



Research Article

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 ant nutritional component can cause partial paralysis and have been known to kill and wipe out 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. Observations of feeding patterns of porcupines on cassava roots, defense mechanisms in cassava and macro level results on cassava metabolism were hereby used to explain the molecular epigenetic link of cyanogenesis of cassava. The results explain the exogenous release and its subsequent removal of HCN during cassava processing. It shall form the basis for the selection and improvement of cassava products for food security.

Keywords: Food security, Processing, Value addition, Starch, Ethanol, Cuttings.

Abbreviations: HCN-Hydrogen cyanide, PRR-Phytophthora root rots, E-Enhancers, P-Promoters.

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 [1]. It produces edible root tubers which form staple food for inhabitants in developing world mainly in the tropical and subtropical countries [2]. It is a very drought and acid soil tolerant crop which although it has thousands of cultivars, it can be classified as either bitter or sweet cassava [3,4]. The existence bitterness indicates physical warning of the presence of a poisonous substance which scientifically has been identified as Hydrogen Cyanide (HCN) [5]. Although cassava cultivars are clonally propagated through cuttings and hence are expected to differ little genetically. Studies have surprisingly however, shown that they have wide variations in HCN concentration ranging from 1 to 2,000 mg/kg [6,7]. The HCN is an ant nutritional component which can cause partial paralysis and have been known to kill and wipe out families in Africa [8]. 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.

The Statement of the Problem

Furthermore, there has not been significant consensus of the positive correlation between bitterness and HCN level in cassava. For example, Bokanga and Bradbury, (1994) [9] 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 the production of cassava HCN are

not widespread outside Africa, hence the causes of high HCN production should be some unique practices done in Africa [10]. Although, studies have shown that no cassava cultivar, lacks cyanogenic glycosides because each has a way of protecting itself depending on the level and frequency of threats [11]. The roots and leaves of cassava contain highest amounts of two cyanogenic glucosides referred to as linamarin and lotaustralin [12-14]. The two are broken down by an enzyme called linamarase to produce HCN [15]. However, leaves have higher cyanogenic glycoside levels of 5.0 g linamarin per kg of fresh weight, whereas roots have about twenty times lower than leaves [11].

Reconciling the Experiential and Research Knowledge of Cassava Bitterness

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. 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. These include 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. Hence, the objective of this study was to explain what actually happens at the molecular level before the phenotypic observation is made. This shall reconcile experiential



practices with experimental knowledge concerning the molecular basis of bitterness in cassava.

The Foundations for Unlocking the Mechanisms of HCN Production

The cyanogenic glycosides are enzymatically hydrolyzed by beta-glycosidase 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 [16]. Studies have shown that HCN producing plants should remain relatively free of damage by general herbivores, but can still be attacked by specialists like porcupines which have through experience known how to overcome the HCN defenses in cassava [17-20]. These are herbivores which have devised HCN minimizing mechanisms. 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 is disrupted [11].

Separation and Mixing of Glucoside and Linamarin in Cassava

In some plants, the separation of the substrate and cyanogenic enzymes is at the subcellular level while in others like sorghum is at the tissue level [21]. For example, in rubber trees, the endosperm contains linamarin but the linamarase is located in the apoplast [22,23].

In cassava leaves, linamarin is located in the vacuoles, while the enzyme linamarase is localized to cell walls and laticifers almost 8-fold [24-27]. 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 -glycosidase, linamarase, are actually present in all cassava organs except seeds. An explanation of why this substance is located in the cell wall is that it serves as signal to detect and transmit any significant physical interference from exogenous attackers who are trying to gain entry into the cell. However, if they enter the cell or a strong enough, then the cell triggers another chemical defense against them endogenously. 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 by almost all consumers [28]. The nature and amount of preformed pathogen inhibitors are influenced by the environment, genotype and age of the plant [29,30].

The Possible Epigenetic Link of HCN Release in Cassava

Studies by White, et al. [11] suggested that, the molecular basis for the absence of hydroxynitrile lyase, which catalyzes 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*) [31]. Later studies could however not fully establish whether linamarin is transported apoplastically between shoots and roots or between root cells [11].

Other similar studies further point to the epigenetic expression due to biotic stress, for example, the expression of the rice per 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, in subsequent cassava generations [32,33]. 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 heliotropic responses making it to obtain maximum light. The leaves also droop at bright noon light to protect it from excess UV light [34]. It is no surprise if it has internal mechanisms to protect itself from predators including root pathogens like Phytophthora Root Rots (PRR) Phytophthora spp. [35].

Cyanogenic glycosides are used by many plants to defend themselves [36]. They also regulate the plant-insect interactions [37]. 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. Three, glycosides, cyanohydrins and hydrogen cyanide are known as cyanogens.

Recent studies have also associated production of HCN to abiotic stresses like dry spells which encourage water stress, increased weeds in the cassava farm, soil characteristics (which might mean deficiency or toxicity of particular elements), the age of the plant, piece meal mechanical harvesting (indicating disturbance of ground around cassava plants) and cassava branch pruning [38].

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 [3]. The correlation between the harvesting method and amount of HCN has not so far been investigated.

Method

Area of Study

This study was done at Mua Hills in Machakos County, in Kenya, East Africa, which lies at latitude: 1.45 South and longitude: 37.21 East with an altitude up to 1967.00m/6453.41ft. The area has red soils which are loosely packed. The cassava is grown in ridges of soils. The temperatures are between 18-25°C in a day. The area is near a game reserve with numerous wild animals including nocturnal animals like the African porcupines (*Hystrix cristata*).

The lower side towards Kapiti plains usually has farmers planting cassava but the farmers are harassed by porcupines from the nearby game reserve. The study aimed at providing molecular explanation of the macro level experimental results of cassava studies with experiential practices by farmers and consumers by utilizing plant molecular epigenetic findings. Systematic observation on nocturnal predators on cassava was done. These predatory patterns of porcupines on cassava were recorded observation in form of photos

for detailed analysis. The study also focused on literature on cassava on world wide scale and analyzed and explained some previous results based on recent findings and observations.

Results

Porcupine Studies on Cassava HCN

Our studies with porcupines and cassava farms at the border between the in Mua Hills and the Kapiti plains showed that when a porcupine dug the roots of a particular cassava and consumed a portion of its root, the porcupine did not return to the same plant the next day.

These puzzled researchers, who thought that the economics of rejecting ready food and sacrificing to dig for fresh one could, have meant a life and death affair. The most likely explanation was that the cassava produced a fatal HCN to deter the porcupine from coming to back to the same plant to finish its left-over food. Furthermore, the porcupine could have the ability detect the presence of HCN. This could be via the sniffing and detecting of high dangerous concentrations of HCN. This detection made it to go and dig another plant further from the first one. This is because ground disturbance could trigger HCN production in neighboring cassava plants (Figure 1).



Note: (A) while it tasted the next plant (B) to examine the level of HCN due to its disturbance. The explanation of the above behavior of porcupine was probably due to the cassava plant accumulating more HCN as an internal defense mechanism.

Figure 1: Portion of Cassava root left by a porcupine.

Studies on 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 were 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 Kathonzwani 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 HCN poisoning 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 [39].

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).

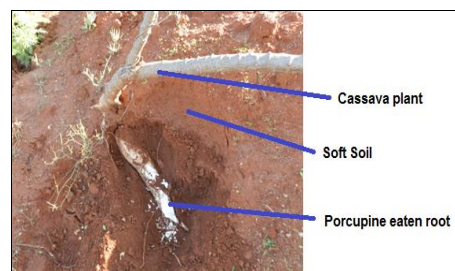


Figure 2: Cassava tuber exposed due to disturbance by animals and humans. This shows that little disturbance by animals and humans can reduce the probability of cassava plants producing lethal doses of HCN. The soft soil above enabled a porcupine to at least eat a portion of the cassava before the plant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

HCN Studies on Food Products

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. Cassava is stored under ambient temperatures, the cyanide levels drops by about 30% after four days [39]. Other studies in Africa showed 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 [40].

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 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 [41]. 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. 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 amino acid 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*, *Eucalyptus polyanthemos* and in the legume *Phaseolus lunatus* [42-44].

The number of cyanogenic glycosides varies in different plant tissues, organs, species and environmental conditions where it grows [45]. 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 [46-48].

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 favorable 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 (Figure 3).

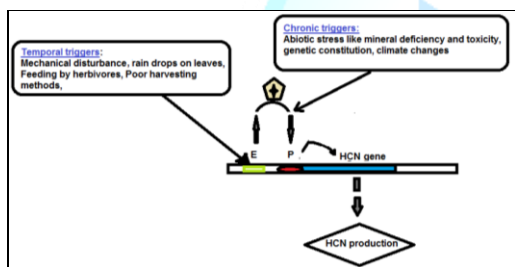


Figure 3: The postulated molecular epigenetic control of HCN production at the gene level.

Temporal HCN production caused mainly by external mechanical factors act on upstream Enhancers (E) of the DNA. This could be via simple nucleosome configurations. When this stimulation passes a certain threshold, in respect of minimizing metabolism expenditure, it triggers transcription factors to regulate cytosine methylation of demethylation causing the activation of Promoters (P) which now increases the internal chronic HCN production to cause bitterness in a cassava variety.

References

1. Allem AC. The closest wild relatives of cassava (*Manihot esculenta*) (1999) *Euphytica* 107: 123-133. <https://doi.org/10.1023/A:1026422229054>
2. Burns A, Gleadow R, Cliff J, Zacarias A and Cavagnaro T. Cassava: The drought, war and Famine crop in a changing world (2010) *Sustainability* 2: 3572-3607. <https://doi.org/10.3390/su2113572>
3. Utomo WH, Wargiono J and Islami T. Cassava production and utilization in Indonesia (2006) ISTRC Conference, India.
4. Wheately CC, Orrego JI, Sanchez T and Granados E. Quality evaluation of cassava core collection at CIAT. In Roca AM and Thro AM (Ed) (1993) *Proceedings of the First International Scientific Meeting of Cassava Biotechnology Network*, CIAT, Colombia 379-383.

5. King NLR and Bradbury JH. Bitterness in cassava: Identification of a new apiosyl glucoside and other compounds that affect its bitter taste (1995) *J Sci Food Agric* 68: 223-230. <https://doi.org/10.1002/jsfa.2740680214>
6. Cardoso AP, Mirione E, Ernesto M, Massaza F, Cliff J, et al. Processing of cassava roots to remove cyanogens (2005) *J Food Comp Anal* 18: 451-460. <https://doi.org/10.1016/j.jfca.2004.04.002>
7. Centro InternationL de Agricultura Tropical (CIAT) Annual report. Improved Cassava for the developing world (2007) 39-40.
8. [Cassava Cyanide Diseases and Neurolathyrism Network \(CCDNN\) \(2011\) NEWS.](https://doi.org/10.1002/jsfa.2740680214)
9. Bokanga M and Bradbury JH. ACIAR Report; Cassava Cyanide: Improved Techniques for estimation and influence of environment on concentration (1994) Australian center for international agricultural research, Australia, pp 11.
10. Rosling H. Cassava toxicity and food security. A review of health effects of cyanide exposure from cassava and of ways to prevent these effects (1987) Report for UNICEF, Sweden 1-40.
11. Wanda LBW, Arias-Garzon ID, Jennifer MM and Richard TS. Cyanogenesis in Cassava. The Role of Hydroxynitrile Lyase in Root Cyanide Production (1998) *Plant Physiol* 116: 1219-1225. <https://doi.org/10.1104/pp.116.4.1219>
12. Sinha SK and Nair TVR. Studies on the variability of cyanogenic glucoside content in cassava tubers (1968) *Indian J Agric Sci* 38: 958-963.
13. Jorgensen K, Bat S, Busk PK, Sorenson C, Olsen CE, et al. Cassava plants with depleted cyanogenic glucoside content in leaves and tubers. Distribution of cyanogenic glucosides, their site of synthesis and transport and blockage of the biosynthesis by RNA interference technology (2005) *Plant Physiol* 139: 363-364. <https://doi.org/10.1104/pp.105.065904>
14. Cereda MP and Mattos MCY. Linamarin, the toxic compound of cassava (1996) *J Venom Anim Toxins* 2. <https://doi.org/10.1590/S0104-79301996000100002>
15. Uyoh EA, Udensi O, Natui V and Urua I. Effect of different processing methods on cyanide content of garri from four cultivars of cassava (2007) *J Food Agri Environ* 5: 105-107.
16. Poulton JE. Localization and Catabolism of Cyanogenic Glycosides. In *Cyanide Compounds in Biology* (1988) *Ciba Found Symp* 140: 67-71. <https://doi.org/10.1002/9780470513712.ch6>
17. Schappert PJ and Shore JS. Cyanogenesis, herbivory and plant defense in *Turnera ulmifolia* on Jamaica (1999) *Ecosci* 6: 511-520. <https://doi.org/10.1080/11956860.1999.11682560>
18. Viette M, Tettamanti C and Saucy F. Preference for acyanogenic white clover (*Trifolium repens*) in the vole *Arvicola terrestris*: II. Generalization and further investigations (2000) *J Chem Ecology* 26: 101-122. <https://doi.org/10.1023/A:1005441528235>
19. Glander KE, Wright PC, Seigler DS, Randrianasol B and Randrianasol V. Consumption of cyanogenic bamboo by a newly discovered species of bamboo lemur (1989) *Am J Primatol* 19: 119-124. <https://doi.org/10.1002/ajp.1350190205>
20. Ferreira C, Parra RP and Terra WR. The effect of dietary plant glycosides on larval-glucosidases from *Spodoptera frugiperda* and *Diatraea saccharalis* (1997) *Insect Biochem Molecular Bio* 27: 55-59. [https://doi.org/10.1016/S0965-1748\(96\)00069-0](https://doi.org/10.1016/S0965-1748(96)00069-0)
21. Wajant H, Riedel D, Bent S and Mundry KW. Immunocytological localization of hydroxynitrile lyases from *Sorghum bicolor* L. and *Linum usitatissimum* L (1994) *Plant Sci* 103: 145-154. [https://doi.org/10.1016/0168-9452\(94\)90202-X](https://doi.org/10.1016/0168-9452(94)90202-X)
22. Poulton J. Cyanogenesis in plants (1990) *Plant Physiol* 94: 401-405. <https://doi.org/10.1104/pp.94.2.401>
23. Selmar D. Transport of cyanogenic glycosides: uptake of linustatin by *Hevea cotyledons* (1993) *Planta* 191: 191-199. <https://doi.org/10.1007/BF00199749>



24. Mkpog O, Yan H, Chism G and Sayre RT. Purification, characterization and localization of linamarase in cassava (1990) *Plant Physiol* 93: 176-181. <https://doi.org/10.1104/pp.93.1.176>
25. Pancoro A and Hughes MA. In situ localization of cyanogenic glucosidase (linamarase) gene expression in leaves of cassava (*Manihot esculenta*, Crantz) using non-isotopic riboprobes (1992) *Plant J* 2: 821-827. <https://doi.org/10.1111/j.1365-313X.1992.tb00152.x>
26. White W, McMahon J and Sayre RT. Regulation of cyanogenesis in cassava (1994) *Acta Hort* 375: 69-78. <https://doi.org/10.17660/ActaHortic.1994.375.4>
27. White W and Sayre RT. The characterization of hydroxynitrile lyase for the production of safe food products from cassava (*Manihot esculenta*, Crantz), In DL Gustine and HE Flores (Ed) (1995) *Phytochem Health Curr Top Plant Physiol* 15: 303-304.
28. Sayre R, Beeching JR, Cahoon EB, Egesi C, Fauquet C, et al. The biocassava plus program: biofortification of cassava for Sub-Saharan Africa (2011) *Annu Rev Plant Biol* 62: 251-272. <https://doi.org/10.1146/annurev-arplant-042110-103751>
29. Price KR, Johnson IT and Fenwick GR. The chemistry and biological significance of saponins in food and feeding stuffs (1987) *Crit Rev Food Sci Nutr* 26: 27-135. <https://doi.org/10.1080/10408398709527461>
30. Davis RH. Glucosinolates in Toxic Substances in Crop Plants, DMello JP, Duffus CM and Duffus JH (Ed) (1991) Royal Society of Chemistry, United Kingdom, pp-202-225.
31. Wajant H and Mundry KW. Hydroxynitrile lyase from Sorghum bicolor: a glycoprotein heterodimer (1993) *Plant Sci* 89: 127-133.
32. Chinnusamy V and Zhu JK. Epigenetic regulation of stress responses in plants (2009) *Curr Opin Plant Biol* 12: 133-139. <https://doi.org/10.1016/j.pbi.2008.12.006>
33. Chitwood DH and Timmermans MCP. Small RNAs are on the move (2010) *Nature* 467: 415-419. <https://doi.org/10.1038/nature09351>
34. El-Sharkawy MA. International research on cassava photosynthesis, productivity, ecophysiology, and responses to environmental stresses in the tropics (2006) *Photosynthetica* 44: 481-512. <http://dx.doi.org/10.1007/s11099-007-0067-4>
35. Edison S. Plant protection problems in cassava in India, Howeler RH (Ed) (2002) *Proceedings 7th Regional Cassava Workshop*, Thailand, pp-264-270.
36. Francisco LA and Pinotti MHP. Cyanogenic glycosides in plants (2000) *Brazilian Archives Bio Tech* 43: 487-492. <https://doi.org/10.1590/S1516-89132000000500007>
37. Zagrobelny M, Bak S, Rassmusen AV, Jørgensen B, Naumann CM, et al. Cyanogenic glucosides and plant-insect interactions (2004) *Phytochemistry*, 65: 293-306. <https://doi.org/10.1016/j.phytochem.2003.10.016>
38. Imakumbili MLE, Semu E, Semoka JMR, Abass A and Mkamilo G. Farmers' perceptions on the causes of cassava root bitterness: A case of konzo-affected Mtwara region, Tanzania (2019) *PLoS ONE* 14: e0215527. <https://doi.org/10.1371/journal.pone.0215527>
39. Dolodolotawake U and William GLA. Cyanide content of cassava and cassava products in some Pacific Island countries (2011) *Professional and Technical Reports*, The University of The South Pacific, pp-3-5. <http://repository.usp.ac.fj/id/eprint/4852>
40. Hidayat A, Zuraida N and Hararida I. The Cyanogenic Potential of Roots and Leaves of Ninety Nine CASSAVA cultivars (2002) *Indonesian J Agri Sci* 3: 25-32. <http://dx.doi.org/10.21082/ijas.v3n1.2002.p25-32>
41. Alonso-amelot M, Avila NJL, Lisday D and Bastidas O. A Hydrogen cyanide release during feeding of generalist and specialist lepidopteran larvae on a cyanogenic plant, *Passiflora capsularis* (2006) *Physiological Entomol* 31: 307-315. <https://doi.org/10.1111/j.1365-3032.2006.00528.x>
42. Alonso-Amelot ME and Oliveros A. Kinetics of the natural evolution of hydrogen cyanide in plants in neotropical *Pteridium arachnoideum* and its ecological significance (2005) *J Chem Ecol* 31: 315-331. <https://doi.org/10.1007/s10886-005-1343-z>
43. Goodger JQD, Capon RJ and Woodrow IE. Cyanogenic polymorphism in *Eucalyptus polyanthemus* Schauer subsp. *Vestita* L. Johnson and K. Hill (Myrtaceae) (2002) *Biochem Sys Ecol* 30: 617-630. [https://doi.org/10.1016/S0305-1978\(01\)00141-7](https://doi.org/10.1016/S0305-1978(01)00141-7)
44. Ballhorn DJ, Lieberei R and Ganzhorn JU. Plant cyanogenesis of *Phaseolus lunatus* and its relevance for herbivore-plant interaction: the importance of quantitative data (2005) *J Chem Ecol* 31: 1445-1473. <https://doi.org/10.1007/s10886-005-5791-2>
45. Gleadow RM and Woodrow IE. Temporal and spatial variation in cyanogenic glycosides in *Eucalyptus cladocalyx* (2000) *Tree Physiol* 20: 591-598. <https://doi.org/10.1093/treephys/20.9.591>
46. Fokunang CN, Tomkins PT, Dixon AGO, Tembe EA, Salwa B, et al. Cyanogenic potential in food crops and its implication in cassava (*Manihot esculenta* Crantz) production Pakistan (2001) *J Biological Sci* 4: 926-930. <https://doi.org/10.3923/pjbs.2001.926.930>
47. Wajant H and Pfizenmaier K. Identification of potential active site residues in the hydroxynitrile lyase from *Manihot esculenta* by site-directed mutagenesis (1996) *J Biol Chem* 271: 25830-25834. <https://doi.org/10.1074/jbc.271.42.25830>
48. Yemm R and Poulton J. Isolation and characterization of mandelonitrile lyase from mature black cherry (*Prunus serotina*) seeds (1986) *Arch Biochem Biophys* 247: 440-445. [https://doi.org/10.1016/0003-9861\(86\)90604-1](https://doi.org/10.1016/0003-9861(86)90604-1)