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THESIS

EFFECT OF SUPPLEMENTAL IRRIGATION ON REDUCING CYANIDE CONTENT OF CASSAVA VARIETY KASETSART 50

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A Thesis Submitted in Partial Fulfillment of
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Cassava's cyanogenic potential, exacerbated during drought period, remains a challenge to optimizing its production and consumption. This research investigated how supplemental irrigation during dry season could reduce the cyanide content in the highly-cyanogenic Kasetsart 50 (KU50).

KU50 stakes were planted on May 2009 at Khao Hin Son Research Station, Inseechandrastitya Institute for Crop Research and Development, Kasetsart University, Chachaengsao province. A split-plot in randomized complete block design was used, with 3 harvest periods (6, 9, 12 months after planting, MAP) as main plots, 3 irrigation treatments (**T0** control, rain-fed no irrigation; **T1,** 30mm/month, 10-10-10 mm split; and **T2** 60mm/month, 20-20-20 mm split) as subplots, and 4 field replicates. Following the major rainy season, monthly irrigation via sprinkler system commenced on November'09. Crops were irrigated every 10^{th}, 20^{th} and 30^{th} of the month. Root samples harvested at 6, 9 and 12 MAP were analyzed for *total cyanide*, *non-glucosidic cyanide* (NGC), and *bound cyanide* content in whole root, peel and parenchyma.

Results showed that roots harvested at 9MAP had the lowest bound and total cyanide content and had the highest starch content. T2 yielded the lowest NGC, bound and total cyanide and the highest starch content. Thus, irrigating with 60mm per month and harvesting at 9 months after planting guarantee the lowest cyanogenic content and the highest starch percentage in cassava roots.

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Student's signature Thesis Advisor's signature

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Johazel Hular Bograd

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Symbol/Abbreviation

CG	cyanogenic glycoside
DAP	days after planting
DM	percent dry matter
Glc	glucose
HCN	hydrogen cyanide; cyanide content
HNL	hydroxynitrile lyase
HP	harvest period
KU50	Kasetsart 50 cassava cultivar
MAP	months after planting
MC	moisture content percentage
NGC	non-glycosidic cyanide
PLNT HT	plant height
ppm	parts per million
PRO	protein content, percentage
ROOT YD	root yield
TOP WT	top weight
T0	Control treatment; no supplemental irrigation
T1	Treatment 1; 30mm/month supplemental irrigation (10mm/application)
T2	Treatment 2; 60mm/month (20-20-20 mm split)
# RT	number of bulking roots
% R STA	starch percentage, measured with Reimann Scale
% L STA	starch percentage, measured in the lab

EFFECT OF SUPPLEMENTAL IRRIGATION ON REDUCING CYANIDE CONTENT OF CASSAVA VARIETY KASETSART 50

INTRODUCTION

Cassava (*Manihot esculenta* Crantz), also known as *tapioca, manioc, mandioca, yucca, kamoteng-kahoy, and kayu* in different parts of the world, is a perennial, starchy, tropical root crop originating in Brazil (Pohl, 1827), and is being cultivated in over 90 countries in Africa, Latin America, the Caribbean, and Asia, providing food and livelihood to over 500 million people in these developing countries (Teles, 2002). As an agronomic crop, cassava's popularity has increased exponentially, with world production tripling in the last 20 years (FAO, 2006), transforming this once "poor man's food" from a food security crop into a "cash crop" with significant "green" or environmental applications.

Cassava, regarded as a multi-purpose plant, is utilized as a raw material in various food and non-food industries. The high-starch content of its roots renders cassava an excellent crop for ethanol fuel production (TTDI, 2007). Cassava roots are processed into chips and pellets for animal feeds, while cassava starch and its derivatives are utilized in alcohol, pharmaceutical, textile, paper, wood, adhesives, leather, and other chemical industries.

Cassava's differentiating values are hardiness, or its ability to recover from damage by insect pests and diseases, and adaptability to adverse agricultural conditions, such as drought, infertile soils, and low-input, low-technology production systems. However, a major obstacle to optimizing the cassava production is its cyanogenic potential, which is exacerbated under drought conditions (Sriroth *et al*, 2001), making it toxic and potentially lethal for animal and human consumption if inadequately processed. Furthermore, such cyanogenic quality of cassava has significant economic implications as it increases production costs and reduces value of the crop as a commodity.

Cyanogenic plants contain c*yanogenic glycosides* (CG), which are chemical compounds naturally-occurring in over 2000 plants (*e.g.* bamboo shoots, lima beans, almonds, etc.) and, on their own, are relatively non-toxic (Sayre, 1998). CG's have been suggested as a defense mechanism for these plants. When the cassava root is ruptured during harvest or maceration, the CG (mainly *linamarin*) comes in contact with and is hydrolyzed by a beta-glucosidase (*linamarase*), yielding a ketone (*acetone cyanohydrin*), which spontaneously or enzymatically decomposes into *free hydrogen cyanide* (HCN), which is toxic to both animals and humans (Bradbury, 2002).

FAO (2009) reports the acute lethal dose of hydrogen cyanide for human is 0.5 to 3.5 mg per kilogram of body weight. For an adult human, consumption of 50-100 mg, or 2mmol of HCN equivalents within 24 hours can be fatal (Bradbury *et al*, 1999). The cyanogenic glycosides present in all cassava have been shown to be higher than the maximum levels recommended for food by the FAO, which is 10mg HCN equivalents per kg dry weight. Therefore, cassava foods must be thoroughly processed to remove cyanogens before consumption. The residual cyanogens, linamarin and acetone cyanohydrins, are the apparent source of CN-toxicity to animals and human (Bradbury *et al*, 1999).

All breeds of cassava contain cyanide at varying levels and varying degrees of potency (Alves, 1998). Cultivars with cyanide content of <100 mg/kg of fresh weight (FW) are regarded as "sweet" variety, while those with 100-500 mg/kg FW are "bitter" variety (Wheatley *et al*, 1993). Generally, sweet cassava can be made safe to eat by peeling and thorough cooking. However, detoxifying bitter cassava roots requires extensive processing, usually requiring several days of peeling, grating, pressing and drying (Chotineeranat *et al*, 2006). Lack of knowledge on cassava cultivars and cyanide content and unfortunate circumstances, such as war and famine, in the impoverished communities make proper detoxification of cassava roots difficult and sometimes impossible. During droughts, cassava-associated CN poisoning is rampant in poor countries that consume this root crop as a staple part of their diet. Teles (2002) enumerated the detrimental effects related to cyanide accumulation in the body as acute cyanide intoxication, tropical ataxic neuropathy (TAN), iodine

deficiency/goiter, Konzo and death. High incidence of Konzo, an irreversible paralysis of the legs which occurs mainly in children and young women (Bradbury *et al*, 2010) have been reported in central and southern Africa during times of drought and famine. In the Philippines, incidents of cyanide-poisoning from cassava have been reported (CNN, 2005). Children are particularly at risk of cyanide poisoning because of their smaller body size and lower capability of auto-detoxification.

With 500 million people depending on cassava for daily nutritional input, this research recognizes the biological and economic imperative to reduce cassava's cyanogenic potential. This experiment attempts to reduce cassava cyanide content at the field with improved agronomic practice, specifically with supplemental irrigation, *i.e.* irrigation water meant to augment the crop water requirement when the rain water is not sufficient.

This research used the most popular cultivar in Thailand, Kasetsart 50 (KU50) (Chotineeranat *et al*, 2006), as its test variety. Covering 57% of total cassava-planted area in the country, KU50 is also being grown extensively in Vietnam, Indonesia and Philippines under the name KM94 (TTDI, 2006). Furthermore, it has one of the highest HCN-contents among the bitter varieties, with 1,427.2 ± 481.6 mg HCN per kg dry weight for fresh cassava roots harvested at 6 MAP (Chotineeranat *et al*, 2006).

OBJECTIVES

Overall Objectives

1. To determine the effect of supplemental irrigation on reducing the cyanide content in roots of cassava cultivar, Kasetsart 50.

Specific Objectives

1. To determine how total cyanide content, non-glycosidic cyanide (NGC), and bound cyanide (linamarin) are individually affected by the application of water treatments (0, 30, 60 mm).

2. To demonstrate the effect of harvest period (6, 9, 12 MAP) on total cyanide content, NGC, and bound cyanide.

3. To assess how cyanide content in whole root and in parenchyma are affected by water treatment and harvest period.

4. To establish correlation between water treatment and yield/productivity, and between harvest period and yield/productivity.

5. To study the effects of water treatments and harvest period on some chemical properties (ash, fat and protein content) of the starch extracted from root samples.

LITERATURE REVIEW

1. Cassava Production and Distribution

Cassava is widely cultivated in the tropics for its edible storage roots. In traditional farming, cassava is an ideal crop for small farmers because it can be used for both human food and animal feed. The Food and Agricultural Organization reports Nigeria, Brazil, Thailand, Congo and Indonesia as the top 5 producers of cassava in the world, with total world production reaching 167-190 million metric tons of fresh roots each year (FAO, 2009). Thailand is the number one exporter of cassava products, with a market share of 90% of the world tapioca trade, producing mainly chips and pellets for animal feeds and other industrial use, while the European Union is the largest importer (CIAT, 2009).

Table 1 summarizes the general usage of cassava as a percentage of total production in the three continents where it is being cultivated. Africa consumes domestically almost 90% of its production while Asia consumes only half and exports about a fourth of its total produced cassava. The Americas utilize domestically all its cassava production for food and for feeds.

Table 1 Uses of cassava by continent, as percentage of total annual production (%)

PRODUCING REGION	Food	Feed	Industry	Export	Waste
Africa	88.7	1.4	0.1	0.1	9.5
Asia	55.3	2.9	8.6	26.9	6.3
Americas	42.4	33.4	9.6	0.1	14.0

Source: FAOSTAT (1997)

1.1 Africa

Almost all cassava grown in Africa is for human consumption, from which 30% is consumed after peeling, cleaning and boiling, while 70% is processed into a wide variety of food products including dry chips, flour, cooked or fermented pastes, roasted or steamed granules, cakes, bread, pudding, and beverages (FAO, 1997).

There are different food products made from cassava (**Table 2**), such as *foofoo* or *fufu* in Ghana, Nigeria and Congo; *kwanga* or *chikwanghe* in Congo; *bobolo* or *miondo* in Cameroon; and the most popular product in West Africa, *gari*, a free-flowing, granular, fermented and gelatinized cassava product (Ugwu and Ay, 1992). Young tender cassava leaves are also consumed as food, usually pounded and boiled for 15 to 30 minutes then seasoned with various ingredients to taste.

Cassava is a security crop in civil war-ridden countries like Uganda, Rwanda, Burundi, Angola, Mozambique, Sierra Leone, Guinea and Liberia, while in Tanzania and Malawi, cassava has been the only source of food during severe droughts (Bokanga, 1994).

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1.2 Americas

Casabe or cassava bread is the main staple in the diet of many people in the Amazon Basin and the Caribbean basin, especially in Guyana, Surinam, and Venezuela, while *Farinha* remains a traditional favorite in rural Brazil (FAO, 1997). And while the demand for fresh cassava remains high in low-income groups, consumption of fresh roots is declining in urban areas, where fermented starch (sour starch or *polvilho azedo*) is becoming increasingly popular in urban fast-food outlets and other food industry.

1.3 Asia

Cassava was first introduced in Asia by European explorers who obtained it from South America (Onwueme, 2002). Since the 1960s, Asian countries, especially Thailand and Indonesia, have grown cassava for processing into value-added export products, offering Asian cassava farmers a more stable source of income (FAO, 1997).

Thailand, Asia's largest cassava producer and world's leading exporter of tapioca products, exports to Europe nearly 90 percent of its 18-22 million tons of annual production (Kanto *et al*, 2000), mainly in the form of chips and starch (Maneepun, 1997).

While Indonesia produces roughly the same amount as Thailand, about 70% of its cassava production goes to domestic human consumption, either in fresh or dry forms, and a smaller percentage is exported (Wargiono *et al*, 1995).

India is the third largest producer of cassava in Asia, with the annual production being the highest yield per hectare among all other Asian producers (Padmaja *et al*, 1992). Production is concentrated in the states of Kerala, where rainfall is sufficient at 1800-2000mm/year, and Tamil Nadu, where annual rainfall is only 900mm and where supplemental irrigation is being practiced. Planting season for rainfed crop is in October or November, while irrigated crop is planted in January to March.

China, the fourth largest cassava producer in Asia, utilizes cassava mainly for industries such as in starch and monosodium glutamate production (Fang, 1992; Shu-Ren, 1996), as well as ethanol, glucose and fructose production (Yinong *et al*, 1995).

The average yield for cassava production in the Philippines is 8.4 tons per hectare and is one of the lowest in Asia, in spite of it being the third source of dietary

carbohydrates, after rice and corn (Den *et al*, 1992). Production is divided almost equally among food, feed and industrial uses, is one of the lowest while only 12 percent of the cassava production in Vietnam is consumed as food, mainly as boiled roots (Onwueme, 2002).

Vietnam processes about 60 percent of its cassava into cassava flour, mainly for the animal feed industry (Dang *et al*, 1996) and about 20% for the starch industry, primarily for domestic food processing and for textiles and paper industry. Only 10-20% is for human consumption (Ngoan *et al*, 1995).

2. Cassava Roots Utilization

2.1 Non-Food Applications

Cassava's high starch content lends itself as a versatile raw material in several industries, earning its reputation as a multi-purpose plant. TTDI (2007) enumerates the diverse uses for cassava starch, chips and pellets in various industries, such as in compound feed industry; in alcohol making; in ethanol/gasohol production; in citric acid production; in food and beverages (e.g. native and modified starches used in production of instant noodles, sago, seasonings, sauces, beverages, monosodium glutamate, artificial sweeteners, glucose and high-fructose syrups); in textile industries (i.e. modified cassava starch increases yarn's tensile strength and silkiness, and reduces fluffiness); in paper industry (i.e. modified starch enhances the density of the paper and increases the smoothness of the paper surface); in glue/adhesives industry; in plywood industry (i.e. glue from starch is used to improve strength and quality of plywood); in pharmaceutical industry (i.e. starches contribute to the solid content of pills and tablets and other pharmaceutical products); and in bio-degradable packaging production (i.e. replaces petroleum-based plastics and foams).

2.2 Food Uses

Among the starchy staples, cassava yields about 40% higher carbohydrates than rice and 25% more than maize, making it the cheapest source of calories for both human nutrition and animal feeding (de Vries *et al*, 1967; Martin, 1970; No.jima and Hirose, 1977; Kawano *et al*, 1978, Cock, 1982; Nyerhovwo, 2004).

More than two-third of the total production of cassava is used as food for humans, with lesser amounts being used for animal feed and industrial purposes (Nwokoro, 2004). Nigeria alone currently produces over 14 million tonnes annually, representing about 25% of subSaharan Africa's output (Ayodeji, 2005).

In Africa, close to 90 percent of cassava produced is used as food, with very little percentage is used for animal feed, and even less for export and industries (CIAT, 2009). There is a multitude of cassava-based food products found all over the world. A general review has been compiled by Lancaster *et al* (1982). Jones (1959) has reviewed the foods made from cassava in Africa. Several authors have reviewed the different uses of cassava foods in individual countries.

Table 2 Popular foods from cassava produced and consumed various countries.

Name of Product	Country	Product Description
Chips	Nigeria Cameroon Benin Togo Ghana	Small pieces of sun-dried cassava sometimes fermented and marketed before being ground into flour. The flour is mixed into paste with hot water to form a thick, sticky mass known as "*fufu*" in West Africa or "*ugali*" in East Africa.
Gari	Nigeria Ghana Togo Benin Cameroon	A dry, fermented and gelatinized coarse meal. It is mixed into a paste with hot or cold water and eaten with soups or stews. Also used as snack when mixed with milk and sugar
Farinha	Brazil	A yellowish coarse meal very similar to *gari*. It is used in many Brazilian dishes, especially in the north-east region.
Attieke	Côte d'Ivoire	Attieke, a fermented, pre-gelatinized meal generally consumed with milk or meat and vegetables, resembles wet "*cuscus*". It swells much less than *gari* and *farinha*.
Cassava bread	Haiti, Dominican Rep. Venezuela	A white, flat, circular, light textured bread baked from moist cassava pulp. Thickness varies from 1 to 5 mm and diameter from 10 to 90 cm. Called *casabe* in Spanish, *cassava* in French and *beiju* in Portuguese
Chicouangue	Congo Zaire, Central African Rep.	A pre-gelatinized cassava paste usually in the form of balls wrapped in leaves. In Congo and Zaire, it is steamed before being sold.
Baton	Cameroon, Congo Zaire, Gabon	Basically a fermented and pounded cassava mash but with wide regional variation. Often shaped as 30-50 cm long and 2 to 4 cm diameter sticks, tied in leaves for cooking and may be eaten alone or with a side dish.
Fufu	Cameroon, Congo Zaire	The name used for the paste made from cassava starch, flour and grated roots.
Cassava Pudding	Philippines	Peeled and grated cassava roots are mixed in with condensed milk, coconut milk and sugar then baked in the oven for about 25 minutes.

Source: Muchnik and Vinck (1984)

3. Agronomic and Botanical Characteristics

3.1 Agronomy and Cropping System

In Asia, cassava is usually grown as a mono crop especially in Thailand, Malaysia and Indonesia where it is being grown in commercial scale. There are places in Thailand, Philippines, Kerala, and Java where it is being intercropped with ground nut, rice, maize and vegetables, or grown under plantations of coconut or rubber.

As opposed to tuberous roots or *tubers*, cassava roots are the main storage organ of the plant and are considered a true root and therefore cannot be used for vegetative propagation (Alves, 1998). Cassava propagation may be carried out sexually, through a true seed, or vegetatively, by stem-cuttings or stakes, although almost all commercial propagations are carried out vegetatively (Leihner, 1984a).

Traditionally, commercial stakes are derived from stems that grow from viable buds of the cassava plant. Depending upon growth habit, climate, and soil conditions, the number of stakes obtainable from a single mother plant in a year ranges from three to 30 (Leihner, 1984b), which is considerably less than the propagation rate of other commercial crops propagated through true seed. In 1976, Cock devised a system that produced 12-14,000 stakes after one year by using small two-node cuttings from which a number of successively growing shoots are obtained, rooted in boiled water and planted in the field. In 1985, Cock successfully produced 100-300,000 commercial stakes from cassava leaves excised with their axillary buds, transferred to a mist chamber for sprouting and formation of root propagules, which are then transferred to a peat pot and 2-3 weeks later, to the field.

For plants grown from stakes, a fibrous root system develops (CIAT, 1984), whereby the basal-cut surface and, occasionally, the buds under the soil, give rise to adventitious roots, from which only about 3-10 fibrous roots start to bulk and become storage roots (Ramanujam, 1990). While such eventual shift in function results in diminished ability of a storage root to absorb water and nutrients

(Ramanujam *et al*, 1987), most of the fibrous roots remain thin and continue to function in water and nutrient absorption (Cock, 1984).

Three distinct tissues comprise a mature cassava storage root: periderm (bark), peel (cortex) and parenchyma (central pith). The parenchyma, which is composed of xylem vessels radially-distributed in a matrix of starch-containing cells (Wheatley and Chuzel, 1993), is the edible portion of cassava (Alves, 2002). The peel layer, comprises sclerenchyma, cortical parenchyma and phloem, constitutes approximately 10-20% of total root fresh weight (Barrios and Bressani, 1967); 0.5% to 3.0% of root weight is the periderm - a thin layer of cells on the outermost part of the root, which usually sloughs off as the storage root's growth progresses (O'Hair, 1990).

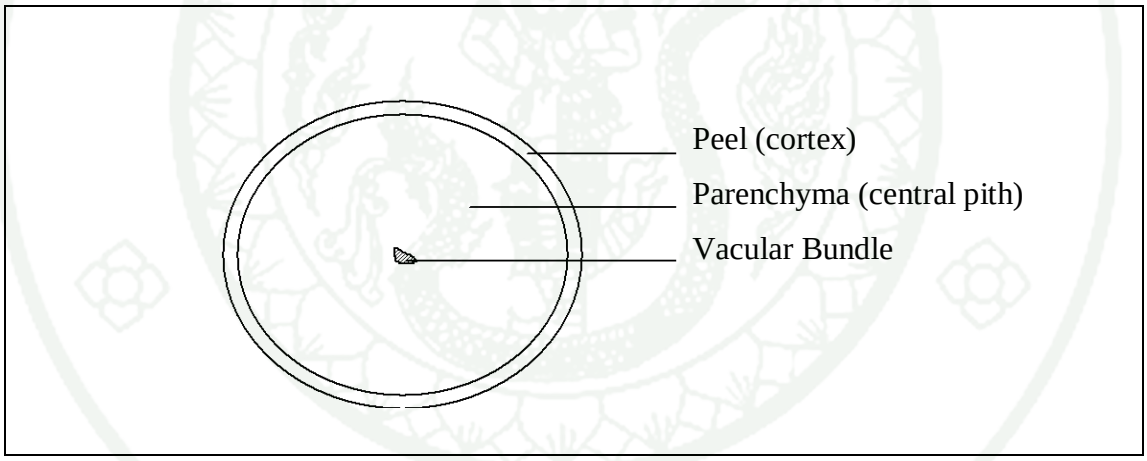

Figure 1 Transversal section of a cassava root.

Source: O'Hair (1990)

Total root fresh weight comprises approximately 85% parenchyma, 10-20% peel, and 0.5-3.0% periderm. The peel is usually not considered suitable for human consumption, but finds use as swine feeds. The central pith or parenchyma is the edible portion; it constitutes the bulk of the root and is primarily a storage parenchyma composed of a multitude of xylem vessels. A thin layer of cambium mainly responsible for the root expansion surrounds the storage parenchyma whose

cells accumulate large starch granules. At the centre of the parenchymal tissue, the primary xylem is organized in a fibrous vascular bundle.

3.2 Plant Growth and Development

Cassava, being a perennial shrub, can grow indefinitely, undergoing distinct developmental phases during its life cycle (Ramanujam, 1990). Such phases include vegetative growth, storage of carbohydrates in the roots, and dormancy periods brought about by severe climatic conditions (Tavora, *et al*, 1995). The occurrence and duration of each phase depend on varietal characteristics, environmental conditions, and cultural practice. These developmental phases are observable in terms of number of days after planting (DAP).

3.2.1 Emergence of sprouting (5-15 DAP)

This involves emergence of adventitious roots from the basal cut surface of the stake, followed by occurrence of sprouting, and concluded by emergence of small leaves (Conceicao, 1979).

3.2.2 Leaf development and root system initiation (15-90 DAP)

The effect of photosynthesis on plant growth becomes apparent as true leaves begin to expand, fibrous roots grow and replace the first adventitious roots, and root differentiation commences, with a few fibrous roots beginning to bulk and transforming into storage roots (Cock *et al*, 1979).

While the rest of the fibrous roots remain thin and continues to function in water and nutrient absorption, at this stage, the storage roots account for 10-15% of total dry matter (Alves, 1998)

3.2.3 Stem and leaves development/ canopy establishment (90-180 DAP)

This is the stage of most active vegetative growth stimulated by the canopy's maximum ability to intercept most of the incident light (Ramanujam, 1985). While the storage root continues to bulk, the canopy size and dry matter (DM) partition to leaves and stems reach their maximum (Howeler and Cadavid, 1983; Ramanujam, 1985; Tavora *et al*, 1995), and the branching habit and plant architecture are clearly manifested (Veltkamp, 1985).

3.2.4 High carbohydrate translocation to roots (180-300 DAP)

Dry matter (DM) accumulation in storage roots reach a maximum during this stage, with the bulking of the storage roots being faster than ever caused by the accelerated photoassimilate partition from leaves to roots (Boerboom, 1978; Tavora *et al*, 1995; Peressin *et al*, 1998). The stem becomes lignified, leaf senescence increases, and leaf fall rate is hastened during this stage (Conceicao, 1979).

3.2.5 Dormancy (300-360 DAP)

At this stage, almost all the leaves will have fallen as leaf production as well as vegetative growth decline (Alves, 1998). Since maximum DM partition to the roots is attained at this stage, only translocation of starch to root is maintained. The cassava 12-month life-cycle is completed. This can be followed by a new vegetative growth period, more DM accumulation in the roots, and possibly dormancy again (Cock, 1976).

3.3 Factors Affecting Cassava Physiology

There are several factors that directly and indirectly affect the physiology and growth of cassava roots, as well as quality and quantity of starch derived from these roots. This research focuses its review on factors that are deemed essential and relevant in the scope of this study.

3.3.1 Soil

Cassava can grow in almost all soil types but it is usually grown in marginal lands, as the most fertile lands are reserved for rice (Onwueme, 2002). The soil is usually an ultisol (Thailand, Indonesia, China and Philippines) and some inceptisols, entisols and alfisols (some parts of Java and southeast Thailand) (Howeler, 1998). Soil for growing cassava is usually characterized as being acidic, with pH ranging from 4.5 to 6.5, and with low organic matter and undulating terrain which increase the possibility for erosion.

3.3.2 Soil Mineral Nutrition and Fertilization

Cassava is known as a "scavenger crop" for its unparalleled ability to extract from the soil more nutrients than most crops, leaving the already marginal, infertile, acidic, and undulating soil it was planted in even poorer than before (Howeler, 2002). Islam *et al* (1980) explains cassava's adaptability to poor or degraded soils where most crops would fail is due to its tolerance to low pH, high levels of exchangeable aluminum (Al) and low concentrations of phosphorus (P) in the soil solution.

Under commercial condition, cassava extracts from the soil the following nutrients: nitrogen 253 kg. ha^{-1}, phosphorus 28 kg. ha^{-1}, potassium 250 kg. ha^{-1}, calcium 42 kg. ha^{-1}, magnesium 29 kg. ha^{-1} (Kobayashi *et al*, 2002). Fertilizer applications should be made only as a supplement to the nutrients already found in the soil at planting time, thus soil sampling and analysis is necessary. Soils containing less than 0.06% of exchangeable K should be supplied with 90-120 kg/Ha of K2O. Excessive N applications will promote foliage growth at the expense of root production. A urea application of 100-150 kg/Ha is recommended at post-planting time if N deficiency symptoms are observed in the foliage.

3.3.3 Temperature

Plant growth, more specifically sprouting, leaf formation, leaf size, storage root formation and storage root size, is significantly influenced by temperature (Alves, 2002). While cassava growth is favorable under annual mean temperatures ranging from 25-29ºC (Caonceicao, 1979), this versatile crop can tolerate conditions from 16 to 38 ºC (Cock, 1984). DM partitioning does not change much when cassava is cultivated under different temperatures, thus the main effect of temperature is on biological production, associating higher temperatures with a greater crop growth rate (CGR) and high photosynthetic rate (Cock and Rosas, 1975). A related experiment by El-Sharkawy, *et al* (1992) verified that photosynthetic rate is directly proportional to temperature, with his 3 sample cultivars reaching their maximum potential photosynthesis at 30-40 ºC.

The results of the work by Keating *et al* (1982) and Githunguri *et al* (2007) showed that crop growth rate (CGR) exhibited a linear response to temperature, thus cassava grown with higher mean temperature and solar radiation had higher CGR. In the study by Githunguri in Kenya, cassava grown in Mokwa, with much higher temperature and solar radiation than Ibadan, had the highest CGR. Conversely, the cassava grown in Minjibar, which received the highest mean solar radiation and mean temperature but lowest rainfall, had the lowest CGR, emphasizing the suggestion that moisture or water significantly influences CGR.

3.3.4 Planting Period

The period of planting does not seem to directly influence plant growth, but it does govern the overall state of the plant (Howeler, 1983). Previous observations have shown that root development is poor when plants are subjected to water-restraining conditions early in their establishment. Santisopasri *et al* (2000) disclosed that crops planted after the rainy period have lower root yield and lower starch content compared with the ones planted during the rainy period. Moreover, the findings revealed that planting period also affected the starch quality, with swelling

power and peak viscosity significantly reduced, and pasting temperature higher in starch extracted from roots subjected to initial water stress, compared with that from of "un-stressed" roots.

While cassava can be planted throughout the year, plant growth only commences when water is available, and plants cultivated after the rainy season rarely grow throughout the dry period (Cock *et al*, 1979). In the study conducted by Pardales and Esquibel (1996), the number of adventitious roots formed when the plants are continuously watered is greater compared with when drought conditions are imposed (64 and 38, respectively).

3.3.5 Water

Irrigation is required when rainfall is insufficient to compensate for the water lost by evapotranspiration. The primary objective of irrigation is to apply water at the right period and in the right amount. For all crops and for each month of the growing season, the irrigation water need is calculated by subtracting the effective rainfall from the crop water need. Moreover, the irrigation water need is the difference between the crop water need and that part of the rainfall which can be used by the plants (effective rainfall).

Climatic parameters that contribute to calculations of irrigation requirements are maximum and minimum temperatures, maximum and minimum air humidity, sunshine hours, solar radiation and rainfall.

In any agricultural country, crop water needs and irrigation needs of the most commonly grown crops will already be known to the farmers. The Department of Agriculture and Agricultural Extension maintains records of such data rendering it unnecessary for individual farmers to determine the crop and irrigation water need for a particular crop at a particular planting season.

Cassava's reputation of hardiness and its ability to thrive in marginal lands has increased its popularity exponentially among small farmers over the last 20 years. Although it is a drought-tolerant crop, water is of paramount importance especially during the critical stages of root initiation and tuberization (Cock *et al*, 1979), which take place 1-5 months after planting (Alves, 1998). Water deficit during at least 2 months of this period can reduce storage root yield from 32 to 60% (Connor *et al*, 1981; Porto *et al*, 1988). Hence, growth and yield are decreased by prolonged dry periods, and percent reduction in storage root yield depends on the duration of the water deficit and by the sensitivity of a particular growth stage to water stress (Santisopasri *et al*, 2000; Sriroth *et al*, 2001).

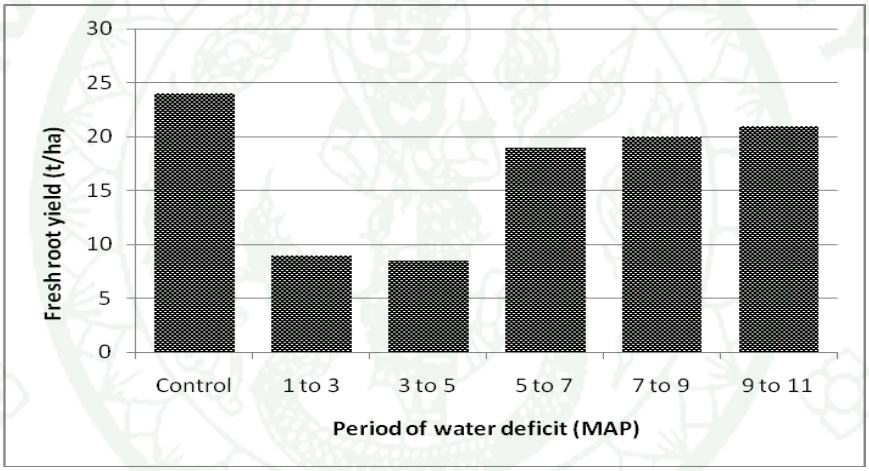

Figure 2 Effect of water deficit during different growth periods on cassava root yield.

Source: Connor *et al* (1981)

Kobayashi *et al* (2002) recommends cassava water requirements to be 1500 mm of water well-distributed throughout the year. The impact of water stress on cassava yield and starch quality was clearly demonstrated in the research by Santisopasri (2000), showing the root yield of samples with initial water stress (IWS) being significantly lower than that of without IWS, and the starch content

significantly lower as well (1.2-3.5% and 20.4 -25.9%, respectively) at 6 months. These results are in concurrence with earlier findings that root development is poor and the growth is retarded for plants subjected to water-limiting conditions early in their establishment (Pardales and Esquibel, 1996). Conversely, depriving plants of water during the later stages does not lead to severe reduction in root starch content seen in plants subjected to initial water stress (Sriroth *et al*, 2001).

Moreover, drought stress reduced size, number of roots and fresh yield of cassava roots (Osiru *et al*, 1995), as well as dry matter (Baker *et al*, 1989). Studies by Githunguri *et al* (2007) showed cassava crop growth rate (CGR) in wetter zones of Kenya (in Mokwa and Ibadan) was almost twice that of those grown in the drier zone (in Minjibar), implying that moisture played a significant role in influencing CGR. Moreover, relative growth rate (RGR) of cassava seedlings also decreased under higher and longer drought stress (Nwosu and Onofeghara, 1992; Githunguri, 2007).

Samutthong (2007) studied how supplemental irrigation during the dry season could increase growth and yield of cassava cultivar Huay Bong 60 in the Chachaengsao province. Using different volumes of irrigation water – 30mm, 45mm, 60mm, and varying the frequency of application – once, twice, or thrice per month, it was concluded that 60mm per month, split thrice (20, 20, 20 mm per application) produced the highest yield and greatest harvest index, significantly higher than the other treatments. As anticipated, control (no supplemental irrigation) produced the lowest yield. Yield differences were not significant for 30 and 45 mm treatments, thus it was recommended that if cassava irrigation is needed and is feasible in the dry period, watering at the rate of 30mm every month, split twice, is sufficient. Moreover, if water availability is not a limiting factor, and if the greatest yield and maximum net income are desired, watering cassava at the rate of 60mm per month, split thrice, was recommended.

Apart from crop yield and productivity, the impact of water stress is evident in the cyanide content in roots, which is the only non-starch components

significantly affected by growth and harvest conditions ($P<0.05$) (Santisopasri *et al*, 2000; Sriroth, *et al*, 2001; Chotineeranat, 2006). Cyanide content in water-stressed plants was higher compared with plants provided with ample water. HCN content for peeled roots subjected to IWS and harvested at 6 and 8 MAP were 96.6-253.8 and 70.3-119.1 µg/g, respectively, while HCN-content for peeled roots without water stress harvested at 10 and 12 MAP were 99.7-284.9 and 7.5-34.3 µg/g (Chotineeranat, 2006). These findings conform to the report by CIAT (1990) that HCN-content is concentrated at the roots during drought conditions and that water deficit may retard the transport mechanism of essential minerals for plant growth.

Furthermore, previous studies have shown that in years of low rainfall or drought, total cyanide content in roots increase significantly compared with normal year (Bokanga *et al*., 1994; Ernesto *et al*, 2002; Cardoso *et al*, 1999); flour samples belonging to the safe level (<40ppm) declined from 51-67% to 16-26% on an average year, and cases of acute intoxication in communities where flour samples were collected increases (Cardoso, 2005).

A previous study by Howeler (1985) showed a correlation between potassium application and cyanide content in cassava, thus recommending application of potassium on the field to lower the HCN-content of the roots.

3.3.6 Harvest Date and Plant Age

Cassava roots can be harvested at any time of the year, with some farmers harvesting as early as 6MAP, or as late as 18 to 24MAP. The food quality of roots, particularly the starch content, increases with time up to an optimal period of 12 to 15MAP, after which there is a loss of quality, mainly due to increased lignifications (Asaoka *et al*, 1992). However, during the dry season, the mealy texture of boiled cassava root is often lost, and roots can no longer be used for this purpose. Furthermore, Relative Growth Rate (RGR) decreases with age, implying that sink capacity influenced RGR, as most sinks like cassava roots and leaves have an optimum capacity (Githunguri, 2007; Akparobi *et al*, 1998).

Many studies confirm that environmental conditions during early plant development and immediately before root harvest significantly impact cassava production efficiency, as manifested in root yield and in the quality of starch extracted from these roots (Bokanga *et al*, 1994; Cardoso *et al*, 1999; Santisopasri *et al*, 2000; Sriroth *et al*, 2001; Ernesto *et al*, 2002; Chotineeranat, 2006). More specifically, the organoleptic properties of boiled or processed roots, and the physico-chemical properties of extracted starch are affected by plant age at harvest and the environment conditions during growth (Moorthy and Ramanujam, 1986; Asaoka *et al*, 1992; Chotineeranat, 2006).

Moreover, the extent and severity of the environmental impacts, such as water-limiting conditions, on cassava depends on the plant growth stage (Santisopasri *et al*, 2000), as mature plants respond differently to water stress from younger plants. The effect of drought conditions to mature plants is manifested in reduction of starch content; as opposed to young plants which ultimately failed develop.

The effect of root age is also clearly manifested in cyanogens content of cassava roots, which typically have higher HCN in younger roots than in older ones. Roots that are 6 months old contained very high amounts of cyanogenic compounds (1,427.2 ± 481.6 mg HCN per kg dry weight for fresh KU50 cassava roots; Chotineeranat et al, 2006). Roots with different ages (*i.e.* 6, 8, 10, 12 months old) exhibited different chemical compositions and cyanide contents, which consequently produced flours containing different levels of cyanide content. Fresh roots with high HCN produced flour with high HCN.

4. Cyanide and Cyanogenesis

4.1 Cyanide Definition

Cyanide is any chemical compound that contains the cyano group (C≡N) (IUPAC, 1987), which consists of a carbon atom triple-bonded to a nitrogen atom

(Greenwood and Earnshaw, 1997). While many cyanide-containing compounds are highly toxic, some are innocuous. Nitriles, which do not release cyanide ions, and hexacyanoferrates - ferrocyanide and ferricyanide, where the cyanide is already tightly bound to an iron ion, have low toxicities, while most other cyanides are fatally poisonous (Miessler and Tarr, 2004). The most dangerous cyanides are hydrogen cyanide (HCN) and salts derived from it, such as potassium cyanide (KCN) and sodium cyanide (NaCN) (Senning, 2006).

4.2 Cyanogenesis in Plants

Cyanogenesis, the ability of plants to produce the toxic hydrogen cyanide (HCN) (Vetter, 2000), exists in over 2000 plant species belonging to more than 100 families (Jones, 1998). Examples of such cyanogenic plants are lima beans, bamboo shoots, almonds, seeds and stones of apples, mangos and peach (Bradbury *et al*, 1991; Bourdoux *et al*, 1982).

Cyanogenic plants neither produce nor store HCN at any stage of plant growth, but rather produces complex compounds, mainly cyanogenic glucosides, but in some case lipids, which may break down to produce HCN (Anon, 2004). Plants also produce the enzymes that break down the cyanogenic compounds but they are both always stored separately inside plant cells. It is only when the plant is damaged, and the structural integrity of the plant cells is destroyed that the enzyme acts on the cyanogenic compounds to produce cyanide.

4.3 Cyanogenesis in Cassava

Although cassava is the third most important food source in the tropical world after rice and maize, and provides calories for over 160 million people of Africa (Polsen and Spenser, 1991), its food value is greatly compromised by the endogenous presence of cyanogenic glucosides. However, processing methods such drying and ensiling have been found to be effective ways of reducing its toxicity in cassava products.

All breeds and all organs, except seeds, of cassava contain cyanogenic glucoside (Alves, 1998). This association with cyanide is the single most important constraint in the expansion of cassava utilization.

Numerous research and experiments deal with the negative impact of cassava cyanogenesis and with ways of addressing this issue. However, a number of studies also discuss the benefits and, to some extent opportunities, of cyanide-content in cassava. It has been suggested that high cyanogens in cassava varieties may be related to higher productivity (Bellotti and Riis, 1994; Dufour, 1994), and better resistance to pests and diseases, as cyanogenic glucosides like linamarin are the plant's defense mechanism (Bradbury, 1999). Highly cyanogenic varieties generally provide higher starch yield compared with sweet varieties, and if processed properly could yield low-cyanide content flour (Chotineeranat, 2006). Moreover, bitter varieties may also be the preferred material for preparation of certain food stuff.

4.3.1 Enzyme and Substrate Compartmentalization

Cassava produces two cyanogenic glucosides, linamarin and lotaustralin, in about 10 to 1 ratio (McMahon, et al, 1995). In cassava plant cells, linamarin, which is synthesized in the leaves and transported to the roots (Wheatley and Chuzel, 1993), are stored inside the vacuoles in the cytoplasm, while the linamarase enzyme is located in the cell wall outside the cytoplasm (Mpkong *et al*, 1991). Santana (2002) reported the absence of linamarase messenger ribonucleic acid (mRNA) in the roots of 2 cassava cultivars, which may suggest that root linamarase is not synthesized in roots but is transported to the roots through the laticifer system.

Hence, the compartmentalization of linamarin in the vacuole, and linamarase and hydroxynitrile lyase (HNL) in separate sites prevents the formation of toxic CN in undamaged cells (Bradbury, 1999). Oke (1994) also reports that linked glucosides are not toxic to the plants which contain them. It is only when cassava tissues are bruised and the cellular structures are disrupted that the cyanogenic

glucosides come in contact with linamarase, and cyanogenesis ensues (de Bruijn, 1983; Bradbury and Holloway, 1988).

4.3.2 Cyanogenesis Mechanism

Cassava roots and leaves contain cyanides in two different forms: 1) cyanogenic glucosides (CG), such as linamarin and lotaustraline, which are also referred to as "bound" cyanide, and 2) non-glycosidic cyanide (NGC); *acetone cyanohydrin* and *hydrogen cyanide* (HCN), also termed as "free" cyanide. Free cyanide comprises 8%-12% of the total root cyanide (Cereda and Matos, 1996).As illustrated in Fig. 3, when the cassava root is ruptured during harvest or maceration, the CG (linamarin) comes in contact with and is hydrolyzed by a beta-glucosidase (linamarase), yielding an unstable cyanohydrins intermediary ketone (acetone cyanohydrins (Wheatley and Chuzel, 1993), which spontaneously (at temperatures > 35 C or pH > 5) or enzymatically (catalyzed by hydroxynitrile lyase, HNL) decomposes into free hydrogen cyanide, which is a volatile poison (Yeoh, 1973; Cooke and Coursey, 1981).

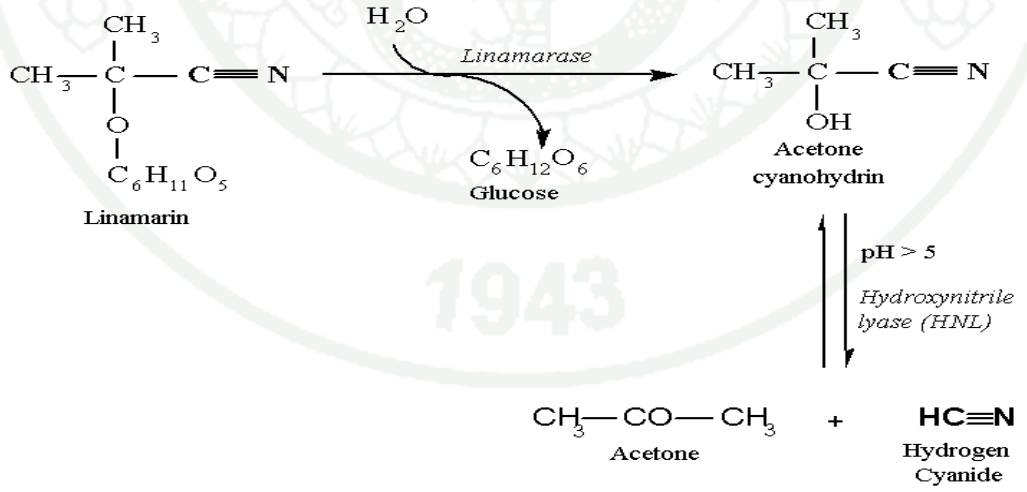

Figure 3 Enzymatic hydrolysis of linamarin

Source: Yeoh (1973)

4.3.3 Types of Cyanide

1) Bound Cyanide or Cyanogenic Glycosides (CG)

On their own, cyanogenic glucosides are relatively non-toxic and an unlikely source of cyanide exposure in humans (Mlingi, 1992; McMahon, *et al*, 1995). Nonetheless, it has been shown that some linamarin may be absorbed by the body and may break down to yield hydrogen cyanide if suitable glucosidases are provided by the microflora present in the gut (Rosling, 1994).

The most abundant CG in cassava is *linamarin* (85%), with lesser amounts of *lotaustralin*. Total CG depends on cultivar, environmental condition, cultural practices and plant age (McMahon *et al*, 1995; Sriroth *et al*, 2001; Chotineeranat *et al*, 2006). Linamarin, which accounts for more than 80% of the cassava cyanogenic glucosides, is a ß-glucoside of acetone cyanohydrin and ethyl-methyl-ketone-cyanohydrin (White *et al*, 1998). Linamarin ß-linkage can only be broken under high pressure, high temperature and use of mineral acids, while its enzymatic break occurs spontaneously in the presence of its (endogenous) hydrolyzing enzyme linamarase (McMahon *et al*, 1995). Linamarin is present in all parts of the cassava plant, being more concentrated on the roots and leaves. The structure of linamarin is shown in **Fig. 3**.

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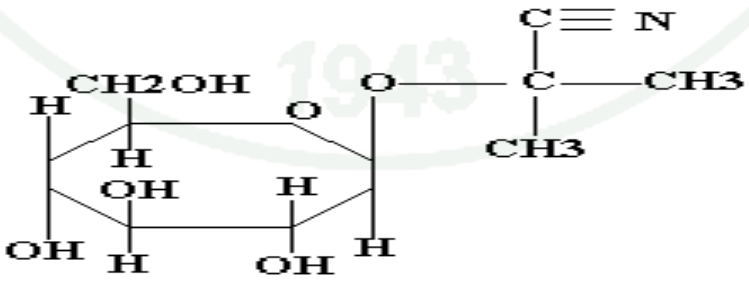

Figure 4 Linamarin structure.

Source: McMahon *et al* (1995)

2) Non-Cyanogenic Glucosides (NGC)

The term "free cyanide" is used by some authors to refer to hydrocyanic acid, and by others to the sum of hydrocyanic acid and cyanohydrins (Oke, 1983). While some authors use the term "bound cyanide" to refer to cyanogenic glucosides, others may use it to refer to hydrocyanic acid bound to albumin and other blood proteins as part of *in vivo* cyanide detoxification processes (White and Sayre, 1995). The term cyanogen refers to any of these three compounds.

Once HCN is produced, it will dissipate in the air, as its boiling temperature is 25.7^{o}C. In damaged plant tissues, including processed roots and leaves, it is possible to find non-hydrolysed cyanogenic glucosides, cyanohydrins and traces of HCN. Hydrolysis of linamarin yields an unstable hydroxynitrile intermediate, acetone cyanohydrins (plus glucose) which spontaneously decomposes to acetone and HCN at pH >5.0 or temperatures >35°C, and can be broken down enzymatically by HNL (Cutler and Conn, 1981; Yemm and Poulton, 1986; Wajant and Mundry, 1993; Wajant *et al*, 1994; White *et al*, 1994; White and Sayre, 1995; Zheng and Poulton, 1995; Hasslacher *et al*, 1996; Wajant and Pfizenmaier, 1996).

3) Total Cyanogen Content

Total cyanide content represents the maximum amount of HCN that could be obtained from a sample (Cooke *et al*, 1978). It therefore represents the cyanogenic potential (sometimes abbreviated as CNP) of the sample and is usually expressed as mg HCN-equivalent per 100 g, or per kg of sample, taking care to specify whether the value is expressed on fresh or dry weight basis (Chotineeranat *et al*, 2006). In total cyanide determination, Essers' method is commonly used for its simplicity, safety and low cost of reagents used (Essers *et al*, 1993). However, some modern laboratories, such as the Cassava Starch Technology Research Unit (CSTRU), have somewhat adapted and modified this method for ease of handling samples.

De Bruijn (1971) analyzed 67 cassava varieties, the cyanogenic potential varied from 31 to 630 mg/kg in the root (fresh weight) and from 540 to 1450 mg/kg in the leaves (fresh weight). Similar ranges of cyanogenic potential were found in larger collections of varieties at the International Institute of Tropical Agriculture (IITA) in Nigeria (851 genotypes) and at the Centro Internacional de Agricultura Tropical (CIAT) in Colombia (560 genotypes) (Bokanga, 1994). From these studies, no correlation was found between the total cyanogenic potentials of roots and leaves. (Bokanga, 1994; Cooke *et al*, 1978). An investigation by Yeoh and Oh (1979) also showed no significant relationship between the amount of cyanide in the leaf and pulp, leaf and peel, and pulp and peel.

Moreover, previous studies using a diverse range of cassava varieties showed no correlation between total cyanide content and the amount of linamarase in root tissues (Bradbury and Egan, 1991; Nambisan and Sundaresan, 1994). Linamarase activity in storage roots and its relationship to total cyanogens content have been investigated using diverse range of cassava varieties (Bradbury and Egan, 1991; Nambisan and Sundaresan, 1994). Iglesias et al (2002) reaffirmed previous studies which showed lack of correlation between cyanogens content and linamarase activity in the storage roots (Bradbury and Egan, 1991; Nambisan and Sundaresan, 1994), and also no correlation between cassava leaf cyanogen content and its linamarase activity (Mkpong *et al*, 1990). Iglesias (2002) noted that many clones exhibited low cyanogens and relatively high enzyme activity, which is potentially useful for human consumption and animal feeds.

4.3.4 Distribution of Cyanide in the Root

Cyanide levels depend on the particular cultivar, growing conditions, (*i.e*. soil type, humidity, temperature) and the age of the plant. Cyanogenic glucosides are not uniformly distributed in the various tissues of cassava plants (De Bruijn, 1971).

The highest proportion of HCN is found in the peels and the cortex layer immediately beneath the peels (Hahn, 1984; Onwueme, 1978). It is for this reason the cassava root is always peeled before being processed or consumed. Peeling removes the cortex and the outer periderm layer adhering to it. The lowest concentration is in the central pith or parenchyma, ranging anywhere between 1- 1550 ppm (Alves, 1998).

Younger tissues contain more total cyanide than older ones (Chotineeranat *et al*, 2006). In the root, the section closest to the stem (proximal) contains more total cyanide than the middle and distal sections; there is a shallow longitudinal gradient from the proximal to the distal end (Wheatley *et al*, 1993). From the peel side of the central pith to the centre of the root, the cyanogenic glucosides gradient is more pronounced; the concentration of cyanogenic glucosides is greatest in the outermost 2-3 mm layer and drops sharply towards the centre (Kojima *et al*, 1983).

4.3.5 Bitter versus Sweet Variety

Currently, there does not exist an acyanogenic breed of cassava, although unconfirmed reports of its existence surfaced prior to 1940 (de Bruijn, 1983; Bradbury and Holloway, 1988), but roots containing 1-2 ppm have been identified (Bradbury *et al*, 1991; Bourdoux *et al*, 1982). At the other extreme are reports of total cyanide levels of 1090 and 1550 ppm in Tanzania (Mlingi and Bainbridge, 1994), mean total cyanogen of 1100 ppm in India (Nambisan, 1994), and 454 ppm in the Amazon (Dufour, 1994).

The organoleptic descriptors 'sweet' and 'bitter' are often used to characterize cassava varieties. Although earlier reports have associated bitter/sweet varieties with high/low levels of cyanogenic glucosides (Bolhuis, 1954), a cause-effect relationship has not been established (Coursey and Haynes, 1970; Coursey, 1973; Pereira *et al*, 1981). While a bitter compound, to which the bitterness is attributable, other than the cyanogenic glucosides, has been isolated and identified

(King and Bradbury, 1996), the association of bitterness to toxicity still endures among cassava farmers, particularly in Africa (Chiwona-Karltun, 2006).

Cassava plants are arbitrarily classified into low- and high-cyanide varieties depending on the cyanogen content of their roots: low-cyanide/"sweet" varieties have roots containing less than 100mg HCN-equivalent per kg (fresh weight), and the roots of high-cyanide/"bitter" varieties contain > 100mg HCN-equivalent per kg fresh weight (Hahn and Keyser, 1985; Wheatley *et al*, 1993)

This classification is not unrelated to the toxicity classification proposed by Bolhuis (1954) in which cassava roots containing up to 50 mg HCN-equivalent per kg are considered innocuous, 50 to 100 mg HCN-equivalent per kg are considered moderately poisonous, and above 100 mg HCN-equivalent are considered dangerously poisonous.

In so-called sweet cassava, the parenchyma contains only a small amount of cyanogens, so that after peeling, these roots can be safely boiled and eaten (Bradbury and Holloway, 1988). The bitter taste of bitter cassava is very largely due to linamarin (King and Bradbury, 1995). High cyanide parenchyma roots must be processed before consumption to reduce the amount of toxic cyanogens to a safe level. The World Health Organization (WHO) has set the safe level of cyanogens in cassava flour at 10ppm (FAO/WHO, 1991), while the acceptable limit in Indonesia is 40 ppm (Damardjati *et al*, 1993; Djazuli and Bradbury, 1999).

Wide fluctuations in cyanide content in the different cultivars could be attributed to their genetic make-up although environmental conditions, such as mineral nutrition, fertilizer applications, shading of plants, and water stress could also affect the level of cyanide in cassava (De Bruijn, 1973; Hughes, 1973).

4.3.6. Implications of Cyanogenesis on Society

Cassava cyanogenesis has major implications in the economics of production and, more importantly, in the safety issues of consumption. The toxicity of hydrogen cyanide to humans, typically expressed as the concentration or dose that is lethal to 50% of the exposed population (LC50 or LD50; FAO, 2006), is dependent on the nature of the exposure. The LC50 for gaseous hydrogen cyanide is 100-300 parts per million, thus inhalation of cyanide in this range results in death within 10-60 minutes, with death coming more quickly as the concentration increases such that inhalation of 2,000 ppm HCN causes death within one minute (White and Sayre, 1995). The LD50 for ingestion is 50-200 milligrams, or 1-3 milligrams HCN per kilogram of body weight (Zheng and Poulton, 1995; Hasslacher *et al*, 1996). For contact with unabraded skin, the LD50 is 100 milligrams HCN per kilogram of body weight (Wajant and Pfizenmaier, 1996).

The long term ingestion of large quantities of poorly processed cassava has also been shown to cause severe disorders in several areas of Africa (Osuntokun, 1972; Ermans *et al.*, 1983; Mozambique Ministry of Health, 1984; Cliff *et al.*, 1985; Howlett *et al*, 1990), while exposure to lower levels of CN can also cause a variety of symptoms, such as vomiting, nausea, palpitations, headaches, and impaired vision (Bokanga *et al*, 1994).

1) Cassava toxicity and consumption safety

For millions of consumers, well-processed cassava is a staple food with no associated negative effects and reports on the toxic effects of cassava are relatively rare in comparison with its wide use as a staple. It should be emphasized that populations growing bitter cassava usually know how to process cassava into safe products (Dufour, 1994), and cases of toxicity and fatality are rare and tends to be associated with agroecological disasters such as severe droughts (Howlett *et al*, 1990), with civil strife (Cliff, 1994) and with economic disturbances (Banea *et al*, 1992).

However, while the hydrogen cyanide is readily removed during processing of cassava, the presence of residual linamarin and its breakdown product (acetone cyanohydrin) in cassava-based food products has been a cause for concern because of their possible effects on health (Auriga and Koj, 1975). Significantly, high and continuous consumption of cassava has been associated with various diseases and nutritional disorders such as tropical ataxic neuropathy in Nigeria (Osuntokun, 1972), goiter and cretinism in Zaire (Ermans *et al*, 1983), and spastic paraparesis or konzo in Mozambique, Tanzania, and Zambia (Mozambique Ministry of Health, 1984; Cliff *et al*, 1985; Howlett *et al*, 1990). Bokanga has published a comprehensive review on the CN-associated chronic disorders (Bokanga *et al*, 1994).

And while the toxicity of cyanogenic glucosides has been thoroughly investigated by Bokanga *et al*, (1994), Maduagwu (1989), Oke (1983), Bourdoux *et al* (1982) and Barrett *et al* (1977), the relative toxicity of cyanohydrins has not been extensively investigated, except for Fomunyam *et al* (1985), who have speculated on the nature of cyanohydrins absorption and subsequent degradation in the digestive system. Moreover, it has been shown in humans that a substantial part of the ingested linamarin will be absorbed and excreted intact in the urine (Brimer and Rosling 1993). Its toxic role remains speculative but one is certain that the cyanide liberated from linamarin is the primary cause of toxicity in cassava.

It is believed that in humans, linamarin can be broken down by linamarase found in the bacteria that reside in the intestinal track resulting in release of hydrogen cyanide. Fortunately, humans can readily neutralize about 10 mg of cyanide by a reversible reaction with methemoglobin fraction in the red blood cells (Lundguist *et al* 1985). Rodanese can further convert majority of the cyanide to less toxic thiocyanate, which is then excreted in the urine (Oke, 1983; Delange *et al*, 1994).

2) Implications of Cassava Production Systems on the Environment

Cassava processing can have negative effects on the environment in the form of unpleasant odors and solid waste created by all forms of cassava processing, the quality and quantity of which vary a lot depending on factors such as plant age, time after harvesting and type and sophistication of industrial equipment (Howeler, 1991). Solid wastes can come in the form of peelings, fibrous by-products from pulping, and starch residues. The long-term and broad-based impact on the environment is generally minimal and corrigible with proper waste treatment and with other relevant production technologies.

HCN released from cassava roots during processing will be present in cassava peel, in press water in *gari* and *farinha* production, in waste water, and in vapor and water sprays when using high-speed graters (Howeler, 1999).

Starch extraction from cassava roots requires large amounts of water, with the residual containing small amounts of starch, proteins and hydrocyanic acid, which when released into bodies of water could cause detrimental effects (Sobrinho, 1975). In particular, the residual starch can cause rapid growth of bacteria, resulting in a depletion of oxygen and harmful effects on aquatic life, while the dissolved hydrocyanic acid has been reported to cause fish kill (Cooke and Maduagwu, 1978). Such environmental problems stem from inefficient production methods and lack of anti-pollution standards by small-scale starch factories.

4.3.7 Addressing Cyanogenesis in Cassava

Agronomic research has been geared towards breeding new cassava varieties of low cyanogen content, that produce zero-level cyanogen cassava products, without sacrificing productivity (Yeoh and Bradbury, 1996). While scientific efforts are underway, breeding for low cyanogens cassava variety is labor-intensive, time-consuming and success is not a guarantee (Iglesias *et al*, 2002).

Meanwhile, understanding the biology and mechanism of cyanogenesis is the key to determine the suitable processing method for a particular cassava variety. Furthermore, a critical means of promoting safe cassava food consumption is providing relevant information, such as the level of residual cyanogens in the food products being sold in the market.

1) Suitable Processing

Peeling results in a great reduction the cyanogenic potential of the raw material as the peel represents about 15 percent of the root weight, and its cyanogen content is usually 5 to 10 times greater than parenchyma. However, the peel also contains large amounts of the enzyme linamarase which is important in the detoxification of cassava during processing. For instance, grinding cassava roots without removing the peel, as is done in the manufacture of the Brazilian farinha, ensures an almost total elimination of cyanogens from cassava (Lancaster *et al*, 1982).

The initial step of several processes for the preparation of foods from cassava is pulping, either by grating or by crushing freshly harvested cassava roots. In general, methods which use grating and crushing are very effective in reducing cyanide content because the mashed wet parenchyma disintegrates the cassava tissues, ensuring intimate contact between linamarin and linamarase, and promoting rapid breakdown of cyanogenic glucoside to hydrogen cyanide gas, which volatilizes into the air (Cardoso, 2005).

Typically, the next processing step is pressing the grated pulp to reduce its moisture content. Pressing methods come in different levels – from the manual (like the *´tipiti'* used by the Amerindians for two millennia, and heavy stone placed on top of bags filled with cassava mash used in Africa) to the industrialized hydraulic presses used in Brazil, providing pressures of up to 25 kg/cm^2 (Jesus *et al*, 1986). The moisture content of the mash is reduced from 60-70 percent to about 50 percent (Nartney, 1981). The cake obtained after pressing needs to be broken down into granules. This can be done manually or mechanically by passing it again in a

grating machine. The powdery granules obtained can then be further processed into the desired products.

Reducing cassava moisture to a point where all physiological reactions and microbial growth are inhibited (at 14 percent moisture content, corresponding to 0.70 water activity) can tremendously increase the short shelf-life of cassava roots (Jensen, 1979). The removal of moisture from cassava roots can be accomplished either by sun drying or in an oven, usually reducing moisture content to 8-12 percent (Williams, 1979). Shorter drying time actually ensures higher quality chips, in terms of starch content and whiteness of the chip, but the cyanogenic potential of cassava decreases with longer drying time (Cereda *et al*, 1996).

However, sun drying, as a stand-alone process, or heap fermentation do not encompass intimate enzyme-substrate contact because the peeled roots are usually cut longitudinally in half with most plant cells remaining intact, and the linamarin and linamarase still stored separately (Mkpong *et al*, 1990). Linamarin retained after sun drying was still 25-33% (Cooke, 1983). Furthermore, boiling cassava parenchyma results in minimal enzymatic breakdown of linamarin due to heat denaturation of linamarase at 100C (Nambisan and Sundaresan, 1985). Cooke (1983) found that after 25 minutes of boiling fresh cassava chips in water, 45% of linamarin was retained. Losses of cyanide by steaming, baking or frying are much smaller than on boiling because of the stability of linamarin in neutral or weak acid conditions to temperatures of 100^{o} C (Bradbury *et al*, 1991). Hence, all these methods of cooking cassava parenchyma are only suitable for sweet cassava.

2) Having Suitable Analytical Methods

Today, many procedures for the determination of linamarin in cassava are available. Many of them are based on the hydrolysis of the linamarin by linamarase, followed by spectrophotometric determination of the cyanide liberated (Cooke, 1978; Bradbury *et al* 1991, Essers *et al*, 1993; Brimer, 1994, Yeoh and Tan

1994a, 1994b). Cooke (1978) showed that enzyme-based assay provided a more accurate estimation of linamarin content in cassava.

More sophisticated methods such as linamarin sensors based on potentiometric determination of cyanide (Yeoh, 1992; Yeoh and Truong, 1993) as well as amperometric sensors based on inhibitory effect of cyanide on redox enzymes (Tatsuma *et al*, 1996a, 1996b), which are not only rapid but could also detect small amounts of linamarin, have been developed. . This is an attempt at creating a fast-response biosensor that can estimate the cyanogenic potential directly from root tissues, and has been used successfully to determine the cyanogenic potential of cassava varieties grown in the Philippines.

However, developing simple, rapid and inexpensive methods of analysis will be relevant to the food industries and research organizations dealing with cassava (Yeoh, 1992; Yeoh and Truong, 1993; Yeoh, 1993; Yeoh and Tan, 1994a, 1994b; Tatsuma et al 1996a, 1996b; Yeoh and Egan 1997; Egan *et al*, 1998; Tatsuma *et al*, 2000), such as the enzyme-based dipstick (Yeoh and Egan 1997; Egan *et al*, 1998; Yeoh *et al*, 1998).

Bradbury (1999) developed a simple picrate method that adequately measures total cyanogens content, as well as the amounts of the various cyanogens present (linamarin, acetone cyanohydrins and cyanide), and that may be used by non-chemists in developing countries to monitor their own cassava roots and products. This picrate method produced comparable results to the intrinsically more accurate acid hydrolysis method (Bradbury and Egan, 1994; Bradbury *et al*, 1991), which is tedious and expensive to use, and necessitates equipments that are not readily available in most developing countries. This picrate method was tested in studies in Mozambique in 1996 (Cardoso *et al*, 1998) and in 1997 (Ernesto *et al*, 1999), and in Indonesia (Dzajuli and Bradbury, 1999) and has yielded comparable results to the more sophisticated acid hydrolysis method. Nambisan (1999) has also developed a method based on the use of cassava latex. Crude enzyme preparation from latex from leaves and petioles must be put in deep freeze at -20^{o}C.

MATERIALS AND METHODS

Materials

1. Planting Material

Kasetsart 50 (KU50) was used as the test cultivar for this experiment because of its economic significance and popularity, covering about 57% of total cassava-planted area in the kingdom (Bradbury, 2008), and for its high cyanide content, with total cyanide content ranging at 1,427.2 ± 481.6 mg HCN per kg dry weight, for fresh cassava roots harvested at 6 MAP (Chotineeranat *et al*, 2006).

KU50, developed in 1992 by the Agronomy Department of Kasetsart University, is a cross between Rayong 1 hybrid seeds and Rayong 90 (Rojanaridpiched *et al*, 1992). KU50 is now being grown extensively in Vietnam, Indonesia and Philippines under the name KM94 for its high yield, high dry matter content, and adaptability to unfavorable conditions.

2. Equipments

2.1 Soil sampling and soil analysis materials;

2.2 Equipments for soil preparation, experimentation lay-out, basal fertilizer application treatment, cassava cultivation and cultural practice;

2.3 Data collection, harvesting and yield data collection materials;

2.4 Cassava root starch analysis, proximate, cyanide extraction and cyanide-content determination materials and implements;

3. Chemicals

3.1 On the Field

3.1.1 Paraquat was applied at the rate of 450cc/rai at 1 MAP, before application of fertilizer.

3.1.2 NPK fertilizer 15-7-18 was applied at the recommended rate of 50 kg per rai for the experimental plot with area of 2,880 sq meters (12 x 10 x 24 m).

3.1.3 Glyphosate (at 250cc/rai) was initially sprayed on September 2009, and then repeated only when necessary.

3.2 In the Laboratory

While the colorimetric assay, first devised by Epstein (1947), and subsequently modified by Joegensen (1955), is a sensitive and reliable method for inorganic cyanide assay, it tended to not achieve complete hydrolysis of cyanogenic glucosides, and therefore, under-estimate.

The enzymic method for cassava cyanide determination developed by Cooke (1978) represents a major progression in cyanide assay methodology, differentiating between *total cyanogens* (glucosidic cyanide, cyanohydrins and free HCN) and *non-glycosidic cyanogen* (cyanohydrins and free HCN), and is more accurate and reproducible than previous techniques in terms of the colorimetric procedure used, as well as in terms of sample preparation.

Presently, it is possible to distinctively quantify hydrocyanic acid, cyanohydrins and cyanogenic glucosides due to modifications of the Cooke's method (O'Brien *et al.*, 1990; Essers *et al.*, 1993).

3.2.1 Cyanide assay

Solutions were prepared every 3 days and frozen until required. Colorimetric reagents were prepared just before using them.

1) Acid Extraction Medium: 0.1M orthophosphoric acid in water

a) Ethanol/acid extraction medium: 0.1M orthophosphoric acid containing 25% volume ethanol

b) Buffer A: pH 4.0, 6.0 and 7.0 prepared from 0.1 M H3PO4 and 0.1 M Na3PO4

c) Buffer B (Cooke, 1979): pH 6.0 and 7.0 prepared from 0.1M H3PO4, adjusted with 5M NaOH

d) Linamarase preparation: enzyme was dissolved in buffer A (pH 6.0) to give an activity of about 5 EU/ml.

2) Colorimetric Procedure

a) Chloramine T reagent (General Purpose Reagent grade)

b) Bispyrazolone (GPR grade), 3-methyl-1-phenyl-5-pyrazolone (GPR grade)

c) Linamarase enzyme

d) Assay Procedure: Buffer solutions pH 4.0, pH6.0, pH 7.0, 0.2M NaOH.

Methods

1. Field Experiment

Field experiments were conducted at the Khao Hin Sorn Research Station, Inseechandrastittaya Institute for Crop Research and Development, Kasetsart University, in Chachaengsao province. It lies on 13°44'37" north of the equator and 101° 30'36" east of the Greenwich meridian (Wikimapia, accessed 2011). This location places it in the tropical humid climate. The experiment was conducted between May 2009 and May 2010.

1.1 Cultural Practices

Weeding by hand-pulling and application of Paraquat (450 cc/rai) were carried out 1 month prior to planting of stakes. The KU 50 stakes were vertically planted in May 2009, using 1 X 1 meter spacing, as per the cultural practice in Thailand for cassava. At 1 MAP, NPK fertilizer (15-7-18) was applied in the recommended rate of 50 kg per rai for the experimental plot with area of 2,880 sq meters (12 x 10 x 24 m). Glyphosate (at 250cc/rai) was initially sprayed on September 2009 at latter growth stages, when necessary.

1.2 Experimental Design

A split-plot in randomized complete block design was used with 4 replications, having the harvest period, **HP** (6, 9, 12 months) as main plot, and irrigation treatments, **IT** (**T0** control, rain-fed no irrigation; **T1,** 30mm/month, 10-10-10 mm split; and **T2** 60mm/month, 20-20-20 mm split) as sub-plot.

There are a total of 48 plots, with 12 x 5 m dimension per plot (total of 60 stakes planted per plot, including the border plants). The border plants are provided to line the plots to take care of sub lateral flow in each plot.

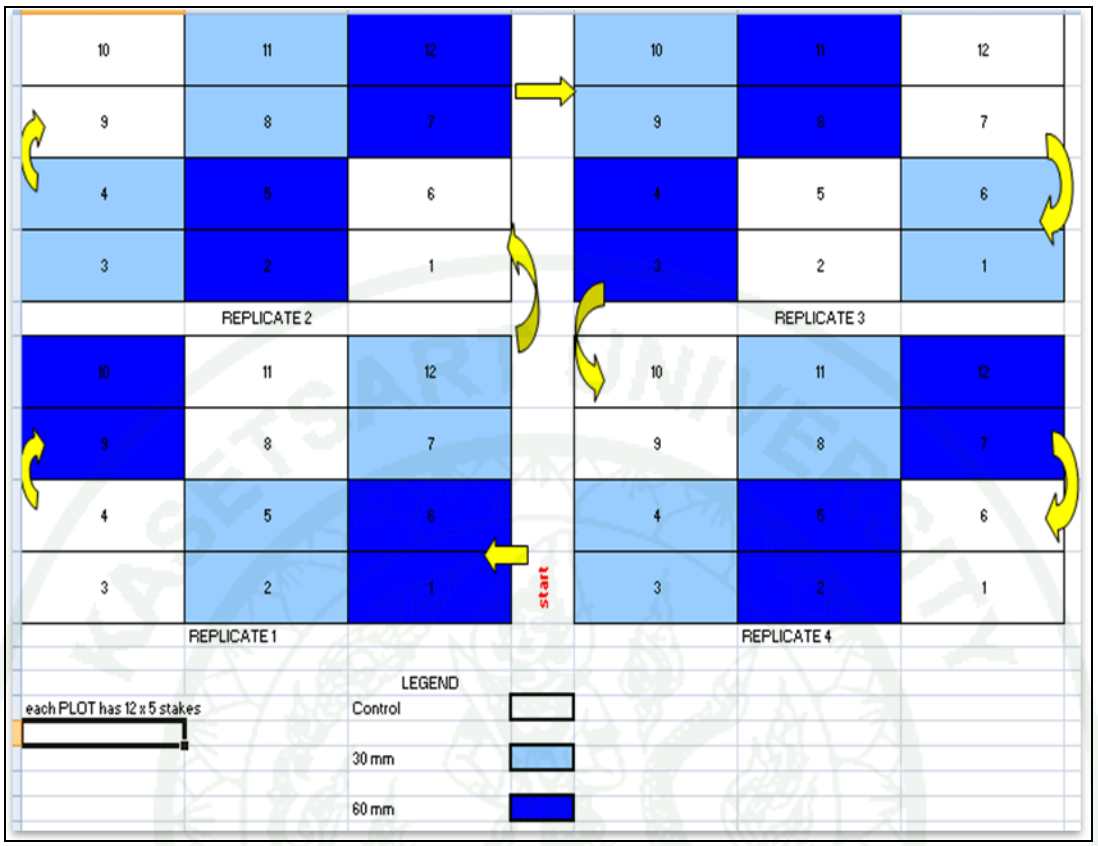

Figure 5 Experimental plot irrigation plan.

Figure 5 shows a schematic diagram of the field plots. Color scheme represents the irrigation treatment, with white boxes representing control plots; light blue for T1 (30mm per month); dark blue for T2 (60mm per month). Yellow arrows were used by the researcher and farm laborers as guide for marking the plants/plots.

1.3. Data Collection and Analysis

1.3.1 Soil sampling and soil analysis

Soil sampling and analysis was done at the beginning of the experiment. Doing an initial soil analysis allows for detection of P, K, Ca, Mg and Zn deficiencies, while soil pH indicates whether Al and/or Mn toxicity or micronutrient deficiencies are likely to occur. Soil analyses usually determine the amount of

available or exchangeable nutrient as this part of the total soil nutrient is best correlated with plant uptake. These available fractions are usually determined by shaking the soil sample with certain extracting solutions and determining the amount of nutrient in the extract.

Representative soil samples were taken in the areas that appear to be uniform in terms of plant growth and previous management. About 10 to 20 subsamples are taken in zigzag fashion across the whole area. These subsamples are thoroughly mixed together and then about 300-500 grams are air dried or dried at about 65 C in a forced-air oven. This combined sample is then finely ground, screened and sent to the Soil Science Department laboratory, Kasetsart University (Bangkhen) for analysis.

Table 2 shows the results of the soil sample analysis of the research planting area. The research plot has a sandy loam texture and is moderately drained, is acidic (pH 4.6) and has liming requirement of 269. It contains moderate amount of phosphorus, low organic matter (1.07%), low calcium and magnesium, and very low potassium.

Table 3 Soil physical and chemical properties of the experimental site in Khao Hin Sorn Research Station, Chachaengsao Province.

pH	Lime req	Particle Size distribution			Texture	OM		Phosphorus		Calcium		Magnesium		Potassium	
		% Sand	% Silt	% Clay		%	Rate	mg/ kg	Rate	mg/ kg	Rate	mg/ kg	Rate	mg/ kg	Rate
4.6	**269**	**70**	**20**	**10**	**SL**	**1.07**	**L**	**14**	**M**	**104**	**L**	**12**	**L**	**20**	**VL**

Source: Soil Science Department, Kasetsart University, Bangkhen (2010)

1.3.2 Monthly Plant Data

Data such as days to emergence, days to flowering, and days to harvesting were recorded. Plant heights were measured and leaf-size/canopy architectures were noted on a monthly basis for the course of the plant's 12-month life cycle for growth measurement.

1.3.3 Harvest Data

Yield and yield components, such as number of roots, weight of stock, weight of top part, starch content using Reimann scale, were recorded at every harvest. Aside from cyanide content, other physico-chemical components such as starch content, protein, carbohydrates, ash and fat contents were analyzed at every harvest.

1.4 Treatment Implementation and Timing

For this experiment, supplemental irrigation started in November 2009, at 6MAP, following the major rainy season. Supplemental irrigation was administered via a sprinkler system. Three treatments are used in this research – T0 (control; no supplemental irrigation, 0mm), T1 (30 mm/month), and T2 (60 mm/month).

Starting at 6MAP, water treatments were administered thrice a month - every 10^{th}, 20^{th} and 30^{th} of the month, irrigating 10mm and 20mm per time for T2 and T3, respectively. Estimation of water volume to be sprinkled per time was carried out by drip pan for field plot sprinkle irrigation. Through several trials and adjustment of the sprinkler mechanism, it was established that 10 mm is sprinkled in 30 minutes on the average, while 20mm was sprinkled in 60 minutes.

Climatic parameters that contribute to calculations of irrigation requirements are maximum and minimum temperatures, maximum and minimum air humidity, Sunshine hours, Solar radiation and rainfall. These parameters have been

found during a campaign of data collection at Nyagatare District at Nyagatare and Matimba weather stations.

1.5 Harvesting

Harvesting of samples was at 6MAP, 9MAP and 12MAP. Laboratory analysis of cyanide-content in root samples was done within 24 hours of harvest to minimize volatilization of free HCN as well as post-harvest spoilage of root samples.

Assay for Total Cyanide Content and Non-Glycosidic Cyanide (NGC) Content were carried out for root peel, parenchyma, and whole root (with peel intact). Data from these assays will be used to compute for other components such as Bound Cyanide. For this research, free cyanide was not determined due to the difficulty in measurement of this highly-volatile and minute cyanogen component.

Of all the major root crops, cassava has the shortest postharvest and shelf life (Ghosh *et al,* 1993), with rapid physiological deterioration rendering the roots inedible within 24-72 hours after harvest (Alves, 2002). Blue, brown and black pigments, considered as condensed tannins, form as polyphenolic compounds in the roots oxidize to quinine-type substances, which are complexed with amino acids, and then deposited in the vascular bundles appearing as colored pigments (Wheatley and Chuzel, 1993). The rate of deterioration of harvested cassava depends upon the varietal characteristics, physiological state of the root upon harvest, and the condition and duration of storage (Ghosh *et al*, 1988).

Laboratory analysis for root moisture content and root starch content were also performed at every harvest.

2. Laboratory Analysis for Cyanide Content

Analysis for cyanide content was performed at the Cassava Starch Technology Research Unit (CSTRU) in Kasetsart University, Bangkhen, Thailand.

2.1 Sample Extraction

The standard procedures for sample extraction for fresh cassava roots were followed. Roots were peeled, then chopped into transverse discs of 2cm thickness. Discs from the proximal, central and distal parts of the root were selected, and then cut into 1cm cubes, which were randomized thoroughly before sampling.

Samples of about 50 grams of diced root were homogenized in 160 ml extraction medium for 2 minutes. Using a further 20ml of extraction medium, the homogenate was washed on to a glass-fibre filter (Whatman GFA), and the extract was collected under vacuum. The same procedure was carried out for peel, parenchyma and whole roots sample preparations.

2.2 Assay Procedure

Cyanogens were assayed in duplicate using the following formats:

2.2.1 Total Cyanide Content Assay

An aliquot of extract (0.1 ml) was added to 0.4 ml pH 7.0 buffer A in a stoppered Quickfit test tube, wherein the linamarase preparation (0.1ml) was added. After 15 minutes incubation at 30 Celsius, 0.2 M NaOH (0.6 ml) was added, followed by Buffer A (2.8 ml; pH6.0). Aliquots were assayed as in the colorimetric procedure aforementioned.

2.2.2 Non-Glycosidic Cyanogens Assay

Extract (0.1ml) was first mixed with 0.4ml pH 4.0 Buffer A, and an excess of 0.2 M NaOh (0.6 ml) was added. After 5 minutes it was diluted with a further 2.9 ml buffer A (pH4.0), after which aliquots were assayed colorimetrically.

2.2.3 Colorimetric Procedure

Chloramine T reagent (0.2 ml: 0.5% w/v) was added to 4 ml buffered extract in a stoppered Quickfit test tube and mixed well. Tubes were placed in an ice/water bath for 5 minutes, after which pyridine/pyrazolone reagent (0.8 ml) was added in a fume cupboard. After 90 minutes, the absorbance at 620 nm was determined. Duplicate analyses were performed and blanks containing extraction medium were run for each analysis.

Total, NGC and Free cyanogens were first calculated using an adaptation of the equation proposed by Cooke (1979).

(1) $$Cyanide\ (mg.kg^{-1} as\ HCN) = \frac{10 \times V' \times A_{620}}{A_{equiv} \times DW}$$

(2) $$V' = V + \left(\frac{MC \times FW}{100}\right)$$

Where V is the volume of extraction medium used (ml), FW is the fresh weight of the sample (g), M is the percentage moisture of the sample, V' is the volume adjusted to include sample moisture, A_{620} is the mean absorbance recorded at 620 nm wavelength, DW is the dry weight of sample (g), and A_{equiv} is the absorbance corresponding to 1 μg HCN derived from standards. Thus, NGC and Total HCN can be computed as:

(3)

$$NGC_{peel/par} = \frac{\left[V + \left(\frac{FWsample \times MC\%}{100}\right)\right] \times (A_{620} \times DF) \times 100}{Slope \times FW_{sample} \times (100 - MC)}$$

Where;

V	volume of extraction media (150 ml)
FW$_{sample}$	fresh weight of approximately 50 grams for parenchyma, 30 grams for peel
MC%	moisture content of sample
***A*$_{620}$**	the mean absorbance of 3 determinations, recorded at 620 nm wavelength
DF	dilution factor; 10 for parenchyma, 20 for peel
Slope	the absorbance corresponding to 1µg HCN derived from KCN standards.

Substituting the values derived from the formula (3), NGC and Total HCN for whole root can be computed as;

(4)

$$NGC_{Root} = \left[\left(\frac{Peel\ FW_{sample}}{Root\ FW_{sample} \times NGC_{peel}}\right) + \left(\frac{Par\ FW_{sample}}{Root\ FW_{sample} \times NGC_{par}}\right)\right]$$

(5)

$$Total\ HCN_{Whole\ Root} = \frac{PAR_{DW}}{ROOT_{DW} \times HCN_{Par}} + \frac{PEEL_{DW}}{ROOT_{DW} \times HCN_{peel}}$$

From the assays for Total Cyanide Content and Non-Glycosidic Cyanide (NGC), Cyanogenic glucoside or Bound Cyanide was calculated as total cyanogens minus NGC.

(6) $$Bound\ Cyanide = Total\ HCN - NGC$$

3. Statistical Analysis

Using General Linear Model (GLM) univariate analysis test for between-subjects of the SPSS computer package, field data and laboratory results were submitted to analysis of variance through F-test and, when there was significance in means, were then compared in pairs through the test of least significant difference (LSD). All tests were performed at 5% level. Among the variables studied, Pearson correlations were calculated between evaluated parameters, and tested through t-test.

RESULTS AND DISCUSSION

Results

In whole roots, statistical results show harvest period, **HP**, has a significant reducing effect on NGC ($P < .05$). While the difference in means for bound and total cyanide are not statistically significant, closer inspection of lab data shows that there was an observable decline in both bound and total cyanide content from 6MAP to 9MAP, but an increase between 9MAP and 12MAP.

In peel, HP has a strong inverse correlation with cyanide content. Table 4 shows NGC, bound and total cyanide content in peel significantly declined with root age ($P < .05$). In parenchyma, roots harvested at 9MAP had the lowest bound and total cyanide content, 12MAP had the highest. While not statistically significant, lab data shows a considerable increase in total cyanide content from 9MAP to 12 MAP (from 560.3 ± 75.5 to 873.8 ± 190.9 mg HCN per kg dry weight).

Table 4 NGC, bound, and total cyanide content in cassava peel, parenchyma and whole root harvested at 6, 9, and 12 months after planting (mg HCN/kg dry weight).

Root Part	Harvest Period	NGC	BOUND	TOTAL
Peel	**6**	169.2 ± 19.8	2397.1 ± 403.1	2566.3 ± 397.9
	9	259.8 ± 123.1	1726.0 ± 195.7	1985.7 ± 300.3
	12	96.6 ± 39.4	1265.2 ± 357.4	1361.8 ± 351.3
	Sig.	.000	.000	.000
Parenchyma	**6**	102.9 ± 17.6	513.2 ± 107.2	616.2 ± 123.9
	9	99.1 ± 3.0	461.2 ± 73.5	560.3 ± 75.5
	12	60.6 ± 13.0	813.3 ± 185.2	873.8 ± 190.9
	Sig.	ns	.050	ns
Whole Root	**6**	113.8 ± 17.0	761.9 ± 115.2	1477.73 ± 314.90
	9	132.8 ± 24.8	691.1 ± 44.4	1023.28 ± 179.75
	12	80.9 ± 12.9	884.5 ± 197.1	950.34 ± 81.72
	Sig.	.039	ns	ns

Data are means ± S.D. of three laboratory determinations.
ns, not significant

The effect of irrigation treatments, **IT**, on cyanide content cannot be isolated from the effect of harvest period, thus the interaction of the two factors (IT x HP), on the NGC, bound and total cyanide content in whole roots, in peel, and in parenchyma was analyzed (Table 5). Tests between subject effects confirm the significance of the interaction between IT and HP ($P < .05$). In whole roots, bound and total cyanide content were lowest at 9MAP, while linamarin content was highest at 12MAP. Moreover, T2 yielded the lowest NGC, bound and total cyanide, but T1 yielded the highest overall cyanogen content albeit not significantly different from T0.

Table 5 NGC, bound and total cyanide content in cassava whole roots subjected to supplemental irrigation treatments T0, T1 and T2 at 6, 9 and 12 months after planting (mg HCN/kg dry wt.).

Harvest Period	Treatment	NGC	BOUND	TOTAL
6	**T0**	113.8 ± 17.0	761.9 ± 115.2	875.7 ± 108.5
	T1	98.8 ± 5.9	880.6 ± 42.6	979.4 ± 46.1
	T2	88.2 ± 4.5	845.6 ± 120.9	933.8 ± 118.6
9	**T0**	132.8 ± 24.8	691.1 ± 44.4	823.8 ± 61.6
	T1	156.7 ± 22.7	692.8 ± 85.3	849.6 ± 102.3
	T2	117.1 ± 12.5	511.9 ± 77.4	629.0 ± 87.3
12	**T0**	80.9 ± 12.9	884.5 ± 197.1	965.4 ± 188.5
	T1	92.0 ± 22.1	879.3 ± 144.7	971.3 ± 159.1
	T2	62.4 ± 21. 0	823.6 ± 37.0	885.9 ± 49.5

Data are means ± S.D. of three laboratory determinations.

For the IT x HP interaction effect on the proportion of NGC and bound cyanide content in whole roots, irrigating at the rate of T2 and harvesting at 9MAP yields the lowest NGC and bound cyanide. Supplemental irrigation's reducing effect on cyanide content was most evident at 9MAP, when T2 yielded the lowest NGC, bound and total cyanide, compared to T1 and T0 which were not statistically different (Fig. 6).

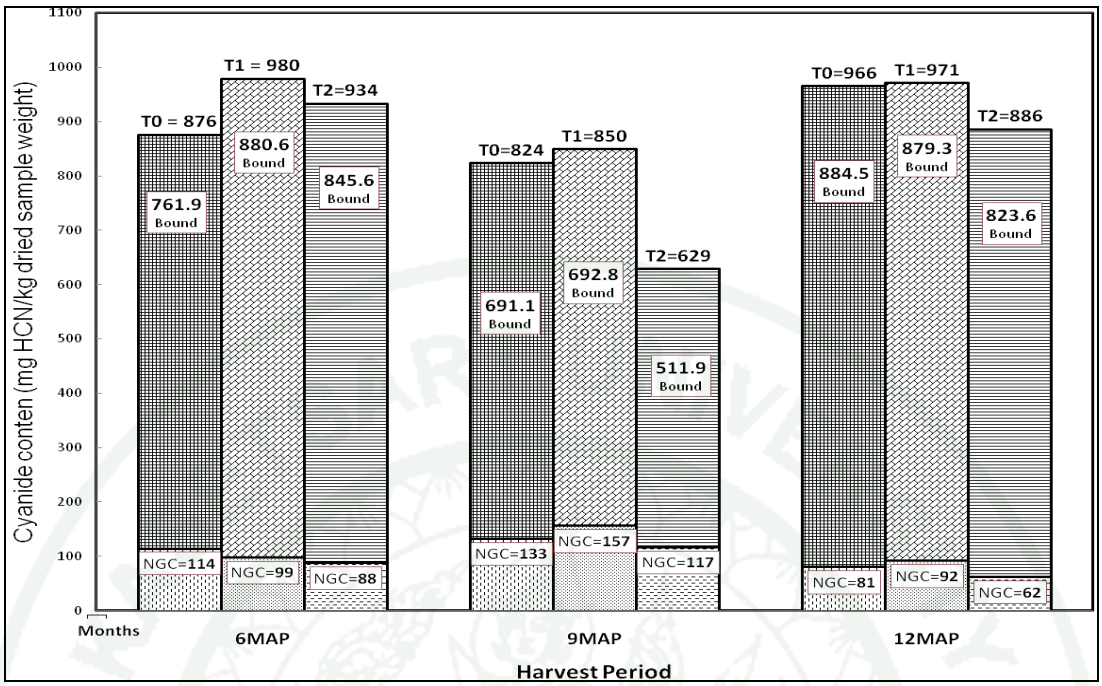

Figure 6 NGC and bound cyanide content in whole roots subjected to supplemental irrigation treatments T0, T1, T2 at harvest periods 6, 9, 12 months after planting.

In the interaction effect of harvest period and irrigation treatment (H x I), bound and total cyanide content for parenchyma were lowest at 9MAP, and highest at 12MAP. The same trend was observed in whole root, with 9MAP yielding the lowest bound and total cyanide contents. In peel, bound and total cyanide showed a steady decline over time, with 6MAP having the highest means, and12MAP the lowest.

On the effect of harvest period (6, 9, 12MAP) on yield, harvest index, and starch chemical properties of KU50 whole root (Table 6), statistical results show that harvest period has a very strong positive correlation with (p=.01) and has a significant positive impact on crop growth rate components such as plant height, weight of leaves & stem, and root yield or total root weight (p=.05). Hence, the highest root yield and biomass was attained at 12MAP. However, starch content, measured in the field (using Reimann Scale) and in the lab, was highest at 9MAP (p=.05), while number of

bulking roots showed no real correlation with HP. Fat (%) exhibited low positive correlation (p=.05), while ash and protein contents have no real correlation to HP.

Table 6 Effect of harvest period (6.9, 12 MAP) and irrigation treatments (T0, T1, T2) on plant height, top weight, root yield, starch content, moisture content, ash, fat and protein in KU50 whole roots.

HP	PLT HT[a] (cm)	TOP WT[a] (ton/rai)	YIELD[a] (ton/rai)	#RTS[a] (/plot)	RSta[a] (%)	MC[b] (%)	LSta[b] (%)	ASH[b] (%)	FAT[b] (%)	PRO[b] (%)
6	165.69	2.43	5.05	165.5	27.89	59.17	84.17	1.92	0.28	1.73
9	192.49	2.41	5.67	146.4	29.44	60.76	84.31	1.97	0.31	1.70
12	252.13	3.87	9.80	157.4	25.47	64.15	77.17	1.97	0.41	1.55
x̄	203.43	2.90	6.84	156.4	27.60	61.36	81.88	1.96	0.33	1.66
LSD	38.23	0.84	2.33	ns	2.26	3.43	3.79	ns	0.13	ns
IT	**PLT HT[a]** (cm)	**TOP WT[a]** (ton/rai)	**YIELD[a]** (ton/rai)	**#RTS[a]** (/plot)	**RSta[a]** (%)	**MC[b]** (%)	**LSta[b]** (%)	**ASH[b]** (%)	**FAT[b]** (%)	**PRO[b]** (%)
T0	200.74	3.09	6.24	150.33	26.23	62.87	80.53	2.07	0.36	1.84
T1	202.44	2.79	6.94	156.00	28.05	60.86	81.96	1.89	0.31	1.61
T2	207.13	2.84	7.34	163.00	28.51	60.34	83.15	1.90	0.33	1.52
x̄	203.44	2.90	6.84	156.44	27.60	61.36	81.88	1.96	0.33	1.66
LSD	ns	ns	13.09	ns	2.26	3.79	3.79	ns	ns	0.28

(HP) harvest period; (PLT HT) plant height; (TOP WT) top weight ton/rai; (YIELD) root yield ton/rai; (#RTS) number of bulking roots per plot; (RSta) %starch content measured by Reimann Scale; (MC) %moisture content; (LSta) % starch content determined in lab; (PRO) % protein content.

[a] Data represent means of four field replicates

[b] Data represent means of three laboratory determinations

ns, not significant

In the test for the effect of irrigation treatments, **IT**, only root yield, starch content, moisture content, and protein are statistically significant (p=.05). Higher supplemental irrigation yields a significant increase in starch content (P<.05) but a significant decrease in protein content (P<.01).

Table 7 Plant height, top weight, root yield, starch content, moisture content, ash, fat and protein in KU50 whole roots subjected to treatments T0, T1, T2 at 6, 9 and 12 months after planting.

HPxIT	PLT HT[a] (cm)	TOP WT[a] (ton/rai)	YIELD[a] (ton/rai)	#RTS[a] (/plot)	RSta[a] (%)	MC[b] (%)	LSta[b] (%)	ASH[b] (%)	FAT[b] (%)	PRO[b] (%)
6 x T0	161.38	2.31	4.57	150.75	27.55	59.28	83.60	1.95	0.26	1.76
6 x T1	170.95	2.50	5.35	168.25	28.16	58.37	84.51	1.86	0.25	1.74
6 x T2	164.75	2.48	5.22	177.50	27.95	59.85	84.40	1.96	0.34	1.69
9 x T0	193.21	2.73	5.41	140.75	27.43	62.44	82.99	2.11	0.38	1.86
9 x T1	190.5	2.11	5.58	144.25	29.80	59.03	84.93	1.89	0.26	1.68
9 x T2	193.75	2.34	6.032	154.25	31.10	60.82	84.93	1.92	0.30	1.55
12 x T0	247.63	4.17	8.75	159.50	23.73	66.91	75.01	2.17	0.46	1.90
12 x T1	245.88	3.76	9.90	155.50	26.20	65.19	76.43	1.93	0.41	1.43
12 x T2	262.88	3.69	10.77	157.25	26.48	60.38	80.06	1.84	0.34	1.33

(HP) harvest period; (PLT HT) plant height; (TOP WT) top weight ton/rai; (YIELD) root yield ton/rai; (#RTS) number of bulking roots per plot; (RSta) %starch content measured by Reimann Scale; (MC) %moisture content; (LSta) % starch content determined in lab; (PRO) % protein content.

[a] Data represent means of four field replicates

[b] Data represent means of three laboratory determinations

ns, not significant

Discussion

To properly approximate how much water or moisture the plant has received during the research period, the average and total rainfall within the vicinity of the research during the same period must also be reported. For each of the crops grown on an irrigation scheme the crop water need is determined, usually on a monthly basis; the crop water need is expressed in mm water layer per time unit, in this case mm/month. Thus, based on Crop Water Requirement, on a monthly basis the effective total water is equal to total rainfall (for that month) plus supplemental irrigation.

Cassava is commonly grown in areas receiving <800 mm rainfall per year, with a dry season of 4-6 months, where tolerance to water deficit is an important attribute. Depending on the rainfall condition of that year, some parts of the country, including the Northeast Region where the research took place, could have an annual rainfall of 400 to 1200 mm. In such drier climatic condition, while crop production in the rainy season may be possible albeit unreliable, rainfall is often not sufficient to cover the water needs of the crops, and crop production in the dry season is only possible with irrigation.

Table 8 Daily average and total monthly precipitation (mm) at Khao Hin Sorn District, Chachaengsao Province (April 2009- May 2010).

	Apr 09	**May**	**June**	**July**	**Aug**	**Sept**	**Oct**	**Nov** [a,b]	**Dec**	**Jan**	**Feb** [c]	**Mar**	**Apr 10**	**May** [d]
Avg RF	5.87	7.68	2.87	7.45	5.52	12.4	7.06	1.17	0	0	1.32	1.77	0.37	0.58
Total RF	176	238	86	231	171	372	219	35	0	0	37	55	11	18

Source: Khaohinsorn Research Station (2010).

a, start of monthly supplemental irrigation

b, 1st harvest at 6MAP

c, 2nd harvest at 9 MAP

d, 3rd harvest at 12 MAP

Thailand is considered to have humid climate, having more than 1,200 mm of rain per year. Table 8, recorded by Khaohinsorn Research Station, shows the total precipitation of the vicinity of the experimental field (April 2009 - May 2010) of 1,649 mm, with the highest rainfall in September (372mm) and the lowest in December and January, with zero precipitation (Fig. 7).

Rainy season in Thailand commences in April and lasts until October. Total rainfall recorded for the major rainy season was 1,493mm, before the crop entered the drought period.

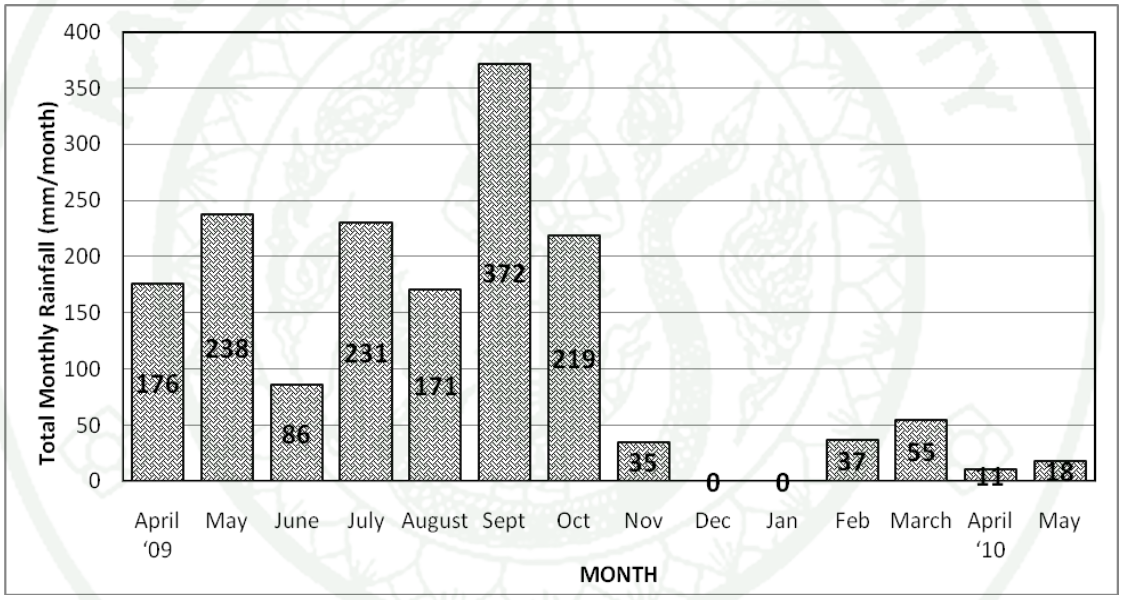

Figure 7 Total monthly rainfall (mm) from April 2009 to May 2010 in Khao Hin Sorn District, Chachaengsao province.

Source: Khaohinsorn Research Station (2010)

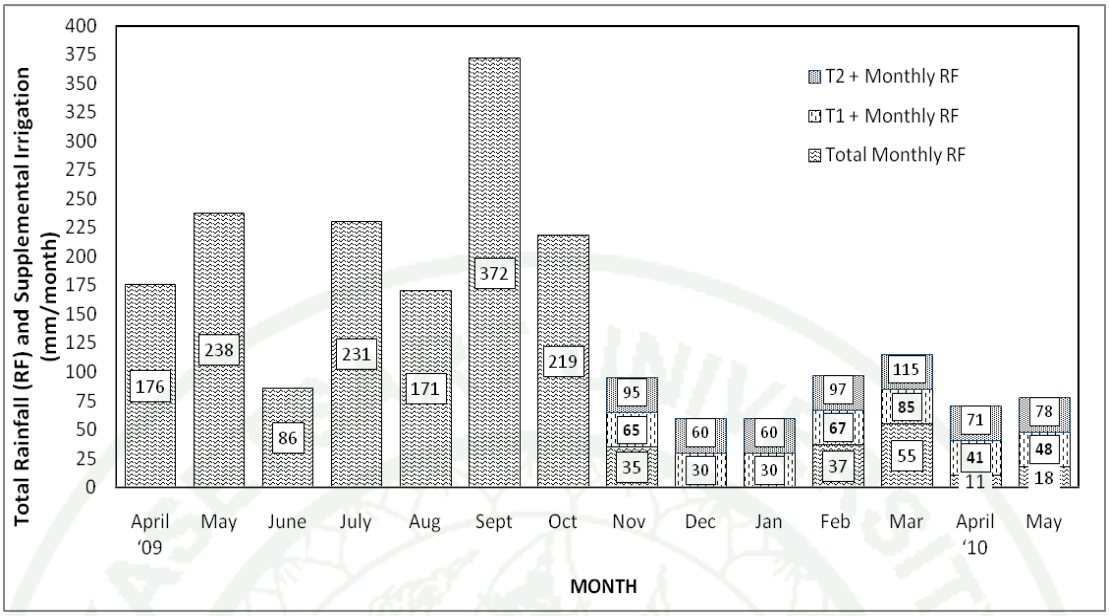

Figure 8 Total monthly rainfall and supplemental irrigation from April 2009 to May 2010 in Khao Hin Sorn District,.Chachaengsao province.

Source: Khaohinsorn Research Station (2010)

Application of treatments (supplemental irrigation) began on the 10th of November following the irrigation schedule previously discussed in the methodology (Fig. 8). After the first harvest, the plants enter a drought period, with December and January recording zero precipitation. The plants derived their crop water requirement solely from the supplemental irrigation. February until May saw a few occurrences of rain, providing some added moisture to the soil and crop. Harvest 2 was at the end of February 2010 (9MAP), with the onset of rain immediately following the drought period. Harvest 3 was at the end of May 2010 (12MAP).

To determine the accurate effect of harvest period on cyanide content and other crop growth components, other factors must be kept constant. As past and prevailing environmental factors, *i.e.* as monthly rainfall, could not be made constant all throughout the experiment, the researcher conceded to using data from control treatment (T0, N=36) to compare the means for analyzing effect of harvest period.

Not taking into account the effect of supplemental irrigation, results showed that HP has a significant effect on NGC ($P < .05$). However, a significant effect of HP on bound and total cyanide could not be established, presumably due to the wide variations in cyanide content among whole root, peel and parenchyma.

1. Effect of harvest period on HCN-content in whole root and parenchyma

To determine the effect of 'harvest period only' on cyanide content, data from control treatment (T0, N=36) were used to compare the means.

Table 4 shows that the plant age of cassava roots upon harvest has a significant effect on NGC ($P < .05$). Thus, the older the root is upon harvest, the lower the NGC-content is in the root. This is in concurrence with the findings of Chotineeranat (2005) which showed that roots harvested at 6 and 8 MAP contained very high amounts of cyanogenic compounds (1, 427. 16 ± 481.57 and 1, 259.47 ± 186.84, respectively), presumably due to the environmental conditions during plant and root development, especially at 8 MAP when drought stress is high (Sriroth *et al*, 1999; Santisopasri *et al*, 2001).

While statistical analyses show that harvest time does not appear to have a significant effect (at 95% level of significance) on bound cyanide content and on the total cyanide content of the whole root, closer inspection of lab data shows that there was a significant decline in bound cyanide content (and consequently on total cyanide content) from 6MAP to 9MAP, but not between 9MAP and 12MAP.

The same table shows the effect of plant age on the cyanide content in peel only. Onwueme (1978), Hahn (1984), Nambisan and Sundaresan (1994), and Bokanga (1994), asserted that the highest proportion of HCN, ranging from 900 to 2000 ppm, is found in the peel and the root cortex layer immediately beneath the peel. It is for this reason cassava roots are always peeled before being processed or consumed as food, as peeling removes the cortex and the outer periderm layer adhering to it, along with the high amount of cyanide content imbedded in the peel.

The inverse correlation between plant age and cyanide content in peel is evidenced in the above results. It was clearly demonstrated that the older the plant is upon harvest, the lower the total cyanide content is and, more specifically, the less linamarin is in the peel. Hence, harvest period has a significant effect on all cyanogen components in the peel ($P < .05$), as NGC, bound cyanide, and total cyanide content in the peel declined with root age.

For parenchyma, as in the case of whole roots, statistical analysis revealed that plant age at harvest has no significant effect on cyanide content. Lab results unexpectedly showed that bound cyanide content in parenchyma was highest at 12MAP, which is in argument with the findings for peel and whole root of the same samples. Such variations in cyanogens content have been reported by Cooke (1978), Cooke *et al* (1978), Bradbury *et al* (1991), Yeo and Truong (1993) and Yeoh *et al* (1998). These previous studies have attributed such cyanide-content discrepancy among peel, parenchyma and whole root to the longitudinal and transverse gradients in linamarin content within the roots.

Furthermore, analysis of all sample means shows that cyanide content is highest in peel, followed by whole root, and lastly by parenchyma (peel > whole root > parenchyma). This corroborates with the generalization by Yeoh and Oh (1979) that cyanide content is highest in the leaf (approximately 6 times more than in parenchyma), followed by peel, then parenchyma.

2. Effect of irrigation treatments on cyanide Content

There does not appear to be a correlation in cyanide content among different plant parts. The statistical results were non-significant, indicating that there was no definite relationship in the level of cyanide between peel and parenchyma, or peel and whole root, or parenchyma and whole root. This corroborates with the study conducted by Yeoh and Oh (1979) on the correlation between cyanide content in leaf, peel and parenchyma.

The decline in total cyanide content over the three harvests is most readily apparent for T2 and T0, with values showing a decrease over time. Running univariate analysis and post-hoc tests revealed that T1 had the highest Total, while T2 and T0 were not statistically different; peel has 3 times as much total HCN-content compared to parenchyma; roots harvested at 6MAP had the highest total HCN, but 9 and 12MAP were not statistically different.

For bound cyanide content, T1 yielded the highest value yet again, while T2 had the lowest, albeit not significantly different from T0; peel has 3.3 times more linamarin-content than parenchyma; roots harvested at 6MAP had the highest bound cyanide content, but 9MAP showed the lowest content, although not significantly different from 12MAP.

For Non-glycosidic cyanide content, T1 recorded the highest value, T2 having the lowest but not statistically different from T0; peel has twice more NGC than parenchyma; roots harvested at 9MAP had the highest NGC-content, followed by 6MAP, then 12 MAP, with statistically different means.

Finally, among the three measured cyanogens, statistical results reveal that supplemental irrigation has significant reduction effect NGC- content only. The findings for the case of total cyanide content and bound cyanide content were statistically inclonclusive.

3. Effect of harvest period on root yield, yield components and starch properties

Statistical analysis shows a very high positive correlation between harvest period and crop growth rate components such as plant height, plant weight, and root yield (at .01 level of significance). As to be expected, the more mature the plant is at harvest, the higher the dry matter accumulation is in the storage roots as well as in the leaves and stems. Crop growth rate is an important index of agricultural productivity, denoting to the rate of dry matter production per area of land as well as a measure of the plant's efficiency to produce biomass over time (Ekanayake, 1996). For this experiment, the highest root yield and biomass was attained at 12MAP.

Conversely, the starch content percentage, separately measured using Reimann Scale at the field and dry oven determination in the laboratory, showed an inverse correlation with plant age (significant at 99%). The high starch content at 6MAP is to be expected, as harvest immediately followed the major rainy season, contributing to high water availability for the roots. This reaffirms the assessment of Santisopasri et al. (2001) that environmental conditions, especially water availability, during early plant development and immediately before harvest, strongly influence cassava's production efficiency, including yield and starch quality.

Starch content was at its maximum around 9 MAP, coinciding with the late drought period, and just continuously decreased until the last harvest at 12MAP. This is in concurrence to Santisopasri's (2001) experiment, elucidating that starch content decreases during period of rain following the late drought period as the plant physiologically adjusts to the changing growing condition. After the drought period, the plant enters a period of rapid growth and expediently forms new leaves, which could explain the increase in plant height and plant weight, possibly utilizing reserved starch as the source of energy for this process (Chotineerat, et al., 2006).

Number of bulking roots showed no real correlation to harvest time. Presumably since all samples were planted under the same initial environmental conditions, and since root formation and tuberization takes place around 5MAP

(Ramanujam, 1990), the number of adventitious roots formed was not significantly different among the samples. Fat percentage exhibited low positive correlation to harvest time (at .05 level of significance), while ash and protein contents appear to have no real correlation to harvest time, although raw data showed a slight increase in these properties over the three harvests, albeit not statistically significant.

4. Effect of irrigation treatments on root yield, yield components and starch properties

Supplemental irrigation does not have a significant influence (at .05 level of significance) on the measured plant and starch components at 6MAP following the major rainy season. With only 1 month of administration of the water treatments, sample means at first harvest were not significantly different. This is evidenced by the analogous moisture content from samples from T0, T1 and T2 at 6MAP (59.28, 58.37, and 59.85%, respectively).

While results showed that increasing irrigation also increases starch content percentage in roots, water treatment appears to have no significant effect on root yield, and yield components (plant height and plant weight), except for number of bulking roots, which showed a low positive correlation to water treatments (significant at 90%). These results were not as definitive as that of Samuthong's (2007), which successfully demonstrated that providing supplemental irrigation of 60mm per month (20, 20, 20 mm split) produced the highest yield and greatest harvest index, which were significantly higher than that of the other treatments, and were 74.2% higher than control (T0).

Laboratory results and statistical analysis show a strong negative correlation between water treatment and protein content (at 99% significance level). This finding corroborates with previous studies by Gebeyehu *et al* (2010) on the effects of drought stress on protein patterns in soybeans, and by Chotineeranat (2006) on KU50 cassava. The former also noted the significant positive correlation of protein content to cyanide content, which was established to be high during drought period. Neslihan-Ozturk *et al* (2002) assert that water deficit induces expression of proteins that are directly or indirectly related to stress. Certain stress-induced proteins are pivotal to the plant's adaptive response to drought (Riccardi *et al*, 1998), among which are implicated in the ions uptake and compartmentalization (Lisse *et al*, 1996), and in photosynthesis-related function and in cellular structure protection (Neslihan-Ozturk and Bartels, 1996).

CONCLUSION AND RECOMMENDATIONS

Conclusion

Cassava's reputation as a drought-tolerant crop is irrefutable. However, the cyanide-content in cassava, which is exacerbated in periods of drought, impacts the economics of its production and safety of consumption. The results of this study showed that for total cyanide, bound cyanide, and NGC, roots harvested at 9MAP contained the lowest levels of cyanide. In terms of irrigation treatments, roots subjected to T2 (60mm) yielded the lowest cyanogen content, while T1 (30 mm) yielded the highest means.

With all factors being equal, the highest water volume, T2, was expected to cause the most reduction effect in cyanide-content in peel, parenchyma and whole roots, T1 having the median effect, and T0 (control) having the least impact. While this was not always the case, with T1 yielding the highest overall cyanogen content, albeit not significantly different from T0, these results would imply that perhaps water volume treatments (30 and 60 mm/ month) used in this study were not sufficiently high to effect a significant reduction in cyanogenic potential in the root samples. And while the reduction effect of supplemental irrigation was only statistically significant for NGC content in whole roots, peel and parenchyma ($P<0.05$), closer inspection of lab data reveals observably lower cyanide content at 9 and 12 MAP when more water was used.

Moreover, supplemental irrigation significantly increased starch content ($P< 0.05$) and number of bulking roots, although did not significantly affect root yield, plant height and plant top weight. Protein content was significantly reduced ($P<0.01$) by supplemental irrigation thus corroborating reports that drought stress induces expression of proteins that are directly or indirectly related the plant's adaptive response to drought (Riccardi, et al., 1998). Thus, in the absence of drought, such water stress-related proteins were not expressed.

As was expected and proven in the results, the more mature the plant is at harvest, the higher the dry matter accumulation is in the storage roots as well as in the leaves and stems. Hence, the highest root yield and biomass was attained at 12MAP.

Conversely, the starch content percentage did not improve with age. Starch content was at its maximum at 9 MAP, coinciding with the late drought period, and then declined towards 12 MAP.

In conclusion, administering ample supply of water, particularly during dry season, should mitigate the cyanogenic potential of cassava roots. If the economic gains outweigh the cost of irrigation, the rate of 60 mm per month or higher is recommended, along with selection and planting of a suitable cassava cultivar (must be low-cyanide variety if planted for food). Moreover, it is recommended to harvest the roots at 9MAP, when bound and total cyanide content in parenchyma and whole root are at the lowest, and starch content is at its highest.

Recommendations

As was reported in previous studies, cyanide content in cassava is water-dependent, with cyanide content increasing in periods of drought. Hence, administering ample supply of water, particularly during dry season, should mitigate the cyanogenic potential of cassava roots. If the economic gains derived from producing and processing lower-cyanide content cassava outweigh the cost of irrigation, then application of water treatment at the rate of 60 mm per month or higher is recommended.

Limiting factors notwithstanding, and based on the aforementioned results, this research recommends selection of a suitable cassava cultivar (must be low-cyanide variety if planted for food), plus supplemental irrigation at 60 mm per month or higher, and harvesting the roots at 9MAP, when bound cyanide and total cyanide content in parenchyma and whole root are at the lowest.

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APPENDIX

Appendix Table 1 Chemical Composition and Cyanide Content of KU50 Whole Roots Planted Before Rainy Season.

HT	Chemical Composition (%)					
	Fat	**Protein**	**Crude Fiber**	**Ash**	**CHO**	**Cyanide Content (ppm Dry Basis)**
6	0.14 ± 0.07	2.81 ± 0.02	2.54 ± 0.05	2.72 ± 0.07	91.77 ± 0.04	1, 427. 16 ± 481.57
8	0.21 ± 0.03	2.30 ± 0.02	1.93 ± 0.02	1.98 ± 0.01	93.58 ± 0.01	1, 259.47 ± 186.84
10	0.14 ± 0.07	1.83 ± 0.02	1.79 ± 0.02	2.41 ± 0.04	93.83 ± 0.11	799.90 ± 94.38
12	0.08 ± 0.01	1.41 ± 0.01	2.59 ± 0.04	2.52 ± 0.08	93.42 ± 0.12	533.72 ± 49.07

HT, harvest time

CHO (carbohydrates) = 100% - (Fat + Protein + Crude Fiber + Ash)

Source: Chotineeranat *et al* (2006)

Appendix Table 2 Chemical Composition and Cyanide Content of KU50 Cassava Flour (Extracted from above root samples).

HT	Chemical Composition (%)					
	Fat	**Protein**	**Crude Fiber**	**Ash**	**CHO**	**Cyanide Content (ppm Dry Basis)**
6	0.17 ± 0.04	0.77 ± 0.01	2.08 ± 0.16	0.91 ± 0.08	96.07 ± 0.04	18.82 ± 4.07
8	0.25 ± 0.02	0.80 ± 0.02	2.04 ± 0.10	1.10 ± 0.06	95.82 ± 0.20	15.51 ± 0.90
10	0.16 ± 0.02	1.10 ±0.01	1.52 ± 0.05	1.12 ± 0.02	96.09 ± 0.08	0.71 ± 0.00
12	0.06 ± 0.00	0.54 ±0.05	1.49 ± 0.03	1.24 ± 0.02	96.67 ± 0.04	1.31 ± 0.06

HT, harvest time

CHO (carbohydrates) = 100% - (Fat + Protein + Crude Fiber + Ash)

Source: Chotineeranat *et al* (2006)

Appendix Table 3 Remaining HCN content of various cassava products during processing.

Food item	**Detoxification stage**	**Remaining HCN**	
		Mean (mg/kg)	**(percentage)**
Mpondu	Fresh leaves	68.6	100.0
	Washed leaves (cold waler)	63.9	93.1
	Dried leaves	66.1	96.3
	Boiled leaves (15 min in water)	3.7	5.4
	Boiled leaves (30 min in water)	1.2	1.7
	Boiled cassava		
	Fresh roots (sweet)	10.7	100.0
	Boiled roots (20 min In water)	1.3	12.1
Fufu	Fresh roots (sweet and bitter)	111.5	100.0
	Soaked roots (3 days)	19.4	17.4
	Dried roots (3 days)	15.7	14.1
	Uncooked fufu (flour and water)	2.5	2.2
	Cooked fufu	1.5	1.3
Fuku	Fresh roots (sweet)	25.5	100.0
	Uncooked fuku (heated)	4.2	16.4
	Cooked fuku	1.2	4.7
Gari	Mash	90.1	100.0
	24 h fermentation	73.2	81.2
	48 h fermentation	55.3	61.3
	48 h pressing	36.0	40.0
	Roasting	25.8	28.6
Lafun	Mash	16.5	100.0
	5 day soaking	35.9	21.8
	5-day soaking + 48 h drying	25.5	15.5
	5-day soaking + 96 h drying	19.6	11.9

Source: Bourdoux *et al* (1982); Oke (1984)

Appendix Table 4 Types of waste and their environmental impact of various unit operations used in cassava processing.

Unit operation	Type of waste generated	Expected environmental impact
1. Washing	Organic matter, soil.	Little impact.
2. Retting	Cyanide diffused into rivers, ponds or back-water. Organic matter.	High HCN concentration in the waste water can be a problem if used directly on land. Dissipation is rapid if passed to waterways. Organic matter is a problem, causing high BOD and COD, and eutrophication of waterways and foul odors.
3. Peeling	Peels with high fibre and high cyanide content.	Can contaminate ground water supply during rain. Foul odor. Cyanide is a problem if used as a feed.
4. Squeezing	Effluent with high content of cyanide and organic matter (mainly starch).	High HCN may kill plants if effluent is allowed to run out on land. Dissipation should be rapid if released into waterways. Organic content may contaminate ground water supply and cause eutrophication of surface water and foul odor.
5.Drying and cooking	Cyanide vapors, ash (from firewood).	Cyanide vapor is not likely to be a problem unless processing is done in an enclosed space.
6. Sieving	Fibrous waste.	If exposed to rain, the seepage of organic material from stored waste could contaminate the ground water
7. Sedimenting	Starch residue. Waste water.	Foul odor Organic matter is a problem, causing high BOD and COD, and eutrophication of water ways.

Source: Nweke (1992)

Appendix Table 5 Tests of Between-Subjects Effects: TOP WEIGHT

Source	Sum of Squares	df	Mean Square	F	Sig.
Corrected Model		11	54.450	7.203	.000
Intercept	9607.267	1	9607.267	1270.880	.000
Harvest	535.796	2	267.898	35.438	.000
Water	19.909	2	9.954	1.317	.287
Harvest * Water	29.731	4	7.433	.983	.435
Rep	13.519	3	4.506	.596	.624
Error	181.429	24	7.560		
Total	10387.650	36			
Corrected Total	780.383	35			

a. R Squared = .768 (Adjusted R Squared = .661)

Appendix Table 6 Tests of Between-Subjects Effects: PLANT HEIGHT

Source	Sum of Squares	df	Mean Square	F	Sig.
Corrected Model		11	4469.282	53.609	.000
Intercept	1489888.094	1	1489888.094	17871.279	.000
Harvest	46979.955	2	23489.977	281.763	.000
Water	262.571	2	131.285	1.575	.228
Harvest * Water	649.684	4	162.421	1.948	.135
Rep	1269.895	3	423.298	5.077	.007
Error	2000.826	24	83.368		
Total	1541051.023	36			
Corrected Total	51162.929	35			

a. R Squared = .961 (Adjusted R Squared = .943)

Appendix Table 7 Tests of Between-Subjects Effects: ROOT YIELD

Source	Sum of Squares	df	Mean Square	F	Sig.
Corrected Model		11	495.066	21.661	.000
Intercept	53322.507	1	53322.507	2333.017	.000
Harvest	5077.536	2	2538.768	111.079	.000
Water	235.744	2	117.872	5.157	.014
Harvest * Water	96.506	4	24.127	1.056	.400
Rep	35.943	3	11.981	.524	.670
Error	548.534	24	22.856		
Total	59316.770	36			
Corrected Total	5994.263	35			

a. R Squared = .908 (Adjusted R Squared = .867)

Appendix Table 8 Tests of Between-Subjects Effects: NUMBER OF BULKING ROOTS

Source	Sum of Squares	df	Mean Square	F	Sig.
Corrected Model		11	495.066	21.661	.000
Intercept	53322.507	1	53322.507	2333.017	.000
Harvest	5077.536	2	2538.768	111.079	.000
Water	235.744	2	117.872	5.157	.014
Harvest * Water	96.506	4	24.127	1.056	.400
Rep	35.943	3	11.981	.524	.670
Error	548.534	24	22.856		
Total	59316.770	36			
Corrected Total	5994.263	35			

a R Squared = .908 (Adjusted R Squared = .867)

Appendix Table 9 Tests of Between-Subjects Effects: STARCH PERCENTAGE (REIMANN SCALE)

Source	Sum of Squares	df	Mean Square	F	Sig.
Corrected Model		11	495.066	21.661	.000
Intercept	53322.507	1	53322.507	2333.017	.000
Harvest	5077.536	2	2538.768	111.079	.000
Water	235.744	2	117.872	5.157	.014
Harvest * Water	96.506	4	24.127	1.056	.400
Rep	35.943	3	11.981	.524	.670
Error	548.534	24	22.856		
Total	59316.770	36			
Corrected Total	5994.263	35			

R Squared = .908 (Adjusted R Squared = .867)

Appendix Table 10 Tests of Between-Subjects Effects: Total Cyanide Content (Dry Basis)

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	5.035E7	29	1736275.297	43.821	.000
Intercept	1.569E8	1	1.569E8	3959.769	.000
Harvest_time	933313.598	2	466656.799	11.778	.000
Treatment	1006331.426	2	503165.713	12.699	.000
Root_P	4.252E7	2	2.126E7	536.552	.000
Harvest_time * Treatment	485582.097	4	121395.524	3.064	.021
Harvest_time * Root_P	3767425.877	4	941856.469	23.771	.000
Treatment * Root_P	891078.059	4	222769.515	5.622	.000
Harvest_time * Treatment * Root_P	682199.299	8	85274.912	2.152	.040
Rep#	67187.522	3	22395.841	.565	.640
Error	3090542.090	78	39622.334		
Total	2.103E8	108			
Corrected Total	5.344E7	107			

a. R Squared = .942 (Adjusted R Squared = .921)

Appendix Table 11 Report: Effect of Harvest Period on HCN Content of Whole Root

6, 9, 12		Dry_Total	Dry_NGC	Dry_Bound
1	Mean	1477.7317	122.8200	1354.9117
	N	12	12	12
	Std. Deviation	1090.84179	41.76383	1056.21046
	Variance	1189935.813	1744.217	1115580.529
	Std. Error of Mean	314.89890	12.05618	304.90170
2	Mean	1023.2767	147.9958	875.2817
	N	12	12	12
	Std. Deviation	622.66123	104.08223	529.84643
	Variance	387707.013	10833.111	280737.236
	Std. Error of Mean	179.74682	30.04595	152.95349
3	Mean	950.3400	76.0342	874.3042
	N	12	12	12
	Std. Deviation	283.07377	28.62281	277.14020
	Variance	80130.758	819.265	76806.692
	Std. Error of Mean	81.71636	8.26269	80.00349
TOTAL	Mean	1150.4494	115.6167	1034.8325
	N	36	36	36
	Std. Deviation	759.61407	71.58759	718.10240
	Variance	577013.535	5124.783	515671.054
	Std. Error of Mean	126.60234	11.93126	119.68373

Appendix Table 12 Report: Effect of Harvest Period on HCN Content of PEEL

6, 9, 12		Dry_Total	Dry_NGC	Dry_Bound
1	Mean	2797.0475	172.2967	2624.7525
	N	12	12	12
	Std. Deviation	745.70271	71.20626	705.02506
	Variance	556072.526	5070.332	497060.333
	Std. Error of Mean	215.26583	20.55548	203.52320
2	Mean	2061.7108	297.6017	1764.1092
	N	12	12	12
	Std. Deviation	602.48754	121.76766	510.13583
	Variance	362991.240	14827.364	260238.565
	Std. Error of Mean	173.92317	35.15130	147.26353
3	Mean	1473.6225	113.1017	1360.5208
	N	12	12	12
	Std. Deviation	455.48974	56.55831	421.53892
	Variance	207470.902	3198.842	177695.064
	Std. Error of Mean	131.48856	16.32698	121.68781
TOTAL	Mean	2110.7936	194.3333	1916.4608
	N	36	36	36
	Std. Deviation	809.65544	115.51679	761.43516
	Variance	655541.939	13344.128	579783.496
	Std. Error of Mean	134.94257	19.25280	126.90586

Appendix Table 13 Report: Effect of Harvest Period on HCN Content of PARENCHYMA

6, 9, 12		Dry_Total	Dry_NGC	Dry_Bound
1	Mean	616.1675	92.9450	523.2225
	N	4	4	4
	Std. Deviation	123.89669	30.64104	100.05669
	Variance	15350.390	938.873	10011.340
	Std. Error of Mean	61.94835	15.32052	50.02834
2	Mean	560.3325	91.5925	468.7400
	N	4	4	4
	Std. Deviation	75.47201	17.66976	62.60720
	Variance	5696.024	312.220	3919.662
	Std. Error of Mean	37.73600	8.83488	31.30360
3	Mean	823.8425	60.5575	763.2825
	N	4	4	4
	Std. Deviation	239.71527	13.01761	236.53720
	Variance	57463.409	169.458	55949.845
	Std. Error of Mean	119.85763	6.50881	118.26860
TOTAL	Mean	666.7808	81.6983	585.0817
	N	12	12	12
	Std. Deviation	188.23939	25.13043	192.14399
	Variance	35434.070	631.538	36919.314
	Std. Error of Mean	54.34003	7.25453	55.46719

Appendix Table 14 Report: Effect of Water Treatment on HCN Content (6MAP)

0,30,60		Dry_Total	Dry_NGC	Dry_Bound
0	Mean	1477.7317	122.8200	1354.9117
	N	12	12	12
	Std. Deviation	1090.84179	41.76383	1056.21046
	Std. Error of Mean	314.89890	12.05618	304.90170
	Variance	1189935.813	1744.217	1115580.529
1	Mean	1428.0292	109.2975	1318.7308
	N	12	12	12
	Std. Deviation	989.74553	48.48781	961.01314
	Std. Error of Mean	285.71492	13.99722	277.42060
	Variance	979596.213	2351.067	923546.247
2	Mean	1499.8075	134.7958	1365.0108
	N	12	12	12
	Std. Deviation	1179.31547	81.63521	1111.64416
	Std. Error of Mean	340.43905	23.56605	320.90403
	Variance	1390784.972	6664.307	1235752.742
Total	Mean	1468.5228	122.3044	1346.2178
	N	36	36	36
	Std. Deviation	1058.24553	59.10313	1014.71863
	Std. Error of Mean	176.37426	9.85052	169.11977
	Variance	1119883.606	3493.179	1029653.892

Appendix Table 15 Report: Effect of Water Treatment on HCN Content (9MAP)

0,30,60		Dry_Total	Dry_NGC	Dry_Bound
0	Mean	1023.2767	147.9958	875.2817
	N	12	12	12
	Std. Deviation	622.66123	104.08223	529.84643
	Std. Error of Mean	387707.013	10833.111	280737.236
	Variance	179.74682	30.04595	152.95349
1	Mean	1391.7633	221.7933	1169.9700
	N	904196.533	17927.687	672280.128
	Std. Deviation	274.49902	38.65196	236.69251
	Std. Error of Mean	958.6600	156.6875	801.9717
	Variance	12	12	12
2	Mean	692.83657	94.21556	617.18105
	N	480022.515	8876.571	380912.445
	Std. Deviation	200.00469	27.19769	178.16482
	Std. Error of Mean	1124.5667	175.4922	949.0744
	Variance	36	36	36
Total	Mean	770.92397	113.77328	667.27123
	N	594323.760	12944.360	445250.893
	Std. Deviation	128.48733	18.96221	111.21187
	Std. Error of Mean	904196.533	17927.687	672280.128
	Variance	274.49902	38.65196	236.69251

Appendix Table 16 Report: Effect of Water Treatment on HCN Content (12MAP)

0,30,60		Dry_Total	Dry_NGC	Dry_Bound
0	Mean	950.3400	76.0342	874.3042
	N	12	12	12
	Std. Deviation	283.07377	28.62281	277.14020
	Std. Error of Mean	80130.758	819.265	76806.692
	Variance	81.71636	8.26269	80.00349
1	Mean	1298.2025	117.2942	1180.9108
	N	12	12	12
	Std. Deviation	573.39751	55.62690	524.93326
	Std. Error of Mean	328784.706	3094.352	275554.925
	Variance	165.52560	16.05810	151.53518
2	Mean	1078.1708	67.6225	1010.5467
	N	12	12	12
	Std. Deviation	328.36295	27.70954	308.38480
	Std. Error of Mean	107822.229	767.819	95101.184
	Variance	94.79022	7.99906	89.02302
Total	Mean	1108.9044	86.9836	1021.9206
	N	36	36	36
	Std. Deviation	428.52460	44.22587	395.99658
	Std. Error of Mean	183633.329	1955.928	156813.294
	Variance	71.42077	7.37098	65.99943

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