



Evaluation of postharvest physiological deterioration of cassava (*Manihot esculenta*) genotypes

S Sowmyapriya¹, MK Kalarani², K Manivannan³, C Mohan⁴

¹ Department of Crop Physiology, Palar Agricultural College, Vellore, Tamil Nadu, India

² Department of Crop Physiology, Tamil Nadu Agricultural University, Coimbatore, Tamil Nadu, India

³ Department of Vegetable Crops, Krishna College of Agriculture and Technology, Madurai, Tamil Nadu, India

⁴ Department of Crop Protection, Sugarcane Research Station, Melalathur, Vellore, Tamil Nadu, India

Abstract

Cassava (*Manihot esculenta*) is the well most important tuber crop in the tropics and sub tropics due to its drought tolerance, ability to grow in any soils and resistance to herbivore, cassava is well suited for cultivation by small and big farmers. However, it is short shelf life of the tuber due to Postharvest Physiological Deterioration (PPD) which limits cassava's economic and industrial potential. Postharvest Physiological Deterioration in cassava tuber is rapid, begins within 5 to 24 hrs after harvest and its induce oxidative burst of Reactive Oxygen Species (ROS) which leads to produce hydrogen cyanide (HCN). The visible signs of deterioration are vascular streaking with a blue green discoloration that cause to be the tubers un edible and unmarketable. The present study was undertaken to evaluate 35 cassava genotypes for PPD tolerance. Tubers from different cassava genotypes were evaluated at 1,2,3,4 and 5 days after harvest for PPD. PPD was observed and cyanide and starch were analyzed each and every day. Two genotypes viz., CI-850 and YTP-1 showed their supremacy in recording low levels of PPD 9.7 and 11.3 percent respectively even five days after harvest. These genotypes can be used as novel donor sources in breeding programmes aimed for developing PPD tolerant genotypes.

Keywords: cassava tuber, postharvest physiological deterioration, hydrogen cyanide, starch, shelf life

Introduction

Cassava (*Manihot esculenta* Crantz) is the most widely cultivated tuber crop in the tropics. Worldwide, it is the sixth most important crop in terms of production and is a major staple food crop to over 800 million populations in the world. Cassava has emerged as a multipurpose crop for the 21st century it could be one of the most climate change-resilient crops and a food secure future for millions of people (El-Sharkawy, 2014) [2]. Temperature rises of between 1.2 and 2 degrees celsius by 2030, combined with changes in rainfall patterns. "Using a combination of 24 climate prediction and crop suitability models, compared the expected impacts of climate change on the production of cassava and other staples potato, maize, bean, banana, millet, and sorghum, would leave cassava outperforming the other crops (Salcedo *et al.*, 2010) [11]. Cassava is a survivor and it's like the Rambo of the food crops. So it has the tolerance to extreme environments, such as drought, high temperature and poor soil has earned its name as a

"Famine reserve crop" and it can reduce nutrition demands and decrease climate risk".

World production of cassava in 2013-14 was estimated at over 277 million tones and is expected to increase further due primarily to both higher demand as human food and its value as raw material for industrial purposes. In cassava cultivation, India ranks 25th in area, 11th in production and 1st in pr

ductivity (34.95 ton ha⁻¹) (pro@tnau.ac.in). India exports several forms of cassava products like raw cassava tuber, starch and sago (Figueiredo *et al.*, 2013) [4]. However, the short shelf life of the tuber (24 hours) limits cassava's economic and industrial potential. Even though cassava has been already identified as a superior and high productive crop for future

climate change condition, the short storage life of cassava tuber is directly linked to an endogenous physiological process known as Postharvest Physiological Deterioration (PPD) which is considered to be a complex procedure linked to enzymatic stress response to wounding (Andres Salcedo and Dimuth Siritunga, 2011) [1]. So, during the period of higher demand or supply to the market, storage becomes the major problem. Put another way, it is leading to large foregone opportunities in farmer's income and rural socioeconomic development.

PPD in cassava is rapid, begins within 24 to 48 hrs after harvest and can result in losses in the range of 40 – 60 per cent of the total expected economic value of the crop (Morante *et al.*, 2010) [8]. Postharvest deterioration occurs in two phases, primary and secondary deterioration. The primary deterioration starts from the central vascular bundles of the root, spreads to the adjacent storage parenchyma and subsequently stored starch undergoes structural changes is known as postharvest physiological deterioration of which the produces cyanogenic glycosides, which break down to release cyanide (HCN) when cells are mechanically damaged and which also inhibit mitochondrial respiration which leads to oxidative burst of Reactive Oxygen Species (ROS) production and causes PPD. The visible signs of deterioration are vascular streaking with a blue or black discoloration that renders the tubers unpalatable and unmarketable (Reilly *et al.*, 2004; Soad *et al.*, 2010) [10, 14]. This initial deterioration is physiological and biochemical and does not involve microorganisms (Uarrota *et al.*, 2014) [16]. The secondary deterioration is due to infection with microorganisms leading to fermentation and softening of the root tissue (Garcia *et al.*, 2013; Sanchez *et al.*, 2013; Zidenga, 2011) [5, 12, 18]. PPD is much more

important economically than secondary microbial deterioration because, the visible colouration of the root is used as an indication of its reduced quality making the crop difficult to sell. The short shelf life of cassava roots severely limits marketing options by increasing losses and overall marketing costs. PPD is the challenging factor for cassava growers, processors and consumers. With this background, the present study was initiated to evaluate PPD, HCN content and starch content in tubers of 34 cassava genotypes.

Materials and Methods

Thirty four cassava genotypes, TCMS 1, TCMS 2, TCMS 3, TCMS 4, TCMS 5, TCMS 6, TCMS 7, TCMS 8, TCMS 9, TCMS 10, TCMS 11, TCMS 12, TCMS 13, TCMS 14, TCMS 15, TCa 12-1, TCa 12-2, TCa 12-3, TCa 12-4, Ca 12-5, TCa 12-6, TCa 12-7, TCa 12-8, TCa 12-9, TCa 12-10, PDP -1, PDP-9, CI-823, CI-850, CMR 100, H740/92, MVD, H226 and YTP-1, from the Tapioca and Castor Research Station, Yethapur, and farmers field of Salem and Namakkal districts, Tamil Nadu, India. Cassava tubers from 12 month old plants were harvested and carefully removing the roots from the soil while avoiding any wounding per cent. Tuber peduncles were removed and tubers were stored under ambient conditions. After 24 hours, cut the transverse sections at 50% of the total length from each root starting from the proximal end. A slice was cut from the distal end of each transverse section. Digital images for each slice were analyzed using Image analyzer (WinDIAS 3.2). PPD score for each slice was assigned according the following equation:

$$\text{PPD (\%)} = [\text{Total area of sliced tuber} - \text{Non deteriorated area}] \times 100$$

The PPD (%) is the percentage of deterioration per slice. The deteriorated and total areas were measured in pixels. Percentage of PPD for each cassava genotypes was obtained by averaging each independent PPD percentage from biological replicates (the average of slices 50% of total length and five different tubers of same genotype) and estimated one to five days at the 24 hrs intervals.

Total hydrogen cyanide (HCN) content of tuber was estimated by the method of Indian Standard, IS: 4706 - 1978 Part II and expressed as ppm. Whatman No.1 filter paper was cut into strips of 10-12 cm long and 0.5 cm wide and saturated them with alkaline picrate solution (dissolve 25 g sodium carbonate and 5g picric acid in one litre of water). One gram of the tuber sample was homogenate in 25 ml of water with 3-4 drops of chloroform. Sample was placed in 100 ml of flask and saturated filter paper was placed in the hanging position. Mixture was incubated at room temperature for 20-24hours. The sodium picrate accumulated in the filter paper is reduced to reddish compound in proportion to the amount of hydrocyanic acid evolved from the tuber sample. Placing the paper in the clean test tube containing 10 ml distilled water and compared it with standards at 625 nm.

Results and Discussion

PPD score was recorded at 24 hours interval up to five days period of storage. In 24 hours after harvest, PPD was found to show an increasing trend in all the genotypes ranging from 0.27 to 31.98 percent on first day, 1.78 to 52.85 percent on second day, 5.81 to 74.17 percent on third day, 8.91 to 81.98 percent on fourth day and 9.81 to 91.72 percent on fifth day. Result of PPD scoring of 34 genotypes revealed CI-850 (9.81%) as the most tolerant followed by YTP-1 (11.76%) and H740/92 (16.13%) and H226 was found to be the most susceptible (91.72%) followed by MVD

(90.21%). Comparing the mean of five days storage performance of different genotypes with reference to PPD, CI-850, YTP-1, and H740/92 showed only 10 to 20 percent PPD. TCa 12-7, TCa 12-2, TCa 12-1, TCa 12-10, TCa12 5, TCMS 15 and TCMS 13 recorded 20 - 50 per cent of PPD. In TCMS 1, TCMS 2, TCMS 3, TCMS 8, TCMS 10, TCMS 12, TCMS 13, TCMS 14, TCa 12-3, TCa 12-4, TCa 12-8, TCa 12-9, TCa 12-6 and CMR 100 observed 50 to 80 percent of PPD. Among the genotypes CI-850 performed differentially with the significant increase in shelf life of cassava tubers. Significant interaction was observed between genotypes and storage periods (Fig. 1). The 34 genotypes included in this study showed similarity in some of these changes, whereas in others drastic differences indicated the influence of genetic variation. Particularly relevant was the rate of progress for PPD percent, with final values around 90% for roots from H226 and only 10% for those from CI-850 at one to five days after harvest. The limited occurrence of vascular streaking in the roots from CI-850 makes it possible to evaluate alternatives for their post-harvest handling. Similar results were observed by Salcedo *et al.* (2010) [11] in comparative evaluations of PPD among different cassava genotypes and within the same genotypes has revealed a considerable variation in degree of development and severity of PPD. PPD response is under genetic control but it is also influenced by environmental factors. The activation and intensity of cassava PPD is closely related with mechanical damages (Sanchez *et al.*, 2013) [12]. The first symptoms of PPD appear in areas where the root peel has been damaged or in the proximal and distal ends of the root, which are the most susceptible zones to physical damage. PPD usually starts at the site of injury, making internal tissues more exposed to oxidation and microbial attack by pathogens responsible for rotting (Uarroda *et al.*, 2014) [16].

The HCN content of different cassava tuber ranging from 3.87 to 51.02 ppm was found on 24 hours after harvest and an increasing trend was observed day by day in all the genotypes ranging from 7.89 to 176.78 ppm on second, 9.49 to 376.72 ppm on third, 10.87 to 521.58 ppm on fourth and 12.5 to 783.67 ppm on fifth day after harvest. This indicated that there was a differential response of the genotypes to the period of storage. Irrespective of the genotypes, first day after harvest had only minimum effect on PPD with lesser accumulation of HCN content. Among the genotypes, low level of HCN accumulation was found in CI-850 (12.54 ppm) followed by YTP-1 (17.99 ppm) and H740/92 (32.67 ppm) even on five day after harvest. Remarkable accumulation of HCN was observed in H226 (783.67 ppm) followed by MVD (711.90 ppm), TCMS 4 (678.97 ppm), TCMS 5 (642.11 ppm), TCMS 7 (621.71 ppm) on five days after harvest (Table 1). In the present study, there is a strong positive association was observed between PPD and cyanide concentration. This was corroborated with previous reports (Sanchez *et al.*, 2013) [12]. In this investigation, the PPD-susceptible genotypes obtained peak at HCN concentration on third day after harvest but tolerant genotypes showed their peak accumulation only five days after harvest. This might be due to cassava tissues accumulate cyanogenic glucosides (linamarin 95% and lotaustralin 5%). Progressive accumulation of linamarin and subsequent degradation by enzyme linamarase to form HCN in cassava tubers might be the reason for obtaining increasing trend of HCN from first to fifth day after harvest (Solarte *et al.*, 2013; moller *et al.*, 2010) [15, 7]. (Fig. 2).

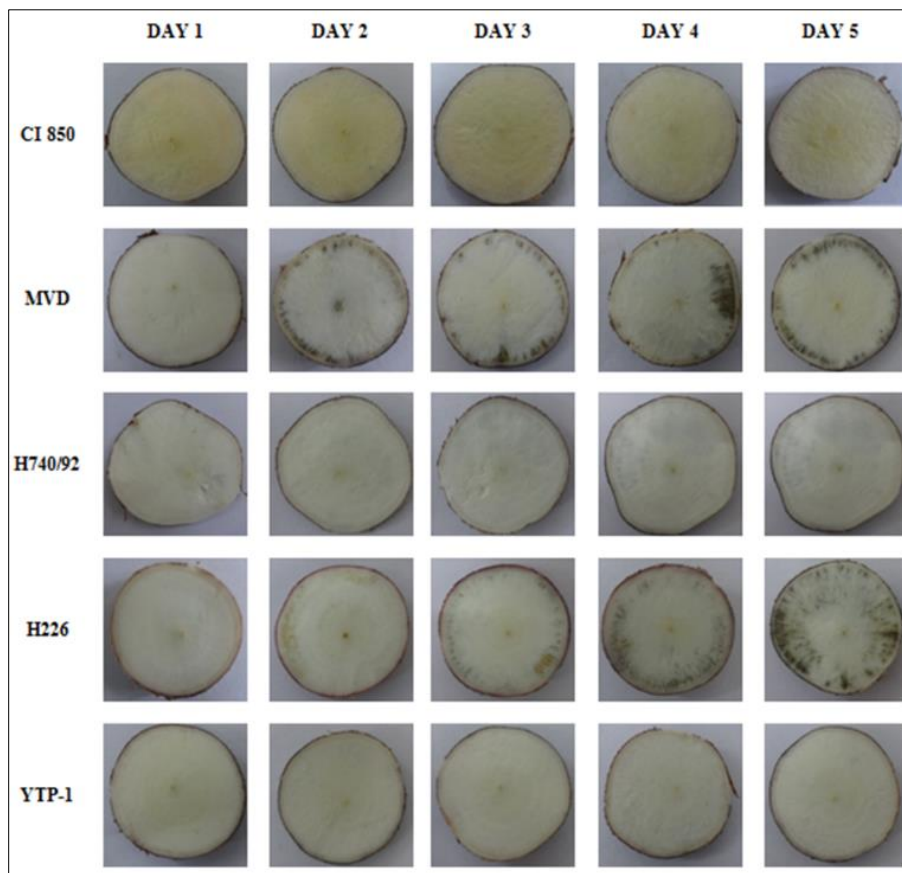


Fig 1: Postharvest Physiological Deterioration (PPD in %) of fresh cassava tuber during storage

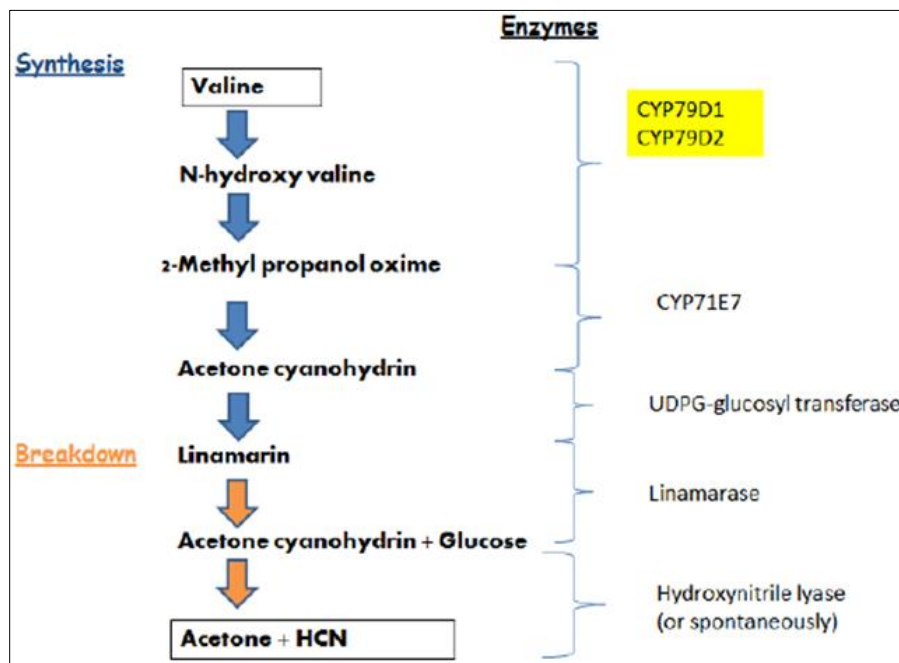


Fig 2: Linamarin biosynthesis and breakdown. Linamarin is synthesized from valine by two cytochrome P450s *CYP79D1* and *CYP79D2*. Breakdown of linamarin releases cyanide (Siritunga and Sayre, 2003).

Table 1: Hydrogen cyanide (HCN) content (ppm) on fresh weight basis in 34 cassava genotypes during postharvest physiological deterioration

Genotypes	Day-1	Day-2	Day-3	Day-4	Day-5	Mean
TCMS 1	16.03	89.21	176.21	267.81	376.11	185.07
TCMS 2	33.67	125.77	221.44	311.78	489.24	236.38
TCMS 3	22.45	111.77	189.67	298.43	489.12	222.29
TCMS 4	42.41	147.87	311.52	435.12	678.97	323.18
TCMS 5	30.95	131.73	231.21	376.88	642.11	282.58
TCMS 6	17.61	109.56	196.89	308.92	512.23	229.04
TCMS 7	27.67	124.55	321.42	471.76	621.71	313.42
TCMS 8	9.08	43.18	84.31	113.63	161.52	82.34
TCMS 9	36.74	142.09	278.11	373.41	589.29	283.93
TCMS 10	11.56	41.77	98.65	133.61	184.44	94.01
TCMS 11	35.21	138.66	217.86	421.25	611.22	284.84
TCMS 12	14.62	92.67	187.26	289.07	428.81	202.49
TCMS 13	5.92	21.53	38.99	87.48	108.54	52.49
TCMS 14	13.78	65.89	137.08	178.44	288.66	136.77
TCMS 15	6.02	23.62	42.71	84.43	112.62	53.88
TCa 12-1	8.32	19.67	56.78	81.05	134.17	60.00
TCa 12-2	5.86	18.81	35.72	78.31	93.42	46.42
TCa 12-3	12.38	52.41	124.23	156.21	269.51	122.95
TCa 12-4	8.65	31.55	68.12	91.24	147.78	69.47
TCa 12-5	6.78	25.61	51.72	91.88	132.62	61.72
TCa 12-6	5.82	11.36	13.65	24.87	32.65	17.67
TCa 12-7	4.78	9.45	14.75	16.27	21.87	13.42
TCa 12-8	15.67	71.26	144.76	212.76	321.22	153.13
TCa 12-9	11.25	48.65	117.66	137.71	204.65	103.98
TCa 12-10	8.31	28.33	62.83	98.38	141.76	67.92
PDP -1	25.69	118.45	196.78	292.67	543.81	235.48
MVD	47.36	145.61	289.27	389.12	711.9	316.65
CI-823	51.02	157.29	238.64	379.83	688.29	303.01
YTP-1	4.01	8.43	10.47	15.67	17.99	11.31
CMR 100	15.74	67.98	165.32	227.87	342.06	163.79
H740/92	6.81	8.21	10.69	27.81	32.67	17.24
PDP-9	27.89	117.34	184.77	387.95	571.83	257.96
H226	33.87	176.78	376.72	521.58	783.67	378.52
CI-850	3.59	7.89	9.45	10.87	12.54	8.87
MEAN	18.46	74.56	144.28	217.47	338.21	158.60
SEd	0.1512	0.6369	1.2167	1.8202	2.8902	
CD (5%)	0.2999	1.2634	2.4133	3.6104	5.7328	

In this present investigation, high cyanide variety is underlined by the name as H226 reaches a dangerously poisonous unsafe level of HCN on three days after harvest. Due to the probable health problems associated with dietary exposure to cyanide, one of the major aim of research on cyanogenic crops is the generation of tolerant plants with reduced levels of cyanogens (Nyirenda *et al.*, 2010) [9].

Irrespective of the genotypes, starch content in cassava tuber was found in decreasing trend from first to fifth day after harvest. Degradation of starch was severe on fifth day after harvest and severity was different from genotype to genotype. H226 and YTP -1 showed more starch content of 28.94 and 27.79 percent respectively on first day after harvest but on fifth day after harvest H226 had lowest starch content (2.78%) and CI -850 showed its supremacy in maintaining its starch content of 21.17 percent (Table 2) followed by YTP -1 (21.66%). Many research groups have sought for a long time to develop PPD-tolerant cassava (Morante *et al.*, 2010) [8]. The loss of starch in cassava roots over

time diminishes the relevance of PPD tolerance for the starch industry. Gradual changes in the functional properties of starch from stored roots, though there may be alternatives for starch modification in different genotypes (Sanchez *et al.*, 2013) [12]. The starch content was drastically reduced in H226 from first to fifth day after harvest even though it is denoted as starch rich genotype. But CI- 850 and YTP-1 were maintained their starch content with only one to two percent reduction even on fifth day after harvest. The high levels of PPD in tuber from H226 may be related to the higher sugar levels found in the tuber at 24 hours after harvest. This might be due to rapid conversion of starch into sugar in PPD susceptible genotypes but in tolerant genotypes the changes occur slowly. Salcedo *et al.* (2010) [11] reported that one to two days after harvest there is a rapid increase in total sugar content of roots accompanied by a decline in starch content and dry weight encourages the entry of microorganisms leading to subsequent decay of the root.

Table 2: Starch content (%) on fresh weight basis in 34 cassava genotypes during postharvest physiological deterioration

Genotypes	Day-1	Day-2	Day-3	Day-4	Day-5	Mean
TCMS 1	27.53	22.3	17.54	14.38	9.58	18.27
TCMS 2	27.54	19.98	14.82	10.86	5.89	15.82
TCMS 3	26.43	20.89	17.21	12.25	6.82	16.72
TCMS 4	28.43	22.12	16.15	10.19	5.94	16.57
TCMS 5	27.71	21.34	15.67	12.55	7.26	16.91
TCMS 6	26.29	17.54	12.84	8.04	5.33	14.01
TCMS 7	19.32	16.59	10.92	7.86	5.68	12.07
TCMS 8	28.32	22.18	18.2	15.24	10.32	18.85
TCMS 9	27.07	21.32	14.65	9.49	4.19	15.34
TCMS 10	24.71	20.45	16.68	13.62	7.88	16.67
TCMS 11	27.12	20.42	15.75	10.99	5.31	15.92
TCMS 12	21.11	16.45	11.85	8.29	5.61	12.66
TCMS 13	20.78	19.11	16.24	13.25	9.33	15.74
TCMS 14	27.23	23.56	18.89	14.13	8.07	18.38
TCMS 15	23.55	21.77	18.66	15.96	11.32	18.25
TCa 12-1	20.45	18.56	14.89	11.78	7.98	14.73
TCa 12-2	21.49	19.51	16.52	13.63	10.61	16.35
TCa 12-3	26.89	21.43	17.46	13.6	5.99	17.07
TCa 12-4	26.21	20.52	16.55	14.39	11.04	17.74
TCa 12-5	24.77	22.76	19.09	15.43	11.25	18.66
TCa 12-6	22.41	20.87	17.88	15.1	11.45	17.54
TCa 12-7	25.21	24.98	22.11	19.25	15.89	21.49
TCa 12-8	28.44	22.75	18.59	13.71	8.32	18.36
TCa 12-9	25.88	20.37	16.6	13.61	7.37	16.77
TCa 12-10	19.08	17.43	13.46	10.3	8.19	13.69
PDP -1	26.06	20.67	15	10.22	6.43	15.68
MVD	28.12	21.78	17.09	12.99	5.89	17.17
CI-823	28.33	21.56	15.89	10.53	4.92	16.25
CI-850	24.56	23.21	22.18	21.87	21.17	22.60
CMR 100	22.56	17.21	12.61	8.45	4.89	13.14
H740/92	25.18	24.44	23.58	21.98	20.87	23.21
PDP-9	24.29	19.22	14.56	10.45	6.76	15.06
H226	28.94	18.21	10.54	4.38	2.78	12.97
YTP-1	27.78	25.17	23.78	22.62	21.66	24.20
MEAN	25.29	20.78	16.60	12.98	8.88	16.91
SEd	0.1775	0.1435	0.1150	0.0897	0.0618	
CD (0.05)	0.3521	0.2847	0.2280	0.1780	0.1225	

Conclusion

This study provides further evidence of PPD tolerance in CI-850 and YTP-1 as well as insight into the implications of deploying PPD-tolerant cassava genotypes. The genotypes have different genetic tolerances to postharvest physiological deterioration and resistance to microbial deterioration. The results presented here confirm that there is a relationship between the accumulation of HCN and the visible symptoms of PPD. However an understanding of the biosynthesis and regulation of metabolites in cassava is required to fully understand their involvement in post-harvest physiological deterioration of cassava.

References

- Andres Salcedo, Dimuth Siritunga. Insights into the Physiological, Biochemical and Molecular Basis of Postharvest Deterioration in Cassava (*Manihot esculenta*) Roots. American Journal of Experimental Agriculture. 2011; 1(4):414-431.
- El-Sharkawy MA. Global warming: causes and impacts on agroecosystems productivity and food security with emphasis on cassava comparative advantage in the tropics/subtropics. Photosynthetica. 2014; 52(2):161-178.
- FAO. Championing the cause of cassava, 2000. Available at <http://www.fao.org/english/newsroom/highlights/2000/000405-e.htm>. Verified March, 24, 2009.
- Figueiredo PG, Marina Aparecida, Moraes-Dallaqua, Silvio José Bicudo, Yomei Tanamatil. Starch accumulation in cassava roots: Spatial and temporal distribution. African Journal of Agricultural Research. 2013; 8(46):5712-5715.
- Garcia JA, Sánchez T, Ceballos H, Alonso L. Non-destructive sampling procedure for biochemical or gene expression studies on post-harvest physiological deterioration of cassava roots. Postharvest Biology and Technology. 2013; 86:29-535.
- Indian Standard, Methods of Test for Edible Starches and Starch Products. Part II, Chemical Methods, IS : 4706 (Part II), 1978.
- Moller BL. Functional diversifications of cyanogenic glucosides. Current opinion in plant biology. 2010; 13:338-47.
- Morante N, Sanchez T, Ceballos H, Calle F, Perez JC, Egesi C, et al. Tolerance to Postharvest Physiological Deterioration in Cassava Roots. Crop Science. 2010; 50(4):1333-1338.

9. Nyirenda DB, Chiwona-Karltun L, Chitundu M, Haggblade S, Brimer L. Chemical safety of cassava products in regions adopting cassava production and processing – Experience from Southern Africa. *Food Chem Toxicol.* In Press, Corrected Proof, 2010.
10. Reilly K, Gomez-Vasquez R, Buschmann H, Tohme J, Beeching JR. Oxidative stress responses during cassava post-harvest physiological deterioration. *Plant Molecular Biology*, 2004, 669-685.
11. Salcedo A, Del Valle A, Sanchez B, Ocasio V, Ortiz A, Marquez P, *et al.* Comparative evaluation of physiological post-harvest root deterioration of 25 cassava (*Manihot esculenta*) accessions: visual vs. hydroxycoumarins fluorescent accumulation analysis. *African Journal of Agricultural Research*. 2010; 5:3138-3144.
12. Sanchez T, Dufour D, Moreno JL, Pizarro M, Aragón IJ, Domínguez M, *et al.* Changes in extended shelf life of cassava roots during storage in ambient conditions. *Postharvest Biology and Technology*. 2013; 86:520-528.
13. Siritunga D, Sayre R. Generation of cyanogen-free transgenic cassava. *Planta*. 2003; 217:367-373.
14. Soad AL, Bayoumi Michael G, Rowan John R, Beeching Ian, Blagbrough S. Constituents and secondary metabolite natural products in fresh and deteriorated cassava roots. *Phytochemistry*. 2010; 71:598-604.
15. Solarte ME, Victor Ocasio-Ramirez, Annete Figueroa, Eduardo González, Dimuth Siritunga. Expression Profiling of Genes Associated with Cyanogenesis in Three Cassava Cultivars Containing Varying Levels of Toxic Cyanogens. *American Journal of Plant Sciences*. 2013; 4:1533-1545.
16. Uarrota VG, Rodolfo Moresco, Bianca Coelho, Eduardo, Costa Nunes, Peruch LKM, *et al.* Metabolomics combined with chemometric tools (PCA, HCA, PLS-DA and SVM) for screening cassava (*Manihot esculenta* Crantz) roots during postharvest physiological deterioration. *Food Chemistry*. 2014; 161:67-78.
17. Wheatley C, Schwabe W. Scopoletin involvement in postharvest deterioration of cassava roots (*Manihot esculenta* Crantz). *Journal of Experimental Botany*. 1985; 36:783-791.
18. Zidenga T. Cyanide metabolism, postharvest physiological deterioration and abiotic stress tolerance in cassava (*Manihot esculenta* Crantz) (Ph.D. thesis). Ohio State University, 2011.