was covered with a PVC film to prevent water loss. The proximal cut surface was exposed to low humidity conditions for seven days, the first time interval routinely used for PPD evaluation. At seven days after harvest, seven transverse sections were made at 2 cm intervals from the proximal end of the root section. Physiological deterioration manifests as discolouration of the vascular tissues and storage parenchyma. The extent of the vascular discolouration is a measurement of the susceptibility of the genotype to PPD. Each of the seven sections was scored on a scale of 0 to 10 (where 0 = 0% deterioration, 2 = 20% deterioration and 9 = 90% deterioration) and average percentage deterioration determination for each genotype was done.

Data analysis

Agrobase (2000), SAS (2002) and Sigmaplot 10.0 (2007) statistical programmes were used for analysis of variance, correlation, and frequency distributions of phenotypic classes. Only

genotypes which had complete data from the three replications were used. Since roots per plant, root weight and fresh and dry root yield data were not normally distributed, data sets were transformed by the square root method using the formula: $y = \sqrt{(x+0.5)}$. Percentage dry matter content and protein content were transformed by the square root method using the formula: $y = \sqrt{(x)}$, where y is the resulting transformation and x the data point.

The SAS correlation (proc corr.), univariate (proc univariate) and regression (proc reg) procedures were used to estimate correlation and regression coefficients between different parameters. Yield, yield components, and quality traits were subjected to simple ANOVA and estimates of broad sense heritability determined using Agrobase (2000).

Principal component analysis (Iezzoni and Pritts, 1991) was used to investigate the relevant traits contributing to the phenotypic variation among genotypes. Sigmaplot 10.0 was used to plot the histogram of different yield and quality components.

5.3 Results

A relatively high number of roots per plant was obtained (average 5.53), with genotype B_1P_2 - 251 having the highest number of 16.50. The average commercial sized storage roots were 1.20 with genotype B_1P_2 - 190 having the highest number of 9.00 commercial sized roots. Highest root weight was recorded for genotype B_1P_2 - 2. Recorded dry matter content ranged from 10.83 in B_1P_2 - 218 to 50.51 in B_1P_2-109. The highest fresh root yield was recorded in B_1P_2 - 2 (58.59 ton ha^{-1}), highest dry root yield was recorded in B_1P_2 - 2 (22.31 ton ha^{-1}) while the highest protein content was recorded in B_1P_2 - 248 (9.61%: Table 5.2).

Table 5.2: Range of values for agronomic traits of 225 progenies of a cassava backcross population in CIAT, Palmira in May 2007

Variables	Minimum	Maximum	Average	SD[a]	LSD[b]	Skewness
Rtplt[c]	0.16	16.50	5.53	2.47	2.47	0.66
ComRt[d]	0.00	9.00	1.20	1.44	11.94	2.21
Rtwt[e]	0.03	1.20	0.20	0.08	0.09	3.27
FRY[f]	0.26	58.59	8.97	5.91	5.42	1.98
DRY[g]	0.09	22.31	3.50	2.27	2.08	1.85
HI[h]	0.01	0.88	0.33	0.13	0.11	0.15
DMC[i]	10.83	50.51	39.34	4.14	5.29	-0.82
PPD[j]	0.00	72.57	13.92	14.86	2.53	1.81
PC[k]	0.77	9.61	2.71	1.06	11.44	0.87

[a]Standard deviation; [b]Least significant difference; [c]Roots per plant; [d]Commercial roots; [e]Root weight (kg); [f]Fresh root yield (ton ha^{-1}); [g]Dry root yield (ton ha^{-1}); [h]Harvest index (0-1); [i]Dry matter content (%); [j]Post harvest deterioration (%); [k]Protein content (%)

Simple correlations were performed among different yield and quality traits (Table 5.3). Dry root yield was highly correlated ($p \leq 0.0001$) with number of commercial sized storage roots, roots per plant, harvest index, root weight, and fresh root yield, while dry matter content and root weight were highly correlated with fresh root yield and harvest index, respectively. Harvest index was highly correlated with number of commercial sized storage root and roots per plant ($p \leq 0.0001$). Post harvest physiological deterioration was negatively correlated with roots per plant, harvest index and dry matter content. Protein content was highly significantly ($p \leq 0.0001$) negatively correlated with root weight, fresh root yield, and dry root yield, and negatively correlated with number of commercial sized roots, roots per plant, dry matter content and post harvest physiological deterioration.

Table 5.3: Simple correlation coefficient matrix of yield components and quality traits for a cassava backcross population evaluated in CIAT in 2007

	ComRt[a]	Rtplt	HI	Rtwt	FRY	DRY	DMC	PPD
Rtplt[b]	0.49****							
HI[c]	0.38****	0.59****						
Rtwt[d]	0.23ns	0.00ns	0.36****					
FRY[e]	0.55****	0.79****	0.69****	0.59****				
DRY[f]	0.54****	0.78****	0.66****	0.56****	0.97****			
DMC[g]	0.04ns	0.06ns	-0.14ns	0.01ns	0.07****	0.11ns		
PPD[h]	0.07ns	-0.15ns	-0.03ns	0.11ns	0.07ns	0.12ns	-0.27ns	
PC[i]	-0.08ns	-0.16ns	0.01ns	-0.35****	-0.36****	-0.36****	-0.09ns	-0.14ns

[a]Commercial roots; [b]Roots per plant; [c]Harvest index (0 - 1); [d]Root weight (kg); [e]Fresh root yield (ton ha^{-1}); [f]Dry root yield (ton ha^{-1}); [g]Dry matter content (%); [h]Post harvest deterioration (%); [i]Protein content (%); ****$p \leq 0.0001$; ns=not significant

The effects attributed to replication were highly significant (Table 5.4). Genotype contributed at least 60.67% to the total sum of squares in number of roots per plant, harvest index, root weight, fresh root yield, dry root yield, dry matter content, and protein content.

The relative contribution of the various traits to the genotype performance was explained by principle component analysis (Table 5.5). The first seven principal components explained most of the variation and accounted for 99.54% of the total variation. The first principal component accounted for 44.09% of the variation. Most of the variables were positively correlated, which is an indication that they are all contributors to total variation, except protein content. Based on the PC1 coefficients, five variables made a major contribution (commercial roots, roots per plant, harvest index, fresh root yield, and dry root yield). PC2 explained 13.99% of the total variation, with major contribution from root weight, dry matter content, post harvest physiological deterioration, and protein content. PC3 explained 13.99% of the total variation with major contribution from roots per plant, root weight, dry matter content, and post harvest physiological deterioration. PC4 explained 9.47% of the total variation and had major contribution from post harvest physiological deterioration, harvest index, and protein content. PC5 explained 8.39% of the total variation and had major contribution from root weight, dry matter content, and post harvest physiological deterioration. PC6 explained 6.68% of the total variation and had major contribution from commercial roots, and harvest index. PC7 explained 3.53% of variation with protein content contributing the most.

Table 5.4: Sum of squares table of yield parameters and quality traits in a cassava backcross population at CIAT, Colombia in 2007

Source of variation	df[a]	Sum of squares						
		Rtplt[b]	HI[c]	Rtwt[d]	FRY[e]	DRY[f]	DMC[g]	PC[i]
Replication	2	0.89	0.07	0.07	16.68	7.01	7.35	0.21
Genotype	214	101.92	9.18	0.80	352.20	123.00	38.14	38.10
Error	424	58.81	2.51	0.62	136.20	47.74	30.62	25.43
Total	638	161.62	11.76	1.50	505.09	117.76	76.12	63.76
F value		3.46****	7.30****	2.77****	5.37****	5.40****	2.94****	2.98****

[a]Degrees of freedom; [b]Roots per plant; [c]Harvest index (0 - 1); [d]Root weight (kg); [e]Fresh root yield (ton ha^{-1}); [f]Dry root yield (ton ha^{-1}); [g]Dry matter content (%); [h]Post harvest physiological deterioration (%); [i]Protein content (%); ****$p \leq 0.0001$

Relative importance of the contribution of various yield and quality related traits to yield and quality improvement was assessed using frequency of distribution in the genotypes (Figures 5.2a to i). The distribution frequencies of commercial root had a skewness value of 1.94, roots per plant had a skewness value of 0.47, harvest index 0.02, root weight 0.39, fresh root yield 0.64, dry root yield 0.85, dry matter content - 0.92, post harvest physiological deterioration 1.81, and protein content 0.96. All of these traits showed normal distribution.

Analysis of variance (Table 5.6) indicated that genotype and replication were highly significant ($p \leq 0.0001$) for all traits evaluated. Moderate to high heritability estimates were obtained for the different traits. In the B_1P_2 population, estimates for heritability in commercial roots was 0.58, roots per plant 0.71, harvest index 0.86, root weight 0.55, fresh root yield 0.76, dry root yield 0.76, dry matter content 0.49, post harvest physiological deterioration 0.69 and protein content 0.61. Heritability for dry matter content was the lowest.

Table 5.5: Principal component coefficients of the various traits with principles of the various yield and quality related traits evaluated in a cassava backcross population at CIAT, Colombia in 2007

Traits	PC1[a]	PC2	PC3	PC4	PC5	PC6	PC7
ComRt[b]	0.33	-0.05	0.27	-0.04	-0.08	0.84	-0.28
Rtplt[c]	0.38	-0.27	0.32	-0.29	-0.24	-0.23	0.25
HI[d]	0.36	-0.26	0.05	0.34	0.24	-0.36	-0.69
Rtwt[e]	0.29	0.37	-0.40	0.29	0.45	0.17	0.22
FRY[f]	0.49	0.03	0.02	-0.04	0.06	-0.09	0.27
DRY[g]	0.48	-0.04	0.11	0.00	-0.05	-0.07	0.26
DMC[h]	-0.06	0.33	0.65	-0.22	0.60	-0.09	0.04
PPD[i]	0.05	0.54	0.36	0.55	-0.50	-0.12	0.01
PC[j]	-0.18	-0.55	0.26	0.58	0.20	0.14	0.42
Eigenvalue	3.96	1.25	1.20	0.85	0.75	0.60	0.31
Percent total variance	44.09	13.99	13.99	9.47	8.39	6.68	3.53
Cumulative	44.09	58.08	71.47	80.94	89.33	96.00	99.54

[a]Principal component; [b]Commercial roots; [c]Roots per plant; [d]Harvest index (0-1); [e]Root weight (kg); [f]Fresh root yield (ton ha^{-1}); [g]Dry root yield (ton ha^{-1}); [h]Dry matter content (%); [i]Post harvest deterioration (%); [j]Protein content (%);

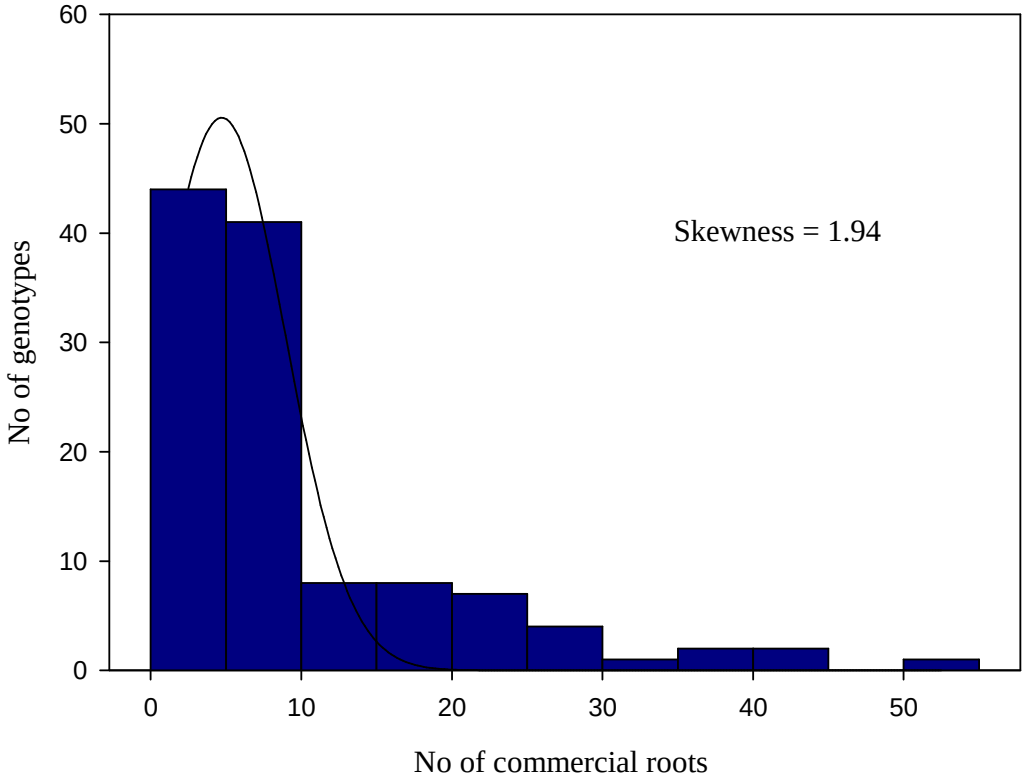


Figure 5.2a: Frequency distribution of number of commercial roots in a segregating cassava backcross population

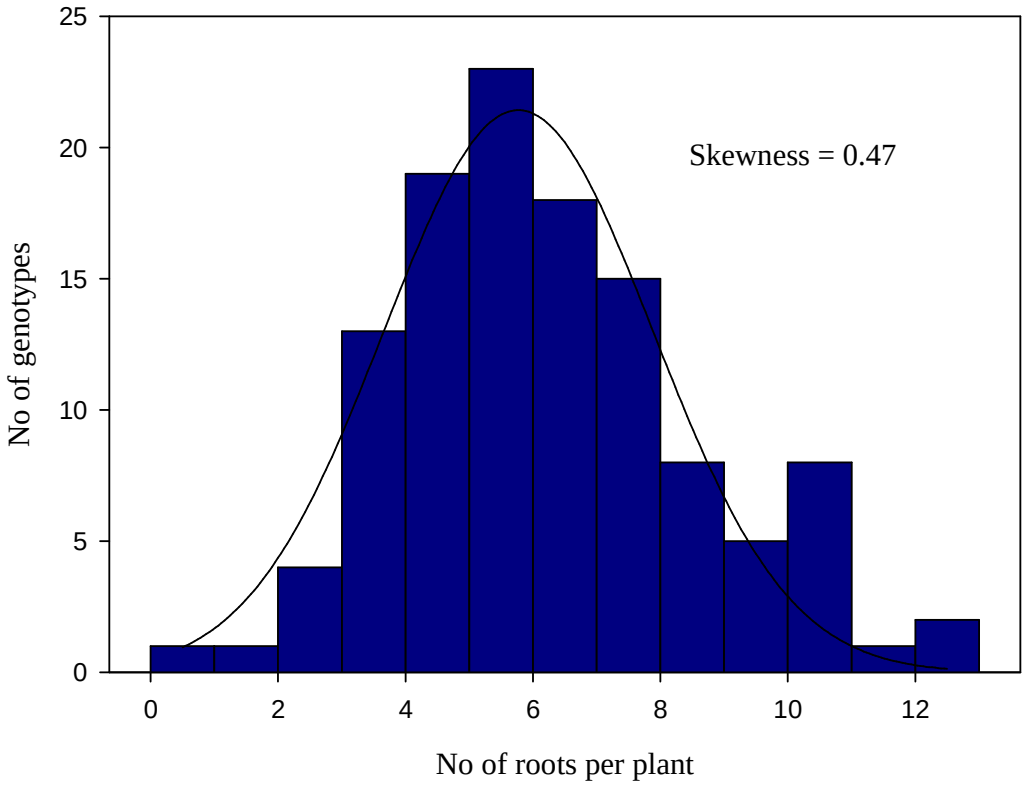


Figure 5.2b: Frequency distribution of the roots per plant in a segregating cassava backcross population

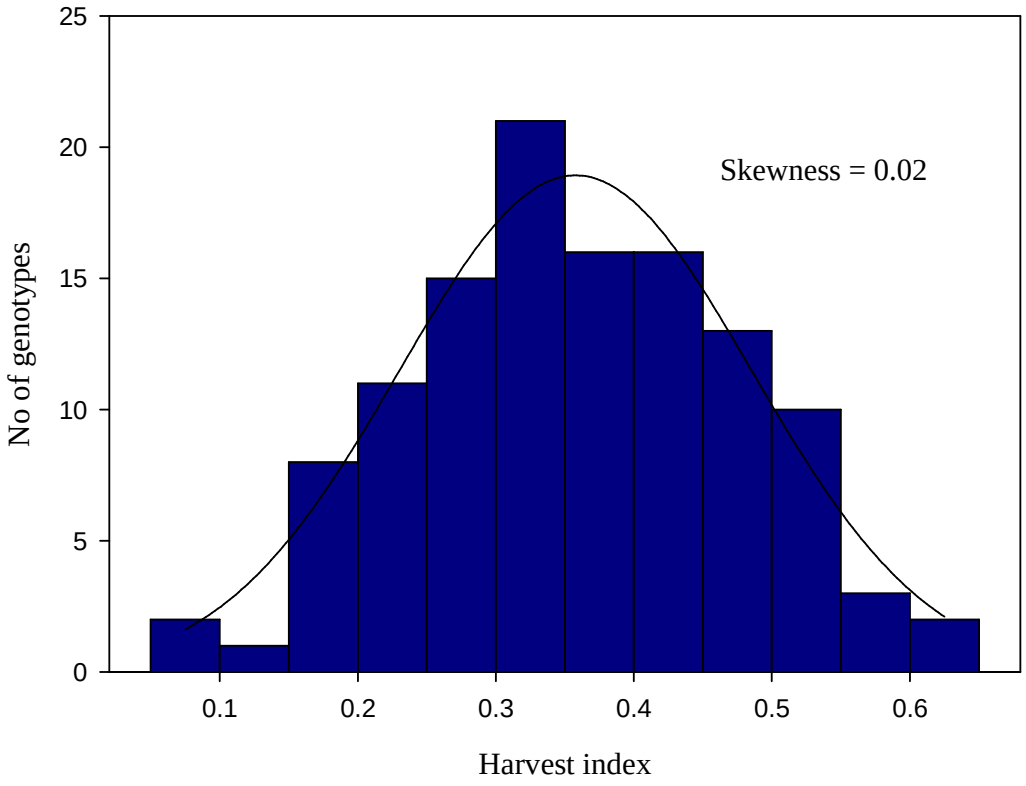


Figure 5.2c: Frequency distribution of harvest index in a segregating cassava backcross population

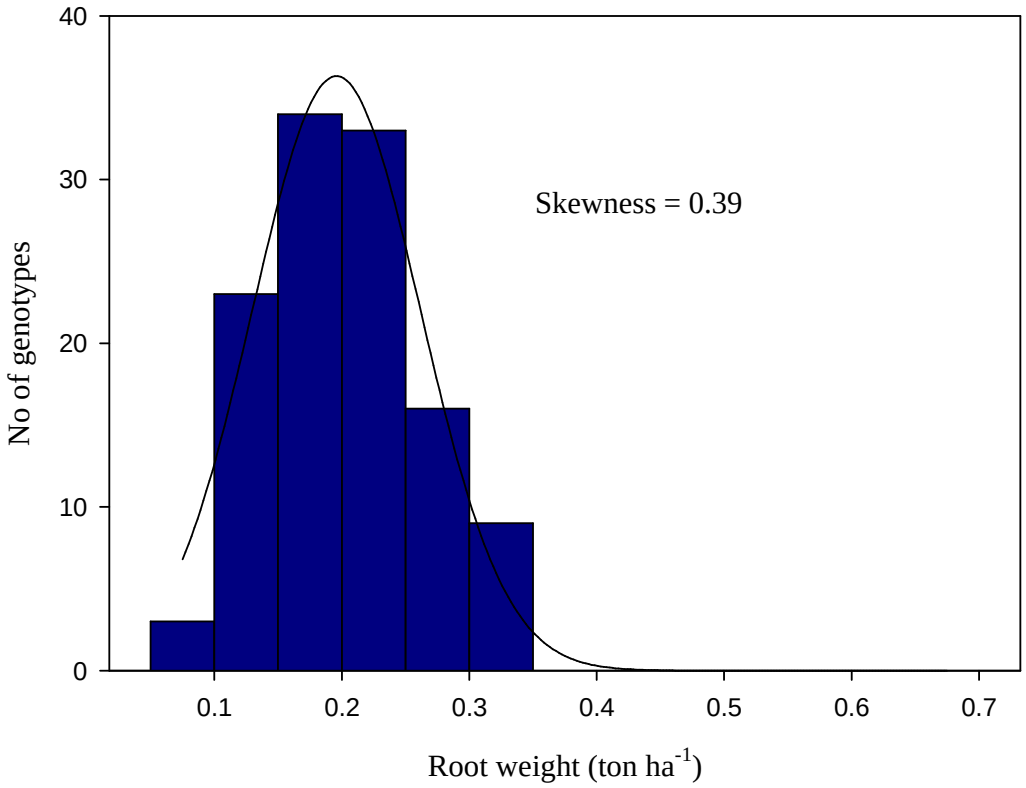


Figure 5.2d: Frequency distribution of root weight in a segregating cassava backcross population

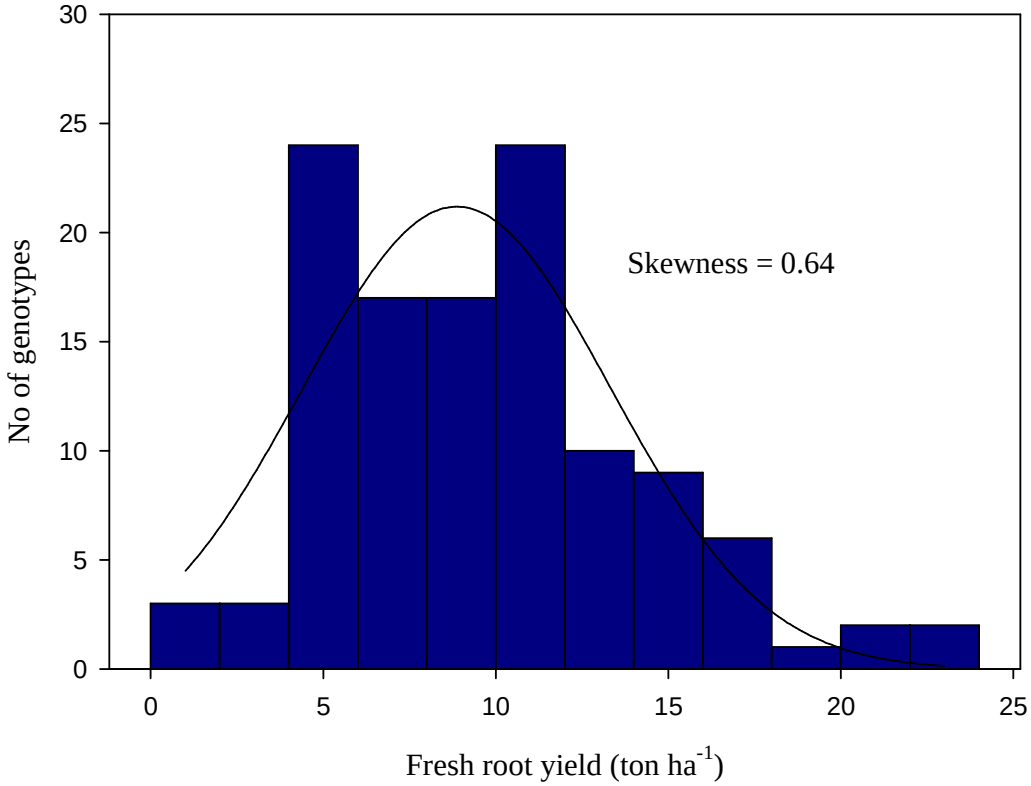


Figure 5.2e: Frequency distribution of fresh root yield (FRY) in a segregating cassava backcross population

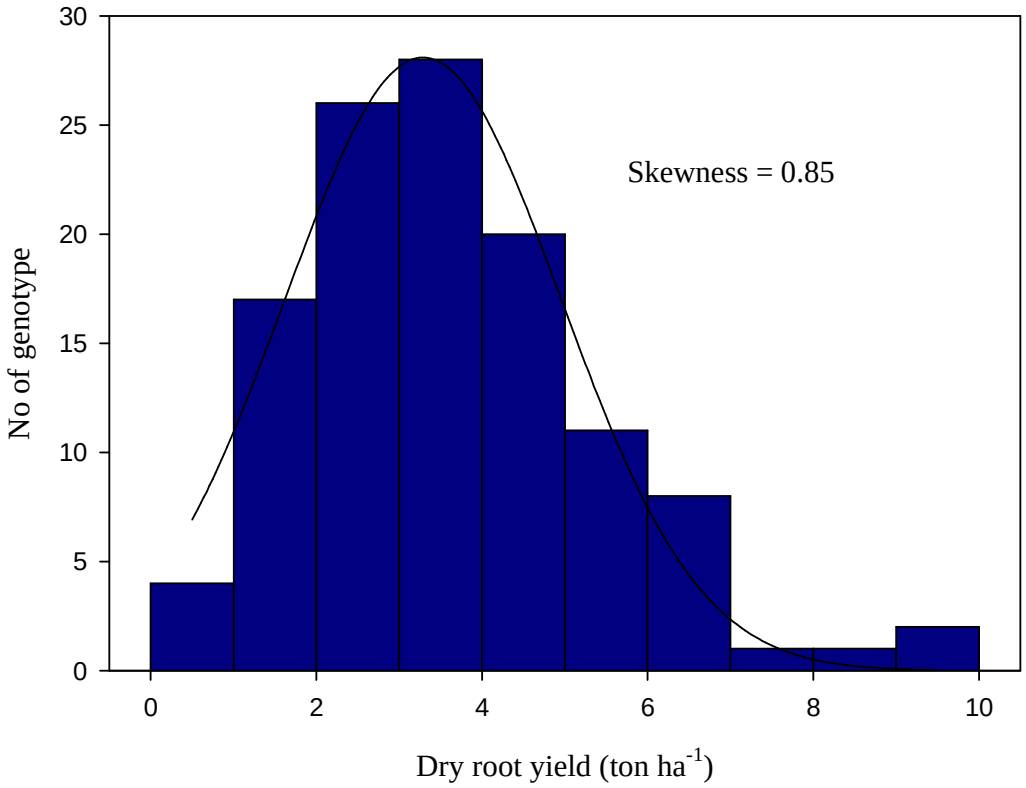


Figure 5.2f: Frequency distribution of dry root yield in a segregating cassava backcross population

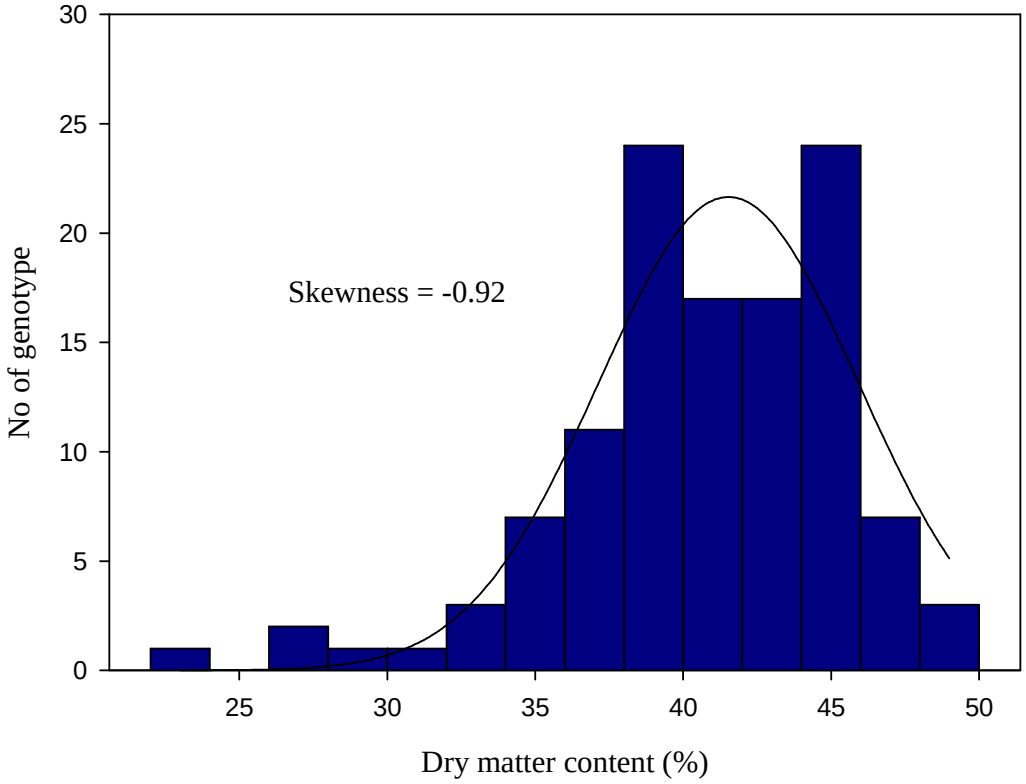


Figure 5.2g: Frequency distribution of the percentage dry matter content in a segregating cassava backcross population

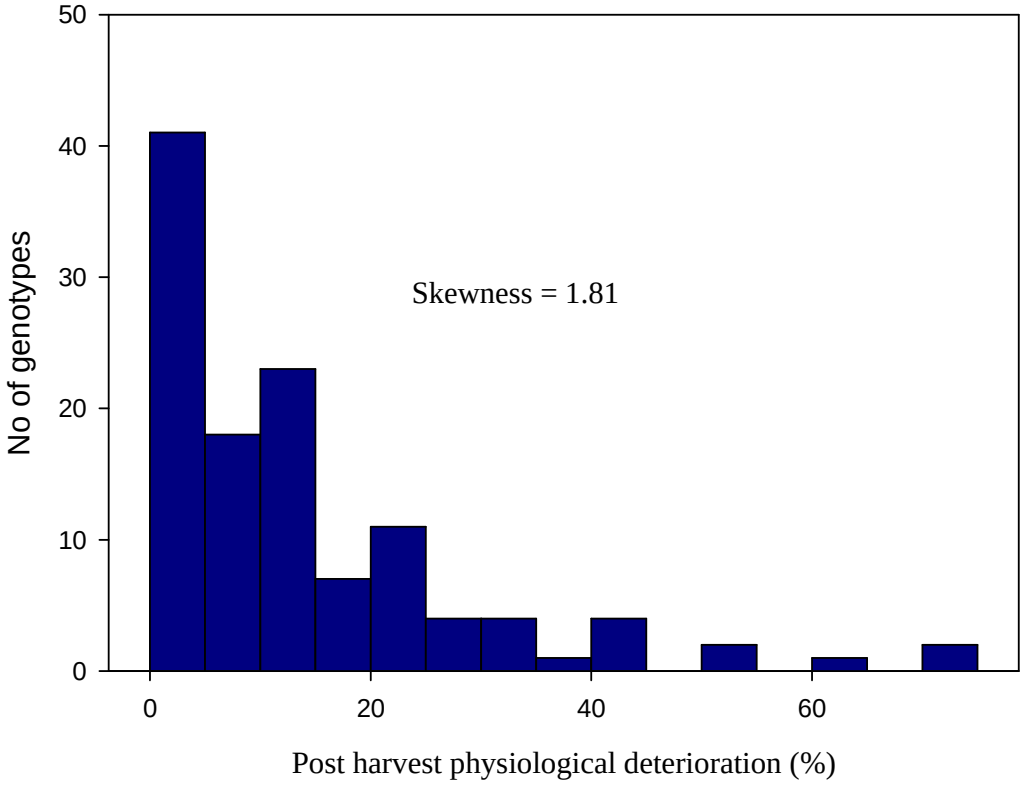


Figure 5.2h: Frequency distribution of the percentage post harvest physiological deterioration (PPD) in a segregating cassava backcross population

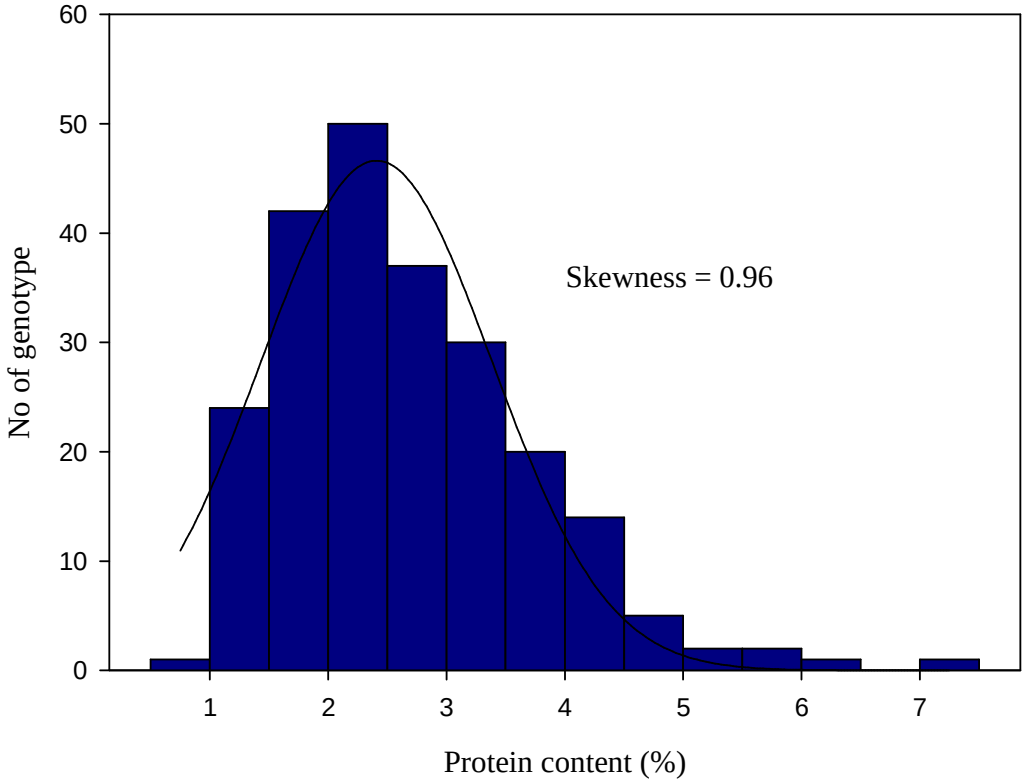


Figure 5.2i: Frequency distribution of the percentage protein content in a segregating cassava backcross population

Table 5.6: Analysis of variance (ANOVA) of yield parameters and quality traits in the B_1P_2 population evaluated at harvest at CIAT, Colombia in 2007

Source of variation	df[a]	Mean squares								
		ComRt[b]	Rtplt[c]	HI[d]	Rtwt[e]	FRY[f]	DRY[g]	DMC[h]	PPD[i]	PC[j]
Replication	2	91.1****	15.9****	0.04****	0.11****	442.05****	69.76****	481.66****	15.72****	116.24**
Genotype	213	188.2****	11.6****	0.04****	0.01****	69.31****	10.28****	30.74****	11.56****	185.86****
Error	426	78.7	3.4	0.01	0.01	16.24	2.39	15.46	3.54	72.31
H[l]		0.58	0.71	0.86	0.55	0.76	0.76	0.49	0.69	0.61

[a]Degrees of freedom; [b]Commercial roots; [c]Roots per plant; [d]Harvest index (0 - 1); [e]Root weight (kg); [f]Fresh root yield (ton ha^{-1}); [g]Dry root yield (ton ha^{-1}); [h]Dry matter content (%); [i]Post harvest physiological deterioration (%); [j]Protein content (%); [l]Broad sense heritability; ****$p \leq 0.0001$

5.4 Discussion

Despite the world-wide importance of cassava (*M. esculenta* Crantz), cassava cultivars have low protein content (Anonymous, 1968; Nassar and Dorea, 1982). Efforts have been made in the past to introgress these traits from wild progenitors but failed during the backcross (Asiedu *et al.*, 1992). The low protein content in the roots of cassava can be attributed to the selection methods adopted by cassava breeders where emphasis has not been placed on protein content as a part of the selection criteria (CIAT, 2004). Storage root proteins have proved to be an important target for cassava breeders, and cassava geneticists using MAS and genetic engineering, because of the role of protein in determining the nutritional quality of storage roots (Zhang *et al.*, 2003).

This finding is progress toward the introgression of protein content and high dry matter content, where high dry matter content of 50.51% in B_1P_2 - 109 is accompanied by high protein content (9.61%) in B_1P_2 – 248 from the same family. It is good to mention that the high heritability(data not shown) from the parent to the offspring of the *Manihot esculenta* ssp *flabellifolia* (Appendix1) used as the grandparent is an indication though the high dry matter content and protein are not from the same progeny in the backcross population in this experiment. The two parents (CW 198 - 11 and MTAI - 8) that were used to generate this B_1P_2 population, were different, with their values of 11.20 and 2.30 for protein content, and 33.24% and 44.96% for dry matter content. From the wild relative (OW 230), the dry matter content and protein content can be as high as 46.12% and 10.50%. The selection of these parents was based on the overall objective of this research goal with the identification of quantitative trait loci for single trait in a segregating population. In chapter 8 it will be noted that the QTL was identified for this single trait not multiple trait as it might have been for other crops with multiple traits objective. These results differ from what was reported by Ceballos *et al*. (2006) with the highest protein content of 7.20% in an unreplicated trial of a wide range of local Neo-tropical varieties and higher than what was reported by Chávez *et al*. (2005) with the highest protein content of 8.72% of the same materials in an unreplicated trial.

Results from simple statistics showed that the percentage dry matter content in this introgression (10.83% to 50.51%) was in the range of the past documentations with between 20.65% to 45.33% in Ojulong *et al.*(2008b), 31.10% to 36.30% in Jaramillo *et al.* (2005), 10.72% to 57.23% in Chávez *et al*. (2005), 28.10% to 38.10% in Iglesias *et al.* (1994), 20.00% to 47.20% in Magoon *et al.* (1973), 19.20% to 66.40% in Rajendran and Hrishi (1982), which is far higher than that documented by Ceballos *et al*. (2006). In their study, percentage dry matter content ranged from 25.7% to 44.0%.

Ceballos *et al*. (2006) reported that the correlation between dry matter and protein contents in the roots was r = - 0.37, suggesting that clones with higher protein content tended to have lower levels of dry matter content. This is contrary to what was found in this study. Although there was a negative correlation, it was not significant.

Simple correlation analysis showed that all traits (commercial roots, roots per plant, harvest index, root weight, and fresh root yield) contributed to economic yield. Contrary to what was reported by Kawano *et al*. (1998) and Ojulong *et al*. (2008b) that association was detected between dry matter content and fresh root yield at the early stage, this contrast from there work might be as a partial result of other genes affecting this stage of introgression in the backcross population.

The contribution of genotype sum of squares to total sum of squares in yield and quality traits was significant, which indicated a large genetic component. This is in agreement with a report by Ceballos *et al*. (2006) which provides strong evidence to support the hypothesis of a genetic origin of protein content in the cassava root. The possibilities of further increasing the range of protein content in the root are therefore encouraging (Steel and Torrie, 1960; Dudley, 1974; Gomez and Gomez, 1984; CIAT, 2003; Ceballos *et al.,* 2006).

To be able to improve a trait by conventional breeding, breeding populations should result in a number of individuals that depart from the mean, expressed as positive skewness. The more

skewed, the greater the potential. Distribution frequencies of all the traits indicated that genetic improvement can be achieved by crossing and selecting superior individuals. The potential of improvement differed between crosses as shown by differences in the level of skewness among traits. The skewness value of 1.81 is in agreement with the value reported by Chávez *et al.* (2005) who worked on landraces and improved clones at CIAT with a skewness of 1.74, which was asymmetrical with a longer tail to the right and concentration of frequencies around low post harvest physiological deterioration values.

High broad-sense heritability was obtained for fresh root yield, dry root yield, dry matter content, post harvest physiological deterioration, root weight, harvest index, roots per plant, commercial roots and protein content, which is in agreement with the findings of Pérez *et al*. (2002), Okogbenin (2004), Ceballos *et al.* (2004) and Ojulong *et al*. (2008b) but low heritability was seen for post harvest physiological deterioration. Heritability is an indication of the ease with which a trait can transfer to the progeny (Kang, 1994).

Results from this study are indeed promising. Perhaps the most relevant benefit from this protein introgression would be in improving the nutritional status of millions of people who depend heavily on cassava as a food security crop in the developing world.

Chapter 6

Introgression of genes for whitefly resistance from F_1 inter-specific hybrids into cassava at CIAT

6.1 Introduction

Cassava (*M. esculenta* Crantz) is an important source of cheap food in all of sub Saharan Africa (Horton *et al*., 1984; Dahniya, 1994). The crop is widely grown by resource-poor farmers who consume the fresh or processed roots and generate income from the sale of the products. Cassava is a hardy crop and can thrive in the poor soils usually found in the marginal areas of the world. Cassava originated in south and central America (Leone, 1977) and was introduced into Africa in the 18th and 19th centuries (Jones, 1959). The crop was rapidly adopted by farmers in Africa because of its low input resource requirements and relative ease of cultivation and processing (Hahn *et al*., 1979).

Cassava leaves contain 5.1% to 6.9% protein (Onwueme, 1978; Oomen and Grubben, 1978; Gomez and Valdivieso, 1985). In Zaire, cassava leaves are the basic vegetable, being the cheapest and richest source of protein. Cassava leaves are widely consumed as a vegetable in other countries in Africa (Lutaladio and Ezumah, 1981). Current agricultural statistics do not show the level of cassava leaf production, but experience from several countries indicates that millions of tonnes of cassava leaves are harvested and used as a vegetable by many African families, providing protein, vitamins, and minerals (Dahniya, 1994).

The most widespread cassava disease of economic importance in Africa is cassava mosaic disease (CMD, Akano *et al*., 2002; Balyejusa Kizito *et al*., 2005; Ogbe *et al*., 2006). In the 1990s a major setback was suffered in cassava production due to this disease (Zhou *et al*.,

1997; Okogbenin *et al*., 1998; Otim-Nape *et al*., 2000; Balyejusa Kizito *et al*., 2005; Ogbe *et al*., 2006). Cassava yields are severely reduced by pests and diseases that are worsened by the fact that it is a long season crop, which exposes it to infestation or infection by a host of pests and pathogens in all growing areas (Egesi *et al.*, 2007b). CMD is caused by at least four geminiviruses of the genus *Begomovirus* (Family Geminiviridae) and is transmitted by the whitefly (Russell, 1978; Thresh *et al*., 1994; Wool *et al*., 1994; Bellotti *et al.*, 1999; Bellotti and Arias, 2001; Akano *et al*., 2002; Ariyo *et al.*, 2002; 2004).

Closely related to low yield is the problem of pests, which pose a serious threat to the increased production of cassava (Herren, 1981; IITA, 1990) and a potential threat to Latin American cassava production (Akano *et al.*, 2002). Whiteflies are considered one of the world's major agricultural pests, attacking a wide range of crop hosts and causing considerable crop losses. As direct feeding pest and virus vector, whiteflies cause major damage in agro-ecosystems based on cassava (Bellotti and Arias, 2001). Whiteflies, especially in the Neotropics, cause direct damage to cassava by feeding on the phloem of the leaves. This causes symptoms such as chlorosis and leaf fall, which result in considerable reduction in root yield if prolonged feeding occurs. Yield losses resulting from *Aleurotrachelus socialis* and *Aleurotrachelus aepim* activity (Vargas and Bellotti, 1981; Farias, 1994; Bellotti *et al.*, 1999) are common in Colombia and Brazil.

Cassava cultivars are sometimes deficient in economically important characters such as resistance to pests (Nassar and Dorea, 1982; Nassar and Grattapaglia, 1986). This can be attributed to the mode of evolution of the species and modifications of the allogamy system of the plant (Nassar and O'Hair, 1985). Lost genes can be restored to the gene pool of the cultigen by inter-specific hybridisation with wild relatives which possess these genes (Nassar *et al.*, 1986). The objective of this study was to introgress genes from wild progenitors of cassava for increased whitefly resistance genes into commercial cassava.

6.2 Materials and methods

The B_1P_2 population used for the present study was derived from an inter-specific hybrid which was crossed to a cassava parent. An inter-specific F_1 (CW 198 - 11) used as the female parent, for the development of B_1P_2 population was developed at CIAT, Cali, Colombia (CIAT, 2002). It has a wild progenitor with high root protein content, high dry matter content, and disease resistance to various cassava diseases and pests. The male parent (MTAI - 8), a successful cultivar with high dry matter content and good tuber formation, resulted from the breeding programme at the Thailand Agricultural Research Centre, Thailand (CIAT, 1990). The pedigree of these parents is given in Chapter 5.

Stem cuttings from the Corpoica evaluation trial described in Chapter 4 were used to establish this replicated experiment. The field trial was conducted in CIAT, Palmira, experimental plot number P^2N in 2006, at Palmira in Valle del Cauca Department (elevation 965 m, $3^o49'$N, $76^o36'$W), located in the mid altitude tropics of Colombia. The site has bimodal rainfall, although there are yearly variations, with peaks usually between March - June and October - December. The soil in Palmira is a fertile alluvial clay loam. Meteorological data at the location during experimentation are presented in Chapter 5 (Table 5.1). Field plot layout was a randomised complete block design, with three replicates of 12 blocks, involving 225 genotypes of the B_1P_2 population.

The total area of the trial was 5989 m^2, comprising of eight plants per genotype, with border plants in the hedges. Planting was on ridges at a spacing of 0.7 m x 1.4 m. The plants were not fertilised or sprayed with insecticide, but weeded when necessary. Traits that were evaluated are yield, quality traits, pest infestation and infection. The traits were measured on the seven internal plants and means were calculated. Details of the yield traits and pest infestation and infection evaluated in the study are described below.

Harvesting was carried out at 10 months after planting (10 MAP). Seven plants were harvested and their storage roots were weighed to determine yield. Sub samples of roots of various sizes, depending on the genotype yield, were taken on genotype basis for dry matter content (DMC) determination. DMC assessment was done by peeling and oven drying root samples for 48 hours after which the weight difference between the fresh weight and dry weight is measured and the percentage dry matter is calculated. Percentage dry matter content was determined as described in Chapter 5.

The dry root yield was then calculated as: %DMC X fresh root yield. The harvested plants were assessed for their number of storage roots per plant. The aerial part (stems and leaves) of the plants were weighed for fresh shoot weight determination. Harvest index was computed as the ratio of root yield to the total harvested biomass per genotype on fresh basis.

Data analysis

Agrobase (2000), SAS (2002) and Sigmaplot 10.0 (2007) statistical programmes were used for data analysis. Only genotypes which had three complete replications were used. Since roots per plant, root weight and fresh and dry root yield data were not normally distributed, data were transformed by the square root method using the formula: $y = \sqrt{(x+0.5)}$. Percent dry matter content and protein content were transformed by the square root method using the formula: $y = \sqrt{(x)}$, where y is the resulting transformation and x the data point.

The SAS correlation (proc corr.), univariate (proc univariate) and regression (proc reg) procedures were used to estimate correlation and regression coefficients between different parameters. Yield, yield components and pest evaluation were subjected to simple ANOVA. Agrobase (2000) was used for estimating broad sense heritability. Sigmaplot 10.0 was used to plot the histogram of different yield and pests components.

Field screening of the B_1P_2 family for resistance to whiteflies was done at CIAT headquarters where the natural whitefly population is high and damage levels are significant so as to distinguish susceptible cultivars (Figure 6.1). The evaluation was done during the dry period of the growing season when the population build up for the whiteflies is high. Whitefly adult and nymph feeding damage is most noticeable on the young, tender apical leaves of the cassava plant. Feeding induces a yellow to green mottled appearance and twisted or curled leaves, eventually resulting in chlorosis and defoliation. Field evaluations of the B_1P_2 used a population scale combined with a leaf damage scale (Table 6.2).

Figure 6.1: Field screening of a cassava backcross population (B_1P_2 family) for incidence and severity of whitefly

Resistance screening using natural *A. socialis* populations is done primarily at two field sites in Colombia: the first field site is Nataima, Tolima, in cooperation with CORPOICA. *A. socialis* populations at this site have consistently been at moderate to high levels for nearly

15 years, offering an opportunity for sustainable research over a long period. The second field was at CIAT headquarters, Palmira, Valle del Cauca (where this field evaluation was conducted). Initially, *A. socialis* populations at CIAT were low. Since 1994, however, populations have increased dramatically and are presently higher than in Tolima (Bellotti and Arias, 2001).

Table 6.1: Population and damage scales for evaluating a cassava backcross population ($B_1P_{2)}$ for resistance to whiteflies

***Population scale**	
1	no whitefly stages present
2	1-200 individuals per cassava leaf
3	201-500 individuals per cassava leaf
4	501-2000 individuals per cassava leaf
5	2001-4000 individuals per cassava leaf
6	>4000 individuals per cassava leaf
***Damage scale**	
1	no leaf damage
2	young leaves still green but slightly flaccid
3	some twisting of young leaves, slight leaf curling
4	apical leaves curled and twisted; yellow-green mottled appearance
5	same as 4, but with sooty mold and yellowing of leaves
6	considerable leaf necrosis and defoliation, sooty mold on mid and lower leaves and young stems

*Scale adapted from Bellotti and Arias (2001)

6.3 Results

The 227 genotypes from the B_1P_2 family were evaluated in three replications at CIAT headquarter fields in Colombia for whitefly infestations (Figure 6.2). Of these, 13.3% were considered susceptible with damage ratings above 3.5 (Table 6.2). The remaining 86.7%,

with damage ratings below 3.5, were considered promising. The most promising resistance was for damage ratings (17.8% of the genotypes) below 2.0.

In the three evaluations, B_1P_2 - 10, B_1P_2 - 79, B_1P_2 - 312, B_1P_2 - 98, B_1P_2 - 176, B_1P_2 - 168, B_1P_2 - 176, B_1P_2 - 246, B_1P_2 - 248, B_1P_2 - 25, B_1P_2 - 89, B_1P_2 - 311, and B_1P_2 - 64 consistently expressed the highest level of resistance across replications. Additional genotypes that have expressed moderate to high levels of resistance include B_1P_2 - 107, B_1P_2 - 136, B_1P_2 - 153, B_1P_2 - 17, B_1P_2 - 174, B_1P_2 - 177, B_1P_2 - 194, B_1P_2 - 197, B_1P_2 - 218, B_1P_2 - 219, B_1P_2 - 220, B_1P_2 - 224, B_1P_2 - 227, B_1P_2 - 229, B_1P_2 - 231, B_1P_2 - 238, B_1P_2 - 24, B_1P_2 - 255, B_1P_2 - 26, B_1P_2 - 47, B_1P_2 - 310, B_1P_2 - 273, B_1P_2 - 269, B_1P_2 - 322, B_1P_2 - 40, B_1P_2 - 303, B_1P_2 - 281, B_1P_2 - 314, B_1P_2 - 78, B_1P_2 - 45, B_1P_2 - 1, B_1P_2 - 114, B_1P_2 - 160, B_1P_2 - 163, B_1P_2 - 195, B_1P_2 - 228, B_1P_2 - 253, B_1P_2 - 93, B_1P_2 - 99, B_1P_2 - 326, B_1P_2 - 61, B_1P_2 - 95 (Figure 6.2).

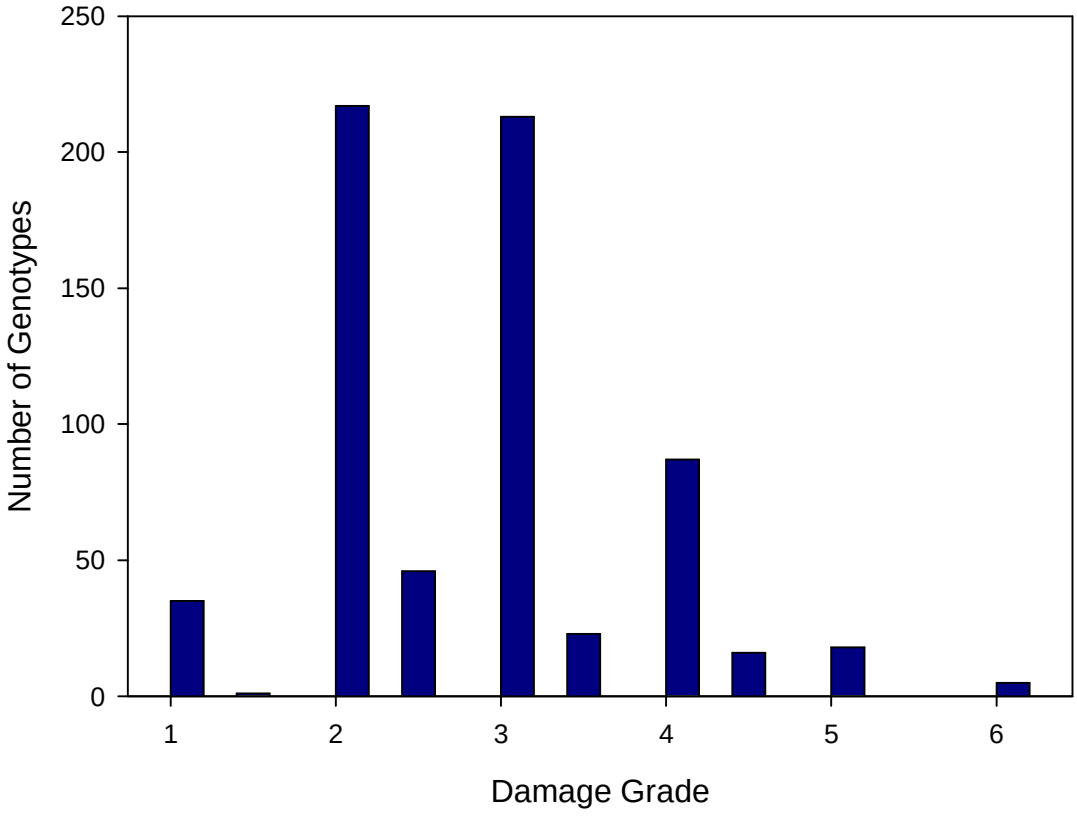


Figure 6.2: The distribution of the damage of whiteflies on a cassava backcross population evaluated at CIAT for resistance to whiteflies [damage scores are based on 1 (no damage) to 6 (severe damage) rating scale]

Low severity of whiteflies was recorded in the field despite the high pest pressure at the location of screening, which was due to low precipitation and high evaporation in 2007. This meteorological condition favours whitefly development (Table 6.1). Surface damage was low for all developmental stages of the pest with a skewness of 0.55 for adult whiteflies, 0.12 for eggs, - 0.37 for nymphs, 0.24 for pupa and a skewness of 0.52 for superior, 0.97 for middle, and 1.16 for the lower part of the plant for severity (data not shown). The distribution of severity of whiteflies was asymmetrical with a long tail to the right, and concentration of frequencies around low damage grade level of whiteflies. This indicates that fewer genotypes were susceptible to the whitefly in the B_1P_2 (Figure 6.3).

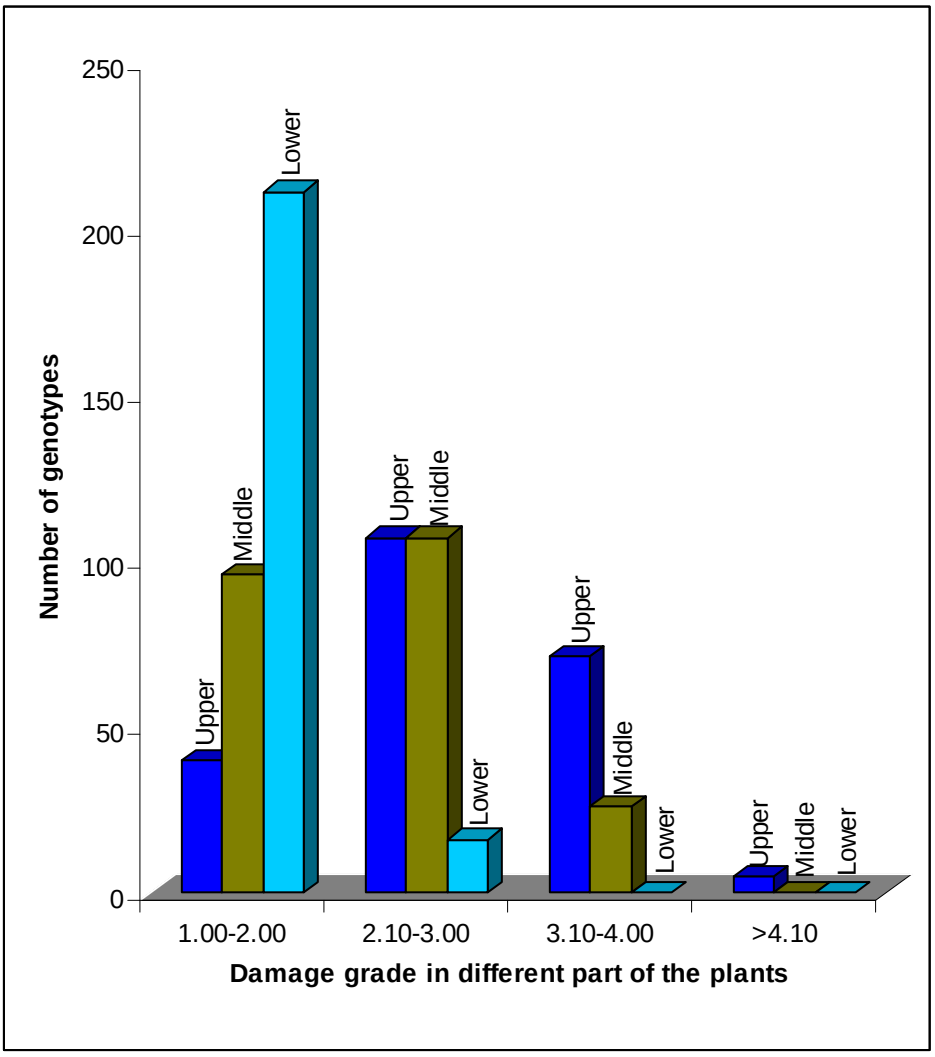


Figure 6.3: Frequency distribution of different degrees of damage done to different parts of a cassava backcross population

General linear model analysis showed genotypes to be highly significant for all yield and pest characteristics evaluated (Table 6.3). Block was highly significant at p≤0.0001 for roots per plant, harvest index and severity. There were no significant differences in the replications of yield, yield related traits and pest severity damage evaluated. This suggested similarity in performance across replications. High broad-sense heritability was estimated for yield, yield related traits, and pest severity damage. This is based on an assumption that genetic and environmental contributions to genotypes variance changes at different rates, if genetic variation is distributed randomly.

Table 6.2: General linear model table of yield and severity grade of whiteflies evaluated on a cassava backcross population at CIAT, Palmira, Colombia in 2007

Source of variance	df[a]	Mean square				
		Rtplt[b]	RtWt[c]	HI[d]	FRY[e]	Severity
Block	11	12.81****	0.78ns	0.02****	47.81ns	6.94****
Rep[f]	2	3.84ns	0.76ns	0.01ns	56.46ns	0.09ns
Genotype	223	11.31****	1.10****	0.04****	67.29****	1.09****
Error	424	3.10	0.24	0.01	14.70	0.41
CV[g]		31.93	42.98	22.53	42.98	23.41
Heritability[h]		0.70	0.56	0.85	0.76	0.30

[a]Degrees of freedom; [b]Roots per plant; [c]Root weight (kg); [d]Harvest index (0 - 1); [e]Fresh root yield (ton ha^{-1}); [f]Replication; [g]Coefficient of variation; [h]Broad-sense heritability; **** P≤0.0001; ns=not significant

There was no significant correlation between the yield and yield related traits evaluated with the grade of damage that was done to the genotypes by whitefly at all parts of the plants evaluated (high severity, medium severity, and low severity), but high severity (DSup) was positively correlated ($p \leq 0.0001$) with medium severity (DMed) and low severity (DBajo). There were highly significant correlations ($p \leq 0.0001$) between the yield and yield related traits measured (Table 6.4). There were positive correlations between the pest severity damage on the superior part of the plant ($p \leq 0.0001$) with adult incidence, egg number, nymph incidence, and pupa incidence. Adult population of the flies were highly significantly ($p \leq 0.0001$) correlated with the egg number, nymph population, pupa population, and highly significant ($p \leq 0.0001$) with the severity damage on the superior part of the plant. Lower part severity damage of the flies were highly significantly ($p \leq 0.0001$) correlated with egg number on the leaf surface, nymph population on the leaf, nymph population on the middle part of the plant, pupa population the lower part of the plant, and high and medium severity damage (Table 6.5).

Table 6.3: Phenotypic correlation for yield related traits and whitefly damage grade in a cassava backcross population (B_1P_2 family) evaluated at CIAT, Palmira, Colombia in 2007

	Variables					
	Rtplt[a]	RtWt	HI	FRY	DSup	DMed
RtWt[b]	0.75****					
HI[c]	0.61****	0.66****				
FRY[d]	0.75****	1.00****	0.66****			
DSup[e]	0.08ns	0.04ns	0.14ns	0.04ns		
DMed[f]	0.06ns	0.02ns	0.09ns	0.02ns	0.69****	
DBajo[g]	0.05ns	0.002ns	0.01ns	0.002ns	0.24****	0.14ns

[a]Roots per plant; [b]Root weight (kg); [c]Harvest index (0 - 1); [d]Fresh root yield (ton ha^{-1}); [e]Superior severity (1 - 6); [f]Middle severity (1 - 6); [g]Below severity (1 - 6); ****$p \leq 0.0001$; ns=not significant

Table 6.4: Correlation between incidence and severity of the population of whiteflies on the B_1P_2 family evaluated at CIAT, Palmira, Colombia in May 2007

	Variables								
	UAdl[a]	UEgg	Unph1	UPul1	MNp2	MPul2	APul3	Sup	Med
UEgg[b]	0.76****								
Unph1[c]	0.34****	0.54****							
UPul1[d]	0.04ns	0.16*	0.05ns						
MNp2[e]	0.02ns	0.09ns	0.32****	-0.57****					
MPul2[f]	0.36****	0.33****	0.13**	0.26****	-0.01ns				
APul3[g]	-0.02ns	0.05ns	0.36****	-0.23****	0.48****	0.04ns			
Sup[h]	0.18****	0.36****	0.37****	0.47****	-0.01ns	0.37****	0.10ns		
Med[i]	0.13*	0.27****	0.16****	0.54****	-0.21****	0.47****	-0.07ns	0.69****	
Bajo[j]	0.08ns	0.18****	0.24****	0.01ns	0.23****	-0.01ns	0.36****	0.24****	0.14****

[a]Adult population on the leaf surface; [b]Eggs number on the leaf surface; [c]Nymph population on the leaf surface; [d]Pupa population on the leaf surface; [e]Nymph population on the middle part of the plant; [f]Pupa population on the middle part of the plant; [g]Pupa population on the lower part of the plant; [h]Superior part severity damage, [i]Middle severity damage; [j]Lower part severity damage; * P≤0.05, **P≤0.01, ****P≤0.0001.

6.4 Discussion

Whitefly borne germiniviruses occur in all main cassava-growing areas of Africa, where it has been ranked as the most important vector-borne disease of any food crop (Geddes, 1990), which has become the object of extensive research (Thresh *et al.,* 1994; Fregene *et al.,* 2000; Bellotti and Arias, 2001; Akano *et al.,* 2002; Legg and Fauquet, 2004; Tomkins *et al.,* 2004; Ogbe *et al.,* 2006; Okogbenin *et al.,* 2007; Dixon *et al.,* 2008). The discovery and use of new resistance genes from wild relatives have steadily increased in different crops. Breeders continue to isolate and introgress genes from wild relatives for resistance to pests and diseases of economic important crops (Hajjar and Hodgkin, 2007). Tropical Manioc Selection (TMS) cassava cultivars, developed by the International Institute for Tropical Agriculture using crosses with *M. glaziovii* Műll.Arg., for combating cassava mosaic disease, is one of the major breakthroughs recorded thus far, which have contributed to a 40% yield increment in Nigeria (Nweke, 2004).

Results from this study are indeed promising. From an earlier report by Bellotti and Arias (2001) that screened 5363 clones of cassava from the CIAT gene bank, 73% were susceptible. From the present study only 13.3% of the genotypes were susceptible, which was an indication that some gene introgression had taken place. This result was obtained from the same high pest pressure field of CIAT headquarters which was used for screening. The distribution of whitefly damage grade was asymmetrical with a long tail to the right, with the concentration of frequencies around high resistance severity. This indicated that more of the genotypes from the B_1P_2 population displayed high resistance in the damage grade of 1-3.

The range of broad-sense heritability recorded in this study for yield and severity was relatively high compared to those documented by others working on cassava (Pérez *et al.,* 2002; Okogbenin, 2004; Ceballos *et al.,* 2004; Ojulong *et al.,* 2008b). Going by the definition of heritability given by Kang (1994), the broad-sense heritability recorded for whitefly was high (0.30) which pointed to the fact that introgression has taken place. The

high heritability indicated that *M. esculenta* ssp *flabellifolia* was a good source of resistance gene to whitefly.

Fresh root yield averaged 8.97 ton ha^{-1} across the 225 genotypes ranging from 0.26 ton ha^{-1} to 58.59 ton ha^{-1} (from Chapter 5). No correlation between yield and pest severity suggested that yield was not affected by the severity of the whiteflies in this high pest pressure zone of Colombia. There was a high correlation between yield and yield related traits and likewise, there was a correlation between the severity of the pest in the superior severity, middle severity, and below severity. It is worth noting here that there was a correlation between pest incidence and severity in the B_1P_2 population but this had no effect on the yield, which was contrary to the previous reports of yield losses ranging from 5%, 42% and 79% yield reduction, respectively (Vargas and Bellotti, 1981; Farias, 1994; Bellotti *et al.*, 1999).

The advantage of whitefly resistance introgression is that breeders can combine it with high protein content, good root formation, yield, and high dry matter using a selection index that includes all of these traits, and eliminating whitefly susceptible genotypes. In the case of whitefly resistant genotypes, elimination would reduce the cost of evaluation significantly, and increase selection efficiency. This chapter has confirmed a level of introgression of resistance to whitefly in the B_1P_2 family and special emphasis will be placed on the genotypes that have shown the highest resistance to whitefly and they will be re-evaluated to determine there final status and final selection will then be done. They will be used as parents for breeding purposes.

Chapter 7

Development of a molecular genetic linkage map of cassava based on a B_1P_2 family derived from *M. esculenta* ssp *flabellifolia*

7.1 Introduction

Manihot species examined to date have a chromosome number of 2n = 36 (Magoon *et al*., 1969; Umanah and Hartmann, 1973; Fregene *et al.,* 1994). This high diploid chromosome number reflects the polyploid nature of the genus (Fregene, 1996). On the basis of observed numbers of satellite chromosomes and the karyology of chromosomes at the pachytene stage of meiosis, it has been postulated that *Manihot* species originated through segmental allotetraploidy and allotraploidy from two closely related taxa (Magoon *et al*., 1969; Umanah and Hartmann, 1973). Fregene *et al*. (1994) reported that normal chromosome pairing at meiosis occurs in hybrids of *Manihot* species that are morphologically different and belong to separate primary phylogenetic lineages according to cpDNA studies (Nassar, 2002).

The genetics of cassava are the least understood of any of the major staple crops that feed mankind (Fregene *et al.,* 1997). This discrepancy is due to the heterozygous nature of the crop, its long growing cycle, its low seed yield per pollination, and the limited funding for research on this crop (Fregene *et al.,* 1997; Ceballos *et al.,* 2004). Although the crop is considered to be a segmental allopolyploid (Magoon *et al.,* 1969; Lopez *et al.,* 2005) or an allopolyploid (Umanah and Hartmann, 1973; Saelim *et al.,* 2006) little is know about the diploid ancestors of cassava's 36 somatic chromosomes (Nassar, 2000) nor is the genetic map of the crop saturated (Okogbenin *et al.,* 2006).

The first genetic map constructed by Fregene *et al.* (1997) was predominantly an RFLP marker map. An additional map constructed by Okogbenin *et al*. (2006) in an effort to saturate the cassava genome was based on a F_2 population with which the cassava map is yet

to be saturated and SSR markers were used for this map. These maps have so far provided initial tools for genetic analysis of important traits of cassava (Jorge *et al.,* 2000; 2001; Akano *et al.,* 2002; Okogbenin and Fregene, 2002; 2003; Balyejusa Kizito *et al.,* 2007).

Cassava is native to the New World tropics and a member of family *Euphorbiaceae* (Fregene *et al.,* 1994). Cassava and some 90 other species make up the genus *Manihot* (Rogers and Appan, 1973; Nassar, 2000). Cassava is the only member widely cultivated for its starchy tuberous roots which provide food in nearly all the tropical countries of the world (Hurtado *et al.,* 2008).

Literature describes genetic approaches to mapping polyploid genomes using molecular markers (Wu *et al.,* 1992; Al-Janabi *et al.,* 1993). These approaches attempt to simplify the determination of allelism by analysing a special class of markers known as single-dose restricted fragments (SDRF) (Wu *et al.,* 1992). SDRFs are DNA markers that are present in one parent and absent in the other parent and segregate in a 1:1 ratio in the progeny. They represent the segregation equivalent of an allele at a heterozygous locus in a diploid or an allopolyploid genome or a simplex allele in an autopolyploid (Al-Janabi *et al.,* 1993).

Molecular marker systems have proven to be efficient in overcoming the limitation of traditional breeding methods. The advantage of MAS is that it enables the breeder to eliminate at an early stage the unwanted genotypes which is made possible by the understanding of the genetics of the plant at molecular level. One of the primary objectives of gene tagging efforts in cassava is to provide tools that can increase the cost-effectiveness and efficiency of cassava breeding programmes. The use of new technology for an orphan crop such as cassava will be helpful. The objective of this study was to construct a framework map that will be used in the QTL analysis for root protein of a backcross population of cassava.

7.2 Materials and methods

The mapping population for this study was the B_1P_2 family (227 genotypes) described in Chapter 5. The selection of CW 198 - 11, the female parent used to generate the B_1P_2 mapping population, was based on its high root protein content, high dry matter content, good tuber formation, and resistance to whitefly at the trial location (CIAT). Eight hundred and seventeen SSR markers were screened in the parents (CW 198 - 11 and MTAI - 8) and the four backcross progenies.

Total genomic DNA was isolated from the parents and the four individuals of the B_1P_2 family using a DNA miniprep extraction protocol based on a modified Dellaporta extraction procedure (Dellaporta *et al.,* 1983). Total DNA was extracted from young fully expanded leaves of field grown plants. Leaf samples of 0.15 - 2.0 g was oven dried and ground to powder using a mortar and pestle. The powder was transferred to 1.5 ml Eppendorf tubes using a spatula. The powder was re-suspended in 800 µl of extraction buffer (100 mM Tris-HCl, 50 mM EDTA pH 8.0) and 500 mM NaCl; (pH 8.5) and 50 µl of 20% SDS (1.25% w/v). The ground tissue was shaken vigorously in the buffer and vortexed intermittently for 15 min at 65^{o}C. To this solution, 250 µl of ice-cold 5M potassium acetate was added and the content of the Eppendorf tubes were homogenised by gently inverting them 5 - 6 times. The mixture was incubated on ice for 20 min and centrifuged at 12000 rpm for 10 min. The aqueous solution was transferred to a new 1.5 ml Eppendorf tube and the nucleic acids precipitated by adding one volume of ice-cold isopropanol (approximately 700 µl), and mixed by gently inverting it 8 - 10 times before incubating at - 80^{o}C for one hour, followed by centrifugation at 12000 rpm for 10 min. The resulting supernatant was poured off and the collected pellet was re-suspended in 500 µl of 50 mM Tris-HCl/10mM EDTA (pH 8.0). The precipitation process was repeated by adding one volume of ice-cold propanol, and mixed by inverting it gently 8 - 10 times followed by incubating it at - 80^{o}C for one hour and pelleting by centrifugation at 12000 rpm for 10 min. The supernatant was poured off and the pellet was dissolved in 200 µl of $T_{10}E_1$ (10 mM Tris-HCl/ 1 mM EDTA) overnight at 4^{o}C. The nucleic acids were treated with 8 µl of 10 mg/ml RNase and incubated at 37^{o}C for 20 - 30

min. Electrophoresis and fluorometry were used in determining DNA quality and DNA concentration respectively.

A total of 817 SSR markers now exist for cassava and were the source of markers for screening the parents and the selected four backcross individuals, and only the selected polymorphic markers were screened on the entire backcross population of 227 genotypes with the parents. The 817 markers included a set of 186 SSR markers developed for cassava by Mba *et al*. (2001); another set of 157 SSR markers obtained from cassava root and leaf cDNA (Mba *et. al*., unpublished data). Others included a third set of 156 SSR markers generated from a genomic library by Fregene *et al.* (unpublished data), a fourth set of 140 SSR markers were obtained from a genomic library by Liliana Cano/Martin Fregene (unpublished data), and a fifth set of 178 ESTs markers was generated from a genomic library by Valerie Verdier/Camilo Lopez (unpublished data).

Amplification reactions were carried out in 15 µl volumes containing 10 ng of target genomic DNA, 0.2 µM of each primer, 1X of *Taq* polymerase buffer (50 mM KCl, 10 mM Tris-HCl (pH 8.5), and 0.1 mg ml^{-1} gelatin), 2.5 mM of $MgCl_2$, 0.1 mM of dNTPs and 0.25 U of *Taq* polymerase enzyme. The final volume was adjusted to 15 µl with sterile distilled H_2O. Temperature cycling was done on a PCR MJ Research (PTC 200) thermocycler (MJ Research, Watertown Mass) PCR machine using the following thermal profile: an initial denaturation cycle of 94^0C for 5 min; 35 cycles of denaturation at 94^0C for 30 sec, annealing at 55^0C, 52^0C, 45^0C, or 40^0C for 1 min (depending on the SSR marker) and extension of 72^0C for 1 min and a final extension step of 72^0C for 7 min (Appendix 2).

After PCR amplification, 15 µl of PCR product was mixed with 10 µl of 50% loading dye (formamide: 98% (v/v)). The mixture was denatured at 94^oC for 4 minute, and 10 µl was loaded on 4% polyacrylamide denaturing gels (PAGE). The samples were electrophoresed in 1XTBE at 70 W for 30 minutes to 1 hour (depending on the weight of the marker) using a BioRad sequencing gel rig (BIORAD, California). DNA was visualised by silver staining according to the manufacturer's guide (Promega).

Linkage analysis

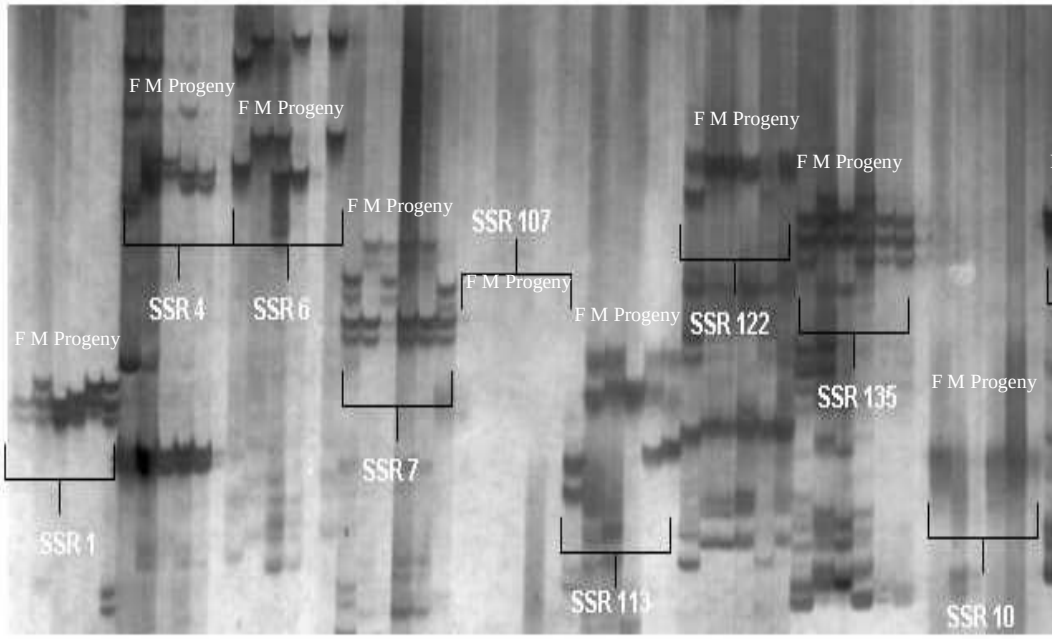
SSR alleles segregating in the mapping population were scored to the expected classes for a B_1P_2 population. Alleles derived from the female grandmother were scored as "A", whereas alleles from the male grandparent were scored as "H". Marker classes at each locus were summarised for all individuals into two different genotype classes expected for a B_1P_2 population and chi square tests for segregation distortion were carried out to compare the observed with the expected 1:1 ratio.

SSR marker data was used to construct a B_1P_2 framework map using the single point genetic linkage analysis computer package MapDisto 1.7.0 (http://mapdisto.free.fr). The "group" command, with $\log_{10}$ of odds ratio (LOD) threshold of 4.0 and a recombination fraction of 0.30 was used to assign SSR markers to linkage groups. Map units, in centiMorgans, (cM) were derived using the Kosambi function (Kosambi, 1944). Maximum likelihood orders of markers were verified by the "ripple" function, and markers were said to belong to the same framework map if the LOD value as calculated by the ripple command was greater or equal to 2.5.

7.3 Results

Of the 817 microsatellite markers screened (Figure 7.1), from the genomic/cDNA library, 49.20% (402) were found to be polymorphic in the parents of the B_1P_2 family and four selected progenies, 145 or 17.75% were monomorphic and the remaining 270 or 33.05% did not amplify (Table 7.1).

From the genomic library (Zarate *et al*., unpublished results), 39.52% were found to be polymorphic in the parents and the selected progeny, 13.17% monomorphic and the remaining 47.29% did not amplify. From ESTs (Libreros *et al*., unpublished results), 38.76% were found to be polymorphic in the parents and the selected progenies, 14.60% monomorphic and the remaining in that group (46.62%) did not amplify (Table 7.1).



F = Female parent; M = Male parent; Progeny = B_1P_2 - 1, B_1P_2 - 111, B_1P_2 - 220, B_1P_2 - 322 respectively; SSR = simple sequence repeat marker used for the screening

Figure 7.1: Silver-stained polyacrylamide gel showing SSR marker alleles in both parents (CW 198 - 11, MTAI - 8) and four selected B_1P_2 mapping progenies in a segregating cassava backcross population

Table 7.1: Percentage polymorphism found with respect to CW 198 - 11, MTAI - 8 and four selected B_1P_2 progenies with the 817 microsatellite markers at the CIAT cassava genetics laboratory

Markers	Total (%)	% Polymorphism detected				
		PolyM[a]	MonoM[b]	NoL[c]	Psize[d]	AnnT[e]
SSRY[f]	41.98	49.20	17.75	33.05	100-500	45.0-62.5
(NS)SSRY[g]	36.23	39.52	13.17	47.29	100-579	40.0-60.0
(ESTs)SSRY[h]	21.78	38.76	14.60	46.62	100-500	52.0-55.0
∑/MEAN	100.00	43.45	15.42	41.12		

[a]Polymorphic markers (%); [b]Monomorphic markers (%); [c]No amplification (%); [d]Product size; [e]Annealing Temperature (^{O}C); [f]Simple sequence repeat yuca (source=genomic/cDNA-Chikelu Mba/Martin Fregene); [g]Non-survivors simple sequence repeat yuca (source=genomic-Angela Zarate/Liliana Cano/Martin Fregene); [h]Expressed sequence tag simple sequence repeat yuca (source=Ests-Valeria Verdie/Camilo Lopéz/Wilson Castelblanco/Martin Fregene

Polymorphic markers that did not segregate in the B_1P_2 population and those that were difficult to score with the trait of interest (protein) were left out of the map. A total of 151 polymorphic microsatellite markers segregating as single-dose markers were chosen for the linkage analysis based on consistency of banding pattern in the 227 genotyping populations, clarity of the gels, and number of amplified fragments. Figure 7.2 shows the segregation of SSR marker SSRY 70 heterozygous in both parents with a shared allele, that are important as 'allelic bridges', used to identify analogous linkage groups drawn based on independent segregation of markers in female and male gametes.

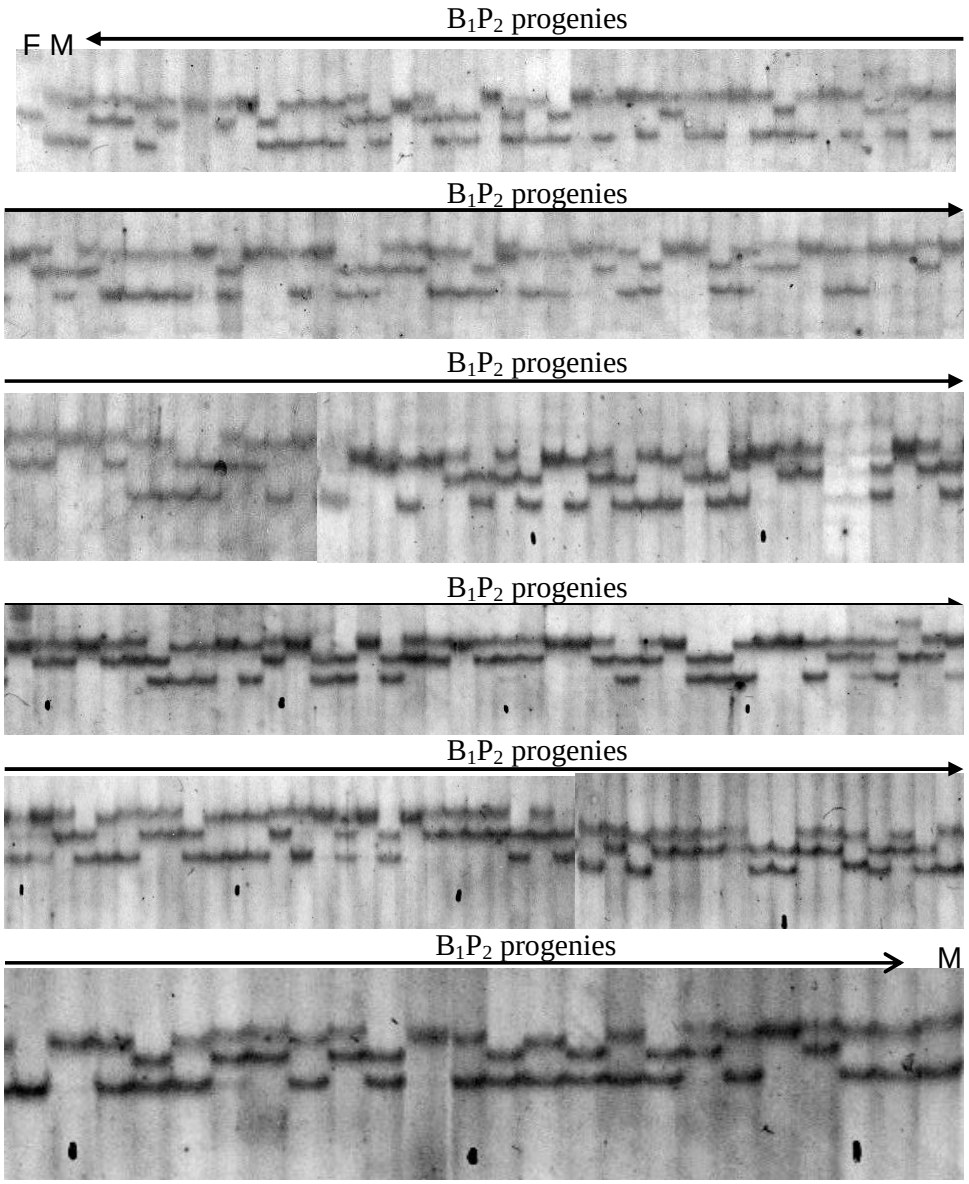


Figure 7.2: **Silver-stained polyacrylamide gel showing PCR amplification using marker SSRY 70 on parents and individuals constituting the B_1P_2 cassava backcross mapping population, F = Female; M = Male; B_1P_2 progenies = genotypes from the B_1P_2 family**

Table 7.2 provides a summary of microsatellite marker distribution on different linkage groups showing the size, number of markers and the average marker interval of each linkage group. The number of linkage group in this map (29) exceeded the haploid number of chromosomes for cassava (n = 18), indicating that the map is not complete.

One hundred and fifty one markers were employed in the linkage analysis and these markers could be assigned to 29 linkage groups, which had two - seven markers, and a linkage group length varying from 7.39 cM to 119.45 cM (Figure 7.3a - d). The linkage map of the B_1P_2 population spanned a total of genetic distance of 1249.55 cM (Kosambi cM), with 46 markers remaining unlinked. The average markers distance was 16.69 cM, with intervals between loci ranging from 4.43 cM to 33.46 cM (Table 7.2).

Table 7.2: Linkage group size, number of markers, and the average marker interval per linkage group of a cassava backcross (B_1P_2) linkage map

Linkage group	Size (cM)	No. of markers	Average marker interval (cM)
1	44.77	3	14.92
2	32.01	2	32.01
3	7.39	2	7.39
4	80.00	6	13.33
5	43.84	4	10.96
6	10.90	2	10.90
7	62.61	6	10.43
8	29.45	2	29.45
9	23.78	3	7.92
10	63.00	7	9.00
11	51.05	7	7.29
12	83.01	7	11.85
13	81.86	5	16.37
14	28.84	4	7.21
15	16.98	2	16.98
16	27.27	2	27.27
17	20.92	4	5.23
18	20.21	2	20.21
19	119.45	6	19.91
20	19.71	2	19.71
21	64.67	4	16.17
22	25.23	2	25.23
23	109.91	6	18.31
24	13.30	3	4.43
25	63.58	4	15.89
26	15.40	2	15.40
27	30.08	2	30.08
28	33.46	2	33.46
29	26.87	2	26.87
∑/mean	1249.55	105	16.69

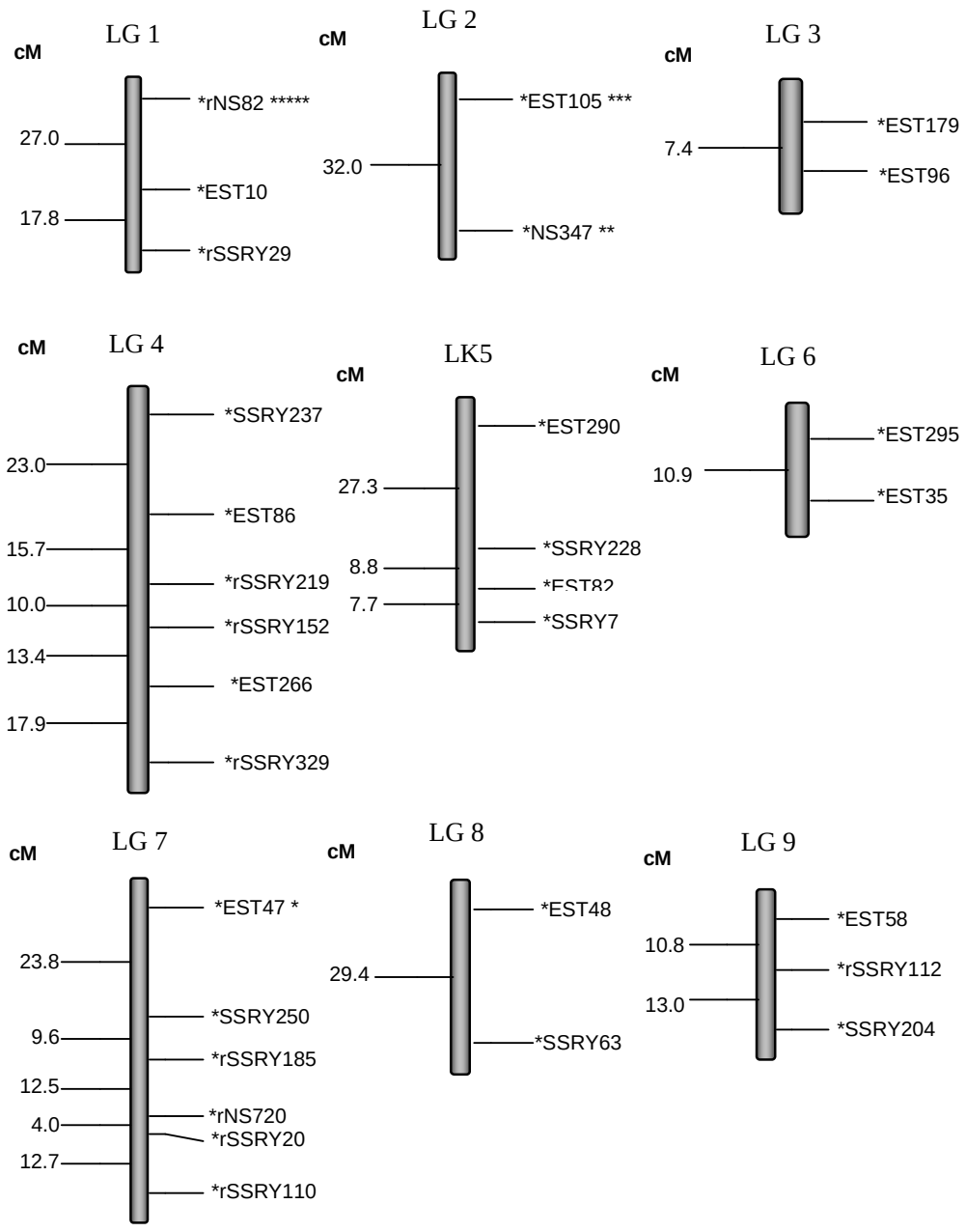


Figure 7.3a: A genetic linkage map (LG 1 – LG 9) of a cassava backcross based on a B_1P_2 family and SSR markers, cM = centiMorgan, LG = Linkage group, *r=code for mirror marker, *, **, *=probability**

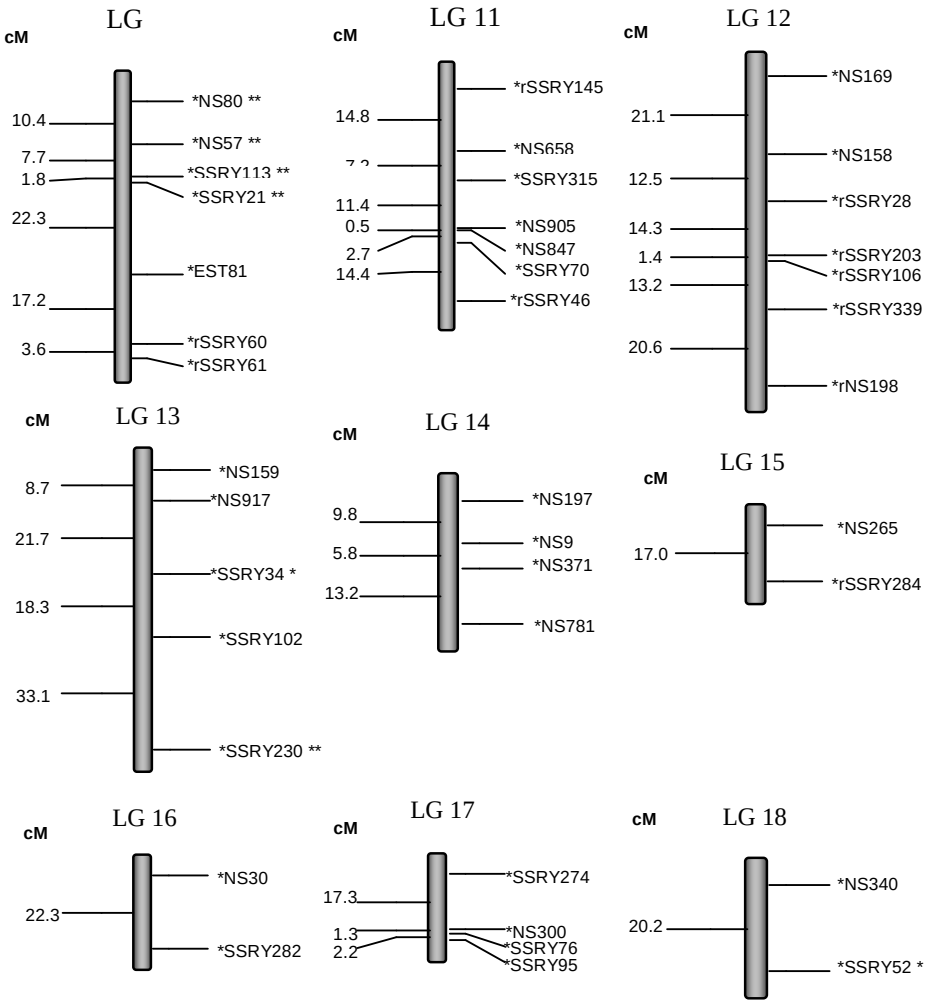


Figure 7.3b: A genetic linkage map (LG 10 – LG 18) of a cassava backcross based on a B_1P_2 family and SSR markers cM = centiMorgan, LG = Linkage group, *r=code for mirror marker, *, **, *=probability**

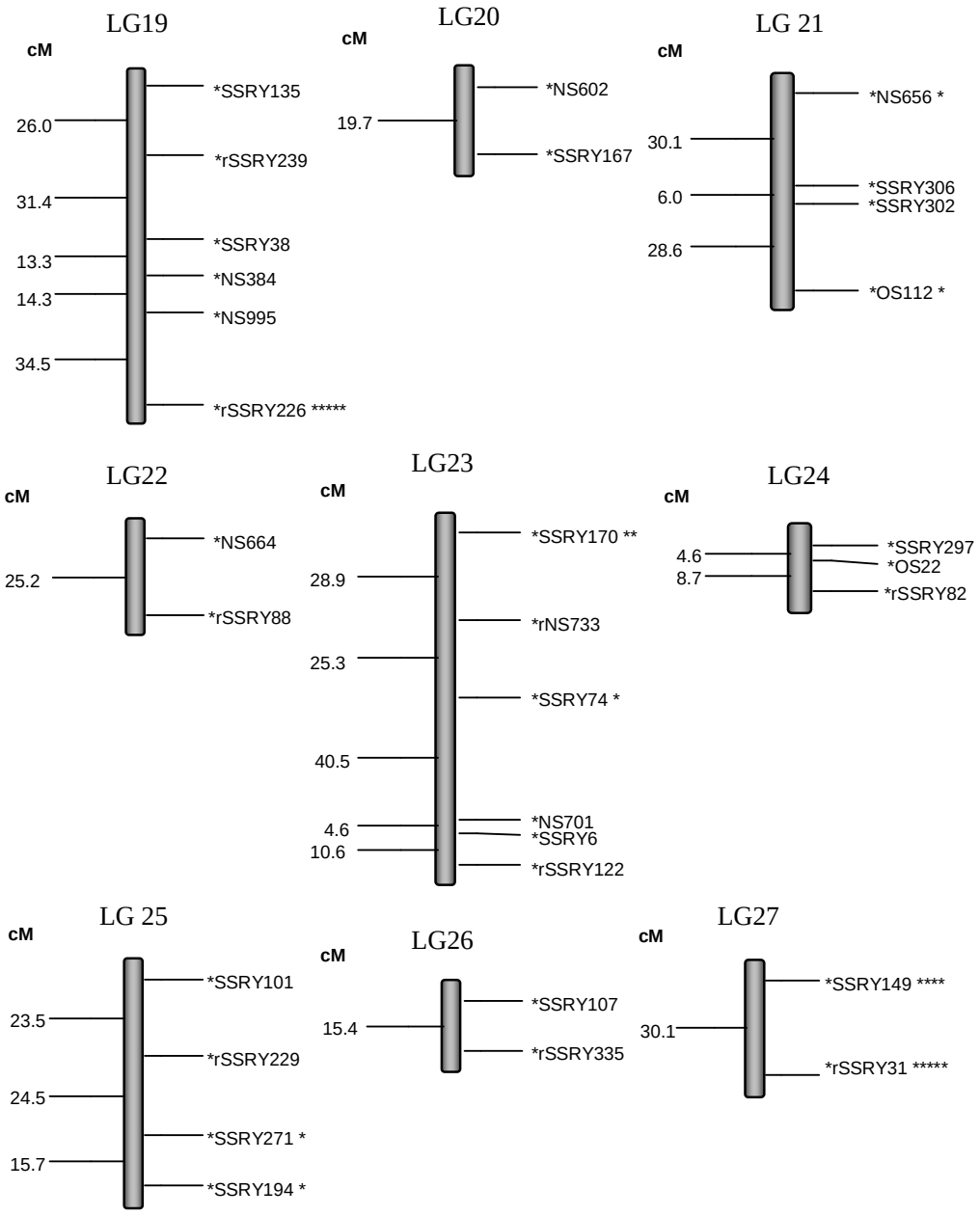


Figure 7.3c: A genetic linkage map (LG 19 – LG 27) of a cassava backcross based on a B_1P_2 family and SSR markers cM = centiMorgan, LG = Linkage group, *r=code for mirror marker, *, **, *=probability**

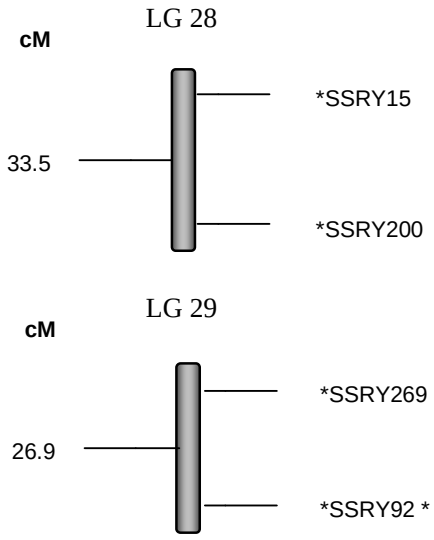


Figure 7.3d: A genetic linkage map (LG 28 – LG 29) of a cassava backcross based on a B_1P_2 family and SSR markers cM = centiMorgan, LG = Linkage group, *r=code for mirror marker, *, **, *=probability**

7.4 Discussion

Microsatellite markers have been the markers of choice for cassava because of its simple application, and are advantageous to applied plant breeding. They are co-dominant, easily assayed and detect high levels of polymorphism and have become highly valuable markers to breeders for the purposes of genome and QTL mapping (Morgante and Olivieri, 1993; Roa *et al.*, 2000; Okogbenin *et al.*, 2006).

Forty-three percent polymorphism observed in the parents of the B_1P_1 family and four selected progeny is not surprising and is comparable to results of other species (Winter *et al.*, 1999). Okogbenin *et al*. (2006) constructed the first PCR based genetic linkage map of cassava that contained only SSR loci but acknowledged that their map required further saturation. The expected number of 18 linkage groups for a comprehensive linkage map of cassava (2n = 36) was exceeded, suggesting it is not complete. In this newly developed SSR map from a B_1P_2 population, similar findings were observed. The number of the linkage map was exceeded which left room for

further saturation of the map, but most of the markers in this map are new additions to the previous cassava map.

Clustering of markers on some groups, compared to others, suggested that recombination did not occur uniformly across the cassava genome. Non randomness of recombination frequency has been reported for several other crops (Bonierbale *et al*., 1988; Causse *et al*., 1994; Lagercrantz and Lydiate 1995; Castiglioni *et al*., 1999; Ogundiwin *et al.,* 2008).

The genetic maps of cassava in the F_1 population were done using the RFLP based markers and constructed by Fregene *et al.* (1997). The mapping of 200 SSR markers in the F_1, the development of an SSR based F_2 (Okogbenin *et al.,* 2006) and BC_1 genetic map is expected to improve QTL mapping efficiency for complex traits in cassava, selection of genotypes with minimum alleles from the wild relatives and high protein content to hasten the introgression efforts. If a genetic map already exists it is helpful to first use markers that are already well distributed over the genome before using unmapped markers. The genetic maps constructed by Fregene *et al.* (1997), Mba *et al.* (2001), Okogbenin *et al.* (2006) and the B_1P_2 reported in this chapter used the same set of SSR markers but not sharing similar cassava parents. The length of the cassava genome based on genetic mapping in an F_1 cross is estimated to be about 1610 cM (Fregene *et al.,* 1997). Another map based on an F_2 population was estimated to be about 1236.7 cM (Okogbenin *et al.,* 2006). The F_1 and F_2 map of this species differed from the B_1P_2 map with respect to marker type and number, genome coverage and marker density. The F_1 map spans 931.6 cM with 168 markers (predominantly RFLP), the F_2 map spans 1236.7 cM with 100 SSR markers compared to the B_1P_2 map of 1249.55 cM with 105 SSR markers.

A number of the markers mapped onto the F_1 and F_2 derived maps were not polymorphic in the B_1P_2 map, illustrating the need for screening more SSR markers when dealing with B_1P_2 as compared with the F_1 and F_2 population. Nine markers were found to be common to the F_1, F_2 and B_1P_2 maps. This B_1P_2 population developed from high protein lines, holds great potential for the detection of a QTL of agronomic interest in view of MAS. This map will complement genetic analysis in cassava and should provide us the additional opportunity to estimate genetic effects of

QTL. Development of a B_1P_2 map provides a different generation to study the QTL and their genetic effects.

In marker-assisted breeding, co-dominant markers such as SSR are effective in identifying desirable genotypes at early stages of selection. Therefore, this B_1P_2 map will be used for the study of QTL controlling protein content in the root of cassava.

Chapter 8

Quantitative trait loci (QTL) mapping of protein content in a B_1P_2 family derived from *M. esculenta* ssp *flabellifolia*

8.1 Introduction

The genetics of cassava is one of the least understood of the major staple crops that feed mankind (Fregene *et al.,* 1997). Limited research funding and several biological constraints impede the elucidation of the genetics of cassava traits. Such constraints include a long growth cycle, poor seed set, allogamy, allotetraploidy and severe inbreeding depression on selfing. The long crop cycle also considerably lengthens the time required for the development of new improved varieties (Kawano *et al.,* 1998; Fregene *et al*., 2001a).

Attempts have been made to improve the protein content of cassava roots through conventional breeding methods involving hybridisation at the inter-specific levels as well as by induced polyploidy and mutation (Mahungu, 1987; Asiedu *et al.*, 1992). Screening of a large germplasm collection of about 1400 varieties from India showed no significant variability in protein content (Hrishi and Jos, 1977). However, Ceballos *et al.* (2006) reported a wide variation in the protein values in the roots after screening cassava germplasm from the global collection held at CIAT. They concluded that because of the large variability, protein in cassava roots is largely controlled by genetic factors and can be improved.

Most agronomical important characteristics such as yield and quality traits are inherited quantitatively (Zhuang *et al*., 1997; Okogbenin and Fregene, 2002). The combined influence of quantitative loci and the environment produce the phenotype observed for these traits (Geldermann, 1975; Okogbenin and Fregene; 2003). In many cases, the effects of the environment mask those of the genotype, to the extent that the phenotype provides an imperfect measure of the genetic potential of the plant. To deal

with this problem, elaborate field testing techniques have been developed (Allard, 1960). These techniques have been useful in the development of new crop varieties, but at the cost of much time and effort. For many crops, the development time of an improved variety often exceeds 10 years (Tanksley *et al.,* 1989; Okogbenin *et al.*, 2007; Dixon *et al.,* 2008).

For most quantitative traits, little is known about the number, chromosomal position, action or individual and interactive effects of genes controlling their expression (Tanksley *et al*., 1989). While the theory and techniques of quantitative genetics have proved useful in the study of quantitative traits, these characters continue to be more difficult to manipulate in breeding programmes compared to single gene traits. If complex or quantitative traits could be resolved into their individual genetic components, it might be possible to deal with these characters with the efficacy of dealing with single gene traits (Wu *et al.,* 1992).

One of the main uses of DNA markers in agricultural research has been in the construction of linkage maps and identification of markers linked to regions of the genome controlling traits of interest (Mohan *et al.,* 1997; Collard *et al.,* 2005). DNA markers are widely accepted as potentially valuable tools for cassava improvement (Fregene *et al.,* 2001a; 2006; Okogbenin *et al.,* 2008; Hurtado *et al.,* 2008) and other crops like rice (Mackill *et al.,* 1999), wheat (Eagles *et al.,* 2001; Chee *et al.,* 2001), maize (Tuberosa *et al.,* 2003; Holding *et al.,* 2008; Wassom *et al.,* 2008), barley (Thomas, 2003), soybean (Panthee *et al.,* 2005), bean (Blair *et al.,* 2006) and others species (Barone, 2004; Kelly *et al.,* 2003; Snowdon and Friedt, 2004). In cassava, molecular genetic markers have been identified, linked to resistance to diseases (Akano *et al*., 2002; Jorge *et al*., 2000), yield and yield components (Okogbenin and Fregene 2002; 2003), morphological traits (Okogbenin and Fregene 2002), and root quality traits (Balyejusa Kizito *et al.,* 2007).

Protein content in cassava is variable (Phuc *et al.,* 2000; Chapter 5). The major protein in cassava is highest in glutamic acid and lowest in methionine (Sreermamurthy, 1945; Close *et al.,* 1953) with cystine and cysteine involved in the detoxification of cyanide (Osuntokun *et al.,* 1968). Several efforts have been made in

other crops to identify QTL that are responsible for protein in crops like maize (Wu *et al.,* 2002; Holding *et al.,* 2008; Wassom *et al.,* 2008), rice (Zhang *et al.,* 2008), wheat (Chee *et al.,* 2001), and soybean (Panthee *et al.,* 2005).

Wild relatives of crops harbour useful genes, which can be introgressed to improve domesticated cultivars (Zhou *et al.,* 2003; Aluko *et al.,* 2004; Fregene *et al.,* 2006; Ogundiwin *et al.,* 2008). Significant advances have been made both in the molecular technologies and hybridisation procedures available for breeding and cultivar development that allow for the incorporation of more distantly related taxa (Prescott-Allen and Prescott-Allen, 1986; Hajjar and Hodgkin, 2007). Tanksley and McCouch (1997) pointed to the potential role of genome mapping in efficiently utilising the genetic diversity of wild relatives which would result in new gene discoveries and use.

The use of molecular markers to introgress a target region of the genome saves time (Frisch *et al.,* 1999). It has been shown in several crops that the tremendous genetic potential locked up in wild relatives can be made available through advanced backcross quantitative traits loci mapping schemes (Tanksley and McCouch, 1997; Chee *et al.,* 2001; Blair *et al.,* 2006). Wassom *et al.* (2008) using BC_1-derived S_1 lines in maize, reported that QTL detected by composite interval mapping explained 46.9%, 45.2%, 44.3%, and 17.7% of phenotypic variance for oil, protein, starch, and mass, respectively and 17.5%, 22.9%, 40.1%, and 28.7% for oil, protein, starch, and yield, respectively, in topcross hybrids.

The objective of this study was to identify QTL controlling protein content in the root, to study the influence of the environment on the expression of protein content and to determine the effects of each QTL in the first backcross derivatives of *M. esculenta* ssp *flabellifolia,* using SSR markers from the linkage map, and phenotypic measurements.

8.2 Materials and methods

The mapping population for this study was the B_1P_2 family described in Chapter 5. The selection of CW 198 - 11, the female parent used to generate the B_1P_2 mapping population, was based on its high root protein content, high dry matter content, good tuber formation, and resistance to whitefly at the trial location (CIAT).

The field trial described in Chapter 7 which was repeated in 2008 at CIAT and Quilichao locations was used for the QTL mapping population. A total of 225 genotypes of the B_1P_2 population with the parents were used. The trial was harvested at 10 MAP and evaluated for protein content in the roots in three environments in Colombia in the 2007 and 2008 cropping season.

Means of the protein contents of plant harvested in the B_1P_2 progenies were calculated using Microsoft Excel. Variance components were determined using the type III sum of squares of the ANOVA. Spearman's rank correlation coefficients of protein content were calculated across locations to determine the extent or magnitude to which protein content in the root is controlled by the same genes in different environments. Frequency distribution of the mean protein content of backcross population data were examined with Sigmaplot (10.0).

For molecular marker analysis, total genomic DNA isolation was done using the DNA miniprep extraction protocol based on a modified Dellaporta *et al*. (1983) extraction procedure as described earlier in Chapter 7. Simple sequence repeat (SSR) marker analysis was conducted as described in Chapter 7.

QTL mapping was based on 105 markers from the genetic map of the B_1P_2 population (Chapter 7). Each of the markers was tested for normal Mendelian segregation using chi-square tests with a significance level of 0.05. Adjusted means of protein content were employed in single marker analysis for QTL mapping of the B_1P_2 segregating population using the software package MapDisto (Lorieux, 2007). Simple interval mapping and composite interval mapping was conducted using the computer package Window QTL Cartographer version 2.5 (Wang *et al.*, 2007). The cassava genome was

scanned for the presence of a QTL at 2.0 cM intervals. A LOD score of 2.5 was chosen as the minimum to declare the presence of a QTL (Rector *et al.*, 1998). In this programme, threshold values for significance were declared at p = 0.05 and were estimated from 1000 permutations of the data for the trait (Churchill and Doerge, 1994). The resulting R^2 represents the proportion of the phenotypic variance explained.

8.3 Results

The distribution of protein content in individuals of the backcross population in different environments is presented in Figure 8.1a-c. The range of the protein content of the mapping population at CIAT Headquarters in 2007 (CIAT 2007), CIAT Headquarters in 2008 (CIAT 2008) and CIAT sub-station, Quilichao in 2008 (Quilichao 2008) were from 0.77% to 9.61%, 0.17% to 9.11% and 0.69% to 7.75% respectively. The frequency distribution for the trait showed continuous distribution as expected for quantitative traits (Churchill and Doerge, 1994). The protein trait does not fit a normal distribution with a skewness of 0.87 for CIAT 2007, 0.88 for CIAT 2008 and 1.28 for Quilichao 2008 which is a good indicator that the trait is quantitatively inherited. The phenotypic data for the protein QTL were not transformed for normality to normalize data to avoid misrepresentation among individuals and thus reducing the ability of detecting QTL.

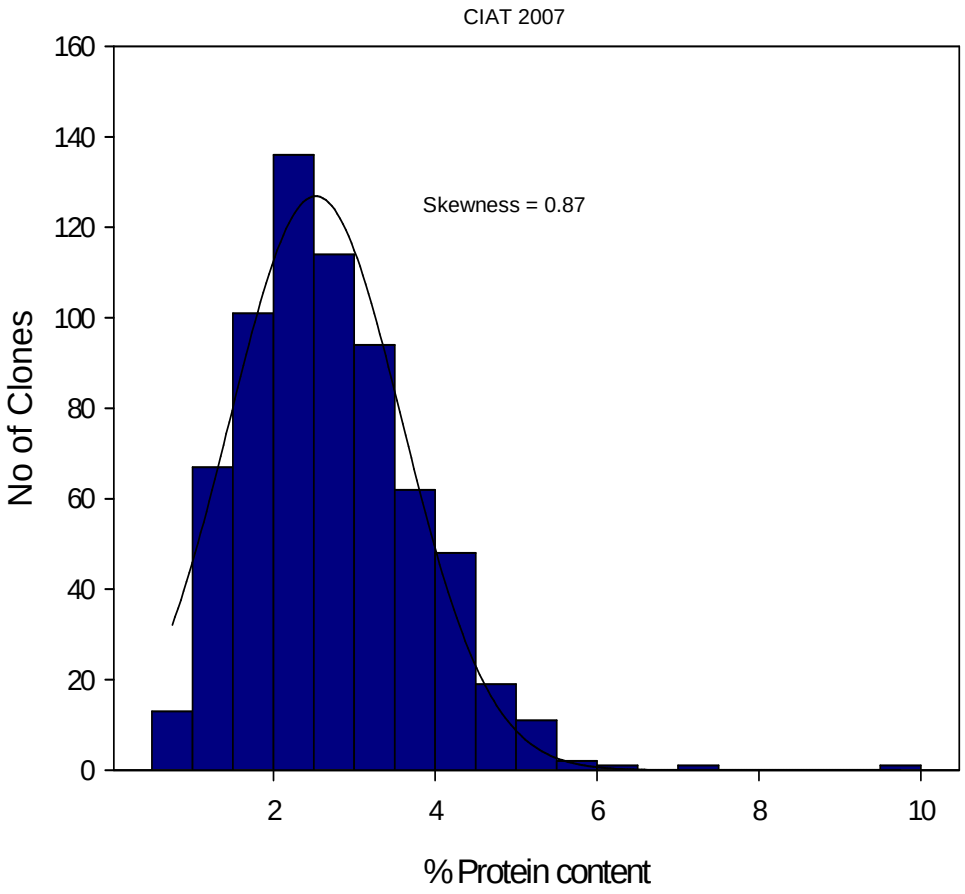


Figure 8.1a: Frequency distribution of the mean protein content of a cassava backcross population at CIAT, Colombia during the 2007 cropping season

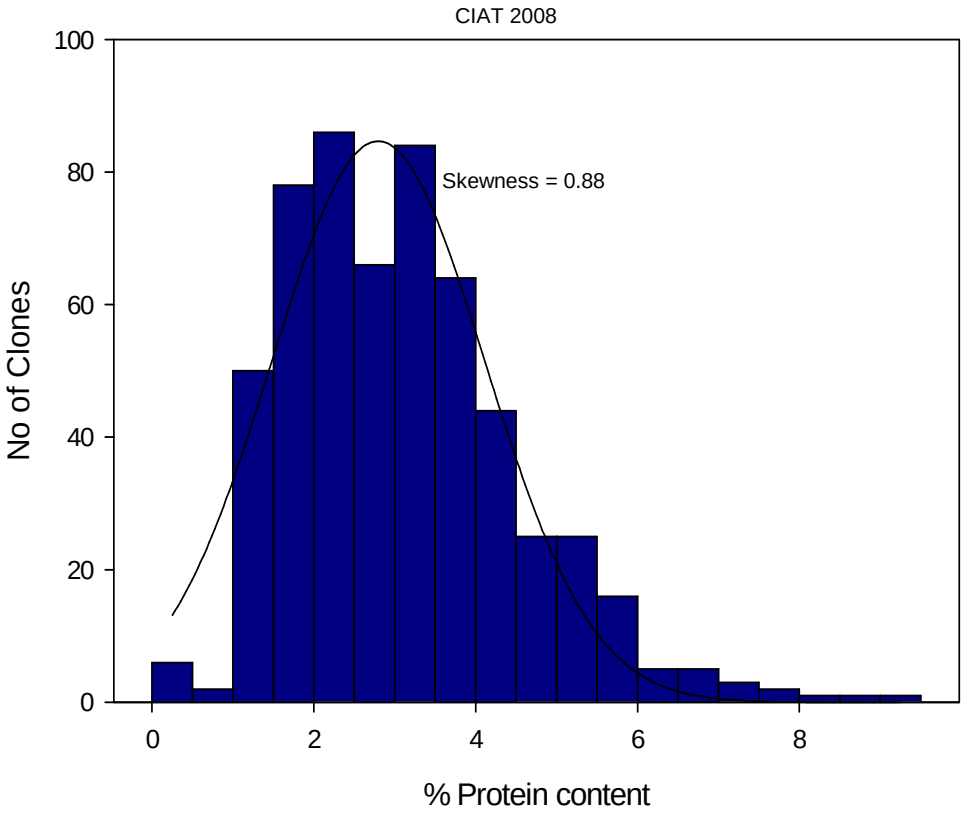


Figure 8.1b: Frequency distribution of the mean protein content of a cassava backcross population at CIAT, Colombia during the 2008 cropping season

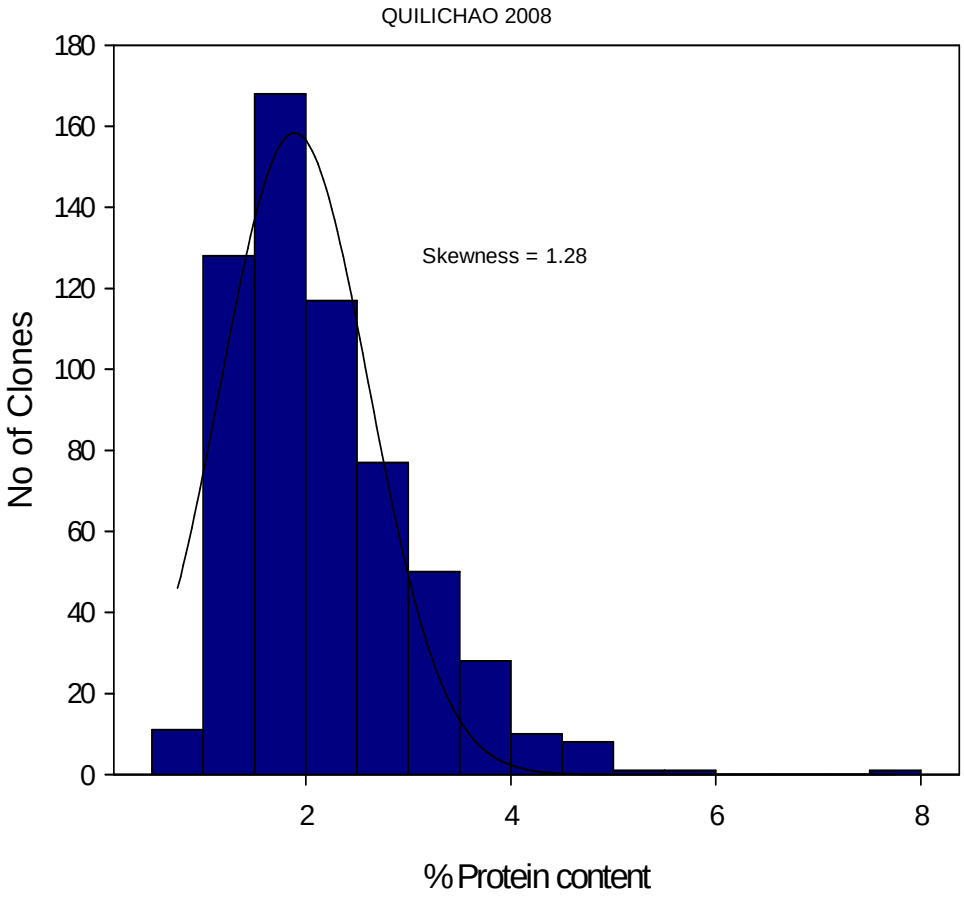


Figure 8.1c: Frequency distribution of the mean protein content of a cassava backcross population at the CIAT station in Quilichao, Colombia during the 2008 cropping season

The analysis of variance combined over two years and in three environments revealed highly significant differences ($p<0.0001$) among clones for protein content in the roots and provided evidence for genetic origin of high root protein content in (Table 8.1).

Environmental influence on protein content was highly significant and there was no correlation between clones and the environment. In CIAT 2007, mean of the protein content in the population was 3.12%; CIAT 2008 mean was 3.19% and Quilichao 2008 mean was 3.08% (Figure 8.1a-c). Genotypic correlation of protein content in all

environments were significant ($p<0.001$, $p<0.05$) between CIAT 2007, CIAT 2008 and Quilichao 2008 but not significant between CIAT 2008 and Quilichao 2008 (Table 8.2).

Table 8.1: Analysis of variance for protein content in roots from a cassava backcross population in three environments in Colombia between 2007 and 2008

Source of variation	Df	Sum of squares	Mean square	F-value	Pr >F
Clones	274	45.60	0.17	1.84	<0.0001
Error	544	49.13	0.09		
Total	818	94.73			

CV = 19.23%; data has been transformed by the √ (%protein) function

Table 8.2: Genotypic rank correlation coefficients between root protein content in a cassava backcross population in three environments in Colombia between 2007 and 2008

	CIAT 2007[ψ]	CIAT 2008
CIAT 2008	0.27****	
Quilichao 2008	0.26****	0.13*

[ψ] = these are the locations and year of the experiment; *p<0.05, ****p<0.0001

Out of the 105 SSR markers used for the detection of QTL in this mapping population, 21 markers showed varying degrees of segregation distortion on 14 chromosomes (Table 8.3). Five of the markers (rNS82, EST105, rSSRY226, rSSRY31 and SSRY149) had χ^2 values of 23.68, 13.02, 33.64, 41.82 and 15.61.

Table 8.3: **χ^2 values and chromosome location of microsatellite markers showing segregation distortion among 225 cassava backcross lines derived from the cross CW 198 - 11 X MTAI - 8**

Marker	Chromosome	χ^2	Probability F	Chromosome position
rNS82	1	23.68	0.0000	0.00
EST105	2	13.02	0.0003	0.00
NS347	2	8.64	0.00328	32.00
EST47	7	4.35	0.037	0.00
SSRY113	10	10.77	0.00103	18.00
NS57	10	7.88	0.00501	10.3
NS80	10	6.69	0.0097	0.00
SSRY21	10	9.53	0.00202	19.80
SSRY230	13	9.16	0.00247	81.60
SSRY34	13	4.05	0.04407	30.4
SSRY52	18	4.93	0.02643	20.20
rSSRY226	19	33.64	0.00000	119.30
NS656	21	5.49	0.01909	0.00
OS112	21	4.65	0.03097	64.60
SSRY170	23	9.25	0.00236	0.00
SSRY74	23	5.94	0.01476	54.30
SSRY194	25	5.59	0.01803	63.6
SSRY271	25	4.74	0.02946	47.9
rSSRY31	27	41.82	0.000000	30.10
SSRY92	29	6.08	0.01364	26.90

A total of four putative QTL were identified on linkage group 6, 7, and 23 respectively using MapDisto (Figure 8.2). Composite interval mapping using Windows Cartographer programme, was consistent with results from MapDisto and revealed linkage groups 7 and 23 as having QTL and detected another QTL on linkage group 13 (Figure 8.3). All the QTL for protein content in the root showed a LOD score above 2.5. QTL *protg.7* found on linkage group 7 in interval EST47 -

rSSRY110, accounted for 15% of the phenotypic variance for protein content in the trial at CIAT 2007 location with a LOD of 3.8. QTL *protg.13* was found on linkage group 13 in interval NS59 - SSRY230 and explained 15% of phenotypic variance in the Quilichao 2008 experiment with LOD 3.0. QTL *protg.23* on linkage group 23 located between markers NS664 and rSSRY88, accounted for 24% and 25% of phenotypic variance in CIAT 2008 and Quilichao 2008 with LOD score of 3.58 and 5.30 respectively (Table 8.4). In Figure 8.2 where MapDisto was used to detect QTL, a QTL was detected for CIAT 2007 location on linkage group 23 but in a further check using CIM, phenotypic variance in CIAT 2007 was 0.00% with LOD score of 0.00. The QTL detected on linkage group 6 using MapDisto that could not be detected using CIM, was eliminated in further reports.

The three QTL (*protg.7, protg.13* and *protg.23*) all showed additive gene action with values of 6.20, 3.21, and 4.14, 4.52 respectively. QTL *protg.23* identified in linkage group 23 cut across locations and years using MapDisto but in Windows QTL cartographer, QTL were detected in two locations. The additive effects in these three QTL showed that all came from the CW 198 - 11 parents, which is also the protein donor parent, suggesting that these QTL are associated with protein content and are not artefacts. The use of threshold values by permutation with a significance level of 0.05 for declaration of an association between marker loci and QTL using composite interval marker eliminated markers with no significant effect and provided an approximate location for QTL affecting protein content (Figure 8.3).

Table 8.4: Quantitative trait loci for protein content in the root of a cassava backcross mapping population

Trait	QTL[a]	Linkage group	Marker Interval	LOD[b]	R^2	Additive	Gene action	Allelic source
Protein	protg.7	LK.7	EST47 - rSSRY110	3.8	0.15	6.20	additive	CIAT 2007
	protg.13	LK.13	NS58 - SSRY230	3.0	0.15	3.21	additive	Quilichao 2008
	protg.23	LK.23	NS664 - rSSRY88	0.00	0.00	0.00	additive	CIAT 2007
				3.58	0.24	4.14	additive	CIAT 2008
				5.30	0.25	4.52	additive	Quilichao 2008

[a]Quantitative trait loci; LG = Linkage group; [b]Logarithm of odds ratio

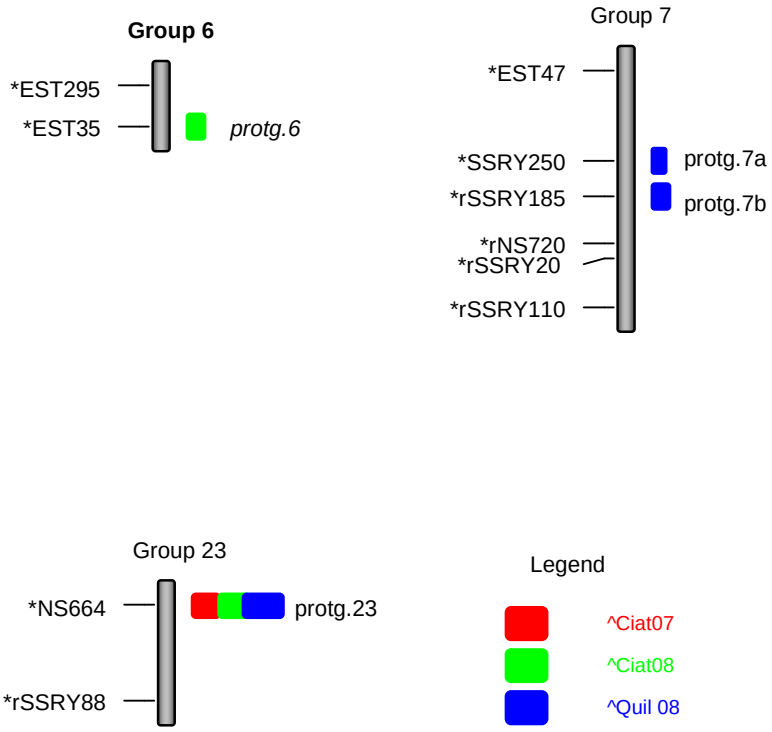


Figure 8.2: The likelihood plots of QTL associated with root protein content of cassava in the B_1P_2 population

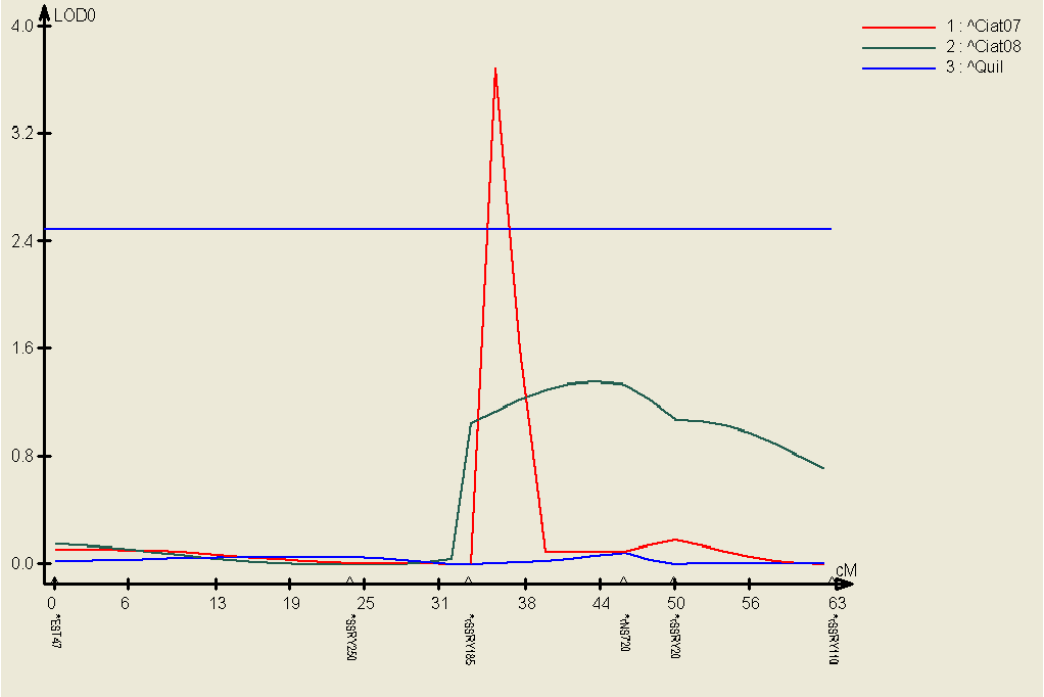


Figure 8.3a: Quantitative trait loci scan for linkage group 7 associated with protein content in a backcross population of cassava

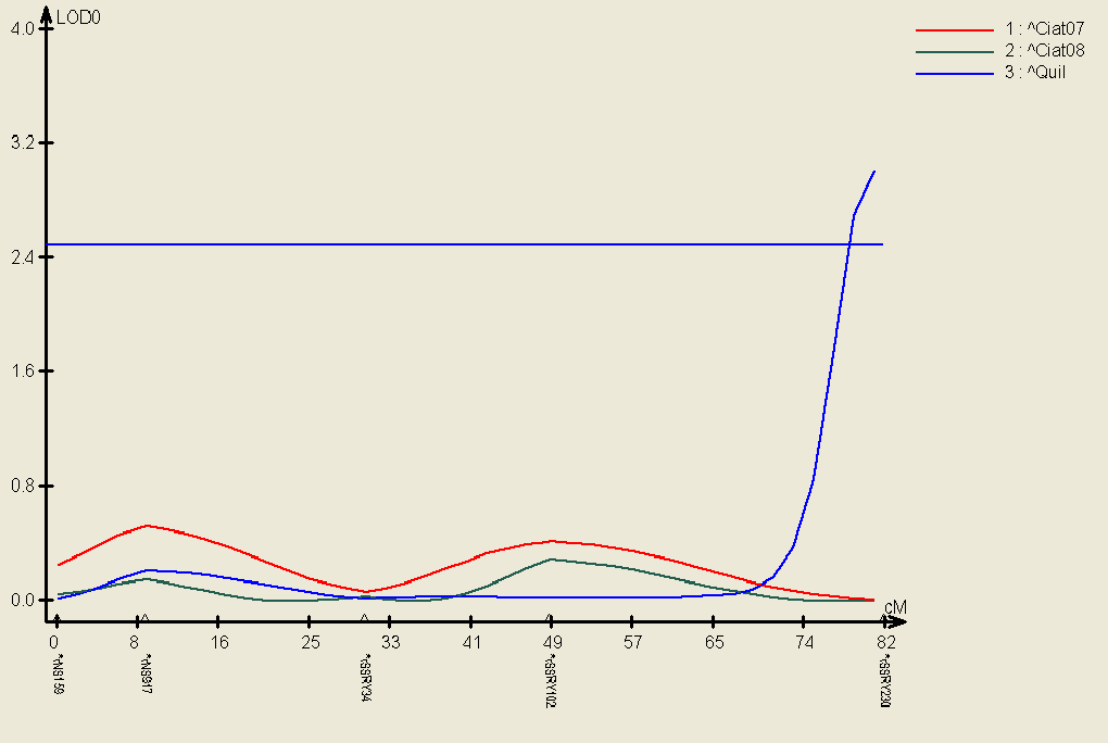


Figure 8.3b: Quantitative trait loci scan of linkage group 13 associated with protein content in a backcross population of cassava

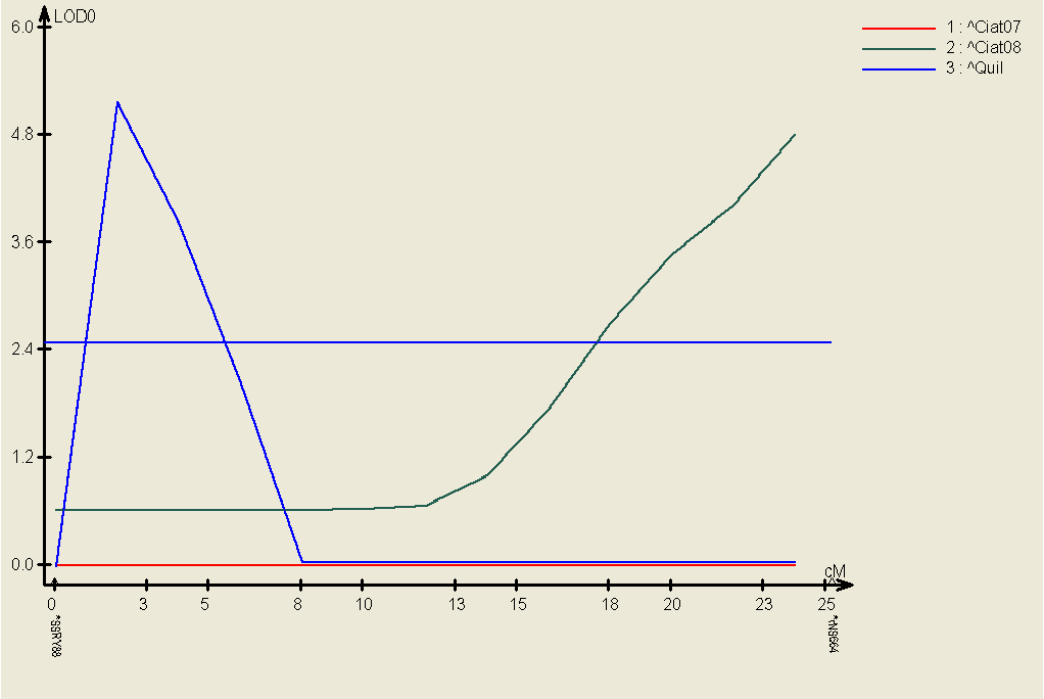


Figure 8.3c: Quantitative trait locus scan of linkage group 23 associated with protein content in a backcross population of cassava

8.4 Discussion

New technology in the area of molecular biology has made positive contributions to improvement and more efficient breeding (Fregene *et al.*, 1997; DeVries and Toenniessen, 2001; Okogbenin and Fregene, 2002; Okogbenin *et al.*, 2006; Balyejusa Kizito *et al.*, 2007; Okogbenin *et al.*, 2008). Wild *Manihot* germplasm offers a wealth of useful genes for the cultivated *M. esculenta* species, but its use in regular breeding programmes is restricted by linkage drag and a long reproductive cycle (Nassar, 2000; Fregene *et al.*, 2006; Hajjar and Hodgkin, 2007).

A QTL approach was used in this study to identify the genes that control protein content in the root of cassava. Three different QTL were identified on linkage groups 7, 13, and 23 in three locations. A QTL was identified in more than one location, which suggested stability across environments. Okogbenin and Fregene (2002) in their earlier studies of early root bulking of cassava reported a total of 18 and 27 QTL controlling the traits in the two experiments reported. In another report by Okogbenin and Fregene (2003) a number of QTL was detected in more than one trial: 29 QTL in two trials and 11 QTL in three trials for different productivity traits. Balyejusa Kizito *et al*. (2007) reported two QTL for cyanogenic glucoside potential and six QTL for dry matter content from an S_1 population of cassava in a single environment. Okogbenin *et al.* (2008) identified three QTL each for dry root yield, fresh foliage and harvest index, which explained phenotypic variances of 33.0%, 43.5%, and 36.0% respectively in a single environment. Jorge *et al.* (2001) identified eight QTL that were involved in the resistance of *Xanthomonas axonopodis* pv *manihotis* pathogen population from an intra-specific population cross between two non-inbred cassava where one QTL, located on linkage group D of there map was observed over two crop cycles. In another backcross population, Wydra *et al.* (2004) reported 11 markers, which explained between 16.0% and 33.3% of phenotypic variance of area under disease progress curve for cassava bacterial blight from different locations in Africa.

Results from Chapter 5 revealed broad-sense heritability for protein of 0.61, an indication that the protein trait is heritable compared to yield (Dixon *et al.,* 1994a; Kawano *et al.,* 1998; Benesi *et al.,* 2004; Okogbenin, 2004; Ceballos *et al.,* 2006; Balyejusa Kizito *et al.,* 2007). The three QTL detected showed additive effects suggesting this gene action plays a more important role for the protein trait in the backcross population of cassava. Cach *et al.* (2006) have reported that additive effects play an important role in dry matter content. Likewise Balyejusa Kizito *et al.* (2007) identified QTL for cyanogenic glucosides in their population with additive effects.

QTL for protein content in cassava found in this study is comparable with that reported for durum wheat (Chee *et al.,* 2001), soybean (Panthee *et al.,* 2005), and maize (Holding *et al.,* 2008; Wassom *et al.,* 2008). The magnitude of QTL is similar to that identified in previous cassava studies for early yield and root quality (Okogbenin and Fregene, 2003; Baylejusa Kizito *et al.,* 2007). These results indicate that the population structure employed and coverage of markers was optimum.

From the breeding point of view, the three QTL found for high protein content can be the basis for marker-assisted introgression of the high root protein trait into adapted gene pools that have low protein content (Chávez *et al.*, 2005; Ceballos *et al.,* 2006). Use of high protein alleles for cultivar improvement would allow for selection of breeding materials with high protein concentration above the current cassava value in available commercial cassava cultivars.

Summary

Keywords: Cassava, *Manihot esculenta* ssp *flabellifolia,* inter-specific cross, introgression, protein, yield, pest, molecular markers (SSR), molecular map, quantitative trait loci (QTL)

Manihot esculenta ssp *flabellifolia* possesses valuable genetic variability for protein and disease resistance genes that were introgressed into cultivated cassava. FLA 444- 5 with a protein content of 10.5% (dry weight basis) was hybridised with cassava through controlled crosses and backcrossed to cassava to obtain the B_1P_2 family used in this study. The inter-specific hybrids had protein values ranging from 0.77% - 11.25%, which indicated introgression of the trait.

Application of *in vitro* seed germination, overcame the bottleneck of seed dormancy and insufficient planting materials for replicational trials at an early stage of evaluation. High positive and negative correlations were estimated in the B_1P_2 backcross family for yield traits, quality traits, and pests. Moderate to high heritability values were estimated for commercial roots, roots per plant, harvest index, root weight, fresh root yield, dry root yield, dry matter content, post harvest physiological deterioration, and protein content. Protein content and dry matter content were negatively correlated, but not significantly. Location and year did not show any significant difference in protein yield by genotype. Evaluation of whitefly in this study showed a high number of genotypes (86.70%) being resistant to infestation and infection in this Colombian high pressure pest region.

SSR markers (817) were screened in the parental lines and four B_1P_2 progenies, 402 polymorphic markers were identified; of which 105 were used for map construction. The genetic map consisted of 29 linkage groups spanning 1249.55 cM with average marker interval of 16.16 cM. A total of three QTL (*protg.7, protg.13* and *protg.23)* controlling protein were identified in the three different environments, with one QTL present across all three environments. The individual effects of alleles at these QTL ranged from 15% to 25% of the phenotypic variance explained.

Opsomming

Sleutelwoorde: Cassava, *Manihot esculenta* ssp *flabellifolia*, interspesifieke kruisings, introgressie, proteïen, opbrengs, insekte, molekulêre merkers (SSR), molekulêre kaart, kwantitatiewe eienskap lokusse (QTL)

Manihot esculenta ssp *flabellifolia* besit waardevolle genetiese bronne vir proteïen en insekweerstandsgene wat ingekruis is in gekultiveerde cassava. FLA 444-5 met ‘n proteïeninhoud van 10.5% (droë gewig basis) is met kommersiële cassava gekruis deur beheerde kruisings om die B_1P_2 familie te kry wat gebruik is in hierdie studie. Die interspesifieke basters het proteïenwaardes gehad wat gewissel het tussen 0.77% - 11.25%, wat aandui dat die eienskap wel oorgedra is. Toepassing van *in vitro* saad ontkieming, het die bottelnek van saad dormansie en die gebrek aan plant materiaal vir gerepliseerde proewe in die vroeë fases van evaluasie oorkom. Hoë positiewe en negatiewe korrelasies is in die B_1P_2 terugkruisingfamilie gevind vir opbrengs en verwante eienskappe, kwaliteitseienskappe en insekweerstand. Gemiddelde tot hoë oorerflikheid is gevind vir kommersiële wortels, wortels per plant, oesindeks, wortelmassa, vars wortelmassa, droë wortelmassa, droëmateriaal opbrengs, na-oes fisiologiese agteruitgang, en proteïeninhoud. Proteïeninhoud en droëmateriaal inhoud was negatief gekorreleer, maar nie betekenisvol nie. Lokaliteit en jaar het geen betekenisvolle verskille tussen genotipes uitgewys nie. Evaluasie van witvlieg besmetting het ‘n groot getal genotipes (86.70%) met weerstand uitgwys in die Kolombiese hoë besmettingsdruk area waar die proewe geplant is.

SSR merkers (817) is geëvalueer met die ouerlyne en vier B_1P_2 populasies. Polimorfiese merkers (402) is geïdentifiseer, waarvan 105 duidelike segregasie getoon het. Hierdie merkers is vir die konstruksie van 29 voorlopige koppelingsgroepe met 227 genotipes wat oor 1249.55 cM strek met ‘n gemiddelde merker interval van 16.69 cM. ‘n Totaal van drie QTL (*protg.7, protg.13* and *protg.23)* wat proteïeninhoud beheer, is geïdentifiseer in die terukruisingspopulasie in twee verskillende omgewings. Die individuele effekte van allele by hierdie QTL het tussen 15% - 25% van die fenotipiese variasie verklaar.

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List of abbreviations

Abbreviation	Full meaning
ABC-QTL	Advanced backcross - quantitative trait loci
AFLP	Amplified fragment length polymorphism
ANOVA	Analysis of variance
APul	Pupa population on the lower part of the plant
Bajo	Lower
B_1P_2	Backcross one family name nomenclature
BC	Before Christ
BC_1	Backcross one
Bp	Base pair
Cal	Calories
CBB	Cassava bacterial blight
CIAT	Centro Internacional de Agricultura Tropical
Cm	centimeter
cpDNA	Chloroplasmic DNA
CM	Cassava mealybug
CF	Crude fibre
CMD	Cassava mosaic disease
CNp	Cyanogenic potential
cDNA	Complementary DNA
cM	Centi Morgan
CIM	Composite interval mapping
ComRt	Commercial Root
CW	CIAT F_1 name nomenclature
CV	Coefficient of variation
CORPOICA	Corporación Colombiana de Investigación Agropecuaria
CMG	Cassava mosaic germiniviruses
DNA	Deoxyribonucleic acid

^{o}C	degrees Celsius
dNTP	2'-deoxynucleoside 5'-triphosphate
DMC	Dry matter content
DRY	Dry root yield
df	Degrees of freedom
DSup	Damage at the superior surface
DMed	Damage at the middle part of the plant
DBajo	Damage at the lower part of the plant
EMBRAPA	Empresa Brasileira de Pesquisa Agropecuária
EST	Expressed sequence tags
ECM	Expectation/conditional maximisation
EDTA	Ethylenediaminetetraacetate
F_1	First filial generation
F_2	Second filial generation

Abbreviation	**Full meaning**
FRY	Fresh Root Yield
GA_3	Gibberellic acid-3
G X E	Genotype by environment interaction
g	gram(s)
HCl	Hydrochloric acid
HCN	Hydrogen cyanide
H_2O_2	Hydrogen peroxide
ha	Hectare(s)
HI	Harvest Index
IITA	International Institute of Tropical Agriculture
IPGRI	International Plant Genetic Resources Institute (Now Biodiversity)
KCl	Potassium Chloride
kg	Kilogram
kb	Kilobite

L	Litre
LOD	Log_{10} of odds ratio
LSD	Least significant difference
M	metre(s)
Mg^{2+}	Magnesium ion
mRNA	Messenger RNA
MAS	Marker assisted selection
MAP	Months after planting
mg	Milligram
mm	Millimetre(s)
min	Minutes
mo	Month
m^2	square metres
MNp	Nymph population on the middle part of the plant
Mpul	Pupa population on the middle part of the plant
Med	Middle
mM	milliMolar
M	Molar
mtDNA	mitochondrial DNA
µl	microlitre(s)
µM	microMolar
NAA	1-Naphthaleneacetic acid
NaCl	Sodium Chloride
N	Nitrogen
NPK	Nitrogen:Phosporus:Potassium
ns	Not significant
nm	nanometre(s)
PAGE	Polyacrylamide denaturing gels

Abbreviation	Full meaning
PCA	Principal component analysis
PCR	Polymerase chain reaction
PCs	Principal Component Analysis
pH	power of Hydrogen
PC	Protein content
PPD	Post harvest physiological deterioration
QTL	Quantitative trait loci
RFLP	Restriction fragment length polymorphism
RAPD	Random amplified polymorphic DNA
RtWt	Root weight
Rtplt	Roots per plant
Rep	Replication
Rpm	Revolution per minute
S_1	Selfing of first generation
SDRF	Single dose restricted fragment
Sup	Superior
SD	Standard deviation
SDS	Sodium dodecyle sulphate
SRT	Single row trial
SNP	single nucleotide polymorphism
SAS	Statistical analytical system
SSCP	Single strand conformation polymorphism
STS	Sequence tagged sites
SCAR	sequenced characterized amplified regions
SSR	simple sequence repeats
SSRP	Simple sequence repeat polymorphism
SSRY	Simple sequence repeats yuca
Spp	subspecies
TDFs	Transcript derived fragments

T	ton
Taq	Thermus aquaticus
TE	Tris/EDTA
TBE	Tris/Boric acid/EDTA
Tris-HCl	Tris[hydroxymethyl]aminomethane hydrochloric acid
TMS	Tropical Manioc Selection
UAdl	Adult population on the leaf surface
U	Unit(s)
UEgg	Egg number on the leaf surface
Unph	Nymph population on the leaf surface
UnPul	Pupa population on the leaf surface
VNTR	Variable number of tandem repeats
v/v	Volume by volume
W	Watt
W_a	Weight in the air
Abbreviation	**Full meaning**
W_w	Weight inside water
w/v	Weight by volume
Whfly	White fly
Yld	Yield

Appendix 1: Selected accessions of open pollinated *M. esculenta* ssp *flabellifolia*

No	Clone	Mother	% DMC[a]	%PC[b]	%CF[c]	%Ash	%Amylose
1	OW 54- 1	FLA 444- 8	33.286979	8.07	5.04	2.972652	10.95
2	OW 54- 2	FLA 444- 8	44.572127	8.66	8.075	-	10.32847
3	OW 54- 3	FLA 444- 8	32.995882	5.41	5.405	3.236246	11.24088
4	OW 54- 4	FLA 444- 8	31.444819	10.32	3.57	2.554745	-
5	OW 54- 5	FLA 444- 8	30.664794	5.02	5.015	2.140584	12.29927
6	OW 54- 6	FLA 444- 8	47.695334	10.46	16.5	2.312139	-
7	OW 54- 7	FLA 444- 8	37.5	7.46	7.455	2.993744	-
8	OW 54- 8	FLA 444- 8	44.766585	7.8	41.64	2.267574	10.54745
9	OW 56- 1	FLA 444- 2	50.560957	4.78	42.05	1.105583	12.26277
10	OW 56- 2	FLA 444- 2	33.835052	9.45	13.15	2.389595	14.34307
11	OW 57- 1	FLA 444- 4	50.015446	6.64	31.57	2.559913	-
12	OW 57- 2	FLA 444- 4	36.566009	8.2	3.825	2.013423	-
13	OW 57- 3	FLA 444- 4	39.572778	4.49	3.465	1.739539	12.37226
14	OW 57- 4	FLA 444- 4	30.047564	6.42	4.52	2.212806	11.09489
15	OW 57- 5	FLA 444- 4	29.602446	3.5	4.045	2.498048	14.30657
16	OW 57- 6	FLA 444- 4	35.7493	6.57	4.425	0.897989	-
17	OW 57- 7	FLA 444- 4	31.309362	4.75	2.415	2.621628	12.15328
18	OW 58- 1	FLA 444- 5	37.288136	2.68	10.82	1.210287	13.43066
19	OW 58- 2	FLA 444- 5	34.228953	5.07	6.03	2.133744	-
20	OW 58- 3	FLA 444- 5	36.07344	3.7	8.605	1.340996	13.28467
21	OW 58- 4	FLA 444- 5	46.438884	4.28	18.76	1.420548	12.55474
22	OW 58- 5	FLA 444- 5	33.451163	4.17	15.53	1.845308	11.86131
23	OW 58- 6	FLA 444- 5	57.318741	5.53	41.34	2.180685	-
24	OW 58- 7	FLA 444- 5	29.816514	6.23	10.88	2.279006	12.15328
25	OW 58- 8	FLA 444- 5	40.413471	3.6	2.63	1.575006	15.80292
26	OW 58- 9	FLA 444- 5	61.373874	2.24	46.1	1.478415	11.86131
27	OW 58- 10	FLA 444- 5	32.916003	6.25	4.485	2.156281	14.05109
28	OW 59- 1	FLA 444- 6	23.764045	8.99	5.15	2.130109	11.53285
29	OW 60- 1	FLA 444- 9	31.862745	8.79	7.47	2.315789	10.10949

30	OW	61-	1	FLA	433-	1	39.097104	5.85	36.95	3.296703	-
31	OW	61-	2	FLA	433-	1	49.807115	5.76	36.96	3.390773	-
32	OW	61-	3	FLA	433-	1	49.081365	5.77	24.63	2.115768	-
33	OW	61-	4	FLA	433-	1	32.642487	5.21	6.21	1.380104	-
34	OW	61-	5	FLA	433-	1	40.278746	5.84	15.61	2.142677	12.22628
35	OW	61-	6	FLA	433-	1	28.4375	8.28	3.88	2.187325	-
36	OW	62-	1	FLA	433-	2	28.007392	8.91	5.74	2.233429	-
37	OW	62-	2	FLA	433-	2	27.038043	13.08	-	2.744149	-
38	OW	62-	3	FLA	433-	2	30.21316	5.56	8.38	1.877435	12.26277
39	OW	62-	4	FLA	433-	2	41.880342	7.93	-	2.678571	-

No	**Clone**			**Mother**			**% DMC[a]**	**%PC[b]**	**%CF[c]**	**%Ash**	**%Amylose**
40	OW	62-	5	FLA	433-	2	47.51462	6.36	33.03	4.347826	-
41	OW	63-	1	FLA	433-	4	37.954164	5.14	22.63	1.709402	14.92701
42	OW	63-	2	FLA	433-	4	40.677966	7.12	31.94	2.358491	-
43	OW	63-	3	FLA	433-	4	32.433405	5.61	24.56	2.803738	-
44	OW	64-	1	FLA	432-	1	27.915633	10.5	6.51	2.013423	-
45	OW	64-	2	FLA	432-	1	29.080404	5.82	6.355	2.258065	13.21168
46	OW	64-	3	FLA	432-	1	35.350782	2.42	7.145	2.721088	16.67883
47	OW	64-	4	FLA	432-	1	48.610498	2.55	37.77	1.904762	-
48	OW	64-	5	FLA	432-	1	29.463807	6.51	5.17	1.904762	11.86131
49	OW	64-	6	FLA	432-	1	45.276074	3.65	4.134	1.639344	12.84672
50	OW	64-	7	FLA	432-	1	40.097121	4.12	18.07	2.325581	-
51	OW	64-	8	FLA	432-	1	46.340641	4.89	43.95	2.839117	-
52	OW	64-	9	FLA	432-	1	36.335761	4.46	58.17	2.747253	-
53	OW	65-	1	FLA	432-	2	45.111691	6.75	27.91	1.492537	-
54	OW	65-	2	FLA	432-	2	38.817006	6.05	24.09	2.463054	-
55	OW	65-	3	FLA	432-	2	29.102564	6.89	20.6	3.225806	-
56	OW	65-	4	FLA	432-	2	37.409639	6.24	16.85	4.697987	-
57	OW	65-	5	FLA	432-	2	42.421915	4.41	28.5	1.333333	-
58	OW	65-	6	FLA	432-	2	30.273224	6.37	20.3	-	-
59	OW	65-	7	FLA	432-	2	30.67911	3.76	13.67	1.470588	-
60	OW	65-	8	FLA	432-	2	42.971888	6.12	-	2.92887	-
61	OW	66-	1	FLA	430-	5	45.467836	8.17	15.84	1.169591	13.32117

62	OW	66-	2	FLA	430-	5	53.154876	6.97	-	1.831502	11.38686
63	OW	66-	3	FLA	430-	5	66.124837	6.5	41.53	3.875969	-
64	OW	66-	4	FLA	430-	5	53.208292	4.94	49.17	2.542373	-
65	OW	66-	5	FLA	430-	5	34.27762	9.06	15.52	2.857143	13.68613
66	OW	66-	6	FLA	430-	5	35.641026	7.48	23.4	2.793296	14.12409
67	OW	70-	1	FLA	443-	3	36.708145	7.36	4.49	1.574803	-
68	OW	70-	2	FLA	443-	3	33.652008	6.59	-	1.694915	-
69	OW	79-	1	FLA	439-	2	56.511976	5.34	16.27	1.766784	-
70	OW	79-	2	FLA	439-	2	68.476128	1.28	42.48	0.818331	-
71	OW	79-	3	FLA	439-	2	59.59241	0.85	32.73	0.873362	16.49635
72	OW	79-	4	FLA	439-	2	68.200549	1.46	29.13	0.921659	-
73	OW	79-	5	FLA	439-	2	37.422467	0.82	41.2	1.242236	17.44526
74	OW	79-	7	FLA	439-	2	44.457617	3.11	46.45	1.069519	16.42336
75	OW	79-	8	FLA	439-	2	67.797784	3.1	33.42	1.587302	14.67153
76	OW	80-	1	FLA	439-	3	81.70347	1.9	37.27	1.492537	-
77	OW	80-	3	FLA	439-	3	78.317433	0.85	34.81	1.176471	12.40876
78	OW	80-	4	FLA	439-	3	62.834979	0.58	33.56	1.477833	15.87591
79	OW	80-	5	FLA	439-	3	65.768725	2.53	33.97	1.260504	12.84672
80	OW	80-	6	FLA	439-	3	75.052854	1.91	31.33	0.561798	-
81	OW	80-	7	FLA	439-	3	80.058224	1.38	39.6	0.869565	-
82	OW	80-	8	FLA	439-	3	81.029619	3.39	41.76	1.081081	-
83	OW	80-	9	FLA	439-	3	79.508971	1	45.3	0.892857	10.94891
84	OW	81-	1	FLA	439-	5	82.214765	0.82	44.77	0.515464	-
85	OW	81-	2	FLA	439-	5	51.251158	3.93	56.25	1.020408	-
86	OW	81-	3	FLA	439-	5	73.701299	1.16	42.51	0.505051	-
87	OW	81-	4	FLA	439-	5	70.657277	1.66	40.26	1.530612	-

No	**Clone**			**Mother**			**% DMC[a]**	**%PC[b]**	**%CF[c]**	**%Ash**	**%Amylose**
88	OW	81-	5	FLA	439-	5	61.626248	1.13	31.89	0.526316	16.05839
89	OW	81-	6	FLA	439-	5	77.348551	2.23	41.43	1.583113	11.82482
90	OW	81-	7	FLA	439-	5	79.237947	3.19	33.3	0.625	13.35766
91	OW	81-	8	FLA	439-	5	40.293742	2.58	53.6	2.427184	17.37226
92	OW	82-	1	FLA	434-	3	87.84029	4.63	36.67	1.271186	-
93	OW	82-	2	FLA	434-	3	82.450331	6.13	33.58	1.923077	-

94	OW	82-	3	FLA	434-	3	61.710037	6.19	-	0.8	-	
95	OW	82-	5	FLA	434-	3	52.610442	8.44	17.71	2.762431	-	
96	OW	82-	6	FLA	434-	3	75.35545	7.51	32.04	2.55102	-	
97	OW	82-	7	FLA	434-	3	75.335121	2.15	30.97	1.428571		14.16058
98	OW	82-	8	FLA	434-	3	60.939431	8.26	20.41	2.424242	-	
99	OW	82-	9	FLA	434-	3	69.408369	8.61	32.92	2.459016	-	
100	OW	83-	1	FLA	434-	2	70.408163	5.48	43.68	2.777778	-	
101	OW	83-	2	FLA	434-	2	66.543438	4.92	57.54	3.773585	-	
102	OW	89-	1	FLA	436-	4	67.362722	5.27	35.86	1.639344		14.0146
103	OW	90-	1	FLA	435-	9	68.732908	8.19	37.56	1.149425	-	
104	OW	90-	2	FLA	435-	9	28.718428	9.76	3.9	2.459016		14.08759
105	OW	90-	3	FLA	435-	9	78.372591	5.53	27.1	1.156069	-	
106	OW	90-	4	FLA	435-	9	52.397408	8.57	9.665	1.190476	-	
107	OW	179-	1	FLA	423-	4	25.904453	11.11	5.955	3.151261		14.70803
108	OW	179-	2	FLA	423-	4	49.71385	5.5	33.99	2.083333		15.91241
109	OW	179-	3	FLA	423-	4	24.667584	9.25	4.705	2.55814		11.86131
110	OW	179-	4	FLA	423-	4	27.183559	8.73	4.56	2.272727		13.43066
111	OW	179-	5	FLA	423-	4	28.619718	9.62	4.695	2.028398	-	
112	OW	179-	6	FLA	423-	4	24.733317	10.56	5.375	2.590674		13.21168
113	OW	179-	7	FLA	423-	4	29.170195	11.08	6.52	2.4		9.744526
114	OW	179-	8	FLA	423-	4	44.706473	10.44	24.01	2.155172	-	
115	OW	179-	9	FLA	423-	4	35.034014	10.11	16.71	2.903811		15.25547
116	OW	180-	1	FLA	423-	5	24.686011	8.28	3.515	2.356902		15.18248
117	OW	180-	2	FLA	423-	5	43.352029	7.63	4.01	1.632653	-	
118	OW	180-	3	FLA	423-	5	32.913029	8.63	24.05	2.708333		13.9781
119	OW	180-	4	FLA	423-	5	27.474794	13.5	5.38	-		14.56204
120	OW	181-	1	FLA	423-	6	41.600634	9	13.55	3.082852	-	
121	OW	181-	2	FLA	423-	6	32.81106	7.25	5.645	-		11.67883
122	OW	181-	3	FLA	423-	6	21.134296	10.19	4.985	3.321033		14.23358
123	OW	181-	4	FLA	423-	6	34.932415	6.28	2.67	1.718213		12.18978
124	OW	182-	1	FLA	423-	7	39.562389	7.06	4.78	1.643836		14.12409
125	OW	182-	2	FLA	423-	7	29.379085	6.88	-	2.822581		15.40146
126	OW	182-	3	FLA	423-	7	34.081676	7.06	5.5	2.185792		14.67153

127	OW	182-	4	FLA	423-	7	35.928144	9.56	13.76	2.267003	13.68613
128	OW	182-	5	FLA	423-	7	60.247168	6.75	29.85	2.614379	-
129	OW	182-	6	FLA	423-	7	54.125737	9.38	19.38	3.395062	-
130	OW	182-	7	FLA	423-	7	38.512793	8.31	20.9	3.571429	14.12409
131	OW	182-	8	FLA	423-	7	32.880804	9.4	3.885	2.416918	14.08759
132	OW	183-	1	FLA	423-	8	33.728421	6.07	7.105	2.439024	12.11679
133	OW	183-	2	FLA	423-	8	42.020592	6.66	21.85	2.173913	12.9562
134	OW	183-	3	FLA	423-	8	44.978632	5.9	3.12	1.654846	-
135	OW	183-	4	FLA	423-	8	21.436004	14.59	3.61	3.319502	9.708029
No	**Clone**			**Mother**			**% DMC[a]**	**%PC[b]**	**%CF[c]**	**%Ash**	**%Amylose**
136	OW	183-	5	FLA	423-	8	31.645928	6.91	5.27	2.237136	14.45255
137	OW	183-	6	FLA	423-	8	34.036383	7.72	7.025	1.811594	14.12409
138	OW	183-	7	FLA	423-	8	47.056118	7.64	24.98	2.222222	15.36496
139	OW	185-	1	FLA	423-	10	34.680233	7.52	4.04	2.647658	13.9781
140	OW	185-	2	FLA	423-	10	28.971963	11.77	6.965	3.563474	17.18978
141	OW	186-	2	FLA	426-	3	38.493186	10.45	4.84	2.119461	14.08759
142	OW	186-	5	FLA	426-	3	40.768163	11.54	5.97	-	14.45255
143	OW	186-	6	FLA	426-	3	55.474453	8.89	6.845	2.134146	-
144	OW	186-	7	FLA	426-	3	58.319605	8.79	22.34	2.748414	14.59854
145	OW	187-	1	FLA	427-	1	57.954545	10.54	-	-	-
146	OW	187-	2	FLA	427-	1	41.432887	8.77	3.955	1.62037	14.70803
147	OW	187-	3	FLA	427-	1	37.813953	7.24	3.51	1.854305	10.87591
148	OW	187-	4	FLA	427-	1	43.773428	9.11	6.725	2.155887	13.21168
149	OW	187-	5	FLA	427-	1	33.976834	9.24	5.49	1.968504	13.39416
150	OW	187-	6	FLA	427-	1	35.445626	7.42	2.62	2.071563	-
151	OW	189-	1	FLA	427-	3	45.035294	8.41	4.92	1.215278	14.48905
152	OW	189-	2	FLA	427-	3	34.843305	6.68	-	-	13.61314
153	OW	189-	3	FLA	427-	3	44.518403	,	-	-	12.73723
154	OW	190-	1	FLA	427-	4	41.484943	10.5	6.02	1.856148	15
155	OW	190-	3	FLA	427-	4	48.257165	9.52	9.265	1.182033	13.21168
156	OW	190-	4	FLA	427-	4	71.275072	10.09	37.05	1.932367	-
157	OW	190-	5	FLA	427-	4	63.875089	8.47	28.67	1.851852	-
158	OW	190-	6	FLA	427-	4	72.872789	7.97	35.22	2.586207	-

159	OW	191-	1	FLA	427-	5	81.25	6.78	-	2.341137	-
160	OW	191-	2	FLA	427-	5	52.631579	7.62	6.33	1.304348	-
161	OW	191-	3	FLA	427-	5	43.635487	8.72	10.54	1.671309	15.40146
162	OW	192-	1	FLA	427-	7	33.50545	7.84	2.82	1.949318	-
163	OW	195-	1	FLA	429-	1	58.739537	8.13	30.49	1.587302	14.34307
164	OW	195-	2	FLA	429-	1	55.834829	7	30.33	1.629328	14.19708
165	OW	195-	3	FLA	429-	1	51.106314	5.74	8.46	1.718213	12.62774
166	OW	195-	4	FLA	429-	1	44.481481	5.63	8.79	2.554028	12.88321
167	OW	200-	1	FLA	429-	8	44.516428	7.35	14.74	2.04461	14.92701
168	OW	200-	2	FLA	429-	8	83.858268	6.05	24.45	2.506964	-
169	OW	200-	3	FLA	429-	8	39.707835	9.29	6.965	2.555366	-
170	OW	201-	1	FLA	430-	1	64.233577	3.19	43.54	1.762115	-
171	OW	201-	2	FLA	430-	1	55.503948	8.83	12.17	2.054795	-
172	OW	202-	1	FLA	430-	2	45.662848	10.47	19.63	1.766784	17.62774
173	OW	202-	2	FLA	430-	2	71.785714	5.8	40.25	1.277955	-
174	OW	203-	1	FLA	430-	5	83.417722	4.29	45.7	1.449275	-
175	OW	203-	2	FLA	430-	5	77.637131	9.87	-	-	-
176	OW	204-	1	FLA	434-	2	75.329567	9.41	33.87	4.487179	-
177	OW	206-	1	FLA	435-	2	73.651126	6.29	32.91	2.321429	-
178	OW	206-	2	FLA	435-	2	41.785526	6.79	7.21	1.807229	-
179	OW	208-	1	FLA	435-	10	40.65	8.02	-	1.968504	-
180	OW	208-	2	FLA	435-	10	91.67	7.19	11.31	1.456311	14.93
181	OW	208-	3	FLA	435-	10	71.334586	7.43	34.5	2.168022	-
182	OW	208-	4	FLA	435-	10	66.453674	10.4	-	2.020202	-
183	OW	209-	1	FLA	436-	1	57.274296	10.7	27.24	2.309469	-

No	**Clone**			**Mother**			**% DMC[a]**	**%PC[b]**	**%CF[c]**	**%Ash**	**%Amylose**
184	OW	209-	2	FLA	436-	1	57.45165	10.68	7.105	3.340757	-
185	OW	209-	3	FLA	436-	1	58.359822	7.71	39.56	1.777778	16.13139
186	OW	209-	4	FLA	436-	1	59.71564	8.03	26.2	1.724138	15
187	OW	212-	1	FLA	436-	5	69.097222	4.78	28.28	1.25	16.78832
188	OW	212-	2	FLA	436-	5	67.90378	4.97	36.28	1.37931	15.94891
189	OW	212-	3	FLA	436-	5	44.305902	6.73	14.14	1.061571	-
190	OW	212-	4	FLA	436-	5	81.848552	5.03	37.41	0.763359	-

191	OW	212-	5	FLA	436-	5	74.838246	4.88	34.38	1.502146	14.41606
192	OW	212-	6	FLA	436-	5	51.630941	6.45	22.82	1.574803	15.58394
193	OW	213-	1	FLA	437-	1	51.156463	5.69	13.68	1.282051	12.37226
194	OW	213-	2	FLA	437-	1	61.925602	3.16	23.78	1.973684	12.81022
195	OW	213-	3	FLA	437-	1	55.636034	5.94	14.84	1.012146	14.23358
196	OW	213-	4	FLA	437-	1	44.744274	2.61	7.12	1.5625	15.94891
197	OW	213-	5	FLA	437-	1	53.430924	1.87	14.03	1.187648	14.89051
198	OW	213-	6	FLA	437-	1	44.176404	3.99	11.84	1.467505	15.62044
199	OW	213-	7	FLA	437-	1	58.27381	4.03	26.18	1.624815	14.05109
200	OW	214-	1	FLA	437-	2	62.566845	4.41	22.65	1.152074	14.85401
201	OW	214-	2	FLA	437-	2	47.576396	3.91	16.06	1.890359	17.37226
202	OW	214-	3	FLA	437-	2	76.963351	3.38	33.86	1.001669	-
203	OW	214-	4	FLA	437-	2	36.932797	2.16	19.96	2.625298	-
204	OW	215-	1	FLA	437-	3	39.046579	1.27	14.36	2.513966	19.51
205	OW	215-	2	FLA	437-	3	44.421344	5.62	14.58	1.204819	13.94161
206	OW	215-	3	FLA	437-	3	40.648601	6.8	23.85	1.470588	15.07299
207	OW	217-	2	FLA	437-	6	55.58325	1.62	45	1.075269	14.63504
208	OW	217-	3	FLA	437-	6	34.769231	3.82	40.12	1.75	14.9635
209	OW	217-	4	FLA	437-	6	57.784615	1.47	40.35	2.813299	15.21898
210	OW	217-	5	FLA	437-	6	61.151961	5.02	28.86	1.907357	-
211	OW	220-	1	FLA	438-	2	37.822071	2.96	25.93	1.386482	15.69343
212	OW	220-	2	FLA	438-	2	46.029237	7.39	10.73	1.116071	12.9562
213	OW	220-	3	FLA	438-	2	48.160103	6.35	25.96	1.626016	14.16058
214	OW	220-	4	FLA	438-	2	32.807731	2.48	33.64	2.34375	-
215	OW	220-	5	FLA	438-	2	28.585757	2.69	28.7	2.10084	19.70803
216	OW	220-	6	FLA	438-	2	45.805266	1.96	16.1	2.189781	15.76642
217	OW	221-	1	FLA	438-	3	56.516937	1	17.16	1.075269	-
218	OW	221-	2	FLA	438-	3	59.393346	1.3	23.25	2.017291	-
219	OW	222-	1	FLA	439-	1	81.186094	3.54	41.49	2.307692	-
220	OW	222-	2	FLA	439-	1	67.409293	1.95	19.56	1.605505	-
221	OW	222-	3	FLA	439-	1	46.36099	1.5	28.16	2.173913	16.05839
222	OW	222-	4	FLA	439-	1	82.088566	1.07	39.35	0.911162	-
223	OW	222-	5	FLA	439-	1	74.71868	1.65	37.79	1.094891	13.72263

224	OW	222-	6	FLA	439-	1	72.788732	1.74	24.07	0.806452	15
225	OW	222-	7	FLA	439-	1	68.516285	2.21	26.96	1.677852	15.40146
226	OW	222-	8	FLA	439-	1	70.841724	5.15	26.84	1.917808	-
227	OW	223-	1	FLA	439-	2	64.402317	7.1	28.35	1.213592	-
228	OW	223-	2	FLA	439-	2	65.208333	6.9	37.38	1.162791	-
229	OW	224-	1	FLA	439-	3	61.643836	6.77	38.9	0.976563	13.10219
230	OW	224-	2	FLA	439-	3	68.003646	6.87	35.53	1.278772	-
231	OW	224-	3	FLA	439-	3	61.060433	6.54	40.52	0.966184	13.24818
No	**Clone**			**Mother**			**% DMC[a]**	**%PC[b]**	**%CF[c]**	**%Ash**	**%Amylose**
232	OW	224-	4	FLA	439-	3	65.048137	4.67	38.29	0.990099	-
233	OW	225-	1	FLA	439-	4	72.188906	4.33	42.98	1.079137	16.05839
234	OW	225-	2	FLA	439-	4	66.555556	7.22	38.26	2.247191	-
235	OW	227-	1	FLA	441-	1	70.209581	7.15	33.71	1.846966	15.32847
236	OW	227-	2	FLA	441-	1	65.250737	6.93	38.09	2.048417	14.56204
237	OW	227-	3	FLA	441-	1	71.523179	5.64	28.73	1.634877	16.86131
238	OW	227-	4	FLA	441-	1	79.569267	3.72	29.68	1.519757	-
239	OW	228-	1	FLA	441-	2	72.969792	6.67	33.88	1.315789	-
240	OW	228-	2	FLA	441-	2	46.424602	7.94	38.02	1.506024	15.94891
241	OW	228-	3	FLA	441-	2	69.968051	7.11	24.22	2.09205	-
242	OW	228-	4	FLA	441-	2	53.220339	8.2	26.12	1.953125	14.30657
243	OW	229-	2	FLA	441-	3	50.217707	2.59	41.64	2.28013	14.67153
244	OW	229-	3	FLA	441-	3	89.655172	3.34	-	1.973684	-
245	OW	229-	4	FLA	441-	3	56.468531	3.57	27.84	0.986842	16.38686
246	OW	229-	5	FLA	441-	3	72.007722	2.31	-	2.970297	-
247	OW	229-	6	FLA	441-	3	62.698053	1.72	37.58	1.703163	15.94891
248	OW	229-	7	FLA	441-	3	67.715736	5.79	21.82	1.792115	-
249	OW	229-	8	FLA	441-	3	58.92961	8.55	26.34	1.333333	15.21898
250	OW	230-	1	FLA	441-	5	46.123651	10.49	18.91	2.39726	14.81752
251	OW	230-	2	FLA	441-	5	35.045396	9.2	6.09	1.449275	14.78102
252	OW	230-	3	FLA	441-	5	36.596874	10.5	8.005	3.546099	14.67153
253	OW	230-	4	FLA	441-	5	36.570145	10.34	3.67	1.957586	14.37956
254	OW	230-	5	FLA	441-	5	32.994546	9.14	3.13	1.934236	14.08759
255	OW	230-	6	FLA	441-	5	32.444155	8.55	2.575	2.237522	14.56204

256	OW	231-	1	FLA	444-	7	38.021779	7.16	2.7	1.44	14.37956
257	OW	231-	2	FLA	444-	7	35.256888	7.98	2.605	1.906158	13.43066
258	OW	231-	3	FLA	444-	7	30.312372	11.84	3.145	1.983664	13.9781
259	OW	231-	4	FLA	444-	7	33.272469	11	5.66	1.818182	14.19708
260	OW	231-	5	FLA	444-	7	39.269761	5.19	2.445	2.262443	11.67883
261	OW	231-	6	FLA	444-	7	32.820629	8.27	2.14	1.67364	13.35766
262	OW	232-	1	FLA	445-	2	63.152346	4.22	40.19	2.173913	16.05839
263	OW	232-	2	FLA	445-	2	34.549098	8.13	42.89	3.205128	15.9854
264	OW	232-	3	FLA	445-	2	45.623342	7.7	3.75	1.634877	-
265	OW	232-	4	FLA	445-	2	40.126382	5.52	3.83	1.707317	13.46715
266	OW	234-	1	FLA	496-	1	57.183557	7.91	32.99	1.812689	15.94891
267	OW	234-	2	FLA	496-	1	38.552136	5.41	2.165	2.094241	15.51095
268	OW	234-	3	FLA	496-	1	62.017167	6.01	44.76	3.144654	12.9562
269	OW	234-	4	FLA	496-	1	31.997795	8.94	3.415	2.233903	14.78102
270	OW	235-	1	FLA	508-	1	35.457516	12.07	5.84	1.90678	13.94161
271	OW	235-	2	FLA	508-	1	17.876802	8.32	9.065	2.578797	-
272	OW	235-	3	FLA	508-	1	28.290115	10.12	2.925	2.909091	12.22628
273	OW	235-	4	FLA	508-	1	29.533301	11.27	5.02	2.247191	11.56934

[a]Dry matter content (%); [b]Protein content (%); [c]Crude fibre (%)

Appendix 2: Information on the 817 sequence repeat markers used for the screening of the parents and four selected backcross progenies of cassava

S/No	Name	Sequence	Product size	Annealing temperature(°C)
1	SSRY1	F: GCAGCTGCCGCTAATAGTTT	197	45
		R: CCAAGAGATTGCACTAGCGA	-	
2	SSRY2	F: CGCCTACCACTGCCATAAAC	167	55
		R: TGATGAAATTCAAAGCACCA	-	
3	SSRY3	F: TTAGCCAGGCCACTGTTCTT	247	55
		R: GCGAGGTTCAAATATGCGAT	-	
4	SSRY4	F: ATAGAGCAGAAGTGCAGGCG	287	55
		R: CTAACGCACACGACTACGGA	-	
5	SSRY5	F: TGATGAAATTCAAAGCACCA	173	55
		R: CGCCTACCACTGCCATAAAC	-	
6	SSRY6	F: TTTGTTGCGTTTAGAAAGGTGA	298	45
		R: AACAAATCATTACGATCCATTTGA	-	
7	SSRY7	F: TGCCTAAGGAAAATTCATTCAT	250	45
		R: TGCTAAGCTGGTCATGCACT	-	
8	SSRY8	F: AGTGGTTTGAGAAGACTGGTGA	288	45
		R: TTTCCAAAATGGAACTTCAAA	-	
9	SSRY9	F: ACAATTCATCATGAGTCATCAACT	278	55
		R: CCGTTATTGTTCCTGGTCCT	-	
10	SSRY10	F: CGTTTGTCCTTTCTGATGTTCT	153	55
		R: TGCAATGCAGTGAACCATCT	-	
11	SSRY11	F: TGTAACAAGGCAAATGGCAG	265	55
		R: TTCTTGTGTCGTGCAACCAT	-	
12	SSRY12	F: AACTGTCAAACCATTCTACTTGC	266	55
		R: GCCAGCAAGGTTTGCTACAT	-	
13	SSRY13	F: GCAAGAATTCCACCAGGAAG	234	55
		R: CAATGATGGTAAGATGGTGCAG	-	
14	SSRY14	F: TTTGCATCGATTCCATCATC	300	55
		R: TTGACCTTAGCACATTTAAGGATTC	-	
15	SSRY15	F: TGAAAGCCTGCATTCAAACA	215	55
		R: TGATGCAGGTAGCAAGGATG	-	

16	SSRY16	F: GCACTGCAAAAATATCATCTTGA	218	55
		R: CTGGAAAGATGGGACGTGTT	-	
17	SSRY17	F: CTTAGAAAAGAAATTGCATGTGAG	277	55
		R: TGTCTGATCAAGCTGGTGACA	-	

S/No	Name	Sequence	Product size	Annealing temperature(°C)
18	SSRY18	F: GTGCTGCAAGGCGATTAAGT	198	55
		R: GCTACAACTGATAGTTGCATGCTT	-	
19	SSRY19	F: TGTAAGGCATTCCAAGAATTATCA	214	55
		R: TCTCCTGTGAAAAGTGCATGA	-	
20	SSRY20	F: CATTGGACTTCCTACAAATATGAAT	143	55
		R: TGATGGAAAGTGGTTATGTCCTT	-	
21	SSRY21	F: CCTGCCACAATATTGAAATGG	192	55
		R: CAACAATTGGACTAAGCAGCA	-	
22	SSRY22	F: CTTGCCACTAGAACAGCCAC	299	45
		R: GGCGTGGACTAACCTGTTCT	-	
23	SSRY23	F: GCGAGGTTCAAATATGCGAT	247	45
		R: TTAGCCAGGCCACTGTTCTT	-	
24	SSRY24	F: CTTTCACATGATTGCAGCGT	100	45
		R: GGATTATCCACTTCTCCAAATGTT	-	
25	SSRY25	F: TGGCTACATGATAGCAACATCAA	296	55
		R: CGCATGGTTTGTCTCGTTTA	-	
26	SSRY26	F: TGCTAATTGCAGGAAATAGGAT	121	55
		R: GCAGCTTTTTAGCATAACAATCAA	-	
27	SSRY27	F: CCATGATTGTTTAAGTGGCG	277	55
		R: CCATTGGAGAACTTGGCAAC	-	
28	SSRY28	F: TTGACATGAGTGATATTTTCTTGAG	180	55
		R: GCTGCGTGCAAAACTAAAAT	-	
29	SSRY29	F: TGGTAGCTTTTGAATATCTGATGG	281	55
		R: TGCCAACCAAACCATTATAGAC	-	
30	SSRY30	F: CCATCCACTAGAAACTTTAAAAGCA	220	55
		R: CAACTCAGCGGAGCTTTTTC	-	
31	SSRY31	F: CTTCATCACGTGTTAATACCAATC	188	55
		R: ATTGTTGTGGTTGCAGGACA	-	
32	SSRY32	F: CAAATTTGCAACAATAGAGAACA	298	55
		R: TCCACAAAGTCGTCCATTACA	-	

33	SSRY33	F: AACTCTTTTGACTGAAGATGCTGA	273	55
		R: CATGATTACCGCCAAGGCT	-	
34	SSRY34	F: TTCCAGACCTGTTCCACCAT	279	55
		R: ATTGCAGGGATTATTGCTCG	-	
35	SSRY35	F: GCAGTAAAACCATTCCTCCAA	282	55
		R: CTGATCAGCAGGATGCATGT	-	
36	SSRY36	F: CAACTGTTTCAACCAACAGACA	134	55
		R: ATTCTCGTGAACTGCTTGGC	-	

S/No	Name	Sequence	Product size	Annealing temperature(°C)
37	SSRY37	F: ATGGCAAAAGATCGAGCAAC	187	55
		R: GGCCAGTAATTCCTCAAGGC	-	
38	SSRY38	F: GGCTGTTCGTGATCCTTATTAAC	122	55
		R: GTAGTTGAGAAAACTTTGCATGAG	-	
39	SSRY39	F: TCAATGCATAGGATTTTGAAAGTA	293	55
		R: AATGAAATGTCAGCTCATGCT	-	
40	SSRY40	F: TGCATCATGGTCCACTCACT	231	55
		R: CATTCTTTTCGGCATTCCAT	-	
41	SSRY41	F: TATCACAATCGAAACCGACG	271	55
		R: TTTTCCAACAATCTGATACTCGT	-	
42	SSRY42	F: TTCCTCCAAAGTTATCTAGAACCA	221	55
		R: CAATCCTTGTAGTAGCCAGTCTCA	-	
43	SSRY43	F: TCAGACGTTGATACCTCACTTCA	255	55
		R: CCAGAGCATGGTCTTTCTGA	-	
44	SSRY44	F: GGTTCAAGCATTCACCTTGC	194	55
		R: GACTATTTGTGATGAAGGCTTGC	-	
45	SSRY45	F: TGAAACTGTTTGCAAATTACGA	228	55
		R: TCCAGTTCACATGTAGTTGGCT	-	
46	SSRY46	F: TCAGGAACAATACTCCATCGAA	268	55
		R: CGCTAAAGAAGCTGTCGAGC	-	
47	SSRY47	F:GGAGCACCTTTTGCTGAGTT	244	55
		R: TTGGAACAAAGCAGCATCAC	-	
48	SSRY48	F: AGCTGCCATGTCAATTGTTG	178	55
		R: TCATAAAGCTCGTGATTTCCA	-	
49	SSRY49	F: TGAAAATCTCACTGGCATTATTT	300	55
		R: TGCAACCATAGTGCCAAGC	-	

50	SSRY50	F: CCGCTTAACTCCTTGCTGTC	271	55
		R: CAAGTGGATGAGCTACGCAA	-	
51	SSRY51	F: AGGTTGGATGCTTGAAGGAA	298	55
		R: GGATGCAGGAGTGCTCAACT	-	
52	SSRY52	F: GCCAGCAAGGTTTGCTACAT	266	55
		R: AACTGTCAAACCATTCTACTTGC	-	
53	SSRY53	F: CCATGCAGTAGTGCCATCTTT	138	55
		R: ATTTTCACCAACCGCAACTC	-	
54	SSRY54	F: GCGACTTTCTGGATGGATTC	151	55
		R: TGCAAATGACAAATAACCATCTC	-	
55	SSRY55	F: GCAATTTGCAAAGACATACCA	145	55
		R: TGTGGAGCTTGATTTTGCAG	-	

S/No	Name	Sequence	Product size	Annealing temperature(°C)
56	SSRY56	F: AACTCTTAATGGCTAAAATTATTGATG	137	55
		R: TTTTAGTTTAGTTTAGTTAGTTGCGCT	-	
57	SSRY57	F: TGTCATTGTCTGTTGACCATTT	293	55
		R: TAACCTGCCAAGAACAAGGC	-	
58	SSRY58	F: GAAGGACAAGCAAAGAAGCAA	217	55
		R: TGGAATCCAATATTGATGACTAAGA	-	
59	SSRY59	F: GCAATGCAGTGAACCATCTTT	158	55
		R: CGTTTGTCCTTTCTGATGTTC	-	
60	SSRY60	F: CGGCCACCAACTCAAATAAC	137	55
		R: TTGCAATGATATCAACGGCT	-	
61	SSRY61	F: GGCTGCTTTACCTTCTACTCAGA	233	55
		R: CAAGAACGCCAATATGCTGA	-	
62	SSRY62	F: CATTCTCCAGGAAAGTCATTTTG	250	55
		R: AGCTCATGCCATACAAGCAA	-	
63	SSRY63	F: TCAGAATCATCTACCTTGGCA	290	55
		R: AAGACAATCATTTTGTGCTCCA	-	
64	SSRY64	F: CGACAAGTCGTATATGTAGTATTCACG	194	55
		R: GCAGAGGTGGCTAACGAGAC	-	
65	SSRY65	F: CATCGCCAAATCGTCAAGTA	299	55

		R: TGATGCCATGCATTTCACTT	-	
66	SSRY66	F: ATCTCAGCTTCCAACTCTTTCAGT	261	55
		R: CGAAATGCTTGGAGACAGGTATAG	-	
67	SSRY67	F: AGTTTGCACCACCTTTTTCC	278	55
		R: TGTCAAGTGATGAGCTGCTG	-	
68	SSRY68	F: GCTGCAGAATTTGAAAGATGG	287	55
		R: CAGCTGGAGGACCAAAAATG	-	
69	SSRY69	F: CGATCTCAGTCGATACCCAAG	239	55
		R: CACTCCGTTGCAGGCATTA	-	
70	SSRY70	F: CGCTATTAGAATTGCCAGCAC	249	55
		R: CGCTTGTTGTATCCATTGGC	-	
71	SSRY71	F: TGATGCAGGTAGCAAGGATG	217	55
		R: TGAAAGCCTGCATTCAAACA	-	
72	SSRY72	F: AAGCATCAGTGGCTATCAACA	141	55
		R:TTTTGCTGTGCTATTTCTGAGC	-	
73	SSRY73	F: AAGTTGATGGTTCTGAATCTGGA	265	55
		R: ACAGTGATTGAGCGAGGCTT	-	
74	SSRY74	F: TTGCTCGAATTCCACACAAT	114	55

S/No	Name	Sequence	Product size	Annealing temperature(°C)
		R: GGTCAGGTGAGTAATAAAGAACAGTG	-	
75	SSRY75	F:TCTGGTAAACCTACTAGTGCTCCA	284	55
		R: TTCATGCACGTCCTGATACA	-	
76	SSRY76	F: AAAGGAAGCAACCTTCAGCA	273	55
		R: CATGATTTGGATTTTGGAATGA	-	
77	SSRY77	F: CAGGAGGTGGCAGATTTTGT	275	55
		R: GCATGTTCCACCTGCATAAG	-	
78	SSRY78	F: TGCACACGTTCTGTTTCCAT	248	55
		R: ATGCCTCCACGTCCAGATAC	-	
79	SSRY79	F: CAAACCAATGGTCATGCTGT	210	55
		R: CAGCATCAGAAAGACAAAAACAA	-	
80	SSRY80	F: TTCCTGGAAATGTCCTTAGATG	299	55
		R: TGGCACATGCAACAATTAGC	-	
81	SSRY81	F: GGCGATTTCATGTCATGCTT	204	55
		R: TGATTTTCTGCGTGATGAGC	-	

82	SSRY82	F: TGTGACAATTTTCAGATAGCTTCA	211	55
		R: CACCATCGGCATTAAACTTTG	-	
83	SSRY83	F: TGGCTAGATGGTGATTATTGCTT	239	55
		R: TGCTTACTCTTTGATTCCACG	-	
84	SSRY84	F: TTCCTTTCATTCATCCTGGC	203	55
		R: AGAACTTCATGCACACAAGTTAAT	-	
85	SSRY85	F: AAGGTGGCAGCACTTTTCTG	292	55
		R: AGAATACTATACGGACTACATGCCA	-	
86	SSRY86	F: GACACCTGCTGATTCCGAG	296	55
		R: TTGCCACATAGCAGAATCCTT	-	
87	SSRY87	F: CTCATCTCATGAAGAACTTGTGC	102	55
		R: AGAGCACGCATTGTGCATTT	-	
88	SSRY88	F: CCAAGTCCTCACCTCCAAAG	243	55
		R: CCTTGATGTGGCCAAAGTG	-	
89	SSRY89	F: AGTTGAGAAAACCTTGCATGAG	120	55
		R: GGCTGTTCGTGATCCTTATTAAC	-	
90	SSRY90	F: AGGTTATGGCGGTGGCAG	193	55
		R: GCGATTTTGCGAATTACCAC	-	
91	SSRY91	F: GTCTGCATGGCTCGATGAT	300	55
		R: TGCCTGCTTCATATGTTTTTG	-	
92	SSRY92	F: CCAATGCTCAGTTTGACAACTC	171	55
		R: TCGGCTTAAGGTATGAACGC	-	

S/No	Name	Sequence	Product size	Annealing temperature(°C)
93	SSRY93	F: TTTGTTGCTCACATGAAAACG	289	55
		R: CAGATTTCTTGTGGTGCGTG	-	
94	SSRY94	F: AGGATGGACTTGGAGATGGA	268	55
		R: GGTGGAAGTAAGGCTGTTAGTG	-	
95	SSRY95	F: CATGATTTGGATTTTGGAATGA	282	55
		R: CAAAAGAAGCAACCTTCAGCA	-	
96	SSRY96	F: CTTTACCTGCATGCCATTGA	149	55
		R: CTCCATGTTATCCAAGGTTGC	-	
97	SSRY97	F: GAGCAATCAAATTCAACAGCA	194	55
		R: AAGCCGAAGCTTATGAAGGA	-	
98	SSRY98	F: ACCAATCCAAGCTGCAAATC	209	55
		R: GTGATTGGTAGTGGTGGCCT	-	

99	SSRY99	F: ATCAAGGCGCAAAAGTCAAT	192	55
		R: CTTGCTTTGGTTCCAATTATTTA	-	
100	SSRY100	F: ATCCTTGCCTGACATTTTGC	210	55
		R: TTCGCAGAGTCCAATTGTTG	-	
101	SSRY101	F: GGAGAATACCACCGACAGGA	213	55
		R: ACAGCAGCAATCACCATTTC	-	
102	SSRY102	F: TTGGCTGCTTTCACTAATGC	179	55
		R: TTGAACACGTTGAACAACCA	-	
103	SSRY103	F: TGAGAAGGAAACTGCTTGCAC	272	55
		R: CAGCAAGACCATCACCAGTTT	-	
104	SSRY104	F: AGGCCATGGCAATTACTGAA	258	55
		R: TTCTTGATATGCGCAACAGC	-	
105	SSRY105	F: CAAACATCTGCACTTTTGGC	225	55
		R: TCGAGTGGCTTCTGGTCTTC	-	
106	SSRY106	F: GGAAACTGCTTGCACAAAGA	270	55
		R: CAGCAAGACCATCACCAGTTT	-	
107	SSRY107	F: CCATTTTCTCTTGCTTCTGTCA	120	45
		R: TGGTTTGAAGTCCTATAAAATCCTT	-	
108	SSRY108	F: ACGCTATGATGTCCAAAGGC	203	55
		R: CATGCCACATAGTTCGTGCT	-	
109	SSRY109	F: TGCTAATTGCAGGAAATAGGAT	125	55
		R: GCAGCTTTTTAGCATAACAATCAA	-	
110	SSRY110	F: TTGAGTGGTGAATGCGAAAG	247	55
		R: AGTGCCACCTTGAAAGAGCA	-	
111	SSRY111	F: GCATCTTACATCCAGAATACTGCT	235	55
		R: GAAGGAATGCCTGGCTTAAA	-	

S/No	Name	Sequence	Product size	Annealing temperature(°C)
112	SSRY112	F: CGCAAGGTAAATCGGAGCTA	117	55
		R: ACAATCAAAGGAGTCGTGTAATC	-	
113	SSRY113	F: TTTGCTGACCTGCCACAATA	187	45
		R: TCAACAATTGGACTAAGCAGC	-	
114	SSRY114	F: AACAGGAAGGAAAATCAAGCC	167	55
		R: TCAACTGCAGATTCATTCAAGA	-	
115	SSRY115	F: CAACCGCTTTCGATGGTATT	296	55
		R: TGCCATCACAATTTTGCCTA	-	

116	SSRY116	F: CGTTTTCCTGTTAAATCTTGCAT	167	55
		R: TAGAGCAGCTGCAAAGCAAA	-	
117	SSRY117	F: TAAAGTTTGGCATGCCTGTG	142	55
		R: GCAAATGTGTTTTCAATATAAGGC	-	
118	SSRY118	F: TAGAGCAGCTGCAAAGCAAA	169	55
		R: TCGTTTTCCTGTTGAAATCTTG	-	
119	SSRY119	F: AACATAGGCATTAAAGTTTGGCA	155	55
		R: GCAAATGTGTTTTCAATATAAGGC	-	
120	SSRY120	F: TCACCGTTAATTGTAGTCTGCG	139	55
		R: GCGAGGTTCAAATATGCGAT	-	
121	SSRY121	F: CCAGAAACTGAAATGCATCG	168	45
		R: TGGAATTGTTGTCTGGATCG	-	
122	SSRY122	F: AAGCCAATTGTTGTGAGTTGC	273	45
		R: GGTGCTTGGTTTATGCCTGT	-	
123	SSRY123	F: AGCAGATCCAAATCACTGAAA	136	55
		R: TTCAACAATAAAGCTCAGAAAGAG	-	
124	SSRY124	F: CTGCTGGACGGAGGATTCTA	146	55
		R: TGGCATCAATTTTTGCTTCA	-	
125	SSRY125	F: CAGGACATGACGCAATTCTG	247	55
		R: GCATGTTAGAAGTTTTTGCAATTT	-	
126	SSRY126	F: AATGGATCATGTTCAATGTCTTC	245	55
		R: TTGAAATACGGCTCAAGCTC	-	
127	SSRY127	F: CTTCGGCCTCTACAAAAGGA	130	45
		R: GCTGAACTGCTTTGCCAACT	-	
128	SSRY128	F: CAGGACATGACGCAATTCTG	243	45
		R: GCATGTAGAAGTCTTTGCAATTATG	-	
129	SSRY129	F: CTTTTTGCCAGTCTTCCTGC	205	55
		R: AATGGATCATGTTCAATGTCTTC	-	
130	SSRY130	F: GGTCCCTGATAGTTGATAATGGAT	223	55
		R: CTTTTTGCCAGTCTTCCTGC	-	

S/No	Name	Sequence	Product size	Annealing temperature(°C)
131	SSRY131	F: CATTGTTCAGCAAACACTGGA	111	45
		R: GCGAGAGATGTTGCTATTGCT	-	
132	SSRY132	F: CTTTTTGCCAGTCTTCCTGC	196	55
		R: TGTCCAATGTCTTCCTTTCCTT	-	
133	SSRY133	F: AGCATGTCATTGCACCAAAC	295	45
		R: CGACTGCATCAGAACAATGC	-	
134	SSRY134	F: TCCACAAAGATAAGCTAAGCG	213	55
		R: GCAAGTTCAAAAGGAGCAGC	-	
135	SSRY135	F: CCAGAAACTGAAATGCATCG	253	45
		R: AACATGTGCGACAGTGATTG	-	
136	SSRY136	F: CGACTGCATCAGAACAATGC	296	55
		R: AGCATGTCATTGCACCAAAC	-	
137	SSRY137	F: TAGTTAGCTCGGTTCGTCCG	157	55
		R:TTTTGATAGATCAAGAGAGTTTTTGAA	-	
138	SSRY138	F: AGAATGTCTCTTTATTCTTGACAATTT	129	55
		R: TTCAGGAAACATGCACAAACA	-	
139	SSRY139	F: AAAAAGTGACAGAGTTCCGCTC	129	55
		R: CAGATTCTTCAAGCCAAATGTC	-	
140	SSRY140	F: CAGTGAGCAGAAACTAAAAACATTG	212	55
		R: GGCACTTTGGAAAGGAAGAG	-	
141	SSRY141	F: TCCAAAATCTTGGTCATTTTGA	262	55
		R: TGCTGTGATTAAGGAACCAACTT	-	
142	SSRY142	F: CTTTTTGCCAGTCTTCCTGC	206	55
		R: AATGGATCATGTTCAATGTCTTC	-	
143	SSRY143	F: GCTCATGAACTGAGCCTTCA	153	55
		R: AGCAGATCCAAATCACTGAAA	-	
144	SSRY144	F: TAATGTCATCGTCGGCTTCG	117	55
		R: GCTGATAGCACAGAACACAG	-	
145	SSRY145	F: GATTCCTCTAGCAGTTAAGC	143	55
		R: CGATGATGCTCTTCGGAGGG	-	
146	SSRY146	F: TTCCCTCGCTAGAACTTGTC	139	45
		R: CTATTTGACCGTCTTCGCCG	-	
147	SSRY147	F: GTACATCACCACCAACGGGC	113	45

		R: AGAGCGGTGGGGCGAAGAGC	-	
148	SSRY148	F: GGCTTCATCATGGAAAAACC	114	45
		R: CAATGCTTTACGGAAGAGCC	-	
149	SSRY149	F: AGCAGAGCATTTACAGCAAGG	500	55
S/No	Name	Sequence	Product size	Annealing temperature(°C)
		R: TGTGGAGTTAAAGGTGTGAATG	-	
150	SSRY150	F: CAATGCAGGTGAAGTGAATACC	175	45
		R: AGGGTGCTCTTCAGAGAAAGG	-	
151	SSRY151	F: AGTGGAAATAAGCCATGTGATG	182	45
		R: CCCATAATTGATGCCAGGTT	-	
152	SSRY152	F: CTCTAGCTATGGATTAGATCT	233	55
		R: GTAGCTTCGAGTCGTGGGAGA	-	
153	SSRY153	F: TTCCAGAAAGACTTCCGTTCA	117	45
		R: CTCAACTACTGCACTGCACTC	-	
154	SSRY154	F: ACAATGTCCCAATTGGAGGA	318	45
		R: ACCATGGATAGAGCTCACCG	-	
155	SSRY155	F: CGTTGATAAAGTGGAAAGAGCA	158	55
		R: ACTCCACTCCCGATGCTCGC	-	
156	SSRY156	F: TTCAAGGAAGCCTTCAGCTC	160	55
		R: GAGCCACATCTACTCGACACC	-	
157	SSRY157	F: TGTTCTTGATCTTCTGCTGCA	500	45
		R: TGATTGTGGACGTGGGTAGA	-	
158	SSRY158	F: CCTTACTTGTGTTTCTTACTGACAAG	224	55
		R: CCAAGTCCTCACCTCCAAAG	-	
159	SSRY159	F: CTTATCCTGTCCCCTCCACC	159	45
		R: GACAATTGCATAGGAAGCACA	-	
160	SSRY160	F: CTGGCTCTTCCAGACACCTT	151	55
		R: GGCAAGAGAAGCCATAAAGC	-	
161	SSRY161	F: AAGGAACACCTCTCCTAGAATCA	220	55
		R: CCAGCTGTATGTTGAGTGAGC	-	
162	SSRY162	F: TTTAGTTAGTTGCGCTAGCTTCC	126	55
		R:AACTCTTAATGGCTAAAATTATTGATG	-	
163	SSRY163	F: TCATGATGCTATTCCAAGTGTG	231	55
		R: AGGCCTCCAACAATTAGCCT	-	
164	SSRY164	F: TCAAACAAGAATTAGCAGAACTGG	187	45

		R: TGAGATTTCGTAATATTCATTTCACTT	-	
165	SSRY165	F: AAATGAGTTGCAAAGGCCAA	243	55
		R: GGTAAACAAATGATGTGGTGTTC	-	
166	SSRY166	F: AATAACAACAAGAGTTGTGGAAAAA	244	55
		R: TATCCATGACTGTGATGCGG	-	
167	SSRY167	F: AAAATTGGATGGGACCGTTT	183	55
		R: AAGGAAAGGGAGAAATCAAAGA	-	

S/No	Name	Sequence	Product size	Annealing temperature(°C)
168	SSRY168	F: ACAGCCACACTTGTTCTCCA	277	45
		R: CTGCAATCTCCAACAGCAAC	-	
169	SSRY169	F: ACAGCTCTAAAAACTGCAGCC	100	55
		R: AACGTAGGCCCTAACTAACCC	-	
170	SSRY170	F: TCTCGATTTGGTTTGGTTCA	299	55
		R: TCATCCTTGTTGCAGCGTTA	-	
171	SSRY171	F: ACTGTGCCAAAATAGCCAAATAGT	291	55
		R: TCATGAGTGTGGGATGTTTTTATG	-	
172	SSRY172	F: TCCAACTGGCTTAACTTGAGG	201	55
		R: TTTAGTTTTTGAAACAATGATGAAA	-	
173	SSRY173	F: TGTAAATATGCAAAGAAGCACGA	281	55
		R: TACCTTTGGTGGAGTTTGCC	-	
174	SSRY174	F: AACAAAACCATTTTCATGTTGA	136	55
		R: TTGCATACTCATCTCCATCTTCA	-	
175	SSRY175	F: TGACTAGCAGACACGGTTTCA	136	55
		R: GCTAACAGTCCAATAACGATAAGG	-	
176	SSRY176	F: TGGCTAAATTATTGATGTTTTAGTGT	112	55
		R: TTTTTCAAAATAGAGGGACCAA	-	
177	SSRY177	F: ACCACAAACATAGGCACGAG	268	45
		R: CACCCAATTCACCAATTACCA	-	
178	SSRY178	F: GGCCCGTAAGGTTTACAGAG	104	55
		R: CTGCAAAAACACGATCCCTT	-	
179	SSRY179	F: CAGGCTCAGGTGAAGTAAAGG	226	55
		R:GCGAAAGTAAGTCTACAACTTTTCTAA	-	
180	SSRY180	F: CCTTGGCAGAGATGAATTAGAG	163	55
		R: GGGGCATTCTACATGATCAATAA	-	
181	SSRY181	F: GGTAGATCTGGATCGAGGAGG	199	55

		R: CAATCGAAACCGACGATACA	-	
182	SSRY182	F: GGAATTCTTTGCTTATGATGCC	253	55
		R: TTCCTTTACAATTCTGGACGC	-	
183	SSRY183	F: TGCTGTGATTAAGGAACCAACTT	221	55
		R: TTAACTTTTTCCAGTTCTACCCA	-	
184	SSRY184	F: TCATCCCAAAAATACCTCTAACA	163	55
		R: CTCCGACAAGCATGTGAATG	-	
185	SSRY185	F: GAAGAAGACGGTTAAAGCAAGTT	243	55
		R: ATGCCAGTTTGCTATCCAGG	-	
186	SSRY186	F: GCTTTGTGTAAACAACCTCGC	297	55
		R: AATGACCATGCCAACACAAG	-	

S/No	Name	Sequence	Product size	Annealing temperature(°C)
187	SSRY 187	F: TGGGTCATCTGCCCATAACT (20)	160	60.34
		R: CCAGGAAGTTACGAGCTTGG (20)	-	
188	SSRY 188	F: GCCTCGAATTAGGCTCCACT (20)	198	60.73
		R: AGCCAATGCCGACATACAGT (20)	-	
189	SSRY 189	F: GATGACTGGTCTCAAGTGGTGA (22)	185	60.16
		R: AAGAAGAGCTCCTCCTGCAA (20)	-	
190	SSRY 190	F: TGGCAGGGTTACCTTTGTGT (20)	164	60.41
		R: AACCAGTTAAGGTTGGCTCAAA (22)	-	
191	SSRY 191	F: TTTCATGAACAACTTTTCTGGGTA (24)	186	59.91
		R: TGTTGGCTGAGAAAGCAAGA (20)	-	
192	SSRY 192	F: GCCAATGCTCCATTTTCAAT (20)	183	59.91
		R: TTTCCAATCCACCATTTGCT (20)	-	
193	SSRY 193	F: CGAAGCTCCGCCTCAGTAT (19)	218	60.51
		R: TGGAATCGGTTACCAGAAGTG (21)	-	
194	SSRY 194	F: CTGGTAACCGATTCCACACC (20)	196	60.23
		R: GCAAGCTCTCCAATGACCTT (20)	-	
195	SSRY 195	F: CGCTTACAACACCACCTTCA (20)	186	59.76
		R: GCTTGATCTCAGCCATGTCA (20)	-	
196	SSRY 196	F: CCACACTACCAGCCCAAAAT (20)	188	59.85
		R: CTTTTGCCATGACCTGGTTT (20)	-	
197	SSRY 197	F: TGCCATTACCAAGTGATGATCT (22)	209	59.46
		R: CACAAAGCCACTCAAATCCA (20)	-	
198	SSRY 198	F: AATTCCCGGGATATCGTC (18)	219	57.17

		R: CGCAAACTCTCTTTTTCACTCC (22)	-	
199	SSRY 199	F: TCTCCATATCCTTGGATTCGT (21)	205	58.47
		R: GCCGACCATAAATCTGGAAA (20)	-	
200	SSRY 200	F: CCAACTCACCTTTCAACCAGA (21)	205	60.13
		R: GAAGCCAACCCATCATCTTC (20)	-	
201	SSRY 201	F: CCAAGGTGGAGGTCAAGAAA (20)	197	60.08
		R: CTCCACATTACAGCCAACTGA (21)	-	
202	SSRY 202	F: CGATTTATTTCGGGATGTGG (20)	191	60.15
		R: TTTAGAGCTGGGACCTCCTG (20)	-	
203	SSRY 203	F: AGGAAGCTGTCCCCTATTCA (20)	246	58.74
		R: TACCAGCAAGACCATCACCA (20)	-	
204	SSRY 204	F: TCTTTGCTGGTGAGTTTTGC (20)	182	59.05
		R: GAATGCCTCCCAAACATCTC (20)	-	
205	SSRY 205	F: CTGTCTTGATTCCGGCAACT (20)	201	60.25
		R: GCAAGTCGTTGCCTACCTTG (20)	-	

S/No	Name	Sequence	Product size	Annealing temperature(°C)
206	SSRY 206	F: AAGGACGGAGTTGTCTCCTG (20)	219	59.3
		R: CCAAACGAGCAATATTAGGTGA (22)	-	
207	SSRY 207	F: TGCCCTTCACCAGCTAGTTT (20)	199	59.88
		R: ACAATTCCTTTCGCCTTGAA (20)	-	
208	SSRY 208	F: AGGACGAGGATGACGATGAA (20)	198	60.62
		R: TCACCATTCTCTTCTGGTTCAA (22)	-	
209	SSRY 209	F: ACGAGGATGAAGACGGTGAA (20)	195	60.66
		R: TCCTCTCAATAGAGCCTCAACC (22)	-	
210	SSRY 210	F: TTGCCGGCTTTTCTGATTAC (20)	219	60.21
		R: GACCAGTTGCTGTTCGTCAA (20)	-	
211	SSRY 211	F: TTCACCTCTGCTTCGAGTGA (20)	202	59.7
		R: CATGCTTTTGGTTCCAGTGA (20)	-	
212	SSRY 212	F: GACCTGCTGATGCTTCGATT (20)	238	60.37
		R: ATCACCAACACCACCATCAC (20)	-	
213	SSRY 213	F: GGGTAGCTTAGCCTGCTCAC (20)	199	59.1
		R: GCACTAACTGCCCTTTGCAT (20)	-	
214	SSRY 214	F: ATTCGCGTACGCAGATTCTT (20)	234	59.87
		R: GCCACAGGCTAAGGAAACAA (20)	-	
215	SSRY 215	F: GTTGATGAGCTGTGGCATTG (20)	204	60.27

		R: CCTAGACGAAGTGGGTCGAA (20)	-	
216	SSRY 216	F: CTGAACAGGAGCTCCCTCAC (20)	210	59.99
		R: CCATCTCCCTCGACAATCTC (20)	-	
217	SSRY 217	F: ATGAAGAAGTCCAGCGGAGA (20)	181	59.95
		R: CCTGCAATGCACATACATGA (20)	-	
218	SSRY 218	F: ATAGCTTTTTCCAGCCCTTG (20)	203	58.47
		R: CAGAATCCTGAAATGCTTAGCC (22)	-	
219	SSRY 219	F: CGAGAACAACAGGGTTCTACA	201	57.87
		R: GCTCTCTTGGGGAGGTGTCT	-	
220	SSRY 220	F: TGCTCTCTGATCTTGCACTAGC	194	59.93
		R: GTTGCATCAATGCCTTCAGT	-	
221	SSRY 221	F: TGGATAGAGGGGGTTTTGTTT	207	59.69
		R: GCAAAGCCCCATTAAATACG	-	
222	SSRY 222	F: TAAGGCAACGGGAAACAGAG	213	60.24
		R: GCAATATTCTTCCCAGCGAAT	-	
223	SSRY 223	F: GCATCTGTTTCCTGATTGTTTG	167	59.61
		R: CGGGGTTTGGAAATCAGTAA	-	
224	SSRY 224	F: TCCCCTCCAACCCTATCTTT	241	59.76
		R: CCCCAATAAATTTTAACCTCAA	-	

S/No	Name	Sequence	Product size	Annealing temperature(°C)
225	SSRY 225	F: GAAAATTGCAGAGGCTGCT	234	58.19
		R: TTGCACCTGAGTTCCATCAG	-	
226	SSRY 226	F: AACTGGAGTAGAGAAACTGGAGGA	219	59.82
		R: AACGTTTCGCCGATTACAAG	-	
227	SSRY 227	F: CCCATCAATGGAAACCTCAC	203	60.17
		R: CCAAGGTTGTTGGGTAAGGA	-	
228	SSRY 228	F: TTTCTTATCCTCCGCTATCCA	208	58.8
		R: TGGCAGAGATTTTGAGACGA	-	
229	SSRY 229	F: AGTCCAGCCTCTTCCTTCGT	199	60.39
		R: TGATCAGCGAAATCGTGGTA	-	
230	SSRY 230	F: CCATCTCTCTCCCTCTGCAA	185	60.49
		R: AAATGGCAGGAGATTGATGC	-	
231	SSRY 231	F: GGGCGCCTATTACTGTGAAA	199	60.1
		R: CCACAGAAGAACACCAAACTG	-	
232	SSRY 232	F: TGACTCTTCCTCTTCCTCTTGC	182	60.13

		R: GCGGTGTATAGACCCCATCT	-	
233	SSRY 233	F: CGAAACGATCGAAGTTCCAC	207	60.64
		R: TGTGGCCATCACACTCATTT	-	
234	SSRY 234	F: TTGCCAGAACCCTAGGAGTAA	196	58.84
		R: TGTCCCTAGGAAGGTTGCTG	-	
235	SSRY 235	F: CAGCTTTGCCATCCAATTTT	216	60.07
		R: CAGCAAAATGACATGAGTGTATCTC	-	
236	SSRY 236	F: TGAAAAACGTTCCTTCCCTTT	185	59.97
		R: CCGTATCCTATGGCAACACC	-	
237	SSRY 237	F: AAAGGAAAGGAAACCATTCTCA	185	59.11
		R: TTCTTCATGCAAGCAATTTCA	-	
238	SSRY 238	F: GGCCTTAAGCCACCATTCTA	216	59.18
		R: GCTTCTGCGAATTCGTTTCT	-	
239	SSRY 239	F: TGCATTTCCCTGGGTGTAAG	208	60.88
		R: TTTCTCAATAGACAGACGAGCA	-	
240	SSRY 240	F:TCGGCTTTTAACATCCTTCG	181	60.2
		R: AGCTAGGAGCAACGCAGTTC	-	
241	SSRY 241	F: GTAGGGCAATGGTGATTGGT	201	59.68
		R: ACCCAGCCATACACTAGCAA	-	
242	SSRY 242	F: TGGGTTCGAAAACAGCAAAC	201	61.04
		R: TAATGCCTGGAGGGTAATGG	-	
243	SSRY 243	F: GAGAGAGTACGTCACAGAGATCG	180	58.23
		R: TCCAGCAGGATAAACATCCA	-	

S/No	Name	Sequence	Product size	Annealing temperature(°C)
244	SSRY 244	F: TGAGTTTTGCCAGGTCTATCTTT	209	59.32
		R: TGCAGCAAGGATGAACAAGA	-	
245	SSRY 245	F: CTTGTTCATCCTTGCTGCAT	200	58.88
		R: CACTTGATTTGCAGCCTCTG	-	
246	SSRY 246	F: TGTAGGGCTGAGCAAGTTCC	194	60.4
		R: TCCCCTCAGATGCTCAAGAC	-	
247	SSRY 247	F: CCAGATCCAAACACCCTGTA	166	58.41
		R: AAACAGCTCACATGGCCTTC	-	
248	SSRY 248	F: TGTTTGCTGAGTGCAGTCCT	247	59.62
		R: CCTCGTACAGAAAACTCAAGCA	-	
249	SSRY 249	F: TGGAAATTGTCTTCGCAGAAT	180	59.7

		R: TGTGCATGCTCTTTTCCTTG	-	
250	SSRY 250	F: GATCGGATGTCTGAGGAGGA	197	60.16
		R: AATTGGAAGGGAAAGCCAAA	-	
251	SSRY 251	F: AGTCTCTACAGCCAAGTAGCATCT	213	57.98
		R: GCGTTGACGACGTCCATAAT	-	
252	SSRY 252	F: GACGCGAGAACTGACAAGTTT	221	59.53
		R: CCCACCTCCGAAATCACTAA	-	
253	SSRY 253	F: GCTGATGGAGGAGTAGCAGTG	186	60.03
		R: TCCAGAGGGAGAGATCTGACA	-	
254	SSRY 254	F: TGTCAGATCTCTCCCTCTGGA	220	59.93
		R: GACGCATCTCCTGCACAATA	-	
255	SSRY 255	F: ACCAGCACTGTTGTGTCCAG	183	59.78
		R: GAAGAGATGCGACGATGGTT	-	
256	SSRY 256	F: GGTGGTGGAGGTCCTGATTA	201	59.78
		R: AGAACAAAGGGCTCCATTCA	-	
257	SSRY 257	F: CATGAATGGAGCCCTTTGTT	193	59.93
		R: CTTGGCTGAGGCCTTTCAT	-	
258	SSRY 258	F: TCCTGCAACTCTGTTTTCCA	214	59.41
		R: TTCTCGGGGAGTTTCAAAAG	-	
259	SSRY 259	F: GCATTCTCTTTTCTCCTTTCTCA	217	59.15
		R: CGGAGAAGTTGGATTTCAC	-	
260	SSRY 260	F: CGGATTCCCGGATATCGTC	206	62.5
		R: GCAATGGAATCAATCCCTGA	-	
261	SSRY 261	F: CAGAGACTTCTCCGCCTGTT	172	59.6
		R: TCAAAAGAGAGACGCACAGGT	-	
262	SSRY 262	F: TTCAGTTTCAGGAGACAGAAAGG	132	59.91
		R: CTCGACATTCCCTTCACTTTG	-	

S/No	Name	Sequence	Product size	Annealing temperature(°C)
263	SSRY 263	F: CAAAGTGAAGGGAATGTCGAG	187	59.72
		R: TTCCAGGAATGTACTGCACAA	-	
264	SSRY 264	F: AACAACGAAAGGTGGCAGAT	207	59.6
		R: TTGGATCAGAGGAGCCAATC	-	
265	SSRY 265	F: CTGCTCCACGCTGTTTATCA	211	60
		R: GCTGCTGGTCAAAAGAGTCC	-	
266	SSRY 266	F: TCTGGTTTTCCACAGGGAAC	200	59.94

		R: TCGTTAGGCCCAATTCGTAG	-	
267	SSRY 267	F: TGCTTCCAATCACTCCCTTC	196	60.2
		R: ACTTTTTGGTGGGTGGTGTG	-	
268	SSRY 268	F: GCAATATCTTCTTGGAGTTCAATTCT	199	60.36
		R: CCAATATAAGCGGCGTCATT	-	
269	SSRY 269	F: AATAGTTTCAGGCAAGGGTGA	413	58.71
		R: TCAATCACAAGCCAGACACA	-	
270	SSRY 270	F: CGAAGCTCCGCCTCAGTAT	206	60.51
		R: CCAGAAGTGGACCAAGTGAGA	-	
271	SSRY 271	F: CTGGTAACCGATTCCACACC	195	60.23
		R: GCAAGCTCTCCAATGACCTT	-	
272	SSRY 272	F: ACTCGTGGGATCTTCCCTTT	193	59.93
		R: CTGGAACCTCTGGCTCTCAC	-	
273	SSRY 273	F: TCCTCCATCAATTCAGACCA	282	59.01
		R: TCCTAAATCCCATAATACCCAGT	-	
274	SSRY 274	F: TTCTTCCCCGTTCACGAAT	220	60.45
		R: TCAGCGACATCTCTCTTCCA	-	
275	SSRY 275	F: AGGCTGTGGTGGACAAGTTC	203	60.16
		R: TTCCATCTTCAGCCTTGCTT	-	
276	SSRY 276	F: TGAACACTTTCCCACCACCT	243	60.4
		R: ATAGAATCCGGACCCAAACC	-	
277	SSRY 277	F: GGTTTGGGTCCGGATTCTAT	186	60.02
		R: CTGAGTGGTGATTCCGCTAA	-	
278	SSRY 278	F: GTGCATGGAAGCTCTCAACA	186	59.99
		R: CTTTCTCCAGCTCGTTCCAC	-	
279	SSRY 279	F: ACGCGTGGGTTTTATGCTT	169	60.52
		R: GGTCCTGCCTTGCACTGTAT	-	
280	SSRY 280	F: TGTGCATGGAGAGATTGACAG	175	59.85
		R: AAGTCGTTTATTGCCGATGC	-	
281	SSRY 281	F: TCTCGCTTTATTCCCCAATC	183	59.11
		R: CTTGTACGAGCATCCCCATT	-	

S/No	Name	Sequence	Product size	Annealing temperature(°C)
282	SSRY 282	F: CCATCCCCAACAAACAAGAT	196	59.65
		R: TCCAACACTACGCTGTGTTTCT	-	
283	SSRY 283	F: AAAAGCGTGGGCTTTTGA	220	59.4
		R: TGGAACTCAGTGAAGCGATG	-	
284	SSRY 284	F: TCCTTCCAAAGCCAGACTTG	194	60.37
		R: GGAACATTTTAGCGGTCAGG	-	
285	SSRY 285	F: CCATCCAATAAAGCGTCGTT	200	59.96
		R: TCAATAGTTGCTGCCCACAT	-	
286	SSRY 286	F: ATCCACAATCCTCCCTACCC	205	60.01
		R: TGGGCCCAGAGATAGAGTGT	-	
287	SSRY 287	F: GGCTTTGTTGTGGTTGTTGA	206	59.59
		R: CATGAATTAGAAACCAAGGGAAG	-	
288	SSRY 288	F: TGGTTTAAGGTTTGGGCGTA	177	60.35
		R: TCAGGCATCCCTAATATTTCTTTC	-	
289	SSRY 289	F: AACACCCAACAGCAACATCA	192	60.01
		R: ATCTTCTTGGTGGGTCATGG	-	
290	SSRY 290	F: AAAGGCCTTCGTTGGAGTTTA	201	60.12
		R: ATGCCGATGCTTTCAGGTAT	-	
291	SSRY 291	F: AAAGGACCCTCCCTCTTTCC	207	60.79
		R: TGGGTTCTGGATTTGTTCAAG	-	
292	SSRY 292	F: CTTCTGTACTGTATCCGTTCACTAAT	221	57.24
		R: GAGCTCCATGCGAACAGATT	-	
293	SSRY 293	F: TCCATCTCCAGGCTCTCCT	193	59.89
		R: GTCCCATGCCCTGTTAGAGA	-	
294	SSRY 294	F: TGCCAAAAGAGAAAGCCAAG	194	60.49
		R: CACCCATTCCTTTGATGCTC	-	
295	SSRY 295	F: GCTTAGCTTATCCTTGGACCTTG	209	60.6
		R: AGACAAGCACCCACCAGAAT	-	
296	SSRY 296	F: TCAATTTGTTTTTCCCTGCTG	195	60.1
		R: TTTTCTGAGGCTGGTGTCCT	-	
297	SSRY 297	F: CCCAGGCACAGGTACTCTCA	199	61.27
		R: TTGTGCTCCAATGAAAATGG	-	
298	SSRY 298	F: GCCTTGTTCTGATCCATGCT	181	60.23

		R: TGCCTGAATCAGTGTCTTCAA	-	
299	SSRY 299	F: TCTTTTTCACAGAACCAACTGAA	206	58.91
		R: ATGCGAAACAGAGAGGAGGA	-	
300	SSRY 300	F: ATAGAAGGGCCCGAGACTGT	208	60.1
		R: CCATTTCCAGGCATTTCATC	-	

S/No	Name	Sequence	Product size	Annealing temperature(°C)
301	SSRY 301	F: GAACGCTTCAACGGCATAAT	198	60.1
		R: CCAATGCCAACAACACTTCTT	-	
302	SSRY 302	F: CAAACCCAAACCCAGAGCTA	225	60.1
		R: CGCTTGCCTTCTCTTTCTCT	-	
303	SSRY 303	F: GCATCATCACCATTTTTCTTTG	195	59.46
		R: TGGAAGGTGTTAGCAACTGTG	-	
304	SSRY 304	F: GACGCGTGGGCTCAGTTA	198	60.99
		R: TTAGCCACGGAGTCTGGTTC	-	
305	SSRY 305	F: AAAACACAATCAAATCCCTCA	215	57.05
		R: AAGCTTGGCTAAGGTTCTGC	-	
306	SSRY 306	F: GGACAGCCTCGTCATTTCAC	201	60.67
		R: CGGAGTGCTCCTCTCCATTA	-	
307	SSRY 307	F: AGGGTTACCACTCGCCATTA	200	59.45
		R: ACCATGAAAGCCCACCAATA	-	
308	SSRY 308	F: TTCGGCTCGTTAAGTCTCGT	388	60.02
		R: CGAATTTTGATCGAATTTAGTTTCA	-	
309	SSRY 309	F: TCCTCCTCCCTCTTCAGATTC	218	59.76
		R: GCCACAGGCTAAGGAAACAA	-	
310	SSRY 310	F: GACGCGTGGGCTTCAACT	196	62.41
		R: TCAAGGGGAGCAACAGTAGTC	-	
311	SSRY 311	F: GCCAACAGGAATCCTTCTGTA	202	59.18
		R: GAAACCAATGCAGTTTCACAA	-	
312	SSRY 312	F: TGATTGCAGCAGAAAGCAAG	204	60.28
		R: ATGGAAGGCTTGAAGTGGTG	-	
313	SSRY 313	F: ATAGCACCCCACCACCTGTA	204	60.25
		R: GCGAACTTTGCAGCTATTGA	-	
314	SSRY 314	F: CAAACATTGGCAAAGCTTCA	188	59.85
		R: GCTCTCTCAAGCGCAGATTT	-	
315	SSRY 315	F: TGAGAAACATAAACCGTTTTCAA	192	58.71
		R: CAGCATGGAAGCAAAATCAA	-	
316	SSRY 316	F: GAGCTTTACGGTCTCTGTCTCTG	195	59.71
		R: ACATCGTGAGGTCCAAGAGG	-	
317	SSRY 317	F: CCTCTGCTACTGGCTATTTTCA	224	58.68

		R: CGAAGAAGCCTCATTCCTGA	-	
318	SSRY 318	F: CACGCGTCCGTCTACATCT	201	59.87
		R: GTAATGGAGGGCGGTACTCA	-	
319	SSRY 319	F: CCAATCCATCCTTCCTCTCA	170	60
		R: AGGAAGCAAAGACGACCTGA	-	

S/No	Name	Sequence	Product size	Annealing temperature(°C)
320	SSRY 320	F: GTTGATGAGCTGTGGCATTG	204	60.27
		R: CCTAGACGAAGTGGGTCGAA	-	
321	SSRY 321	F: TTTTCCTTCCTCCAAGTCCA	200	59.64
		R: CGACAACACCTTCCTCCATT	-	
322	SSRY 322	F: GTCTGCGCTGAGCAGTCTC	175	60.03
		R: GAGTGAGACGACGAAACGTG	-	
323	SSRY 323	F: GCTTGCTCAGCCTTGAGTATT	189	58.76
		R: TTGCCATGGCTACAAAAGAA	-	
324	SSRY 324	F: CGCTTACAACACCACCTTCA	206	59.76
		R: GCTTGATCTCAGCCATGTCA	-	
325	SSRY 325	F: AGCCAAAAACCATACCCACA	230	60.23
		R: GCTATTGCTGTGTGGTCCAG	-	
326	SSRY 326	F: GTCAGGCGGTCACCAAAC	220	60.1
		R: ATCAGGAGGAGCTTGACAGC	-	
327	SSRY 327	F: CCCGTCTTAATCCTCCATCA	194	59.89
		R: CATCTTCGAGAAATGGACTCG	-	
328	SSRY 328	F: GCCCAATTACCAAAAGCTGA	231	60.07
		R: CTGCTGCCACCACTCTGATA	-	
329	SSRY 329	F: CACCCATTTCATTTCCGATT	211	59.62
		R: GTGCGTTTCCTGCTTTTTGT	-	
330	SSRY 330	F: CCACCATCATCATCGTCATC	193	59.72
		R: TTCTTCTTCTTCCCCATTGC	-	
331	SSRY 331	F: TCAGATCCCTCGGTTCTCAG	198	60.34
		R: TGCTTTGCTTCTCAAGTCCA	-	
332	SSRY 332	F: CAGGCTCGAGGTCTTCTTTG	225	60.13
		R: CCACCCCATCTTCAACATTT	-	
333	SSRY 333	F: TACTTTGGGCCTTCCTTTCA	180	59.68
		R: GGTTGGAGGAGCCATAGGTT	-	
334	SSRY 334	F: GGACGCGTGGGAAGAAAT	208	61.02

		R: GGGGATACACCATGAAGCAG	-	
335	SSRY 335	F: ATGGTGCACAGATTGACAGG	201	59.55
		R: CCACTCCTCAAACCCACAAA	-	
336	SSRY 336	F: CAGTCTCTAACGATCCCCTCA	188	59.29
		R: CATTTTCAGCAGCCTTTTCC	-	
337	SSRY 337	F: TCGCTCACAAAAACAATCCA	207	60.23
		R: AGATCTTTGCACGTTCACCA	-	
338	SSRY 338	F: CTCTACTCGGCATGGATTGG	209	60.61
		R: AGCTCCGCTAAAACACATGC	-	

S/No	Name	Sequence	Product size	Annealing temperature(°C)
339	SSRY 339	F: CGCACCAACCTCATTTATCC	213	60.33
		R: GGCTTCACAGCCGTAAAAGT	-	
340	SSRY 340	F: AAGAAAACGCAACCCCTTCT	186	60.11
		R: ACAGCTTCCCTCAGGTTTCC	-	
341	SSRY 341	F: CTTTAGCCTCTGCCTCCTCA	188	59.71
		R: CATGAACTGAGCCTTCGACA	-	
342	SSRY 342	F: TCTGCTTTTGGCTGGAATTT	194	59.82
		R: GGTTCGAAGCATTCTATGGTTA	-	
343	SSRY 343	F: CACTCTTGTGCAGAACTTTGCT	186	59.72
		R: CTCAATCCGCTCCATCTCTC	-	
344	NS6	F: TTCCATCCAAGCTCCTTCAG	296	55
		R: GCTCGCATATTCCCATCAAT	-	
345	NS9	F: AAGACTTTCCATCTTCAATTATTTTT	-	55
		R: ATGGCATTTCTGCAGCTCTT	-	
346	NS10	F: GTCATTACGGCGAATCTGCT	202	55
		R: CGATTTTGCGAATTACCACC	-	
347	NS16	F: GAGCATTCAACCTCTCTGGG	197	50
		R: ACGTCGATAATGGCAAGACC	-	
348	NS22	F: CAGCCAAATCAACATCCCTT	298	55
		R: CAAGCCCCATCATCATTTTC	-	
349	NS23	F: CCATAAGGGAAGGAACACCTC	294	50
		R: CCGCTATGGGAGCAGACTAT	-	
350	NS30	F: AGCTGACTCCCACCACTGTC	188	55
		R: CACAAGACAAAGCAAGGCAA	-	
351	NS33	F: TCACCTCACAGCCAAGAGAA	249	55

		R: GAGCTTGCTCACCGGACTTA	-	
352	NS36	F: GACCATCCAAACTAGCACATCA	299	55
		R: GCACCAGAACTAGGGATGGA	-	
353	NS37	F: TGAGTGCGATGAGAACGTAA	229	55
		R: GTGCATCCTTACCAATCCTG	-	
354	NS40	F: GCTGTTACGGCCAGAGTAGA	175	55
		R: GATGTCTGAAATCCCTCTCTTT	-	
355	NS51	F: CGCATTGGACTTCCTACAAA	262	45
		R: TGTGGAATTGTGAGCGGATA	-	
356	NS53	F: CCAACGTATGGAATGTGCTG	242	55
		R: CCTAAGTTGTTAGCCAGTGATTAGA	-	
357	NS57	F: ACCAAAATCTCCACACCCTG	-	55
		R: CAACAATTGGACTAAGCAGCA	-	

S/No	Name	Sequence	Product size	Annealing temperature(°C)
358	NS59	F: CTCCTGCCAAAATTGACCAC	-	55
		R: CATGAGCATATCCCTCCTCA	-	
359	NS69	F: TGTGGATGCCATGACTGATT	-	55
		R: TGTGGAATTGTGAGCGGATA	-	
360	NS72	F: TGTAACAGGTGGAAGTGCGA	207	55
		R: TCAGGACCAATATTACCTACACCA	-	
361	NS73	F: CAGATAGGCGTTCCCCAATA	266	55
		R: TTCAAAGTTTAATGCCGATGG	-	
362	NS74	F: TCGCTGTATGCAATACTTCGTT	229	55
		R: TAGTGTTGGGGACTCTTTCG	-	
363	NS76	F: ATGCAGTCAACTGTCCAACG	-	55
		R: TGCCTCTGCAATATGAGCTG	-	
364	NS77	F: GGACGCACAGTATTCTCCAC	579	55
		R: GATAATGGCAAGACCGGA	-	
365	NS78	F: AGCAATGCCTTGATCTTGAG	379	55
		R: AAGATGGCAATTCAAGCAAG	-	
366	NS80	F: GTGTGAAGCCACGCTGTAAA	288	55
		R: ATCAGGTCCATTGTTTGCCT	-	
367	NS82	F: AATTGAATTTTTCTCAGCACTGT	203	55
		R: TGTCGCCCACTTACATTTCA	-	
368	NS92	F: ACTTCATTGGTGCTGGTGCT	276	55

		R: AGCGTTTAGCACGTCAGAGC	-	
369	NS97	F: TTAAAAGCACCTGTGGGTCC	262	55
		R: GATACCCACAAGCCCAAAGA	-	
370	NS109	F: AACTGCAAACAAAGCCGAAG	-	55
		R: TCCTTGGCATAGCCAAAATC	-	
371	NS119	F: GGGAAGTGAGCAGAGACTGG	-	55
		R: GATGGTGGTGATGATGATGC	-	
372	NS124	F: CCCACGCTTCTGCTCTTTTA	219	55
		R: AGGCATACCGCCATGATTAG	-	
373	NS128	F: AAAGGACAGCGCTACCAGAA	283	55
		R: GATCGCTTCACCTTCCTCAG	-	
374	NS136	F: GACTATTTGTGATGAAGGCTTGC	-	55
		R: GGTTCAAGCATTCACCTTGC	-	
375	NS142	F: TCACTCAGCAGCTCTACCCA	159	50
		R: CCACCTCCACCTTACTATCCA	-	
376	NS144	F: GTAGATTTCGGAGCGCCTTC	102	50
		R: GCACCGTTAGAAGCGAAGTT	-	

S/No	Name	Sequence	Product size	Annealing temperature(°C)
377	NS147	F: GCTGATGTCTATTGCACACGA	292	50
		R: CAACGTTCTCTCGTAAATGGTG	-	
378	NS149	F: TCTTGCTCAAGGGCTCAAAT	299	55
		R: TTTGATTCCACGAAATCTAGAGAA	-	
379	NS158	F: GTGCGAAATGGAAATCAATG	166	55
		R: TGAAATAGTGATACATGCAAAAGGA	-	
380	NS159	F: TATGGAGCAAAGTCAGCCCT	264	55
		R: GTCCATGCACATGCCACTAC	-	
381	NS160	F: CAATAAGAGTATAACCATTACCTGTG	129	55
		R: ATGCATCTTCCTGGTTTTGT	-	
382	NS162	F: ATTATTATTGTGAAGCAATGTCA	131	55
		R: CCAGTAAGCCTAAGCACGAT	-	
383	NS166	F: ACAAAAGCAATCAGGCAAGC	277	55
		R: TTGTGCACCATGAAACCATT	-	
384	NS169	F: GTGCGAAATGGAAATCAATG	319	55
		R: GCCTTCTCAGCATATGGAGC	-	
385	NS170	F: TTCCTTTACAATTCTGGACGC	285	55

		R: AGTCGGGAGTTGGAACCTCT	-	
386	NS174	F: TTAGGAGGTGTGTCCCATCC	283	55
		R: CTCAATTCATTAAAGCGCGG	-	
387	NS176	F: TGTGATACAGGGTGCTTTGC	269	55
		R:AACTAGGGAAAGTTCTGAAAGTAGAGA	-	
388	NS178	F: TTACAGGTGCCCGATGTGTA	184	55
		R: CGTTCGAGTTGCATTCATTC	-	
389	NS185	F: AGTTAAGGGCCAATTCCTGC	-	55
		R: CCTTTCTGATGTTCTCTCTGCAT	-	
390	NS186	F: CCACAATCCTTGTAGTAGCCAG	226	55
		R: TGCAGTATTCCTCCAAAGTTATC	-	
391	NS189	F: TGGGCTGTTCGTGATCCTTA	104	55
		R: CATGAGTTTAAAAATTATCACATCCG	-	
392	NS190	F: CCAAGCAACCATCATTCAGA	178	55
		R: TTCATGTGTGGGTTTCCTCA	-	
393	NS192	F: GACTTCCAGACGGGATGTGT	264	55
		R: ATCATGTTACACAAACAATATCAGC	-	
394	NS193	F: TTGGGGGCTTTAAGTTGTTG	258	55
		R: AAAGCCCATCCCCTCTATGT	-	
395	NS194	F: TGGTAAGGTTCTTATTTTTTGAG	191	55
		R: TCCTTCATTTCAGCAATCTT	-	

S/No	Name	Sequence	Product size	Annealing temperature(°C)
396	NS197	F: TGTAAAATTTAAGACTTTCCATCTTCA	-	50
		R: ATGGCATTTCTGCAGCTCTT	-	
397	NS198	F: TGCAGCATATCAGGCATTTC	196	55
		R: TGGAAGCATGCATCAAATGT	-	
398	NS207	F: AGTCGGGAGTTGGAACCTCT	287	55
		R: TTCCTTTACAATTCTGGACGC	-	
399	NS208	F: TCCTGGTTTTGTCCTTGTTGT	131	45
		R: GCGTGGGCTAACCAATAAGA	-	
400	NS210	F: AAAGGGAGATTTGCAGAGCA	251	55
		R: TGGCTTTGGTTGTATGTGGA	-	
401	NS216	F: TTTTGAACAGAACACCATCCC	179	55
		R: CCCCTTCAGGTCGTCACTAA	-	
402	NS217	F: TCAGCTGTATGTTGAGTGAGCA	226	55

		R: AGGGAAGGAACACCTCTCCTA	-	
403	NS231	F: ATAAGAAAGCAAGGCGCAGA	-	50
		R: GTGGAGACCGAGCAAAACAT	-	
404	NS235	F: CCAAAACATAGGGAGCGAAA	210	55
		R: AATTATGGGCAGGAGAAGCC	-	
405	NS242	F: ACGCCTTAAGTCGGAAGTCA	280	45
		R: GCACTAATCAATATTCCACACCA	-	
406	NS248	F: TCAGAAGTGCTCTAGCTTGTCC	254	50
		R: CATTTGATAGGCAACGCTCA	-	
407	NS254	F: TTGCTTCAAGGGTGGAAAAG	287	50
		R: CAAGGAGAGACATGCCTGGT	-	
408	NS255	F: TCAAGAGGATCCGTAGACCC	118	50
		R: CGCGTGGACTACAAGAACAA	-	
409	NS260	F: TCAGCTGTATGTTGAGTGAGCA	224	50
		R: AGGGAAGGAACACCTCTCCTA	-	
410	NS265	F: CTCCATTGCCGTGGATTAAC	285	55
		R: GCACAATACGCGAATCTTCA	-	
411	NS267	F: ACACGCAACAAATCAACCAA	164	55
		R: CCGCTCCAGGTGCTTTTAC	-	
412	NS270	F: ATCAGGCAGAGGAGAGACGA	260	55
		R:GAACAGAGAGAGAAATGATAGTCTAGT	-	
413	NS271	F: AAACCACCAATCAGTCCAGC	216	55
		R: ATGTCTAATTGAAGGAGAGGATTC	-	
414	NS272	F: TGTAAAATTTAAGACTTTCCATCTTCA	174	55

S/No	Name	Sequence	Product size	Annealing temperature(°C)
		R: ATGGCATTTCTGCAGCTCTT	-	
415	NS287	F: GGACCCAAGTCATCATCAGG	171	55
		R: TTAGCCCTATGTCATCCGGT	-	
416	NS290	F: CCAAGCAACCATCATTCAGA	106	45
		R: TCATAAAGCTCGTGATTTCCA	-	
417	NS294	F: CGAAAGTCCTTGAAGCAACA	263	55
		R: CAATTAAGGCAGACGGAGGA	-	
418	NS295	F: TGTAAAATTTAAGACTTTCCATCTTCA	281	45
		R: AATACCCCTGACATCCCCTC	-	
419	NS300	F: TGAGAATTGTTGATTCCCAAGTT	294	55

		R: GCCAGGCTTTCCTGTGATAG	-	
420	NS301	F: TGGGCTGTTCGTGATCCTTA	106	45
		R: CATGAGTTTAAAAATTATCACATCCG	-	
421	NS306	F: AAGACCCACCAGAAAGCTGA	300	55
		R: CAATTTCCAATGTGGTCTCAAA	-	
422	NS307	F: GCGCGTGGACTAACCATTA	143	45
		R: GCATGCATCTTCCTGGTTTT	-	
423	NS308	F: GGAAATTGGTTATGTCCTTTCC	-	55
		R: CGCATTGGACTTCCTACAAA	-	
424	NS313	F: TGCTGGGGAACTAGTGTGGT	147	55
		R: GCAACTTCAAAGGCTGAAGG	-	
425	NS315	F: GTGTGCGGGCATGCAG	247	55
		R: CCATATGCACAGGCCCTACT	-	
426	NS319	F: TTCTAAAGGTTGATTAAAGCTCTGTG	164	55
		R: AGGGAAGGAACACCTCTCCTA	-	
427	NS323	F: CCTGGCAGAGAACTGGAGAC	265	55
		R: ATGGTGCATGCTCAAATGTC	-	
428	NS327	F: TCAGCTGTATGTTGAGTGAGCA	220	55
		R: AGGGAAGGAACACCTCTCCTA	-	
429	NS340	F: GCCAGCAAGGTTTGCTACAT	241	55
		R: TTGCTAAAAATCCCTGGACC	-	
430	NS341	F: TCTTTGATGAGACCAAGCCA	267	55
		R: GCACAGACAAACAACCAAGC	-	
431	NS342	F: TCAGCTGTATGTTGAGTGAGCA	275	45
		R: TGCAGGTCGACTCTAGAGGAT	-	
432	NS346	F: CTGCAAAGTCGATGCCTACA	290	55
		R: TCTCCATCAGCAGTGCAAAC	-	
433	NS347	F: AAAGGGAGATTTGCAGAGCA	250	55

S/No	Name	Sequence	Product size	Annealing temperature(°C)
		R: TGGCTTTGGTTGTATGTGGA	-	
434	NS349	F: CACTGCGTACAAGCAACACC	263	55
		R: GCAAAAGTGAAAAGGACGGA	-	
435	NS350	F: TCTGGAGCATCAAACTGCTG	220	55
		R: GGTGTTGCTTGTACGCAGTG	-	
436	NS356	F: CAATAGTATTACATGTCCTGCATACG	263	50
		R: CGCATTTTGCTTGCAGATTA	-	
437	NS371	F: CCAGAGCTATGTGCAGGCTT	149	50
		R: TGTAAAATTTAAGACTTTCCATCTTCA	-	
438	NS376	F: TCAAGACCCTTGCTTTGGTT	205	55
		R: GGACTATCAAGGCGCAAAAG	-	
439	NS379	F: TTTTGCCTTCCTCTTAGCCA	232	50
		R: TGATCCCAAGGATCTTCCAG	-	
440	NS381	F: TGTAAAATTTAAGACTTTCCATCTTCA	209	50
		R: GCGTGGACTAACAAAGCCTC	-	
441	NS384	F: CATGAGTTTAAAAATTATCACATCCG	106	55
		R: TGGGCTGTTCGTGATCCTTA	-	
442	NS391	F: TCTCAACCTCAGAATGTTCCAA	132	55
		R: ATTTCCTGCACCCGGATAA	-	
443	NS395	F: GAAACTGCAGGGAACTGTCC	216	55
		R: GGTTTCAACCCCCAGAGAGT	-	
444	NS562	F: TGTGACTGAGGTTGGATGGA	127	45
		R: AACACCAGCAAAATTGCACA	-	
445	NS568	F: CCTGCCTACTGTTCACCTCA	207	55
		R: CGCATGCACTTGACCAATTA	-	
446	NS576	F: ATGAGTGAGAAATCTGCCGC	147	55
		R: GAGAGGAAGGAAGTTAGAAATCCA	-	
447	NS584	F: TTTTATATGCGAGCGTATACGTG	188	50
		R: CAGATAGGCGTTCCCCAATA	-	
448	NS587	F: GAAATGCTTCTGTTAAGCAACATG	166	50
		R: AATGGACTTCTCACGCTGCT	-	
449	NS602	F: AGTGGATGTATTTGTGTTTTG	127	55
		R: GCCTTTGTCCAGTCCATAGG	-	

450	NS615	F: TGCTTGGGCCATCTCTACTC	173	50
		R:CGCGTGGACTAACAGTTTTG	-	
451	NS619	F: TGTAAGCTGAACGGCTTTCAT	103	55
		R: TCAAATGAAGGTGAACTACTCT	-	

S/No	Name	Sequence	Product size	Annealing temperature(°C)
452	NS622	F: TAAGTCGCGCAAATCCTTCT	147	55
		R: AGCCCACAACAACTGTGTGA	-	
453	NS644	F: AACGTGGAAGGCCAGTAGAA	296	55
		R: TGTCCTCCAAACTCCCAGAC	-	
454	NS656	F: AAGAACCCAACGCATTTGTC	197	55
		R: TGCCTCTAAGAAGATTGGAAGC	-	
455	NS658	F: CATGATGGCCCGAAGATAGT	-	55
		R: TCGTTGGAGCCATTACATTTC	-	
456	NS664	F: GGGTGCCAAACTCTCATTGT	300	50
		R: GGTGAGAGCCTAACCTGTGC	-	
457	NS667	F: GAATGCATAACATGAAAACAGG	217	55
		R: TGGAGGTGGAGCTTAGGAGA	-	
458	NS689	F: AGGATGATGATGAGACAAGAAGA	144	55
		R: CAGACTGGACTTGAACTTTCACT	-	
459	NS693	F: GCCTATTTGTGACCACGCTT	152	55
		R: TTATTAGGCGACTTGCTCTGG	-	
460	NS701	F: TCTCTTGTTCATTTGTTGCGTT	296	55
		R: TCCATTTGAGCCAAAATTTTATT	-	
461	NS713	F: ATGGCATTTCTGCAGCTCTT	200	50
		R: GGCGCGTGGACTACAAGTAT	-	
462	NS717	F: GCCAAATCGCCAAGGTAATA	-	55
		R: GGTGAGTGATAAGGTTACGGC	-	
463	NS720	F: CCATTACTTACACATTGGACTTCCT	157	55
		R: GGAAATTGGTTATGTCCTTTCC	-	
464	NS725	F: AAAACGAAAATCATGCCCAG	155	55
		R: TGCTATTCCAAGTGTTTGCC	-	
465	NS733	F: TCTAGTGGTATCAGTGGAAATGG	293	50
		R: AAGGACTGGCAACGTGAAAT	-	
466	NS743	F: GGCCAATTTTTTCTATATATATGTTTT	376	50
		R: CGTGCGCACACAATTGGC	-	

467	NS772	F: CCACACCTGGTCTTTCCTGT	153	55
		R: AATCACATAATGAGTATAAATAAATG	-	
468	NS774	F: AACCCGCAGAGAATCATGG	124	55
		R: TCTCTTGCTTCTGTCACAACG	-	
469	NS780	F: TTCTTGTCTAAAGATACATACACATGC	-	50
		R: TGCAAATAAGATGAAGAATGTTTGA	-	
470	NS781	F: CACCCAATTCACCAATTACCA	205	50

S/No	Name	Sequence	Product size	Annealing temperature(°C)
		R: GAAGACGACGATGGATAGCTG	-	
471	NS847	F: CAAACTTAAACTCCGTCCGC	-	55
		R: TTGGCCTGTAAGGTTCCATC	-	
472	NS882	F: GCGTAAAGAAACTGATGAAGGA	308	55
		R: GGGAGGAACTTGGCTTTCTC	-	
473	NS890	F: TAAATTGGGGGTTCTTGCTC	324	55
		R: TGCTTACTCTTTGATTCCACG	-	
474	NS898	F: GCATCTTCCTGGTTTTGTCC	127	50
		R: CAATAAGAGTATAACCATTACCTGTG	-	
475	NS899	F: AATGACCATGCCAACACAAG	298	55
		R: CACCGTCAAGAGGATTTGGT	-	
476	NS905	F: CAAACTTAAACTCCGTCCGC	283	55
		R: TTGGCCTGTAAGGTTCCATC	-	
477	NS909	F: GCCAGGCTTTCCTGTGATAG	343	55
		R: TGTGATACAGGGTGCTTTGC	-	
478	NS911	F: TGTTGTTCAGACGATGTCCAA	127	50
		R: TTGAAGCAGTTATGAACCGT	-	
479	NS912	F: GAGAACTCAACCCCATACC	356	55
		R: AAGGGACACGACTTGGTCAC	-	
480	NS917	F: TCAGGGCCATTGAAAAATGT	273	55
		R: CCTGTGCAGTATTGAGCGTG	-	
481	NS928	F: GATACCCACAAGCCCAAAGA	283	55
		R: GACCCACCCATCCACTAGAA	-	
482	NS933	F: TGCTACATAACAGTCATTATTCA	228	55
		R: TGCCTTTCTCTGTCAAGCCT	-	
483	NS945	F: GCAAGGCTCCATTAAAAGTCC	394	55
		R: TGTTTGAAATAGTGTTGCTTCTTGA	-	

484	NS946	F: GCAAGGCTCCATTAAAAGTCC	182	55
		R: TTGTATGCTTGCTGACTGGAG	-	
485	NS948	F: TTTTGCCTTCCTCTTAGCCA	235	55
		R: TGATCCCAAGGATCTTCCAG	-	
486	NS955	F: AGCTGCGTGCCTACAAGTG	242	55
		R: TTGAAAGTGTGCTGTTTGAAGTC	-	
487	NS960	F: AAAGCCCGCATTCAAACAC	216	55
		R: TGATGCAGGTAGCAAGGATG	-	
488	NS963	F: TTTTTGTCTGCTGCATATGTTT	121	55
		R: GAAGAAACCACCCAAGTGGA	-	
489	NS964	F: AAGGGACACGACTTGGTCAC	118	55

S/No	Name	Sequence	Product size	Annealing temperature(°C)
		R: TGGTTAAAATTTCTTTTGTGAACTG	-	
490	NS977	F: TTCCTCGCATGGCAGAAG	300	55
		R: GACCCTTTGCCGTTACTCAT	-	
491	NS978	F: TGTTGGCCATATTTCCCATT	238	55
		R: TTGAACACACTTGGCCAGAA	-	
492	NS980	F: TGATCCCAAGGATCTTCCAG	234	50
		R: TTTTGCCTTCCTCTTAGCCA	-	
493	NS982	F: AGGTGTGTCCCATCCCTTC	300	50
		R: TGTGCATATTGAAATCTCAGACTC	-	
494	NS983	F: GCTTCAAACATCAAACCCTAAC	279	55
		R: TCTGCAGATGCAACAAATCC	-	
495	NS995	F: CATGAGTTTAAAAATTATCACATCCG	105	55
		R: GGGCTGTTCGTGATCCATA	-	
496	NS1002	F: GACGGTTGTCGCCTTGTC	213	50
		R: AAACCAATCAAACTTGCAACC	-	
497	OS22	F: TGTGACAATTTTCAGATAGCTTCA	161	55
		R: TTTGTTGGGAAAAGACGAGC	-	
498	OS112	F: TGGGTGCCATTTGTAGTTGA	289	55
		R: TCCTCGCATTATGTCACACAC	-	
499	OS312	F: TCTGCGCTGTTCATCAAATC	223	50
		R: TGCTGGGAGGAAGTAGGAGA	-	
500	NS1003	F: TGCAATTGTAAGGGC CAAAT	270	52
		R: AATTTGGAGCTCAAGCGATG	-	

501	NS1004	F: ACAGATGTTGAGGGGATGCT	250	52
		R: TGAAAAATGATG TTGAAGCGA	-	
502	NS1005	F: ATG CTAAATTAATGGCGGA	-	-
		R: TGTTTATGAAGCATATCAAAAT	-	
503	NS1006	F: AACTTGCGTCCCAAAGTGTT	275	55
		R: TCCTATACAGTGCTGTCCGC	-	
504	NS1007	F: AGCACCTTGGGCAGCTTCT	300	55
		R: TCTCCTAATGGTGCGTTCAA	-	
505	NS1008	F: AAATGGCCTAGAAATCCATGA	275	52
		R: AACCCACTCAAGTGTCTCCG	-	
506	NS1009	F: CGTTCCACCAAGAAAATGGT	-	-
		R: TTGCATGAATCAGAAGCAATG	-	
507	NS1010	F: TAGCGATTGCATTTTACCCC	500	55
		R: ACTGCAAAGCCCTTGAGAGA	-	
508	NS1011	F: AGGCTGTTCAGTCAACCTGG	150	40

S/No	Name	Sequence	Product size	Annealing temperature(°C)
		R: ACTAATGCCTCTGCTTTCC	-	
509	NS1012	F: TGTTGATACAATCTAAATGTAGCCTTC	350	55
		R: TGTTGAATCCCACATTGGTG	-	
510	NS1013	F: CATGTTACCTTGCAGCGTGT	300	40
		R: GAGACACACGCATCCTCAGA	-	
511	NS1014	F: TTTTCCAATTGCTTTGGTGA	225	52
		R: TCCCTCTATTTTGACGATCCA	-	
512	NS1015	F: CCCTTTCCTTTGGTTTGGTT	150	40
		R: ATGATATCGGCTCGGTTCAG	-	
513	NS1016	F: CTGAAAGGGAATTTCATGCC	375	55
		R: TGGACTTCGTAATTTTCTGCAC	-	
514	NS1017	F: TTGGATTCCAGTGATAGCCC	275	52
		R: TTCTCATTTTGAGCCATGACC	-	
515	NS1018	F: GTGCCATGGCTTTGCTATCT	400	45
		R: AGAACATTTCCAGCACACCC	-	
516	NS1019	F: CTGGAGAAGACCACCCAGAA	225	60
		R: AAGCAGTGGCTCAGTTTGGT	-	
517	NS1020	F: TCCAAGCCTCAAAACTTGCT	300	55
		R: GAGAAAGACTCAGGTTCGGC	-	

518	NS1021	F: TCACAATGAAGCCCAGTGAA	275	55
		R: TTGTATCTGAGCCTTGCGTG	-	
519	NS1022	F: GCTTAGGTGGACCCATTTGA	150	40
		R: AATCATTATAAGGCGTGGCG	-	
520	NS1023	F: ATTTAATGCACCCCCAACG	400	45
		R: TCCTCTCCTCAAGCTCCACT	-	
521	NS1024	F: CGCATTTGCATGAGACCTAC	-	-
		R: GCTGGAGTATCATGTCCCGT	-	
522	NS1025	F: TGGATGTTTACGGACTCAAAA	175	40
		R: AGCACTCCAAACAAGACCAAA	-	
523	NS1026	F: ATGGCCAGCTTGTCAGCTAT	250	45
		R: CTGCGTGCATCAATCAGACT	-	
524	NS1027	F: GTTATATTATAGTAGTAGAACTTA	-	-
		R: CGATCAGATCTAAATACAGCC	-	
525	NS1028	F: GGGGATGGAGTAAATACGCA	350	55
		R: TTCCCCAAATATCACTTCTGC	-	
526	NS1029	F: GATTTGCACAGAGGCAGTGA	350	55
		R: TTTCTGTTTTGGGTCAAGGG	-	
527	NS1030	F: AAAGCATTGGCCACTTGGTA	300	55

S/No	Name	Sequence	Product size	Annealing temperature(°C)
		R: ACCAGATGTAGTGTTCCCCTT	-	
528	NS1031	F: TCATTGTTGGTGCTGATGGT	375	40
		R: CAACCCCCATTTGTATTGGT	-	
529	NS1032	F: TGGATTATTTCCTTTCCACCA	425	40
		R: CTTTTCTCTAGGGAACGCGA	-	
530	NS1033	F: GGGGTTCCAACCAGAGTTTT	300	45
		R: GTCGATGCCCCTGTTACTGT	-	
531	NS1034	F: GCCAGCGTCAACCTCTTTAC	210	45
		R: GGTGCCAGATGAGACAACCT	-	
532	NS1035	F: TTCCTTGCTTCTCAAGGCAT	225	60
		R: TGCTCCTGCTGTACTGGTTG	-	
533	NS1037	F: TTGGAAGTCAGGCTCCTTGT	200	45
		R: TATCCCGTCAATGCAATCAA	-	
534	NS1038	F: TCATGGTGAATGCCAGAGTAG	175	40
		R: TACCACATGGTGGCAGCAC	-	

535	NS1039	F: TATTCAAGAACCGGGCAGAC	250	45
		R: CGGTCGCAAATAACGAAAAC	-	
536	NS1040	F: CGCCTCTCAACCCAATAAGA	300	45
		R: TAGAAATCTGAGCCACCGCT	-	
537	NS1041	F: TTGGATGATGATTAGGCTCG	350	52
		R: TCGATCTACTTTGTTTTGATTGG	-	
538	NS1042	F: CCGGGTCGCTCTATACCTATC	225	40
		R: AAAAATTGAAACGAATGGAAAAAG	-	
539	NS1043	F: CTCACCATGGCTCATTCTCA	275	55
		R: AAAGCCTGCAAAGAAAACCA	-	
540	NS1044	F: TCTCCCAGAATGGCAGAAAC	350	52
		R: TTGGAGCTTTGAGGTTCAAGA	-	
541	NS1045	F: GTGTTGTGCCCCTTGCTTAG	375	55
		R: AAAAAGGGTGGCGGATAATG	-	
542	NS1046	F: CCTTCCTACCCATCAAGCCT	425	52
		R: AATAAATTGGTGATGGCTGAA	-	
543	NS1047	F: TATGCACATTGCCTCCAAAA	300	55
		R: AACTCAACCCCTCCCATTTC	-	
544	NS1048	F: ATGGTTGTTGGGTGATCCAT	160	45
		R: AAAGCCCAATGAGCAGAAAC	-	
545	NS1049	F: TCTTGCCCATTTGGAAAATC	225	45
		R: CCTTCTGGGCAAATTGAAAA	-	
546	NS1050	F: TATGAACCAGCGACAGCAAC	350	45

S/No	Name	Sequence	Product size	Annealing temperature(°C)
		R: GACACATTATCCAGGTCGGC	-	
547	NS1051	F: TCCAGATTTCCAACAAAGGC	100	40
		R: GGAGCTGAGCTGATACATTGG	-	
548	NS1052	F: GAGTGTTGTCCGGCAGTTTC	300	55
		R: CCATTCCATGGGTTTTGTTT	-	
549	NS1053	F: TGATGCTCATGTTCAGCTCC	220	52
		R: TTTTGAAAGGATGCCAGCTT	-	
550	NS1054	F: CTGGTGATGGTGGGAAAAAT	175	55
		R: ACCCAACCATGAGAAGCAAC	-	
551	NS1055	F: CTAGAAGGTTCCGACATCCG	350	52
		R: TTGGGAATAGTGATTGTTAGAATTG	-	

552	NS1056	F: GCTTAAACTCATTGGGCTGC	380	53
		R: TTCCTTTGGCTCTCCCTGTA	-	
553	NS1057	F: TTCCTTTGGCTCTCCCTGTA	200	53
		R: CTCAACCCTGAAGGACCAAA	-	
554	NS1058	F: GGGTCACACACAAAAGAACG	175	40
		R: TCTCAAAATGTGGAGACCCC	-	
555	NS1059	F: TGAACCCTACGGACCCTAAA	180	45
		R: TCAGGGTATTGATCCACCACT	-	
556	NS1060	F: GGTGAGCTACAAGAGCCCAG	150	53
		R: GCCAAATTTTGACAGAGGGA	-	
557	NS1061	F: ATGCTTTTGCAAGCCACATT	375	53
		R: GAAATGGGCGAGCTTTTCT	-	
558	NS1062	F: CCTTGCATATTCCCACCTTG	400	52
		R: CGTACTGCCCAGGAAATAGC	-	
559	NS1063	F: TGCATACAAAACTGCCCTCA	200	56
		R: AAGTTTGGGAATGCAAACTG	-	
560	NS1064	F: TGTTTGATTTGCTGGGTTGA	450	52
		R: GGCCAGAAATTAAGGGCTTC	-	
561	NS1065	F: TCGGGCCTCACTGATGTAGT	450	52
		R: ATATGTGCCTTGTCGCATGA	-	
562	NS1066	F: AAAGCAATACACGCCAAAGG	450	53
		R: GGCCAAAACTACACAAAGGG	-	
563	NS1067	F: GGCGATGAATTTGTGTGAGA	450	53
		R: TGATGATTCTCCCTTGGTTCT	-	
564	NS1068	F: TCAAGATCCTATTCTTGCTCCC	425	40
		R: AAGGGAATAAACCCCTTTAATTG	-	
565	NS1069	F: TCCACTCTAGATTTTTCCTCCC	450	53

S/No	Name	Sequence	Product size	Annealing temperature(°C)
		R: TGGCATAGTAGATGGGGCTT	-	
566	NS1070	F: GGTCTGTAATCAAGTTTAGGTTTGTG	450	53
		R: TCATGGCTTTGACTAACATCTTTC	-	
567	NS1071	F: CCCTGGAGGAAGGTTATGGT	-	-
		R: TGTCGCTCATATTGTTTCGG	-	
568	NS1072	F: GCCTATTCAAATTGCATCCC	275	52
		R: GGCATTTGAAGAATGGAGGA	-	

569	NS1073	F: ACAGGGTGATTTGGTGGTGT	350	40
		R: TTCGGGTCGTCACAATACAA	-	
570	NS1074	F: TTGGCTTGAATTGAGGACTTTT	350	53
		R: AAAAAAAATTAAGCCAGCAT	-	
571	NS1075	F: AAGGGCGATGAGGAACTGTA	250	40
		R: TTTCTCAAAACATTTATTGCATTC	-	
572	NS1076	F: GCTAGGGCATGTCCATGAGT	375	53
		R: TACATTGCAAGGGCAAATGA	-	
573	NS1077	F: TGCCTTTGAGTTAACTTCTTATTCG	425	55
		R: CCTTGGCTACTTTTCTGTCACC	-	
574	NS1078	F: CTCTGTTTTTGGGTGTGCAA	375	52
		R: GACGCCATTCTTTTCTCAGC	-	
575	NS1079	F: GATCAAGCGCTTACCACCAT	325	53
		R: ACCTCCCACAACATCCAAAC	-	
576	NS1080	F: ATTGCTTACCCACCATCACC	175	55
		R: TGGATGGGAAAATGGTTCAT	-	
577	NS1081	F: TCATTGTTGGTGCTGATGGT	225	53
		R: CGGGTTTTCAGACAGGTCAT	-	
578	NS1082	F: AAGGCTTCCCAAGAATTAAACC	275	52
		R: AGAGGACACAATGGAGGTGA	-	
579	NS1083	F: TTTCCGTACCAGGGTTTGAG	270	45
		R: TGATTCAAAGCGAAGGGGTA	-	
580	NS1084	F: GAACCTGGTTTCTGCCAAAG	250	52
		R: TGGAGCTTGGAGGATCTACG	-	
581	NS1085	F: CCGCCGTCTCGATTCTATTA	350	52
		R: GACAGTGCACGAGCAAGAGA	-	
582	NS1086	F: AGACCTCAGGAACCCATCCT	350	52
		R: GGCAGAAACTCAGCTCCAAC	-	
583	NS1087	F: GTTCCACAGCATGGGCTACT	425	52
		R: TGGGCATCAAAGTTTACATCA	-	
584	NS1088	F: GCCCCTGGCACTATGTAAGA	275	52

S/No	Name	Sequence	Product size	Annealing temperature(°C)
		R: GAGGGGTGAGCTAAAAAGCC	-	
585	NS1089	F: ATGTTGCATGCATGGGTAGA	325	52
		R: TCATCCTAAACTAGATCCCGAA	-	
586	NS1090	F: TTCTCAAGCATATAGGGGCA	400	52
		R: ATTTTCGGGCAAAGGTGAA	-	
587	NS1091	F: CCAGTTCCAGGATTTGAAGC	150	40
		R: ATCCATGCTCCTGTTTTGCT	-	
588	NS1092	F: CACACCTCCCGCAGTCTAAT	350	40
		R: GGATCTGTCATGTCTTCACTCCT	-	
589	NS1093	F: CCAAAACACAAACAGCGAGA	375	40
		R: GCTTTCGTTTTATGCTTGCTT	-	
590	NS1094	F: GCAATGAGCTTAAGATCGGC	240	40
		R:GGCAGCGTCAGATCAGTACA	-	
591	NS1095	F: CCTCAAAGAGGGAGCAGAG	375	52
		R: GTCTTTACGATCGTGGGCAT	-	
592	NS1096	F: CCCGATAGCACATCAGTGAA	350	40
		R: ACTGGGCTGAATTCCAGTGT	-	
593	NS1097	F: TCGCTTTTAGCTTCCCTTTTC	375	55
		R: TTAGTTGCACGGCTTACGTG	-	
594	NS1098	F: CTACTAATAATAATTATCAGCACA	275	40
		R: AGAATGAAACTTAATCATCAGAA	-	
595	NS1099	F: GAGTTCGAGAATGTGCGTGA	225	55
		R: ATTTCTTTCTGCGCAAGCAT	-	
596	NS1100	F: AGGGGGCTCCTATGAAAGAA	325	55
		R: TGGTGGTTCCACAGGACTTT	-	
597	NS1101	F: CAATTGATACCTTATCGCACTTT	350	55
		R: CGGCTTAATCCATGCTTTTT	-	
598	NS1102	F: ATTTTCCGGGTGGGAATAAC	150	40
		R: ATCTTAAGGCGCTGTTTGGA	-	
599	NS1103	F: TTGCTTTGCTTTTTGCATGA	125	40
		R: TCATGTTTAATTTCTTAATTTTAATGT	-	
600	NS1104	F: AAAGAATGGGGTGGGTTTTC	-	-
		R: TATGGTTTTCACGACCATGC	-	

S/No	Name	Sequence	Product size	Annealing temperature(°C)
601	NS1105	F: TAACCATCCTAACCCCACCA	175	45
		R: ATGATGAAGCTCGACAGCCT	-	
602	NS1106	F: GGACGTGCGAACGAAGTTAT	370	45
		R: GCAGAAGCATAAGCAGAAGTAGAA	-	
603	NS1107	F: CATGGAGAACCCCAATGAAT	150	55
		R: CCAAAGAAAACTGTTCTCCCC	-	
604	NS1108	F: CGATTTTCGGGTCGTTACAA	100	52
		R: AAGCCTAACCAAAATTAAATAGATGA	-	
605	NS1109	F: TGTGCATGGTACGAGGGTTA	300	55
		R: CACACGCAATTCAATCCATC	-	
606	NS1110	F: GACATACTTAACAGTACATTG	-	-
		R: TTGGATATCCTTATAAGGTGGT	-	
607	NS1111	F: ATATGTGCCTGTGGTGGGTT	250	40
		R: TCAAATTCGATACCTCGCAA	-	
608	NS1112	F: GTGTCGCGTCTTAATCAGCA	400	45
		R: CCAAGTTTAATGTGGACGTAGC	-	
609	NS1113	F: ACCATTCCATTCTGGGCATA	325	55
		R: GGTGGGGACTGCTATTCTGA	-	
610	NS1114	F: TATCCTCTGGATGGACCTGC	100	40
		R: AGCGAGTTTTTCTCCCCATT	-	
611	NS1115	F: CAAGGAATGATCAACTCGCA	150	55
		R: ACGTTCTGGCACTTTGGAAT	-	
612	NS1116	F: AAACATGCATTCTACCCCCA	325	55
		R: TGGCCAGCCACTATAAAAGG	-	
613	NS1117	F: TGGAAATTTGGAATGTGGGT	350	55
		R: ATGGATGGAAATTCGAGTGC	-	
614	NS1118	F: GAACATGTGTTAAAAGGATTACCA	425	52
		R: AATGTTTGTGTTATGGCGCA	-	
615	NS1119	F: CTCCACTCCTGCCACGTATT	425	45
		R: GGGACAGTGATGGTCTTCGT	-	
616	NS1120	F: CGAATCCAACCAAGGTCCTA	150	45
		R: AATGACCTCAACTAGCACACA	-	
617	NS1121	F: TTTTGCTCCCTCACATGAAA	425	55
		R: TGCTGTTGAGGAGCTGTTGT	-	

618	NS1122	F: AAACATGCATTCTACCCCCA	325	52
		R: AGCCCACCTATACAGGGTCC	-	
619	NS1123	F: CTCACTGATGCTCATTCGCT	300	45
		R: ACAGAGAAAATCCGCCATTG	-	
620	NS1124	F: TTCCTCCTCCTTTTCCTCGT	125	40
		R: GAAACCCAGCTCCAAAAAGA	-	
621	NS1125	F: GCAGCATGCAAGCTTTACAA	350	52
		R: AGATCTTTCGCAAGTCGGAG	-	
622	NS1126	F: CTCCAGAGAGTGTCCTTGAGC	240	40

S/No	Name	Sequence	Product size	Annealing temperature(°C)
		R: TTTAGCCATCCATTCCAAGC	-	
623	NS1127	F: CAATTCTTAAGCCAGCCTGC	175	40
		R: TTCATGTCTTTCTTCAAGGTGA	-	
624	NS1128	F:CCAGGATCTTATGGACCCAA	200	40
		R: TGATCTCGGTGCAGAATGAG	-	
625	NS1129	F: TGGCTCATTGACGAGATCAG	275	40
		R: CAGAATTACAGCAGGCGGAT	-	
626	NS1130	F: TGTGGCACCATATTTCCTGA	375	52
		R: CAGAGCAAAGGTTTAGGCGA	-	
627	NS1131	F: TGGCAGAAACTCAGCTCCTT	350	52
		R: TGAGCAAGTTTTGGGAGCTT	-	
628	NS1132	F: AAACCACCCTGGTAGCCTCT	-	-
		R: TCAAAAATAAAGGGATAAAATTAAGG	-	
629	NS1133	F: CCGAACCTGGTTTCTACCAA	325	52
		R: CAAGCTCTCCCAACTTCGAG	-	
630	NS1134	F: CGTGAATTGCAGTAAAGCTCC	325	52
		R: TGTATACTTGCCCCACTTGC	-	
631	NS1135	F: ACTTCAATGTGAACCCTGCC	230	52
		R: AAACCCTAACCCAGGGAATG	-	
632	NS1136	F: TAGCGAAGGACCTCAGCATT	225	55
		R: CCCGAGTGAAGATGTGGAGT	-	
633	NS1137	F: CAGAAGCTTGGCTCCCTATG	300	52
		R: CAAGCTCTCCCAACTTCGAG	-	
634	NS1138	F: CCTCAACCTACCCTCAACCA	325	52
		R: TGAGAAGGGTGAAGAGGTGG	-	

635	NS1139	F: ACCCATTTGATGGCAACTGT	175	52
		R: CCGAGCTCCCTCCATAGAAT	-	
636	NS1140	F: CCCCTGGCACTATGACAGAT	270	52
		R: TGCCTTCACTCTAGCCGATT	-	
637	NS1141	F: CTGATGATTGCAAGGTGTGG	350	52
		R: TTGGTGCATAAAGGGGAGAG	-	
638	NS1142	F: ATCACACGAGTCTTACGCCC	175	60
		R: ATGCTCAAGAAGCAGGCAAT	-	
639	NS1143	F: GGCTTTGGTCCGTATYYGAA	300	52
		R: ATGGTGTCTCCTTCCACGT	-	
640	(ESTs)SSRY1	F: CCAATTTTGGCACCTGGTAA	-	55
		R: CAAAACCAGTGAATGCAAAAA	-	
641	(ESTs)SSRY2	F: AAAACCATGACTGCCGAGAC	-	52

S/No	Name	Sequence	Product size	Annealing temperature(°C)
		R: TGCTTTGCTTTGACCTACCA	-	
642	(ESTs)SSRY3	F: CGATGATCATTATGGCGATG	-	55
		R: TGGCGAACTCGCTTACTTTT	-	
643	(ESTs)SSRY4	F: GGCTGTTCCAAATGCAAGAT	-	55
		R: ACGAACTCGAAATCGTCGTC	-	
644	(ESTs)SSRY5	F: AATGCAATTTGCTGCTTCCT	-	55
		R: CCAAGGGAAGTCCAGAAGAA	-	
645	(ESTs)SSRY6	F: TCTCTCCAGCAGACGAAACA	-	55
		R: GTACCCTCAAGCCCATCAGA	-	
646	(ESTs)SSRY7	F: GGACAAGCTCACCGAGAAAA	-	55
		R: TGCAGCTCAATTCCTTCTCC	-	
647	(ESTs)SSRY8	F: GAAGAAGCCTGGTCTGTTGG	-	55
		R: GGGACTGTGGTGTCCTCTTC	-	
648	(ESTs)SSRY9	F: AACGGTCCAAATCGTCAAAC	-	55
		R: CTCGATGTCAGCAACGAGAG	-	
649	(ESTs)SSRY10	F: ACCAGCTCCTGTCGACAACT	-	55
		R: CAGCACCATTTCTTCCCCTA	-	
650	(ESTs)SSRY11	F: AAGAGCTTTAGGCGGTCACA	-	55
		R: TTGGGGTTCTCCCTTAATCC	-	
651	(ESTs)SSRY12	F: AAAAATGATCCAACATCTAGCAA	-	55
		R: TTGGGGCCCGATAATAAGAT	-	

652	(ESTs)SSRY13	F: AGCATAGGAACCTGCGTCTC	-	55
		R: TCCAGCTGTAGCTGTTGTGG	-	
653	(ESTs)SSRY14	F: TCGATGCCCTTATTGGTAGG	-	55
		R: GGCAGAGCTGGATTCATCA	-	
654	(ESTs)SSRY15	F: GAGCAATTTCCACCACCATC	-	55
		R: CGGAATGGTCAATACCCTTG	-	
655	(ESTs)SSRY16	F: TCGAAGCATTCTGTGTGTCC	-	55
		R: TCATCCATAAGATCATCTCTGTTACTG	-	
656	(ESTs)SSRY17	F: GGCCATGCTCGTTTTAGAAG	-	55
		R: CACCCCATTGTTCTTTGACA	-	
657	(ESTs)SSRY18	F: AGTAGGTTTTCGCGGTCTCC	-	55
		R: ACCTCCAGAAGGTCGTCGT	-	
658	(ESTs)SSRY19	F: GAGAAGTGGTTGGGGTTTCA	-	55
		R: TCAAGAGCTGGACTTGAGGAA	-	
659	(ESTs)SSRY20	F: CGTCAGCCCATGAAATCTCT	-	55
		R: GCAAAGACAAAGAGGCATCC	-	
660	(ESTs)SSRY21	F: CAAAGCATAACCGCGAATTT	-	55

S/No	Name	Sequence	Product size	Annealing temperature(°C)
		R: AGTGCCCGATTATTGGAGTG	-	
661	(ESTs)SSRY22	F: CCAACCCACAACCTTCTGAT	-	55
		R: AATCCAATCCCCTTGGACTC	-	
662	(ESTs)SSRY23	F: GCGGTGAAGATCTTGGTTTT	-	55
		R: ATGCTCGTGCTCTTCTCGTT	-	
663	(ESTs)SSRY24	F: AGGAAAAAGAGGCTGGAGGA	-	55
		R: ACCTACGGAATCCCAGAAGC	-	
664	(ESTs)SSRY25	F: CATTTTCTTCACTTAACCCAACTG	-	55
		R: CAAGCCCTGAATAGTGACCA	-	
665	(ESTs)SSRY26	F: AAATCTCAAAACGCCACCAC	-	55
		R: AGTCAACGAACAGCGGAAAC	-	
666	(ESTs)SSRY27	F: TTGGATGTATAAAAACGCAGAA	-	55
		R: GAATATCATCCCAGGCTTCG	-	
667	(ESTs)SSRY28	F: ACAGCCACTTGCCTCATCTT	-	55
		R: GACACGATCGTTGCGAGATA	-	
668	(ESTs)SSRY29	F: AGCACCCTTCCACTTTATGC	-	55
		R: CTGAGGAGCAGAGCCGTTAC	-	

669	(ESTs)SSRY30	F: CTCTTCTTGTGCCCAACTCC	-	55
		R: TGGCTAAGGTCCTCGACTGT	-	
670	(ESTs)SSRY31	F: TCGAAGCATTCTGTGTGTCC	-	55
		R: AATTAATTAAGCGACATGAACAAGA	-	
671	(ESTs)SSRY32	F: TCAGGCTCAATCACAAGCAC	-	55
		R: TGCATGCTCTGTTCTGCTTT	-	
672	(ESTs)SSRY33	F: ATAATTGCTGCGACCACCAT	-	55
		R: GCTGCTCAGCCATGGATATT	-	
673	(ESTs)SSRY34	F: TCTTCCTCGTCTTCCAGTGAA	-	55
		R: ATTTCTTCATCGCCGTCATC	-	
674	(ESTs)SSRY35	F: CACTGCTTCCCCCTTCTGTA	-	55
		R: GCCACGCCCATTCTTATATC	-	
675	(ESTs)SSRY36	F: TTCAGCAAAGCTGCAGAAGA	-	55
		R: GATCCCAGAAAATGGCAAGA	-	
676	(ESTs)SSRY37	F: GAGGGAGTCTGCTTCTGCTT	-	55
		R: AAGCTCAGAATACAACGGAAAAA	-	
677	(ESTs)SSRY38	F: GACTCTGCGTCGCATTGTTA	-	55
		R: CCCACAAAAACCACTCCATC	-	
678	(ESTs)SSRY39	F: CAGTCCAAATCCAGCCATTT	-	55
		R: TGGAGCTTGCTCATCAGAGA	-	
679	(ESTs)SSRY40	F: GAAGCCTCGATGCCCTTATC	-	55

S/No	Name	Sequence	Product size	Annealing temperature(°C)
		R: ATCATCCCCTTGGCTAGGAC	-	
680	(ESTs)SSRY41	F: CAGCGTCTCTGCGTCAATAA	-	55
		R: AGTCGACGATGAGGAAGACG	-	
681	(ESTs)SSRY42	F: TTAGTCCTCAAGCAGCAGCA	-	55
		R: GTCTTCTGAAATCGGCAGGA	-	
682	(ESTs)SSRY43	F: CAGCAGAGCTTTGCCTAACC	-	55
		R: AACAAGCACCCAACCCAATA	-	
683	(ESTs)SSRY44	F: CATAAACGCGGTCCAAAAAT	-	55
		R: ACGAAAATGCGGATTACAGC	-	
684	(ESTs)SSRY45	F: GCCGGTAAACGGTAAAATCA	-	55
		R: AATCAAAGAGACGGCGAAGA	-	
685	(ESTs)SSRY46	F: AAGAGGAAGGGTCCTCTCATTC	-	55
		R: AGGCAAGAGTGGTGGATGAT	-	

686	(ESTs)SSRY47	F: GTGTCGCTGCTAGATCTTGACT	-	55
		R: TCATCAGATCACCACCATCAA	-	
687	(ESTs)SSRY48	F: GCCCCCATAAAAATCACCAT	-	55
		R: GAGAGCAAACAACCAAAGTTGA	-	
688	(ESTs)SSRY49	F: GACCACGTCTCGTCGTTTTA	-	55
		R: TGCCACGACATCTTCTTCTG	-	
689	(ESTs)SSRY50	F: TCTCCCCACACACTCTCTCTC	-	55
		R: GGACCGTATCCAAGCTCATC	-	
690	(ESTs)SSRY51	F: CCTGAGGAACGTGAAGCAAT	-	55
		R: TGAAGATGAGGAGAATGTAAAAGG	-	
691	(ESTs)SSRY52	F: GTCTCCCTCCCTCTCCTCTC	-	55
		R: TACGCAATTGCAAATGATCC	-	
692	(ESTs)SSRY53	F: GGTGCTGCAGAAGAAGTGCT	-	52
		R: CCCCTTTCTTCCACTTTCCT	-	
693	(ESTs)SSRY54	F: GCAAATTGGGGGAATGTTTT	-	55
		R: AAGACACGAAGACGGTTGCT	-	
694	(ESTs)SSRY55	F: TGCCCTCAAATTTTCTCCAT	-	55
		R: TTGGAAGATTCCTTTTCCATAGAC	-	
695	(ESTs)SSRY56	F: TCGCAGCGCTATTACCGTAT	-	52
		R: CGAGGAATGGGAATCAAACT	-	
696	(ESTs)SSRY57	F: GGGTCCTAAGCGACCATCTA	-	55
		R: AATTCAAGTTCAGGCGTGGT	-	
697	(ESTs)SSRY58	F: AGCCATCCAATCCAATCTGA	-	55
		R: TATGCGAGGATAGCAGCACA	-	
698	(ESTs)SSRY59	F: GCGGGGATAGTTGAGTAGCA	-	55

S/No	Name	Sequence	Product size	Annealing temperature(°C)
		R: TACGGTGACGTTGTCGAAGA	-	
699	(ESTs)SSRY60	F: CGACGGCTAAAATTGCATCT	-	55
		R: GCCTTTTGGCAGTTTAGACC	-	
700	(ESTs)SSRY61	F: TTCAATGATGGCTGAGCAAG	-	55
		R: TCGCAGCACTAAACATCTCG	-	
701	(ESTs)SSRY62	F: CCATGGCACAATAACATTGG	-	55
		R: TGGGCGTAGGACAGTAAGAGA	-	
702	(ESTs)SSRY63	F: CCCTTCCCGAGCAAGAAC	-	55
		R: CCTGTATCCAAGCTGCCATAA	-	

703	(ESTs)SSRY64	F: CCAACATGCTTCACCAATCA	-	55
		R: CATTCAGACAACGCAATGTG	-	
704	(ESTs)SSRY65	F: GCACCAGTCAACATTCCTGA	-	55
		R: CATCATTCACAACCCCATGA	-	
705	(ESTs)SSRY66	F: CGCTTACAACACCACCTTCA	-	55
		R: TGACGTCCTTAGCCATCCTC	-	
706	(ESTs)SSRY67	F: TCTCCATATCCTTGGATTCGT	-	55
		R: GCCGACCATAAATCTGGAAA	-	
707	(ESTs)SSRY68	F: TCTCCAGGTTAAGGGGGAAG	-	55
		R: CCTCCCTTCCATCCTCCTAC	-	
708	(ESTs)SSRY69	F: GAACCGCTTCTTCTTTCTCTCTT	-	55
		R: TAAGAATGGCAGGGAAAAGG	-	
709	(ESTs)SSRY70	F: GCCAATTTTGCTGGGTTTAC	-	55
		R: GCTGATGAACCCTTCACGTT	-	
710	(ESTs)SSRY71	F: TGCTTCTAACAGAGGCAGAGG	-	55
		R: GGAGCAAAAGGCAGGAAACT	-	
711	(ESTs)SSRY72	F: CGAACTCGATCAGATTCCACT	-	55
		R: TACGGCCTATCCTCTGATGG	-	
712	(ESTs)SSRY73	F: GTCAGAGCGGACACAACAAC	-	52
		R: AGGAGTCCACCGAGGAAGTT	-	
713	(ESTs)SSRY74	F:GGCAGTGATGCAAGAAATGA	-	55
		R: ATCGTTTTCCCCTTCCAGAT	-	
714	(ESTs)SSRY75	F: TGCGCTTCTCAGGGACTATT	-	55
		R: TTCCCCGATTGTTTGTAGGA	-	
715	(ESTs)SSRY76	F: GAAACGCTCGATCCTACTCG	-	55
		R: AGCAAGGAGGGTAACGACAA	-	
716	(ESTs)SSRY77	F: ATCTCAGGGTGGTCGACAGA	-	55
		R: TGCCAAAGGAGGAGAAAATG	-	
717	(ESTs)SSRY78	F: AAGCCAAGGAAGAAGCACTG	-	55

S/No	Name	Sequence	Product size	Annealing temperature(°C)
		R: CCCTCTTTCAAGGCCAAAAT	-	
718	(ESTs)SSRY79	F: CCACCACCTTGTATCCCACT	-	55
		R: TCTTTGCCATCAACCCTTTC	-	
719	(ESTs)SSRY80	F: TGGTAATGGTCTTTTTGTTGTTAAA	-	55
		R: CACTTGTGCCTCTACCAGGAG	-	
720	(ESTs)SSRY81	F: CCCACATGTACTTGGAAGAGA	-	55
		R: GTTGGTGGACTCGTGGATCT	-	
721	(ESTs)SSRY82	F: GAAGGCCAGACCCAACAATA	-	55
		R: TCGAAGGGCTATGGTGCTAT	-	
722	(ESTs)SSRY83	F: TGGACCTGTTTTCTTTTCCTG	-	52
		R: GCACCCACCACTGAAGTACA	-	
723	(ESTs)SSRY84	F: GTGGCAGAGTCTGTCGCTTT	-	55
		R: TTTCTACTCTCCGGGCATTG	-	
724	(ESTs)SSRY85	F: CGTGGATTCTGGGGATAAAG	-	55
		R: GCAAATCATAGCCGCTCATT	-	
725	(ESTs)SSRY86	F: GAGCGTGGATTCCCTTAAAA	-	55
		R: CATGGGTTCAACTCTGACGA	-	
726	(ESTs)SSRY87	F: CAATTGCGAAGACTGCAAAG	-	55
		R: ACAGCACAGGTTGAAGGAGA	-	
727	(ESTs)SSRY88	F: CACGCAGCCTCTTCTCTCAC	-	55
		R: TCAAGAGATCGTTGTCAGCAA	-	
728	(ESTs)SSRY89	F: GCCATTGATTATGCATAGGATG	-	55
		R: GCCTAGGCCTGTATGGAACA	-	
729	(ESTs)SSRY90	F: GAGCCGGAAAAGTTCACAAA	-	55
		R: GAGTTTCTGGGGGAGGAAAG	-	
730	(ESTs)SSRY91	F: TGGAGAGGCCATTGCTAGTC	-	55
		R: ATGCACAAAAGAAATTTCCAA	-	
731	(ESTs)SSRY92	F: GGAAATTTCAATTCCAGACAGG	-	55
		R: ACGGCGGCACACTAAATATC	-	
732	(ESTs)SSRY93	F: CAGGAAACCCGTGACTTCAT	-	55
		R: GCAATGCACATGATTTTGCT	-	
733	(ESTs)SSRY94	F: TCTGCTTTTGGCTGGAATTT	-	55
		R: TTTCCACAAGCATTCCAACA	-	

S/No	Name	Sequence	Product size	Annealing temperature(°C)
734	(ESTs)SSRY95	F: CCAACATGCTTCACCAATCA	-	55
		R: CATTCAGACAACGCAATGTG	-	
735	(ESTs)SSRY96	F: GCATTCTTCCATGACGTTGTT	-	55
		R: CCTCCGTAAACTCAAAGCAA	-	
736	(ESTs)SSRY97	F: GAGACATTTTGGTGGGTGCT	-	55
		R: CACCTTTGTTTTCCCAGCTT	-	
737	(ESTs)SSRY98	F: CCTCAAGCAAAGCAAAGGAC	-	55
		R: CGGAGTGCTCCTCTCCATTA	-	
738	(ESTs)SSRY99	F: TGCCCTTTTGGCAAACTAAC	-	55
		R: TCCTCCTGGCTGATGAGTCT	-	
739	(ESTs)SSRY100	F: GCGCTTTACAGGCGTTTTTA	-	55
		R: GGTCTTTGCTCCGTCGTTAC	-	
740	(ESTs)SSRY101	F: AACTCGCGCCAAATACAAAC	-	55
		R: CTGCTCTCTCACCCCAGAGT	-	
741	(ESTs)SSRY102	F: GCTTGCTCAGCCTTGAGTATT	-	55
		R: TTGCCATGGCTACAAAAGAA	-	
742	(ESTs)SSRY103	F: GCAGCATGGAAATTTTAAGCA	-	55
		R: GAAATCGGCAGGAGATTGAA	-	
743	(ESTs)SSRY104	F: ATATGCAAGGGCAAGCAAAG	-	55
		R: AGCTAGACAAAGCAGCTCGT	-	
744	(ESTs)SSRY105	F: CCAAAATTATCTGCCATTGCT	-	55
		R: TCATTTCAGATGCACTCAACTCT	-	
745	(ESTs)SSRY106	F: TGTGGTCGATGAACAAGATA	-	55
		R: GGAAGCTTCTAAACTGGTCA	-	
746	(ESTs)SSRY107	F: TCGTATGAAAGAGACACGAA	-	55
		R: TGCTTGATCAACTGAATGTC	-	
747	(ESTs)SSRY108	F: GGAACTCTTTCGCTATCTGA	-	52
		R: CGCTACTTTCTGTCTCAACC	-	
748	(ESTs)SSRY109	F: TCAGCTTGCAATGTATGAAG	-	55
		R: AAGTGGGTGGACATTAGAAA	-	
749	(ESTs)SSRY110	F: CGAGAGAAGTGGTACAGAGG	-	55
		R: AAGATCTCGAGTCTGACGAA	-	
750	(ESTs)SSRY111	F: GCAGAAGCAATCTACTCTGG	-	55
		R: GCCTCAAAACATGGGTAATA	-	

751	(ESTs)SSRY112	F: TCTTCTCTTCCAAAGTCTGC	-	55
		R: TAGAAGGGGAATCAAGAACA	-	
752	(ESTs)SSRY113	F: CCAGTTTGTAAATCCCAAAG	-	55
		R: GCAGCTTCAAATGTCTCC	-	
753	(ESTs)SSRY114	F: AGTATCAGCGAAAACCCATA	-	55
		R: GTGCTCATTCTTTCTGGTTC	-	
754	(ESTs)SSRY115	F: AGCACAGGTTTCATGCTAAT	-	55
		R: TGGACTTGAGGAAGAGACAT	-	
755	(ESTs)SSRY116	F: CTGAAGTGCACAGTCAAGAA	-	55

S/No	Name	Sequence	Product size	Annealing temperature(°C)
		R: CTGAAGTGCACAGTCAAGAA	-	
756	(ESTs)SSRY117	F: CAGAGAGAGAGCCTGAAAGA	-	55
		R: CAGAGCTCCGTAGCTTATGT	-	
757	(ESTs)SSRY154	F: CTTGCTTCAAAGGGTCAATA	-	55
		R: CTTCTGCTGAGAACATCTCC	-	
758	(ESTs)SSRY179	F: CCTTTCCACTTCCATTAACA	-	55
		R: CAAGATcttTGGTTGGTCAT	-	
759	(ESTs)SSRY190	F: TCTAACGTACGGAGTCGTTT	-	55
		R: TGAAGAGAAAGCCAAAGAAG	-	
760	(ESTs)SSRY194	F: CTCTGCCTTTTTCTCTCAAG	-	55
		R: CCCCATACTGTAACTCTTGG	-	
761	(ESTs)SSRY209	F: GCAATGGAAGAAGTTGAGAG	-	55
		R: GAGTCAAGTCTCTTTCATGATTAGG	-	
762	(ESTs)SSRY227	F: CATACAGAAACGGAGAGGTC	-	55
		R: CCTGACTTTCCATCAAAAAC	-	
763	(ESTs)SSRY230	F: ACGCAGAATTCATACTTTCG	-	55
		R: CTTCGGCTATTTCTACCAGA	-	
764	(ESTs)SSRY232	F: TCTCTCTTTCTTTCCCTCAA	-	55
		R: ATCCTTCTCATTCTCAAGCA	-	
765	(ESTs)SSRY235	F: TCCTCTAACGAGTCGAGAAG	-	55
		R: CACAAATGGTGAAGACACAG	-	
766	(ESTs)SSRY236	F: ACAAGGTAGACAAAGGCAGA	-	55
		R: TAATCACCATACCCACCTTC	-	
767	(ESTs)SSRY237	F:GAATCCTCCATCAATTTCAG	-	55
		R: ACCATTGACATCAACCTTGT	-	

768	(ESTs)SSRY238	F: GAGCAACAATTTCTCTGAGG	-	55
		R: GAATTGCCATTATCTCTTGC	-	
769	(ESTs)SSRY239	F: GCAACACCTCTCAAGAAAGA	-	55
		R: TAGGCGAAGCCTAAAAGTAG	-	
770	(ESTs)SSRY244	F: AATGAACGGTCAGAATCTTG	-	55
		R: ATTTCTCCAGTTCCCTTCAG	-	
771	(ESTs)SSRY245	F: TTACTGTTGAGGGATTTGCT	-	55
		R: ATGGTTTGaCATCCAttagC	-	
772	(ESTs)SSRY247	F: ACATCCATGGAAGCAGATAG	-	55
		R: GCCCAATAGAAACAAACAGA	-	
773	(ESTs)SSRY249	F: AGGAGAAAGGGAAGCGTA	-	55
		R: TTTCGAGGAAAGTAACGAAG	-	
774	(ESTs)SSRY250	F: ACAGCAACAGCGTTTAATTC	-	55

S/No	Name	Sequence	Product size	Annealing temperature(°C)
		R: CTTTCCTCCACCTCTCTCTT	-	
775	(ESTs)SSRY253	F: GGTTTTGACTTTTGAGGTGA	-	55
		R: GCTTGTGTTCCACCTGTAGT	-	
776	(ESTs)SSRY258	F: CTTAATTGCGGACTTTTCAG	-	55
		R:ACCCTCCGAAGTTACATAAA	-	
777	(ESTs)SSRY259	F: GAAGCTTCCTTCAAATAGCA	-	55
		R: TGTCAAGATTGTAGGCAATG	-	
778	(ESTs)SSRY260	F: TTGACATCCCCTGTTTCTAC	-	55
		R: GGAAAGAAATGGGAATTAGC	-	
779	(ESTs)SSRY262	F: AAGAAATACAAGCAGGGTCA	-	55
		R: TGCATCAAATGGTACAGAGA	-	
780	(ESTs)SSRY264	F: TCCACTTCAAATCTTCTGCT	-	55
		R: CTCTTTGGTTCTGGAAAATG	-	
781	(ESTs)SSRY265	F: GCTCGTTCATTCATCTCACT	-	55
		R: TTAACTGTGAATGCCCTTCT	-	
782	(ESTs)SSRY266	F: CACGATCATCTAAACCAACC	-	55
		R: GTCATCAGAATCCTCCTCTG	-	
783	(ESTs)SSRY267	F: GAGCTGCTTCCCAAGATAAT	-	52
		R: TCTCCTTCAAAGCAGGTAAG	-	
784	(ESTs)SSRY268	F: AAGCAACTTGTTGGAGACAG	-	55
		R: ACATTCAGACAACGCAATG	-	

785	(ESTs)SSRY269	F: AAAGAAGAAGAAAGGGAAGC	-	55
		R: TTAAATGCTCTTCTCCAAGC	-	
786	(ESTs)SSRY270	F: CAACAACAAAGCTCTTCTCC	-	52
		R: TGATTCCTCGACTTTGCTAT	-	
787	(ESTs)SSRY271	F: GTGGTCTTGTTACTGCCATT	-	55
		R: CCGTATCAAAATCACTCGTT	-	
788	(ESTs)SSRY272	F: CACTCCTTTTCCACAAAACA	-	55
		R: TGTATACCGAGCCTTTGACT	-	
789	(ESTs)SSRY273	F: GGCAATACAGAAGAGGACAC	-	55
		R: CACAAAACCGAACACCATA	-	
790	(ESTs)SSRY274	F: CTGAGCTTCTCCTCCTCTTT	-	55
		R: AGTACGACAACCCACATCTC	-	
791	(ESTs)SSRY275	F: GCATAGCAGAGAGAAGAAGG	-	55
		R: CGAAGTGGATTTATGGGTAA	-	
792	(ESTs)SSRY276	F: AATGAGAGTGGGGTCTTTCT	-	55
		R: CATTTCTATGGAGGGTTCAA	-	
793	(ESTs)SSRY277	F: ATTGAATGGTGAGGATGTGT	-	55

S/No	Name	Sequence	Product size	Annealing temperature(°C)
		R: GAGTTTGACATTGCAGGTCT	-	
794	(ESTs)SSRY278	F: CTTTCTTTCTCTCTCTTTGTAGAGC	-	55
		R: GAAATCGGGGAAGAGGAG	-	
795	(ESTs)SSRY279	F: TACACTCAAATGGGTTTTCC	-	55
		R: GAGACCAGTGATTGTGGTTT	-	
796	(ESTs)SSRY280	F: GGTGAATTTTGGGGTTAGAT	-	55
		R: AGAAGCGGATTGTAAGATCA	-	
797	(ESTs)SSRY281	F: ATGTGGTGGTGATGGTTACT	-	55
		R: AAGAACCTGTGGTTCCTTTT	-	
798	(ESTs)SSRY282	F: TGCTACCTCACCAAAGTTCT	-	55
		R: ACCTTctcAGTTTTctgACG	-	
799	(ESTs)SSRY283	F: GTCGCTGCTAGATCTTGACT	-	55
		R: ATCATCAGATCACCACCATC	-	
800	(ESTs)SSRY284	F: GAGAAGTTTTGCCACCATAG	-	55
		R: CTTTCCAAGCAAACTGGTAG	-	
801	(ESTs)SSRY285	F: TCTAGAGATCTGTTGCGACTC	-	55
		R: TTTGAATCCTCCAACACTTC	-	

802	(ESTs)SSRY286	F: ATATCAATTCTCCCACCACA	-	55
		R: GATCGACATctaATTGCATc	-	
803	(ESTs)SSRY287	F: GAGAACTTGGCAACACACTT	-	55
		R: AGAAACCCCTAGAGTGAAGG	-	
804	(ESTs)SSRY288	F: TGGTACTGAATCTCCTGGAC	-	55
		R: aCGATTGTCCATTCTTTGTG	-	
805	(ESTs)SSRY289	F: CTTTAAAGACGCGAGAACTG	-	55
		R: CACCTCCGAAATCACTAAAA	-	
806	(ESTs)SSRY290	F: TCATCTCTCACAGGAACACA	-	55
		R: TGAAGAAGGAAATCAACACC	-	
807	(ESTs)SSRY291	F: ATCAGTGGATCAGTTTAGGG	-	55
		R: AATCCTGGTACTCCACAATG	-	
808	(ESTs)SSRY292	F: TCTCTCTTCCTTGAACTCTCC	-	55
		R: CATCATAAACTGGTGAGACG	-	
809	(ESTs)SSRY293	F: ATCTCCAGGCTCTCCTGCT	-	55
		R: CAACTTGCAACTCTTGTTCA	-	
810	(ESTs)SSRY294	F: TCCTCTCAATCCCTACCTCT	-	55
		R: TTATCGGGCCATATGTTATC	-	
811	(ESTs)SSRY295	F: CTTCAAGCTCACAAAACACA	-	55
		R: CACGGTAGAAAGACCATAGC	-	
812	(ESTs)SSRY296	F: AGATCACAAGGATCACAAGG	-	55

S/No	Name	Sequence	Product size	Annealing temperature(°C)
		R: GCAGTTGTCAAACACTAGCA	-	
813	(ESTs)SSRY297	F: TTATTTTCCCACTCACCAAC	-	55
		R: GACTCATCATTGGCAGAGTT	-	
814	(ESTs)SSRY298	F: CAGCCAGAGTCTCTTCACTC	-	55
		R: CGATTGTGTAAGGGAGAGAT	-	
815	(ESTs)SSRY299	F: GGACCTGTCCCTCTCTATTC	-	55
		R: CCCTCAATACTGCTCGATAG	-	
816	(ESTs)SSRY300	F: AACttggGTAAATCAActgg	-	55
		R: GGATTAGAGCAGACATTTGG	-	
817	(ESTs)SSRY301	F: GTTGTCCTCCAAAGATCGTA	-	55
		R: GAAAGTAGTCCACCCATCAA	-	