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Possible causes and the molecular basis of hydrogen cyanogenesis production in cassava

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Abstract

Cassava (*Manihot esculenta*), is a major source of carbohydrates after rice and maize providing a basic diet to over half a billion people. It is an annual crop belonging to the family Euphorbiaceae. It produces edible root tubers which form the staple food for inhabitants in the developing world mainly in the tropical and subtropical countries. It is a very drought tolerant crop which is classified as either bitter or sweet cassava. However, it produces hydrogen cyanide (HCN) which is toxic. This antinutritional component can cause partial paralysis and have been known to kill and wipe out whole families in Africa. It is surprising that farmers seem to prefer the bitter varieties as they are starchier, deter pests and wild animals. There have not been adequate studies to evaluate the causes and molecular basis of the production of Hydrogen cyanide by cassava. This paper highlights the causes of HCN in cassava and ways of minimizing it for food security.

Key Words: Food security; Processing; Value addition; Starch; Ethanol; Cuttings

Introduction

Cassava (*Manihot esculenta,* Crantz), is globally the third largest source of carbohydrates after rice and maize providing a basic diet to over half a billion people. It is an annual rustic crop belonging to the dicotyledonous family Euphorbiaceae (Allem, 2002). It produces edible root tubers which form staple food for inhabitants in developing world mainly in the tropical and subtropical countries (Burns et.al. 2010). It is a very drought and acid soil tolerant crop (Java Cassava, 2007) which although it has thousands of cultivars, it can be classified as either bitter or sweet cassava (Wheatley et al., 1993). The bitterness indicates a physical warning of the presence of a poisonous substance which scientifically has been identified as hydrogen cyanide (HCN) (King and Bradbury, 1995). The cassava cultivars differ very little genetically for they are clonally propagated through cuttings, but surprisingly, studies show however that they have a wide variation in the concentration of HCN ranging from 1 to 2,000 mg/kg (Cardoso et al., 2005, CIAT 2007). The HCN is an antinutritional component which can cause partial paralysis and have been known to kill and wipe out whole families in Africa (Cassava Cyanide Diseases & Neurolathyrism Network (CCDNN), 2011). It is surprising that farmers seem to prefer the bitter varieties as they are starchier, deter pests and wild animals. There have not been many studies to evaluate the causes and molecular basis of the production of Hydrogen cyanide by cassava. This study is designed to attempt to do that. Furthermore, there has been no consensus of the positive correlation between bitterness and HCN level in cassava. For example, Bokanga and Bradbury, 1994, found an almost tasteless cassava variety with more HCN (15 mg of HCN per 100g) compared to a slightly bitter variety with 5mg of HCN per 100g. The problems associated with cassava HCN are not widespread outside Africa (Rosling, 1987), hence the causes of high HCN production should be some unique

practices done in Africa. Although, no cassava cultivar, lacks cyanogenic glycosides (Wanda et al., 1998).

The roots and leaves of cassava contain highest amounts of two cyanogenic glucosides (Sinha and Nair, 1968, Jorgensen et al., 2005, Cereda and Mattos, 1996) called linamarin and lotaustralin. The two are broken down by an enzyme called linamarase to produce HCN (Uyoh et al., 2007). However, leaves have a higher cyanogenic glycoside level of 5.0 g linamarin per kg of fresh weight, whereas roots have about twenty times lower. Tribes that traditionally consume cassava have come up with some methods of reducing HCN like soaking, cooking and fermentation, etc. Such tribes have also great ideas of how the HCN is produced by the cassava. This study attempts to reconcile this knowledge with the molecular basis of bitterness in cassava.

Previous studies to explore ways to minimize the cyanide content in cassava and its products had been undertaken but have focused mainly on agronomic factors such as the genotype or cultivar, stress, soil type, fertilization, processing techniques, such as cooking, soaking, fermenting and drying and finally harvest or post-harvest practices such as age at harvest, housing of products, storage time and temperature. The above should be combined with other recent advances in plant defense mechanisms and epigenetic studies. This is can be used to decipher the molecular basis of the cause of HCN production in cassava and enhance safety is this important diet crop.

The mechanisms of HCN production

The cyanogenic glycosides are enzymatically hydrolyzed by beta-glucosidase as the cassava root tissues are squeezed during chewing or in the intestine as they are being broken down by gut microorganisms to release HCN which harmful to the consuming predator or human (Poulton, 1988). Studies have shown that HCN producing plants should remain relatively free of damage by general herbivores (Schappert & Shore, 1999; Viette *et al.*, 2000), but can still be attacked by specialists (Glander *et al.*, 1989; Ferreira *et al.*, 1997). In all studies done, it is becoming clear that cyanogenic glycoside and its corresponding cyanogenic enzymes are localized in different cellular compartments or tissues. Therefore, this prevents mixing and cyanogenesis until the tissues are disrupted (White et al., 1998).

Separation and mixing of glucoside and linamarin in cassava

The separation of the substrate and cyanogenic enzymes in some plants is at the subcellular level while in others like sorghum is at the tissue level (Wajant et al., 1994). For example, in rubber trees, the endosperm contains linamarin but the linamarase is located in the apoplast. (Poulton, 1990; Selmar, 1993).

In cassava leaves, linamarin is located in the vacuoles, while the enzyme linamarase is localized to cell walls and laticifers almost 8-fold (Mkpong et al., 1990; Pancoro and Hughes, 1992; Hughes et al., 1994; McMahon et al., 1995; White et al., 1994; White and Sayre, 1995). These results suggest that for HCN to be released there should be a mechanical disruption strong enough to trigger the mixing. Previous studies have shown that linamarin and its β-glucosidase, linamarase, are actually present in all cassava organs except seeds (McMahon et al., 1995). An explanation of this substances being located in the cell wall is to offer a physical deterrence against attackers who are trying to gain entry into the cell, but if they enter the cell then the cell triggers another chemical defense against them. This seems to be similar to the two lines of defense of animals' cells against pathogens, the latter being antibodies production. The cassava peel which account for 11% -20 % of the root weight is made up of sclerenchyma and phloem cells; it has a high amount of cyanogenic glycoside and is therefore removed during cassava processing (Sayre et al, 2011) by almost all consumers. The nature and amount of preformed pathogen inhibitors are influenced by the environment, genotype and age of the plant (Price et al., 1987; Davis, 1991).

The possible epigenetic link of HCN release in cassava

Studies by White at al., 1998 suggested that, the molecular basis for the absence of hydroxynitrile lyase, which catalyse the last step to release HCN from roots and stems could be attributed to very low steady-state hydroxynitrile lyase transcript levels (relative to leaves), suggesting that hydroxynitrile lyase expression is regulated at a pretranslational level. However, later studies confirmed that the mechanical disruption could be responsible for its release, for example, it could be found in leaves which are always disturbed compared to stem and leaves in studies in cassava, sorghum and flax (*Linum usitatissimum*) (Wajant et al., 1994). Later studies could however not fully establish whether linamarin is transported apoplastically between shoots and roots or between root cells (White et al., 1998). Other similar studies further point to the epigenetic expression due to biotic stress, for example, the expression of the rice *pir* gene is induced during fungal infection. Plants seems to release the enzymes based on some epigenetic memory of the stress using epigenetic processes, like include inherited DNA methylation and histone modifications (Chinnusamy and Zhu 2009), in subsequent cassava generations (Chitwood and Timmermans 2010). Epigenomic control modulates gene expression in response to environmental stimuli through signal transduction and other rapid defenses responses. Cassava has been classified as sweet and bitter cultivars; this demarcation can also be related to the production of defense chemicals (cyanogenesis) by the plants against herbivores and pathogens at the same time. In places with mixed farming the cassava plant might be in close proximity with other plants which attract many microbes, this might make the cassava to produce more defense chemicals than one which is grown in monoculture systems. A cassava in poor soils or harsh environment might also be targeted by pathogens and hence it might produce more toxic defense chemicals. This might explain

some cases of cassava poisoning in east Africa region compared to west African region.

The rapid response of cassava to abiotic stresses

The cassava plant opens its stomata only at low evaporation demand and when water use efficiency is highest. The leaves show heloitropic responses making it to obtain maximum light (Berg *et al.,* 1986). The leaves also droop at bright noon light to protect it from excess UV light. (Catalayud *et al.,* 2000; El Sharkawy, 2006). It is no surprise if it has internal mechanisms to protect itself from predators including root pathogens like *Phytophthora* root rots (PRR; *Phytophthora* spp. (Edison, 2002).

Cyanogenic glycosides are used by many plants to defend themselves (Francisco and Pinotti, 2000). They also regulate the plant-insect interactions (Zagrobelny *et al.,* 2004). There are at least 2500 species of plants that produce cyanogenic glycosides and a corresponding hydrolytic enzyme called beta-glycosidase. The plant-predator protection mechanism occurs when the two produce a sugar and a cyanohydrin which rapidly decomposed to HCN and an aldehyde or a ketone (Hosel, 1981; Moller and Seigler, 1999). Three, glycosides, cyanohydrins and hydrogen cyanide are known as cyanogens (Moller and Seigler, 1999).

The method of harvesting and hydrogen cyanide production

Cassava is generally manually harvested. The stems are cut off 40 - 60 cm above the soil so that the stem portion can be handled when uprooting the tubers. In other cases, harvesting involves digging up the roots (Java Cassava, 2007). The correlation between the harvesting method and amount of HCN has not so far been investigated.

Methodology

This study was done at Mua Hills in Machakos County, Kenya. The lower sides towards Kapiti plains usually has farmers planting cassava but the farmers are harassed by porcupines from the neat by game reserve. The study observed the predatory mechanism of porcupines on cassava and recorded observation in form of photos. The study also focused on literature on cassava on world wide scale. We analyzed and explained some previous results based on recent findings and observations.

Results

Animals studies on Cassava HCN

Our studies with porcupines and cassava farms in Mua Hills in Machakos County, Kenya, showed that when a porcupine dug the roots of cassava and consumed a portion of the root, the porcupine did not return to the same plant the next day. The most likely explanation was that the cassava produced HCN to deter the porcupine from coming to finish its stored food. The porcupine could have the ability to sniff high dangerous concentrations of HCN. This made it to go and dig another plant (Figure 1).

Figure 1: Portion of Cassava root left by a porcupine (A) and prepared another smaller one (B) probably due to the plant accumulating more HCN as an internal plant defense mechanism.

Studies in cassava farms

Our studies showed disturbed cassava farms produced bitter roots, for example when animals passed through a cassava farm the cassava dug from the farm where mostly bitter, when one dug cassava roots immediately after a shower the roots where majorly bitter, also when one hung clothes on cassava plants and latter dug the roots the plants had bitter roots. Surprisingly, when small children struggled to get a cassava root and spent more time trying to get the root out, the roots turned to be bitter.

Our Literature studies found out that two families of Makueni District and another of Kathonzweni District, in Kenya were affected after consuming raw and cooked cassava in August and September 2011. A 4-year-old child died in the first family, the family looked extremely poor and the only meal they had was boiled and raw cassava. In the second family, a child aged 5 died in Makueni District Hospital while continuing with management. Both families

complained of headaches, abdominal pains and discomfort vomiting, general body weakness and some fever. The Health Officer collected the cooked and uncooked cassava and fresh samples from the same plants where cassava was harvested. The cassava tasted bitter as claimed by the family members. The area had experienced drought for the last 3 years (Cassava Cyanide Diseases & Neurolathyrism Network, 2011).

The time required for a cassava to produce HCN

In removing HCN people usually soak the root tubers for 4 hours but that is not sufficient, only 18–24 hours can reduce HCN by 50%. A dose of 0.5 to 3.5 mg per kg of human body weight is enough to show HCN intoxication symptoms like rapid respiration, low blood pressure, headache and dizziness, intestinal pain, vomiting and diarrhea and can result in death. The mechanical shaking of a cassava plant is easily transferred to the roots as the plant has heavy leaves which are close to the ground where the tubers are formed (Figure 2).

Figure 2: Cassava tuber exposed due to disturbance by animals and humans. This shows that little disturbance by animals and humans can reduce the probability of cassava plants producing lethal doses of HCN. The soft soil above enabled a porcupine to at least eat a portion of the cassava before the plant started to epigenetically defend itself.

HCN studies on food products

Literature studies showed that samples from Vanuatu had HCN levels of 26 mg/kg to 78 mg/kg but the flour sample from the same had more cyanide content of 57 mg/kg while the cassava chips had 60 mg/kg. When cassava is stored under ambient temperatures, the cyanide levels drops by about 30% after four days (Dolodolotawake and William, 2011).

Other studies in Africa show a seasonal variation in cassava HCN levels with higher levels in dry conditions with the cassava becoming bitter. The cyanide content was found to be higher in younger leaves compared to older ones (Hidayat et al., 2002).

Discussions and Conclusions

Time dependent production of HCN during the harvest period of cassava

The production of Cyanogenesis in cassava can be seen first as a static protection offered by a particular cultivar's constitutive level of cyanogenic glycosides which causes it to have a certain level of bitter taste. Secondly, it can be viewed as a rapid formation of HCN during a mechanical disturbance or a feeding episode by chewing animals or insects on leaves. The first production of bitter glucosides is cultivar, level of growth and other environmental factors, but the second one is a kind of an epigenetic regulation which is rapid as catalyzed by endogenous enzymes to produce HCN. This can help us to understand why some cassava cultivars are mildly bitter but may not be toxic at the level of HCN production depending of the level of disturbance just before harvesting. Some specialized

predators like insects have enzymes that transform cyanogenic glycosides into harmless substance in their gut (Fitzgerald *et al*., 2002) or may hinder the conversion of glucosides in to HCN in their gut, while those who are not adapted to the toxic have to chew it in a way that make it to release the HCN which is lost into the atmosphere before they swallow (Alonso- amelot et al., 2006). Human being depends of the second method to mechanically reduce the HCN before consuming. Therefore, the various processing techniques of cassava significantly reduces the toxicity of HCN because studies have shown that the proportion of HCN, diffused and ingested, will depend on HCN evolution by the plant's tissue, the speed at which the root tuber is eaten. Amazingly, the HCN is also harmful to the plant; therefore it must be produced at a rapid speed at the time of attack or disturbance. Other noncyanogenic aminoacid precursors have been used by plants to deter predators during seed germination and early seedling growth. This phenomenon has been observed in other plants for example in *Pteridium arachnoideum* (Alonso-Amelot and Oliveros, 2005; Eucalyptus *polyanthemos* (Goodger *et al.*, 2002) and in the legume *Phaseolus lunatus* (Ballhorn *et al*., 2005). The number of cyanogenic glycosides varies in different plant tissues, organs, species and environmental conditions where it grows (Gleadow & Woodrow, 2000).

Normally, human beings have acidic stomach environments that deactivate the β-glucosidase enzyme making the production of HCN not possible. How it is that diversification of diet may reduce HCN poisoning? In humans, HCN is detoxified by the enzyme rhodanese, forming thiocyanate, which is excreted in the urine. However, this detoxification used Sulphur donors, which are derived from Sulphur amino acids from the protein rich food consumed.

The epigenetic link of HCN production and food security

The above studies strongly suggest that cassava uses an internal molecular mechanism to protect itself from herbivores and other enemies. It stays alert by preparing a precursor for HCN which through a rapid epigenetic mechanism it triggers an expression of the genes of HCN metabolic pathway when mechanically disturbed. Some cassava plants do not find it necessary to constantly produce the HCN precursor because they are in favourable conditions for a long time. Hence, as we plan to utilize this dry land resource for food security, we should be aware of this by avoiding the HCN from the cassava plant through careful harvesting, processing and habitat selection.

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