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CHARACTERIZATION OF NOVEL RICE GERMPLASM FROM WEST AFRICA AND GENETIC MARKER ASSOCIATIONS WITH RICE COOKING QUALITY

A Dissertation

by

KARIM TRAORE

Submitted to the Office of Graduate Studies of
Texas A&M University
in partial fulfillment of the requirements for the degree of

DOCTOR OF PHILOSOPHY

August 2005

Major Subject: Plant Breeding

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Approved by:

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August 2005

Major Subject: Plant Breeding

ABSTRACT

Characterization of Novel Rice Germplasm from West Africa and Genetic Marker Associations with Rice Cooking Quality. (August 2005)

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Genetic resource enhancement is the foundation of any good breeding program. Landraces from West Africa, interspecifics between *Oryza sativa* and *Oryza glaberrima* and improved lines from the West African Rice Development Association and other research centers were introduced to the Beaumont Rice Research center for in situ evaluation and characterization. Beside the introduction of seeds, milled samples were also introduced for grain chemistry analysis. Field evaluation combined with physico-chemical and molecular characterization revealed unique characteristics among African germplasm. New rice for Africa (NERICA) lines performed well in the USA environment. Varieties like Nerica 2, Nerica 3, Nerica 4, and Nerica 5 need more attention because of their superior performance in yield and grain quality. Landraces did not perform well due to their height and late maturity and their resulting problems with lodging. The rapid visco analyzer RVA profiles showed that the cultivar Jaya has unusually strong paste viscosity features. Comparing West Africa samples grown in Cote d'Ivoire with those grown in Texas, parameters like AA, ASV, Hot, Cool, and CT were not stongly affected by the environment. According to the Stbk value, cultivars

grown in Cote d'Ivoire will cook softer than when they were grown in Texas. The lack of the environmental effect is somewhat surprising considering the difference in latitude, soil types, weather patterns, and management practices between the two locations. Apparent amylose is a key element to characterize a rice cultivar; however certain varieties like Cocodrie and Dixiebelle have similar apparent amylose content but dramatically different functional qualities. A population derived from Cocodrie and Dixiebelle was developed for genotypic and phenotypic analysis of grain chemistry traits that affect functionality. It was concluded that the amount of soluble amylose in the grain had a significant effect on flour pasting properties, even when total apparent amylose content did not vary. Marker association studies revealed that the *Waxy* microsatellite and the *Waxy* exon 10 SNP markers were associated with soluble amylose content and RVA characteristics. These markers will speed up the development of new rice cultivars with desirable quality characteristics in West Africa and in the USA.

DEDICATION

To my father

ACKNOWLEDGMENTS

I wish to express my sincere gratitude to Dr. Anna M. McClung, my co-chair, for her assistance and sharing with me her numerous experiences. Sincere thanks to Dr. William L. Rooney, my co-chair, for his assistance and daily advice. I will never forget your help in solving the administrative protocols at the university. Sincere thanks to Dr. Edwin Price, member of my committee, who started helping me even before I came to Texas A&M University. I will never forget your interest in all my academic and private life issues. I am thankful to Dr. Lloyd W. Rooney, member, with whom I used to work in the national program of Mali. I will always need your constructive adviice. Many thanks to Dr. Robert G. Fjellstrom, member, who helped me to understand basic knowledge in biotechnology.

I am greatly indebted to the Rockefeller Foundation and the West Africa Rice Development Association (WARDA) for providing funds for my study.

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1. INTRODUCTION

The role of agricultural production is to feed the global population of over 6 billion people, which will likely grow an additional 50% over the next 40 years (United States Department of Commerce 1999). Meeting the nutritional needs of such a large population will require increasing agricultural productivity through a combination of methods. These will most likely include expansion of agricultural production into new areas, development of crops with higher yield potential, vigorous protection of potential yields from losses caused by crop pests, and breeding high quality varieties that meet consumer preferences. Africa produces an average of 14.6 million tons of rough rice per year (1989-1996) on 7.3 million ha, equivalent to 2.6 and 4.6 % of the world's total production and rice area, respectively (FAO 1996). West Africa has the largest planted rice area in Africa at about 3.7 million ha. In much of this region, rice is produced under upland (dryland) conditions in contrast to the rest of the world where irrigated rice predominates. The regional contributions to rice production in Africa are: West Africa (42%); North Africa (32 %); East Africa (24 %); Central Africa (1 %); and Southern Africa (1 %) (WARDA 2000).

One of the major concerns in rice production is grain quality (Nanda 2000). While many components contribute to rice quality, the most important are cooking and

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This dissertation follows the style of Molecular Breeding.

eating qualities. These parameters primarily nvolve the physical and chemical characteristics of starch. The constituents which play important roles in cooking and eating quality are amylose content, gelatinization temperature, and gelling consistency.

Singh et al. (2000) concluded that grain quality is second only to yield as a major rice breeding objective. In the future, grain quality will be even more important as very poor consumers, who depend largely on rice for their daily food, demand higher quality rice (Juliano and Villarreal 1993). However, defining quality is often difficult since it is defined by the end user and their preferences are highly variable (Singh et al. 2000). For example, Middle East consumers prefer long grain, well milled rice with strong aroma while the European community generally prefers long grain rice with no scent because the presence of any scent signals spoilage and contamination (Efferson 1985). In West Africa, quality is based on the type of food people prepare for eating. Long grain and aromatic rice are used with sauces, short and medium grain rice are used in porridge mixed with sugar, salt and milk, and broken rice is used in Senegal, Gambia and Mali as fried rice. Long grain aromatic rice has the greatest demand and is the most expensive rice in local markets.

Today the breeding of high quality rice integrates traditional approaches with molecular genetics technology. In rice, standardized methods for transformation and regeneration make the insertion of transgenes for unique traits possible. The complete sequencing of the rice genome provides potential for developing genetic markers associated with many traits. Thus, gene tagging and marker assisted selection (MAS) using an array of molecular markers are now feasible for breeding (Singh et al. 2000).

Certain varieties are still grown and preferred by indigenous people in Africa due to specific qualities like texture, aroma, digestibility, and grain expansion after cooking even though they have low yield potential. Developing molecular markers that are associated with agronomic and grain quality traits would help breeders to develop new cultivars having improved yield potential as well as cooking and sensory quality traits that are desired by consumers.

An association study using 164 rice accessions conducted by Chen et al. (2004) concluded that a microsatellite marker in the *Waxy* gene and a single nucleotide polymorphism in Exon 10 of the waxy gene (ex10-SNP) were correlated with starch paste viscosity profiles. These profiles are an indicator of cooked rice texture and rice suitability for parboiling and canning processes. An inheritance study is needed to verify genetic relationship of this cereal quality property with the presence of the *Waxy* allele and ex10-SNP and to determine if these are suitable markers for improving sensory and processing quality in rice.

Although rice yield and production are increasing in the USA, there are other challenges for USA rice production:

- Maintain stringent quality criteria for conventional long, medium, and short grain market classes
- Identify novel grain properties that may allow product diversification in the market
- Decrease the cost of production for farmers – for example water usage
- Be able to compete with imports on price and quality.

Characterization of foreign germplasm may help USA rice breeding efforts to identify novel traits and incorporate these traits into USA breeding pools. An evaluation of West African germplasm that has previously not been introduced into the USA may reveal genetic resources that have unique quality traits (i.e. rice with slow digestibility) or are tolerant to reduced water usage (i.e. upland rice).

This study proposes to conduct genotypic and phenotypic evaluation of West African (WA) and USA germplasm for agronomic and quality traits in order to identify characteristics that can benefit rice breeding programs in these regions and to determine genetic marker associations with key cooking quality traits that can be used in rice cultivar improvement programs.

2. LITERATURE REVIEW

2.1. Rice in Africa

2.1.1. History of *Oryza glaberrima* in Africa

Most people are unaware that rice is indigenous to Africa. According to Buddenhagen (1978) "rice is not only Asian, rice is also African". However, the cultivated Asian rice *Oryza sativa* (L) is different from *Oryza glaberrima* Steudel, the African rice that was selected by farmers and has been grown in a diverse range of habitats in West Africa for several thousands years (Carney 2000). *Oryza glaberrima* is thought to be selected from wild annual rice *O. breviligulata* which was derived from the perennial *O. longistaminata* (Khush 1997). According to Portères (1956), *O. glaberrima* originated in the Niger River delta which is located within what is known today as the Mali Republic. The primary center of diversity for *O. glaberrima* is the swampy basin of the upper Niger River. Before the twentieth century, any rice cultivation in Africa was believed to be the result of the Asian influence. However, the Portuguese chronicler Gomes Eanes de Azurara mentioned rice cultivation in Africa when he traveled along the Gambia River in 1453 (Carney 2000). In 1855, the botanist Steudel gave the name *Oryza glaberrima* to the African rice because of the smoothness of the hulls (Chevalier and Roehrich 1914), but he did not mention that the rice had an African origin. Vavilov also failed to recognize West Africa as a center of rice domestication (Carney 2000). In 1914, French scientists began to recognize West Africa as an indigenous and independent center of rice domestication. Additional support came with linguistic evidence where names like *erruz, eruz, arroz, riz,* and *rijst* were commonly used in

regions where rice was not grown before the arrival of Europeans and Arabic people (Carney 2000). However, in other regions where rice was already being produced unique names like *Mano* in Mandinka or *Malo* in Wolof were common for rice. By the 1970s, work done by the early French botanists on *O. glaberrima* became fully recognized by the international scientific community and it was accepted that West Africa was an independent center of rice domestication (Carney 2000). The primary center of domestication is located in the inland delta of the Niger River in Mali and two secondary centers are located along the lower Gambia River and the Guinean highlands, and between Sierra Leone, Guinea-Conakry and Liberia. *O. glaberrima* produced along the Niger and Gambia Rivers was grown under irrigated conditions, whereas that produced in the Guinean highlands was grown under rainfed (upland) conditions.

2.1.2. History of *Oryza sativa* in Africa

Archeological data shows that *O. sativa* was domesticated some 7,000 years ago in Asia (Carney 2000). The exact area of the origin of *O. sativa* and its antiquity is not well documented and is still disputed.

Portères (1956), who discovered two foci of *O. sativa* introduction along the West African coast, concluded that Asian varieties were easily introduced into African rice farming systems because people there were already familiar with the cultivation of rice. Three main sources of introduction of Asian rice to Africa were identified. The first was probably from Malayo-Polynesia in 1-2 B.C. (Buddenhagen 1978). The second source was from Sri Lanka and India via Oman, then Somalia, Zanzibar, and Kilwa

probably 2,000 years ago. The third source was from Portugual about 1,500 A.D. into Senegal, Guinea-Bissau and Sierra Leone (Portères 1950).

2.1.3. Importance of Rice in Africa

Africa produces an average of 14.6 million tones of rough rice per year (1989-1996) on 7.3 million ha, equivalent to 2.6 and 4.6 % of the world's total production and rice area, respectively (FAO 1996). Nearly half of the rice production in Africa is located in West Africa where 3.7 million ha are cultivated. The regional contributions to rice production in Africa are: West Africa (42%); North Africa (32 %); East Africa (24 %); Central Africa (1 %); and Southern Africa (1 %).

Rice is the fourth most important grain crop in Africa (DeVries and Toenniessen 2001) following maize, sorghum and millet. However, in countries like Guinea Bissau, Sierra Leone, Cote d'Ivoire, Mali, Liberia, Guinea, and Senegal, rice is the most important source of carbohydrates. Nigeria has the highest production of rice in West Africa, while Madagascar is the leader in Southern Africa, and Tanzania is the main producer in East Africa. According to DeVries and Toenniessen (2001), the annual production in Nigeria, Madagascar, and Tanzania was 3,275,000 tons, 2,447,000 tons and 810,000 tons of rice respectively. Per capita annual rice consumption is very high in some countries like Guinea Bissau (112 kg), Sierra Leone (89 kg), Guinea (73 kg), and Gabon (72 kg). Because of the increased urbanization and relative economic growth, demand for rice in Africa has been increasing at an annual rate of 5.6% since 1962 (WARDA 1997).

Some years ago, rice used to be considered as crop linked to some ethnic groups or genders. Women were the most skillful in rice growing and processing. In Sierra Leone over 1500 rice growing methods were developed. The Temne people discovered that by felling the tidal mangrove forest, they can transplant rice seedlings and grow them when the early rains have cleaned the sea salt which was deposited during the dry season (Buddenhagen 1978). In Guinea, another method was developed by the Baga people. They constructed bundedfields to exclude the sea and allow salt flushing by the early rains. Rice was also transplanted on low ridges built by inverting slices of soil with traditional tools named "Kofi". In Liberia, fences were built using palm fronds to protect upland rice that was intercropped with vegetables against rodents. In West Africa –Mali, *crue (rise of water)* and *decrue (fall of water)* systems were developed to match varieties of correct duration and flood resistance to the rising and falling of floodwaters of the Niger River (Buddenhagen 1978).

Since 1920, three main phases of modernization of rice production were implemented by African farmers. The first phase was the development of large-scale irrigation projects in Mali, Senegal, the Guinea Coast, Madagascar, and Kenya. The second phase dealt with the introduction of agricultural mechanization during the 1950's and 1960's in the Sierra Boli and Southern grasslands developments, in Northern Nigeria, Ghana, and in Guinea. The third phase was the period of intensification of irrigated rice systems, primarily initiated in the 1960's with many small scale schemes were developed, mainly in Cote d'Ivoire (Buddenhagen 1978).

Problems that occurred during these phases included too much emphasis on engineering expertise and too little emphasis on high yielding varieties. The transfer of farmers into a new system of intensive rice cultivation was also a problem. Mechanization had certainly increased the farmer's income, but problems like soil erosion, lack of replacement parts, design inadequacies in Western machines, and shortage of management skills were also present. Also, African farmers were not protected by the political systems of subsidizing found in the European Economic Community and in North America (Buddenhagen 1978). Nowadays, some of these considerations belong to history, as we can now find large areas under irrigation like in Mali (Office du Niger) where 1,000,000 ha that can be fully irrigated. In Cote d'Ivoire, in Nigeria, in Mauritania, in Senegal, more rice is widely grown using irrigation facilities and newly adapted varieties are being developed by National and international research centers.

The increase in rice production was not linked to increase in yield (Figures 1, 2 and 3). Although rice production area has increased to about 70 millions hectares of wetland suitable for rice cultivation in Africa (www.fao.org), there is still a tremendous need for improvement in rice crop production. Over the last five years, Africa has imported 10 times more rice that it has exported (Fig. 4). However, advances in rice production will not come from increases in cultivated land alone; as effort also needs to be put into yield and grain quality. Problems like deforestation and soil erosion must also be considered, because they are linked with increases in production area and can have large destructive effects.

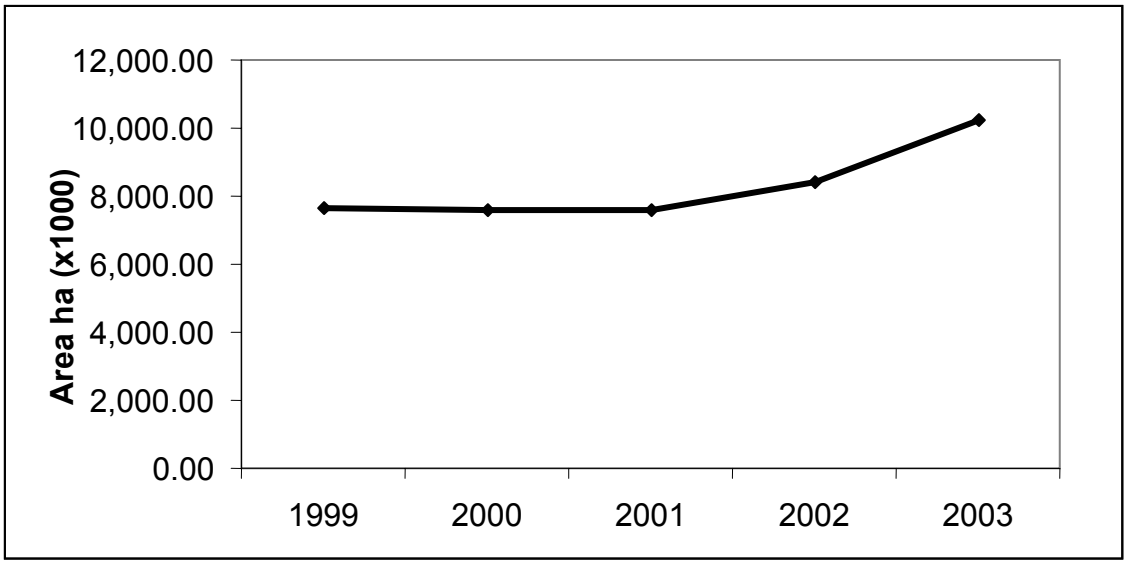


Fig. 1. Rice paddy area cultivated from 1999 to 2003 in Africa (source: FAOSTAT 2004).

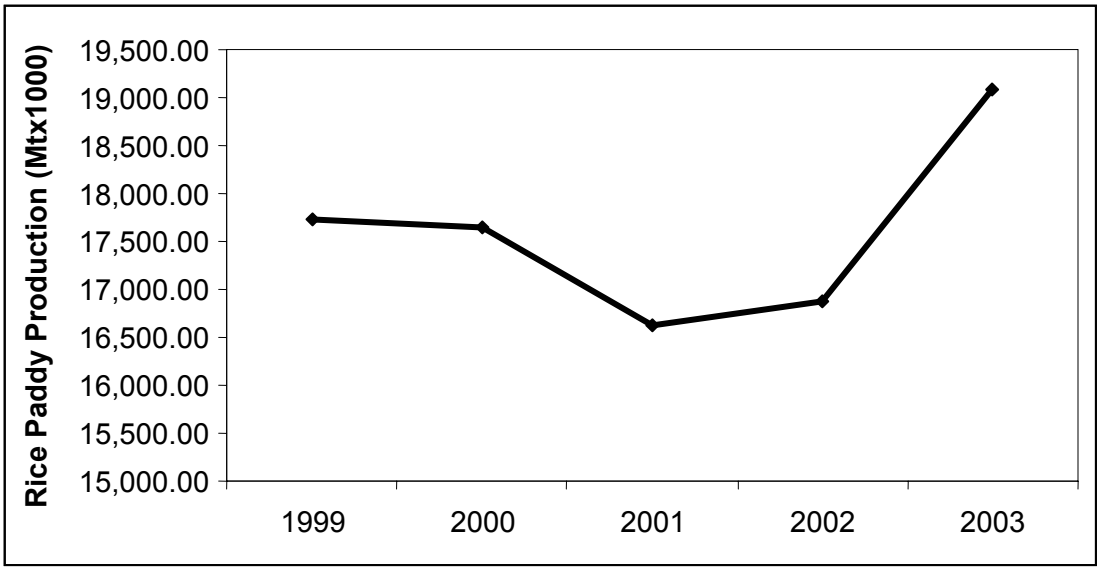


Fig. 2. Rice paddy production (Mtx1000) in Africa from 1999 to 2003 (source: FAOSTAT 2004).

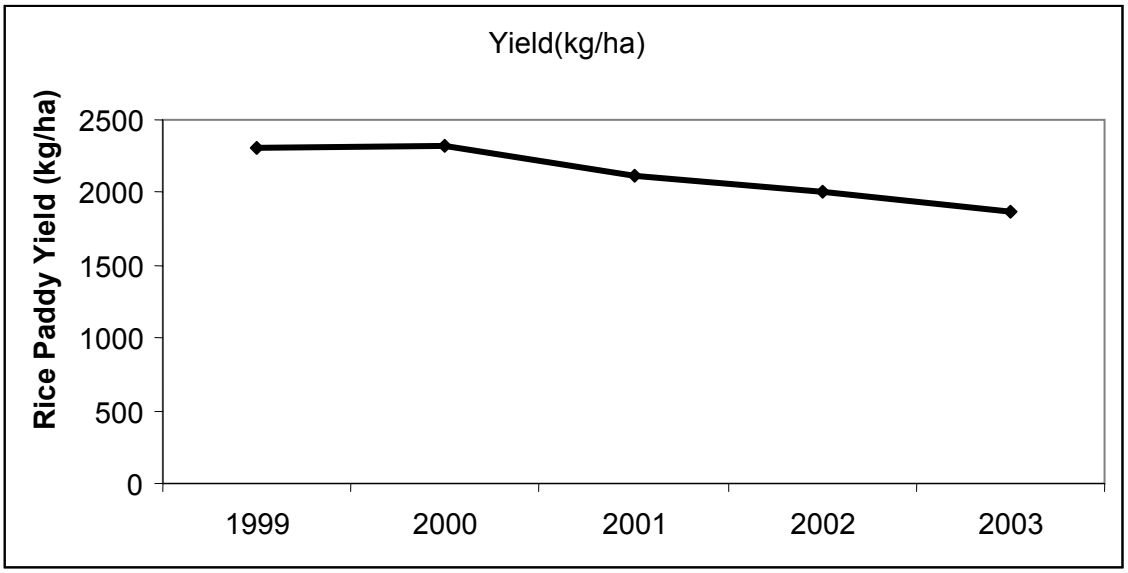


Fig. 3. Rice paddy yield (kg/ha) from 1999 to 2003 (source: FAOSTAT 2004).

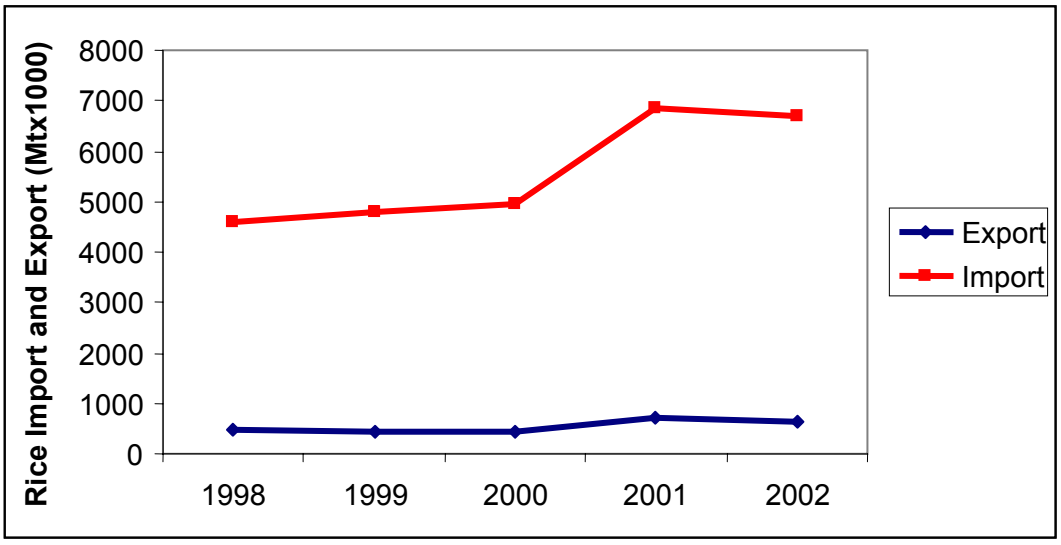


Fig. 4. Rice import and export (Mtx1000) from 1998 to 2002 (source: FAOSTAT 2004).

2.2. Rice Ecosystems in Africa

The total arable land in Africa is 637 million ha (Okigbo 1982) with 68% of this being set aside in reserves. Africa has ample potential to extend its agricultural and rice production. The rainfed (upland) ecosystems accounts for 60% of the total rice area. According to FAO African Regional Office (www.fao.org), the wetlands in tropical sub-Saharan Africa have a total area of 240 million ha. The wetlands can be subdivided into four categories: the coastal wetlands (16,500,000 ha), inland basins (107 million ha), river floodplains (30 million ha), and inland valleys (85 million ha). The inland basins and inland valleys constitute 45 and 36% of the wetlands in Africa and they have great potential of rice production development in Africa. In Africa, a large amount of land borders rivers like the Gambia, Niger, Benue, Congo, Zambia, Limpopo, Tana, White and Blue Nile and Chari Rivers. This land is well suited for rice production since these rivers have well-developed large floodplains in their central and lower stretches. Scientists have grouped the different systems of rice culture in Africa in various ways (Chabrolin 1976 and 1977). The West Africa Rice Development Association (WARDA) has done tremendous work since its creation in 1971 in developing rice cultivars and technology for these different systems (WARDA 1993, 1998).

2.2.1. Dryland Ecosystem

Upland rice is produced using natural rainfall, without any artificial irrigation, and, thus, the major constraint on production is the amount of rainfall (Charpenter 1978; Poehlman and Sleper 1995). The upland rice area is characterized by soils with low water-retention capacity, and poor fertility. The immediate negative consequences of the

rice cultivation in this area are rapid deforestation and destruction of watersheds. This area is the most extensive rice ecosystem in Africa. It is predominant in West Africa and covers 57% of the total area and accounts for 44% of the total regional production (WARDA 1993). The major producing nations in this area are: Sierra Leone, Cote d'ivoire, Liberia, Guinea-Bissau and Nigeria. The average yield is only 1 ton/ha due to soil acidity, weeds, and rodents which can cause severe yield losses. Farmers applying moderate inputs can achieve yields of 2.5 tons/ha. The system may be further improved by the introduction of grain-legumes to the system. The West Africa Rice Development Association has already initiated rotation systems in Cote d'ivoire (WARDA 1997) where it was found that *Crotalaria anagyroides* rotation can increase rice yields by 50%. Crop residue management, use of water-harvesting methods, erosion control methods, and the use of tolerant varieties to biotic and abiotic constraints may improve rice production in the dryland ecosystem.

2.2.2. Lowland/Hydromorphic Ecosystem

The total area covered by the inland valleys system in Africa is 130 million ha (WARDA 1993). From this area, 19 million ha (15%) is within West Africa. The lowland/hydromorphic ecosystem occurs from the mid-slope to the valley bottom of the topo-sequence. Three sources of water are available for the ecosystem. The first source is from direct rainfall, the second is from high water tables, and the third is from surface water. This ecosystem is characterized by fluctuating water tables due to the cyclical swelling and receding water levels of rivers during the rain seasons. Sometimes, the depth of the water can reach 50 to 100 cm (Poehlman and Sleper 1995). Problems found

to this ecosystem include iron toxicity due to a proper lack of drainage; excess standing water/water inundation that can carry away the harvest; and weeds. The system can be improved by the use of rice varieties tolerant to iron toxicity. The average yield in this ecosystem is 1.5 to 5 tons/ha.

2.2.3. Mangrove Swamps Ecosystem

Mangrove swamps cover an area of 1.2 million ha and only 193,000 ha have been developed (WARDA 1993). This ecosystem is found mainly along the West African coast. The major problems dealing with this ecosystem is the high salinity levels due to saltwater intrusion brought by the tidal waves. There is a salt-free period of over 4 to 6 months that allows the crop to grow. Other problems include crop lodging, acidity, iron toxicity, aluminum and manganese toxicity, and disease. In this ecosystem, the average yield is 1 to 2.2 tons/ha.

2.2.4. Irrigated Ecosystem

Rice in the irrigated ecosystem is grown using water supplied by ground irrigation to supplement rainfall such that the standing depth of water in a field is typically 15 cm (Poehlman and Sleper 1995). The irrigated ecosystem is the most reliable production system of rice cultivation in Africa. The vast area in the West Africa lacks fully developed irrigation system. A gradual increase in the 231, 000 ha observed in the irrigated ecosystem in 1980-84 is expected (WARDA 1993). Some African countries have large amounts of irrigated land planted to rice, especially Egypt, Niger, Mauritania with 100%, and Madagascar with 31% of its area irrigated (WARDA 1993). The major problems linked to the irrigated systems are: nutrient deficiencies, acidity,

weeds, diseases (including Rice yellow Mottle Virus RYMV), blast, sheath rot, and bacterial leaf blight) and insects (including gall midge, and stem borers). According to Barr et al. (1975), yield lost due to insects can reach 33.7%.

A yield of 5 to 7 tons/ha can be achieved by progressive farmers employing moderate to high input management (fertilizer, pesticide, seed, and water management) (WARDA 1993) in this ecosystem.

2.2.5. Deepwater/Floating Rice Environment

Deepwater rice ecosystems are characterized by flooding that can reach a depth of 1 to 5 m during the rainy seasons. Usually, the leaves of the rice float on the surface of the water and the rice is characterized by an extensive elongation of the stems. (Poehlman and Sleper 1995). Typical floating rice systems are found where water can exceed 100 cm. The deepwater and floating rice systems are generally marginalized. According to WARDA (1993), the production area was estimated to be 630,000 ha in 1970. During the last 20 years (WARDA 1993), the control of river flooding due to the construction of dams and also the frequent drought periods in West Africa are factors that have contributed to decreasing area of the deepwater/floating systems. The average yield in these systems is around 0.7 and 0.9 t/ha. The major constraints are water control, low yielding varieties, and low fertilizer use efficiency (WARDA 1997).

2.3. Principal Constraints to Rice Cultivation in Africa

Demand for rice in West Africa has been growing at the rate of 6% per year since 1973 (Nwanze 1996). Increased consumption is due both to population growth and to the increased proportion of rice in the West African diet. In an attempt to keep pace with

demand, production of rice in West Africa has been expanding rapidly, growing at 5.1% per year, faster than any of the other principal staple food crops. Despite the growth in regional production already achieved, imports of rice have grown at the alarming rate, averaging 9% a year for two decades. Over 60% of the cultivated rice area in West Africa is found in the upland and rainfed lowland type ecosystems (WARDA 1993). As a low-input system, upland ecosystems tend to not use fertilizer and rely solely on rain. Under these conditions, diseases are considered a major problem as well. Lowland systems use water from rainfall, subsurface water tables, and surface water. By using fertilizer and controlling pests, lowland areas have the potential to produce far greater yields than the upland ecosystem (WARDA 1993).

While breeding rice varieties that are adapted to meet some of the basic climatic and edaphic (drought, nutrient poor or toxic soils) constraints of the African continent has helped, the yield of rice per unit area is only 49% of the global average. Unlike more developed regions of the world, insects and diseases result in a larger drain on yields and the problems may persist longer. The principal constraints dealing with rice cultivation in Africa can be categorized into biotic and non biotic constraints.

2.3.1. Water/Drought

According to Moormann and Veldkamp (1978), rice growth needs 600 mm of rain to complete its growth. Water is a limiting factor in upland/dryland rice cultivation areas but soil characteristics also play an important role in these areas. Varietal traits that can avoid drought are very important. Recent release of early maturing progenies from interspecific crosses between *O. glaberrima* and *O. sativa* named New Rice for Africa

(NERICA) will give farmers the chance to grow rice in areas having limited rainfall (DeVries and Toenniessen 2001). Traditional varieties like 'Moroberekan' are still used in large areas for their adaptation.

2.3.2. Weeds

Herbicides are rarely used in Africa, and most farmers do not use mechanized equipment to culitvate rice. Considering these factors, weeds are considered as the greatest yield reducing factor in areas where rice is directly seeded (WARDA 1997). Weeds can reduce productivity from 25 to 40% and total crop failure is possible if weeds are left uncontrolled (WARDA 1999). WARDA reported that 27-37% of the entire labor for rice cultivation is due to weed control. According to DeVries and Toenniessen (2001), rice is a small plant as compared to sorghum or millet or maize. It is slow to establish a full canopy and, therefore suffers severe damage from competition with weeds for light, moisture, and soil nutrients. Certain varieties among the NERICA selections have droopy leaves that can help shade out weed competition in upland areas. The variety 'IG10' is an *O. glaberrima* type that has this phenotype, but its yield is low. Proper water management in irrigated conditions can help control weeds. Nowadays, in most of the irrigated systems (mainly in Mali), rice is transplanted into flooded fields, which greatly reduces weed competition, but this is a labor intensive technique.

2.3.3. Blast Disease

Among the fungal diseases that attack rice, blast (caused by *Magnaporthe grisea* Cav), is the most serious affecting rice in Africa. The variability of the pathogen has caused research on resistance to be complicated (Mackill and Bonman 1992). Rice blast

can result in a significant yield loss of about 70-80% under severe conditions (Ou 1985). A significant reduction in kernel bulk density and rice yield was noted (Candole et al. 2000). Unfilled and fissured kernels were also identified due to the effect of blast. More than 20 blast resistance genes have been identified through different techniques and mainly through molecular genetic analysis (Wang and Leung 1999). The airborne spores of the rice disease produce lesions on leaves that are able to spread quickly (Poehlman and Sleeper 1995). Most of the traditional varieties grown in Africa have resistance that prevents massive outbreaks (WARDA 1997). However, newly developed varieties for the different ecologies suffer from blast attacks. The use of resistant cultivars is the most appropriate approach for managing blast disease. The cultivar Moroberekan, which originated in West Africa, is one of the most widely used cultivars as source of resistance to blast (Barman et al. 2004).

2.3.4. Insects

Among the different insects damaging the rice crop in Africa are the African rice gall midge, stemborers, leaf feeders, and spider mites. The African rice gall midge (*AfRGM*) (*Orseolia oryzivora* Harris and Gagné) (Diptera: Cecidomyiidae), is an endemic pest whose current range is confined to the continent of Africa (Umeh and Joshi 1993). Until 1980, this pest had been incorrectly identified as the Asian rice gall midge, *Orseolia oryzae* (Wood-Mason), but close examination showed that these two species are distinct based on morphological characteristics (Harris and Gagné 1982) and that all previous records of *O. oryzae* in Africa were actually specimens of *O. oryzivora*. The gall midge had generally been considered a minor pest in Africa, but shortly before the

midge was correctly identified; unusual levels of infestations were recorded in West Africa (Feijen and Schluten 1983). In the late 1980's, the African rice gall midge continued to change from an occasional nuisance to the most important pest of rice in some areas of Africa (IITA 1991), capable of causing total yield loss in outbreak situations (Ukwungwu et al. 1989). It can attack and damage rice in a variety of cultivation types, but is mainly considered a pest of lowland (swamp) rice that is rainfed or irrigated. Currently, African rice gall midge is found in 20 countries across Africa, and its severity as a perennial rice pest is now considerable (WARDA 2001).

Crop losses have been evaluated under both caged and field conditions Under cage conditions, every 1% in damaged tillers resulted in a 2% loss of yield (Nacro et al. 1996). Farmer-managed field trials suggest an even bleaker scenario, with a 2.9% loss of yield for each 1% increase in midge infestation (Williams et al. 1999a). Insecticides are also used to control the gall midge, but systemic insecticides (those transported throughout plant tissues) must be used since the midge is hidden in plant tissue for most of its life cycle. Repeated applications of carbamate or organophosphorus insecticides are often necessary to control African rice gall midge at two or three week intervals (Williams et al. 2002). However, because of costs, environmental and health risks, and the destruction of natural control agents of the midge, insecticides may not be a practical or sustainable solution for African farmers. Cisadane (= Faro 51) and BW 348-1 are varieties with partial resistance to the gall midge, and these are often used in known areas of high gall midge activity. Neither variety is high-yielding, nor is the resistance strong, but Cisadane has shown up to a 26% yield improvement versus susceptible

varieties (WARDA 1998). Varieties from *O. glaberrima* germplasm like TOG 7106, TOG 7206, and 7442 have shown excellent resistance to AfRGM in West Africa.

2.3.5. Grain Quality

Although rarely mentioned in Africa as a constraint, rice quality is considered the second most important problem following yield. However, even varieties with high yield are rejected by consumers because of their poor cooking and nutritional quality. Rice production in Africa is becoming more market oriented where quality becomes a major issue. Rice is generally consumed as a whole grain and physical properties, cooking quality, and sensory traits are very important. Grain quality is composed of components such as nutritional aspects, appearance, cooking quality, and eating quality (Tan et al. 1999). These quality aspects are related to the physical-chemical properties and the history and cultural values of the people who are consuming a particular type of rice. In some areas of West Africa, rice which has a short cooking time and slow digestibility is preferred by consumers. Aroma is another main component that is desired by consumers in West Africa. Cooking yield, kernel expansion, and cooked texture are important factors in breeding rice for African consumers. In some parts of Africa like South West Mali, Guinea, Sierra Leone, North of Cote d'ivoire, parboiled rice is preferred whereas the Senegalese prefer broken rice for a common dish known as fried rice with fish "*Chebu jen*".

2.4. Parameters Which Define Rice Grain Quality

2.4.1. Rice Market Classes

Most of the rice produced in the world is consumed as a whole grain and therefore the grain physical and chemical characteristics are very important. There are different market classes of rice that are defined by a matrix of traits which include grain dimension, grain chemistry, and grain appearance. *Long Grain Rice* has kernels which are 3 to 4 times longer than their width and relatively high amylose content (>20%) which causes the grains to remain separate when they are cooked. In the USA, certain long grain cultivars (e.g., Rexmont, Dixiebelle) with a high amylose (>24%) are recommended for canning purposes (Webb 1991). *Medium Grain Rice* has kernels 2 to 2.9 times longer than their width and an amylose content that is relatively low (16-18%). *Short Grain Rice* has grain that is almost round with the kernels being 1.9 times longer than their width. Kelly (1985) reported that medium and short grains are used for products that are served cold. *Glutinous Rice* is also called *Sweet* or *Waxy Rice* and the kernels are completely opaque white. The grain is essentially composed of amylopectin and no amylose. *Aromatic Rice* possesses a natural flavor that is similar to buttered popcorn in aroma. The most popular types of aromatic rice are Basmati from India and Pakistan and Jasmine from Thailand. *Arborio Rice* is another specialty rice originally from Italy that has very large and bold kernels with large chalky centers. In addition to being consumed as a whole grain, rice flour and starches are also used in the ingredients industry. The primary chemical components of the grain are starch, protein and lipids. These components determine how the rice whole grain, flour, or starch can be used.

2.4.2. Components of Quality

2.4.2.1. Hull and Bran Color

Hull color does not mean a lot in producing regular white milled rice, but it influences parboiled rice (Luh 1991). Produced under the same conditions, a light-colored (straw) hull gives lighter-colored parboiled end product than dark-hulled varieties of rice. Bran (pericarp) color plays a similar in parboiled products. To remove the influence of bran color and produce a very white product, millers must apply deep milling which may also reduce the yield.

2.4.2.2. Grain Dimensions, Weight and Uniformity

According to (Adair et al. 1973; USDA 1989b), grain dimensions are among the most important factors affecting the processing, drying, handling, and grading of rice. Deep creases can result in bran streaks after milling. Non-uniform grain size results in kernels being over- or under-milled. Sharp-pointed extremities on kernels will break off during milling as will large germs (embryos) on kernels, resulting in reduced milling yields. According to Adair et al (1973), milled grain rices are grouped into long grain with (6.61-7.5 mm) length and 3.1 and more as length-width ratio. The medium grains have a length of 5.51 to 6.6 mm and a length-width ratio of 2.1 to 3. The short grains have up to 5.5 mm length and a length to width ratio of 2.0 and less.

2.4.2.3. General Appearance

Factors contributing to general grain appearance are grain size, shape, uniformity, vitreousness, translucency, chalkiness, and color, damaged and imperfect

kernels (Luh 1991). Intensive work is done by breeders to select for bright, clear, translucent kernels (Luh 1991).

Chalkiness in rice can be defined as" white belly", "white core", "white back", "germ tip", or "immature" (Webb 1991). Chalkiness is undesirable and results in reduce milling yield. Many factors contribute to grain chalkiness including moisture level, immature kernels, weather conditions, and cultural practices as well as varietal characteristics (Kushibuchi and Fujimaki 1975).

2.4.2.4. Milling Yield

Milling yield of rice is considered to be the most important component of quality (van Ruiten 1985; Adair et al. 1973; Spadaro et al. 1980). Whole-kernel (head) yield is defined as the amount of intact whole kernels that are three quarters or more in length. Total milled rice is the amount of whole kernels (head rice) and any other sizes of broken kernels from a defined quantity of rough rice. The milling properties of rice are are largely affected by grain shape (Bashyam et al. 1984). The critical moisture content below which the grain is susceptible to breakage during milling is 12-16% (Juliano and Perez 1993). Degree of milling is defined as the extent of bran layer and germ removal from the endosperm. Three main classes of milling are found: well milled, reasonably well milled, and lightly milled. Many methods of measure exist, but none of them is widely used (Webb and Stermer 1972). However, the degree of milling (i.e. the amount lipids and protein left on the milled kernel) can affect cooking properties of rice flour.

According to Hosney (1998), one percent (1%) change in breakage can cause a $100,000 difference in profit for an average-sized rice mill.

Genotype and environment are the two main factors that influence rice quality. According to Webb (1985) the different characteristics determining rice quality include hull and pericarp color, grain size and its shape, grain weight, uniformity, appearance, milling properties, kernel chalkiness, translucency, color, cooking, eating and processing quality, cleanliness, soundness, purity.

2.4.2.5. Rice Cooking and Eating Quality

According to Zhang and Yu (2000) and Setiningsih et al. (2003), cooking and eating quality are the most important components of rice quality. It is very difficult to define quality because it involves objective and subjective criteria. Based on Kaosa-ard and Juliano's findings (1991), country and culture result in different preferences for rice quality. The primary constituents of cooking and eating quality are: amylose content (AC), gelatinization temperature (GT), gel consistency (GC), grain appearance, cooked grain elongation, and fragrance of cooked rice.

2.4.2.6. Amylose Content

Sanjiva Rao et al (1952) were the first to identify that there was a possible relation between amylose and rice quality. Amylose content, also called apparent amylose or amylose-to-amylopectin ratio, is the most important element influencing the cooking quality of rice (Bao et al. 2001). The amylose content of rice is known to play a crucial role in determining its cooked texture and processing functionality.

Zhang et al. (2003) reported that amylose content varied among grains from the same panicle. It varied also between outer and inner layers of the same grain. Grains harvested from the upper position, the first branches or early flowers tend to have higher

amylose content. The trends appeared to be the same for both *Japonica* and *Indica* rice types. Amylose content was found not to be affected by timing of harvest or length of grain storage (Juliano 1971; Muraue 1997).

The *Waxy* gene encodes the enzyme granule bound starch synthase (GBSS). According to Smith et al. (1997), GBSS controls amylose synthesis in the grass family and typical cereal grains contain about 20-30% amylose and the remainder, 70-80%, is amylopectin (Preiss 1990).

According to Sano (1984), the final product of GBSS is amylose. Juliano (1971) concluded that rice germplasm can be classified in different groups based on the amylose content. Waxy rice has 1-2% amylose and non-waxy has more than 2% amylose content. The non-waxy rice is then divided into different groups which are: very low amylose (2-10%), low amylose (10-20%), intermediate amylose (20-25%) and high amylose (>25%). According to Bollich and Webb (1973), many USA consumers prefer long grain rice that cooks dry and flaky. This type of rice is characterized by intermediate amylose content. The short and medium-grain types of rice are preferred by some USA consumers, and these rices have an amylose content of 15 to 20%.

Numerous studies indicate that amylose production in rice, maize, and potato depends on the activity of the GBSS protein (Tsai 1974, Echt and Schwartz 1981, Sano 1984, Rohde et al. 1988), encoded by the *Waxy* gene. In their studies, Delrue et al. (1992) conclude that GBSS also plays a role in the production of amylopectin. Patron et al. (2002) concluded that amylose is synthesized by GBSSI in cereals, which is an isoform of starch synthase of about 60 kD.

In cereals, the majority of the endosperm is made up of starch, comprised of long linear primarily-unbranched polysaccharide chains of glucose called amylose and branched polysaccharide chains of glucose called amylopectin. Waxy endosperm mutants have starch consisting of amylopectin only, with no amylose. Waxy rice is also called glutinous rice or sweet rice. This type of rice does not expand in volume, is very sticky and is very soft when cooked. Rice with intermediate amylose called non-waxy or non-glutinous rice is firm when cooked. High amylose rice becomes hard when cooked (Rao et al. 1952; Williams et al. 1958; Sood et al. 1983). Two types of alleles in the *Waxy* gene have been identified in non-glutinous rice. Rice lines carrying the Wx^a allele are mostly found with indica rices and they have high amylose content. The Wx^b allele is primarily found in japonica type rice and it contains low amylose (Lanceras et al. 2000). Villareal and Juliano (1993) found GBSS is less active in japonica which has the Wx^b allele than in indica with the Wx^a allele. Starch granules of similar AC showed the lower content of waxy gene product in japonica compared to indica rice (Juliano 1980). The Wx^{op} allele was discovered by Mikami et al. in (1999). It characterizes opaque endosperms which look like waxy rice. Amylose content may reach 10% in opaque endosperms contrary to no amylose in waxy rice.

Umeda et al. (1991) compared *Wx* genes of *O. glaberrima* and *O. sativa*. They found two different peaks: the regions corresponding to exons in each of the *Wx* genes were CG rich, while the regions corresponding to introns were AT rich. Umeda and colleagues (1991) studied the waxy genes in nonwaxy and waxy strains of *O. glaberrima*. They concluded that the waxy mutant had the *Wx* gene with a single base

substitution in exon 10. It had a premature termination codon in the coding region for the GBSS protein. The results showed that *O. glaberrima* or *O. sativa* had a deletion (insertions) of 12-bp sequence in intron 11, TGCAA sequence was reiterated. Contrary to the results found by Sano (1984), Umeda et al. (1991) concluded that there were no differences in nucleotide sequences of *Wx* genes of *O. glaberrima* and *O. sativa*. The sequences of their *Wx* genes were similar. Wang et al. (1990) came out with similar results. Watanabe and al. (2002) declared that Asian rice *O. sativa* had larger range of amylose than the African rice *O. glaberrima*. The interspecifics (Asian x African) had an amylose content 14% lower than that of their African parent. A wider range of distribution of amylose was found in the interspecifics compared to theirs parents (African and Asian). Watanabe and colleagues found there were more interspecifics with low amylose content.

According to Harrington et al. (1997), amylose and amylopectin are glucan polymers. The linear chain α (1-4) is found in amylose. Based on Martin and Smith results in 1995, it was concluded that amylopectin is formed when an α (1-6) linkage is made by starch branching enzyme (SBE) between the reducing end of one glucan chain and the C6 of a second glucose residue. Contrary to amylose, amylopectin is a highly branched glucan chain. Starch branching enzymes were grouped into two families according to their structure and functions (Burton et al. 1995; Fisher et al. 1966a; Martin and Smith 1995; Preiss 1991). Family A includes rice SBEIII, maize SBEII, and pea SBEI, and SBE2.1 and 2.2 for *Arabidopsis*. In family B, rice SBEI, maize SBEI, potato SBE, cassava SBE, and pea SBEII are included. In the studies of Yano et al. (1985) and

Mizuno et al. (1993), two isoforms of SBE (SBEI and SBEIII) known as Rice Branching Enzyme (RBE) were identified in rice. When rice is lacking SBEIII, it has exceptional high amylose content (29-35%). Very high amylose level is created by the amylose extender gene (*ae*). A proportion of 70% of amylose is found with the phenotype of *ae* maize mutant. (Scanlon et al. 1994). According to Juliano (1980), the *ae* mutant EM 129 of Kinmaze which had 35% amylose content had the typical Wx^a allele. The molecular weight based on faint was MW 60,000 waxy gene protein band.

Juliano and Pascual (1980) concluded that temperature can cause a variation of 6% in apparent amylose content. The occurrence of chalk in rice grain was increased by high temperature during specific stages of grain development (Tashiro and Ebata 1975; Tashiro and Wardlaw 1991). It was declared that high temperatures during grain development can cause a decrease in amylose content (Lisle et al. 2000). Larkin and Park (1999) concluded that amylose content of *Japonica* rice increases when the grain develops at relatively cool temperatures, 15-20 ^{o}C. There was no clear relationship between temperature and amylose in *Indica* varieties (Asaoka et al. 1984; Wang and Wessler 1998).

Several methods are known to determine the amylose content of starch. However the most widely used is the calorimetry method of the blue amylose-iodine complex. It is economical and fast (Juliano et al. 1987). The problem encountered with this method is its interference with lipids. Lipids are bound to the amylose and to determine the true amylose we need a defatting process. Takeda et al. (1987) concluded that long chains of amylopectin could also increase the apparent amylose content in exceptional situations.

It is more correct to call the defatted amylose “apparent“ amylose. According to Williams et al. (1958), the iodine-blue value is a good index to quantify amylose content.

In 1968, Juliano and colleagues declared that blue –iodine did not correlate with amylose when the amylose content is higher than 30%. Blue-iodine value was then considered as a measure of the dissolved amylose (Bhattacharya et al. 1972). The water solubility of the amylose was then considered to make the difference of blue-iodine value. Three classes of solubility were found with high amylose content varieties: 40, 50, and 60%. Intermediate and low-amylose varieties of rice had a solubility of 55 to 65% (Bhattacharya et al. 1972, 1982). Hot-water insoluble amylose or insoluble amylose is found by subtracting the soluble amylose from the total amylose. The soluble amylose was extracted at 96 ^{o}C. The term apparent amylose (AA) was used by many scientists (Takeda et al. 1987, 1989). Reddy et al. 1993 would rather use the term amylose equivalent (AE) which was more appropriate to him. They declared that soluble AE represented the true amylose of rice starch. The insoluble AE was the blue iodine influence due to the amylopectin. The iodine affinity and amylose content is inflated because long chains of amylopectin can form a helical complex with iodine. Takeda et al. (1987) concluded that the true AC of low-and intermediate AC starches (16-18%) was similar to that of high AC. He said that the iodine affinity was a fact of amylopectin.

Seguchi et al. (2003) concluded that amylose plays an important role in the maintenance of the structures of starch granules. The principal elements that compose starch granules are amylose, amylopectin, lipids, and proteins. When a waxy rice starch

is boiled, the product is viscous and elastic. The swollen trait could be due to the characteristics of amylopectin.

He et al. (1999) said that the "inheritance of grain quality is more complicated than that of other agronomic traits in cereals due to epistasis, maternal and cytoplasmic effects, and the triploid nature of endosperm".

Chen and Zhu (1999) reported that quality characters are controlled by several genetic systems like nuclear genes of maternal plants, nuclear genes of endosperms, and cytoplasmic genes. This fact renders the inheritance of quality characters very complex. The grain of rice is composed of several tissues, the pericarp and testa is diploid maternal tissues; the embryo is diploid hybrid tissues; the endosperm is triploid hybrid tissues. The genetic control of cooking traits (amylose content, Gelatinization temperature, and gel consistency) in the crosses involving indica and japonica had direct genetic effects, maternal effects, and cytoplasmic effects Chen and Zhu (1999).

Different studies showed that high levels of amylose are controlled either by partial or complete dominance. The identification of heterozygotes is not possible with phenotypic data (Bollich and Webb 1973; Mckenzie and Rutger 1983; Pooni et al. 1993; He et al. 1999; Bergman et al. 2001). One major gene and several modifiers were identified with high amylose content incompletely dominant over low amylose content (Seetharaman 1959; Kahlon 1965; Bollich and Webb 1973; Chang and Li 1981; Chauhan and Nanda 1983). Heda and Reddy in 1986 concluded that amylose content is governed by two pairs of genes; high amylose was partially dominant over the low amylose. Tan et al. (1999) reported that amylose content was clearly under the control of

a single locus in elite hybrid rice, Shanyou 63, where high amylose appeared to be a dominant trait. He et al. (1999) found that a major gene and a QTL for amylose content was located on rice chromosome 6 explaining 91.1% of the total variation. Results from McKenzie and Rutger (1983) showed that high amylose was controlled by one gene of major effect and many modifiers. High amylose was dominant over low amylose content in their crosses. Kumar and Khush (1987) reported the same conclusion that high amylose was dominant over low amylose and intermediate amylose content.

Apparent amylose content (AAC) was found to be controlled by an allelic series at one locus with major effects and modifier genes with minor effects (Bollich and Webb 1973; Mckenzie and Rutger 1983; Wang et al. 1995). The results from Stansel (1965) indicated that amylose content was controlled by two complementary genes in some crosses. They found transgressive segregates for high amylose; this indicated that modifiers were present. Pooni et al. (1993) concluded that amylose content is complex and it is transmitted disomically; the expression is in a triploid phase in the endosperm. They concluded that it is not possible to use the standard diploid model to study the genetical control of amylose. Cytoplasmic control of amylose content was clearly defined by Pooni et al. (1993). The dosage effect was studied by Heu and Park in 1976. Different endosperm genotypes were developed, *wxwxwx*, *wxwxWx*, *wxWxWx* and *WxWxWx*. They concluded that the dosage effect of Wx allele had additive action on the amylose content, but amylose content was not directly proportional to the number of *Wx* dose.

Lanceras et al. (2000) reported four QTL for AC. A major QTL was at the *Waxy* locus, which explained most of the variation in AC. Other minor QTL for AC were detected on chromosomes 3, 4 and 7. Lee et al. (2000) concluded that 3 QTL for AC were located on chromosome 1, 6, and 11, with the majority of phenotypic variation in AC explained by the *Waxy* locus on rice chromosome 6.

The $(CT)_n$ repeat number in a microsatellite within the rice *Waxy* gene was found to explain a large amount of the variation of AAC in 89 US nonwaxy rice (Ayres et al. 1997). A total of seven *Wx* microsatellite alleles were identified by Ayres et al. (1997). The $(CT)_n$ polymorphic microsatellite in the waxy gene was identified by Bligh et al. (1995). Low amylose rice from temperate japonica classes known to carry the Wx^b allele had 18 or 19 CT repeats in the *Waxy* microsatellite. Intermediate-amylose types from tropical japonica and carrying the Wx^a had 14 or 20 CT repeats. Indica types with high amylose, carrying Wx^a had 8, 10, or 11 CT repeats. Few cases were found with intermediate amylose content and with 10 or 11 CT repeats (Bao et al. 2002; Olsen and Purugganan (2002). Ayres et al. (1997) concluded that nearly all the entries in the $(CT)_{18}$ group had 14-19% amylose, $(CT)_{14}$ or $(CT)_{20}$ group had 20-23% amylose content, and $(CT)_{11}$ group had an amylose content greater than 23%. Diverse amylose contents were found with the types of rice with the $(CT)_{17}$ and $(CT)_{19}$ classes. Bergman et al. (2001) concluded that waxy rices with high amylose had $(CT)_{10}$ and $(CT)_{11}$ allele, intermediate amylose types had $(CT)_{14}$ and $(CT)_{20,}$ and rice with low amylose content had $(CT)_{17}$ and $(CT)_{18}$. Tan and Zhang (2001) declared that japonica entries had larger CT repeat numbers while indica entries had shorter CT repeats.

Bao et al. (2002) concluded that all the waxy rices are characterized by the AGTTATA sequence at the putative leader intron 5' splice site; they also declared that not all the varieties of the nonwaxy did not have the sequence AGGTATA. Varieties with less than 18% apparent amylose content (AAC) had the sequence AGTTATA (Ayres et al. 1997). They concluded that accessions with $(CT)_{17}$ or $(CT)_{18}$ had only the AGTTATA sequence. Accessions with $(CT)_{19}$ had AGTTATA and AGGTATA together. Bao et al. (2002) found a nonwaxy accession $(CT)_{17}$ with the AGGTATA sequence and AAC of 19% which was an exception.

A new SSR motif (AATT)n in the leader sequence of the *Wx* gene in the first intron at 182 bp downstream of the (CT)n repeat was identified by Wang et al. (1990). Two alleles $(AATT)_5$ and $(AATT)_6$ were found with cultivated rice. The different results showed that the (CT) repeats and the (AATT)n repeats were inversely correlated. Many of the varieties with high amylose content had the $(AATT)_6$ allele, in the other hand varieties with low AC had the (AATT)5 allele. According to Wang et al. (1990); Cai et al. 1998 the (CT)n is located in the first exon of the leader sequence. It is not far from the start site. The (AATT)n motif is located in the intron of the leader sequence.

Sano (1984) concluded that waxy (non-glutinous) phenotypes of rice were under the control of a single recessive gene (*wx*). The two alleles are Wx^a and Wx^b, as referred to above. It was declared that Wx^b was the result of a point mutation (G to T) at the 5' splice junction of the first intron (Wang et al. 1995; Ayres et al. 1997; Bligh et al. 1998; Hirano et al. 1998; Isshiki et al. 1998). During their experiment on indica rice, Mikami et al. (2000) concluded that Wx^b was found to reduce the level of expression of the *Wx* gene

when it is compared with Wx^a. In the indica background, it can be said that the allelic difference plays an important role in variations in amylose content. Mikami et al. (1999) concluded that the W^{op} was responsible for the opaque endosperms. The opaque endosperms were controlled by an allele of the *Wx* gene. The Wx^{op} had a higher level of amylose in the japonica rice than in the indica rice (Mikami et al 1999; Mikami et al. 2000). Dung et al. (2000) found Wx^{in} different from Wx^a, Wx^b or Wx^{op}. The Wx^{in} alleles found in NILs produced rice with intermediate level of *Wx* gene and the amylose contain was 20.3-22.5%. Intermediate amylose content was controlled by a major gene, modifiers were found to be involved. The expression of *Wx* in gene was intermediate between Wx^a and Wx^b.

During seed development, it was declared that amylose synthesis in rice containing the Wx^b allele is affected by temperatures (Asaoka et al. 1984; Inatsu 1979; Sano et al. 1985; Umemoto et al. 1995). The level of *Wx* protein activity is increase under cool temperatures with amylose production increased when seeds mature in cool temperatures. Cool temperatures have no effect on the Wx^a gene (Sano et al. 1991). There is a high correlation between the accumulation of *Wx* protein and the amylose content (Hirano and Sano 1998). Chikubu (1995) reported that of the presence of very high (>30%) amylose greatly reduces rice grain quality. Suzuki et al. (2002) were able to isolate and characterize a rice mutant insensitive to cool temperatures on amylose synthesis. The mutant *coi* isolated by mutagenesis was insensitive to cool temperature.

Okuno et al. (1983) studied an induced mutant of rice named dull mutant. They found that the amylose content of the dull mutant was controlled by a single gene (*du*).

Grain transparency of dull is intermediate between waxy and nonwaxy (Amano 1981; Okuno et al. 1983; Yano et al. 1985). This gene was non-allelic to the *Wx* alleles. The amylose content of the mutant was reduced by half when the *du* alleles was present compared to the non-waxy. In another study conducted by Okuno and Yano (1984), it was declared that the change in the amylose production was not proportional to the dose of *du* alleles. They concluded that the waxy alleles were epistatic to *du* alleles. According to Kumar and Khush (1987), intermediate amylose content was simply inherited. To change the level of amylose content in indica types, the *du* gene can be also used. Dung et al. (2000) concluded that *du2-2* responded differently to Wx^a and Wx^b. It produced chalky endosperms in response to Wx^a, but not with Wx^b. The *du2-2* reduced the amylose content in Wx^a. Dung and colleagues in 2000 concluded that *du2-2* may be used to reduce the amylose content in the indica types of rice carrying the Wxa. This may not alter the endosperm appearance.

Results from Wang et al. (1995) showed that cultivars with high amylose content (20 to 28%) contained both *Wx* protein and mature 2.3 kb Wx mRNA in developing seeds. The cultivars with low (6-16%) amylose content contained 10-fold lower amount of *Wx* protein and mature *Wx* mRNA compared to the high amylose group. The glutinous or waxy group of cultivars had no amylose and only contained the large 3.3 kb *Wx* RNA, with no 2.3 kb transcript. Wang et al. (1995) concluded that the level of *Wx* protein and the amount of amylose content had positive correlation with the level of 2.3 kb Wx mRNA. Wx protein and level of amylose were negatively correlated with the presence of a 3.3 kb *Wx* transcript.

2.4.2.7. Gel Consistency

The gel consistency (GC) test is known to complement the test for amylose (Farias and De la Cruz (1995). It is a measure of cold paste viscosity of cooked milled rice. Cooked rice texture can be defined by GC, mainly if the rice has high amylose (Bao et al. 2001). According to Juliano et al. (1965), amylose was the primary predictor of rice texture, but it failed to describe precisely the texture of certain types of rice. When combined with other tests like GC, best results were found. Rice with the same amylose content can be classed as hard gel consistency (26-40 mm); medium gel consistency (41-60 mm); or soft gel consistency (61-100 mm). The length of gel flow was found to have an inverse relation with amylose content, therefore the long gel corresponded to soft gel and the short gel was noted hard gel (Camgampang et al. 1973; Perez et al. 1979). Khush et al. (1979) reported that with the same amylose content, softer gel consistency rices are preferred by consumers. It was found that among high AC varieties of rice, hard GC had longer amylopectin chain length (Takeda et al. 1987; Juliano et al. 1987).

In their studies, Chang and Li (1981) found that the inheritance of gel consistency was under the control of one gene. They concluded that the short and hard gel consistency was dominant over the long and soft gel consistency. The involvement of one major gene and several minor genes was found by Tang et al. (1989) and Lanceras et al. (2000). They also declared a range of incomplete dominance and the presence of modifiers. The presence of a major gene with several modifiers controlling GC was reported by (Tang et al. 1996; Lanceras et al. 2000). The major QTL was found

near the *Wx* gene. The authors explained the presence of QTL near the Wx gene by the pleiotropy or linkage. He et al. (1999) found two minor QTL on chromosome 6 and 7. Transgressive segregants for GC were found by Harrington et al. (1997). Zaman et al. in 1985 reported the multigenic control of additive effects for GC. Tan et al. (1999) concluded that GC is controlled by a single locus when they found a ratio of 3:1 using a F2:3 populations and RILs fitting a ratio 1:1. Shi et al. (1997) declared that the cytoplasmic effects and maternal additive effects were two components when studying the genetic and genetic x environment contributions. They found small and significant direct additive effects. They also reported direct dominance interaction effects. Tang et al (1991) reported that the differences between the classes of GC hard vs. soft, hard vs. medium, and medium vs. soft gel were under monogenic control. They also noted the presence of modifiers. They found different alleles: gec^a for medium GC and gec^b for soft gel. The two alleles were recessive to the hard GC. The medium GC was dominant over the soft GC. Hard gel was dominant over both medium and soft gel.

2.4.2.8. Gelatinization Temperature

Gelatinization temperature (GT) is the range of temperature wherein at least 90% of the starch granules swell irreversibly in hot water with loss of crystallinity and birefringence (Dela and Khush 2000). The GT ranges from 55 to 79^{o}C. Juliano (1972) found 3 classes which were: low GT (55 to 69^{o}C), intermediate GT (70 to 74^{o}C) and high GT with (more than 74^{o}C). Ghosh and Govindaswamy (1972) declared that the cooking quality of rice is greatly influenced by the GT added to the quality and quantity of starch. In a study conducted by Tomar and Nanda (1985), they declared that the GT

played an important role concerning water uptake, volume expansion and kernel elongation. High GT rice has a final soft texture when overcooked; it elongates less and can be undercooked if the standard cooking procedure is applied. Knowing these side effects, people worldwide prefer rice with intermediate GT. High –GT starches have longer chains compared to low GT (Hizukuri 1985; Tetser and Morisson 1990). It has more crystallites than low-GT. Low GT starches have more amorphous and less crystallite component than the high-GT starches. The different conclusions said that the mechanism controlling GT in the starch may be due to the crystallite perfection.

Hayakawa et al. (1997) found that waxy starch from waxy hexaploid wheat and waxy maize starch swelled more quickly and reached faster the peak viscosity compared to normal starches. The normal starches had higher setback than waxy starches. It was concluded that waxy and low amylose rices had low GT (GT<70) and they had higher glycemic index (GI) compared to intermediate and high amylose rices having intermediate GT (Juliano et al. 1986). Within the high amylose rices with different GT and cooked in optimum cooking water (68-69%), low GT rice had higher GI (91-94%) when it was compared to intermediate GT rice (62-71%). When cooked in excess water (69-70% water) with minimum cooking time (14-20 min), the low GT rice had the same GI as the others.

To measure GT in the laboratory an indirect method measuring alkali spreading value (ASV), based on the method developed by Little et al. (1958), is often used. Entire kernel from milled rice is soaked in 1.5 or 1.7% KOH for about 23 hours. Rice with high GT remains unchanged while low GT rice disintegrates completely and

intermediate GT are partially affected. Panlasigui et al. (1991) found the similar results. A simplified method to determine GT was reported (Bhattacharya et al. 1982a). The equations developed to calculate the GT from ASV was as follow:

y=74.54 -1.40x (r=0.848***, not including waxy rices, the sample size was n=157) or y = 74.80- 1.57x (r = 0.806***, including waxy rices, n=165).

From the equation x = alkali score and y is the GT.

It was concluded from diverse studies that high temperature during growth period increases the GT of rice (Asaoka et al. (1984); Asaoka et al. (1989); Morrison et al. 1987).

Differential thermal analysis (DTA) and differential scanning calorimetry (DSC) are mostly used for thermoanalytical methods ((Daniels 1973; Haines 1995; Harvalkar and Ma 1990; Wendlandt 1974; Wunderlich 1990). DSC was used for the first time by Stevens and Elton (1971).The differential scanning calorimeter (DSC) is used to determine GT (Normand and Marshall 1989; Singh et al. 2000). In the DSC instrument there are two calorimeters heated at a constant rate and maintained at the same temperature. One calorimeter is empty and the second contains the sample, mixture of rice flour and water which is heated at high temperature. The difference in energy required to equilibrate tto study. A mixture of rice and water is put in a sealed pan and placed in a calorimeter. It is heated at high temperaturhe temperature of the empty reference calorimeter and the sample calorimeter is used to determine the peak temperature (Tp), the conclusion temperature (Tc), and the enthalpy of gelatinization (ΔH).

Atwell et al. (1988) defined gelatinization as “the water-mediated disruption of the molecular orders within a starch granule during heating, manifested in granular swelling, native crystallite melting, loss of birefringence, and starch solubilization”. They defined starch retrogradation as the resembling of the starch molecules which were disrupted during gelatinization. It was declared that after cooling of the gelatinized products, the amylose part of the product retrogrades instantaneously while the amylopectin keeps the amorphous state (Miles et al. 1985a, b; Pomeranz 1987; Bean and Setser 1992). It was found that starch gelatinization had two stages (Schoch 1965), the swelling was the first stage followed by the swelling and solubilization of starch molecules. A crystalline part of starch swells slower compared to an amorphous region. The amylopectin fraction was declared responsible for the swelling power (Tester and Morrison 1990).

Through numerous rice chemistry studies, it is becoming apparent that gelatinization temperature is primarily controlled by amylopectin structure. Amylopectin is reportedly synthesized by a combination of enzymes including soluble starch synthase (SSS), ADP-glucose pyrophosphorylase, and starch branching and debranching enzymes (Smith et al. 1997; Myers et al. 2000; Nakamura et al. 2002). Differences in amylopectin among rice varieties are due to the different combinations of their amylopectin chain profiles (long A, short B and long-B type chains). High amylose equivalent (AE) varieties of rice have more long-B and less short chains. Takeda and Hizukuri (1987) concluded that starches from indica had more AA than japonica rices.

They also declared that amylopectins from indica rices had longer chains than japonica rice amylopectins.

Jane et al. (1999) analyzed amylopectin structure in relation to other physicochemical properties of starches from different botanical origins. The results showed that starches with relatively short average amylopectin branch chain lengths (e.g., degree of polymerization 11-16 in waxy rice), when compared to relatively long amylopectin branches (e.g., degree of polymerization 18-21 in wheat and barley), displayed low gelatinization temperatures. Nakamura et al. (2002) characterized the structure of amylopectin in the endosperm of Asian rice to determine the relationship between amylopectin structure and starch physicochemical properties. The results indicated that almost all rice amylopectin could be classified into L- or S-types. The L-type amylopectin was different from the S-type amylopectin in that the numbers of short α-1,4-glucan chains of degree of polymerization (DP) ≤ 10 were less than 20% of the total α -1,4-glucan chains of DP ≤ 24. No significant difference between both types was found in the proportion long chains of DP ≥ 25. Among the 129 rice varieties examined, only a single variety (‘Khauk Yoe’), belonging to the tropical japonica rice group, had an intermediate (M-type) amylopectin structure. The proportion of amylopectin chains with DP ≤ 10 was negatively correlated with the onset temperature of starch gelatinization, whereas no correlation was observed between amylose content and starch thermal properties.

According to Asaoka et al. (1984) and Inouchi et al. (2000), high temperatures increase the amount of long amylopectin (B-type) in rice. Reddy et al. 1993 declared that

it was amylopectin, and not amylose which primarily determines the eating quality of rice. They concluded that soluble AE had no relation with rice texture, and therefore, amylose content plays a little role concerning the eating quality of rice. The insoluble AE, associated with amylopectin, is a reflection of the fine structure of amylopectin. It is an excellent index of rice quality.

Various reports are available on the genetics of GT or ASV. Stansel (1965) declared the presence of two loci when he used medium x high GT rices. A bimodal curve was found by Puri and Siddiq (1980) when he made the crosses between medium and high GT, in a cross between medium x medium and high x high he reported a unimodal curve. Heda and Reddy (1986) found bimodal and unimodal curves. McKenzie and Rutger (1983) found bimodal frequency distributions for ASV. They came out with a single gene of major effect. The authors suggested a simple genetic control for alkali spreading value in crosses with high GT and the bimodal was found with intermediate x intermediate. Polygenic nature of inheritance was also mentioned. The interaction of two pairs of major genes with duplicate gene action and cumulative effect was reported by Tomar and Nanda (1984). Dominance of low digestibility over high digestibility was reported by Heu and Choe (1973) when he studied the ASV with crosses indica x japonica. They concluded that the ASV is controlled by a single gene. Hsieh and Wang (1988) mentioned the case of dominant and additive genes. Additive gene action was also reported by Somrith in 1974.

He et al. (1999) after using a QTL mapping declared that the major gene named *Alk*, on chromosome 6 controlled ASV. Tan et al. (1999) concluded that the ASV was

under the control of the *Wx* gene. Tan et al. (2001) detected a segment of chromosome 11 that played a role in the determination of GT. Bao et al. (2004) reported that rice starch thermal traits were mainly controlled by the *Alk* gene. They found a major QTL on chromosome 6 that was significant for To, Tp, and Tc. Other QTL on chromosome 1, 7, 10 had effects on To, Tp, and Tc. Two minor QTLs on chromosome 1 and chromosome 7 controlled ΔH. The authors suggested that ΔH might have similar genetic basis as swelling volume.

Gene mapping analysis by Umemoto et al (2002) showed that the soluble starch synthase IIa (SSSIIa) gene is located at the *Alk* locus on rice chromosome 6. It appears that different alleles of the SSSIIa gene are responsible for the differences in cultivars with different amylopectin chain lengths. The activity of SSSIIa appears to be reduced in cultivars with short chains of amylopectin, which causes them to have a lower gelatinization temperature, which in turn causes them to have more disintegration in alkali solutions (Umemoto et al. 2004).

Because there are so many genes controlling amylopectin synthesis, it is not surprising that the genetics controlling GT are complex. Incongruous results are obtained when analyzing inheritance arising from different rice genotypes carrying different alleles of starch synthesis genes. It is also understandable that the relationship between AC and GT can become confused in separate studies using different rice genotypes since AC is controlled by the *Wx* gene, which displays linkage to the SSSI and SSSIIa genes. The correlation has been reported to be significantly negative (Tetens et al. 1997) or null (Bhattacharya et al. 1999; Nakamura et al. 2002) in different studies. Although GBSS

encoded by the *Wx* locus can play a significant role affecting GT, the *Alk* locus plays the largest role in determining GT when both factors are variable in the parents of a genetic cross (He et al. 1999).

2.4.2.9. Starch Pasting Properties

Paste viscosity parameters are known to play an important role in defining the cooking, eating, and processing quality of rice (Bao et al. 1999). Brabender Amyloviscograph and Rapid ViscoAnalyzer (RVA) are commonly used to measure the pasting properties of starch.

Dengate (1984) defined the RVA terms cited below:

The peak viscosity (P) was defined as the highest viscosity obtained during pasting at a programmed heating to 95^0C at 1.5^0C min^{-1};

The peak viscosity temperature (PT), was defined as the ease of cooking that was indicated by the apparent viscosity at 95^0C in relation to the peak viscosity;

The paste stability (H) was defined as the resistance to breakdown; it was defined by the apparent viscosity after 20-60 min of cooking at 95^0C. It measured the stability of paste during the cooking period;

The setback or cold paste viscosity (C) defined by the apparent viscosity of the paste.

Champagne et al. (1999) used the following definitions:

Pasting temperature defined as the temperature at the initial viscosity increase;

Peak was the maximum viscosity attained during heating and holding cycles immediately after the cycle was at 95^0C;

Peaktime was defined as the time required reaching the peak;

Trough was the minimum viscosity after the peak was reached;

Final viscosity was the viscosity at the end; it was the amylograph cool paste viscosity;

Breakdown was the difference between peak and trough;

Setback was the difference between final viscosity and trough.

Sowbhagya and Bhattacharya (2001) defined the items from the viscosity values as seen below, where total set-back and relative set-back are new terms used by the authors:

Peak viscosity (P) was defined as the maximum value of viscosity reached during heating for 43.3 min and cooking for 20 min. It is in Brabender units BU. The viscosity reached the maximum peak at 95^0C;

Initial viscosity V95i (BU) was obtained when the temperature reached 95^0C for the very first time;

Viscosity value (VP)+10 (BU) was obtained 10 min later after P was reached;

Hot-paste viscosity (H) = V95f in BU was defined as the final viscosity at the end of cooking at 95^0C; it is also called the lowest viscosity.

Cold-paste viscosity (C) in BU was the value attained when the paste cooled at 50^0C;

Time of heating tp (min);

Breakdown (BD) was defined as the difference between peak viscosity and hot-paste viscosity (P-H) in BU;

Set-back (SB) was the difference between cold paste viscosity and peak viscosity C and P = C-P;

Total set-back = C-H = BD+SB in BU;

Relative set-back (%) = BD/SBt = (P-H) x 100/(C-H) (%);

Mazurus et al. al. (1957) defined three important descriptors for rice amylograph viscosity characteristics which were peak, hot paste, and cool paste viscosities. They defined peak viscosity as the maximum viscosity of cooked rice flour at 95^0C; Limpisut et al. (2002) defined the peak viscosity as the maximum viscosity value attained when the starch paste was heated. The hot paste viscosity was defined as the minimum viscosity of the rice flour at 95^0C. The viscosity of the cooked rice flour at 50^0C defined its cool paste viscosity. Low breakdown viscosity is defined as the difference between peak and hot paste viscosity by Limpisut et al. (2002). The setback viscosity was defined by Mazurus et al. (1957) as the difference between cool paste and peak viscosities; Limpisut et al. (2002) defined it as the rise and fall in the viscosity when the cooling cycle was ending.

Bao et al. (1999) also defined the rice paste viscosity parameters in the terms cited below: the peak was the first peak viscosity after gelatinization; the hot paste was the paste viscosity at the end of the 95^0C holding period; the cool paste viscosity was the paste viscosity recorded at the end of the test; the break down was the difference between peak and hot paste; the difference between the cool paste and the peak gave the setback; the difference between the cool paste and the hot paste defined the consistency.

According to Limpisut et al. (2002) the pasting temperature (PT) was the temperature which indicated an initial increase in viscosity; the peak viscosity (PV) was defined as the maximum viscosity during the heating cycle; the breakdown viscosity (BD) was the difference between the peak viscosity and final viscosity at 95^0C; the

setback viscosity (SB) was the difference between final viscosity and peak viscosity at 50^0C ; the consistency viscosity (CC) was the difference between final viscosity at respectively 50^0C and 95^0C.

Bollich et al. (1980) found that the low breakdown was very important for canning rice cultivars. The retrogradation tendency of the rice starch was defined by the setback. Long, medium and waxy grain types were well differentiated by the setback viscosities values. Positive values were found with typical long grain types, medium and waxy types had negative values of setback. Varavinit et al. (2002) concluded that waxy rice flour had the highest peak viscosity among the different starches they studied. The amylose rice with 28% amylose had the highest setback, it was followed by rice with (18% amylose), and the lowest value for setback was found with the waxy rice (5% amylose). Low amylose starch was characterized by low degree of retrogradation (Varavinit et al. 2003) which is the changes that occur during the cooling and storage of gelatinized starch. Waxy rice or low amylose rice had high breakdown, while medium and high amylose rice starches were characterized by low breakdown. Rice types with higher breakdown had higher peak viscosity (Varavinit et al. 2003). The breakdown was able to group rice types into low amylose in one hand and medium and high and the second hand, but it was not possible to differentiate medium amylose from high amylose rices. Starch granules of rice with high breakdown were easy to disintegrate after heating. The extra stickiness of waxy rice was due to this high breakdown.

Watanabe et al. (2002) concluded that amylose content had more pronounced effects on viscosity than protein when they were studying the crosses between *O. sativa*

x *O. glaberrima*. The authors declared that African rice had rice texture more rigid compared to the interspecifics which had a stickier aspect.

Tester and Morrison (1990) concluded that pasting properties of starch were affected by amylose and lipid contents. They are also affected by branch-length distribution of amylopectin. It was found that amylopectin enhanced swelling and pasting whereas swelling was inhibited by amylose and lipids. Jane et al. (1999) reported from their studies that waxy cereal starches had lower pasting temperatures, higher peak viscosity, and lower set-back viscosity compared to the normal starches. The findings were not true for *du* waxy maize and *ae* waxy maize. The authors also found that the amylose-lipid complexes in normal starches (maize, rice, wheat, and barley) increased the pasting temperatures, and the resistance to shear-thinning of starch pastes. Potato starch was found to have very high peak viscosity because of its high content in phosphate monoester (Lim et al. 1994; McPherson and Jane 1999). Due to the absence of phospholipids, the tuber and root starches had lower pasting temperatures, lower resistance to shear-thinning, and lower set-back viscosities (Lim et al. 1994). Compared to potato and normal maize, the starch paste of green of green leaf canna had no breakdown in viscosity during the holding time at 95^0C. It had very high set-back viscosity. This quality of green leaf canna was probably due to his high amylose content and high proportions of very long amylopectin branch chains. A very high set-back was also recorded with mung bean because of its high amylose content. Waxy rice had the lowest value of pasting temperature (Varavinit et al. 2003) the medium and high amylose rices had the pasting temperature of more than 90^0C. The authors concluded

from their study that low amylose rice had the highest peak viscosity and breakdown, but the lowest setback and pasting temperature were recorded with low amylose rice. Noosuk et al. (2003) reported that the waxy rice had the higher swelling volume with higher viscosity compared to medium and high amylose rice.

Barber (1972) and Juliano (1985) concluded that ageing of rice increased its hydration and paste viscosity. In 1978, Indudhara et al. (1978) found that ageing decreased the hydration and viscosity of rice. Sowbhagya and Bhattachaya (2001) reported that the paste breakdown decreased with time of storage while the setback increased. The setback was considered to be an indication of cooked rice hardness after cooling and retrogradation. Cold-paste and hot-paste viscosity ratio did not change during the time of storage whereas other viscogram indices changed over time. Ramesh et al. (2000) concluded that newly harvested rices absorbs less water, and therefore swells slowly. There is a loss of solids and the cooked rice becomes sticky and lumpy. In the case of aged rice, the grains elongate well, solid loss is reduced and as a result the cooked rice is less sticky and is fluffy. Barber (1972) reported that storage of milled rice resulted in a higher paste viscosity due to the increase in free fatty acids (FFAs). Reece and Blakeney (1996) concluded that these ideas were not verified with waxy rice types. The authors reported that the interaction between FFA and amylose was important and it increased the viscosity of cooked rice. The FFA/amylose complexes were considered as potential sources of resistant starch.

In 1997, Gravois and Webb reported that peak viscosity (PKV), hot paste viscosity (HPV) and cool paste viscosity (CPV) were controlled by a single locus with

additive effects. Bao et al. (1999) concluded that the viscosity profiles of rice were controlled by the direct effects of seed, the cytoplasmic effects and the maternal effects. They found additive co-variance for the setback viscosity (SBV), cytoplasmic variance was detected for hot paste viscosity (HPV), cool paste viscosity (CPV) and consistency viscosity (CSV). Bao et al. 1999 found 2 QTLs (*qPKV-2* and *qPKV-12*) controlling the peak viscosity on chromosome 2 and chromosome 12. Two QTLs were identified for hot paste viscosity; they were *qHPV-6-1* and *qHPV-6-2* both located on chromosome 6. Three QTLs were identified for cool paste viscosity; they were called *qCPV-6-1* on chromosome 6, *qCPV-1* on chromosome 1 and *qCPV-6-2* on chromosome 6. The authors identified 5 QTLs for breakdown viscosity which were *qBDV-6* on chromosome 6 and *qBDV-1, qBDV-5, qBDV-7* and *qBDV-12* on chromosome 1, 5, 7, and 12 respectively. Five QTLs were found for consistencies which were *qCSV-6-1* in the interval of *wx* gene; it was located on chromosome 6; *qCSV-1*, and qCSV-6-2 and *qCSV-7* located on chromosome 1, 6, and 7 respectively. Finally Bao et al. (1999) found 4 QTLs for setback viscosity which were *qSBV-1* on chromosome 1, *qSBV-5-1* and *qSBV-5-2* on chromosome 5, and *qSBV-6* located on chromosome 6.

Larkin et al. (2003) concluded that *Waxy* locus had significant effects on RVA parameters. They reported that the high amylose, Wx^a allele found at the Rexmont waxy locus was associated strongly with amylose content, higher cool paste viscosity, high setback viscosity value and low breakdown. A higher peak viscosity, lower hot and cool paste viscosity, higher breakdown and lower setback were recorded with the heterozygous Wx^aWx^b compared to Wx^aWx^a. Their results also showed that soluble

starch synthase had a minor role on RVA parameters. The rice branching enzyme 1 (RBE1), rice branching enzyme 3 (RBE3), and the starch debranching enzyme were not correlated with the different pasting parameters.

Chen et al. (2004) studying a diverse set of rice germplasm, identified that a single nucleotide polymorphism (SNP) in exon 10 of the *Waxy* gene was associated with RVA parameters. Rice cultivars with the Rexmont polymorphism showed an elevated (strong) RVA and the Jodon polymorphism had a lower (weak) RVA curve. The elevated RVA curve is associated with lower starch solids loss, better grain integrity, and firmer cooked product following parboiling and canning processes.

2.4.2.10. Correlation Studies Among Starch Properties

Different studies were conducted regarding the correlations between and among the different characteristics of rice quality with cooking and eating quality.

According to Antonio and Juliano (1974), waxy rices with high GT had a harder texture compared to the ones with low GT. The intermediate-GT waxy rices were more associated with high viscosity than the low-GT ones (Perdon and Juliano 1975). Sasaki et al. (2000) reported a negative correlation between final GT and amylose content, similar correlation was found between gelatinization enthalpy and amylose content.

The authors found that starch with higher amylose content had more amorphous region and less crystalline, the GT and endothermic enthalpy were lowered consequently. Flipse et al. (1996) declared that the increase in viscosity was due to the leached out amylose rearranging into a thin amylose gel; starch with low amylose had less viscosity during cooling. Juliano et al. (1964) found that the setback correlates with

the amylose content, the more positive the setback, the higher the amylose content. He also declared that the final viscosity is related to the softness of the gel, the low final viscosity is correlated with a softer gel.

Glaszmann (1987) found that the higher the apparent amylose content, the harder the gel consistency and the less sticky was the rice. Bao et al. (2000) reported that there is correlation between setback viscosity (SBV) and consistency viscosity (CSV) with apparent amylose (AA). A negative correlation exists between SBV and CSV with the adhesiveness of cooked rice.

There is a strong correlation between intermediate amylose content and intermediate amylograph viscosity (Gravois and Webb 1997). Water absorption, volume expansion, fluffiness and separability of cooked rice are directly related to amylose content (Delwiche et al. 1996; Noosuk 2003). The presence of amylose may reduce the swelling volume of starch granules. Juliano and Pascual (1980) reported that amylose content was positively correlated with volume expansion and water absorption during cooking; it was found negatively correlated with tenderness and stickiness. Amylose content was found positively correlated with ASV (Tan and Corke 2000); it was negatively correlated with GC and swelling volume. The authors concluded that the low amylose content was an indicator of a longer/softer gel and a smaller swelling volume. Waxy starch swells at a rapid speed, but the swollen granules disrupted quickly at low temperature. Therefore the viscosity developed by waxy rice at an early stage cannot be maintained and there is lack of viscosity at the end (Tester and Morrison 1990). There is a negative correlation between amylose content with cohesiveness, tenderness and

glossiness (Juliano 1971). The amount of long-B chains in the molecule had a strong correlation with texture (Ramesh et al. 2000).

Juliano (1985) reported a negative correlation between protein content and adhesiveness. Juliano and Pascual (1980) reported a negative correlation between protein content and GC.

Juliano and Pascual (1980) and Juliano (1985) reported from their study on waxy rice that the hardness of nonwaxy cooked rice had a positive correlation with breakdown, hot and cold paste viscosities and setback. Sowbhagya and Bhattacharya found similar results in 2001. There was negative correlation between amylose content and the peak RVA viscosity (Noosuk et al. 2003).

Ong and Blanshard (1995a, 1995b) reported a positive correlation between the GT and the crystallinity of rice. Fredriksson et al. (1998) reported that amylose content had a negative correlation with the onset and the peak temperature of gelatinization for different starches.

Varavinit et al. (2003) found that onset (To), peak (Tp) and conclusion temperature (Tc) had positive correlation with amylose content in their study of different cultivars of Thai rice. The authors concluded that low amylose rice had the highest peak viscosity and breakdown; it had also the lowest setback and pasting temperature. Ming et al. reported the same conclusion that lower amylose content was associated with higher peak viscosity. Tan and Corke (2002) found similar results; they also concluded that protein content was negatively correlated with peak viscosity and hot paste viscosity; there was negative correlation between swelling volume and cool paste viscosity.

Juliano and Pascual (1980) concluded that peak viscosity was positively correlated with rice consistency and cooked rice hardness; it was negatively correlated with cooked rice stickiness. There was positive correlation between the values of setback and consistency; they correlated positively with cooked rice hardness. A negative correlation was found between hardness and stickiness. Beakdown and final viscosity of rice was correlated (Tan and Corke 2002).

The low solubility of amylose was found associated with the high setback, low breakdown, hard GC, and faster retrogradation (Kongseree et al. 1972; Juliano and Perdon 1975; Maningat and Juliano 1978).

Protein content was positively correlated with Tp and To. GC was correlated negatively with amylograph setback (Cagampang et al. 1973; Perez 1979); it was positively correlated with cooked rice stickiness.

GC was found positively correlated with swelling volume (Tan and Corke 2002); it was negatively correlated with ASV and protein content.

2.4.2.11. Aroma in Cooked Rice

Nijssen et al. (1996) reported more than 200 volatiles compounds in cooked rice. Buttery et al. (1982) identified the popcorn-like smelling 2-acetyl-1-pyrroline (ACPY). It was found to be the key element for the roasty, popcorn-like aroma of freshly baked wheat bread (Schieberle et al. 1985), freshly-pooped popcorn (Schieberle et al. 1990), cooked panda leaves (Buttery et al. 1983), and the smell of tiger's urine (Brahmachary et al. 1990). Buttery et al. (1988) were the first scientist to do a systematic study on the contribution of the different volatiles on a long rice variety from California. Other

studies conducted elsewhere revealed that the field location (Fushimi et al. 1996), the temperature during ripening and drying (Itani et al. 1996), and the storage or aging affected the level of ACPY in scented rice (Laksanalamai et al. 1994; Widjaja et al. 1996). Champagne et al. (1997) concluded that the intensities of desirable and undesirable flavor attributes were higher in rice dried to 15% moisture when it was compared to rice dried to 12%.

Weber et al. (2000) concluded that the pleasant odor of raw or cooked non-aromatic or aromatic rice was controlled by a blend of various volatiles. Most of the volatiles found in aromatic and non aromatic rices were similar, but their proportion differed.

Different techniques to detect aroma were developed by several scientist around the world. The technique of chewing a half of a single seed was developed by Berner and Hoff (1986). Chewing a few seeds was developed by Dhulappanavar (1976). The method of heating of leaf tissues in water and noting the aroma was developed by Nagaraju et al. (1975). Sood and Siddiq (1978) developed the technique of eluting aroma from leaf tissue with 1.7% KOH solution. Evaluating the aroma from both leaf and grain with 1.7% KOH solution developed was a method developed by Pinson (1994).

Inheritance of scent has been worked on by several scientists. A single dominant aromatic gene was discovered in 1938 by Kadam and Pantakar. Jodon (1944) proposed a single gene *(fgr*) control. Different F_2 segregation ratios were reported in different F_2 populations including 1:3 ratios meaning a single recessive aroma gene (Ghose and Butany (1952); Sood and Siddiq (1978); Dong et al. (2001), 15:1 or 9:7 ratios indicating

two dominant genes (Duhulappanavar and Mensinkai 1969; Tripthi and Rao 1979), 37:27 ratios meaning three complementary recessive genes (Reddy and Sathyanarayanaiah 1980), 175:81 ratios indicating four complementary recessive genes (Duhulappanavar 1976), 3:13 ratios indicating a single recessive gene interacting with an inhibitor gene (Tsuzuki and Shimokawa 1990), and 1:3 or 7:9 ratios indicating a single recessive genes or two recessive genes depending on the varieties used (Pinson 1994).

Ahn et al. (1992) reported from a RFLP analysis that the gene for aroma for the variety Della is linked to a single copy DNA clone, RG28, located on chromosome 8 at a distance of 4.5 cM. Similar results were declared by Yano et al. (1991) when he was studying a Bangladesh native variety, Surjarmakhi; Lorieux et al. (1996) when they were studying Azucena from the Philippines. Tomar and Prassad (1997) declared that a dominant aroma gene was located on chromosome 11 in an indica rice landrace, Baspatri. Siddiq et al. (1986) reported two recessive aroma genes in an indica variety, T3 located on the chromosome 5 and 9 respectively.

2.4.2.12. Protein

In addition to amylose and amylopectin, starch granules contain small quantity of diverse components such as proteins, lipids, pentosans, and minerals (phosphorous and silica). The most abundant are protein and lipids (Lineback et al. 1986; Ring 1995). Two types of protein are associated with starch granules: the storage proteins which are gluten and gliadin proteins; the starch granule-associated protein tightly bound the starch granule (Baldwin 2001). Among the starch granule-associated proteins, two were well

studied: the 15 kDa group called friabilin and the 60 kDa most commonly known as waxy protein or starch granule-bound starch synthase isoform I (GBSS I).

Rice is the principal source of protein in most rice eating countries. Protein content can define most of the physicochemical properties of cooked rice (Hamaker and Griffin 1990, 1991; Marshall et al. 1990; Juliano 1993; Hamaker 1994).

Juliano (1985) concluded that rice is unique among cereals because it has a storage protein principally made of glutelin (*oryzenin*), which has a more balanced amino-acid profile compared to the prolamin-rich storage proteins. According to Nanda and Coffman (1979) and Perez et al. (1996), protein content is seriously affected by environmental conditions and the level of fertilizer and growth duration. The degree of milling affects highly the protein content of rice (Perez et al. 1996). Indica rices have a protein content that varies from 4.9 to 19.3% and japonica rices may contain 5.9 to 16.5% (Lin et al. 1993). Japonica rice had 20% prolamin and indica rice had 30% based on Hibino et al (1989) results. Cagampang et al. (1996) concluded that glutelin was the most important fraction in the whole grain. In the bran and polish, albumins and globulins were the most important. Prolamins were evenly distributed in all three fractions. Song et al. (1988) reported that high AC indica rice in Taiwan had 2% more protein content than low AC japonica rice.

According to Juliano (1984), rice protein is insoluble in water, and therefore is difficult to separate during processing. Rice bran and broken rice are the two main sources of rice protein and they have been under-used for many years. According to Juliano (1984), glutelin is extremely insoluble due its hydrophobic bonding, disulfide

linkages, and its high molecular weight of its polymeric shape. Hamada (1997) defined the soluble proteins as the protein that was consecutively extracted with water, salt, alcohol, and acetic acid, and the residue protein was the ones that could not be extracted. Rice starch is found attached to rice protein bodies I and II. Protein body I accounts for almost 20% of the rice endosperm protein is made of prolamin; Protein body II is glutelin and takes about 70% of the rice total endosperm protein. Removal of protein from rice granules had remarkable changes in starch gelatinization (Marshall et al. 1990). Removal of protein also increased the RVA paste viscosity and decreased the pasting temperature (Lim et al. 1999). Teo et al. (2000) reported that the modification of the protein rather than starch was responsible for diverse rheological changes linked to the storage.

Protein content of rice has been studied by many scientists; they found that it is quantitative trait (Singh et al. 1977; Singh and Singh 1982; Kaul 1983; Kambayshi et al. 1984; Sood and Siddiq 1986; Gupta et al. 1988; Shenoy et al. 1991). Protein content was controlled by genetic effects of triploid endosperm, cytoplasm and diploid maternal plant (Shi et al. 1996a). Shi et al. (1999) reported that protein content was controlled by genetic main effects as well as GE interaction effects; they found the embryo interaction effects as effects of triploid endosperm, cytoplasm and diploid maternal plant.

Tan et al. (2001) identified two QTL which had an effect on protein content. One of the two QTL is in the interval of *C952-Wx* on chromosome 6. The second QTL is located on chromosome 7 in the interval *R1245-RM234*. Five QTL were identified by Hu et al. (2004). The major QTL *qRPC* was mapped in the interval of RG-335-RG172a

on chromosome 5; the second QTL *qRPC-7* was mapped in the interval ZG34B-G20 on chromosome 7. The remaining three QTLs with relative small additive effects were mapped on chromosome1, 4, and 6 respectively. Variation in rice protein content has been shown to be quantitatively inherited (Shenoy et al. 1991; Shi et al. 1999).

2.4.2.13. Lipids

Rice fat content consists essentially of unsaturated fatty acids. It has great influence on rice appearance and eating quality (Qi et al. 1983; Chen et al. 1998). Rice contains about 3%. The lipid content decreases when the rice is milled because lipids are more concentrated in the peripheral parts of the grain (Hoseney 1998). Rice fat content has been used as a measure of the degree of milling. The surface lipid content (SLC) is defined as the "ratio of the mass of surface lipids extracted from a sample of milled kernels to the mass of the original sample" (Chen et al. 1998). Milled rice has an average of 0.3 to 0.5% lipids (Hoseney 1998). Compared to barley, wheat and rye, brown rice has more nonpolar lipids and less glycolipids and phospholipids. Rice oil contains *oryzanol* which is a phenolic antioxidant.

Hu et al. (2004) reported three QTLs about rice fat content (RFC). They were mapped respectively on chromosome 1, 2 and 5 and were designated as *qRFC-1, qRFC-2* and *qRFC-3*. The authors concluded that there were epistatic interactions and they were very important component of the genetic basis of RFC. Hu et al. (2004) reported that that the segregation of the double haploid lines that they studied could be largely explained by a few main effects added to many epistatic loci.

The results from Hu et al. (2003) showed a highly negative correlation between rice protein content and rice fat content. Wilcox and Shibles (2001) declared the same conclusions about other crops.

Quality even based on subjective and objective factors is very important for any rice breeding program. Since rice is consumed in Africa as a whole grain, the physical properties, the general appearance, and the cooking characteristics are very important. In West Africa the most important criteria dealing with rice quality is slow digestibility, grain expansion after cooking, and cooking time. Farmers prefer rice that will "stay in the stomach" a long period of time to allow them to work hard for a long period in the field. They also prefer varieties with high volume after cooking (kernel elongation and expansion) to help feed their large families. In Africa, most of the research projects deal with biotic traits (disease and insect resistance) and abiotic stresses like drought, acidity, soil fertility, etc. whereas quality aspects have been largely neglected.

Access to limited water resources is becoming a big issue for US rice producers. Development of rice cultivars that can yield well under a reduced water regime will be a tremendous gain. Once germplasm is identified that is tolerant to reduced water availability and is adapted to the US production environment, it can serve as a resource for breeding efforts, genetic studies, and marker association studies.

Although , phenotypic evaluation methods show the response of cultivars to different environments (the GxE interaction), genotypic marker methods save time and labor, by clearly determining the presence/absence of alleles, and therefore can speed up

the selection process. The use of markers that are associated with quality traits will enhance the rice breeding programs both in the US and West Africa.

Although the proportion of the population in the developing world that is malnourished fell from 46% in the early 1960s to 31% in 1995, "There are still 1.3 billion of the population who go to bed hungry everyday" (Khush 1997).

The population growth in Africa is still very high and demand for rice is increasing daily. People will consume poor quality rice if they do not have a choice but once the economic situation improves the demand for quality rice increases. In this case, varieties with particular names and characteristics are requested. In Mali 'Gambiaka' is the preferred variety because of its grain characteristics and aroma. In 2005, the rice growing area in Mali is expected to increase from 48,000 to 70, 000 ha (IER 1995).

From 1968 to 1989 the average yield in Mali was 2t/ha. From 1989 to 1998, the yield increase in the "Office du Niger", the main rice growing area of Mali was 60% (News bulletin-Le Sahel 1999). Nowadays farmers can reach a yield of 8t/ha. In local markets, some people not familiar with characteristics of Gambiaka will pay elevated prices for another variety because it is sold under the name Gambiaka. In general, consumers in West Africa and in Mali prefer: long and white grain, aroma, high yield after cooking (kernel elongation), and softness after cooling, and ability to store overnight without spoilage. Many works were conducted on the waxy gene and the different SNPs. Ayres et al. (1997) found that (CT)n repeat at the GBSS allele was found to explain a large amount of the variation in amylose content. Bergman et al. (2001) concluded that high amylose rice had (CT)10 and (CT)11, intermediate rice had (CT)14

and (CT)20, and low amylose rice had (CT)17 and (CT)18. Ayres et al. (1997) found that 18% or less amylose has the sequence AGTTATA at the putative intron 5' splice site; higher amylose has the sequence AGGTATA. Larkin et al. (2003) concluded that GBSS locus had significant effects on RVA parameters. They found that an adenine to cytosine tranversion in exon 6 results in the substitution of serine for tyrosine (intermediate amylose).A cytosine to thymine transition in exon 10 results in the substitution of a serine for proline can distinguishes high amylose strong RVA from other high amylose, intermediate amylose, and low amylose. Another silent mutation in exon 9 with a thymine to cytosine transition has no effect on the amino-acid sequence.

Chen et al. (2004) – using a diverse set of germplasm, identified that Exon 10 of the GBSS locus was associated with RVA curve parameters.

Larkin and Park (2003) did not analyze whether it was amylose content alone or some other feature of the Waxy gene that gave rise to the differences in RVA between the progeny lines they studied.

Knowledge of the cooking characteristics and markers associated with key cooking traits will help National breeding programs at developing improved rice cultivars that possess quality traits that are important to consumers and will add value to cultivars grown by farmers. Identification of novel African cultivars by the use of phenotypic characterization for their ability to grown under reduced water use will help US rice producer to lower the cost of rice production.

3. MATERIALS AND METHODS

3.1. Origin of the Experimental Material

Seven "New Rice for Africa" (NERICA) varieties from crosses between *O. sativa* and *O. glaberrima* developed at WARDA and 32 other varieties from the WARDA Genetic Resource Unit, including improved varieties from WARDA, other National programs, and from different international centers, were introduced to the US during the Fall of 2002. (Table 1). Both milled rice samples produced in West Africa and rough rice samples of these germplasm accessions were introduced. The milled rice samples were analyzed directly and the rough rice samples were used for planting in the quarantine greenhouse.

Some varieties did not flower in the quarantine greenhouse in Beaumont and, thus, were not planted in the field experiments. The different basmati types of rice were not planted in the field experiments because they were originally from India and Pakistan, and not West Africa, and have been well characterized in the US already.

Table 1. List of rice varieties introduced from WARDA in 2002.

No	Designation	Type *	Crosses**	Ecology
1	BAKUE DANANE	2	Landrace CI	Upland
2	BASMATI 217	1	India	Rainfed
3	BASMATI 370	1	India	Irrigated
4	BASMATI 6129	1	India	Irrigated
5	BG 90-2(FARO 29) (GR 14) (ROK 28)	1	(Peta 3*TN1)/Remadja	Irrigated
6	BIEU	2	Landrace CI	Upland
7	CG 14	3	O. glaberrima	Upland
8	COCOTE	2	Landrace CI	Upland
9	DANANE	2	Landrace CI	Upland
10	DIGBOBLI	2	Landrace CI	Upland
11	DISSOU	2	Landrace CI	Upland
12	DOIGAMLIN	2	Landrace CI	Upland
13	GAMBIAKA KOKOUN (MALI)	1	Landrace CI	Upland
14	GNANLE GNAN-MAN	2	Landrace CI	Upland
15	GNINNI ZEBA	2	Landrace CI	Upland
16	GNOKOU GNOKOU	2	Landrace CI	Upland
17	HOLLANDAIS	2	Landrace CI	Upland
18	ITA 123 (FKR 28) (TOM1-3) (KADIAKA)	1	Mutant of OS 6	Irrigated
19	JAYA	1	Taichung Native No 1/T141	Irrigated
20	KHAO DAWK MALI 105	1	Thailand	Rainfed
21	LOGNINI COURT	2	Landrace CI	Upland
22	MAHAFIN	2	Landrace CI	Upland
23	MELKIN BARKA	2	Landrace CI	Upland
24	MINMLI	2	Landrace CI	Upland
25	MOKOSSI	2	Landrace CI	Upland
26	MOLOUBA KOLE	2	Landrace CI	Upland
27	PALAHI (KAOLAKA)	2	Sierra Leone	Mangrove
28	PUSA BASMATI	1	India	Rainfed
29	SUPER BASMATI	1	India	Rainfed
30	WAB 450-11-1-1-P31-HB (NERICA5)	4	WAB 56-104/CG 14	Irrigated
31	WAB 450-11-1-P31-HB (NERICA 2)	4	WAB 56-104 /CG 14	Upland
32	WAB 450-I-B-P-160-HB (NERICA 6)	4	WAB 56-104 /CG 14	Upland
33	WAB 450-I-B-P-20-HB (NERICA 7)	4	WAB 56-104 /CG 14	Upland
34	WAB 450-I-B-P-28-HB (NERICA 3)	4	WAB 56-104 /CG 14	Upland
35	WAB 450-I-B-P-38-HB (NERICA 1)	4	WAB 56-104 /CG 14	Upland
36	WAB 450-I-B-P-91-HB (NERICA 4)	4	WAB 56-104 /CG 14	Upland
37	WAB 56-104	1	WAB 56-104 /CG 14	Upland
38	WAB 638-1	1	WAB 56-104 /CG 14	Upland
39	YABLO	2	Landrace CI	Upland

Note: Seeds were not received from PALAHAI (KAOLAKA)

* 1: Improved types
2: Landraces
3: *O. glaberrima* type
4: Interspecifics (*O. glaberrima x O. sativa*)

** CI: Cote d'Ivoire

3.2. Description of the Experimental Field Sites

3.2.1. Beaumont, Texas

The field experiments were conducted at the Texas A&M University System Agricultural Research and Extension Center in collaboration with the USDA-Agricultural Research Service at Beaumont, TX. The center is located at 29°57N and 94°30W. The two field experiments were conducted during the 2004 cropping season. The soil at the station is an Entic Pelludert (fine, montmorillonitic, and thermic), with a sand, silt and clay composition of 3.2, 32.4 and 64.4 respectively (Texas A&M University 1971). The average annual rainfall is 1473 mm.

3.2.2. Mbe-Bouake/Cote d'Ivoire

The rice samples introduced from West Africa were grown in Mbe, Cote d'Ivoire located between 7.5°N and 8.5°N and between 4.5 °W and 5.5°W (WARDA's nd) where the average annual rainfall is 985 mm. The station is characterized by 3 main seasons: a long dry season starting in early November with an end in mid-March; a long rainy season from mid-March to mid-July; a short rainy season from mid-July to mid-August, and an inter-season rainy period which starts in mid-August and ends in October.

3.3. Experimental Objectives and Descriptions

3.3.1. Field Evaluation of West African Cultivars

Two experiments were conducted in the field at Beaumont in 2004.

3.3.1.1. Yield Evaluation of West African Cultivars

Objective*:* The Yield experiment was conducted for genotypic and phenotypic evaluation of West African and US germplasm in order to identify agronomic

characteristics that can mutually benefit WA and US rice breeding programs.

Materials*:* The yield experiment was composed of 26 varieties introduced from WARDA (Table 2) and increased at Puerto Rico following the quarantine growout, and 8 US checks (Table 3). These were evaluated for agronomic characteristics including yield potential, yield components, and other traits associated with adaptation to the environment.

Methods: The experimental plots consisted of 6 rows of 4.57 meters long with a row spacing of 17.8 cm. The experimental design was a Randomized Complete Block Design (RCBD) having four replications. Fertilizer was applied at the rate of 33.7 kg/ha of N-P-K (0-30-0) pre-plant, 56.2kg/ha of urea at planting, 90kg/ha of urea at tillering, and 78.7kg/ha of urea at panicle development stage.

Roundup herbicide was applied prior to planting at a rate of 946 ml/ha. This was followed by Stam 80edf at a rate of 3.37 kg/ha, Bolero at a rate of 2.34 l/ha, and Basagran at a rate of 1.75l/ha applied at the 2-3 leaf stage for broad spectrum weed control. The insecticide Karate Z was applied at a rate of 0.146 l/ha after permanent flood for control of rice water weevil and the fungicide Quadris was applied at a rate of 0.73l/ha at pre-panicle differentiation for control of sheath blight disease.

At harvest, the two central rows, 3.66 meters in length were hand cut and threshed with a mechanical thresher. Moisture was recorded for each individual plot

Table 2. List of rice varieties introduced from WARDA and planted in the yield experiment at Beaumont in 2004.

No	Designation	Type *	Crosses**	Ecology
1	BAKUE DANANE	2	Landrace CI	Upland
2	BG 90-2(FARO 29) (GR 14) (ROK 28)-33390	1	(Peta 3*TN1)/Remadja	Irrigated
3	BG 90-2(FARO 29) (GR 14) (ROK 28)-33392	1	(Peta 3*TN1)/Remadja	Irrigated
4	COCOTE	2	Landrace CI	Upland
5	GNANLE GNAN-MAN	2	Landrace CI	Upland
6	GNINNI ZEBA-33420	2	Landrace CI	Upland
7	GNINNI ZEBA-33423	2	Landrace CI	Upland
8	GNOKOU GNOKOU	2	Landrace CI	Upland
9	ITA 123 (FKR 28) (TOM1-3) (KADIAKA)-33433	1	Mutant of OS 6	Irrigated
10	ITA 123 (FKR 28) (TOM1-3) (KADIAKA)-33434	1	Mutant of OS 6	Irrigated
11	LOGNINI COURT	2	Landrace CI	Upland
12	MAHAFIN	2	Landrace CI	Upland
13	MINMLI	2	Landrace CI	Upland
14	MOKOSSI	2	Landrace CI	Upland
15	MOLOUBA KOLE-33459	2	Landrace CI	Upland
16	MOLOUBA KOLE-33460	2	Landrace CI	Upland
17	WAB 450-11-1-1-P31-HB (NERICA5)	4	WAB 56-104/CG 14	Irrigated
18	WAB 450-11-1-P31-HB (NERICA 2)	4	WAB 56-104 /CG 14	Upland
19	WAB 450-I-B-P-160-HB (NERICA 6)	4	WAB 56-104 /CG 14	Upland
20	WAB 450-I-B-P-20-HB (NERICA 7)	4	WAB 56-104 /CG 14	Upland
21	WAB 450-I-B-P-28-HB (NERICA 3)	4	WAB 56-104 /CG 14	Upland
22	WAB 450-I-B-P-38-HB (NERICA 1)	4	WAB 56-104 /CG 14	Upland
23	WAB 450-I-B-P-91-HB (NERICA 4)	4	WAB 56-104 /CG 14	Upland
24	WAB 56-104	1	WAB 56-104 /CG 14	Upland
25	WAB 638-1	1	WAB 56-104 /CG 14	Upland
26	YABLO	2	Landrace CI	Upland

Note: Selections were made among the varieties BG-90-2, IT 123, Gninni Zeba and Moluba Kole which did not appear homogeneous in Puerto Rico and two phenotypes were chosen and tested in different plots as separate varieties.

Table 3. US adapted materials used as checks in the yield trial conducted at Beaumont in 2004.

No	Designation	Type *	Origin	Ecology
1	COCODRIE	1	Louisiana	Irrigated
2	WELLS	1	Arkansas	Irrigated
3	CHENIERE	1	Louisiana	Irrigated
4	ZHE733	1	China	Irrigated
5	BANKS	1	Arkansas	Irrigated
6	CYPRESS	1	Louisiana	Irrigated
7	SIERRA	1	Texas	Irrigated
8	JASMINE 85	1	Philippines	Irrigated

* **

1: Improved types CI: Cote d'Ivoire
2: Landraces
3: *O. glaberrima* type
4: Interspecifics (*O. glaberrima x O. sativa*)

harvested and the grains were dried to 12% moisture. The dried seed was cleaned and kept in air conditioned storage at room temperature until analyzed.

The following variables were recorded in the field yield experiment:

Vegetative vigor= VG: The vegetative vigor was noted at the tillering stage according to the Standard Evaluation System for Rice (SES) (INGER 1996) using a scale of 1 to 9. A rating of 1 was given to plots with extra vigorous plants, very fast growing, and rapid tiller development compared to the others; 3 was given to vigorous plants; 5 for normal plants; 7 for weak plants; and 9 for very weak plants.

Days to 50% flowering= 50% FL: The number of days from planting until 50% of the main heads in the plots had extruded anthers was recorded on each individual plot.

Plant height= PHGT: The average height of the plants in a plot recorded in centimeters.

Panicle length= PANL: The mean length of 5 randomly selected panicles per plot was measured in centimeters (IRRI 1980).

Panicle exsertion= PANEX: Panicle exsertion was recorded at the maturity on each plot using SES (INGER 1996). A rating of 1 was given to plots with well exserted panicles; 3 was given to moderately well exerted, 5 to just exserted, 7 to partly exserted, and 9 to enclosed panicles.

Leaf length= LFl: The mean length of 5 randomly selected leaves below the flag on 5 plants was measured at late vegetative stage (IRRI 1980).

Leaf width= LFw: The mean width of 5 randomly selected leaves below the flag on 5 plants was measured at late vegetative stage (IRRI 1980).

Lodging= LGD: At maturity, a rating of 1 was given to plots with no lodging; 3 was given to moderately lodged plants; 5 to plots where most plants was moderately lodged; 7 was given to weak plants nearly flat, and 9 to very weak plots where all plants were lodged (IRRI 1980).

Panicle threshability= Thres: Threshability was determined by firmly grasping and pulling the hand over the panicle and estimating the percentage of shattered grains at maturity. Based on SES (INGER 1996), a rating of 1 was for difficult threshability (less than 1%); 3 for moderately difficult (1-5%); 5 for intermediate (6-25%); 7 for loose (26-50%), and 9 for easy (51-100%).

Leaf senescence= LSen: A rating of 1, 5, or 9 was given to plants which had late and slow senescence, intermediate with upper leaves yellowing, and early and fast (all leaves yellow and dead), respectively.

Yield = Yld: Represents the grain yield of the two row plot, dried to 12% moisture and converted to kg/ha.

100SW= represents the weight of 100 seeds (g).

Phenotypic acceptability= PAcp: it was the score given for the overall acceptability of the variety at harvest maturity in the location where it is being grown (INGER 1996). A rating of 1 was for excellent, 3 for good, 5 for fair, 7 for poor and 9 was given to unacceptable varieties.

3.3.1.2. Rice Milling and Quality Experiment

Milled rice samples from Africa (Table 4) were introduced during the summer 2002 and were evaluated in the laboratory for their quality characteristics. In addition, seed were produced as described above so that, Beaumont field experiments could be conducted to compare the accessions for milling and quality traits when grown in the US. The field experiment consisted of 32 varieties, introduced from WARDA and increased at Puerto Rico after the quarantine, and 14 US checks (Table 5).

The experimental plots consisted of 3 rows that were 4.57 m long with a row spacing 17.78 cm. The fertilizer, herbicide, insecticide, and fungicide applications, the experimental design, and the sample harvest and handling methods were the same as previously described. Maturity was noted for the plots as date from emergence to flowering.

The analyses that were conducted on the rice milled samples introduced from West Africa included: Alkali Spreading Value (ASV), Apparent Amylose Content (AA), Rapid Visco Analyzer (RVA), Differential Scanning Calorimetry (DSC), and Cooking time.

The following analyses were performed with the cultivars grown at Beaumont: Alkali Spreading Value (ASV), Apparent Amylose Content (AA), Soluble Amylose (SA), Rapid Visco Analyzer (RVA), Cooking time, Protein, Aroma, Milling percent, and Grain dimensions.

Table 4. List of rice varieties introduced from WARDA and planted for milling and quality experiment at Beaumont in 2004.

No	Designation	Type*	Crosses**	Ecology
1	BAKUE DANANE	2	Landrace CI	Upland
2	DANANE	2	Landrace CI	Upland
3	BG 90-2(FARO 29) (GR 14) (ROK 28)-33390	1	(Peta 3*TN1)/Remadja	Irrigated
4	BG 90-2(FARO 29) (GR 14) (ROK 28)-33392	1	(Peta 3*TN1)/Remadja	Irrigated
5	DIGBOBLI	2	Landrace CI	Upland
6	DOIGAMLIN-MAN	2	Landrace CI	Upland
7	DOIGAMLIN			
8	COCOTE	2	Landrace CI	Upland
9	GNANLE GNAN-MAN	2	Landrace CI	Upland
10	GNINNI ZEBA-33420	2	Landrace CI	Upland
11	GNINNI ZEBA-33423	2	Landrace CI	Upland
12	GNOKOU GNOKOU	2	Landrace CI	Upland
13	HOLLANDAIS	2	Landrace CI	Upland
14	ITA 123 (FKR 28) (TOM1-3) (KADIAKA)-33433	1	Mutant of OS 6	Irrigated
15	ITA 123 (FKR 28) (TOM1-3) (KADIAKA)-33434	1	Mutant of OS 6	Irrigated
16	JAYA	1	TaichungNative N1/T141	
17	LOGNINI COURT	2	Landrace CI	Upland
18	MAHAFIN	2	Landrace CI	Upland
19	MINMLI	2	Landrace CI	Upland
20	MOKOSSI	2	Landrace CI	Upland
21	MOLOUBA KOLE-33459	2	Landrace CI	Upland
22	MOLOUBA KOLE-33460	2	Landrace CI	Upland
23	WAB 450-11-1-1-P31-HB (NERICA5)	4	WAB 56-104/CG 14	Irrigated
24	WAB 450-11-1-P31-HB (NERICA 2)	4	WAB 56-104 /CG 14	Upland
25	WAB 450-I-B-P-160-HB (NERICA 6)	4	WAB 56-104 /CG 14	Upland
26	WAB 450-I-B-P-20-HB (NERICA 7)	4	WAB 56-104 /CG 14	Upland
27	WAB 450-I-B-P-28-HB (NERICA 3)	4	WAB 56-104 /CG 14	Upland
28	WAB 450-I-B-P-38-HB (NERICA 1)	4	WAB 56-104 /CG 14	Upland
29	WAB 450-I-B-P-91-HB (NERICA 4)	4	WAB 56-104 /CG 14	Upland
30	WAB 56-104	1	WAB 56-104 /CG 14	Upland
31	WAB 638-1	1	WAB 56-104 /CG 14	Upland
32	YABLO	2	Landrace CI	Upland

Table 5. US adapted materials used as checks in the milling experiment conducted at Beaumont in 2004.

No	Designation	Type*	Origin	Ecology
1	COCODRIE	1	Louisiana	Irrigated
2	WELLS	1	Arkansas	Irrigated
3	CHENIERE	1	Louisiana	Irrigated
4	ZHE733 (Beaumont sample)	1	China	Irrigated
5	ZHE733	1	China	Irrigated
6	BANKS	1	Arkansas	Irrigated
7	CYPRESS	1	Louisiana	Irrigated
8	SIERRA	1	Texas	Irrigated
9	JASMINE 85	1	Philippines	Irrigated
10	SABER	1	Texas	Irrigated
11	SABER (Beaumont sample)	1	Texas	Irrigated
12	BALDO		Italy	Irrigated
13	BENGAL	1	Louisiana	Irrigated
14	DAWN		Texas	Irrigated

* **

1: Improved types CI: Cote d'Ivoire
2: Traditional types
3: *O. glaberrima* type
4: Interspecific types

Alkali Spreading Value (ASV)

The method used for ASV involved the visual observation of the degree of dispersion of grains of the milled rice after their immersion in 1.5% or 1.7% KOH. The method was developed by (Little et al. 1958). Known cultivar checks were used as guide for scoring the field grown samples. Six kernels of each sample were evaluated for degree of starch dispersion after soaking in the KOH solution overnight at room temperature. A rating of 2 is for no reaction (kernel firm); 3 for whole kernel with slight to moderate collar dispersion; 4 for slightly split or whole kernel with collar surrounding the kernel; 5 for severely split kernel with extreme collar; 6 for kernel with cotton center and 7 for clear center.

Alkali digestion can determine indirectly the gelatinization temperature (GT). A low ASV corresponds to a high GT, and conversely a high ASV corresponds to a low GT. Low amylose content rice generally has a soft gel, high ASV and low GT (Tan et al. 1999). The scoring method was visual and based on the method of Jennings et al. (1979).

***Apparent Amylose (* AA)**

The milled samples were individually ground through a 0.40 mm screen using a cyclone sample mill model no 3010-018 UDY mill (UDY, Fort Collins, Colorado, USA). Samples of 50 mg of milled ground rice was weighed in duplicate and transferred to sample culture tubes and 1 ml ethanol (100%) added. The tubes were shaken gently for approximately 2-3 min. The samples were covered with plastic wrap and was allowed to stand overnight in 1 N NAOH solution (4 ml 1N NAOH). The following day, 45 ml of deionized water were be added to each sample in a tube. The samples were vortexed for 10 to 15 seconds using a Maxi-Mix 1 mixer (type 16700; Barnstead/Thermolyne, Dubuque, Iowa). The solutions were allowed to overnight in 0.1 N NAOH in a room temperature. An auto-analyzer 3 (model AA3; Bran and Luebbe, Roselle, IL) was used to determine the apparent amylose content using an automated analyzer control and evaluation software AACCE Version 5.24 (Bran and Luebbe, Roselle, IL,). Laboratory checks known for high and low amylose content were used: Dixiebelle (25% amylose), Gulfmont (22%), Bengal (17%), and Mochi (0%). The average of two replications were used as the apparent amylose content values.

Soluble Amylose Content and Insoluble Amylose* (SA –IA*)

The milled rice was ground to flour as described above. Fifty (50)mg of milled ground rice were weighed in duplicate and transferred to culture tubes (item no. T3062-8; Fisher Scientific, Norcross, Ga.) and 1ml ethanol (100%) was added. The tubes were placed in a rack, shaken gently for approximately 5s. Thirty ml of deionized water were added to each tube, and the tubes were placed in a water bath heated to at least 98^{o} C for 30 min. The tubes were removed, allowed to cool, and 20 ml of deionized water were added to each tube. Each sample was vortexed for 10 to 15 seconds using a Maxi-Mix 1 mixer (type 16700; Barnstead/Thermolyne, Dubuque, Iowa). From each tube a volume of 12-13 ml were decanted from each culture tube into a coning 15 ml centrifuge tube. The centrifugation was conducted for 5 min at 3,000 rpm. Supernatant from the centrifuged sample were decanted in another corresponding labeled 15 ml coning centrifuging tube. The tube was capped and refrigerated overnight, and samples were analyzed the following day the same way as in apparent amylose experiment.

The insoluble amylose (IA) was calculated by subtracting the soluble amylose from the apparent amylose.

***Rapid ViscoAnalyzer* (RVA)**

The rice samples were milled and ground using the method described previously. Paste viscosity was determined on a Rapid ViscoAnalyzer (RVA) instrument using the American Association of Cereal Chemistry (AACC) (1995) Standard Method 61-02. The RVA 3-D model was used with Thermocline Windows control and analysis software, Version 1.2 (Newport Scientific, Sydney, Australia). The RVA uses 3 g of rice flour in

25 ml water (Juliano 1996). The temperature is at 50^{o}C for 1 min, heating to 95^{o}C at 12^{o}C per min, 2.5min at 95^{o}C. The cooling is 50^{o}C or 30^{o}C at 12^{o}C per min. The heating is at 30^{o}C for 0.9 min for a total running time of 12.5 min. The RVA breakdown, the consistency and the setback at 50^{o}C and 30^{o}C is calculated. The units for all the calculated parameters are in Rapid Visco Units (RVU). One unit RVU=10cp. The viscosity characteristics obtained from the RVA can be described by three important parameters: the peak (first peak viscosity after gelatinization), hot paste (paste viscosity at the end of 95^{o}C holding period), and cool paste viscosity (paste viscosity at the end of the test). Breakdown (Bkdn) is derived from peak minus hot paste viscosity; setback (Stbk) is derived from cool paste viscosity minus peak viscosity values; consistency Viscosity (CSV) is derived from cool paste viscosity minus hot paste viscosity. The different parameters obtained are measured in Rapid Visco Units (RVU).

Differential Scanning Calorimetry **(DSC6)**

Differential scanning calorimetry experiment was conducted using DSC6 (Perkin-Elmer Corp., Norwalk, CT). The software used with the computer was the Pyris series Thermal Analysis Manager Suite N537-0605 Version 4.0. Before any experiment was conducted, the Dixiebelle variety was run as a standard. A sample of 160 mg of rice flour was weighed and 320 µl of deionized water was gently added to it in a polystyrene weighing dish. The sample was mixed with water and a 1- ml syringe was used to collect 0.1ml of mixture and inserted into the instrument. The onset (To), peak (Tp), conclusion (Tc) and enthalpy (ΔH) of gelatinization were calculated automatically by using the

program “Pyris” indicated earlier. The gelatinization temperature range (Tr) can be calculated as [2(Tp-To)] as described by Krueger et al. (1987)

Milled Rrice Cooking Time **(CT)**

Minimum cooking time is defined as “the amount of time in minutes required for gelatinizing the starch in 90% of the kernels” (Ranghino 1966). This is determined by kernels being cooked in excess water and then sampled every minute during cooking, starting at 10-14 minutes, until 9 out of 10 kernels show no ungelatinized starch. Sampling times depends on grain market class. Conventional short and medium grains and jasmine and Toro (soft-cooking) types are sampled starting at 10 minutes whereas conventional long grains (firm-cooking types) sampling starts at 14 minutes.

300 ml of distilled water was dispensed into 400 or 600 ml beaker, several boiling chips were added, and the water was brought to a rolling boil on a hot plate. The timer was set for 10 or 14 minutes. 3-5 grams of kernels were added to the boiling water and the contents were stirred with a long spatula. The beaker was covered with a watch glass. Timing started as the rice was added to boiling water. As the timer went off, 10 grains of rice was scooped with ladle. Excess water was drained off by blotting scoop on paper towels. Ten rice grains were spread on a large glass plate and separated over 7.0 cm square areas using a small spatula. Rice grains were pressed firmly with small glass plate until grains have been completely flattened. Ungelatinized starch appeares as stark white, opaque spots within centers of otherwise transparent grain. The sampling continued at one-minute intervals until reading of 9-10 clear centers was obtained for a sample.

Milled Rice Crude Protein Determination

Each rice sample was weighed in duplicates. The protein content was determined by a nitrogen gas analyzer (model 528; LECO). Between 0.8 to 1 mg were placed into a quartz combustion tube in an induction furnace at 850^{o}C, and flushed with pure oxygen for very rapid combustion. The results were displayed as weighed percentage of nitrogen. A factor of 5.95 was used to convert nitrogen % to protein. The method was based on the Official Method 990.03 (AOAC 1995).

Aroma Content - Quantification of 2-Acetyl-1-Pyrroline by Gas Chromatography with Conventional FID Detection

Bergman et al. (2000) reported rapid solvent method for extracting 2-AP. Extraction and quantification of 2-AP is accomplished by weighing 0.3 gm of 20 mesh ground rice to a 12 x 32 mm crimp top vial. Stock solution (0.5 ml) containing w\v = 458.5 ng\ml TMP in methylene chloride is added to the crimp top vial and vial sealed. Extraction is performed at 85 ^{o}C for 2.5 hours. Vial contents are analyzed by gas chromatography.

The AP concentration is calculated using the formula below:

$$\text{AP concentration} = \frac{\text{Peak area of AP\Peak area of TMP x ng of TMP}}{\text{Weight of rice (g)}}$$

Milling Experiment

Total and whole milled rice were determined for each sample. An initial amount of 125 g of clean rough rice was weighed and the moisture content recorded. The different samples were milled using the McGill#2 mill for 1 minute. The adjusted total

weight at 12% moisture content was determined. Broken kernels were removed using the sieves and then the amount of whole milled rice was determined.

Adjusted weight at 12% = [(100- moisture content of sample)/88]

Percent mill = (Adjusted weight/125)*100

Grain Dimensions

Grain dimensions were measured using the WinSEEDLE scanner machine. Rough rice was dehulled using the Satake dehuller and broken rice was removed using the sieve#10. The seeds were poured into the appropriate tray and the rubber tipped forceps were used to make sure none of the seeds are touching. The lid of the scanner was closed and a digital image was saved of the sample (WinSEEDLE 2005). A resolution of 400 dpi (dots per inch) was used during the scanning. A Regent Positioning System was used to choose the exact area on the scanner where the sample was placed and scanned. The recommended menu filters were used to remove objects with an area less than 3 mm^2 and greater than 200 m^2.

3.3.2. Molecular Genetic Laboratory Experiments

In the biotechnology laboratory at Beaumont, Texas, the DNA was extracted from leaf tissue and from the brown rice using the DNA extraction procedures CTAB and the QIAGEN DNAeasy method respectively. Fingerprinting markers were used to characterize the African parents using the CTAB extraction method. The QIAGEN DNAeasy was used to study the segregating populations Cocodrie/Dixiebelle.

3.3.2.1. Molecular Markers for WA Cultivar Characterization

DNA was extracted from leaf samples from plants grown in quarantine using the CTAB method**.** PCR was used for amplification followed by evaluation for polymorphisms using ABI sequencer.

3.3.2.2. Marker Associations with Cooking Quality Traits

A population was developed by the USDA-ARS Beaumont rice breeding program between Cocodrie (CCDR) and Dixiebelle (DXBL). The two varieties have a similar apparent amylose content of 26%, but present different RVA profiles. Studies need to be conducted to elucidate the differences between CCDR and DXBL regarding the RVA parameters and associations with the *Waxy* microsatellite (RM190), a SNP in exon 10 of the *Waxy* gene and a starch soluble synthase AB26295.

Brown rice was used for DNA extraction using Qiagen Kit. PCR was used for amplification followed by evaluation for polymorphisms using ABI sequencer.

Lipid Extraction from Milled Rice Flour

Petroleum ether and methanol 85% were used respectively to remove free lipid and bound lipid from the rice flour. Hogan et al. (1961) described the method of determining the degree of milling of whole milled rice. The Goldfish extraction apparatus (Lab Conco, MO, and USA) was used. The time for the experiment was 4 hours and 16 hours respectively for petroleum ether and methanol 85% extraction methods. The solvent was collected and evaporated; per cent lipid was calculated as the mass of the extracted lipid divided by the beginning total milled rice mass.

The results were expressed as % surface lipid

1) On an as is weight moisture basis:

$$\frac{[\text{wt (g) of beaker containing extracted lipids - wt of beaker}] \times 100}{\text{Wt (g) of original rice sample}}$$

2) On a dry weight basis:

$$\frac{[\text{wt (g) of beaker containing extracted lipids - wt of beaker}] \times 100}{\text{Wt (g) of original rice sample} * (1 - \% \text{ moisture})}$$

3) On a 12% moisture basis:

$$\frac{[[\text{wt (g) of beaker containing extracted lipids - wt of beaker}] \times 100] \times 0.88}{\text{Wt (g) of original rice sample} * (1 - \% \text{ moisture})}$$

DNA Extraction from Leaf Tissue Using the Cetyltrimethylammonium Bromide CTAB Method

Total DNA was extracted from green leaves dried in an oven at 45^{o}C for 24 hours. 60 mg of dried leaf tissue was weighed on waxed weighing paper, cut into sections as fine as possible with scissors, and transferred into one of the appropriately labeled 2 ml tubes. Five glass beads were added to each vial of leaf tissue and attached to the BioSpec Products Mini-beadbeater. To completely pulverize the sample, the Mini-beadbeater was run for one minute. 900 µl of Ctab extraction buffer was added to each pulverized sample and each sample was briefly vortexed to ensure no air bubbles were attached to the material. The samples with the extraction buffer were placed into a water bath maintained at 65^{o}C for at least 1 hour. The samples were quickly cooled to room temperature and 600 µl of chloroform was added and each tube was immediately capped upon completion. The rack containing the tubes was vigorously shaken fifty times in rapid succession. The tubes were centrifuged for 10 min at 12,000g at room temperature.

After spinning, each tube was uncapped and the top layer of buffer containing extracted DNA was removed with a filtered tip, and placed into the duplicate appropriately labeled tube. The tubes containing the tissue/chloroform was recapped and placed into the designated container with the cap tightly secured. 800 µl of absolute isopropanol was added to each sample containing the DNA extract. Isopropanol causes DNA to precipitate. The solution was mixed well and placed at -20^{o} C for at least 30 min. The samples were be then centrifuged at 13,000 g for 15 minutes. Immediately after centrifugation, the supernatant was removed and the liquid were removed with a pipette using unfiltered tips without dislodging the DNA pellet. 400 µl of 100% ethanol was added into each tube containing the DNA pellet, capped and gently shaken. The samples were left at room temperature for 15-30 min or were refrigerated at -20^{o} C until the next working day. The tubes were removed and placed into a centrifuge at 13,000g at 4^{o} C for 15 minutes. Ethanol washes the DNA. Immediately upon completion of the centrifugation, the ethanol was removed carefully to not dislodge the DNA pellet. The DNA pellets was allowed to air dry or under gently heating at 30-40^{o} C until they were completely dried. Immediately after drying, 500 µl of TE buffer was added to each tube containing the DNA; the samples were allowed to dissolve overnight at 4^{o} C. Before any solution was removed, the tubes were gently vortexed at low speed to assure dissolution and complete mixture of DNA, and briefly centrifuged at 12,000 g (10s or less) to remove any solution from the cap or tube walls. If sediment was seen after centrifugation, it was recommended to spin for 5 min at 12,000g to compact any

precipitate, and the solution containing the DNA was transferred into new tubes appropriately labeled. The samples were stored at 4^{o} C.

DNA Extraction from Seed Using the QIAGEN DNAeasy Plant Mini Kit

Two tubes were labeled for each sample. 20 milled seeds and 5 to 8 glass beads were added to each cone bottom tube. The tube was shaken for 10 minutes at maximum speed and centrifuge for 30s to pull dust from tube top. The seeds and beads were discarded from the remaining bran (approximately 20 mg). 400 µl AP1 buffer and 4 µl RNase stock solution were added to the bran. The solution was vortexed briefly. The tubes were placed into a rack and incubated for 10 min at 65^{o} C, the solution was mixed by inverting the tubes 2-3 times during incubation. 130 µl of AP2 solution was added to each tube, mixed and incubated on ice for 5 min. The solution was centrifuged at maximum speed for 5 minutes. After centrifugation, 400 µl of solution was removed and placed in a shredder column (lavender tube), then centrifuged at maximum speed for 2 min. 350 µl of the flow-through was transferred into a labeled dolphin tube without disturbing the pellet. After the transfer, 525 µl of Buffer AP3/E was added and mixed by pipetting, 650 µl of the solution was pipetted into the white mini spin column. The solution was centrifuged for 1 min at 8,000 rpm. The flow-through was discarded into a beaker. The remaining solution of sample was added to the spin column, and then centrifuged for 1 min at 800 rpm and the flow-through was again discarded into a beaker. The spin column was placed into a new 2ml collection tube (from the kit –no top). 500 µl AW Buffer was added to the spin column, and centrifuged for 2 min at maximum speed. Only the top portion of the spin column was transferred into a labeled

dolphin tube, the lower portion of the column was discarded as well as the flow-through into a beaker. 50 µl of AE buffer (preheated to 65^{o} C) was directly pipetted onto the white portion of the membrane, and allowed to incubate for 5 min. The solution was then centrifuged for 1min at 8,000 rpm. The addition of 50 µl of AE and the incubation for 5 min was repeated in order to get 100 µl of total volume. The spin column was discarded and the liquid was stored.

Polymerase Chain Reaction PCR

All the tubes containing the DNA template including the checks were assembled and quickly centrifuged at 12,000g to remove all moisture from sides and top. The following ratio for PCR were used as described in Table 6, the Epicenter Tfl was used when the DNA was isolated from seed.

Table 6. Composition of the PCR master mix.

Taq		*Tfl*		Amount of water required		
20 µl total mix					*Taq*	*Tfl*
10 x	2 µl	10 x	2 µl	1 µl template	14.7 µl	15 µl
Mg	1 µl	Mg	1 µl	2 µl template	13.7 µl	14 µl
dNTP	0.4 µl	dNTP	0.4 µl	3 µl template	12.7 µl	13 µl
Primer F	0.4 µl	Primer F	0.4 µl	4 µl template	11.7 µl	12 µl
Primer R	0.4 µl	Primer R	0.4 µl			
Taq	0.1 µl	Tfl	0.8 µl			

A total amount of 550 mM Tris pH 9.0, 450 mm ammonium sulfate, 20 mM magnesium chloride was prepared and the total amount of master mix for each primer

was determined. Enough for 2-3 extra samples was prepared. A microcentrifuge tube was labeled appropriately for the volume required. The calculated amount of molecular grade water was added to each master mix tube. The tubes were replaced, capped and placed in the ice container. The forward and reverse primers and the dNTP solution were removed from the freezer to allow thawing. The solutions were not allowed to completely thaw at room temperature because the shelf life was shortened. All the reagents were quickly vortexed before use. The 10x and Mg solutions was completely thawed for correct molarity. Each container was vortexed after complete thawing. The master mix tubes were capped at all times unless adding reagents. The proper amount of 10x and Mg (*Taq*) or 10x (*Tfl*) was added to each mix. The tubes were well mixed after addition. After mixture, the appropriate amount of dNTP solution was added to each mix, followed by an addition of the appropriate amount of forward and reverse primer solution. The solution of master mix was briefly vortexed to mix the reagents. The proper amount of *Taq* or *Tfl* polymerase was added to each tube and was vortexed, but mixed with a pipette or caped and inverted gently. The mixture was not shaken vigorously. The plastic well plate was placed into the plate holder, and both were placed into the cold box. The appropriate amount of master mix was added to each labeled well in the plate, and the appropriate amount of DNA template was added to each well. The solution was mixed by pipetting up and down at least 4 times and then by swirling the solution with the pipette tip. The GeneAmp PCR system 9700 Thermocycler was then prepared for proper run conditions. The conditions are listed in Table 7 for microsatellite markers.

Table 7. Run conditions of the GeneAmp PCR system 9700.

ABI 3100					
1 HOLD	3 TEMP	25 CYCLES		2 HOLDS	
94^{o} C	94^{o} C	55-60^{o} C	72^{o} C	72^{o} C	4 ^{o}C
4.00 min	0:45 sec	0:45 sec	1:00 min	5:00 min	α

The conditions for *Waxy* gene exon 6 and exon 10 and *Alk* gene SNP markers are as follows:

PCR Sample Set-up

The PCR samples are the same as 'normal' PCR with exception of using two labeled forward primers instead of one.

The PCR reaction was performed in10 µl volumes containing 55mM Tris (pH 9.0), 45 mM $(NH_4)_2SO_4$, 2.0 mM $MgCl_2$, 0.2 mM dNTPs, 0.2 µM each of an allelic specific primer (ASP) 1, an ASP 2 (10 µM), and a reverse primer, 1.0 µl DNA extract containing approximately 2.5 ng DNA, and 0.3 U *Tfl* polymerase (Epicentre Technologies, Madison).

The PCR parameters for SNP markers were: initial denaturation at 94 ^{o}C for 3 minutes; followed by 16-23 cycles of amplification (see the following specifications for each SNP marker analyzed) where denaturation was set at 94 ^{o}C for 45 sec, then primer/template annealing starting at the IA temperature and lowered by 0.5 ^{o}C for each cycle of amplification, then primer extension at 72 ^{o}C for 1 min; followed by a final extension at 72 ^{o}C for 3 min and a temperature hold at 4 ^{o}C until the PCR reactants were

analyzed with the ABI 3100 Genetic Analyzer capillary electrophoresis instrument or stored at 4 ^{o}C in a refrigerator prior to capillary electrophoresis. The initial annealing temperature (IA) for the *Alk* (SSSIIa) SNP marker was 67 ^{o}C and the number of amplification cycles was 19-23. The IA for *Waxy* Exon 6 and *Waxy* Exon 10 SNP markers was 65 ^{o}C and the number of amplification cycles was 16-20.

The sequences for the *Alk* SNP marker are CGGGTCGAACGCCGAAAC-HEX for the primer ASP 1, AACGGGTCGAACGCCGAAAT-FAM for the primer ASP 2 and GGCCTCAACCAGCTCTACGC for the reverse primer. The genotypes for the *Alk* SNP marker are 90 (hex) for intermediate and high gelatinization temperature and 92 (fam) for low gelatinization temperature.

For the *Waxy* Exon 6 SNP, CAACCCATACTTCAAAGGAACATC-TAMRA is the sequence for primer ASP 1, AACAACCCATACTTCAAAGGAACATA-FAM is the sequence for primer ASP 2 and AGTCGTTGCAGACGAACACAAC is the sequence for the reverse primer. The *Waxy* exon 6 SNP marker genotypes are 146 (fam) for intermediate amylose and 148 (tamra) for low or high amylose.

The primer ASP 1 GCGGCCATGACGTCTGG-HEX is for *Waxy* exon 10 SNP, the primer ASP 2 is GGCGGCCATGACGTCTGA-FAM and the reverse primer is TCAGGCAATCGAGGCGAAG. The *Waxy* exon 10 SNP marker genotypes are 133 (hex) for weak RVA and 134 (fam) for strong RVA.

Twenty (21) PCR markers were selected and screened for marker association study. The markers were either near starch metabolism like soluble starch synthase (SSS) and starch branching enzyme (SBE) or at a map position with significant effects on starch properties (like amylose content, or RVA pasting properties). The three markers listed in Table 8 were used for markers association studies with the population Cocodrie/Dixiebelle after the screening procedure.

When the PCR 9700 thermocycler is prepared for proper run, and the temperature reaches 94 ^{o}C, the plate was quickly placed into the thermocycler rack and the lid was clamped. The rubber cap must be in place and not detached when clamping. The run is automatic, so the machine was kept the plate at 4 ^{o}C until removed. After completion, the lid of the thermocycler was raised and the run was stopped.

Table 8. PCR primers for molecular marker analysis.

Primers	Annealing Temperature °C	Sequence	Starch metabolism gene	Map Location (chromosome location)
Waxy RM 190	55	5'-CTTTGTCTATCTCAAGACAC-3' 5'-TTGCAGATGTTCTTCCTGATG-3'	Granule-bound starch synthase	6-8.2
exon10 *wx*10	66	5'-GCGGCCATGACGTCTGG-3' 5'-GGCGGCCATGACGTCTGA-3'	Granule-bound starch synthase	6-8.2
AB 26285	55	5'-CTAGCCATGCTCTCGTACC-3' 5'-CAACTTACTGTGACTGACTTGG-3'	Soluble starch synthase I SSI	6-15.3

The rubber cap was allowed to cool before removing the PCR plate. The plates were stored at 4^{o} C for short term and at -20^{o} C for longer term storage.

After use, the rubber cap was placed into a 10% Chlorox solution for 10-15 min, and then it was rinsed in deionized water. The water was replaced several times. They were stored in a dry and clean place.

Sample Preparation for 3100 Sequencer

The molecular weight of the different samples was determined by the use of the 3100 sequencer machine based on the following procedure:

A formamide molecular weight standard (400 HD) solution was made for sample dilution and 20 µl of 400HD ROX was added to the formamide for a full 96 well plate. To each well were added 9 ml of formamide/ROX mixture and 1 µl of PCR reaction agent was added to each well. In case of multiplexing, 1 µl of second PCR was added to the well. The plate was centrifuged to remove any bubbles and samples were denatured on the PCR machine for 2 minutes at 94 degrees. The 96 well plates were assembled into an ABI plate holder. The trays were placed on a sample holder and linked to the 3100 data collection software. The data collection software was stored and samples were analyzed.

3.4. Statistical Analyses

The procedure PROC GLM from SAS Version 9.0 was used to estimate the means differences between the different variables. PROC CORR was used to generate the different correlation tables using SAS Version 9.0. The different histograms and multivariate analyses to differentiate the groups of cultivars was done using STATVIEW SAS Second edition (1998) with MANOVA. Genotypes for each locus were scored as having Cocodrie, Dixiebelle or heterozygous genotype. The single factor analysis and the multiple locus analyses of variance were generated using PROC GLM SAS Version 9.0. A paired comparison was used with SAS 9.0 to compare means of samples from WA with the same entries grown in Beaumont research station.

4. RESULTS AND DISCUSSION

4.1. Replicated Yield Trial Conducted at Beaumont

4.1.1. Comparison Among Varieties

Results from the yield trial conducted at Beaumont demonstrated that the cultivars were significantly different for all traits (Tables 9 and 10). As might be expected, the highest yielding varieties evaluated in Beaumont were developed for production in the US. Two of these were Cocodrie and Wells which are the two most widely grown rice cultivars in the southern US at this time (Table 10). Zhe733 was the highest yielding cultivar in this study. It was developed in China but because of its high yield in US environments, it is currently being used in a high yield introgression program conducted by USDA-ARS ((Rutger J.N, USDA-ARS Dale Bumpers National Rice Research Center, Stuttgart, AR, pers. comm.). BG90-2-92, an irrigated variety from Sri Lanka, produced comparable yield to these high yielding cultivars although it flowered over 40 days later (Table 10). In addition, Nerica 5 and Nerica 2 were not significantly different from Cocodrie in yield potential although they flowered one week earlier. This demonstrates that these West African cultivars are adapted to the US and may be new genetic resources that may be useful in US breeding programs.

Until about 5 years ago, Cypress was the most widely grown cultivar in the southern US rice growing region. The West African cultivars Nerica 4, Nerica 7, Nerica 3, WAB56-104, and Bakue Danane were not significantly different from Cypress in yield potential (Table 10). These varieties may be interesting genetic resources for use in US breeding programs.

The poorest yielding lines, Mokossi, Gnanle Gnan, Yablo, and Logninni Court, were characterized as being very late in flowering, very tall, susceptible to lodging, and had very long and wide leaves (Table 10).

Table 9. Mean square of the different parameters for the yield trial conducted at Beaumont.

Mean Square								
Source of variation	df	Yld	100SW	VG	Til	50%FL	PHGT	LGD
Replication	3	191820.4	0.019	2.56	1.23	11.50	10.35	0.79
Varieties	33	118587550**	0.42**	5.91**	6.20**	1033.43**	2845.01**	14.58**
Error	87	257073	0.007	0.78	0.49	4.75	27.89	1.11

Table 9. Continued.

Mean Square								
Source of variation	df	Thresh	Lsen	Pacp	Exser	PanL	LeafL	LeafW
Replication	3	0.16	13.28**	1.10	0.03	1.99	32.08	1.37
Varieties	33	1.12**	8.55**	246.80**	5.21**	11.35**	1058.46**	13.09**
Error	87	0.12	1.84	0.84	0.03	3.12	20.79	0.85

Table 10. Means of the different parameters for agronomic traits evaluated in the yield trial conducted at Beaumont.

No	Varieties	Yld (kg/ha	100SW(g)	VG	FL50 % days	PHGT (cm)	LGD	Thres
8	ZHE733	7404.0 a	2.9 c	5 f	59 o	98.3 mn	6 c	2 bcd
22	COCODRIE	7004.2 ab	2.4 klmn	3 bcde	74 l	99.2 mn	1 a	3 f
2	BG 90-2-92	7004.2 ab	2.3 mn	1 a	120 a	108.5 jkl	1 a	3 f
30	Wells	6986.9 ab	2.7 de	4 def	75 kl	107.0 jklm	1 a	2 ef
25	Nerica 5	6229.6 bc	2.3 n	4 def	69 mn	109.2 jkl	3 b	2 bcd
27	Nerica 2	6200.8 bc	2.6 efghi	5 f	68 n	110.0 jkl	3 b	2 bcd
26	Cheniere	6081.6 c	2.1 o	3 bcde	76 jkl	98.2 mn	1 a	3 f
34	Jasmine	6052.8 c	2.6 efghij	1 a	92 g	123.0 hi	1 a	3 f
18	Banks	5974.0 c	2.4 jklm	4 def	78 ijk	111.2 jk	1 a	3 f
14	Sierra	5933.6 c	2.9 c	3 bcde	75 kl	95.5 n	1 a	1 a
33	Nerica 4	4563.1 d	2.8 c	4 def	73 l	99.0 mn	1 a	1 ab
3	WAB 56-104	4540.1 d	3.2 b	5 f	69 mn	127.0 h	1 a	2 bcd
29	Nerica 7	4480.5 d	3.5 a	5 f	72l m	154.2 efg	4 bc	2 bcd
4	CYPRESS	4457.4 d	2.2 n	3 bcde	79 ij	105.7 klm	1 a	3 f
31	Nerica 3	4251.7 ed	2.9 c	5 f	73 l	100.5 lmn	1 a	1 bc
10	Bakue Danane	4063.4 edf	1.9 p	4 def	99 f	156.7 def	1 a	3 f
11	Cocote	3498.3 egf	1.8 p	3 bcde	98 f	158.2 efd	1 a	3 f
15	Gnokou – Gnokou	3456.0 egf	2.6 efgh	3 bcde	106 cd	161.2 bcde	1 a	2 ef
28	Nerica 6	3344.5 gf	2.7 de	4 def	88 h	146.7 g	1 a	3 f
16	ITA 123-33	3302.2 gf	2.7 de	1 a	101 ef	128.0 h	1 a	3 f
5	WAB 638-1	3106.2 gh	1.8 p	4 def	101 ef	151.5 fg	1 a	3 f
13	Gninni Zeba 23	3063.9 hg	2.5 hijkl	3 bcde	105 de	162.5 bcde	1 a	2 ef
7	Moluba kole 59	3029.3 gh	2.5 hijkl	4 def	80 i	165.3 bcd	1 a	2 ef
32	Nerica 1	2987 gh	2.6 gef	4 def	74 l	116.0 ij	1 a	2 cde
20	Mahafin	2963.9 gh	2.5 fghij	4 def	103 de	162.0 bcde	1 a	2 bcd
17	ITA 123-34	2946.6 gh	2.4 ijkl	1 a	102 ef	126.0 h	1 a	3 f
6	Gninni Zeba 20	2801.2 gh	2.4 ijkl	1 a	104 de	164.7 bcd	1 a	2 cde
21	Minmli	2337.3 hi	2.5 ghijk	5 f	110 b	147.0 g	1 a	3 f
24	Moluba kole 60	2272.0 hi	2.6 def	3 bcde	109 bc	170.2 ab	1 a	2 def
23	Mokossi	1535.8 ij	2.4 ijkl	3 bcde	104 de	169.0 abc	6 c	3 f
12	Gnanle Gnan	1422.4 j	2.4 ijkl	2 abc	102 ef	168.7 abc	6 c	3 f
9	Yablo	1284.0 j	2.7 de	2 abc	106 cd	160.0 cdef	5 bc	3 f
19	Logninni Court	1109.7 j	2.3 n	2 abc	118 a	176.5 a	9 d	3 f
	Average	4150.0	2.5	3	90	134.0	2	2

Yld= yield (kg/ha) PHGT= Plant height (cm)
100SW= weight of 100 seeds (g) LGD= lodging
VG= vegetative vigor Thres= Threshability
FL50%= 50% flowering date
Means with the same letter within the same column are not statistically different.

Table 10. Continued.

No	Varieties	Lsen	Pacp	PANEX	PanL(cm)	LeafL(cm)	LeafW(mm)
8	ZHE733	9 d	7 g	3 c	22.3 g	34.2 jk	14.3 hijkl
22	COCODRIE	5 cd	3 bcd	3 c	23.3 defg	42 hij	13.8 jkl
2	BG 90-2-92	1 a	3 abc	3 c	24.5 defg	57.5 ef	13.8 kl
30	Wells	5 cd	2 ab	3 c	28.2 ab	40.0 hij	13.1 l
1	BG 90-2-90	1 a	3 abc	3 c	23.8 defg	55.3 f	13.4 l
25	Nerica 5	6 d	5 efg	3 c	23.5 defg	38.5 ijk	15.4 fghij
27	Nerica 2	6 d	4 cde	1 a	24.3 defg	41.1 hij	15.7 efghi
26	Cheniere	5 cd	3 bcd	3 c	25.9 abcdef	41.9 hij	14.2 ijkl
34	Jasmine	5 cd	5 cde	3 c	26.2 abcde	43.9 ghi	19.8 a
18	Banks	5 cd	1 a	3 c	23.2 defg	47.3 gh	15.8 efgh
14	Sierra	5 cd	3 abc	3 c	23.8 defg	33.2 k	16.7 defg
33	Nerica 4	5 cd	3 abc	3 c	22.6 gf	43.8 ghi	15.3 ghijk
3	WAB 56-104	5 cd	4 cde	1 a	24.1 defg	44.2 ghi	13.7 l
29	Nerica 7	5 cd	5 efg	1 a	24.5 defg	50.3 fg	15.6 fghi
4	CYPRESS	4 bcd	3 abc	3 c	24.3 defg	51.6 fg	14.7 hijkl
31	Nerica 3	5 cd	3 bcd	3 c	23 efg	40.2 hijk	14.7 hijkl
10	Bakue Danane	5 cd	4 cde	1 a	24.8 cdefg	66.0 d	18.1 bcd
11	Cocote	5 cd	5 cde	1 a	24.2 defg	66.6 d	18.8 ab
15	Gnokou – Gnokou	1 a	3 abc	1 a	23.15 defg	77.1 bc	18.4 abc
28	Nerica 6	4 bcd	3 bcd	3 c	22.35 g	68.9 d	16.5 defg
16	ITA 123-33	4 bcd	5 efg	4 d	25.7 abcdef	51.7 fg	18.4 abc
5	WAB 638-1	5 cd	5 cde	1 a	23.7 defg	65.2 d	18.1 bcd
13	Gninni Zeba 23	2 ab	3 abc	1 a	25.1 bcdefg	77.2 bc	18.1 bcd
7	Moluba kole 59	2 abc	4 cde	1 a	26.4 abcd	63.6 de	17.5 3bcd
32	Nerica 1	3 abc	5 cde	3 c	24d efg	64.0 de	17.6 bcd
20	Mahafin	3 abc	3 abc	1 a	28.7 a	83.9 b	19.8 a
17	ITA 123-34	4 bcd	5 cde	5 e	25.6 abcdefg	57.0 ef	18.1 bcd
6	Gninni Zeba 20	2 ab	3 bcd	1 a	26.0 abcde	79.03b	18.4 abc
21	Minmli	3 abc	5 cde	1 a	23.0 efg	70.5 cd	17.9 bcd
24	Moluba kole 60	2 ab	3 bcd	1 a	28.1 ab	95.1 a	17.3 bcde
23	Mokossi	4 bcd	7 g	1 a	24.5 defg	78.3 b	18.6 abc
12	Gnanle Gnan	5 cd	6 fg	1 a	22.6 gf	69.5 d	18.4 abc
9	Yablo	3 bcd	6 fg	1 a	24.6 defg	79.7 b	17.4 7bcd
19	Logninni Court	4 bcd	7 g	1 a	27.9 abc	83.9 b	17.0 cdef
	Average	4	4	2	24.7	59.0	16.7

Lsen = Leaf senescence
Pacp = Phenotypic acceptability
PANEX = Panicle exsertion
PanL = Panicle length (cm)
LeafL = Leaf length (cm)
LeafW = Leaf width (mm)
Means with the same letter within the same column are not statistically different.

4.1. 2. Comparisons Between Germplasm Groups

African cultivars have rarely been evaluated under US field conditions. Useful traits from the African native rice *O. glaberrima* have been successfully introgressed into sativa germplasm to produce the Nerica cultivars (Jones et al. 1997a, 1997b). The results from this study are the first to compare Nerica and other WA cultivars with US cultivars. Means and Pearson least significant differences (PLSD) between the different groups of cultivars are summarized in Tables 11 to 13.

The germplasm entries were divided into four groups based on their background. The first group (LOC) was composed of the landraces collected from farmers' fields in Cote d'Ivoire. The second group (IMP) was composed of the improved varieties introduced to West Africa through different collaborative research programs, or were developed by WARDA or West African national programs. The third group (INT) was composed of the Nerica cultivars that were derived from an interspecific cross of *O. glaberrima* and *O. sativa*. The fourth group (US) was composed of the USA current commercial cultivars as well as other varieties that possess various grain quality traits that were useful for making comparison with the WA germplasm. In addition, Zhe 733, a cultivar from China is included in this group because it is currently being used in a high yield introgression project in the US (Rutger J.N, USDA-ARS Dale Bumpers National Rice Research Center, Stuttgart, AR, pers.comm).

The US cultivars had significantly higher yield than the other groups as would be expected since they were developed for production in this region (Tables 11 and 12).

The Nerica lines had the same yield potential as the improved lines from WA and all groups had higher yield potential than the landraces from WA (Tables 11 and 12).

The vigor of US and LOC cultivars was similar, however, the INT cultivars had significantly greater vigor than the other groups (Table 12). This indicates that the INT germplasm may be a new genetic resource for improving US cultivars for rapid plant growth and development.

The INT cultivars had similar days to flowering as the US cultivars indicating that they are well adapted to US growing conditions (Table 12). In contrast, the IMP and LOC cultivars flowered about one month later than these cultivars under US conditions (Table 11). Jasmine 85 is a cultivar developed in the Philippines but commercialized in the US, although it is considered a late maturing variety. The IMP and LOC groups averaged 10 days later in flowering than Jasmine 85 indicating that their late maturity would likely be a negative factor in using them in US breeding programs.

The IMP and INT cultivars were similar in height to each other but were over 14 cm taller, on average, than US cultivars (Table 12). However, only Nerica 6 and Nerica 7 were considered very tall cultivars in the INT group while the other Nerica cultivars were similar in height to the US materials. The landrace germplasm sources were taller than any other group (Table 12). Height is an important factor for competing with weed pressure in WA where herbicides are not commonly used and most rice is

harvested manually. The US materials have been developed to have short and sturdy culm which is well suited for the high input production practices and combine harvesting which is used in the US. Thus, the US group was significantly shorter than the other groups and all but two of the US varieties (Banks and Wells) have a semidwarf plant type (Table 12).

Only small differences in lodging were observed among the groups (Table 12). All of the US cultivars, except Zhe733, were rated highly resistant to lodging. Although Zhe733 is semidwarf cultivar, it was as susceptible to lodging as Mokossi, Gnanle Gnan, Yablo, and Nerica 7.

Table 13 shows the correlation among the agronomic traits using cultivar means. High yield was associated with early flowering, reduced plant height, reduced panicle exsertion, lodging resistance, reduced leaf size, and increased leaf senescence. This suggests that the high yielding cultivars are more physiologically efficient and have less biomass. Later maturing cultivars were rated as having high vigor due to their lush vegetative growth as observed in tall plant height, extensive panicle exsertion, and large leaf size.

Table 11. Mean comparison of the different variables for the three West African groups of varieties and the US checks.

	Yld (kg/ha)	100SW (g)	VG	FL50% (days)	PHGT (cm)	LDG	Thres	Lsen	Pacp	PANEX	PanL (cm)	LeafL (cm)	LeafW (mm)
IMP	4342.36	2.59	1.57	101.93	122.21	1.29	2.71	3.29	4.43	3.71	25.00	53.51	16.50
INT	4579.60	2.77	4.14	73.61	119.32	2.14	2.07	4.86	4.86	2.71	23.49	49.56	15.90
LOC	2586.72	2.38	2.92	103.26	162.72	2.48	2.78	3.40	3.40	1.00	25.20	75.68	17.83
USA	6199.17	2.51	2.93	76.39	105.00	1.45	2.61	5.26	5.26	3.00	24.74	42.16	15.93

Table 12. Fisher's PLSD mean differences of the different variables for the three West African groups of varieties and the US checks.

	Yield (kg/ha)		100SW (g)		VG		FL50% (days)		PHGT (cm)		LGD		Thres	
	Mean diff	P	Mean diff	P	Mean diff	P	Mean diff	P-Value	Mean diff	P	Mean diff	P	Mean diff	P
IMP – INT	-237.2	NS	-0.2	NS	-2.5	S	28.3	S	2.8	NS	-0.8	NS	0.6	S
IMP-LOC	1755.6	S	0.2	S	-1.3	S	-1.3	NS	-40.5	S	-1.1	NS	-0.06	NS
IMP-USA	-1856.8	S	0.08	NS	-1.3	S	25.5	S	17.2	S	-0.1	NS	0.1	NS
INT-LOC	1992.8	S	0.4	S	1.2	S	-29.6	S	-43.3	S	-0.3	NS	-0.7	S
INT-USA	-1619.5	S	0.3	S	1.2	S	-2.7	NS	14.3	S	0.6	NS	-0.5	S
LOC-USA	-3612.4	S	-0.1	NS	-0.01	NS	26.8	S	57.7	S	1.0	S	0.1	NS

Table 12. Continued.

	Lsen		Pacp		PANEX		PanL (cm)		LeafL (cm)		LeafW (mm)	
	Mean diff	P	Mean diff	P	Mean diff	P	Mean diff	P	Mean diff	P	Mean diff	P
IMP – INT	-1.5	S	0.1	NS	1.0	S	1.5	S	3.9	NS	0.6	Ns
IMP-LOC	-0.1	NS	-0.2	NS	2.7	S	-0.2	NS	-22.1	S	-1.3	S
IMP-USA	-1.9	S	0.9	NS	0.7	S	0.2	NS	11.3	S	0.5	Ns
INT-LOC	1.4	S	-0.3	NS	1.7	S	-1.7	S	-26.1	NS	-1.9	S
INT-USA	-0.4	NS	0.8	S	-0.2	NS	-1.2	S	7.4	S	-0.02	Ns
LOC-USA	-1.8	S	1.1	S	-2.0	S	0.4	NS	33.5	S	1.9	S

IMP = Improved varieties from WARDA
INT = Interspecifics (O. sativa x O. glaberrima)
LOC = Landraces from Cote d'Ivoire
USA = Checks from USA

S= significant
NS= not significant
P= Probability

Table 13. Correlation coefficients among the different variables using cultivar means in the yield experiment (4 replications).

	100SW	Yld	VG	FL50%	PHGT	LDG	Thres	Lsen	Pacp	PANEX	PanL	LeafL
Yld	0.08											
VG	0.28*	0.15	-									
FL50%	-0.35**	-0.61**	-0.50**	-								
PHGT	-0.13	-0.78**	-0.14	0.68**	-							
LDG	0.10	-0.26**	-0.04	0.12	0.29**	-						
Thres	-0.45**	-0.24**	-0.31**	0.42**	0.33**	0.06	-					
Lsen	0.07	0.33**	0.25**	-0.52**	-0.34**	0.22*	-0.13	-				
Pacp	0.017	-0.35**	-0.08	0.17	0.32**	0.57**	0.13	0.17	-			
PANEX	0.086	0.51**	-0.16	-0.41**	-0.77**	-0.23**	-0.09	0.23**	-0.18	-		
PanL	-.0.05	-0.14	-0.14	0.25**	0.26**	0.07	0.13	-0.12	-0.01	-0.11	-	
LeafL	-0.23	-0.7*	-0.22*	0.77**	0.86**	0.17	0.32	-0.51	0.16	-0.64**	0.28**	-
LeafW	-0.18*	-0.59**	-0.22*	0.51**	0.63**	0.06	0.20	-0.23	0.25	-0.35**	0.12	0.59**

4.2. Physio-Chemical Analyses of Milled Samples Introduced from WARDA

4.2.1. Chemical Analysis Results

These samples were grown and milled in WARDA and then shipped to Beaumont for analysis. They included grain shapes similar to long, medium, and short grain classes; aromatics and non-aromatics; red bran cultivars; chalky and translucent grains. These results provide a means of assessing how the cultivars compared for grain quality traits when grown under WA conditions and in the US. WARDA samples demonstrated a wide range for all parameters measured (Table 14). Bergman et al. (2001) indicated that rice germplasm can be identified as waxy (0-2%), very low amylose (3-9%), low amylose (10-19%), intermediate amylose (20-24%), or high amylose (>24%). Apparent amylose content plays a key role in market class; it is also a key component of the cooking and processing quality of rice (Juliano 1971 and Juliano 1985). Apparent amylose content varied from 15% for Khao Dawk Mali 105 which originated from Thailand, to 26% for CG 14 which is an *Oryza glaberrima* type that originated in West Africa. The high amylose level from CG 14 is an indicator that *Oryza glaberrima* is a genetic resource of genes that will affect cooking and eating properties in rice (Aluko et al. 2004).The results showed that African cultivars possessed the full range of amylose content classes although no waxy classes (0% amylose) were found among the cultivars (Table 14).

Cooking time is a key parameter for West African rice producers. Because firewood is used to cook rice in most regions in West Africa, short cooking time for rice will save labor and reduce deforestation. Many of the Basmati style rice cultivars had

very short cooking times (12-13 minutes) which is likely due to their very thin grains (Table 14). However, the traditional variety Doigamlin which had an apparent amylose content of 25% had a minimum cooking time of 13 minutes. Other cultivars from WA having les than 16 minutes cooking time were BG 90-2, Bieu, Hollandais, Cocote, Danane, Jaya, and Gambiaka Kokoun (Table 14). In contrast, WAB 56-104 which is an improved variety from WARDA had the longest cooking time of 24 minutes (Table 14).

Samples were analyzed with two concentrations of potassium hydroxide and results showed that ASV varied across the complete scale, from 2.3. to 7.0 (Table 14 and 15). ASV can be influenced by growing environment and the results suggest that using the 1.5% concentration are more accurate for the samples that were grown in WA. At this concentration, ASV showed the widest range and was more highly correlated ($r = -0.93$) with gelatinization temperature as measured by the peak DSC value than at the 1.7% concentration ($r = -0.80$, data not shown).

The RVA Peak values varied from 139 or the entry Nerica 5 an interspecific from WA to 362RVU for Jaya which originated from Taiwan and is well adapted to the irrigated system in the Sahel in West Africa (Tables 14 and 15). Both of these cultivars have similar amylose contents but widely differing RVA curves that differ widely. High

Table 14 Grain cooking parameters of 39 African accessions produced at WARDA and analyzed in Beaumont.

West African Accessions	Amylose Content %	Cooking Time Min	ASV 1.5% KOH	ASV 1.7% KOH	Rapid Visco Analyzer Peak	Hot	Cool	Bkdn	Stbk	Init.T	DSC Onset	Delta H	Peak	End
BAKUE DANANE	21.1	19.5	3.9	4.7	226	158	334	67	109	87	70	2.99	75	80
BASMATI 217	21.0	15.7	4.3	5.3	156	140	276	16	120	92	70	3.23	74	80
BASMATI 370	22.8	13.5	4.0	4.8	191	145	298	46	107	87	69	3.06	74	79
BASMATI 6129	21.4	14.0	3.9	4.7	202	161	320	41	117	89	70	3.05	74	80
BG 90-2(FARO 29)(GR 14)(ROK 28)	24.7	15.0	7.0	7.0	214	156	341	58	127	81	61	1.63	66	70
BIEU	24.6	15.5	4.0	5.0	335	196	376	139	41	78	68	2.77	73	79
CG 14	26.1	18.5	3.5	5.0	196	170	277	26	81	86	71	3.16	75	80
COCOTE	22.8	15.8	3.9	4.6	237	174	355	63	118	88	70	3.20	75	81
DANANE	23.3	17.0	3.6	4.7	304	176	337	128	33	80	70	3.16	75	83
DIGBOBLI	22.9	19.5	3.3	4.7	284	182	344	102	60	81	71	3.29	75	81
DISSOU	22.6	19.0	4.0	5.0	222	162	334	60	112	87	69	3.01	74	80
DOIGAMLIN	25.2	13.0	3.9	5.2	283	158	354	124	72	81	71	2.88	74	78
GAMBIAKA KOKOUN (MALI)	24.7	17.6	4.0	5.0	175	160	318	15	144	88	71	3.08	75	80
GNANLE GNAN-MAN	22.7	19.0	2.8	4.8	258	147	315	111	56	84	72	3.39	75	80
GNINNI ZEBA	23.0	19.7	3.3	4.8	242	156	304	86	62	85	70	2.98	75	82
GNOKOU GNOKOU	23.1	19.7	2.6	4.3	237	147	297	90	60	85	70	2.99	75	81
HOLLANDAIS	24.2	15.7	3.4	5.0	230	161	333	69	103	86	70	4.21	75	81
ITA 123 (FKR 28) (TOM1-3)(KADIAKA)	26.0	20.0	6.2	6.3	233	195	365	38	132	86	64	0.58	69	72
JAYA	25.6	17.0	7.0	7.0	363	267	486	96	123	75	62	2.44	67	72
KHAO DAWK MALI 105	15.0	11.9	6.0	6.8	237	171	333	66	97	89	64	2.87	68	74
LOGNINI COURT	23.3	20.8	3.8	4.0	254	162	317	92	63	83	70	3.13	75	81
MAHAFIN	22.6	19.0	3.0	4.8	277	168	327	108	50	82	70	3.17	75	81
MELKIN BARBA	25.8	20.0	4.0	5.1	280	176	396	104	116	78	70	3.25	74	79
MINMLI	22.8	20.0	3.8	4.0	234	151	306	83	72	85	72	2.88	75	80
MOKOSSI	22.8	20.8	2.3	4.4	259	152	318	107	59	83	71	2.57	76	82
MOLOUBA KOLE	22.5	18.0	3.8	4.8	264	175	328	89	65	83	69	2.91	75	80
PA LAHAI (KAOLAKA)	24.8	20.0	3.0	4.0	308	202	380	106	72	81	72	3.33	76	81
PUSA BASMATI	24.2	11.7	6.3	7.0	258	240	419	19	160	89	64	2.29	68	76
SUPER BASMATI	21.5	13.0	4.2	5.7	188	159	307	28	119	90	70	2.77	75	82
WAB 450-11-1-1-P31-HB (NERICA 5)	24.7	20.6	3.1	4.8	139	108	207	31	68	83	72	2.71	76	82
WAB 450-11-1-P-31-1-HB (NERICA 2)	23.6	20.7	3.3	4.3	170	127	256	43	86	83	58	2.83	75	82
WAB 450-I-B-P-160-HB (NERICA 6)	23.5	20.0	4.0	5.8	183	140	288	43	105	88	70	3.03	73	80
WAB 450-I-B-P-20-HB (NERICA 7)	22.2	23.0	3.9	5.2	211	145	282	65	71	85	70	2.77	74	82
WAB 450-I-B-P-28-HB (NERICA 3)	21.7	20.8	3.2	4.5	211	143	294	68	84	85	71	3.02	75	80
WAB 450-I-B-P-38-HB (NERICA 1)	25.3	20.0	4.0	4.0	239	172	332	67	92	80	71	2.90	75	81
WAB 450-I-B-P-91-HB (NERICA 4)	22.6	21.0	3.8	4.3	228	142	295	86	67	81	70	2.85	74	80
WAB 56-104	21.1	24.0	3.4	4.8	154	128	259	27	105	89	70	3.10	76	83
WAB 638-1	20.8	20.7	2.8	4.0	193	158	328	35	135	90	72	2.82	77	82
YABLO	23.6	19.0	3.7	4.6	250	147	305	103	56	82	69	2.21	75	81

Table 15. Summary results of chemical data from introduced milled samples from WARDA.

Variable	N	Mean	Std Dev	Min	Max
AA (%)	39	23.13	1.98	15.00	26.10
CT (min)	39	18.20	3.03	11.70	24.00
ASV	39	3.95	1.10	2.30	7.00
Peak (RVU)	39	233.90	48.90	139.30	362.80
Hot (RVU)	39	163.57	28.47	108.20	267.10
Cool (RVU)	39	324.06	47.52	207.40	485.80
Bkdn (RVU)	39	70.34	33.92	14.70	138.70
Stbk (RVU)	39	90.16	31.08	33.00	160.10
Intemp (RVU)	39	84.56	3.81	75.30	91.70
CS (RVU)	39	160.50	23.86	99.30	220.20
Onset (^{o}C)	39	69.13	3.16	58.32	72.18
DeltaH (j/g)	39	2.88	0.55	0.58	4.21
Peak1(^{o}C)	39	73.94	2.66	65.60	77.06
End (^{o}C)	39	79.75	2.91	69.92	83.08

AA = Apparent amylose
CT = Cooking time
ASV = Alkali spreading value
Peak = Peak RVA
Hot = Hot RVA
Cool = Cool RVA
Bkdn = Breakdown RVA
Stbk = Setback RVA
Intemp = Initial temperature RVA
CS = Consistency RVA
Onset= Onset DSC
DeltaH = Enthalpy DSC
Peak1 = Peak DSC
End = End DSC

RVA peak values and high apparent amylose content are indicators of good parboiling and canning stability. Thus, Jaya can be compared to Dixiebelle, a USA variety grown commercially under contract for the canning and processing industries. The demand for rice of superior quality is becoming a priority for rice breeding programs worldwide.

Juliano et al. (1990) and the divergent RVA curves of Nerica 5 and Jaya indicate that the WA germplasm may have grain chemistry traits that may be useful to US gene pools.

Results from the correlations (Table 16) showed that the CT was negatively correlated with the ASV. This indicates that quick cooking rice has a lower gelatinization temperature (i.e. high ASV score), as is expected. As expected, ASV was also negatively correlated with Onset, Delta H, Peak and End points of the DSC while it was positively correlated with Hot and Cool paste viscosities of the RVA curve (Table 16). Amylose content was not highly correlated with any of the RVA parameters or with ASV. Similar conclusions were made by Mckenzie and Rutger (1983) where they declared that there was no strong relationship between amylose content and mean alkali spreading values in a study where they used six crosses.

Table 16. Correlation coefficients among the different variables using cultivar means from introduced milled samples from WARDA. (n= 2 replications).

	AA	CT	ASV	Peak	Hot	Cool	Bkdn	Stbk	Intemp	CS	Onset	DeltaH	Peak1
CT	0.14												
ASV	0.06	-0.49**											
Peak	0.26	-0.15	0.16										
Hot	0.29	0.36	0.56**	0.73**									
Cool	0.27	-0.37	0.54**	0.79**	0.92**								
Bkdn	0.14	0.08	-0.23	0.82**	0.22	0.36*							
Stbk	-0.00	-0.33*	0.56**	-0.36*	0.25	0.28	0.73**						
Intemp	-0.54	-0.18	-0.05	-0.72**	-0.34	-0.41**	-0.75**	0.50**					
CS	0.19	-0.31	0.41**	0.70**	0.64**	0.88**	0.46**	0.25	-0.40*				
Onset	-0.04	0.28	-0.71**	-0.08	-0.33*	-0.30	0.16	-0.33*	0.14	-0.19			
DeltaH	-0.21	0.02	-0.59**	-0.02	-0.22	-0.19	0.16	-0.25	0.08	-0.11	0.51**		
Peak1	-0.04	0.47**	-0.93**	-0.26	-0.54**	-0.54**	0.07	-0.41**	0.14	-0.43**	0.75**	0.60**	
End	-0.13	0.44**	-0.88**	-0..24	-0.50**	-0.54**	0.06	-0.43**	0.17	-0.48**	0.68**	0.62**	0.93**

The high simple regressions observed between between ASV and Peak1 indicates that gelatinization temperature can be predicted by either the ASV score or the first Peak of the DSC (Fig 5).

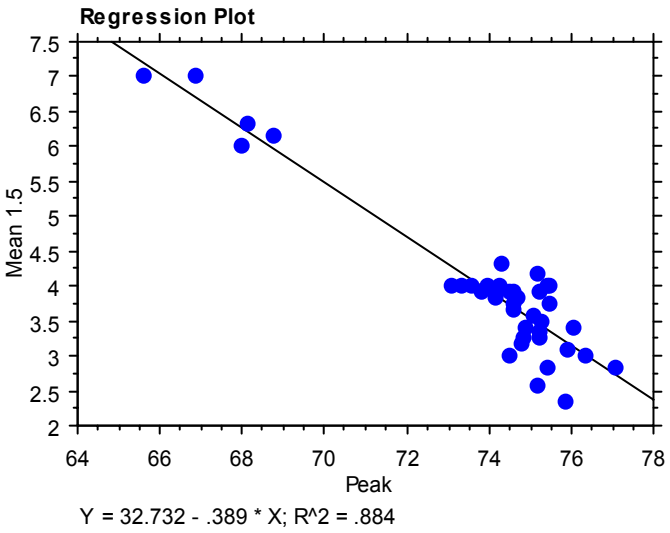


Fig. 5. Linear regression between Peak1 and ASV.

4.2.2. Molecular Analysis Results

Using the RM 190 marker which is a microsatellite marker in the *Waxy* gene, alleles common to those found in U.S. cultivars (105, 118, 122, and 124 bp alleles) were identified, as well as some rare marker alleles (103, 114, and 116 bp) (Fig. 6). The three cultivars that had the 114 bp allele for RM190 were very similar in grain shape and all other quality measurements, but ranged in cooking time by 4.9 minutes. The long grain cultivar Nerica 5 was unusual in that it possessed an amylose content and *Waxy* allele like Dixiebelle (105 bp), a USA firm cooking variety, but had a weak RVA curve similar to L-202, a USA soft cooking cultivar. Larkin et al. (2003) concluded that apparent

amylose content was not a guarantee of a particular type of cooked rice texture. In some cases high amylose varieties did not exhibit the starch pasting parameters associated with high amylose content. Jaya is a chalky medium grain cultivar that has the same amylose content and *Waxy* allele as Dixiebelle but a much stronger RVA curve than Dixiebelle. Bieu is a long grain cultivar with a short (15.5 minute) cooking time and a *Waxy* allele like Lemont (124 bp), however it had a high amylose content and a strong RVA curve like Dixiebelle. Usually low amylose content varieties (14-18%) have soft, sticky texture when they are cooked (Webb 1991); intermediate varieties (19-23% amylose) and high amylose (>23% amylose) have a firm, dry texture after cooking.

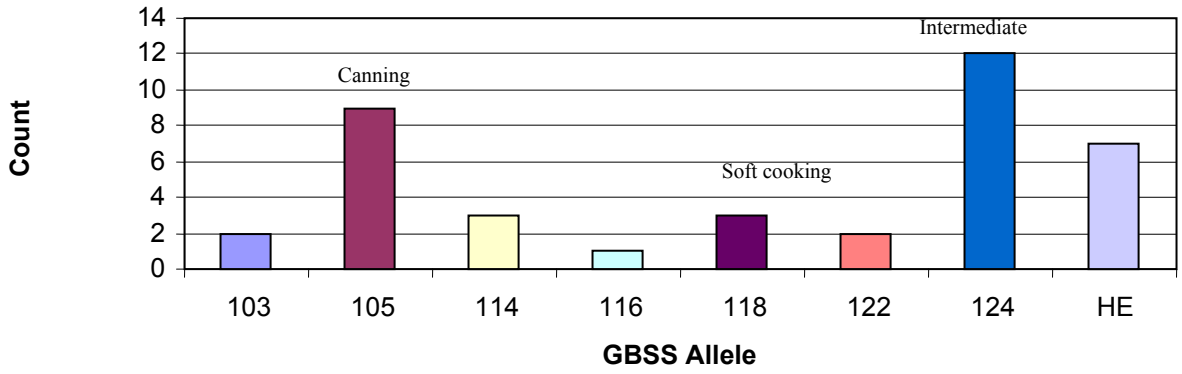


Fig. 6. Distribution of GBSS alleles in WARDA material. HE= heterozygous, the samples were mixtures.

Umemoto et al. (2002, 2004) concluded that soluble starch synthase IIa (SSIIa) is the gene controlling differences in alkali disintegration of rice grains and chain-length distribution of amylopectin among japonica and indica cultivars. The majority of

WARDA materials had intermediate ASV with an *Alk* marker allele of 90bp. The 90bp marker allele is associated with intermediate and high gelatinization temperature. Only 12% of the WARDA germplasm had grain which disintegrated almost entirely in alkali, with an ASV score of 6.8 and each of these had an *Alk* marker allele of 92bp (Fig. 7). The 92 bp allele indicates the presence of a mutation in the *Alk* gene resulting in an inactive SSSIIa protein and low gelatinization temperature. African consumers typically select varieties with intermediate and high ASV. The values of ASV are indirect measures of the gelatinization temperature (GT), and are inversely related to another, where low values of ASV have high GT. Varieties with slow digestion are preferred by African farmers for longer satisfaction.

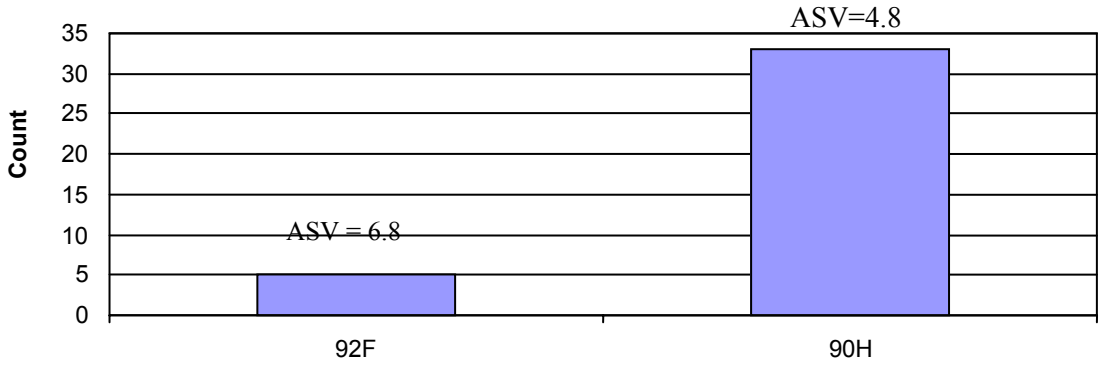


Fig. 7. Distribution of *Alk* alleles in WARDA material.

Previous work conducted by Larkin and Park (2003) showed that single nucleotide polymorphisms (SNP) located at three points in the Waxy gene at exons 1, 6, and 10 were associated with rice starch properties were located at three points in the

Waxy gene, at exons 1, 6 and 10. Their work concluded that for the intermediate amylose cultivar Lemont, an adenine to cytosine transversion in exon 6 results in the substitution of a serine amino acid residue for tyrosine in the GBSS protein. Their results showed that an A-C substitution in exon 6 was observed in all intermediate amylose varieties. Results for the *Waxy* exon 6 marker shows that 58% of the WARDA materials had the 146 bp marker allele, which corresponds to the presence of C at the *Waxy* exon 6 SNP, and all these accessions had intermediate amylose content. It was also seen that 34% of the WARDA accessions had the 148 bp allele, which corresponds to the presence of an A at the exon 6 SNP site, which is associated with either high or low amylose content. The remaining 8% of the WARDA materials were heterozygous for the *Waxy* exon 6 SNP marker (Fig. 8).

In the high amylose cultivar Rexmont, Larkin and Park (2003) indicate that a cytosine to thymine transition in exon 10 produces a serine for proline substitution in the GBSS protein. This exon 10 C-T substitution was related to a strong viscosity curve in high amylose cultivars. High amylose cultivars with soft cooking, like L-202, had a C instead of a T at the exon 10 SNP site. Using the *Waxy* exon 10 marker, the results showed that 72% of WARDA materials had the 133 bp allele, with the presence of a C at this SNP site, while 19% had the 134 bp allele, indicating the presence of a T at the SNP, and 9% were heterozygous (Fig. 9).

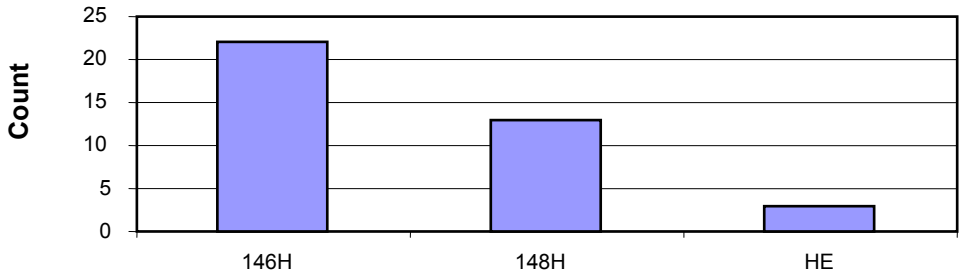


Fig 8. Distribution of exon 6 alleles in WARDA material.

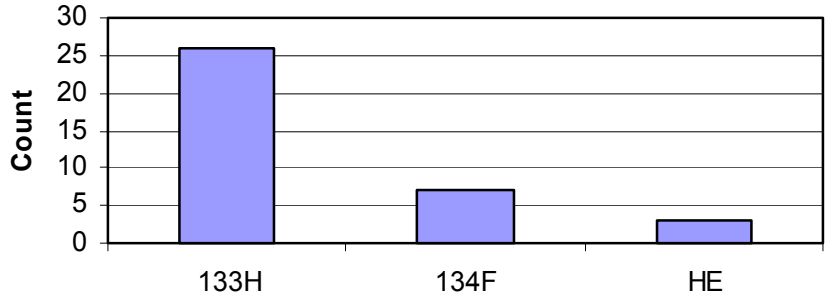


Fig. 9. Distribution of the exon10 alleles in WARDA material.

Comparing the RVA data and the RM190 alleles (Fig. 10), it was found that certain materials from WARDA had atypical combinations of *Waxy* gene markers. CG 14, an *O. glaberrima* type, and the *O. sativa* cultivar Gambiaka Kokoun Mali, Nerica 5 an interspecific, all had the 105 bp RM190 allele (Dixiebelle type) but had a relatively low RVA Peak (blue circle). In contrast, it was found that Basmati 217 and Khao Dawk Mali 105 had low amylose content and a relatively high RVA Peak. In reference to the work done by Larkin et al. (2003), it was found that the Rexmont type W^a with high

amylose had lower Peak viscosity, higher Hot and Cool paste viscosity, lower breakdown and higher setback viscosity whereas types of rice like Toro 2 W^b had the opposite figures. *Indica* rice generally has the W^a and the *japonica* type has W^b.

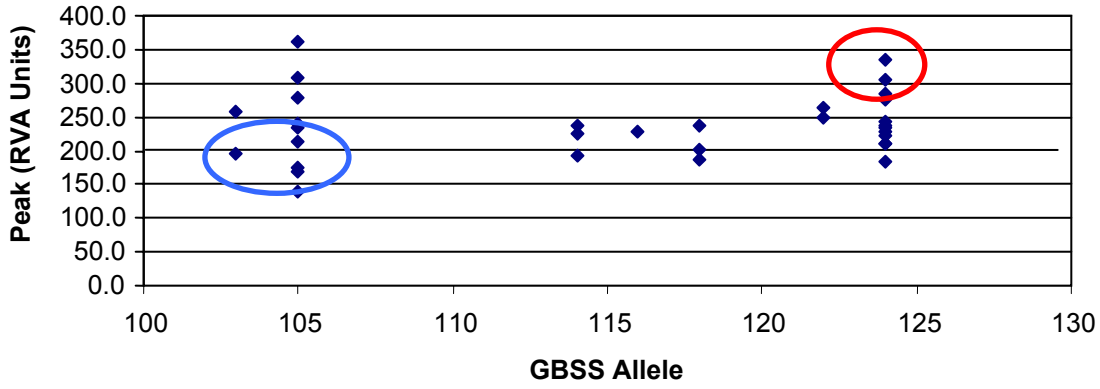


Fig. 10. Range in RVA peak viscosities for WARDA material. (Blue circle represents cultivars with high amylose content and weak RVA and red circle represents cultivars with low amylose content and high RVA. The two cases are atypical situations).

Analysis conducted with the interspecific cross (*O. glaberrima and O. sativa*) accessions showed there were two distinct groups. The first group had RM190 alleles of 103/105 bp, which are similar to the *O. glaberrima* parent (CG 14). The second group had 124 bp allele at RM190, which is similar to the *O. sativa parent* WAB 56-104 (Fig. 11).

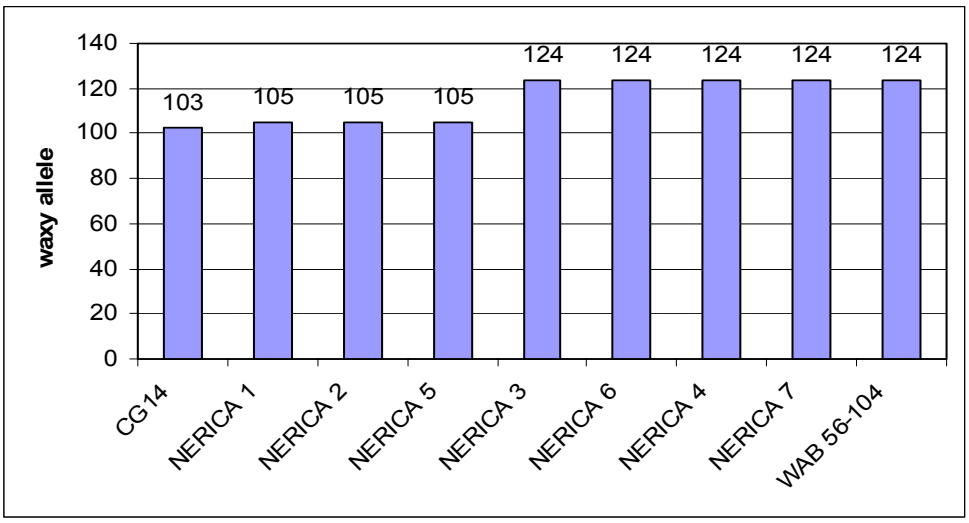


Fig. 11. *Waxy* allele characteristics of *O. glaberrima* derivatives.

4.3. Physico-Chemical Analysis of Cultivars Introduced from WARDA and Grown in US Environment

4.3.1. Milling Characteristics of the West African Cultivars Grown in Beaumont

Analysis of variance showed significant differences between the varieties for milling yield (Table 17). Total milling yield varied from 78% for Gnanle Gnan-Man a landrace from West Africa to 70% for the US check Saber (entry 41). Whole mill yield varied from 70% for Bengal to less than 40% for ZHE733 both checks from the US. Bengal and Nerica 3 had both a superior total and whole milling yield (Table 18). Milling yield depends on many factors including grain shape, grain moisture, the depth of surface grooves and the pressure required to remove the bran (Bhashyan and Srinivas 1984; Juliano and Perez 1993).

Table 17. Mean square of the grain milling and grain dimensions and cooking time.

	Mean Square						
Source of variation	df	Total †	Whole †	Grain length	Grain Width	Grain Length Width ratio	Cooking Time
Replication	2-3	0.67	21.92	0.011	0.01*	0.008	0.50
Varieties	42	13.37**	219.58**	0.76**	0.19**	0.47**	13.69**
Error	84-126	1.53	8.18	0.007	0.001	0.003	0.54

*, ** indicate significance at 5 and 1% respectively.
† Total and Whole were run with 4 replications; the others were run with 3 replications.

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Correlations data (Table 19) show that grain with large diameters takes a longer time to cook (r =0.54**). This indicates that, in addition to the chemical parameters, some physical properties of grain can affect cooking time.

The total mill was highly correlated to the cooking time (r=0.66**) and the grain width (r=0.41**). Whole milling yield was negatively correlated to grain width (r=-0.34**). This may suggest that broader grains are more susceptible to cracking and fissuring. The ratio of grain length over grain width can affect the milling yield. Better whole milling yield was obtained when the grain length over the grain width was high (r=0.34**).

Table 18. Means of the milling and grain dimensions and cooking time of the cultivars introduced from West Africa and grown in Beaumont.

No	Varieties	Total %	Whole %	Grain Length (mm)	Grain Width (mm)	Grain Length Width ratio	Cooking Time (min.)
10	Gnanle Gnan-Man-33416	78 a	55.42 ghij	6.52 qr	2.67 cd	2.43 i	22.26 b
44	Baldo	77.12 ab	55.47 ghij	7.11 ghij	3.08 a	2.30 k	22.40 ab
45	Bengal	76.60 abc	70.37 a	6.51 qrs	2.67 cd	2.43 i	19.90 hijkl
26	Nerica 2	76.52 abc	64.87 bcd	7.17 fghi	2.43 i	2.94 g	22.00 bc
19	Mahafin	76.46 abcd	56.26 ghi	6.13 u	2.63 de	2.32 jk	19.66 ijklm
24	Nerica 5	76.20 abcde	64.10 bcd	6.93 kl	2.23 mn	3.10 de	29.23 bcdefgh
42	ZHE733(BMT)	76.15 abcde	36.90 m	6.69 nop	2.54 fg	2.62 h	21.43 gbcdef
31	Nerica 1	76.02 abcdef	57.40 efgh	6.96 jk	2.40 ijk	2.89 g	20.23 efghijkl
21	Mokossi	76.00 abcdefg	57.62 efg	6.33 t	2.62 de	2.41 ij	20.20 fghijkl
29	Nerica 3	75.97 abcdefg	67.72 ab	7.05 hijk	2.42 i	2.90 g	20.96 bcdefghi
23	Moluba kole	75.82 bcdefg	59.07 efg	6.80 lmn	2.53 fgh	2.68 h	21.66 bcde
32	Nerica 4	75.72 bcdefgh	66.50 ab	7.02 ijk	2.41 ij	2.91 g	21.03 bcdefghi
35	Yablo	75.40 bcdefghi	57.60 efg	6.68 nop	2.72 c	2.45 i	21.46 bcdef
28	Nerica 7	75.15 bcdefghij	56.87 efgh	7.59 bc	2.49 fghi	3.05 ef	23.53 a
38	Sierra	75.12 bcdefghij	64.32 bcd	7.77 a	2.44 i	3.19 cd	20.50 defghijkl
30	ZHE733	75.00 bcdefghij	35.70 m	6.78 lmno	2.56 ef	2.64 h	21.50 bcdef
39	Cheniere	74.95 cdefghijk	66.72 ab	7.35 de	2.10 p	3.49 a	19.33 jklmn
11	GnanleGnan-Man 33417	74.70 cdefghijk	57.65 efg	6.41 rst	2.72 c	2.35 ijk	21.90 bcd
34	WAB 638-1	74.67 cdefghijk	66.15 abc	6.36 rst	2.13 op	2.98 fg	16.76 r
37	COCODRIE	74.57 cdefghijkl	65.00 bcd	7.35 de	2.26 lmn	3.24 bc	21.26 bcdefgh
1	Bakue Danane	74.57 cdefghijkl	66.05 abc	6.17 u	2.12 op	2.90 g	17.50 qr
46	Jaya	74.33 defghijklm	46.03 l	6.50 qrs	2.64 cd	2.46 i	17.73 qr
4	Cocote	74.32 defghijklm	64.42 bcd	6.15 u	2.13 op	2.88 g	17.33 qr
18	Lognini court	74.17 efghijklm	57.02 efgh	6.68 nop	2.54 fg	2.62 h	20.30 efghijkl
33	WAB 56-104	73.92 fghijklm	59.17 efg	7.68 ab	2.46 hi	3.12 de	21.10 bcdefghi
27	Nerica 6	73.85 ghijklm	56.57 fghi	6.65 nopq	2.79 c	2.44 i	20.36 efghijkl
12	Gninni Zeba 33420	73.62 hijklm	58.82 efg	5.68 w	2.88 b	1.97 l	20.60 cdefghijk
13	Gninni Zeba 33423	73.57 ijklm	57.40 efgh	5.69 w	2.92 b	1.94 l	19.83 hijkl
36	CYPRESS	73.42 ijklmn	68.20 ab	7.28 efg	2.22 mn	3.27 bc	19.10 lmnop
16	ITA 123	73.40 ijklmn	51.65 k	6.62 opq	2.48 ghi	2.66 h	19.16 klmno
14	Gnokou Gnokou	73.32 ijklmno	57.77 efg	5.84 v	2.94 b	1.98 l	20.76 cdefghij
15	Hollandais	73.10 klmnop	52.05 ijk	7.14 fghi	2.19 no	3.25 bc	17.76 pqr
5	Danane	73.02 klmnop	61.5 cdef	6.93 kl	2.09 p	3.31 b	16.90 r
17	ITA 123	72.82 klmnopq	52.75 hijk	6.58 pq	2.46 hi	2.67 h	20.00 ghijkl

Means with the same letter within the same column are not statistically different.

Table 18. Continued.

No	Varieties	Total	Whole	Grain Length (mm)	GrainW (mm)	GrainL/W ratio	Cooking Time (min.)
6	Digbobli	72.52 lmnopq	54.25 ghijk	7.20 efgh	2.46 ghi	2.91 g	20.00 ghijkl
20	Minmli	72.47 lmnopqr	61.57 cde	7.30 ef	2.33 jkl	3.12 de	20.73 cdefghij
40	Jasmine	72.35 mnopqr	50.65 k	7.17 fghi	2.29 lm	3.12 de	13.76 t
43	Dawn	71.37 nopqr	61.2 def	7.49 cd	2.11 op	3.54 a	18.16 nopqr
2	BG 90-2	71.32 opqr	61.40 cdef	6.35 st	2.34 jkl	2.70 h	14.93 st
25	SABR	71.22 pqr	67.95 ab	6.75 mno	2.11 op	3.19 cd	17.83 opqr
22	Moluba Kole	70.95 qr	55.02 ghijk	6.90 klm	2.33 kl	2.96 fg	18.33 mnopq
3	BG 90-2	70.92 qr	58.87 efg	6.36 rst	2.33 kl	2.72 h	15.40 s
41	SABR (BMT)	70.75 r	67.52 ab	6.80 lmn	2.13 op	3.18 cd	17.43 qr

Means with the same letter within the same column are not statistically different.

Table 19. Correlation study for cooking time and grain dimensions and milling parameters.

	Cooking Time	Grain length	Grain width	Grain length width ratio	Total
Grain length	NS				
Grain width	0.54 **	-0.41 **			
Grain Length Width ratio	NS	0.76 **	-0.89 **		
Total	0.66 **	NS	0.41 **	NS	
Whole	NS	NS	-0.34 *	0.34 *	NS

4.3.2. Aroma Characteristics of the West African Cultivars Grown in Beaumont

The aromatic compound 2-acetyl-1-pyrroline (2-AP) was determined in the USDA laboratory at Beaumont for six (6) cultivars from West Africa and two US checks. The results showed that Sierra, a US check, had the highest value of 2-AP (1258 ng/g) (Tables 20, 21). It was followed by Bakue Danane and Cocote, both from Cote d'Ivoire in West Africa. Jasmine 85, a check from the US, and Nerica 1, an interspecific, were not statistically different with 494 and 444 ng/g of 2-AP, respectively. West

African consumers value the aroma and WAB 638-1 was released by WARDA for this market. It is considered one of the most expensive rices sold in the market because of its taste and aroma and is also called AKADI meaning it tastes good.

Table 20. Mean square of the aroma content of selected parents from West Africa.

Mean Square		
Source of variation	df	Aroma
Replication	2	5737.097
Varieties	5	377281.081**
Error	10	7445.93

** Significant at 1%

Table 21. Means of the aroma content of selected parents from West Africa.

Varieties	Aroma (2-AP ng/g)
Sierra	1258.83 a
Bakue Danane	1140.00 ab
Cocote	1102.00 ab
WAB 638-1	1075.33 b
Jasmine 125	494.00 c
Nerica 1	444.00 c

Means with the same letter within the same column are not statistically different.

4.3.3. Grain Quality Properties of the West African Cultivars Grown in Beaumont

Grown in the US environment, materials from West Africa showed variability for different traits that define the rice quality. The analysis of variance indicated significant differences between the cultivars (Tables 22, 23). The African materials were characterized by an apparent amylose content that varied from 19.8% for Bakue Danane to 25.3% for Nerica 1.The US materials varied from 10.6% for Bengal to 25.3% Sierra. For the overall experiment, the soluble amylose content varied from 5.6% for Bengal to

16.3% for Cheniere. The insoluble amylose content varied from 4.9% for Bengal to 13% for Jaya. The majority of the cultivars had more soluble amylose than insoluble amylose, but varieties like BG 90-2, Saber, Baldo, and ZHE733 had equal amounts SA and IA whereas Jaya had more IA than SA (Table 23). ASV data showed that it varied from 3.1 for Cocodrie to 7 for Jaya and BG 90-2. The cultivars were characterized by intermediate to low GT. CT varied from 14 minutes for Jasmine 85, a US check, to 23.5 min for Moluba Kole landrace from WA. Rice nutrition content is very important in WA where rice is a staple food. Zhe733, Jaya, Cheniere, and Nerica 2 had the highest protein content (9.0%) in this study whereas Lognini Court had the lowest (5.8%) (Table 23) Regarding the RVA curve, Jaya had the highest peak value (369 RVU) whereas Cheniere had the lowest (183 RVU) even though both had similar amylose contents. Contrary to Rexmont characterized as having a high Peak, a low Bkdn and high Stbk (Larkin et al. 2003), Jaya has a high Peak, a low Bkdn and a low Stbk which is atypical. BG 90-2 had an intermediate Peak, but a high Cool and high Stbk. A high Stbk is a high increase in viscosity during Cooling. The interspecifics had intermediate Peaks and Nerica 2 had relatively high Stbk (58 RVU). WA materials were characterized by intermediate to long grains types with only two cultivars having short grains (Gnokou Gnokou and Gninni Zeba).

Table 22. Mean square of the different variables of the African parents planted at Beaumont for milling and quality analysis.

		Mean Square							
Source of Variation	Df	Heading	AA	SA	IA	ASV	CT	Protein	Peak
Rep.	3	13.18	5.42**	0.90	4.05**	0.24	0.50	2.27	779.24*
Variety	42	748.72**	27.61**	15.33**	7.10**	3.30**	13.69**	2.09**	2554.26**
Error	84	6.41	0.25	0.29	0.40	0.11	0.54	15.09	228.55

Table 22. Continued.

		Mean Square				
Source of Variation	Df	Hot	Bkdn	Cool	Stbk	CSV
Rep.	3	81.80	1009**	199.11	503.27**	59.51
Variety	42	2412.54**	2122.68**	5922.70**	5845.00**	1372.47**
Error	84	147.45	134.09	257.80	144.95	52.25

ASV= Alkali Spreading Value
CT= Cooking Time
GrainL= Grain length
GrainW= Grain Width
GrainWL= Grain Width length ratio
CSV= Consistency Viscosity
*and ** indicate significance at 5 and 1% respectively
Rep. Replication

Table 23. Means of the different parameters of the African parents planted at Beaumont for milling and quality analysis.

No	Varieties	Heading	AA	SA	IA	ASV	CT
1	DANANE	125.0	22.6	13.7	8.9	4.1	16.9
2	LOGNINI COURT	124.0	23.4	15.3	8.0	4	20.3
3	BG 90-2 -33392	121.7	24.5	12.1	12.4	7	15.4
4	BG 90-2 -33390	120.3	24.0	12.0	12.0	7	14.9
5	MINMLI	110.3	22.0	12.9	9.0	3.7	20.7
6	MOLUBA KOLE-33460	107.0	21.8	13.5	8.3	3.5	21.6
7	JAYA	107.0	24.0	10.7	13.3	7	17.7
8	GNOKOU –GNOKOU	105.0	22.5	14.0	8.4	3.2	20.7
9	IITA 33433	103.3	25.2	14.8	10.4	6	19.1
10	GNINNI ZEBA-33423	101.7	22.5	13.7	8.7	3.6	19.8
11	YABLO	101.7	22.4	13.6	8.7	3.9	21.4
12	GNINNI ZEBA-33420	101.7	22.3	14.0	8.2	3.7	20.6
13	GNANLE-GNAN MAN - 33417	101.0	20.7	13.0	7.7	3.7	21.9
14	IITA 123-33434	99.0	25.0	15.3	9.7	5	20.0
15	MOKOSSI	98.0	22.0	12.6	8.3	3.4	20.2
16	MAHAFIN	98.0	21.3	12.7	8.6	3.9	19.6
17	BAKUE DANANE	97.0	19.8	11.3	8.6	3.8	17.5
18	COCOTE	97.0	21.0	11.9	9.0	4.2	17.3
19	WAB 638-1	97.0	20.4	11.1	9.2	4.1	16.7
20	HOLLANDAIS	95.7	22.4	13.9	8.4	3.8	17.7
21	NERICA 6	95.7	22.1	13.9	8.2	4.1	20.3
22	DIGBOBLI	95.0	21.8	13.4	8.3	4.7	20.0
23	JASMINE 85	93.0	15.2	9.0	6.2	6.1	13.7
24	MOLUBA KOLE-33459	91.7	22.6	13.7	8.9	4.6	18.3
25	GNANLE-GNAN MAN - 33416	87.0	21.6	12.5	9.0	3.9	22.2
26	CYPRESS	83.3	21.0	11.9	9.0	3.8	19.1
27	DAWN	82.7	22.0	13.7	8.2	3.5	18.1
28	SABR	82.0	19.7	10.3	9.3	3.9	17.8
29	SIERRA	82.0	25.3	15.7	9.7	4	20.5
30	SABR(BMT)	82.0	20.0	10.0	10.0	3.6	17.4
31	CHENIERE	82.0	25.3	16.3	8.9	3.9	19.3
32	BENGAL	79.0	10.6	5.6	4.9	6	19.9
33	COCODRIE	79.0	25.1	16.1	8.9	3.1	21.2
34	NERICA 7	77.7	21.0	12.4	8.6	3.3	23.5
35	NERICA 1	77.7	25.3	15.0	10.2	3.6	20.2
36	NERICA 4	76.3	20.0	10.8	9.2	3.2	21.0
37	NERICA 3	75.7	20.3	10.8	9.4	3.2	20.9
38	WAB 56-104	73.0	20.6	11.3	9.2	3.9	21.1
39	NERICA 2	72.3	25	14.9	10.0	4	22.0
40	NERICA 5	71.3	24.3	14.3	10.0	3.7	21.2
41	BALDO	70.7	13.0	6.4	6.6	5	22.4
42	ZHE733	64.7	23.6	11.8	11.8	3.4	21.5
43	ZHE733(BMT)	64.7	23.6	11.9	11.6	3.1	21.4
	LSD 5%	4.11	0.81	0.88	1.03	0.54	1.19

LSD= least significant difference

Table 23. Continued.

No	Varieties	Protein	Peak	Hot	Bkdn	Cool	Stbk	CSV
1	DANANE	6.6	302.90	161.1	141.8	294.7	-8.1	133.6
2	LOGNINI COURT	5.8	283.73	135.6	148.1	258.6	-25.1	123.0
3	BG 90-2 -33392	8.4	287.3	197.3	90.0	392.1	104.8	194.8
4	BG 90-2 -33390	8.6	285.0	206.6	78.4	387.6	102.7	181.0
5	MINMLI	8.4	266.2	163.0	103.2	299.3	33.1	136.2
6	MOLUBA KOLE-33460	7.4	275.0	146.9	128.0	280.4	5.4	133.5
7	JAYA	9.0	369.1	285.7	83.4	460.0	90.0	174.3
8	GNOKOU –GNOKOU	7.1	288.8	149.3	139.5	277.6	-11.2	128.3
9	IITA 33433	8.5	243.4	159.3	83.8	313.5	70.1	154.0
10	GNINNI ZEBA-33423	6.4	299.5	158.1	141.3	285.0	-14.5	126.8
11	YABLO	7.2	290.1	143.7	146.4	278.23	-12.0	135.5
12	GNINNI ZEBA-33420	6.5	300.7	150.0	150.6	275.43	-25.2	125.3
13	GNANLE-GNAN MAN -33417	7.2	293.3	136.6	156.7	262.2	-31.1	125.7
14	IITA 123-33434	8.8	241.1	150.2	91.4	307.8	66.2	157.6
15	MOKOSSI	7.3	287.9	153.2	134.7	290.4	2.5	137.2
16	MAHAFIN	6.9	301.6	148.3	153.3	283.7	-17.9	135.3
17	BAKUE DANANE	8.3	301.2	160.6	140.6	317.5	16.2	156.9
18	COCOTE	7.6	318.6	171.4	147.2	320.7	2.1	149.3
19	WAB 638-1	8.6	290.8	158.9	131.8	311.3	20.5	152.3
20	HOLLANDAIS	6.8	301.5	151.8	149.6	299.6	-1.9	147.7
21	NERICA 6	7.2	286.2	162.9	123.3	305.0	18.7	142.0
22	DIGBOBLI	7.1	302.2	159.8	142.3	300.2	-1.9	140.4
23	JASMINE 85	8.2	327.4	138.6	188.8	244.1	-83.3	105.4
24	MOLUBA KOLE-33459	6.8	290.3	151.1	139.1	288.0	-2.2	136.9
25	GNANLE-GNAN MAN -33416	7.3	299.4	143.5	156.0	276.3	-23.1	132.8
26	CYPRESS	8.1	292.0	146.3	145.8	284.8	-7.2	138.6
27	DAWN	7.2	297.1	148.7	148.5	281.8	-15.4	133.1
28	SABR	8.0	290.9	169.8	121.0	314.8	23.9	145.0
29	SIERRA	7.4	307.4	197.2	110.2	377.0	69.6	179.8
30	SABR (BMT)	7.7	295.4	165.0	130.4	307.6	12.1	142.6
31	CHENIERE	9.1	182.9	101.4	81.5	221.0	38.0	119.5
32	BENGAL	8.7	319.0	158.0	161.0	238.7	-80.3	80.7
33	COCODRIE	7.6	230.1	145.7	84.4	297.1	67.0	151.4
34	NERICA 7	8.4	297.1	154.5	142.6	276.1	-21.0	121.6
35	NERICA 1	7.8	267.7	158.4	109.3	311.8	44.2	153.5
36	NERICA 4	8.4	299.2	144.5	154.7	274.1	-25.1	129.6
37	NERICA 3	8.3	293.9	147.4	146.5	271.0	-22.9	123.6
38	WAB 56-104	8.2	299.9	159.7	140.2	285.6	-14.3	125.9
39	NERICA 2	9.0	269.5	147.8	94.7	327.9	58.4	153.0
40	NERICA 5	8.9	269.7	153.2	116.5	295.8	26.1	142.6
41	BALDO	8.0	333.5	183.8	149.7	282.9	-50.5	99.1
42	ZHE733	8.8	321.3	219.7	101.7	383.4	62.0	163.7
43	ZHE733 (BMT)	9.0	302.5	194.6	107.8	358.83	56.3	164.2
	LSD 5%	0.68	24.54	19.71	18.80	26.07	19.54	11.7

LSD= Least significant difference

Correlations among quality traits are presented in Table 24. Late maturity was negatively correlated with milling yield suggesting that kernels in these genotypes may have been poorly developed. (r=-0.42**). ASV value was negatively correlated with the total milling (r=-0.31). Late varieties had high ASV value (r=0.40**). Cook time (CT) and whole milling yield were highly and positively correlated (r=0.65) while CT was negatively correlated with ASV (r=-0.58**). The latter is as would be expected with high ASV being an indicator of low gelatinization temperature. Whole milling yield was negatively correlated with IA content (r=-0.34*) suggesting that the chemical makeup of the grain is also a factor affecting milling yield. Protein content was positively correlated with several RVA parameters [Hot (r=0.30*), with Bkdn it was negatively correlated (r=-0.50**), with Cool (r=0.32*)] but negatively correlated with Bkdn (r=0.50**). This indicates that higher protein content increases the viscosity and would result in harger texture of the cooked rice. Similar results were found by Primo et al. (1962) where they concluded that protein content reduced cooked rice stickiness. The CSV is related to cooked rice hardness (Limpisut et al. 2002) and was positively correlated with AA (r=0.70**), to SA (r=0.37) and IA (r=0.84**). The results indicate that the high insoluble amylose has a greater effect on RVA parameters and consistency than soluble amylose.

Table 24. Correlation coefficients among different variables of the different parameters of the African parents planted at Beaumont for milling and quality analysis.

	Total	Whole	Head	AA	SA	IA	ASV	CT	Protein	Peak	Hot	Bkdn	Cool	Stbk
Whole	NS													
Head	-0.42**	NS												
AA	NS	NS	NS											
SA	NS	NS	NS	0.87**										
IA	NS	-0.34*	NS	0.68**	NS									
ASV	-0.31*	NS	0.40**	NS	NS	NS								
CT	0.65**	NS	0.43**	NS	NS	NS	-0.58**							
Protein	NS	NS	-0.43**	NS	-0.32*	0.40**	NS	NS						
Peak	NS	NS	NS	NS	NS	NS	NS	NS	NS					
Hot	NS	NS	NS	NS	NS	0.64**	0.47**	NS	0.30*	NS				
Bkdn	NS	NS	NS	-0.67**	-0.67**	-0.72**	NS	NS	-0.50**	NS	-0.40**			
Cool	NS	-0.37*	NS	0.41**	NS	0.82**	0.41**	NS	0.32*	NS	0.90**	-0.61**		
Stbk	NS	NS	NS	0.74**	0.42**	0.84**	NS	NS	0.41**	NS	0.52**	-0.93**	0.78**	
CSV	NS	NS	NS	0.70**	0.37*	0.84*	NS	NS	NS	NS	0.57**	-0.68**	0.85**	0.89**

NS= not significant
*, ** significant at 5 and 1% respectively

4.4. Comparative Study of the Chemical Properties of Cultivars Grown in Beaumont and Milled Samples Introduced from West Africa

Chen and Zhu (1999) studied the genetic effects and genotype x environment interactions for cooking quality traits in *Indica-Japonica* crosses. They concluded that the expression of dominant genes is affected by environment factors without changing their directions. Comparing WA samples grown in Cote d'Ivoire with those grown in Texas parameters like AA, ASV, Hot, Cool and CT were not strongly affected by the environment (Tables 25, 26, and 27). The variable most affected by environment was the Stbk which predicts the hardness of the cook rice. The same cultivars produced in Beaumont (BMT) were softer than when they were produced in WA environment. The lack of the environmental effect is somewhat surprising considering the difference in latitude, soil types, weather patterns, and management practices between the two locations. This suggests that cereal quality results from WA are predictive of their performance in Texas.

Table 25. Means of the different variables from cultivars produced in two different locations: West Africa (WA) and Beaumont (BMT).

Parameters	N	WA	BMT	Sdt Err. WA	Stdt Err.BMT
AA	26	23.2	22.3	1.32	1.60
ASV	26	3.8	4.1	1.17	0.97
Peak	26	234.3	292.1	46.43	22.23
Hot	26	159.2	160.4	28.80	28.38
Cool	26	317.4	302.8	48.20	41.14
Bkdn	26	75	130.7	27.64	22.90
Stbk	26	83.0	10.1	28.65	36.86
CT	26	19.5	19.8	2.08	1.95

Table 26. Paired comparisons between the West African and USA environments for the different variables.

Parameters	N	Mean Diff	Sdt Error	T Value	Pr>t
AA	26	0.88	0.15	5.85	<0.0001
ASV	26	0.30	0.11	2.79	0.0099
Peak	26	57.80	7.62	7.59	<0.0001
Hot	26	1.21	4.13	0.29	0.7705
Cool	26	14.55	7.24	2.01	0.0554
Bkdn	26	55.52	5.50	10.09	<0.0001
Stbk	26	72.89	5.61	12.98	<0.0001
CT	26	0.29	0.29	0.99	0.3304

Table 27. Descriptive statistics of the cultivars grown in WA and BMT environments.

	Mean	Std. dev	Min	Max	Variance
AA (WA)	23.18	1.32	20.81	25.97	1.76
AA (BMT)	22.28	1.60	19.80	25.30	2.57
CT (WA)	19.47	2.08	15.00	24.00	4.35
CT (BMT)	19.76	1.95	15.15	23.50	3.80
ASV (WA)	3.8	1.17	2.33	7.00	1.37
ASV (BMT)	4.11	0.97	3.20	7.00	0.95
Peak (WA)	234.32	46.43	139.25	362.83	2156.28
Peak (BMT)	292.12	22.23	242.25	369.10	494.45
Hot (WA)	159.18	28.80	108.16	267.08	829.50
Hot (BMT)	160.40	28.38	135.60	285.70	805.64
Cool (WA)	317.35	48.20	207.41	485.75	2323.248
Cool (BMT)	302.80	41.14	258.60	460.00	1692.50
Bkdn (WA)	75.14	27.64	26.50	127.58	764.12
Bkdn (BMT)	130.66	22.90	83.40	156.35	524.79
Stbk (WA)	83.02	28.65	33.00	134.66	820.88
Stbk (BMT)	10.13	36.86	-33.40	103.75	1358.70

4.5. Chemical and Molecular Studies of the Cocodrie / Dixiebelle Population

4.5.1. Pasting Properties of the Parents Cocodrie and Dixiebelle

The results for the different chemical analyses performed are summarized in Tables 28 and 29. The two parents of the genetic population studied, Cocodrie and Dixiebelle, had similar apparent amylose content (AA). Cocodrie had a value of 25.67% AA and Dixiebelle had 25.87% AA, which was not significantly different. The measurement of soluble amylose (SA) is defined as the amount of amylose soluble in hot-water (Maningat and Juliano 1978). The SA content was different for the two parents with Cocodrie having 14.97% SA and Dixiebelle having 13.80% SA (Table 29). Likewise, Dixiebelle had more insoluble amylose compared to Cocodrie, being 11.94% and 10.70%, respectively. The two parents had similar protein content. Although protein content has been reported to have effects on RVA viscosity curves through water-binding (Martin and Fitzgerald 2002), these present results show that any pasting property differences between Cocodrie and Dixiebelle are not due to differences in their protein content (Table 29). The RVA parameters were significantly different for the parents. Dixiebelle had a higher Peak with 311.05 RVU and Cocodrie had 221.78 RVU. Dixiebelle had 192.53 RVU as the Hot value and Cocodrie had 137.75 RVU. The Cool paste RVU values were significantly different, with 405.88 for Dixiebelle and 290.55 for Cocodrie. Dixiebelle had 90.20 RVU as Stbk value and Cocodrie had 68.78 RVU. Significant differences were found for Bkdn with 123.14 and 84.05 RVU for Dixiebelle and Cocodrie, respectively. Similar RVA results were studied and discussed by Kongseree and Juliano (1972), Juliano and Perdon (1975), and Maningat and Juliano

(1978). They declared that cultivars showing low solubility of amylose had, in general, very high setback and low breakdown, hard gel consistency and faster retrogradation. The initial temperature was significantly different for Dixiebelle and Cocodrie with the respective values of 79.53 and 80.17 RVU, although small in magnitude. Significant difference was found between the two parents in consistency, where the higher value was found with Dixiebelle and the lower value with Cocodrie, having 213.36 and 152.83 RVU, respectively. For the DSC measurements, Cocodrie took more time to reach the Peak (5.90 min) compared to Dixiebelle (5.78 min), while the Hot time and the initial time were similar for them. The remaining DSC parameters also did not show any significant differences (Table 29). Figure 12 shows that Cocodrie has lower RVA curve than Dixiebelle.

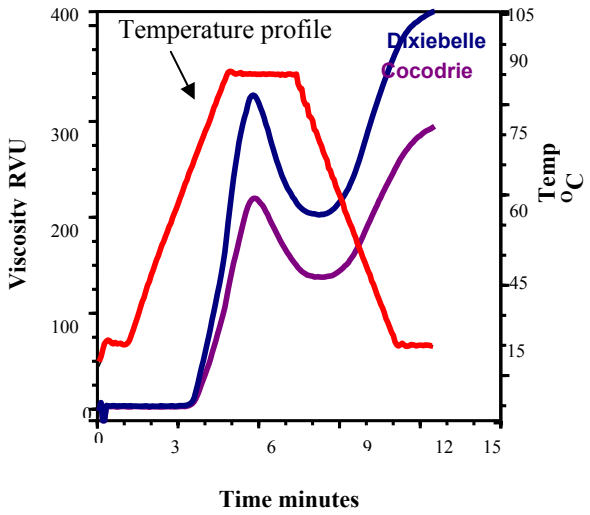


Fig. 12. Comparative RVA curves between Cocodrie and Dixiebelle parents.

Table 28. Mean square of the different parameters for the parents Cocodrie and Dixiebelle.

		Mean Square										
Source of variation	Df	AA	SA	IA	Peak	Hot	Cool	Bkdn	Intemp	CS	Stbk	Onset1
Varieties	1	0.14	4.39**	4.99*	31730.69	10804**	47881.78**	5502.15**	1.44**	13191**	1651**	0.028
Error	13	0.33	0.69	0.23	45.67	28.59	22.91	45.93	0.13	13.56	29.56	0.13
R^2		0.032	0.63	0.39	0.98	0.97	0.99	0.90	0.46	0.98	0.81	0.02

Table 28. Continued.

		Mean Square										
Source of variation	Df	DeltaH1	Peak1	End1	Onset2	Peak2	DeltaH2	End2	Protein	Peaktime	Hotime	Intime
Varieties	1	0.05	0.01	0.69	1.32	0.10	0.0004	0.0001	0.028	0.053	0.0134	0.004
Error	13	0.07	0.06	0.58	0.87	1.02	0.002	0.92	0.31	0.004	0.01	0.001
R^2		0.04	0.01	0.08	0.10	0.007	0.01	0.000009	0.007	0.491	0.085	0.2307

Table 29. Means of the different variables for the parents Cocodrie and Dixiebelle.

	Means											
Varieties	AA (%)	SA (%)	IA (%)	Protein (%)	Peak (RVU)	Hot (RVU)	Cool (RVU)	Stbk (RVU)	Bkdn (RVU)	Intemp (RVU)	CS	Onset1 (^oC)
Dixiebelle	25.87	13.80 b	11.94 a	5.84	311.05 a	192.53 a	405.88 a	90.20 a	123.14 a	79.53 b	213.36 a	72.84
Cocodrie	25.67	14.97 a	10.70 b	5.93	221.78 b	137.75 b	290.55 b	68.78 b	84.05 b	80.17 a	152.83 b	72.93
R^2	0.03	0.63	0.39	0.006	0.95	0.97	0.99	0.81	0.90	0.46	0.98	0.02

Table 29. Continued.

	Mean									
Varieties	DeltaH1 (j/g)	Peak1 (^oC)	End1 (^oC)	Onset2 (^oC)	DeltaH2 (j/g)	Peak2 (^oC)	End2 (^oC)	Peaktime (min)	Hotime (min)	Intime (min)
Dixiebelle	3.06	76.70	82.02	94.58	0.26	99.53	104.21	5.78 b	8.22	3.50
Cocodrie	2.95	76.77	81.58	93.98	0.27	99.7	104.21	5.90 a	8.28	3.53
R^2	0.046	0.01	0.08	0.10	0.01	0.007	0.000009	0.491	0.085	0.23

Means with the same letter within the same column are not statistically different.

4.6. Effects of Petroleum Ether and Methanol Treatments on the Different Chemical Properties of Cocodrie and Dixiebelle Parents

Individual plants of the two parents Cocodrie and Dixiebelle were harvested and treated separately for the different analyses. No significant differences were found among the two parents for the surface lipid content. They had the same degree of milling (Tables 30 and 31).

Previous results from Fitzgerald et al (2003) and Yang and Chang (1999) showed that lipids had an impact on rice-flour pasting parameters. Cooked-rice firmness was associated with the amount of lipid-amylose complex. In the present study, surface lipid had no effect on the changes seen in the different pasting properties of the parents, as the petroleum ether treatment (which removes surface lipids) was not different from the control. The effects of the methanol, which removed bound lipids, were considerable, most notably decreasing the Peak RVA measurement. No interaction was found between the three treatments (untreated, petroleum ether extracted, and methanol extracted flour) and the two varieties for the RVA peak. The treatments affected both varieties the same way by lowering the RVA peak (Figs. 13, 14). The analysis of variance shown in table 32 indicates significant interactions between variety and treatment for Hot, Cool, Stbk, and CSV. However, the magnitude of the variation between varieties (i.e mean square) was over 10 times greater than the interaction with treatments. Results are shown in Figs. 14 and 15. It can be inferred that Cocodrie and Dixiebelle had differences in the amount of bound lipids that were not estimated in this experiment.

The removal of the bound lipids had an effect on the second peak of the DSC (Table 32) measurements. No second peak was observed when the methanol treatment was applied. The results are supported by the conclusions Bergman et al (2004), who declared that superior–processing types of rice had a significant amount of lipid complexed with amylose. Dixiebelle as well as Cocodrie did not show a second peak with the DSC. Marshall et al. (1990) found that different varieties had different thermal properties, and that enthalpy changes were due more to the specific variety than to the treatments for lipid removal. They concluded that removal of rice lipid or rice protein had minor, but quantifiable effect on starch gelatinization. The different results are summarized in table 32 where onset1 (initial temperature), ΔH1 (enthalpy), peak1 and end1 are DSC parameters recorded with the first peak and onset2, ΔH2 (enthalpy), peak2, and end2 are for the DSC second peak.

Table 30. Mean square of the lipid content of the parents Cocodrie and Dixiebelle after the treatment with petroleum ether.

Source of Variation	Df	Mean square
Varieties	4	0.0051
Error	29	0.004

Table 31. Means of the lipid content of the parents Cocodrie and Dixiebelle after the treatment with petroleum ether.

Varieties	Mean
Cocodrie10769	0.22
Dixiebelle10790	0.17
Dixiebelle10820	0.22
Cocodrie10857	0.24
Dixiebelle11036	0.19

Table 32. Mean square of different variables of Cocodrie and Dixiebelle parents after different treatments.

		Mean Square							
Source of Variation	Df	AA	SA	IA	Protein	Peak	Hot	Cool	Stbk
Variety	4	0.33	4.46**	6.08**	2.18**	13138.66**	5582.80**	29968.80**	2772.65**
Treatment	2	8.13**	3.10**	1.23	0.39	10588.98**	1345.58**	27472.95**	3211.67**
Var*trt	8	0.46	0.27	1.02*	0.42*	643.41	306.86**	136.20*	146.44**
Error	30	0.23	0.19	0.43	0.13	384.80	62.73	55.19	24.29

Var= Varieties
Trt = Treatments
* and ** indicate significance at 5 and 1% respectively.

Table 32. Continued.

		Mean Square				
Source of Variation	Df	Bkdn	Peaktime	Hotime	Intime	CS
Var	4	2462.41**	0.08092485**	0.06617063	0.01229651**	9849.99**
Trt	2	5270.32**	2.22279236**	1.1606068950**	0.16973527**	16710.95**
Var*trt	8	67.58	0.01171940	0.01391493	0.00146007*	70.43
Error	30	42.91	0.005	0.01	0.0004	37.95

Var= Varieties
Trt = Treatments
*and ** indicate significance at 5 and 1% respectively.

Table 32. Continued.

		Mean Square								
Source of Variation	Df	Intemp	Onset1	ΔH1	Peak1	End1	Onset2	ΔH2	Peak2	End2
Var	4	1.40**	0.18	0.18*	0.29**	1.25	4.62*	0.004	4.39*	6.89*
Trt	2	21.72**	8.02**	0.22*	8.00**	1.81	134.35**	0.17**	99.02**	70.62**
Var*trt	8	0.08	0.10	0.05	0.08	1.10	6.88**	0.006*	2.81*	4.24*
Error	30	0.06	0.10	0.05	0.06	0.99	0.96	0.001	0.94	1.26

Var= Varieties
Trt = Treatments
* and ** indicate significance at 5 and 1% respectively.

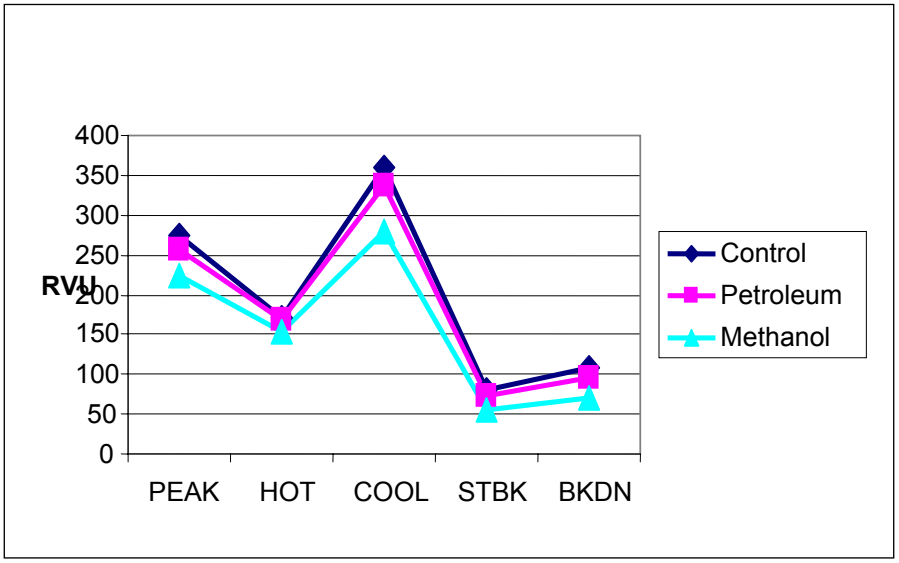


Fig. 13. Effects of the different treatments on the RVA curves using the means of the two parents Cocodie and Dixiebelle.

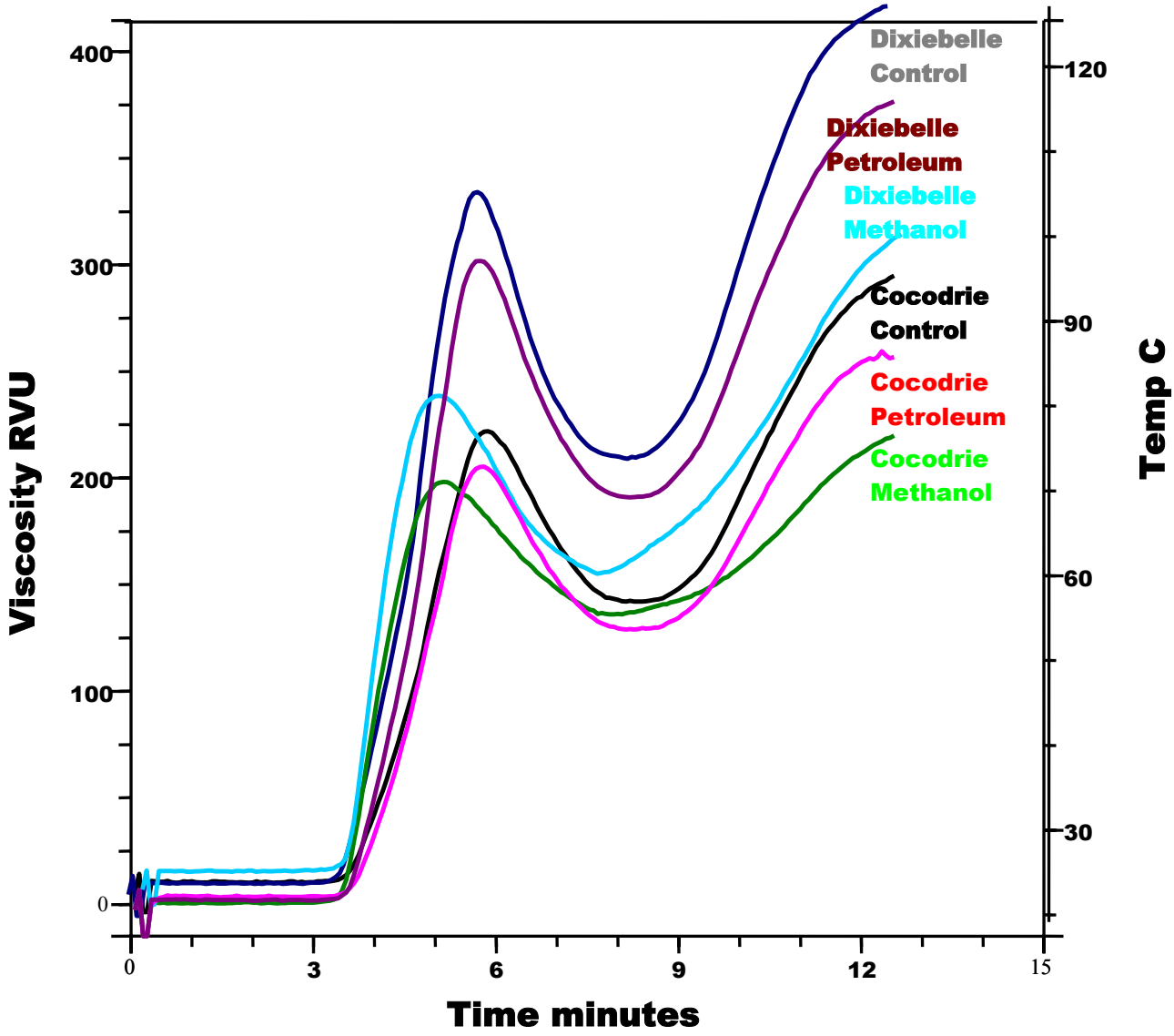


Fig. 14. Effects of the different treatments on RVA curves of the individual parents Cocodrie and Dixiebelle.

4.7. Inheritance of Physico-Chemical Properties in Cocodrie/Dixiebelle Progeny Population

The descriptive statistics showed that the Cocodrie/Dixiebelle population had wide range for the different parameters which were measured (Table 33). Transgressive segregation in both directions was detected in the population. Protein and heading date were skewed, with kurtosis greater than 1. Figure 16 shows the distribution of the different parameters.

Table 33. Descriptive statistics of the population Cocodrie/Dixiebelle.

Variable	N	Mean	Std Dev	Mini	Maxi	Skew-ness	Kurtosis	Dixiebelle *	Cocodrie *
AA	199	25.51	0.52	23.60	27.00	-0.22	0.66	25.87	25.67
SA	199	14.90	1.12	12.50	17.30	-0.04	-0.76	13.80	14.97
IA	199	10.62	1.06	7.70	13.00	-0.17	-0.67	11.94	10.70
Protein	199	5.44	0.57	3.20	7.00	-0.62	1.84	5.84	5.93
Peak	199	245.39	38.42	181.10	324.80	0.37	-1.16	311.05	221.78
Hot	199	147.28	34.65	89.90	224.80	0.32	-1.18	192.53	137.75
Cool	199	335.16	56.06	238.30	447.30	0.17	-1.25	405.88	290.55
Stbk	199	89.78	23.71	34.00	147.00	0.15	-0.64	90.20	68.78
Bkdn	199	98.12	12.51	57.00	131.50	0.26	0.5	123.14	84.05
Intemp	199	79.94	0.66	78.40	81.60	0.15	-0.27	79.53	80.17
CS	199	187.88	25.00	129.30	246.50	0.17	-0.71	213.36	152.83
Heading	199	69.52	3.20	63.00	79.00	0.96	1.02	73	68
Peaktime	199	5.83	0.16	5.50	6.20	0.12	-0.68	5.78	5.90
Hotime	199	8.27	0.11	7.90	8.60	-0.006	0.25	8.22	8.28
Intime	199	3.53	0.06	3.40	3.70	0.09	-0.14	3.50	3.53

AA = Apparent Amylose Intemp = initial Temperature *= Parents
SA = Soluble Amylose CS = Consistency; Intime = Time for initial t^o
IA = Insoluble Amylose Peaktime = time to reach the Peak
Stbk = Setback Hotime = Time to reach the Hot

The correlation Table 34 showed that SA explained was more highly correlated with differences in the apparent amylose content and RVA parameters than IA.

Table 34. Correlation coefficients among different variables in the population of 199 F4 from the cross of Cocodrie/Dixiebelle.

	SA	AA	IA	Protein	Peak	Hot	Cool	Stbk	Bkdn	Itemp	CS	Days	Ptime
AA	0.34**												
IA	-0.89**	0.12											
Protein	-0.05	-0.15*	-0.02										
Peak	-0.72**	-0.22**	0.65**	-0.08									
Hot	-0.81**	-0.24**	0.74**	0.04	0.94**								
Cool	-0.82**	-0.31**	0.71**	0.04	0.94**	0.95**							
Stbk	-0.75**	-0.37**	0.61**	0.25**	0.60**	0.73**	0.83**						
Bkdn	0.019	-0.01	-0.02	-0.37**	0.45**	0.13	0.24**	-0.15*					
Itemp	-0.16*	-0.23*	0.05	0.26**	-0.01	0.10	0.15*	0.37**	-0.33**				
CS	-0.71**	-0.36**	0.57**	0.05	0.80**	0.75**	0.91**	0.87**	0.35**	0.19**			
Days	-0.14*	-0.002	0.15*	-0.22**	0.15*	0.10	0.10	0.0009	0.18*	-0.38**	0.09		
Ptime	-0.73**	-0.29**	0.62**	0.19**	0.66**	0.81**	0.76**	0.72**	-0.2**	0.37**	0.59**	0.06	
Hotime	-0.04	-0.11	-0.01	0.03	-0.06	0.04	-0.04	-0.01	-0.30**	0.20**	-0.16*	-0.07	0.16*

*, ** indicate respectively the significance at 5, and 1%.

When SA and AA were compared, it was concluded that SA had the best linear regression profiles (Fig. 15) with the RVA parameters. When both were compared for Peak, SA had R^2= 0.53 and AA had R^2= 0.052; for Hot it was 0.68 and 0.062; for Cool the coefficients were .67 and .1 respectively for SA and AA; for consistency the R^2 values were 0.50 and 0.131 and for setback it was recorded 0.57 and 0.14, respectively as coefficients values for SA and AA. The correlation table showed that AA and SA are correlated, but strong correlation existed between RVA curves and SA compared to AA and RVA curves for this particular cross where both parents had similar AA.

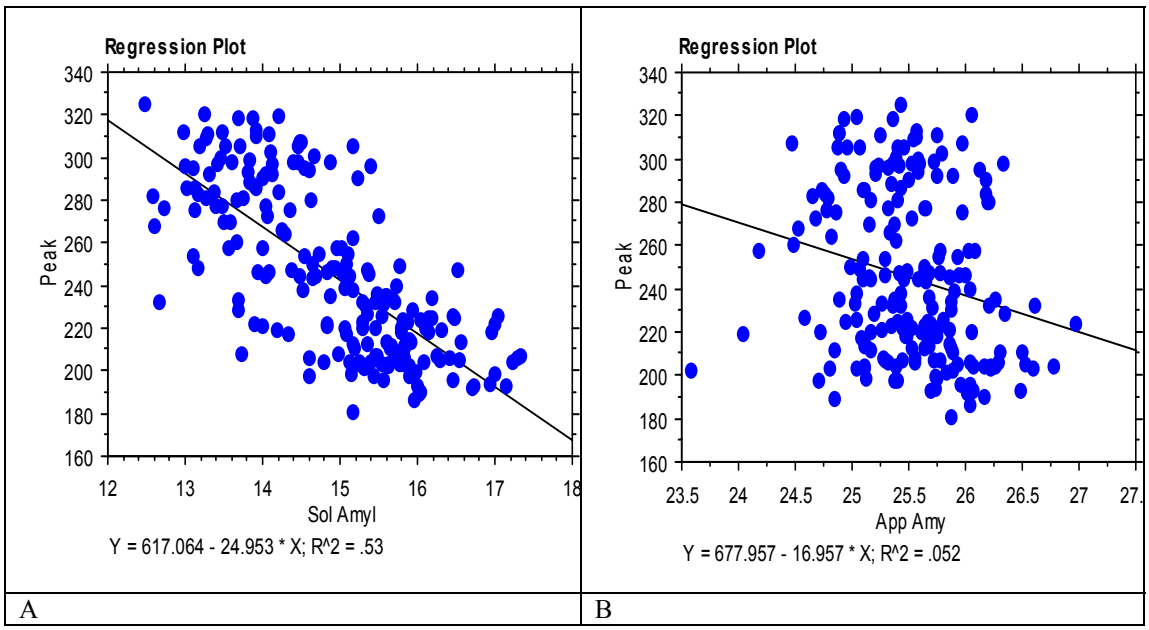


Fig. 15. Comparative simple regression lines between soluble amylose and apparent amylose for selected highly significant parameters (A and B for peak, C and D for hot, E and F for cool, G and H for set back and I and j for consistency viscosity).

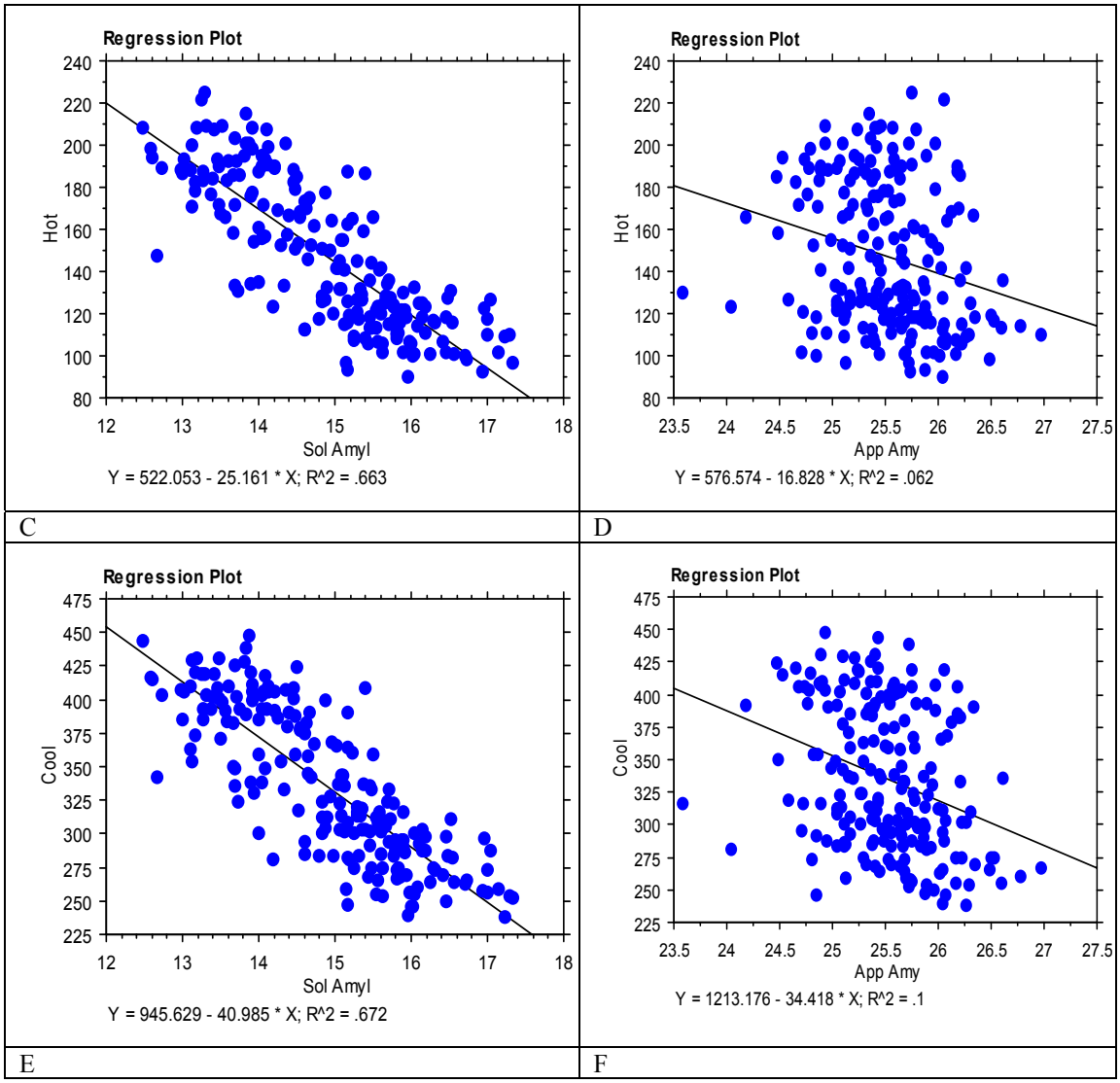


Fig. 15. Continued.

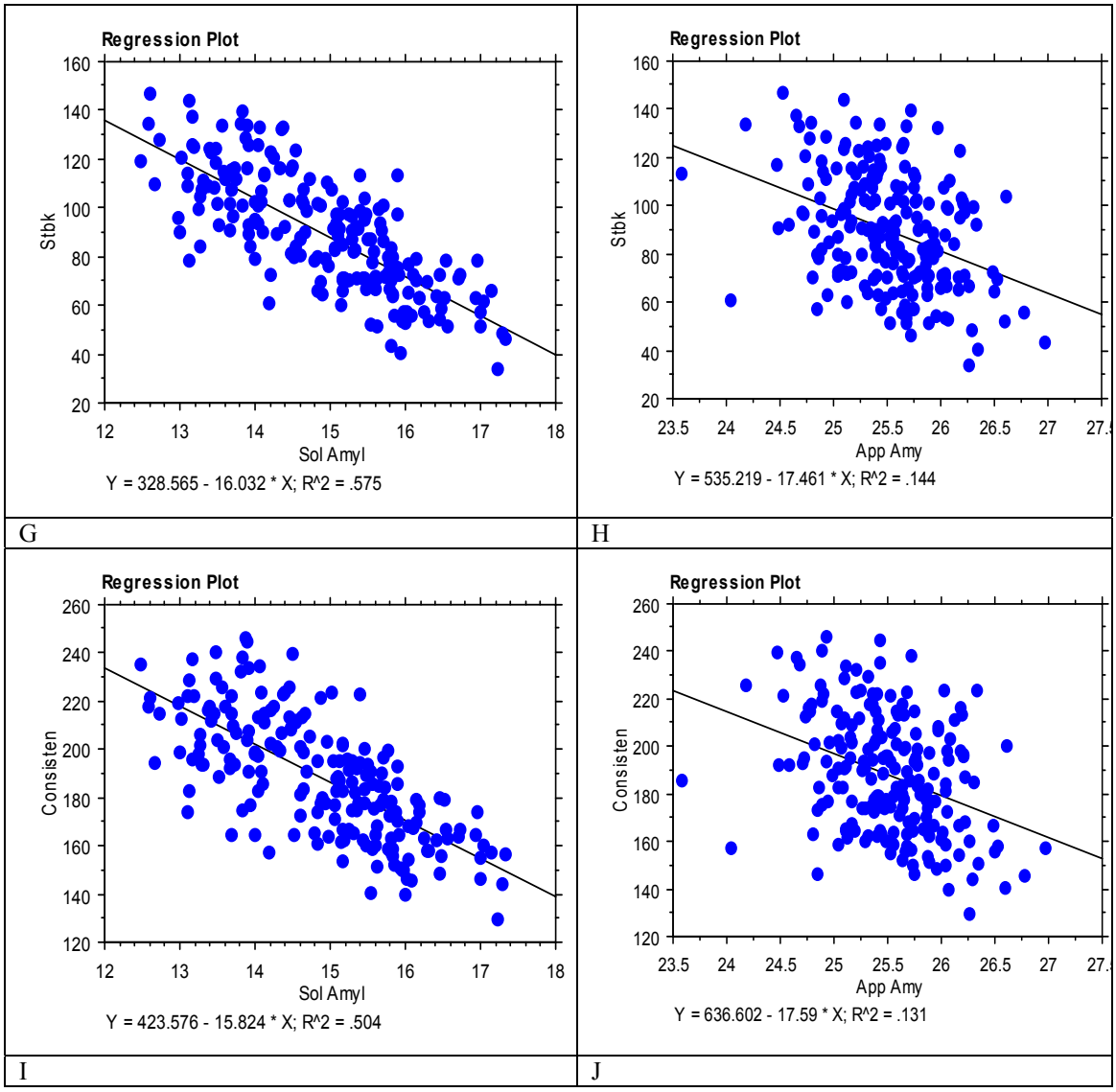


Fig. 15. Continued.

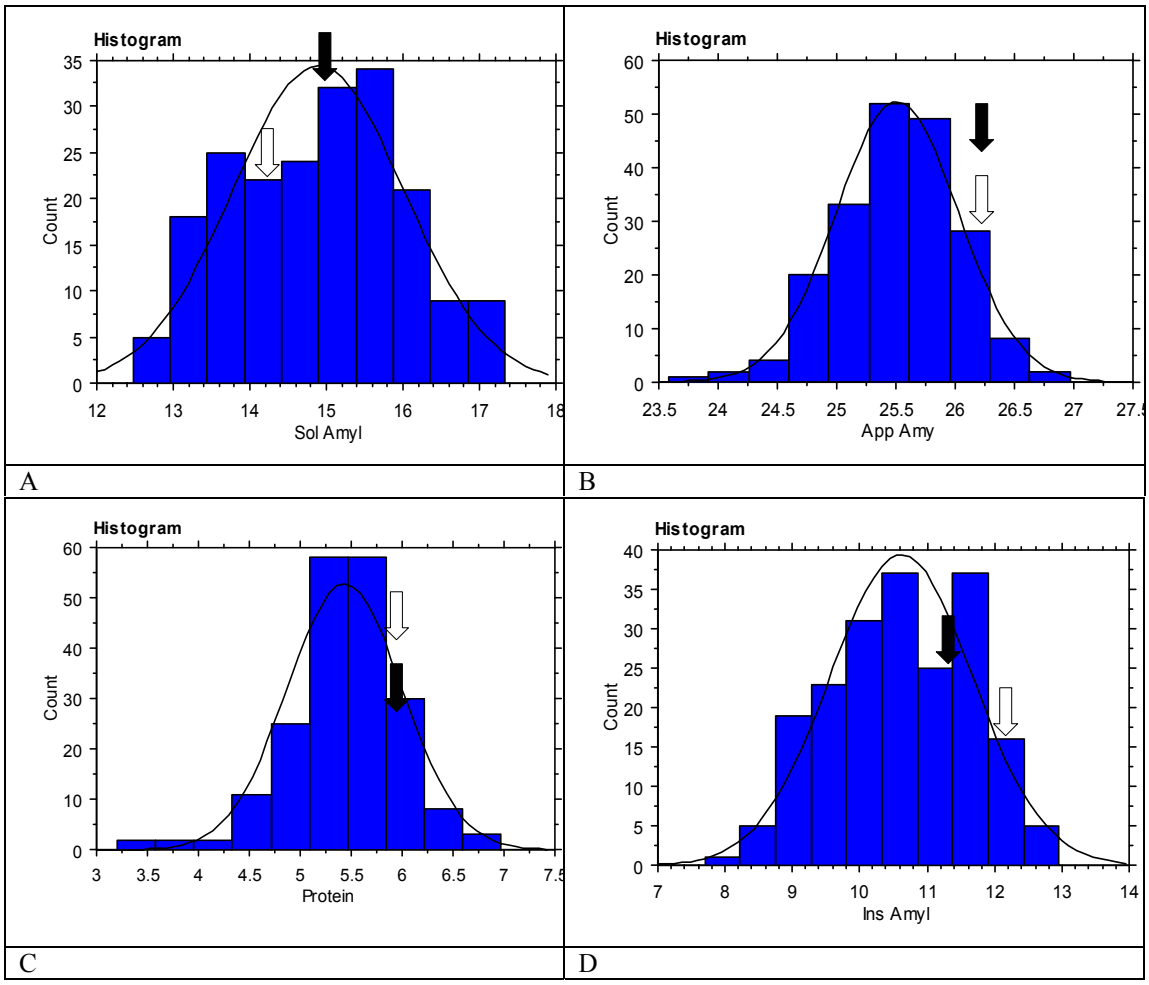


Fig. 16. Histograms indicating the distribution of the different parameters (A= soluble amylose, B= apparent amylose, C= protein, D= insoluble amylose, E= hot paste viscosity, F= peak , G= setback, H=cool, I= breakdown, J= initial temperature, K= initial time, L= heading, M= hot time, N= peak time, O= consistency viscosity) measured in the population Cocodrie and Dixiebelle with the filled arrows as the Cocodrie parent and the empty arrows as the Dixiebelle parent.

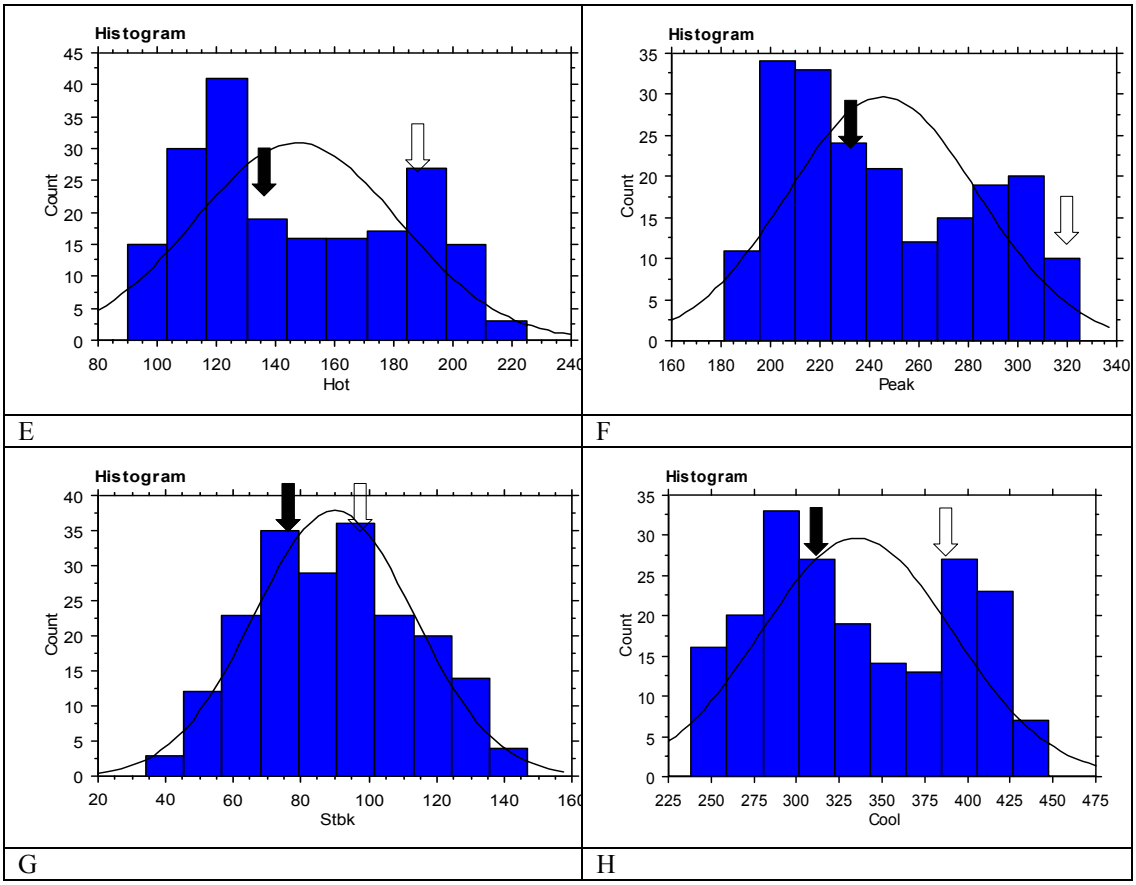


Fig. 16. Continued.

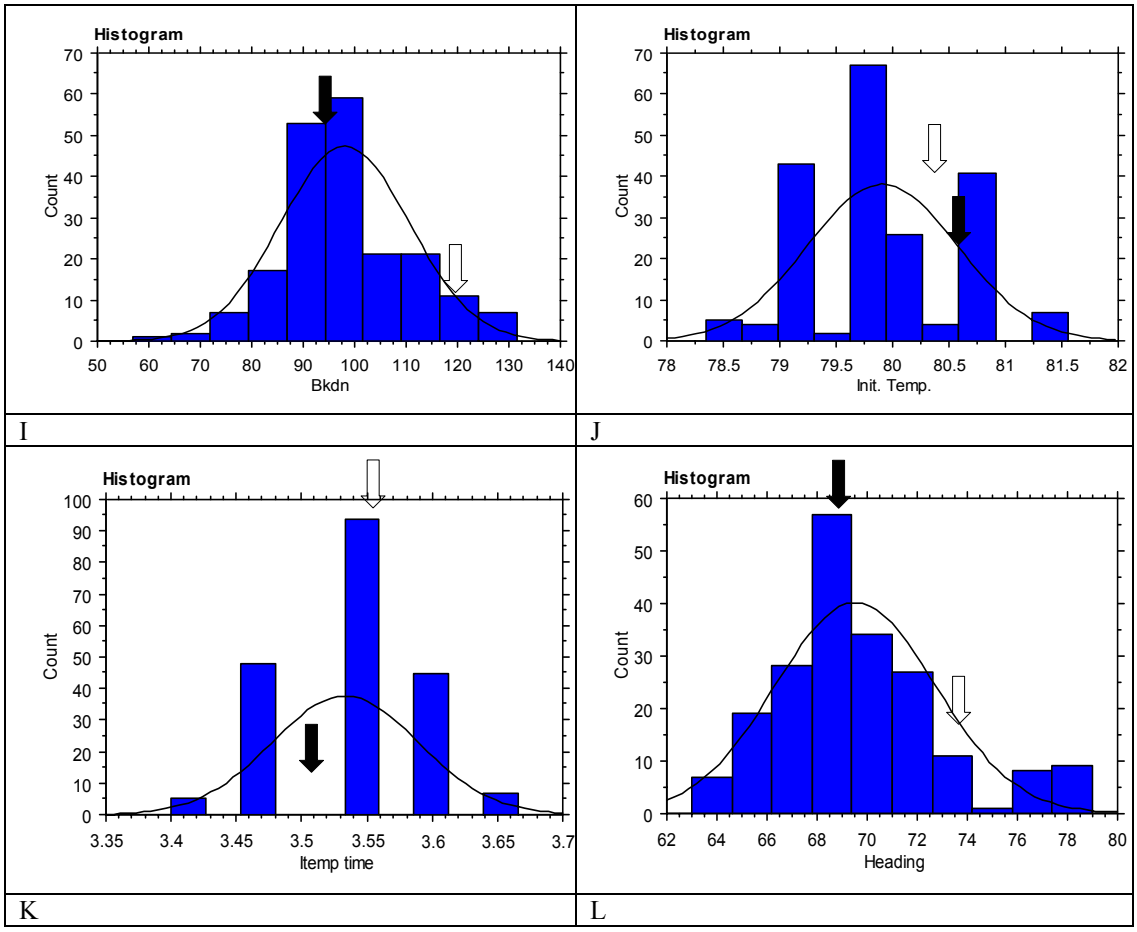


Fig. 16. Continued.

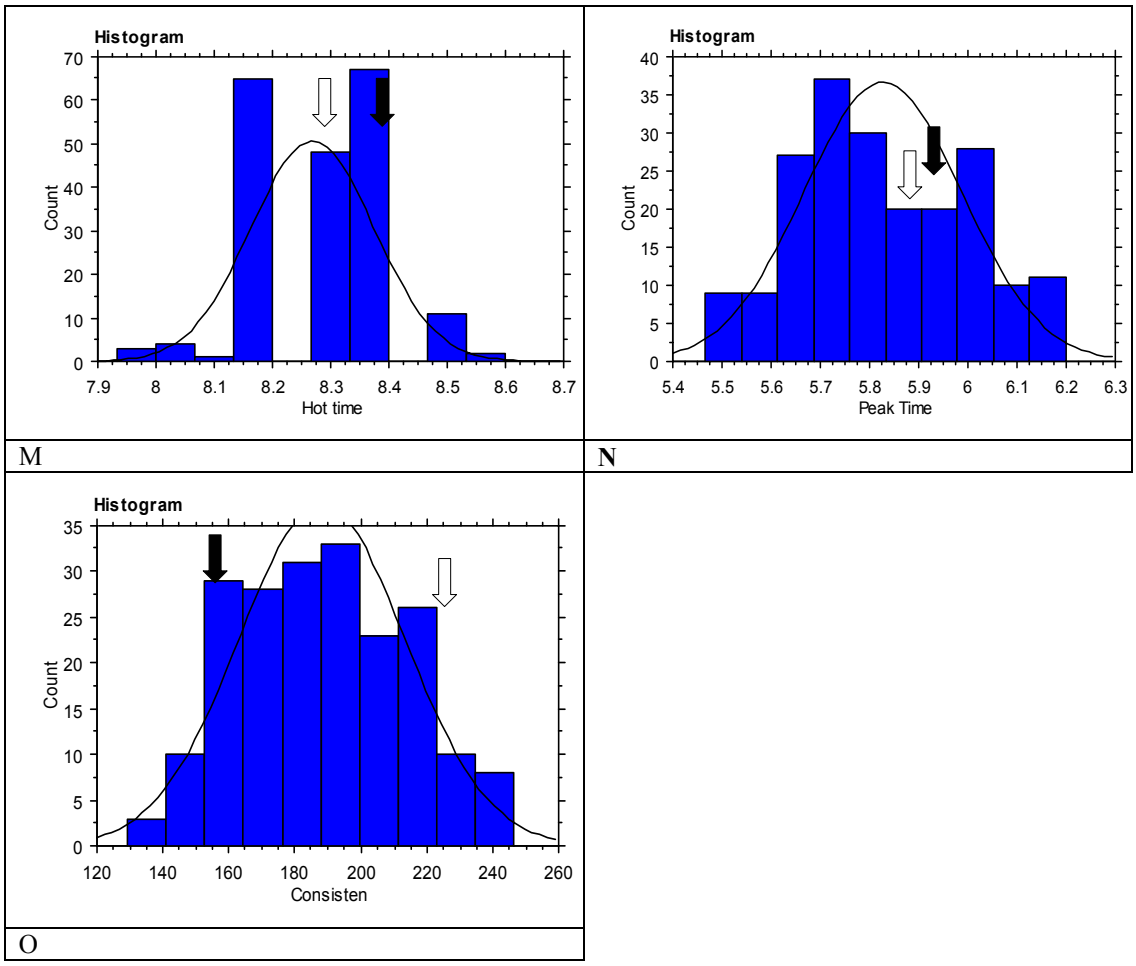


Fig. 16. Continued.

4.8. Marker Associations

The *Waxy* gene encodes the granule bound starch synthase enzyme. Two separate single nucleotide polymorphisms have been identified, one in exon 6 and one in exon 10, that result in amino acid substitutions in the GBSS protein (Larkin and Park 2003). These findings were associated with diverse cultivars with different classes (low, intermediate, and high) of amylose content. Our case concerns a population with similar amylose content, but segregating for the exon 10 SNP in the *Waxy* gene. Three PCR markers, being the *Waxy* microsatellite (RM190), *Waxy* exon10 and AB26295 were used in the study. Single factor analysis was performed for the progenies of Cocodrie/Dixiebelle using SAS 9.0 and the general linear model (GLM) procedure using a DNA marker as a main effect. In this model, three "treatments" are present, which are the progeny with the Dixiebelle allele, the Cocodrie allele, or the heterozygous progeny with both alleles. In the analysis of RM190, the data showed significant differences for the different parameters except for heading, Hot time, initial time and protein. For a total number of 199 individual plants data, it was found that progeny with the 105 bp *Waxy* (RM190) allele (the allele from Dixiebelle) had an apparent amylose content of 25.38% and progeny with the 124 bp allele (from Cocodrie) had an average amylose content of 25.61%. Progeny that were heterozygous for RM190, having both the 105 and 124 bp alleles, had an average amylose content of 25.52%.

The highest value for soluble amylose content was registered with progeny having the 124 bp allele with a value of 15.82%, while the soluble amylose (SA) content was 13.78% for progeny with the 105 bp allele and heterozygotes with both the 105:124

alleles had a SA of 14.82%. The highest value for insoluble amylose (IA) was found with the 105 allele (11.56%), the lowest value was seen with progeny with the 124 allele (9.80%), the heterozygous progeny had 10.68% IA (Table 35).

Based on the data, although there is a significant difference in total amylose content between Cocodrie and Dixiebelle alleles of RM190 there is a much greater difference in their proportion of SA to IA content. The Cocodrie allele had higher SA and consequently had a lower RVA curve. These results are supported by the conclusions of Rhadika Reddy et al. (1993) and Ong and Blanshard (1995a, b). They concluded from their studies that the high-iodine –affinity amylopectin remains in the gelatinized granule. Juliano et al. (1987) concluded that the amylopectin in the gelatinized granule had greater effect on cooked rice hardness than leached amylose. The three types of progeny with different RM190 alleles were significantly different for their RVA curve parameters (Tables 35 and 36). For Peak, Hot, Cool, Stbk, CSV, and Peaktime, all three marker classes were significantly different with the heterozygous class being intermediate to the homozygous classes. The progeny homozygous for the 105 allele had significantly higher breakdown (101.76 RVU) as compared to the heterozygous class (97.28) and the progeny fixed for the 124 allele (95.51 RVU) (Table 36).

No significant differences between the RM190 progeny types were found with heading, peak time, hot time and initial temperature (Table 36). It has been shown that insoluble amylose was associated with very high Stbk, low Bkdn, hard gel consistency,

and faster retrogradation during pasting of rice flour slurry (Kongseree and Juliano 1972; Maningat and Juliano 1978; Juliano and Perdon 1975).

Results from ANOVA for RVA parameters using the *Waxy* microsatellite marker alleles as a main effect show that the additive effects were significant for Peak, Hot, Cool, Stbk, Bkdn, CSV, and Peaktime (Table 36). Peak, Hot, and Cool paste viscosities had R^2>0.80 with the *Waxy* marker, meaning that 80% of the phenotypic variability was explained by the *Waxy* genotype for these traits. However Stbk, CSV, and Peaktime had 43-58% of the phenotypic variance, explained by the *Waxy* microsatellite marker. Peak also had some dominance effects although this was much less than the additive effect (Table 36). In this study, although Bkdn was significantly different among the *Waxy* classes and was primarily controlled by the additive gene action only 5% of the phenotypic variability was controlled by the *Waxy* (Table 36). The RVA profile is a useful indicator of rice eating and cooking quality. Previous works conducted by Gravois

Table 35. Mean square of the different variables for the Cocodrie /Dixiebelle population with the *Waxy* microsatellite marker using single factor analysis.

		Mean Square						
Source of variation	df	AA	SA	IA	Peak	Hot	Cool	Stbk
Waxy ms	2	1.04*	78.00**	60.33**	116548.61**	98433.24**	253426.81**	26737.78**
Error	196	0.25	0.47	0.52	301.96	208.57	588.57	295.07
Contrast								
Additive	1	2.08*	155.75**	120.39**	232946.49**	196828.50**	506468.69**	52458.07**
Dominant	1	0.03	0.002	0.001	1124.02*	660.57	138.03	474.27
R^2		0.04	0.63	0.54	0.80	0.83	0.81	0.48

Note:
Contrast additive = *Waxy* 105 vs *Waxy* 124
Contrast dominant = *Waxy* 105:124 (heterozygous) vs *Waxy* 105 and 124

Table 35. Continued.

		Mean Square							
Source of variation	df	Bkdn	Intemp	CS	Heading	Peaktime	Hotime	Intime	Protein
Waxy ms	2	776.89**	1.41*	36241.03**	26.91	1.38**	0.016	0.001	0.005
Error	196	150.2	0.43	261.36	10.08	0.01	0.01	0.003	0.32
Contrast									
Additive	1	1516.99**	0.22	71815.18**	50.77	2.76**	0.02	0.003	0.006
Dominant	1	60.46	2.67*	195.83	2.048	0.0001	0.005	0.017	0.004
R^2		0.05	0.03	0.58	0.02	0.53	0.01	0.03	0.0001

Means with the same letter within the same column are not statistically different.

Table 36. Means of the different variables for the Cocodrie /Dixiebelle population with the *Waxy* microsatellite marker.

	Mean						
Markers	AA (%)	SA (%)	IA (%)	Protein (%)	Peak (RVU)	Hot (RVU)	Cool (RVU)
Waxy 105	25.38 b	13.78 c	11.56 a	5.44	288.23 a	188.47 a	396.95 a
Waxy 105:124	25.52 ab	14.82 b	10.68 b	5.42	243.70 b	146.43b	337.85 b
Waxy 124	25.61 a	15.82 a	9.80 c	5.43	210.83 c	115.33 c	282.83 c

Means with the same letter within the same column are not statistically different.

Table 36. Continued.

	Mean							
Markers	Stbk (RVU)	Bkdn (RVU)	Intemp (RVU)	CSV	Heading	Peaktime	Hotime	Intime
Waxy105	108.73 a	101.76 a	80.03 a	210.47 a	70.08	5.97 a	8.25	3.53 a
Waxy 105:124	94.15 b	97.28 b	79.71 b	191.42 b	69.76	5.83 b	8.25	3.50 b
Waxy 124	72.00 c	95.51 b	79.96 a	167.50 c	68.94	5.70 c	8.28	3.52 ab

Means with the same letter within the same column are not statistically different

and Webb (1997) on the inheritance of RVA parameters also showed significant additive effects as was seen in this study.

These findings also confirmed the results from Larkin et al. (2003) where they concluded that the *Waxy* locus had a significant effect on peak viscosity, hot paste viscosity, cool paste viscosity, breakdown, and setback viscosity.

Means for the RM190 alleles in Tables 35 and 36 show that the Dixiebelle type progeny with *Waxy* allele 105 had less soluble amylose, higher insoluble amylose, and high RVA curve values relative to the Cocodrie allele 124. Tables 35 and 36 show that the single factor analysis comparing the two homozygous classes of the waxy allele slightly increased the percentage of the phenotypic variability (R^2) explained by the waxy allele as compared to the analysis with all three waxy classes (Table 37). The means for the *Waxy* 105 and *Waxy* 124 microsatellite marker alleles are summarized in Table 38.

Table 37. Mean square of the different variables for the Cocodrie /Dixiebelle population for the homozygous classes of the *Waxy* marker.

		Mean Square						
Source of variation	Df	AA	SA	IA	Peak	Hot	Cool	Stbk
Waxy ms	1	2.07**	155.75**	120.39**	232946.50**	196828.50**	506468.68**	52458.07**
Error	155	0.28	0.44	0.53	297.19	191.54	530.67	261.96
R^2		0.04	0.69	0.59	0.83	0.86	0.86	0.56

Table 37. Continued.

		Mean Square							
Source of variation	Df	Bkdn	Intemp	CSV	Heading	Peaktime	Hotime	Intime	Protein
Waxy ms	1	1516.99**	0.22	71815.18**	50.77	2.76**	0.02	0.003	0.006
Error	155	157.80	0.42	258.33	9.29	0.01	0.01	0.003	0.31
R^2		0.06	0.003	0.64	0.03	0.58	0.01	0.006	0.0001

Table 38. Means of the different variables for the Cocodrie /Dixiebelle population for the homozygous classes of the *Waxy* marker.

	Mean						
Markers	AA (%)	SA (%)	IA (%)	Protein (%)	Peak (RVU)	Hot (RVU)	Cool (RVU)
Waxy105	25.38 b	13.81 b	11.56 a	5.44	288.23 a	188.47 a	396.95 a
Waxy 124	25.61 a	15.82 a	9.80 b	5.43	210.83 b	115.33 b	282.83 b

Means with the same letter within the same column are not statistically different.

Table 38. Continued.

	Mean							
Markers	Stbk (RVU)	Bkdn (RVU)	Intemp (min)	CS	Heading	Peaktime	Hotime	Intime
Waxy105	108.73 a	101.76 a	80.03	210.47 a	70.08	5.97a	8.25	3.53
Waxy 124	72.00 b	95. 51 b	79.96	167.50 b	68.94	5.70 b	8.28	3.52

Means with the same letter within the same column are not statistically different.

The *Waxy* exon 10 SNP is characterized by a substitution of a serine residue for proline (Larkin and Park 2003). The substitution occurred after a cytosine to thymine transition in exon 10 in cultivars with high amylose. Our results showed that the *Waxy* exon 10 SNP and the *Waxy* microsatellite (RM190) marker both can characterize our population. The two results were similar with 1 to 3 % more precision (higher R^2 values) with *Waxy* marker compared to the *Waxy* exon 10 SNP (Tables 39 to 42). Previous works conducted by Larkin and Park (2003) showed a strong association among *Waxy* allele variables (i.e. CT repeat number) with apparent amylose class. However, Larkin and Park did not analyze whether it was amylose content alone or some other feature of the *Waxy* gene that gave rise to the differences in RVA between the progeny lines they studied. Studying progeny from a cross between parents with similar amylose content, our results showed strong association between soluble amylose, insoluble amylose and the RVA profile with the *Waxy* exon 10 mutation.

These results suggest that the SNP at exon 10 in the *Waxy* gene directs a change in the GBSS protein, which in turn alters the proportion of soluble to insoluble amylose in the rice grain. However the GBSS protein difference does not change the total amount of amylose in the grain. It would further appear that this proportional change of insoluble to soluble amylose is associated with differences in the starch paste viscosity measurements observed between Dixiebelle and Cocodrie.

Table 39. Mean square of the different variables for the Cocodrie /Dixiebelle population with the *Waxy* exon10 SNP marker using single factor analysis.

		Mean Square						
Source of variation	Df	AA	SA	IA	Peak	Hot	Cool	Stbk
Exon10	2	1.18*	76.56**	58.02**	112729.13	95577.79	246835.08**	26329.33**
Error	196	0.25	0.48	0.54	340.94	237.71	655.83	299.24
Contrast								
Additive	1	2.35**	153.07**	115.99**	225123.11**	190999.55**	493627.87**	52042.91**
Dominant	1	0.02	0.02	0.008	1091.87	679.47	235.11	313.75
R^2		0.04	0.61	0.52	0.77	0.80	0.79	0.47

Note :
Contrast additive = exon 10 (134 vs exon 10 133)
Contrast dominant = exon 10 (134:133 heterozygous vs exon 10 134 and 133).

Table 39. Continued.

		Mean Square							
Source of variation	df	Bkdn	Intemp	CSV	Heading	Peaktime	Hotime	Intime	Protein
Exon10	2	715.20**	1.66*	35430.25**	27.45	1.37**	0.01	0.01*	0.02
Error		150.84	0.42	269.64	10.07	0.01	0.01	0.003	0.32
Contrast									
Additive	1	1397.38**	0.25	70497.41**	48.68	2.75**	0.02	0.002	0.03
Dominant	1	47.67	3.15	116.52	5.17	0.0002	0.007	0.019	0.008
R^2		0.04	0.03	0.57	0.02	0.53	0.01	0.03	0.0006

Table 40. Means of the different variables for the Cocodrie /Dixiebelle population with the *Waxy* exon10 SNP marker.

	Means						
Markers	AA (%)	SA (%)	IA (%)	Protein (%)	Peak (RVU)	Hot (RVU)	Cool (RVU)
Exon10-134	25.37 b	13.85 c	11.52 a	5.45	286.37 a	184.87 a	394.64 a
Exon10 133:134	25.52 ab	14.86 b	10.65 b	5.42	242.80 b	145.51 b	336.08 b
Exon10 133	25.62 a	15.82 a	9.81 c	5.42	210.92 c	115.39 c	282.94 c

Means with the same letter within the same column are not statistically different

Table 40. Continued.

	Means							
Markers	Stbk (RVU)	Bkdn (RVU)	Itemp (RVU)	CS	Heading	Peaktime	Hotime	Intime
Exon10-134	108.29 a	101.49 a	80.04 a	209.77 a	70.02	5.96 a	8.28	3.53
Exon10 133:134	93.29 b	97.30 ab	79.96 a	190.57 b	68.87	5.83 b	8.25	3.52
Exon10 133	72.02 c	95.55 b	79.68 b	167.56 c	68.91	5.70 c	8.25	3.50

Means with the same letter within the same column are not statistically different

Table 41. Mean square of the different variables for the Cocodrie /Dixiebelle population for the homozygous classes of the *Waxy* exon 10 marker.

		Mean Square						
Source of variation	Df	AA	SA	IA	Peak	Hot	Cool	Stbk
Exon10	1	2.35**	153.07**	115.99**	225123.11**	190999.55**	493627.87**	52042.91**
Error	157	0.27	0.46	0.56	346.70	230.21	626.98	268.87
R^2		0.05	0.67	0.56	0.80	0.84	0.83	0.55

Table 41. Continued.

		Mean Square							
Source of variation	Df	Bkdn	Intemp	CSV	Heading	Peaktime	Hotime	Intime	Protein
Exon10	1	1397.38**	0.24	70497.41**	48.68*	2.75**	0.02	0.002	0.03
Error	157	156.58	0.41	270.34	9.39	0.01	0.01	0.003	0.31
R^2		0.05	0.003	0.62	0.03	0.58	0.01	0.005	0.0006

Table 42. Means of the different variables for the Cocodrie /Dixiebelle population for the homozygous classes of the *Waxy* exon 10 marker.

	Means						
Markers	AA (%)	SA (%)	IA (%)	Protein (%)	Peak (RVU)	Hot (RVU)	Cool (RVU)
Exon10 134	25.37 b	13.85 b	11.52 a	5.45	286.36 a	184.87 a	394.64 a
Exon10 133	25.62 a	15.82 a	9.81 b	5.42	210.92 b	115.38 b	282.94 b

Means with the same letter within the same column are not statistically different.

Table 42. Continued.

	Means							
Markers	Stbk (RVU)	Bkdn (RVU)	Itemp (min)	CS	Heading	Peaktime	Hotime	Intime
Exon10 134	108.29 a	101.49 a	80.04	209.77 a	70.02 a	5.96 a	8.25	3.53
Exon10 133	72. 02 b	95.55 b	79.96	167.56 b	68.91 b	5.70 b	8.28	3.52

Means with the same letter within the same column are not statistically different

A third marker , AB26295, which is a microsatellite marker at the soluble starch synthase I (SSI) gene was used to find association with the parameters studied in our population. Soluble starch synthases and starch branching and debranching enzymes play important roles in the synthesis of amylopectin. The results showed that the soluble amylose, insoluble amylose, and RVA curves were associated with AB26295 (Tables 43 to 46). However, the total phenotypic explanation derived from the single factor analysis (Tables 43 to 46) showed that the R^2 ranged from 16% Stbk to 39% for Hot paste viscosity, although values are lower than those observed with the *Waxy* microsatellite or exon 10 SNP. It can be noted that AB26295 is linked (~10 cM) with the *Waxy* gene, and this linkage most likely explains the association between this marker and RVA parameters. However AB26295 most likely does not play major role in differentiating the two parental types and the heterozygotes apart from each other compared to the *Waxy* microsatellite marker and *Waxy* exon 10 SNP.

Table 43. Mean square of the different variables for the Cocodrie /Dixiebelle population with the AB26295 marker using single factor analysis.

		Mean Square						
Source of variation	Df	AA	SA	IA	Peak	Hot	Cool	Stbk
AB26295	2	0.45	30.41**	24.52**	56690.88**	46866.25**	112115.44**	9376.46**
Error	196	0.26	0.95	0.88	912.76	734.77	2030.52	472.23
Contrast								
Additive	1	0.57	60.77**	48.92**	112538.50**	93473.63**	223082.71**	18729.33**
Dominant	1	0.30	0.002	0.39	259.71	18.65	246.32	0.16
R^2		0.01	0.24	0.22	0.38	0.39	0.36	0.16

Note:
Contrast additive = AB26295 164 vs AB26295 162
Contrast dominant = AB26295 (heterozygous) vs AB26295 164 and AB26295 162.

Table 43. Continued.

		Mean Square							
Source of variation	Df	Bkdn	Intemp	CSV	Heading	Peaktime	Hotime	Intime	Protein
AB26295	2	526.84*	0.36	14023.28**	15.28	0.65**	0.04*	0.004	0.07
Error		152.76	0.44	488.07	10.19	0.01	0.01	0.003	0.32
Contrast									
Additive	1	884.66*	0.41	27728.64**	30.21	1.30**	0.01	0.004	0.05
Dominant	1	140.36	0.34	130.25	0.64	0.003	0.09**	0.004	0.11
R^2		0.03	0.008	0.22	0.01	0.25	0.04	0.01	0.002

Table 44. Means of the different variables for the Cocodrie /Dixiebelle population with the AB26295 marker.

	Means						
Markers	AA (%)	SA (%)	IA (%)	Protein (%)	Peak (RVU)	Hot (RVU)	Cool (RVU)
AB-164	25.59	15.49 a	10.10 c	5.44	219.10 c	123.73 c	298.43 c
AB 162-164	25.43	14.86 b	10.57 b	5.47	249.08 b	149.33 b	339.48 b
AB162	25.46	14.22 c	11.24 a	5.40	273.69 a	173.48 a	375.29 a

Means with the same letter within the same column are not statistically different.

Table 44. Continued.

	Means							
Markers	Stbk (RVU)	Bkdn (RVU)	Intemp (RVU)	CS	Heading	Peaktime	Hotime	Intime
AB-164	79.33	95.37 b	82.00	174.70	69.13	5.73	8.27	3.52
AB 162-164	90.40	99.77 a	79.86	190.15	69.44	5.83	8.22	3.51
AB162	101.60	100.21 a	80.01	201.80	70.02	5.92	8.28	3.55

Means with the same letter within the same column are not statistically different

Table 45. Mean square of the different variables for the Cocodrie /Dixiebelle population for the homozygous classes of AB26295 marker.

		Mean Square						
Source of variation	Df	AA	SA	IA	Peak	Hot	Cool	Stbk
AB26295	1	0.57	60.77**	48.92**	112538.50**	93473.63**	223082.71**	18729.33**
Error	150	0.25	0.88	0.79	826.75	670.88	1819.94	431.81
R^2		0.01	0.31	0.29	0.47	0.48	0.45	0.22

Table 45. Continued.

		Mean Square							
Source of variation	Df	Bkdn	Intemp	CSV	Heading	Peaktime	Hotime	Intime	Protein
AB26295	1	884.66*	0.41	27728.64**	30.21	1.30**	0.01	0.004	0.052
Error	150	152.72	0.45	459.21	10.79	0.01	0.01	0.003	0.28
R^2		0.03	0.005	0.28	0.01	0.31	0.005	0.009	0.001

Table 46. Means of the different variables for the Cocodrie /Dixiebelle population for the homozygous classes of the AB26295 marker.

	Means						
Markers	AA (%)	SA (%)	IA (%)	Protein (%)	Peak (RVU)	Hot (RVU)	Cool (RVU)
AB26295 164	25.59	15.49 a	10.10 b	5.44	219.10 b	123.73 b	298.43 b
AB26295 162	25.46	14.22 b	11.24 a	5.40	273.69 a	173.48 a	375.29 a

Means with the same letter within the same column are not statistically different.

Table 46. Continued.

	Means							
Markers	Stbk (RVU)	Bkdn (RVU)	Intemp (min)	CS	Heading	Peaktime	Hotime	Intime
AB26295 164	79.33 b	95.37 b	79.90	174.70 b	69.13	5.73 b	8.27	3.52
AB26295 162	101.60 a	100.21 a	80.01	201.80 a	70.02	5.92 a	8.28	3.53

Means with the same letter within the same column are not statistically different

In conclusion, phenotypic variability in grain chemistry and functionality parameters observed in progeny from a Cocodrie/Dixiebelle cross was primarily explained by the the *Waxy* microsatellite marker explained most of the phenotypic variability between Cocodrie and Dixiebelle; it was followed by the *Waxy* exon 10 SNP marker, with AB26295 explaining the least amount of variation (Table 47). The use of either *Waxy* microsatellite marker or exon10 SNP will speed up the breeding process in order to screen for cooking properties when the parents used have similar apparent amylose content. Based on these results, soluble amylose was able to differentiate the two parents for their RVA curves whereas apparent amylose content was not.

Table 47. R^2 values indicating the total phenotypic variation (%) explained by markers.

	R^2 Values						
	AA	SA	IA	Peak	Hot	Cool	Stbk
Waxy ms	0.04	0.63	0.54	0.80	0.83	0.81	0.48
Exon 10	0.04	0.61	0.52	0.77	0.80	0.79	0.47
AB26295	0.01	0.24	0.22	0.38	0.39	0.36	0.16

Table 47. Continued.

	R^2 Values							
	Bkdn	Intemp	CS	Heading	Peaktime	Hotime	Intime	Protein
Waxy ms	0.05	0.03	0.58	0.02	0.53	0.01	0.03	0.0001
Exon 10	0.04	0.03	0.57	0.02	0.53	0.01	0.03	0.0006
AB26295	0.03	0.008	0.22	0.01	0.25	0.04	0.01	0.002

5. SUMMARY AND CONCLUSIONS

Different studies were conducted to characterize the novel rice germplasm from West Africa in order to understand the physical-chemical properties and identify molecular markers associated with important rice functional qualities that could benefit both West African and USA breeding programs. The West African germplasm included Nerica cultivars that have been derived from interspecific crosses between *Oryza glaberrima* and *Oryza sativa* as well as upland cultivars that are known for their tolerance to stresses like drought. Thus determining how well these genetic sources are adapted to US rice production systems may benefit and broaden US rice breeding programs.

Results from the yield evaluation experiment showed that BG 90-2, an improved cultivar well adapted in African irrigated rice growing conditions, had the same yield as the top yielding variety from the USA, ZHE733, with 7004.2 kg/ha and 7404 kg/ha, respectively. Because of its high yield potential, Zhe733 is currently being used as an indica donor in an introgression program directed by Dr. Neil Rutger (USDA-ARS, Stuttgart, AR). Although BG 90-2 was very late compared to the USA checks, it could be useful under US organic growing conditions because of its high yield and high tillering ability that can compete with weeds.

Nerica 5 and Nerica 2 ranked immediately following Wells in yield, a predominant commercial cultivar in Arkansas – the state which produces over half of the USA's rice. These two cultivars also had high milling quality, cooking quality

comparable to cultivars that are used by the US parboiling and canning industries, and a grain length: width ratio similar to US long grain. These cultivars appear to offer improved yield potential and genetic that could be beneficial for use in US rice breeding programs.

Four other WA cultivars were also well adapted to the US environment. Nerica 3 and Nerica 4 had yield potential similar Cypress which was the predominant long grain cultivar in southern US for about 10 years during the 1990's. In addition to good yield potential, they were early maturing, short stature, had excellent milling quality, and possessed grain dimensions and cooking quality typical of southern US long grains. WAB 56-104 also had yield potential, early maturity, grain dimensions, and cooking quality similar to Cypress, it was extremely tall indicating that this trait would need to be eliminated in breeding programs for cultivars for the US. Baku Danane and WAB 638-1 also had good yield potential, excellent milling quality, good grain length: width, and typical cooking quality for US long grains. Although they were too tall for production in the USA, both had a very short cooking time that was two to four minutes less than US long grain cultivars. These may offer novel genes for cooking time that could benefit development of rice cultivars for the convenience (quick-cooking) food market.

When entries were divided into four groups (landraces, interspecifics, improved cultivars and USA checks), the group comparison showed that the improved types from West Africa and the interspecifics yielded the same as each other, but less than USA checks. Highly significant differences were found between the other groups. The average yield was 6199 kg/ha for the USA checks, followed by the interspecifics with 4579

kg/ha, the improved cultivars with 4342 kg/ha, and the landraces with 2586 kg/ha. Correlation coefficients showed that in the Beaumont environment, yield was affected mainly by the crop duration and plant height. Highly negative coefficients were recorded between yield and flowering time (r=-0.61) and between yield and plant height (r=-0.78). Lodging was observed with certain landraces, which considerably reduced their yield potential. The Nerica cultivars were very vigorous compared to the US checks, indicating that they could be a source of improvement for US cultivars. Early season vigor is important for weed competition and can help reduce the need for herbicides.

The physical-chemical data showed that samples introduced from WARDA had a wide range for all quality parameters measured. In general, West African consumers dislike glutinous (i.e., waxy) types of rice and no waxy-type rice was found among the WA samples. Several of the WA samples had very high amylose contents with the highest being observed in CG 14, an indigenous *Oryza glaberrima* cultivar, and in Bieu, an upland landrace. The RVA profiles showed that the peak viscosity varied from 139 RVU for the interspecific variety Nerica 5 to 362 RVU for Jaya, a well adapted in African irrigated variety that originated from Taiwan. Jaya has a unique RVA profile and warrants further genetic investigation.

The *Waxy* microsatellite, *Waxy* exon 6, *Waxy* exon 10, and *Alk* markers were used to characterize the rice samples introduced from West Africa. The results showed that *Waxy* microsatellite marker alleles common to those found in USA cultivars were identified in the West African rices, as well as some rare alleles not found in USA cultivars. The long grain cultivar Nerica 5 was unusual in that it possessed an amylose

content and *Waxy* microsatellite allele like Dixiebelle, a USA firm cooking variety, but had a weak RVA curve similar to L-202, a USA soft cooking type. Jaya is a chalky medium grain cultivar that has the same amylose content and *Waxy* allele as Dixiebelle but a much stronger RVA curve. Another unique case was Bieu, which has a long grain and short cooking time (15.5 minutes) with a *Waxy* allele like Lemont, however it had a high amylose content and a stronger RVA curve like Dixiebelle. The *Alk* alleles showed that majority of African samples had intermediate to high gelatinization temperature. The *Waxy* exon 10 marker alleles distribution showed that most of the introduced samples behaved like Cocodrie, a USA variety with high amylose like Dixiebelle, but relatively lower RVA curve. For the *Waxy* exon 6 marker alleles, the 146H (intermediate amylose) types were found in most of the cases. The second class was 148F that varies from high to low amylose content types. Heterozygous types were found with both Waxy exon 6 and exon 10 markers.

The milling data from rice samples grown at Beaumont showed that Gnanle Gan-Man, a landrace from West Africa, had a high total milled rice recovery (78%) followed by Baldo, Bengal, and Nerica 2 which all had approximately 77%, High whole milled rice recovery of approximately 68% was observed in Cypress, Saber and Nerica 3. Cultivars having high concentration of aromatic compound 2-AP were Sierra (1258.83 ng/g), Bakue Danane (1140 ng/g), Cocote (1102 ng/g) and WAB 638-1 (1075.33 ng/g). Although Jasmine 85 is produced in the US for the aromatic rice market, it and Nerica 1 had much lower levels of 2-AP.

Data from samples introduced from West Africa were compared to the samples produced in Beaumont. Growing environment had a surprisingly minor effect on rice cooking quality parameters like AA, ASV, Hot, Cool, and CT. The variable most affected was the Stbk which is an indicator of the hardness of cooked rice. The data showed that the same cultivars grown in Texas cook softer than when they were grown in West Africa.

It was long believed that apparent amylose content was able to explain most of the differences in the cooking quality of rice. Today it is seen that rice cultivars with the same level of amylose behave differently. This was the case of the cultivars Cocodrie developed in Louisiana and Dixiebelle developed in Texas. Results indicated that soluble and insoluble amylose contents contributed more to the differences between cultivars than apparent amylose. Cocodrie, with more soluble amylose (14.97%), cooks softer than Dixiebelle (13.80% soluble amylose).The *Waxy* microsatellite and the *Waxy* exon 10 SNP markers were found to be highly associated with rice functional quality in a segregating population derived from these two cultivars. These markers could be used in a marker assisted selection breeding program to speed up the process of developing rice cultivars with desirable quality traits in US and West African breeding programs. Evaluation of novel germplasm from West Africa allowed identification of Nerica rice varieties as potential sources of novel germplasm that is adapted to the USA environment. Further studies will allow breeding programs to target specific cultivars with specific genes. African cultivars need to be further explored through collaborative projects to identify other useful sources of grain quality as well as adaptation to diverse

ecosystems (upland, lowland, and irrigated). The *Waxy* microsatellite and *Waxy* exon 10 SNP markers are useful molecular markers for rapid and efficient identification of cooking quality traits that can be difficult to separate with only physical-chemical data. These findings should be a basis for new collaborative research program between WARDA and the USDA-ARS rice breeding program for reciprocal benefits.

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