Sveriges lantbruksuniversitet
Swedish University of Agricultural Sciences

Faculty of Landscape Architecture, Horticulture and Crop Production Science

Evaluation of the effects of the waxy starch mutation and environment on yield and starch functional properties of cassava

Amanda Karlström

Master's thesis, Horticultural Science Program, Alnarp 2015

Evaluation of the effects of the waxy starch mutation and environment on yield and starch functional properties of cassava

Effekter av waxy mutationen och miljö på avkastning och stärkelseegenskaper i kassava

Amanda Karlström

Supervisor: Mulatu Geleta Dida, Swedish University of Agricultural Sciences (SLU), Department of Plant Breeding.

Co-Supervisor: Hernán Ceballos, International Center for Tropical Agriculture (Centro Internacional de Agricultura Tropical; CIAT), Cassava breeder, Cassava Program.

Examiner: Tomas Bryngelsson, Swedish University of Agricultural Sciences (SLU), Department of Plant Breeding.

Credits: 30 hec
Level: A2E
Course title: Degree project for MSc Thesis in Horticulture
Course code: EX0544
Subject: Biology
Programme/education: Horticultural Science Program

Place of publication: Alnarp
Year of publication: 2015
Cover art: Cassava roots, Amanda Karlström
Online publication: http://stud.epsilon.slu.se

Keywords: cassava, environment, functional properties, starch, yield penalty, waxy

Sveriges lantbruksuniversitet
Swedish University of Agricultural Sciences

Faculty of Landscape Architecture, Horticulture and Crop Production Science
Department of Plant Breeding

Abstract

Cassava roots are one of the most important sources of starch on a global scale. Two aspects of cassava as a starch crop were studied in this thesis work; 1) determining if the amylose-free (waxy) mutation is coupled with a reduction in yield, and 2) the environmental impact on yield, yield components and starch functional properties of cassava. The waxy trait, resulting from a mutation in the *GBSS* locus, is in several other crops associated with a yield penalty. In this study, siblings from eight full-sibling families, segregating for the waxy trait, were used to determine if the waxy mutation has implications for yield, dry matter content and harvest index in cassava. The only significant effect of starch type was on the dry matter content, with the waxy clones having a 0.8% lower dry matter than their wild type siblings. The environmental effects on cassava yield and starch functional properties were examined by comparing data from two contrasting environments of Colombia; the Cauca river valley and the Caribbean coast. Significant differences were found in yield, dry matter content and harvest index between cassava clones from different environments. There were significant effects of environment on starch pasting properties, solubility and dispersed volume fraction. Starches from the higher temperature, Caribbean coast had an elevated pasting temperature (5°C higher for waxy starches and 3.6° C for wild type), whereas the peak viscosity was lower in starches from this environment (114 cP lower in waxy starch and 205 cP in wild type). The gravimetric method for determining dry matter content in cassava roots was also evaluated.

Abbreviations

BD	Breakdown
CIAT	Centro Internacional de Agricultura Tropical (International Center for Tropical Agriculture)
CPV	Cold paste viscosity
CS	Consistency
DMC	Dry matter content
FRY	Fresh root yield
GBSS	Granule-bound Starch Synthase
HI	Harvest index
HPV	Hot paste viscosity
PT	Pasting temperature
PV	Peak viscosity
SB	Setback

Table of contents

1. Introduction

Cassava, *Manihot esculenta* Crantz,, is a perennial, woody shrub originating from South-America which is mainly cultivated for its tuberous, carbohydrate-rich roots (Piperno and Holst, 1998). It is the second largest source of starch worldwide, after maize, and the largest starch-commodity in international trade (Aiemnaka *et al.,* 2012). The cassava plant is tolerant to drought, low fertility and acidic soils. Cultivation of the crop is characterized by small-scale farming on marginal agricultural lands.

The International Center for Tropical Agriculture (CIAT) have specifically aimed to develop high-value cassava clones, with root characteristics that fit the needs of specific industries. This is done with the incentive to benefit cassava farmers by strengthening markets where the farmers can sell their produce at a better price. Cassava roots with novel starch properties are of interest for the starch industry, and one such novel property are roots containing amylose-free, so called waxy, starch. The lack of amylose in the starch is the result of a recessive mutation in the waxy locus encoding Granule-bound starch synthase (GBSS), the protein responsible for the elongation of amylose. In 2006 a cassava clone carrying the waxy mutation was discovered at CIAT, Palmira and this clone has since been used in the development of commercial waxy cassava varieties (Ceballos *et al.,* 2007). Waxy mutants are also found in other crops such as maize, barley, amaranth, wheat, rice, potato and sorghum (Jampala *et al.,* 2012; Jobling, 2004; Graybosch, 1998, Oscarsson *et al*., 1998; Sanchez *et al.,* 2010). Compared to their wild-type counterparts, waxy starches generally show a higher swelling power, higher peak viscosity and a better freeze-thaw stability (Jobling, 2004; Sanchez *et al.,* 2010). However, the waxy trait have been associated with reductions in yield in several crops, with a yield reduction in maize estimated to around 5% (Oscarsson *et al.,* 1998; Fergason, 2001; Rooney *et al.,* 2005). One of the aims of this thesis is to determine if the waxy trait in cassava is coupled with a yield penalty.

The potential applications of starch is largely influenced by its functional properties, e.g. swelling power, clarity of the starch paste, temperature at which gelatinization is initiated and the maximum viscosity of the starch gel. These properties are affected by the composition and structure of the starch granules, which varies between plant sources (Jobling, 2004). However, the functional properties of a starch can also vary within the same plant species and variety due to environmental factors. Factors which have been shown to affect the functional properties of starch include temperature, precipitation and planting date (Tester and Karkalas, 2001)**.** The end users of a starch, be it waxy or wild type, select starch based on its suitability for their purposes. Differences in functional properties of the starch due to environmental growth conditions can therefore have implications on its end use. Another aim for this study therefore is to compare functional properties of waxy and wild type cassava clones grown in two different locations in Colombia.

Cassava generally exhibits a high degree of environment by genotype interaction, which makes locating breeding trials in the ecological region for which a variety is targeted of increasing importance (Ceballos *et al.,* 2012). Root yield and high dry matter content are important breeding objectives for the majority of cassava end uses. The root yield of cassava clones in early trials is, however, poorly related to their performance in later trials. Therefore harvest index (root yield/ total biomass) is commonly used as an indirect selection criterion for yield during early trials (Kawano, 2003). In this study, data on root yield, dry matter content and harvest index was collected from the waxy clones grown in two locations in Colombia, in order to evaluate the effect of environment on these traits.

2. Objectives

This thesis work was conducted with three main objectives:

- To determine if the amylose-free (waxy) trait in cassava is associated with a yield penalty, by measuring yield properties in full-siblings from eight cassava families segregating for the mutation at the GBSS locus.

- To examine the environmental effects on yield and starch properties of cassava, through comparing data from two contrasting locations in Colombia.

- To assess the reliability of the gravimetric method as a methodology for determining the dry matter content in cassava roots, through a comparison of dry matter contents estimated by the gravimetric method and oven method.

3. Background

3.1 Cassava

Cassava is of large importance as a staple food and energy source in tropical and sub-tropical areas of the world. It is mainly the roots of the plants which are utilized. The roots have an approximate dry matter content of 34%. Around 85% of the root dry matter is constituted of starch (Sánchez *et al,* 2009). The roots are commonly consumed after some form of processing (Montagnac *et al.,* 2009). In 2013 the global production of cassava roots was 277 million tonnes, with yields ranging from 1.3 tonnes/ha in Burkina Faso to 35 tonnes/ha in India (Faostat, 2015). The major regions of production can be seen in Figure 1.

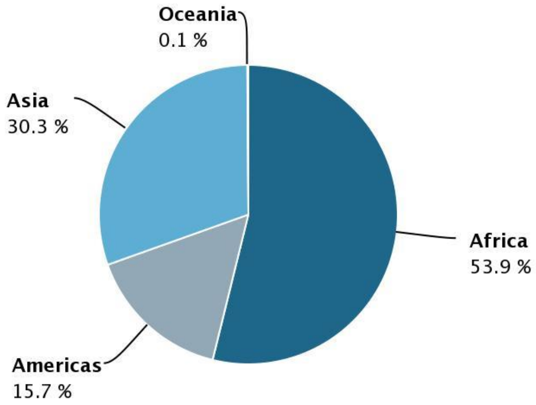

Figure 1. Average production share of cassava roots for the four cassava producing regions during the years 1993-2013. Graphics from Faostat, 2015

Cassava is monoecious and hence predominantly cross-pollinated, and hybrids are obtained either by controlled pollination between two parents or by open pollination, where only the female parent is known. Cassava is propagated vegetatively by stem cuttings, with one plant producing an average of 6-10 cuttings. The stem cuttings are optimally taken at an age of 8-18 months. The propagation method is bulky and the time required to obtain planting material for replicated trials is long, due to the slow replication rate. One selection cycle (from crossing to multiple site trials) typically requires more than five years (Ceballos *et al*., 2012).

According to Ceballos (2012**)** there are six major factors contributing to slow cassava improvements. 1) There has been little influence of technology developed in temperate regions, as seen in maize and other cereals, since cassava is solely a tropical crop.

2) Few varieties have been developed exclusively for industrial purposes. This results in an unreliable supply for the industries, and varieties which are suboptimal for both industry and human consumption. 3) The genetic improvement of cassava is slow due to a long growth cycle and low reproduction rate of planting material. 4) Little investment has been allocated to cassava from governments of low-income countries in the subtropical and tropical region, compared to other crops e.g. cereals. 5) High transportation costs due to large root bulk weight and rapid perishability of roots. 6) A catch 22 situation where there is limited development of industrial markets due to unreliable supply of roots and a low production of roots because of the lack of industrial markets. The cassava program at CIAT are actively working with improving cassava development by selecting cultivars that are specifically aimed at meeting various industrial end-uses, improving the effectiveness of the selection and shortening the selection cycle and by strengthening cassava markets and creating new ones.

3.2 Starch

Starch is a carbohydrate present in various plant tissues and organs of almost all green plants. Globally, the major starch crops are maize, cassava, sweet-potato, potato and wheat (Fuglie *et al.,* 2006**).** It is deposited in starch granules, which in higher plants are synthesized in plastids in both photosynthetic and non-photosynthetic cells (Zeeman *et al.,* 2010). In the photosynthetic tissues the starch granules are formed in the chloroplast during the light hours as a result of carbon fixation. The starch is then degraded during the dark hours and used for leaf transpiration and for sucrose synthesis which is then transported to sink tissues. This foliar starch is termed transitory starch because of the diurnal pattern of build-up and decrease. Starch is also produced in storage tissues (e.g. tubers, roots, fruits or seeds) within specialized plastids (amyloplasts). The degradation of starch in such storage organs provide a source of carbon and energy during seed germination, tuber sprouting, fruit ripening etc. (Preiss, 2004; Bahaji *et al.,* 2014).

Starch granules are composed of two glucose polymers; amylopectin and amylose. Amylopectin is the major starch component, constituting around 70-80% of the granule (Buléon *et al*., 1998). It is a branched molecule made up of glucosyl residues linked end-to-end by α-1,4-glucosidic bonds. The branched structure of amylopectin is because 5% of its glucose units are joined by α-1,6-glucosidic linkages**.** The starch granule also contains around 20-30% of amylose; a smaller, linear molecule in which all glucose units are linked by α-1,4-bonds (Jobling 2004, Zeeman *et al.,* 2010). The differences found in structural organization and physiochemical properties between amylose and amylopectin is largely due to dissimilarities in branching level (Delvalle *et al.,* 2005). The chain-lengths and level of branching of the polymers are highly dependent on the botanical origin of the starch (Tester and Karkalas, 2001).

The starch granule has a lamellar structure due to the organization of amylopectin within the starch granule. The linear parts of the amylopectin form parallel double helices which are packed together in arrays resulting in the crystalline lamellae which are seen in the starch granule. The amylopectin regions which contain branched α-1,6-linkages form alternating, amorphous lamellae (Zeeman *et al.,* 2010). Starch granules also exhibit higher order organization with alternating amorphous and semi-crystalline (from crystalline and amorphous lamellae) zones, resulting in what looks like concentric growth rings. The localization of amylose within the starch granule has not been properly established, but is believed to be within the amorphous regions of the granule (Zeeman *et al.,* 2010). The internal structure of the starch granule is not homogenous but has been shown to vary in amylopectin and amylose composition (Jane, 2006).

Starch granules from different plant sources differ in size, shape, structure and composition, thus leading to differences in response and behaviour when the starches are heated and thereafter cooled (Jane *et al.,* 1999; Peroni *et al.,* 2006). Characteristics such as the degree of packing of amylopectin helices, level of amylopectin branching, size of starch granules and amylose content have impact on the properties of the starch (Peroni *et al.,* 2006).

3.2.1 Starch synthesis

In the initial stage of starch synthesis the glucosyl-residue from an ADP-glucose is added to a pre-existing α-1,4-bound glucose unit**,** both in the synthesis of amylopectin and amylose (Ohdan *et al.,* 2005). The formation of the ADP-glucose (from glucose-1-phophate and ATP) is catalyzed by the enzyme ADP-glucose pyrophosphorylase in either the cytosol, in cereal endosperms, or inside the plastid, in non-cereal storage organs (James *et al.,* 2003). The elongation of the α-1,4-linked glucans are performed by either soluble starch synthases (SS) or a granule bound starch synthase (GBSS) for amylopectin and amylose respectively (Ohdan *et al.,* 2005). There are several isoforms of soluble starch synthases, assembled into four groups according to their sequence homology; SSI, SSII, SSIII, SSIV. The different isoforms have distinct functions in starch formation. The SS isoforms are either soluble in the stroma of the plastid or partly soluble and found in association with the starch granule. Mutants in these isoforms show varying phenotypes with alterations in starch content, starch morphology and, most commonly, in amylopectin chain length distribution (Zeeman *et al.,* 2010; Brust *et al.,* 2014). GBSSI is in contrast to the soluble SS only found in the starch granule, and is responsible for the elongation of the very long glucan chains found in amylose (Wattebled *et al.,* 2002). Mutation or down-regulation of the *Waxy* gene which encodes GBSSI has in many plant species been shown to diminish the amylose content in starch (Okagaki *et al.,* 1991; Muth *et al.,* 2002; Raemakers *et al.,* 2005). In a recent study by Seung *et al* (2015) Arabidopsis plants mutant for the Protein Targeting to Starch enzyme (PTST) showed phenotypes lacking amylose. The role of

PTST in amylose synthesis was shown in this study to be localization of GBSS to the starch granule.

The branched nature of the amylopectin is the result of the activity of the starch-branching-enzyme (SBE) which hydrolyses α-1,4-bonds and transfers the resulting short oligosaccharide to an already existing α-1,4-glucan chain by a α-1,6-linkage (Tester and Karkalas, 2001).

3.2.2 Starch functional properties

As mentioned above, the properties of starch varies between plant sources and is dependent on its physiochemical characteristics. A large part of the starch properties which are important for its utilization is related to the behaviour when heated in water. One key characteristic is the pasting temperature, at which the starch granules start swelling due to diffusion of water into the starch granule and the uncoiling and disassociation of amylopectin helices. This increases the viscosity of the water-starch solution. During the swelling of the granules carbohydrates are leached, primarily amylose but at higher temperatures also amylopectin is lost. If the starch is further heated, viscosity will reach a peak, where after the starch granules will disassociate, become solubilized and loose viscosity. If the starch solution is cooled, the linear starch polymers will aggregate and form a gel (Jobling, 2004). This transition is referred to as the gelatinization of starch. The extent of swelling and amount of carbohydrates solubilized are dependent on heating temperature and the interaction between the carbohydrate chains in the amorphous and crystalline regions of the starch granule. This interaction is influenced by the amylose: amylopectin ratio and distribution, degree and length of branching and conformation of amylose and amylopectin chains (Mat Hashim *et al.,* 1992; Hoover, 2001; Jobling, 2004).

3.2.3 Industrial applications of starch

Starch has a large variety of uses in a wide range of industries. In the food industry it has multiple applications as a food additive, e.g. as emulsifier, stabilizer and glazing or thickening agent. These food additives are used in products such as ready-made soups and sauces, desserts, ice-creams, processed meat and bakery products (Fuglie *et al.,* 2006). The sweetener industry is the largest user of starch globally, while the production of food products constitutes the largest utilization in low-income countries (Fuglie *et al.,* 2006). In addition to being a food and feed source, starch is also used extensively within the industrial production of adhesives, agrochemicals, cosmetic, detergent, paper, plastic, textile, pharmaceuticals and other medical appliances (Ellis *et al.,* 1998).The general trend is that non-food industries increase their share of starch utilization as a country's economy develops and incomes grow (Fuglie *et al.,* 2006).

Starch in its original form only has a few applications, mainly as a thickener or binder. However, the pasting of starch alters its properties which broaden the number of end uses. Starch physiochemical properties which influence the gelatinization are hence of importance for their industrial end-use. Since the physical characteristics, biodegradability and amount and nature of non-carbohydrate constituents varies in starches from different plant sources, they are suited for various applications (Ellis *et al.,* 1998). One example is tuber and root starches, which when processed, form clear pastes with a bland taste, making them suitable for the food industry (Jobling, 2004). Cereal starches are rich in lipids which can influence the starch quality negatively in several ways, e.g. by decreasing water-binding capacity (and thereby the swelling and solubilization), by oxidation which forms undesirable flavours and by rendering starch films and pastes opaque or cloudy (Swinkels, 1985).

3.2.4 Influence of environment on starch properties

The influence of genetic factors on starch properties has been well-studied in many plant species (Zeng *et al.,* 1997; Bogracheva *et al,* 1999; Collado *et al.,* 1999; Beta *et al.,* 2001; Kharabian-Masouleh *et al.,* 2012**).** However, environmental factors have also been reported to affect starch characteristics **(**Hizukuri, 1969; Tester, 1997; Tester *et al.,* 1999; Graybosch *et al.,* 2003; Bao *et al.,* 2004). The environmental factor which has been most widely studied for its impact on starch is growth temperature. The amylose content of various crops has been shown to be affected by growth temperature. In sweet-potato elevated soil -temperatures were shown to increase amylose levels, while higher ambient temperatures decreased it in rice and maize (Hizukuri, 1969; Noda *et al.*, 2001; Tester and Karkalas, 2001). In barley and potato the effect of temperature on amylose content seem to be variable (Hizukuri, 1969; Tester *et al.,* 1997; Tester, 1999). Growth temperatures have also been reported to affect lipid and phosphorous contents of starch (Tester and Karkalas, 2001). Temperature seem to have a consistent effect when it comes to the onset of gelatinization, with a positive correlation between gelatinization temperature and growth temperature reported for rice, wheat, barley and potato (Tester and Karkalas, 2001). Pasting temperatures of soybean, rice, potato and sweet potato were also shown to increase when grown at higher temperatures (Hizukuri, 1969; Noda *et al.,* 2001).

Starch properties have also been studied in relation to crop water supply, although to a lesser extent than temperature. Santisopasri *et al* (2001) studied how cassava starch was affected when plants were exposed to water-stress during the first six months of growth. They found that starch granules from water-stressed plants were smaller, had lower peak-viscosities and higher pasting and gelatinization temperatures. In addition, the swelling power of the starch was reduced after initial water-stress. Water availability during harvest has also been proposed to influence the granule structure and thermal properties of cassava starch (Sriroth *et al.,* 1999; Chatakanonda *et al.,* 2003). The effects of water stress and water management regimes on starch characteristics have also been studied in sorghum and wheat (Taylor *et al.,* 1997; Singh *et al.,* 2008)

3.3 Waxy starches

In a paper published by Collins (1909), a type of maize with a new appearance in the kernel endosperm was described. This maize, which was found in China, had an endosperm which Collins depicted in the following way; "*Instead of being translucent it (the endosperm, authors comm.) is completely opaque, though not in the least approaching the coarse opaque texture of the amylaceous endosperm. The texture suggests that of the hardest waxes, though it is still harder and more crystalline. From this optical resemblance to wax the term cereous or waxy endosperm is suggested*". Later, the appearance of these waxy kernels was explained by their starch composition, which was solely constituted of amylopectin (Eriksson, 1969). The waxy maize starch has characteristics resembling those of tapioca (cassava) starch, a product which was imported in large quantities from South East Asia into the United States until the World War II. Due to the war, tapioca import became impossible and the production of waxy maize expanded in the United States in order to provide starch for industrial applications such as envelope seal gums, remoistening glues and certain food products (Schopmeyer *et al.,* 1943). Waxy starch varieties are now found in both monocots and dicots, including; barley, wheat, rice (often referred to as glutinous rice), sorghum, potato and cassava (Eriksson, 1969; Yamanaka *et al.,* 2004; Ceballos *et al.,* 2007, Muth *et al.,* 2008). The molecular basis for the waxy characteristic of several plant species has been assigned to a mutation in the *Waxy* (*Wx*) gene, encoding GBSS I (Fukunaga *et al.,* 2002; Larkin and Park, 2003; McIntyre *et al.,* 2008). The mutation of the gene has been described to be due to transposable elements, nucleotide substitutions, or sequence insertions or deletions (Fukunaga *et al.,* 2002; Aiemanaka *et al.,* 2012)

Waxy plant tissues are easily detected with iodine since amylose-containing starch binds to iodine in solution which results in a blue staining. The shorter amylopectin chains have a lower iodine-binding ability and waxy starch is therefore stained red-brown by iodine (Denyer *et al.,* 2001).

Waxy starch is of interest to the starch industry due to its functional properties. Compared to wild-type starches, waxy starches generally exhibit a larger swelling power and a higher peak viscosity**.** They have also been reported to have a higher freeze-thaw stability (Takeiti *et al.,* 2007; Sanchez *et al.,* 2010). Thus, the waxy starches have several applications. Waxy or partially waxy wheat are suited for the production of Japanese udon noodles (Graybosch, 1998, Van Hung *et al.,* 2006), whereas waxy maize starch is used as stabilizer and thickener in food products and as an emulsifier in salad dressings (Jobling, 2004).

Waxy starch from wheat has been shown to be more susceptible to mechanical damage when compared to wild-type wheat starch. This has implications on the applications of the starch since damaged starch granules absorb more water and are more susceptible to degradation by α-amylase. These characteristics can be both advantageous and problematic depending on the end use of the starch (Bettge *et al.,* 2000).

3.3.1 Waxy cassava starch

Cassava is the second most important source of starch, after maize, in the world. Nevertheless, cassava starch is a larger international trade commodity, since most maize starch is produced and consumed in the United States (Aiemnaka *et al.,* 2012). In South and South-East Asia cassava is the major starch source and 40% of the cassava produced in the region is used for starch extraction (Fuglie *et al.,* 2006). The cassava starch granules are 16.9-18.0 µm in size and have round to oval shape and low levels of lipids and phosphorous (Hoover*,* 2001; Gomand *et al.*, 2009).

The development of an amylose-free cassava cultivar has long been an objective for breeders and a request from the starch industry. It was first achieved in 2003 (Raemakers *et al*., 2003) through a down-regulation of the *GBSSI* gene, an achievement which has since been repeated (Raemakers *et al*., 2005, Zhao *et al.*, 2011). Field trials with such transgenic, low-amylose cassava have been conducted in Indonesia (Koehorst van Putten *et al.,* 2012). Around the year 2000, CIAT started screening the worldwide cassava germplasm collection for different starch traits, such as low amylose-content. The project did not, however, identify any clones with waxy characteristics (Sanchez *et al.,* 2009). In order to bring forward potential recessive traits, inbreeding was subsequently introduced into the cassava breeding program at CIAT. In 2006 the inbreeding resulted in the discovery of a cassava mutant with a reduced content of amylose and an absence of GBSS I in the root tissue (Ceballos *et al.,* 2007). Aiemanaka *et al* (2012) further showed that the waxy trait in cassava is recessively inherited through Mendelian segregation and suggested that the waxy genotype is due to an indel which causes a premature stop codon or frame-shift mutation in *GBSS I*. The authors also developed two SNP markers that can distinguish *WxWx, wxwx* and *Wxwx* genotypes.
The waxy trait has been introgressed into elite germplasm from CIAT and Thailand. Currently commercial waxy cultivars are being developed for Thailand, Colombia and Brazil (H. Ceballos, CIAT, pers. com.)

Compared to the wild-type cassava starch the waxy starch has a higher peak viscosity, a higher swelling index and a lower solubility (Ceballos *et al.,* 2007; Sanchez *et al.*, 2010). Sanchez *et al* (2010) also reported that freeze-thaw stability for the waxy cassava clone AM 206-5 was better than that of both waxy maize and waxy potato, but equal to the stability of waxy rice.

3.3.2 Yield penalty in waxy crops

Cultivars exhibiting the waxy trait have been associated with lower yields in various crops. Waxy maize hybrids reportedly yield up to 5% less than their non-waxy counterparts (Fergason, 2001). Sorghum waxy lines were on average shown to yield 17% less than the wild-type lines from the same segregating population (Rooney *et al.*, 2005). However, individual sorghum lines with waxy starch were found to be as high-yielding as those with wild-type starch (Rooney *et al.,* 2005; Jampala *et al.,* 2012). Oscarsson *et al* (1998) found that among several barley varieties, with varying content of amylose, protein and β-glucan, the two waxy varieties yielded less and had a lower starch content compared to all other varieties, except high-amylose ones. However, cultivars with different genetic backgrounds were used in this study, the lower yields can therefore not with certainty be assigned to the waxy mutation. In wheat, genotypes with the waxy trait have been shown to produce yields equal to genotypes with wildtype starch (Graybosch *et al.,* 2003). The underlying mechanisms of yield reduction in waxy genotypes have not been established but is believed to be due to a combination of pleiotropic effects, genetic linkage as well as relatively low breeding efforts for improvement of waxy cultivars (Rooney *et al.,* 2005).

4. Materials and methods

4.1 Plant material

The full-sib families (same father and same mother), segregating for the waxy starch trait, were produced by controlled crosses (hand pollinations) as described by Kawano in 1980. All crosses were performed at CIAT, Palmira. The waxy clones had been produced through pollinations between distantly related F1 plants, which were heterozygous for the *Waxy* allele. These F1 plants resulted from crosses between the waxy cultivar AM 206-5 and several elite clones.

The wild-type starch clones used in the environmental effect trial were part of advanced trials of the breeding program at CIAT.

4.2 Design of yield penalty trial

The effects of the waxy trait on yield and yield components were evaluated based on data from a field-trial conducted at CIAT experimental station, Palmira, Valle del Cauca, Colombia. Plants were harvested in May 2015, 11 months after planting. Eight full-sibling families with genotypes producing either waxy (*wx wx*) or wild type (*Wx wx, Wx Wx*) starch were included in the trial. Homozygote and heterozygote wild type plants were not distinguished. The family sizes varied and, therefore, each family was represented by a different number of genotypes (Table 1). However, for each family, an equal number of waxy and wild type genotypes were chosen. Instead of planting all genotypes from a certain family together, they were more or less equally split into three different sets (Table 1). Each set had a combination of about 1/3 of the genotypes from each of the eight families. One set contained a total of 30 waxy and 30 wild type genotypes (except set 3 which only had 27, and therefore 3 additional plants were added). The three sets of genotypes were replicated in four plots (Figure 2). This added up to a total of 360 waxy and 360 wildtype plants. In each plot, planting was done in six rows with five plants per row. The data collected at harvest was fresh root yield (FRY), harvest index (HI, root weight/ total weight of biomass including roots) and dry matter content (DMC). FRY and HI data were collected by bulking the five plants from each row and weighing them together, averages for each plot was then calculated. Also dry matter content was measured by bulking the roots from each row, with two replicated measurements per row (see determination of dry matter content for details). In order to determine that the plants of each plot represented the correct starch type, two roots from each row was sprayed with iodine solution.

Table 1. Distribution within sets of the waxy and wild type material randomly selected from each family

Cross	Number of waxy and wild type genotypes in sets		
	Set 1	Set 2	Set 3
GM 5458	3+3	3+3	4+4
GM 5466	5+5	5+5	5+5
GM 5507	3+3	3+3	2+2
GM 5536	3+3	3+3	2+2
GM 5615	3+3	3+3	2+2
GM 5619	3+3	3+3	2+2
GM 5672	5+5	5+5	5+5
GM 5722	5+5	5+5	5+5
Total	30+30	30+30	27 (+3 add. plants) + 27 (+ 3 add. plants)

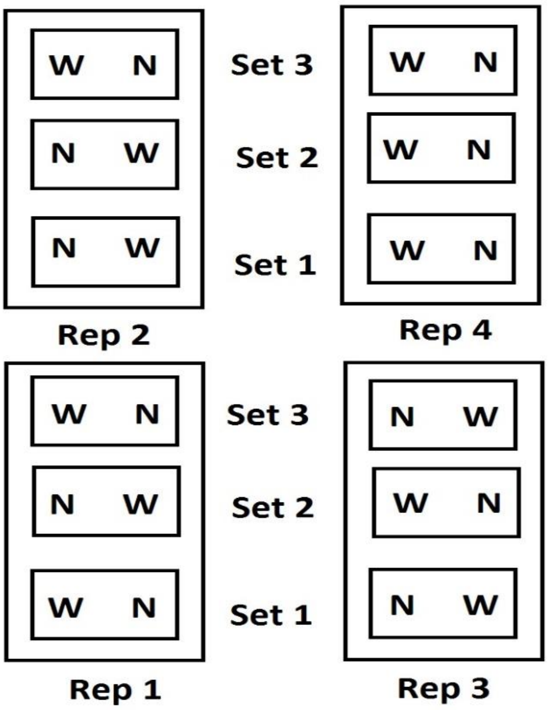

Figure 2. Experimental design for the yield penalty trial associated with the waxy trait in cassava. The same number of waxy (W) and wild-type (N) starch genotypes from eight families were divided into three sets with four replications.

4.3 Design of environmental effect trial

Environmental effects on yield was evaluated in 295 waxy clones, and the effects on starch properties in 40 clones, of which 30 were waxy starch genotypes and 10 were wild type starch genotypes. The clones were grown in three different locations of Colombia. The waxy genotypes were planted in Malambo, Barranquilla, Atlántico and at the CIAT experimental station, Palmira, Valle del Cauca, whereas wild-type starch genotypes were planted close to Sincelejo, Sucre and at CIAT, Palmira. Yield properties were only evaluated for the waxy genotypes. The two Departments Atlántico and Sucre are Caribbean coastal lowland areas characterized by high average temperatures and annual precipitations of (810 and 1100 mm respectively, see Figure 3 for average temperatures**).** Palmira is situated in the inter-Andean valley of the Cauca river. Compared to the climate of the Caribbean coast, Palmira has lower average temperatures and a lower annual precipitation of 1021 mm. Both of the two locations on the Caribbean coast are situated around sea level, whereas Palmira is positioned 1000 m above sea level. Soils of the Caribbean coastal region are dominated by gleysols and cambisols while the Cauca river valley is dominated by luvisols and cambisols (IIASA/FAO, 2015). The waxy cassava clones at Malambo and Palmira were harvested 11 months after planting in April, 2015. Similarily**,** the wild type starch genotypes were harvested 11 months after planting in June in Sucre and in July in Valle del Cauca

Harvest data on yield and yield components were collected from the 295 waxy genotypes grown in both Malambo and Palmira. Plot sizes and experimental design differed slightly between the two locations; plots with six plants per genotype were harvested in Malambo whereas only two plants per genotype were harvested in Palmira. Furthermore, the clones on the Caribbean coast were grown in three replicated plots, but only one replication was available in Palmira. This is because data from Malambo was taken from an advanced yield trial conducted for evaluation and selection based on agronomic performance, whereas the clones grown at the experimental station at CIAT only functioned as a back-up of planting material. Data collected at the harvest of the trials were on HI, FRY and DMC by the gravimetric method.

The starch functional properties analysed on the 40 genotypes were pasting characteristics, swelling power, solubility, dispersed volume fraction and clarity of gel.

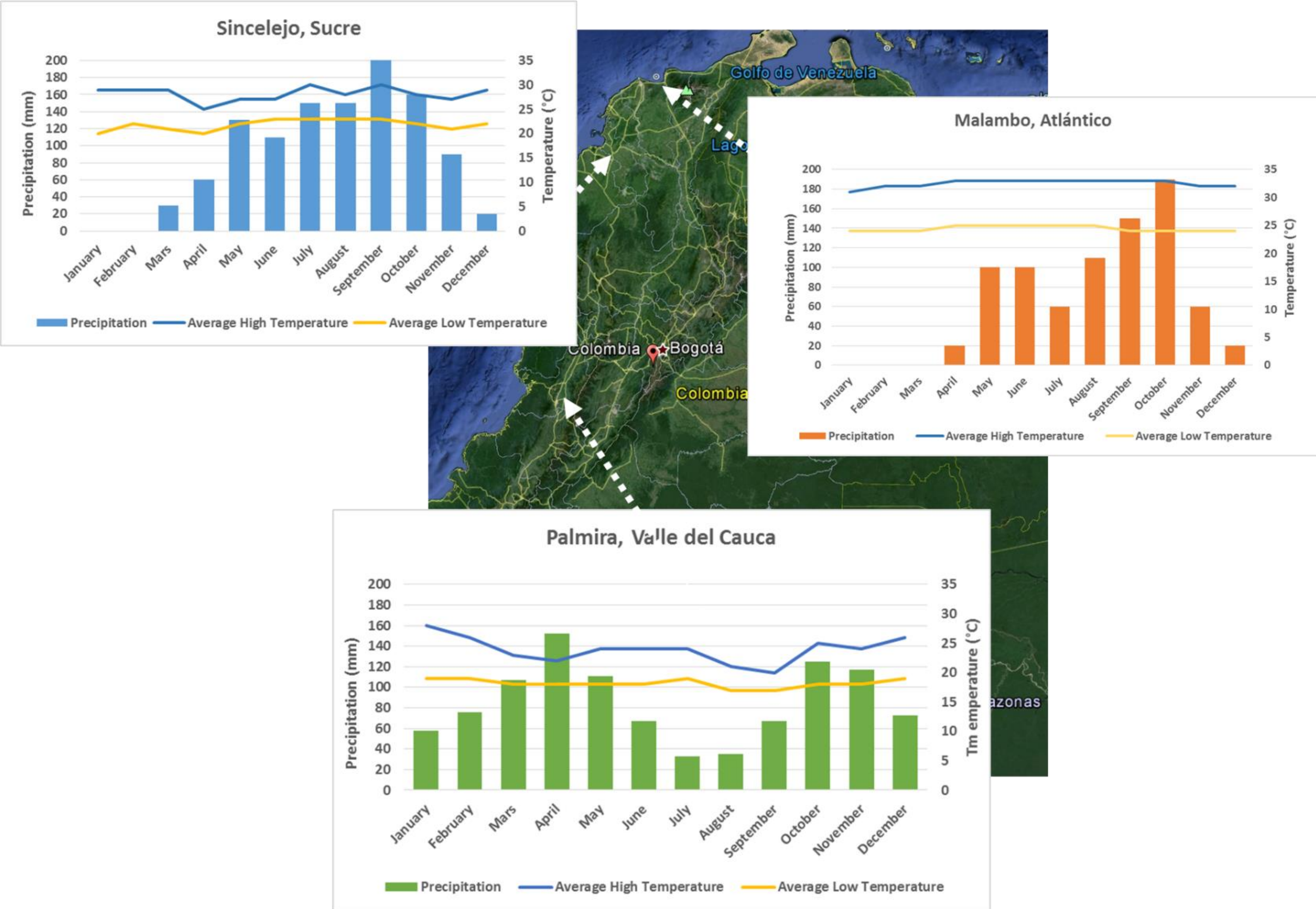

Figure 3. Map showing the three cassava planting locations with graphical presentation of the monthly precipitation and average high and low temperature at each location. Map from Google Earth (2015) and weather data from Weatherbase (2015)

4.4 Fresh root yield and harvest index

Fresh root yield (FRY) and Harvest index (HI) was measured shortly after the cassava plants were harvested. For fresh root yield, all roots from each plot was bulked and weighed together. The FRY, in tonnes per hectare, was then calculated based on the measured weight and planting distance. In adjusted FRY, the loss of productivity because of missing plants is accounted for**.** Roots with symptoms of rotting were discarded and not included in the measurement. Harvest index, was defined as the root weight / total weight of biomass, including roots. To determine HI, all above-ground plant material from the relevant plants was gathered on a jute cloth with straps and weighed together (see Figure 4).

Figure 4. Method of weighing above-ground plant material and roots

Figure 5. Appliances used to measure cassava root weight in air (right picture) and water (left picture) in order to calculate dry matter content.

4.5 Determination of dry matter content

The determination of dry matter content of the roots of all genotypes was conducted using the Specific Gravity Method. The method takes advantage of the relationship between the specific gravity of cassava roots and their dry matter content, and is easily utilized in the field by weighing the fresh weight of samples both in air and water (Toro and Cañas, 2012). Approximately 5 kg of fresh harvested roots from each genotype were collected in marked plastic bags and brought to a weighing station. Each bagged sample was put into a metal mesh basket tied to a cord and weighed with a balance suspended in the air. The same metal basket with roots was then submerged in a large container filled with water while the cord was connected to a balance (see Figure 5).

In order to calculate the dry matter content (in %) the specific gravity was first calculated using formula (1) and the dry matter using formula (2), as described in Toro and Cañas (2012).

$$(1) \qquad \text{Specific Gravity} = \frac{\text{Fresh weight air}}{(\text{Fresh weight air - Fresh weight water})}$$

$$(2) \qquad \%\ \text{dry matter} = (158.26 \times \text{Specific Gravity}) - 142.05$$

In order to verify the accuracy of the specific gravity method four to five roots from each waxy genotype used for the environment effect trial grown in Palmira were sent to the Roots Tubers and Bananas (RTB) quality laboratory, CIAT where dry matter content was measured by heating in oven. The equation which is currently used at CIAT to calculate DMC from specific gravity is taken from Toro and Cañas, 1983**.** Dry matter content from the specific gravity method was then compared to the oven method.

4.6 Starch analysis

Starch extraction and analysis were conducted on the roots of the 40 clones grown in the environmental effect trial. For these 40 genotypes approximately five kilograms of roots from both the Caribbean coast and the Cauca Valley was collected for starch extraction. The extraction and analysis of the starch were conducted in the RTB laboratory at CIAT, Palmira. Starch extraction was performed by laboratory staff using the procedure described in Sanchez *et al* (2010)**.**

Initially the dry matter content of the starch was measured by weighing approximately one gram of each sample in two replicates, followed by an overnight oven treatment at 105°C, which is then followed by measuring the weight of each sample of each replicate.

4.6.1 Pasting properties

Hot paste viscosity profiles of the starch samples were obtained with a Rapid Visco Analyzer model RVA-4 series (Newport Scientific, Australia). Starch suspensions of 5% on dry basis (db) were prepared (1.25 g db starch in 23.75 g distilled water). The temperature profile during which the viscosity was measured was as follows: initial temperature of 50°C, held for 1 min, heating from 50°C to 90°C at a rate of 6°C min^{-1}, holding at 90°C for 5 min and thereafter cooling the gel to 50°C at 6°C per minute. The temperature was then maintained at 50°C for two minutes. The rate of agitation was 960 rpm during the first seconds and thereafter decreased to 160 rpm for the rest of the measurement. Two replicates were conducted for each clone. Parameters obtained from the viscosity profiles are: pasting temperature (PT) which is the temperature at which the swelling of starch commences, peak viscosity (PV), hot paste viscosity (HPV) measured at the end of the 90°C plateau and cold paste viscosity (CPV) measured at 50°C at 19.33 minutes. Three additional parameters were also calculated. Breakdown (BD) which is a measure of the loss in viscosity when the starch granules are disintegrated, and estimated as PV-HPV. Setback (SB), a measure of the degree of starch gelling when the amylose and amylopectin realign into a stable gel structure, estimated as CPV–PV and the consistency (CS) of the gel, estimated as CPV-HPV. Also the ease of cooking, which is the (time of peak viscosity) - (time of pasting temperature) was calculated for all samples.

4.6.2 Swelling power, solubility and dispersed volume fraction

The swelling power and solubility of the starch samples were determined using a 1.2% db starch paste (0,336 g db starch in 27,664 g distilled water). The starch pastes were prepared in the RVA starting at 35°C for 1 min, increasing to 75°C at a rate of 6°C per minute and holding at 75°C for 2.5 minutes. During the first minute the samples were stirred at 960 rpm and thereafter maintained at 160 rpm during the rest of the analysis. The paste was then immediately transferred to 40 ml Falcon tubes**,** left to cool to room temperature and centrifuged at 7000 rpm for 10 minutes. The supernatant and sediment was thereafter collected and weighed (W_{su} and W_{se} respectively) and dried in an oven at 105°C for 24 and 48 hours, respectively, after which they were weighed a second time (D_{su} and D_{se}). Two replicates of every genotype were taken. The obtained values were thereafter used to calculate three parameters: the concentration of soluble material in the supernatant (solubility), the swelling power and the volume fraction of the dispersed starch phase (ϕ).

$$\text{Solubility (\% db)} = 100 \text{ x } \mathrm{D_{su}}/\mathrm{m_{starch}}$$

$$\text{Swelling power } \left({}^{\mathrm{m_{water}}}/_{\mathrm{m_{starch}}}\right) = \frac{\mathrm{W_{se}}\text{-}\mathrm{D_{se}}}{\mathrm{D_{se}}}$$

For calculating the volume fraction of the dispersed phase, first the total volume (cm^3) of the paste is calculated, using a starch specific density of 1.5 g cm^{-3}.

$$V_{paste} = V_{water} + \left(\frac{m_{starch}}{1.5\ g\ cm^{-3}}\right)$$

$$\text{Dispersed volume fraction } (\phi) = \frac{[V_{paste} - (W_{su} - D_{su})]}{V_{paste}}$$

4.6.3 Clarity of gel

The clarity of the starch was measured as the light transmittance of starch pastes according to Craig et al (1989). A total volume of 20 ml 1% db starch solutions were prepared by dissolving the starch in distilled water. The samples were boiled in a waterbath at 97°C (at 1000 m above sea level) for 30 minutes with thorough shaking every five minutes. The transmittance was measured at 650 nm after the samples had cooled to room temperature. Two replicates of every sample were conducted.

4.7 Statistical analysis

Analysis of variance was done for the data generated from the waxy yield penalty trial for each plot (bulked across rows). Because of the trial design, the effect of sets was nested within replicates. The statistical analysis was conducted in SAS Software using the Proc GLM procedure.

Differences between environments in FRY, HI and DMC were analysed in SPSS with a paired samples T-test. Correlations between FRY, HI and DMC were tested with Pearson’s correlation, also in SPSS. Analysis of variance for starch properties was conducted in SPSS using Univariate GLM.

Spearman rank order correlation was used to compare the specific gravity method with the oven method for determining DMC. All mean values are presented with standard deviations.

5. Results

5.1 Yield penalty of waxy mutation

The fresh root yield, harvest index and dry matter content of the waxy genotypes and their wild-type siblings are presented in Table 2**.** There was no significant effect of starch type (waxy, wild-type) on the FRY ($p=0.94$, adjusted FRY ($p=0.88$) and HI ($p=0.33$) across the eight families (Table 3). There was, however, a highly significant difference ($p<0.01$) in dry matter content (DMC) between the waxy and wild type clones. Nevertheless the average difference in DMC between waxy and wild type genotypes was small, only 0.8% (waxy plants having an average of 32.8% and wild-type 33.6%). There was a significant effect of replicate and set for the DMC and HI. For FRY and adjusted FRY there was a significant effect of replicates.

Table 2 Means and standard deviations of FRY, Adjusted FRY, DMC and HI for the waxy and wild type starch cassava full-siblings. Also included is the least significant difference (LSD) at the 0.05 and 0.01 level for the analysis of variance

Starch type	FRY (t/ha)	Adjusted FRY (t/ha)	DMC (%)	HI
Waxy	7.24 (±2.40)	7.57 (±2.58)	32.8 (±1.49)	0.41 (±0.08)
Wild-type	7.40 (±2.73)	7.67 (±33.61)	33.6 (±1.61)	0.38 (0.07)
LSD 0.05	0.91	0.94	0.46	0.02
LSD 0.01	1.21	1.25	0.61	0.03

Table 3 Mean squares from the analysis of variance for FRY, adjusted FRY, DMC and HI in waxy and wild type cassava clones from the same eight full-sibling families

Source of variation	df	Fresh root yield	Adjusted fresh root yield	Dry matter content	Harvest Index
Replicate	3	24.4*	26.8*	7.8**	0.056**
Set (Replicate)	8	9.2	10.3	6.2**	0.013**
Starch type (Set* Replicate)	12	2.9	3.7	6.1**	0.005
Error	96	6.3	6.7	1.6	0.004

*Significant at a 0.05 level of significance

** Significant at a 0.01 level of significance

5.2 Environmental effects

5.2.1 Fresh root yield, harvest index and dry matter content of waxy clones

The waxy genotypes grown in the Caribbean coast and Valle del Cauca had an average FRY of 14.9 (±8.5) and 20.6 (±12.3) tonnes/ha, respectively. Plants grown in Valle del Cauca also produced more roots per unit of biomass, with an average HI of 0.43 (±0.12), compared to plants from the Caribbean coast, which had an average of 0.36 (±0.11). DMC in Valle del Cauca and the Caribbean coast were on average 31.9% (±2.0) and 24.3% (±2.3), respectively. For all three parameters there were highly significant differences between the two environments (p=0.00). Pearson's correlations showed that there was positive but weak associations (see Figure 6) between how well the genotypes performed in the two environments (Pearson correlation FRY: 0.370, HI: 0.154, DMC: 0.206). All correlations were significant at the 0.01 level of significance.

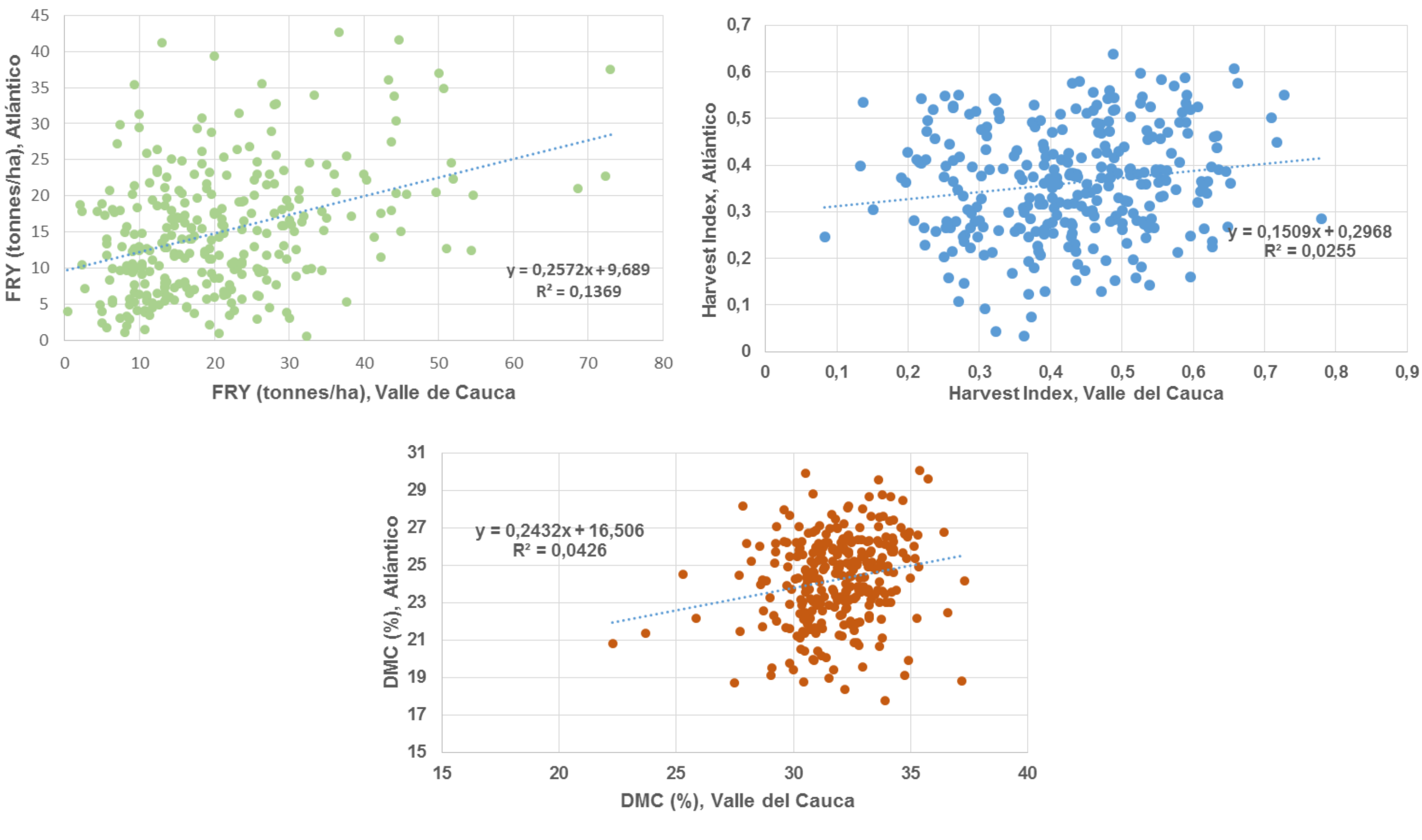

Figure 6. The linear relationship between fresh root yield (FRY), dry matter content (DMC) and harvest index for the 295 waxy cassava genotypes grown in Valle del Cauca and Atlántico

Table 4 Mean values and standard deviations for RVA traits in waxy and wild type starch clones grown in Valle del Cauca and on the Caribbean coast.

Starch type	Pasting temperature (°C)	Maximum viscosity (cP)	Breakdown (cP)	Consistency (cP)	Setback (cP)	Ease of cooking (min)
			Valle del Cauca			
Waxy	66.6 (±0.9)	1122 (±63)	660 (±55)	58 (±10)	-601(±68)	1.3 (±0.2)
Wild-type	67.9(±1.7)	858(±95)	397(±54)	219(±64)	-177(±49)	2.7 (0.4)
			Caribbean coast			
Waxy	71.7(±0.9)	1008 (± 52)	568 (±46)	61(±19)	-507(±45)	1.2 (±0.2)
Wild-type	71.5(±1)	653(±63)	214(±26)	158(±36)	-55(±35)	2.8 (±0.5)

Table 5 Starch paste properties with mean and standard deviation for waxy and wild-type cassava from two environments.

Starch type	Swelling power (g/g)	Water solubility (% db)	Volume fraction of dispersed phase (ϕ)	Paste clarity (%)
		Valle del Cauca		
Waxy	61 (±9)	7 (±2)	0.62 (±0.04)	66 (±4)
Wildtype	29 (±4)	14 (2)	0.33 (±0.03)	63 (±6)
		Caribbean coast		
Waxy	59(±7)	8 (±3)	0.60 (±0.04)	67 (±3)
Wildtype	30 (±4)	14 (±1)	0.31 (±0.02)	62 (±5)

5.2.3 Starch properties

The results of the starch analyses from the two contrasting environments are presented in Table 4 and 5. The pasting properties were influenced by the environment in which the clones were grown (Table 4 and Figure 8). As shown in Table 6 environment had a significant effect on all RVA parameters except on the ease of cooking of both starch types and the consistency of waxy starches. Also genotype and the genotype x environment (G x E) interaction had highly significant effects on most traits, although environment accounted for the larger part of the variation (with the exception of ease of cooking of wild type clones and consistency). Pasting of starches from clones grown on the Caribbean coast commenced at a temperature of 5.1°C (waxy) and 3.6°C (wild-type) higher than starches from Valle del Cauca. Peak viscosity was higher in starches from Valle del Cauca, in average 114 cP greater in waxy starch and 205 cP in wild type. The consistency (CPV-HPV**)** of the starch gels were more affected by environment in the wild type starches compared to the waxy ones. This is visualized in Figure 8, where it can be seen that the cold paste viscosity (at 19.3 min) largely differs between environments for the wild-type, but not for the waxy starches. The waxy cassava starches also have a lower final viscosity compared to the wild-type. Waxy clones were more rapid in reaching their peak viscosity than the wild type starch clones, and therefore needs a shorter cooking time. Starch from the waxy genotype GM4781-3 had very distinct RVA pasting properties, with much lower cold paste viscosities in both environments (data not shown).

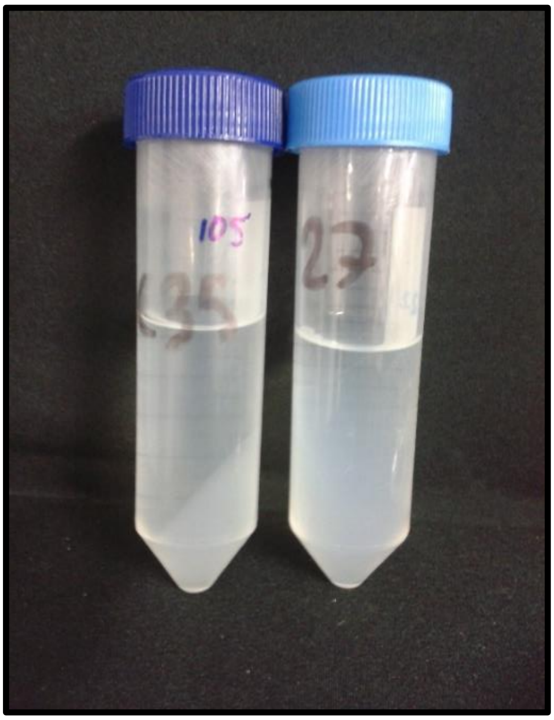

Figure 7. 1.2 % db cassava starch pastes, produced at 75 °C, after centrifugation. To the left a wild-type starch paste with good separation of water and gel. To the right an amylose-free gel.

The 1.2% db waxy starch pastes produced in order to calculate swelling power, solubility and dispersed volume fraction formed a much less dense pellet after centrifugation than pastes from wild type starch (see figure 7**).** The separation of the supernatant from the sediment therefore became less precise for waxy starches, and for two genotypes it was not possible to distinguish the gel from the water phase. These were therefore left out of the analysis. Results for the three mentioned parameters and paste clarity can be seen in Table 5. The paste clarity did not differ much between environments for the waxy clones, the average transmittance in pastes from Caribbean coast being 66.72%, and from Valle del Cauca 66.45%**.** The wild type pastes were less clear and had a 0.8 difference between the average transmittances in the two environments. There was no statistically significant effect of environment on paste clarity for waxy clones (p=0.330), whereas for wild type clones there was (p=0.001) (Table 6). Waxy starches from the Caribbean coast had around 1% higher solubility than those from Valle del Cauca, and the analysis of variance showed a significant effect of the environment on the solubility of the waxy starches. The difference in solubility for wild type starches between environments is statistically insignificant. Swelling power was slightly higher for waxy clones grown in Valle del Cauca compared to those grown in the Caribbean coast (60.98 and 58.79 respectively), while the opposite was shown for the wild type starches (28.71 and 29.66, respectively). Both the average water solubility and swelling power of the waxy starches revealed in this study were higher than those reported for the waxy clone AM 206-5 grown at the CIAT experimental station in Palmira. (Ceballos *et al,* 2007; Sanchez et al, 2010**.**

As can be seen in Table 6, for swelling power, solubility and paste clarity, environment accounted for more of the variation in the waxy clones, while genotype had a larger effect on these parameters in wild type starch

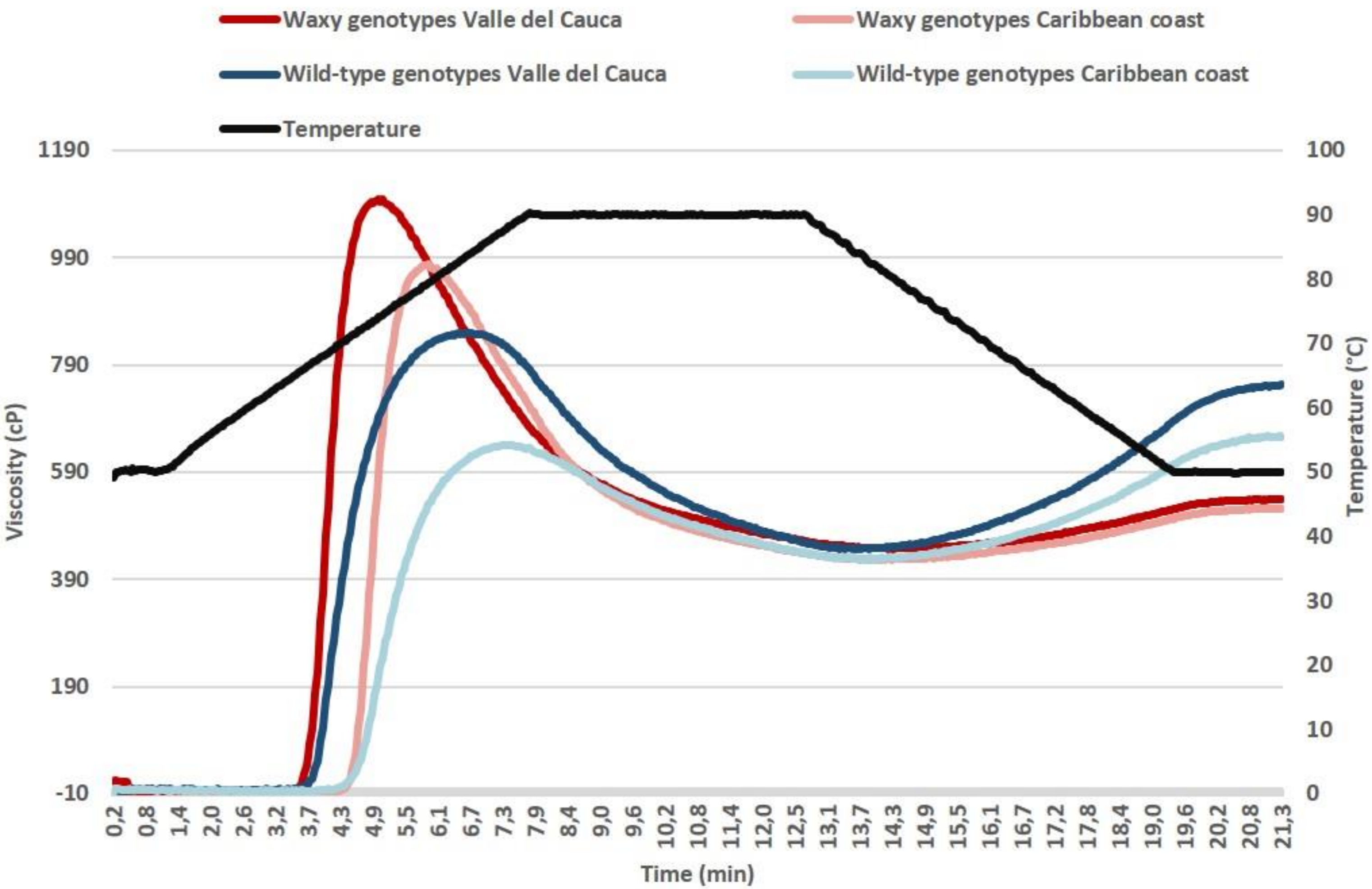

Figure 8. RVA amylograms (at 5 % suspensions) calculated from the mean viscosity values of the 30 waxy and 10 wild type starch genotypes grown in Valle del Cauca and on the Caribbean coast.

Table 6. Mean square values from the analysis of variance for starch properties of 30 waxy and 10 wild-type starch cassava genotypes grown in two environments in Colombia.

Source of variation	d.f.	Pasting temperature	Peak viscosity	Breakdown	Consistency	Setback	Ease of cooking
				Waxy starch			
Genotype	29	1**	11763**	8693**	1780**	14241**	0.4
Environment	1	738**	395432**	254196**	288	271606**	0.7
Genotype x Environment	29	1**	5738**	2941**	900**	2300**	0.3
Error	60	0.08	383	219	100	278	0.3
				Wild-type starch			
Genotype	9	3**	9941**	5052**	5210**	4680**	0.5**
Environment	1	125**	419783**	336723**	37149**	150185**	0.06
Genotype x environment	9	6**	17242**	2208**	5918**	2660**	0.2**
Error	20	0.1	85	107	120	132	0.05

Source of variation	d.f.	Solubility	Swelling power	Dispersed volume fraction	Clarity of gel
			Waxy starch		
Genotype	27 (29[a])	14**	75	0.003**	39**
Environment	1	37**	135	0.006**	2
Genotype x Environment	27 (29[a])	7*	79	0.002**	11**
Error	56 (60[a])	3	45	0.001	2
			Wild-type starch		
Genotype	9	2**	35**	0.003**	70**
Environment	1	0.06	9	0.008**	6**
Genotype x environment	9	6**	28**	0.000**	58**
Error	20	0.3	3	6.6×10^{-5}	0.4

*Significant at a significance level of 0.05
** Significant at a significance level of 0.01
[a] Degrees of freedom for clarity of gel

5.3 Evaluation of the specific gravity method for determining dry matter content

The comparison between DMC of cassava roots estimated by the specific gravity method and by heating in oven is shown in Figure 9. For the majority of clones used in this study, the specific gravity method (Toro and Cañas, 2012) underestimated the true dry matter content. A Spearman rank-order correlation was run to test if the DMC for the clones were in the same order regardless of the methods used. There was a highly significant correlation (r_S=0.848, p=0.000). However, the order of the clones of the two methods was not exactly the same.

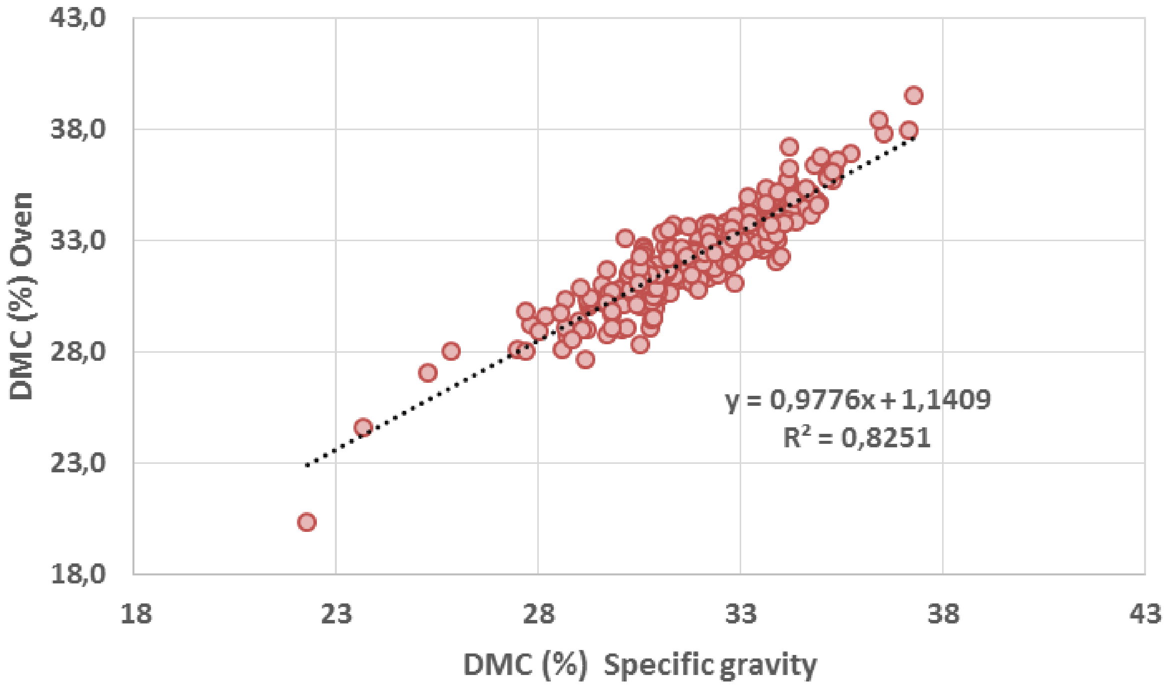

Figure 9. The linear relationship for 295 cassava clones between DMC estimated by the specific gravity method using (equation from Toro and Cañas, 2008) compared to DMC measured by oven method.

6. Discussion

6.1 Yield penalty of the waxy trait

In the eight, cassava full-sibling families segregating for the waxy trait, the waxy mutation did not have a significant effect on the fresh root yield and harvest index of the plants. Fresh root yields of the waxy plants were 21 t/ha and 21.3 t/ha for the wild type. The dry matter contents of the siblings were, however, significantly affected by the starch-type of the roots, with waxy roots having an average of 0.8% lower DMC. Although the dry matter penalty is less than one percent, it can potentially become a quantitatively important loss in large scale processing of cassava starch. However, as for waxy maize starch (Elbehri and Paarlberg, 2001), the waxy cassava is expected to be sold for a premium price, thereby outweighing the lower dry matter yield. One important aspect to take into consideration is that it will be single cassava clones that will be grown as commercial varieties. The average performance of the waxy clones is therefore of less significance, as long as effort is put into developing a few competitively yielding commercial varieties with an adequate dry matter content.

6.2 Environmental effects on yield and starch properties

The performance of the waxy genotypes, regarding yield, harvest index and dry matter content, was not stable across the two environments. This is manifested by the poor correlations of these variables between Valle del Cauca and the Caribbean coast. This is an indicator that selections made in one of the environments have a low predicting value for the other. It must be emphasized that the data from Valle del Cauca is based on unreplicated plots with only two plants, this can have implications for the results particularly for FRY. FRY in cassava has previously been shown to vary substantially with location (Tan and Mak, 1995; Egesi *et al.,* 2007;). In a study by Dixon and Nukenine (2000) environment and genotype by environment interaction were shown to highly affect the production of fresh root yield of cassava grown in multi-locational trials. Selections for fresh root yield of cassava should, conclusively, only be performed in a target environment. The dry matter content was also affected by G x E interactions, however, to a lesser degree. Other previous studies on cassava have reported that the variation found in dry matter content is mainly contributed by the genotype, while G x E had a lower contribution to the total variation **(**Kawano *et al.,* 1987; Tan and Mak, 1995; Benesi *et al.,* 2004). Season (and thereby rainfall) was also shown to have a large effect on DMC. Genotype and environment had an equal contribution to the variation of harvest index in cassava (Tan and Mak, 1995). Although dry matter content and harvest index in previous studies have been shown to depend highly on genotype, environment also influence these traits, as shown in this study. It is therefore advisable to make selections also for these traits in the target environments.

Environment largely affected the pasting properties of cassava starch, and had a significant effect on all parameters except the ease of cooking of both starch-types and the consistency of waxy starches. The onset of pasting required 5.1°C more for waxy, and 3.6°C, for wild-type, when grown in the agro-ecological zone of the Caribbean coast, characterized by higher temperatures and a lower altitude. These results could be explained by the higher growth temperature at the coast, as other studies have reported an increase in pasting and gelatinization temperatures with growth temperature (Hizukuri, 1969; Tester *et al.,* 1999; Noda *et al.,* 2001; Tester and Karkalas, 2001). High growth temperatures have been suggested to contribute to strong intermolecular hydrogen-bonds which resist swelling, thereby increasing the pasting temperature (Tester *et al.,* 1999; Tester and Karkalas, 2001). There was also a substantial environmental effect on the peak viscosity of the starches, with the cassava grown on the Caribbean coast having a 114 cP lower average peak viscosity in waxy starch and 205 cP in wild type. The only RVA parameter for which the waxy and wild type starch responded differently to the change of environment was consistency. Wild type genotypes grown in Valle del Cauca exhibit a higher cold paste viscosity (at 50°C, after 19.3 min) compared to the Caribbean genotypes. One reasonable explanation is that the amylose content of the normal starch is affected by the growth environment. Elevated growing temperatures have in previous studies been shown to affect the amylose content of maize, wheat, rice and potatoes (Tester and Karkalas, 2001). The starch amylose is involved in the gel network formation after heating, and can therefore influence final viscosity (Jane *et al.,* 1999)**.**

There is no complete agreement among previous publications on how starch peak viscosities are affected by elevated temperatures. Noda *et al* (2001), who studied the effect of soil temperature on starch properties of sweet potato found that peak viscosities increased when soil temperatures increased from 15 to 27°C, but remained unchanged when temperature rose from 27 to 33°C. Hizukuri (1969) reported that maximum viscosity was shown to decrease with elevated growth temperatures for potato and sweet-potato, increase for rice and remain the same for soybean seedling starches. The effect of environment on pasting properties has also been studied in other crops. In rice, growth season was reported to have highest effect on the variation found in peak viscosity and hot paste viscosity, although growth year, genotype and genotype by season interaction also had large effects on these variables. However, for setback and breakdown, genotype accounted for the major part of the variation (Bao *et al.,* 2004). In wheat, environment has been shown to have little effect on RVA-variables of flour, as compared to effects of genotype (Graybosch *et al.,* 2003).

The actual differences between environments in solubility, swelling power, dispersed volume fraction and gel clarity were small and would not be expected to affect the utilization of the starches substantially. The difference found in swelling power between waxy and wild type starch are in accordance with earlier reports, where waxy starches have been found to have an increased swelling ability compared to the wild type of the same crop (Sasaki and Matsuki, 1998, Li and Yeh*,* 2001; Sanchez *et al.,* 2010; Zhao *et al.,* 2011). Also the volume fraction of the dispersed phase has been reported to be higher in a waxy cassava compared to wild type starch (Sanchez *et al.,* 2010). Zhao *et al* (2011) reported varying starch solubilities in transgenic waxy cassava compared to the wild-type and suggested that amylose content have little effect on this property. The paste clarity of the waxy clones in this study had a higher transmittance than what has earlier been reported for the ”original” waxy clone AM 206-5 (Ceballos *et al.,* 2007; Sanchez *et al.,* 2010). This is a positive result since colourless starches are attractive to the industry because they do not alter the appearance of the end product.

To the author’s knowledge, there have not been any studies of how crop growth altitude affects starch properties. Since altitude is only one of many factors that could possibly influence the cassava starch functional properties in this study, no conclusions can be drawn.

6.3 Evaluation of the specific gravity method

The equation used at CIAT (from Toro and Cañas, 1983) to calculate the dry matter content from the specific gravity of the cassava roots generally underestimated the true DMC. Nevertheless, what is of importance for breeding purposes is the within ranking of clones, because it is what is used in making selections. Since there was a strong rank-order correlation (r_S=0.885) between DMC retrieved from the two methods, the specific gravity method with the currently used equation can be considered satisfactory.

7. Acknowledgments

I would like to thank everyone who supported and helped me during the work of this thesis. You made it possible for me to realise it. I would like to thank my supervisor Mulatu Geleta Dida for coming with helpful input on my thesis. Thanks to my co-supervisor Hernán Ceballos for making it possible for me to do my thesis at CIAT and for teaching me everything about cassava breeding. Thanks to Fernando Calle for always being positive, teaching me about cassava and supporting me in the field. I also want to thank everyone who was working in the field with the cassava harvests. I am thankful to everyone at the Cassava Program at CIAT for taking me in and making my stay a good one.

Thanks to everyone at the RTB-laboratory at CIAT for helping me with my starch analyses and for having patience with my poor Spanish. I would especially want to thank Monica Pizarro Sanchez and Jhon Larry Moreno Alzate for finalizing the starch analyses.

I am grateful to SLU and SIDA who made this work possible through the Minor Field Studies scholarship.

8. References

Aiemnaka, P., Wongkaew, A., Chanthaworn, J., Nagashima, S. K., Boonma, S., Authapun, J., Jenweerawata, S., Kongsilaa, P., Kittipadakula, P., Nakasathiena, S., Sreewongchaia, T., Wannaratc, W., Vichukitab, V., Becerra López-Lavalled, L.A., Ceballos, H., Rojanaridpiched, C. & Phumichai, C. (2012). Molecular characterization of a spontaneous waxy starch mutation in cassava. *Crop Science*, 52: 2121-2130.

Bahaji, A., Li, J., Sánchez-López, Á. M., Baroja-Fernández, E., Muñoz, F. J., Ovecka, M., Almagroa, G., Monteroa, M., Ezquera, I., Etxeberriac, E. & Pozueta-Romero, J. (2014). Starch biosynthesis, its regulation and biotechnological approaches to improve crop yields. *Biotechnology Advances*, 32: 87-106.

Bao, J., Kong, X., Xie, J., & Xu, L. (2004). Analysis of genotypic and environmental effects on rice starch. 1. Apparent amylose content, pasting viscosity, and gel texture. *Journal of Agricultural and Food Chemistry*, *52*: 6010-6016.

Benesi, I. R., Labuschagne, M. T., Dixon, A. G., & Mahungu, N. M. (2004). Stability of native starch quality parameters, starch extraction and root dry matter of cassava genotypes in different environments. *Journal of the Science of Food and Agriculture*, 84:1381-1388.

Beta, T., & Corke, H. (2001). Genetic and environmental variation in sorghum starch properties. *Journal of Cereal Science*, 34: 261-268.

Bettge, A.D., Giroux, M.J., & Morris, C.F. (2000). Susceptibility of waxy starch granules to mechanical damage. *Cereal Chemistry*, 77: 750-753.

Bogracheva, T. Y., Cairns, P., Noel, T. R., Hulleman, S., Wanga, T. L., Morris, V. J., Ringb, C.L. & Hedley, C. L. (1999). The effect of mutant genes at the r, rb, rug3, rug4, rug5 and lam loci on the granular structure and physico-chemical properties of pea seed starch. *Carbohydrate Polymers*, 39: 303-314.

Brust H., Lehmann T., D'Hulst C., Fettke J. (2014) Analysis of the functional interaction of Arabidopsis Starch Synthase and Branching Enzyme isoforms reveals that the cooperative action of SSI and BEs results in glucans with polymodal chain length distribution similar to amylopectin. Plos One 9:e102364. doi:10.1371/journal.pone.0102364

Buléon, A., Colonna, P., Planchot, V., & Ball, S. (1998). Starch granules: structure and biosynthesis. *International Journal of Biological Macromolecules*, 23: 85-112.

Ceballos, H. (2012) Cassava in Colombia and the world: New prospects for a millennial crop. In: Ospina, B., Ceballos, H., *Cassava in the third millennium, modern production, processing, use and marketing systems.* Cali, CIAT publication 377: 420-426

Ceballos, H., Sánchez, T., Morante, N., Fregene, M., Dufour, D., Smith, A. M., Denyer, K., Pérez, J.C., Calle, F. & Mestres, C. (2007). Discovery of an amylose-free starch mutant in cassava (Manihot esculenta Crantz). *Journal of Agricultural and Food Chemistry*, 55: 7469-7476.

Chatakanonda, P., Chinachoti, P., Sriroth, K., Piyachomkwan, K., Chotineeranat, S., Tang, H.R., & Hills, B. (2003). The influence of time and conditions of harvest on the functional behaviour of cassava starch—a proton NMR relaxation study. *Carbohydrate Polymers*, 53: 233-240.

Collado, L.S., Mabesa, R.C., & Corke, H. (1999). Genetic variation in the physical properties of sweet potato starch. *Journal of Agricultural and Food Chemistry*, 47: 4195-4201.

Collins, G.N. (1909) A new type of Indian corn from China. U.S Dept. Agriculture. *Bureau of Plant Industry Bulletin.* 161:7-30

Craig, S.A.S., Maningat, C.C.,Seib, P.A., Hoseney, R.C., (1989) Starch paste clarity. *Cereal Chemistry,* 66*:* 173-182

Dang, J.M.C., and Bason, M.L. (2014) AACCI Approved Methods Technical Committee Report: collaborative study on a method for determining the gelatinization temperature of milled rice flour using the Rapid Visco Analyser. *Cereal Foods World*, 59: 31–34.

Denyer, K., Johnson, P., Zeeman, S., & Smith, A.M. (2001). The control of amylose synthesis. *Journal of Plant Physiology*, 158: 479-487.

Delvallé, D., Dumez, S., Wattebled, F. *et al* (2005). Soluble starch synthase I: a major determinant for the synthesis of amylopectin in *Arabidopsis thaliana* leaves. *The Plant Journal*, 43: 398-412.

Dixon, A.G.O., & Nukenine, E.N. (2000). Genotype x environment interaction and optimum resource allocation for yield and yield components of cassava. *African Crop Science Journal*, 8: 1-10.

Egesi, C.N., Ilona, P., Ogbe, F.O., Akoroda, M., & Dixon, A. (2007). Genetic variation and genotype× environment interaction for yield and other agronomic traits in cassava in Nigeria. *Agronomy Journal*, 99: 1137-1142.

Elbehri, A., & Paarlberg, P. (2003). Price behavior in corn market with identity-preserved types. In: *American Agrcultural Economics Association Annual Meeting,* Montreal, Canada July 27-30.

Ellis, R.P., Cochrane, M.P., Dale, M.F.B., Duffus, C. M., Lynn, A., Morrison, I. M., Derek, R., Prentice, M., Swanston & Tiller, S. A. (1998). Starch production and industrial use. *Journal of the Science of Food and Agriculture*, 77: 289-311.

Eriksson, G. (1969). The waxy character. *Hereditas*, 63: 180-204.

Faostat (2015) *Food and Agriculture Organization of the United Nations Statistics Division.* http://faostat3.fao.org/home [15-08-2015]

Fergason, V. (2001) High-amylose and waxy corns. In: Hallauer, A.R. (ed), *Speciality Corns,* 2nd ed. Boca Raton, CRC Press LLC.

Fuglie, K.O., Oates, C.G., & Xie, J. (2006) Root Crops, starch and agro-industrialization in Asia. *Social Sciences Working Paper Series (CIP),* International Potato Center (CIP), Lima, Peru.

Fukunaga, K., Kawase, M., & Kato, K. (2002). Structural variation in the Waxy gene and differentiation in foxtail millet [Setaria italica (L.) P. Beauv.]: implications for multiple origins of the waxy phenotype. *Molecular Genetics and Genomics*, 268: 214-222.

Gomanda, S. V., Lamberts, L., Derde, L. J., Goesaert, H., Vandeputte, G. E., Goderis, B., Visserc, R.G.F. & Delcour, J. A. (2010). Structural properties and gelatinisation characteristics of potato and cassava starches and mutants thereof. *Food Hydrocolloids,* 24: 307-317.

Google Earth (2015). https://earth.google.com, [15-08-2015]

Graybosch, R.A. (1998). Waxy wheats: origin, properties, and prospects. *Trends in Food Science & Technology*, 9: 135-142.

Graybosch, R.A., Souza, E., Berzonsky, W., Baenziger, P.S., & Chung, O. (2003). Functional properties of waxy wheat flours: genotypic and environmental effects. *Journal of Cereal Science*, 38: 69-76.

Hizukuri, S. (1969). The effect of environment temperature of plants on the physicochemical properties of their starches. *Journal of the Japanese Society of Starch Science* 17: 73-88.

Hoover, R. (2001). Composition, molecular structure, and physicochemical properties of tuber and root starches: a review. *Carbohydrate Polymers*, 45: 253-267.

IIASA/FAO (2015). Global Agro-ecological Zones (GAEZ v3.0). IIASA, Laxenburg, Austria and FAO, Rome, Italy available at: http://gaez.fao.org [22 August 2015].

James, M.G., Denyer, K., & Myers, A.M. (2003). Starch synthesis in the cereal endosperm. *Current Opinion in Plant Biology*, 6: 215-222.

Jampala, B., Rooney, W.L., Peterson, G.C., Bean, S., & Hays, D.B. (2012). Estimating the relative effects of the endosperm traits of waxy and high protein digestibility on yield in grain sorghum. *Field Crops Research*, 139, 57-62.

Jane, J. L. (2006). Current understanding on starch granule structures. *Journal of Applied Glycoscience*, 53: 205-213.

Jane, J., Chen, Y.Y., Lee, L.F., McPherson, A.E., Wong, K.S., Radosavljevic, M., & Kasemsuwan, T. (1999). Effects of amylopectin branch chain length and amylose content on the gelatinization and pasting properties of starch 1. *Cereal Chemistry*, 76: 629-637.

Jobling, S. (2004). Improving starch for food and industrial applications. *Current Opinion in Plant Biology*, 7: 210-218.

Kawano, K., Fukuda, W.M.G., & Cenpukdee, U. (1987). Genetic and environmental effects on dry matter content of cassava root. *Crop Science*, 27: 69-74.

Kharabian-Masouleh, A., Waters, D. L., Reinke, R. F., Ward, R., & Henry, R. J. (2012). SNP in starch biosynthesis genes associated with nutritional and functional properties of rice. *Scientific Reports*, 2.

Koehorst-van Putten, H. J. J., Sudarmonowati, E., Herman, M., Pereira-Bertram, I. J., Wolters, A. M. A., Meima, H., de Vetten, N., Raemakers, R.G.F. & Visser, R. G. F. (2012). Field testing and exploitation of genetically modified cassava with low-amylose or amylose-free starch in Indonesia. *Transgenic Research*, 21: 39-50.

Larkin, P.D., & Park, W.D. (2003). Association of waxy gene single nucleotide polymorphisms with starch characteristics in rice (Oryza sativa L.). *Molecular Breeding*, 12: 335-339.

Li, J.Y., & Yeh, A.I. (2001). Relationships between thermal, rheological characteristics and swelling power for various starches. *Journal of Food Engineering*, 50: 141-148.

Manners, D.J. (1989). Recent developments in our understanding of amylopectin structure. *Carbohydrate Polymers*, 11: 87-112.

Mat Hashim, D.B., Moorthy, S.N., Mitchell, J.R., Hill, S.E., Linfoot, K.J., & Blanshard, J.M.V. (1992). The effect of low levels of antioxidants on the swelling and solubility of cassava starch. *Starch-Stärke*, 44: 471-475.

McIntyre, C.L., Drenth, J., Gonzalez, N., Henzell, R.G., & Jordan, D.R. (2008). Molecular characterization of the waxy locus in sorghum. *Genome*, 51: 524-533.

Montagnac, J.A., Davis, C.R., & Tanumihardjo, S.A. (2009). Nutritional value of cassava for use as a staple food and recent advances for improvement. *Comprehensive Reviews in Food Science and Food Safety*, 8: 181-194.

Muth, J., Hartje, S., Twyman, R.M., Hofferbert, H.R., Tacke, E., & Prüfer, D. (2008). Precision breeding for novel starch variants in potato. *Plant Biotechnology Journal*, 6: 576-584.

Noda, T., Kobayashi, T., & Suda, I. (2001). Effect of soil temperature on starch properties of sweet potatoes. *Carbohydrate Polymers*, 44: 239-246.

Ohdan, T., Francisco, P.B., Sawada, T., Hirose, T., Terao, T., Satoh, H., & Nakamura, Y. (2005). Expression profiling of genes involved in starch synthesis in sink and source organs of rice. *Journal of Experimental Botany*, *56*(422), 3229-3244.

Okagaki, R.J., Neuffer, M.G., & Wessler, S.R. (1991). A deletion common to two independently derived waxy mutations of maize. *Genetics*, 128: 425-431.

Oscarsson, M., Andersson, R., Åman, P., Olofsson, S., & Jonsson, A. (1998). Effects of cultivar, nitrogen fertilization rate and environment on yield and grain quality of barley. *Journal of the Science of Food and Agriculture*, 78: 359-366.

Peroni, F.H.G., Rocha, T.S., & Franco, C.M.L. (2006). Some structural and physicochemical characteristics of tuber and root starches. *Food Science and Technology International*, 12: 505-513.

Piperno, D.R., & Holst, I. (1998). The presence of starch grains on prehistoric stone tools from the humid neotropics: indications of early tuber use and agriculture in Panama. *Journal of Archaeological Science*, 25: 765-776.

Preiss, J. (2004). Plant starch synthesis. In: Eliasson, A-C., *Starch in Food: structure, function and applications.* Cambridge: Woodhead Publishing Limited. pp 3-56

Sánchez, T., Salcedo, E., Ceballos, H., *et al* (2009). Screening of starch quality traits in cassava (*Manihot esculenta* Crantz). *Starch-Stärke*, 61: 12-19.

Sánchez, T., Dufour, D., Moreno, I.X., & Ceballos, H. (2010). Comparison of pasting and gel stabilities of waxy and normal starches from potato, maize, and rice with those of a novel waxy cassava starch under thermal, chemical, and mechanical stress. *Journal of Agricultural and Food Chemistry*, 58: 5093-5099.

Santisopasri, V., Kurotjanawong, K., Chotineeranat, S., Piyachomkwan, K., Sriroth, K., & Oates, C. G. (2001). Impact of water stress on yield and quality of cassava starch. *Industrial Crops and Products*, 13: 115-129.

Sasaki, T., & Matsuki, J. (1998). Effect of wheat starch structure on swelling power. *Cereal Chemistry*, 75: 525-529.

Schopmeyer, H.H., Felton, G.E., & Ford, C.L. (1943). Waxy cornstarch as a replacement for tapioca. *Industrial & Engineering Chemistry*, 35: 1168-1172.

Seung, D., Soyk, S., Coiro, M., Maier, B.A., Eicke, S., & Zeeman, S. C. (2015). Protein Targeting to Starch is required for localising Granule-bound Starch Synthase to starch granules and for normal amylose synthesis in Arabidopsis. *Plos Biology*, 13: e1002080-e1002080.

Singh, S., Singh, G., Singh, P., & Singh, N. (2008). Effect of water stress at different stages of grain development on the characteristics of starch and protein of different wheat varieties. *Food Chemistry*, 108: 130-139.

Sriroth, K., Santisopasri, V., Petchalanuwat, C., Kurotjanawong, K., Piyachomkwan, K., & Oates, C. G. (1999). Cassava starch granule structure–function properties: influence of time and conditions at harvest on four cultivars of cassava starch. *Carbohydrate Polymers*, 38: 161-170.

Swinkels, J.J.M. (1985). Composition and properties of commercial native starches. *Starch-Stärke*, 37:1-5.

Tan, S.L., & Mak, C. (1995). Genotype× environment influence on cassava performance. *Field Crops Research*, 42: 111-123.

Takeiti, C., Fakhouri, F., Ormenese, R., Steel, C., & Collares, F. (2007). Freeze-thaw stability of gels prepared from starches of non-conventional sources. *Starch-Stärke*, 59: 156-160.

Tester, R.F. (1997). Influence of growth conditions on barley starch properties. *International Journal of Biological Macromolecules*, 21: 37-45.

Tester, R.F., Debon, S. J.J., Davies, H.V., & Gidley, M.J. (1999). Effect of temperature on the synthesis, composition and physical properties of potato starch. *Journal of the Science of Food and Agriculture*, 79: 2045-2051.

Tester, R.F., & Karkalas, J. (2001). The effects of environmental conditions on the structural features and physico-chemical properties of starches. *Starch-Stärke*, 53: 513-519.

Toro, J.C. & Cañas, A. (1983) Determinación del contendio de materia seca y almidón en yuca por el sistema de gravedad específica. In: Domínguez CE, ed. Yuca: Investigación, producción y utilización. CIAT; United Nations Development Programme (UNDP), Cali, Colombia

Toro, J.C. & Cañas, A. (2012) Appendix: Determining dry matter content of fresh roots, using the specific gravity method. In: Ospina, B., Ceballos, H., *Cassava in the third millennium, modern production, processing, use and marketing systems.* Cali, CIAT publication 377, 420-426

Raemakers, C.J.J.M., Schreuder, M.M., Pereira, I., Suurs, L., Vincken, J.P., Jacobsen, E & Visser, R.G.F. (2003) Production of amylose-free cassava plants by genetic modification. In: Fauquet, C.M., Taylor, N.J., (eds). *Cassava: An ancient crop for modern times,* Proceedings of the 5th International meeting of the Cassava Biotechnology Network, 4-9 November 2001, St Louis, MO.

Raemakers, K., Schreuder, M., Suurs, L., Furrer-Verhorst, H., Vincken, J.P., Vetten, N., ... & Visser, R.G. (2005). Improved cassava starch by antisense inhibition of granule-bound starch synthase I. *Molecular Breeding*, 16: 163-172.

Rooney, W.L., Aydin, S., & Kuhlman, L.C. (2005). Assessing the relationship between endosperm type and grain yield potential in sorghum (Sorghum bicolor L. Moench). *Field Crops Research*, 91:199-205.

Zeeman, S.C., Kossmann, J., & Smith, A.M. (2010). Starch: its metabolism, evolution, and biotechnological modification in plants. *Annual Review of Plant Biology*, 61, 209-234.

Zeng, M., Morris, C.F., Batey, I.L., & Wrigley, C.W. (1997). Sources of variation for starch gelatinization, pasting, and gelation properties in wheat. *Cereal Chemistry*, 74, 63-71.

Zhao, S.S., Dufour, D., Sánchez, T., Ceballos, H., & Zhang, P. (2011). Development of waxy cassava with different biological and physico-chemical characteristics of starches for industrial applications. *Biotechnology and Bioengineering*, 108: 1925-1935.

Van Hung, P., Maeda, T., & Morita, N. (2006). Waxy and high-amylose wheat starches and flours—Characteristics, functionality and application. *Trends in Food Science & Technology*, 17: 448-456.

Yamanaka, S., Nakamura, I., Watanabe, K.N., & Sato, Y.I. (2004). Identification of SNPs in the waxy gene among glutinous rice cultivars and their evolutionary significance during the domestication process of rice. *Theoretical and Applied Genetics*, 108: 1200-1204.

Wattebled, F., Buleon, A., Bouchet, B., *et al* (2002). Granule-Bound Starch Synthase I. *European Journal of Biochemistry*, 269: 3810-3820.

Weatherbase (2015) *Homepage*
http://www.weatherbase.com/weather/city.php3?c=CO&name=Colombia
[15-08-2015]