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

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ABSTRACT

Cassava *(Manihot esculenta* Crantz) is an important tropical root crop grown worldwide for food, feed and industrial purposes. Harvested cassava roots quickly deteriorate and loose the shelf-life due to a phenomenon called postharvest physiological deterioration (PPD). PPD symptom starts within 24 hr after harvest, initially as blue black discolouration in the storage parenchyma and quickly spread to entire root. The roots become unfit for consumption within 2-3 days after the harvest in most cases. Identification of delayed PPD genotypes in cassava will help breed superior varieties tolerant to deterioration with long shelf-life. Low molecular weight phytochemicals produced during PPD are reported to have significant role in PPD development. We analyzed the biochemical changes associated to secondary metabolites in 61 cassava genotypes during storage and evaluated the relationship with PPD. PPD evaluation was done visually at specified intervals by taking transverse sections at 25, 50 and 75% along length of roots from proximal to distal end and the roots were categorized into different PPD classes based on the visual scoring. Root morphological, starch, and carotene content had no direct correlation with PPD. The HPTLC chromatographic data on phytoconstituents of methanolic extract of cassava roots and its relation with PPD symptoms were analyzed and polymorphic bands were assessed for grouping the genotypes based on PPD expression levels. Cluster analysis revealed a close association between PPD expression and phytochemical constituents of stored roots and this can help to categorize the genotypes based on PPD.

Key words: Cassava, Evaluation, Postharvest physiological deterioration, Storage roots

Harvested cassava *(Manihot esculenta* Crantz*)* roots deteriorate quickly and they can't be stored satisfactorily for more than two to three days under ambient conditions. This phenomenon is unique to cassava roots and is known as Postharvest Physiological Deterioration (PPD). The extent of PPD damage and speed of symptom development in roots depends on the genotypic as well as the environmental conditions. (reviewed by Ravi and Aked 1996, Reilly *et al.* 2004). The PPD is considered one of the main postharvest constraints for farmers, traders, food and starch industries. The short shelf-life of harvested roots makes the long distance transport of cassava for marketing or industrial purpose unviable either due to loss in transit or low quality raw material for food processing industry. Cassava root PPD limits the expansion of cassava production in developing countries, and has become one of the major constraints compared to other root crops, due to root discounting, waste and added costs (Wenham 1995, Westby 2002, Reilly *et al.* 2004).

Cassava roots develop blue/black vascular streaking initially during PPD, followed by discoloration of storage parenchyma with brownish or blackish occlusions (Reilly *et al.* 2007). The roots become unpalatable and unfit for consumption after two to three days. Changes in many enzymes of secondary metabolism, cell wall modification and wound healing were reported (Ravi and Aked 1996). The PPD of cassava roots is an active and complex process involving gene expression and protein synthesis. Phenolic compounds associated with PPD include scopoletin, scopolin, esculin, proanthocyanidins, (+) - catechin and (+) gallocatechin (Tanaka *et al*. 1983, Rickard 1981, 1985; Wheatley and Schwabe 1985). Scopoletin has a crucial role in the development of physiological deterioration of cassava root (Wheatley and Schwabe 1985). When applied to freshly cut roots, scopoletin produced intense and rapid discoloration of the tissue. Scopoletin, identified as a fluorescent compound (Rickard 1982), is absent or has very

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low occurrence in fresh roots. However, during the first 24 to 48 hr after harvest its concentration increases 150 to 200-fold and is then followed by the accumulation of scopolin and esculin (Uritani *et al.* 1983). A second smaller increase of hydroxycoumarins has been reported at 4-6 days after harvest with a differential accumulation amongst genotypes with low and high susceptibility to PPD (Buschmman *et al.* 2000).

The expected benefit for developing varieties with delayed PPD using molecular breeding technologies were estimated to the tune of approximately $2.9 billion for Nigeria, $855 million for Ghana and $280 million for Uganda, major cassava producing countries in Africa over 20 year period (Rudi et al. 2010). Since PPD is a complex trait, linked to multiple genes and quantitative trait loci (QTLs), transgenic approach to delay PPD is difficult to achieve. Conventional breeding potentially could produce cultivars with resistance to PPD by using recurrent selection methods (Salcedo and Sritunga 2011). Low molecular weight phytochemicals produced during PPD are reported to have significant role in PPD development (Uarrota *et al* 2014). Accumulation of secondary metabolites particularly hydroxycoumarin is closely related with PPD and hence cassava genotypes need to be evaluated for the variation in the production of phenolic intermediates during storage. Chemo characterization of genotypes involving metabolomic techniques coupled with chemometric analysis can be carried out quickly and reliably and can be employed successfully to screen them (Schulz and Baranska 2007) This would help in breeding varieties for delayed PPD. We analyzed the biochemical changes in 61 cassava genotypes during storage and evaluated the performance in terms of PPD expression as well as secondary metabolite accumulation to identify their correlation for shelf- life.

MATERIALS AND METHODS

Sixty one cassava genotypes comprising of released varieties, improved cassava clones and exotic genotypes (Table1) were grown 1 m apart at the ICAR-CTCRI, Thiruvananthapuram research farm during 2013-14. Harvest was done manually with special care not to cause any injury to the roots. Commercial-size roots were selected and placed on shelves in a well ventilated room. Root peduncles were removed and entire roots were stored after recording morphological measurements. The roots were left untouched until sampling is done at 5, 10 and 15 and 20 days after harvest.

Cassava roots were stored for 20 days in a well ventilated room to study the development of PPD. The average temperature during the storage period was 28.0°C, with a range from 26 to 31°C. Average relative humidity at 7:00 hr was 73%. PPD evaluation was done at specified intervals by taking transverse sections at 25, 50 and 75% of the total length of the roots, starting from the proximal end. A slice (0.3 cm average thickness) was cut from the distal end of each transverse section. Digital pictures of each slice were obtained using scanner (HP scanjet G2410).

Table 1 Classification of cassava genotypes based on visual scoring of PPD symptoms

PDD category	Genotypes
Low	9S-7, 9S-98, 11S-31, 11S-86, 11S-14, CE63-3, CI43-2, CR43-2, CR54-A5, Sree Athulya, Kalpaka.
Moderate	11S-4, 11S-8, 7 IV D-7, CI-896, , 9S–127, CO-2, CR 43-7, CE-775, CR24-4, IMS 2-8, S-1284, H226 and 57-6 Sreevijaya, CR35-8
High	9S-3, 9S-56, 9S-174, 9S–172, 9S–286, 9S–132, 11S-3, 11S-7, 11S-11, 11S–22, 11S–26, 11S–53, 11S-40, 11S-271, 8W5, C4 D7-1, CE-7B6, CE-185, CI-4, CI-273,CI-800, CI-891, CI-889, CO-3, CR20 A2, CR21-10, CR52 A4, CR54 A3, CR54- A19, CR59-8R, CR-786, SB 56, PDP-1, Sreeprabha, Vellayani Hrshwa and Sreerekha
Extreme	CR43-11, CR59-4, CR-775, IH 5/15, IRS 2-1, Sree Harsha, Sree Padmanabha, 7 IV C4 and CR35-8

Three independent evaluations of PPD were carried out under laboratory conditions. For scoring of PPD expression, the roots of each genotype were randomly chosen and cut into three equal parts transversely from proximal end to distal end and visually scored in a scale of 1 to 5 following Salcedo *et al.* (2010) with modifications. The scoring of PPD intensity comprised of, no damage (score 1), up to 25% damage (score 2), 26-50% damage (score 3), 51-75% (score 4) and fully damaged root slice (score 5). The mean PPD score for each root was calculated by averaging the scores for the 3 transverse sections.

Starch was determined by titrimetric assay using potassium ferricyanide with methylene blue as indicator of end point of titration following the method by Moorthy and Padmaja (2002). Carotene content was determined by taking 5 g from randomly selected root tissue of cassava genotypes and extraction with petroleum ether, as described by Iglesias *et al.* (1997). The quantification was done by measuring the absorbance at 455 nm using a Perkin-Elmer Lamda 25 UV-VIS spectrophotometer.

Roots of cassava accession at 10 days after storage (20 g each) were peeled then cut into approximately 0.5 cm^3 cubes and dried in oven at 55°C for 48 hr and crushed to fine powder. The root powder (5 g) was extracted with 10 ml hot methanol (60°C) thrice and the extracts were pooled. The extracts were filtered and evaporated under reduced pressure at concentrated to 2 ml and subjected to TLC. A Camag HPTLC system comprising of Linomate V automatic sample applicator, Hamilton Syringe, Camag TLC Scanner-3, Camag Win CAT software, Camag Twin trough chamber and stationary phase precoated silica gel 60F 254 were used. The mobile phase comprised of Toulene: Ethylacetate: Formicacid: Methanol: Water (6: 5: 0.5: 3: 0.5). The solvent was run for 80 mm, band length 6 mm, slit dimension 6.00 × 0.30 mm. The Chromatogram was visualized using UV light 366nm and scanned at 340nm

and 290nm using TLC Scanner III CAMAG. The plate was subsequently derivatized using 1% vaniline/H_2SO_4 for visualization.

All statistical analyses were carried out using RStudio statistical package (RStudio 2014). Analysis of variance was used to evaluate the differences among cassava genotypes. The relationship between visual PPD scores and root characters were assessed using correlation. Chromatogram of each genotype was scored by the presence (1) or absence (0) of each band noted. Presence and absence of bands were entered in a binary data matrix. Based on chromatographic band spectra, cluster analysis was performed using R studio statistical package. Dendrogram was constructed using Ward's method using squared Euclidean distance.

RESULTS AND DISCUSSION

Mean root length of cassava genotypes was 34.9±7 cm. The accession 11S-4 had the longest root (51 cm), whereas, Sree Padmanabha had the shortest roots (20.7 cm). Mean root fresh weight was 752±280 g. The highest root fresh weight was observed for CR59-8 (2078.3 g), whereas CR35-8 had the lowest (288.3 g). Average carotene and starch content were 0.34±0.1 µg/g and 82.1±4% respectively. Highest carotene content was recorded in CI-896 (0.78 µg/g) which had pale yellow root tissue. In general, in the present study, the genotypes of cassava did not have appreciable amount of carotene content in root tissues as compared to high carotene levels observed in deep yellow fleshed varieties (Sánchez *et al.* 2006).

The genotypes categorized based on PPD score are presented in Table 1. Genotypes such as 9S-7, 9S-98, 11S-31, 11S-86, 11S-14, CE63-3, CI43-2, CR43-2, CR54-A5, CR59-8R, Sree Athulya and Kalpaka showed low PPD scores with lower discoloration and streaking compared to the rest of the genotypes. Roots of these genotypes were intact even at tenth day of storage and free from any foul smell which normally develops in cassava when PPD intensity is high. The progress of PPD in genotypes such as 11S-4, 7 IV D-7, CI-896, CO-2, CR 43-7, CR54- A19, CR24-4, IMS 2-8, S-1284 and Sree Vijaya was moderate. These genotypes were lesser susceptible to microbial deterioration compared to genotypes with high and extreme PPD. Genotypes such as CR43-11, CR59-4, CR-775, IH 5/15, IRS 2-1, Sree Harsha and Sree Padmanabha had high scores of 3 and 4 even at fifth day of storage and rapidly deteriorated with increased susceptibility to microbial attack.

There was no correlation between the root morphological traits and PPD severity. Carotene and starch content of root did not influence the PPD in the genotypes studied. β-carotene is a quencher of ROS (reactive oxygen species) produced during PPD and β-carotene has been reported to extend the storage life of cassava roots by up to 4 weeks (Sayre *et al.* 2011). Gloria and Uritani (1984) reported that a low in β-carotene in the root tissues of cassava hastened the PPD. Chavez *et al.* (2000) reported that carotenoid content in the root above 5 mg per kg fresh weight delayed the PPD symptom development. In the present study, carotene contents in roots of genotypes were below the threshold level and hence did not exert any positive effect on modulation of ROS and PPD. We found no correlation for root carotene content with PPD (Fig 1). Carotenoids have been related in the literature to delay or reduce postharvest deterioration in cassava roots (Morante *et al.* 2010, Sánchez *et al.* 2006). However, there was a weak negative correlation for root length and starch content with PPD in the genotypes studied. Oirschot *et al.* (2000) reported negative correlation between PPD and sugar/starch ratio, in contrast with Wheatley and Gomez (1985), who found no correlation between PPD and starch content.

Earlier studies indicated that visible symptoms of PPD

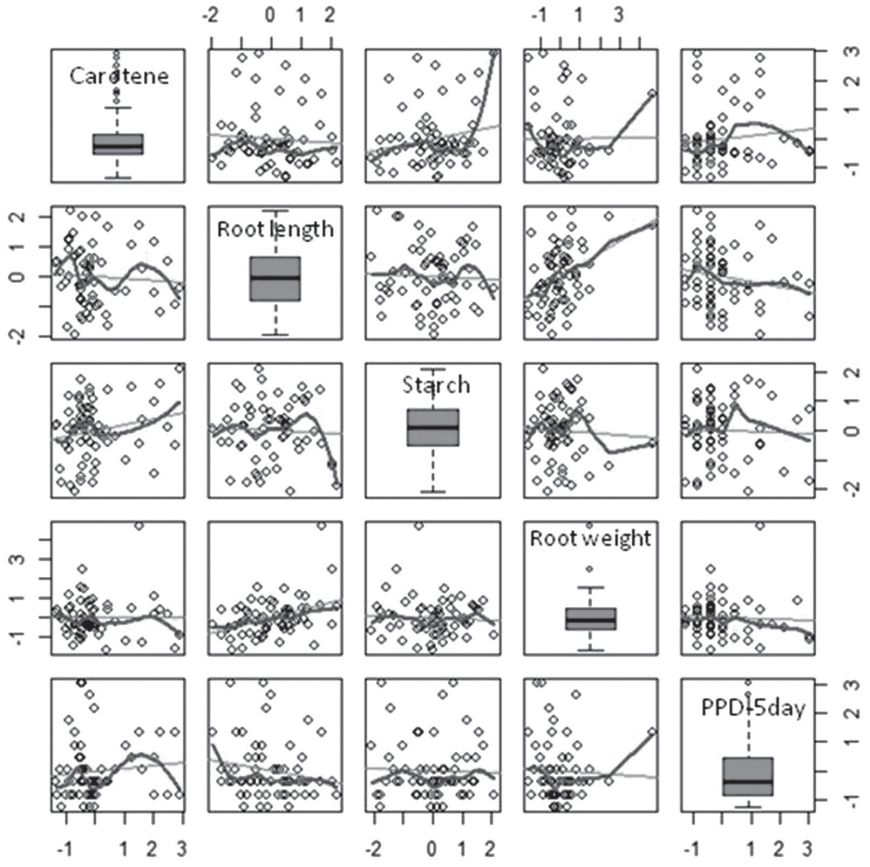

Fig 1 Correlation matrix showing the relationship of cassava root characters, carotene and starch content with PPD after 5 days of storage at room conditions. The data was standardized and centered before performing the correlation.

in intact cassava roots appeared after 5 days in most cases (Hirose *et al.* 1984 and Salcedo *et al.* 2009) and hence our initial PPD assessment on cassava genotypes was carried out on fifth day of storage by visual scoring. Since the roots were kept without additional cut other than detaching them from main stem, the PPD was noticed in most of the genotypes studied at the first sampling which was done on 5th day of storage. The PPD progressed in most cases from the proximal portion of the root to distal end unless the tips are damaged during harvesting or handling. The scores of PPD in genotypes on 10th day of storage revealed that the PPD progressed quickly between 5 and 10 days of storage. Roots of several genotypes deteriorated rapidly and PPD score reached to 3-4 during this period. Albeit there were differences among genotypes, severity of PPD scoring was not statistically significant. Variations in degree of development and severity of PPD among the cassava genotypes and within the same genotype have been reported (Buschmann *et al*. 2000). Kawano and Rojanaridpiched (1983) reported that PPD was affected by the environment where the plants were grown.

PPD of cassava starts with mechanical damage caused when the roots are detached from the plant and is closely related to wounding (Booth 1975). The cassava root is a storage organ for photosynthates and since it is not a propagule with reproductive function there is no biological need to repair wounds. Peaks of ROS as well as increased activity of antioxidative enzymes that modulate ROS have been detected during deterioration (Reilly *et al*. 2001). Signal molecules synthesized or released during wound response trigger protective or defensive responses locally as well as systemically. These signals induce the production of defensive enzymes and secondary metabolites such as glucanases and chitinases, phytolaexins and antioxidants and help wound repair and sealing molecules (such as callose, lignin and suberin) (Buschmann *et al.* 2000).

When HPTLC chromatographic data of methanolic extract of cassava roots with PPD symptoms were analyzed, several polymorphic bands corresponding to phyto-constituents were observed (Fig 2). The phytochemical profile was utilized for classifying the PPD response of cassava genotypes. The individual components of the profile were not identified; however their chromatographic performances (UV absorbance, RF values) were studied. The intense fluorescence revealed that these compounds were mainly, phenols, coumarins and flavonoids. Secondary metabolites like scopoletin and its O-glucoside scopolin with high UV fluorescence were reported to accumulate to

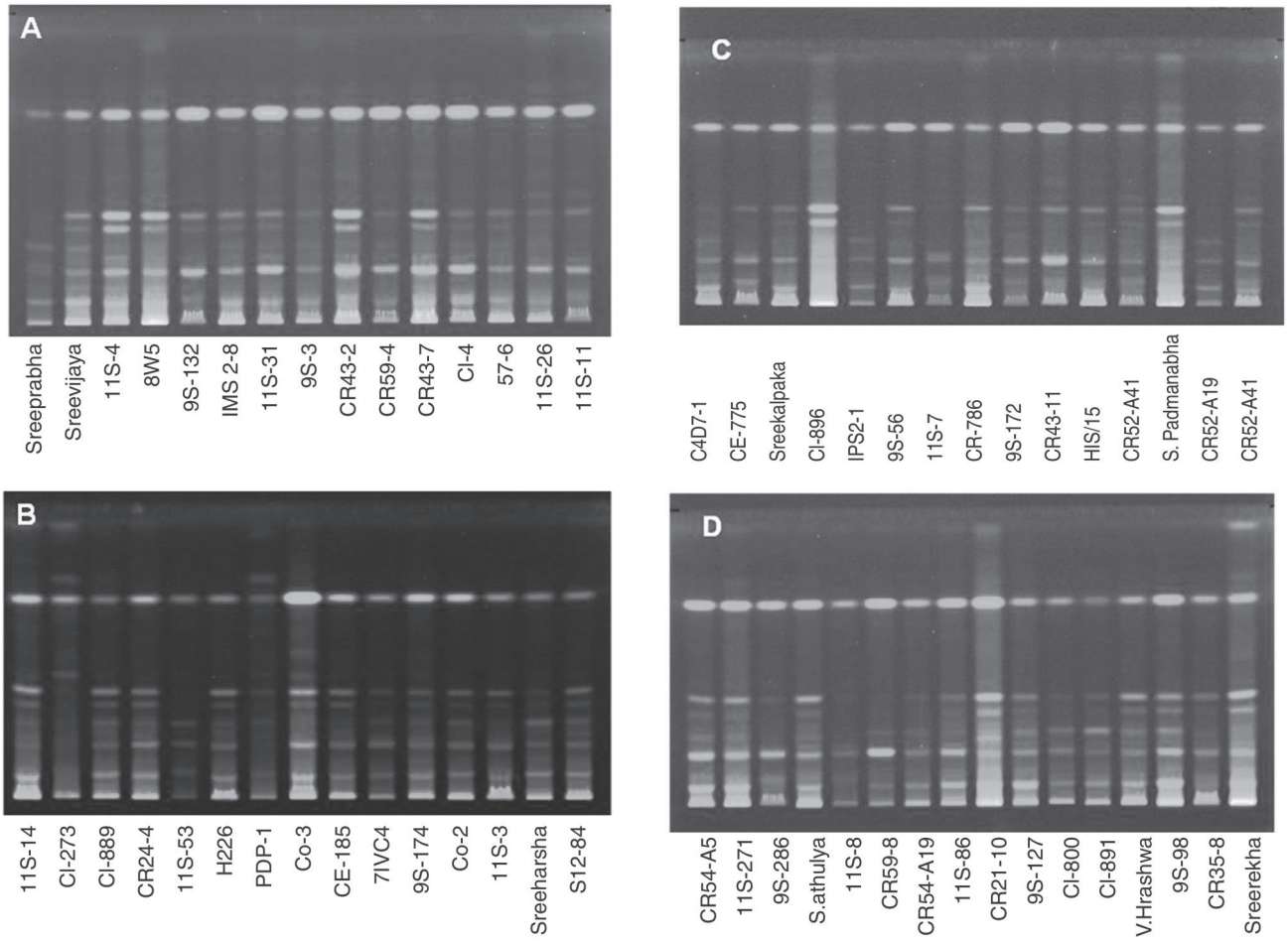

Fig 2 Thin layer chromatogram of phytochemical profile of methanolic extract of 61 cassava genotypes (A-D) at 10 days of storage. The image was taken using UV light of 350 nm before derivatization.

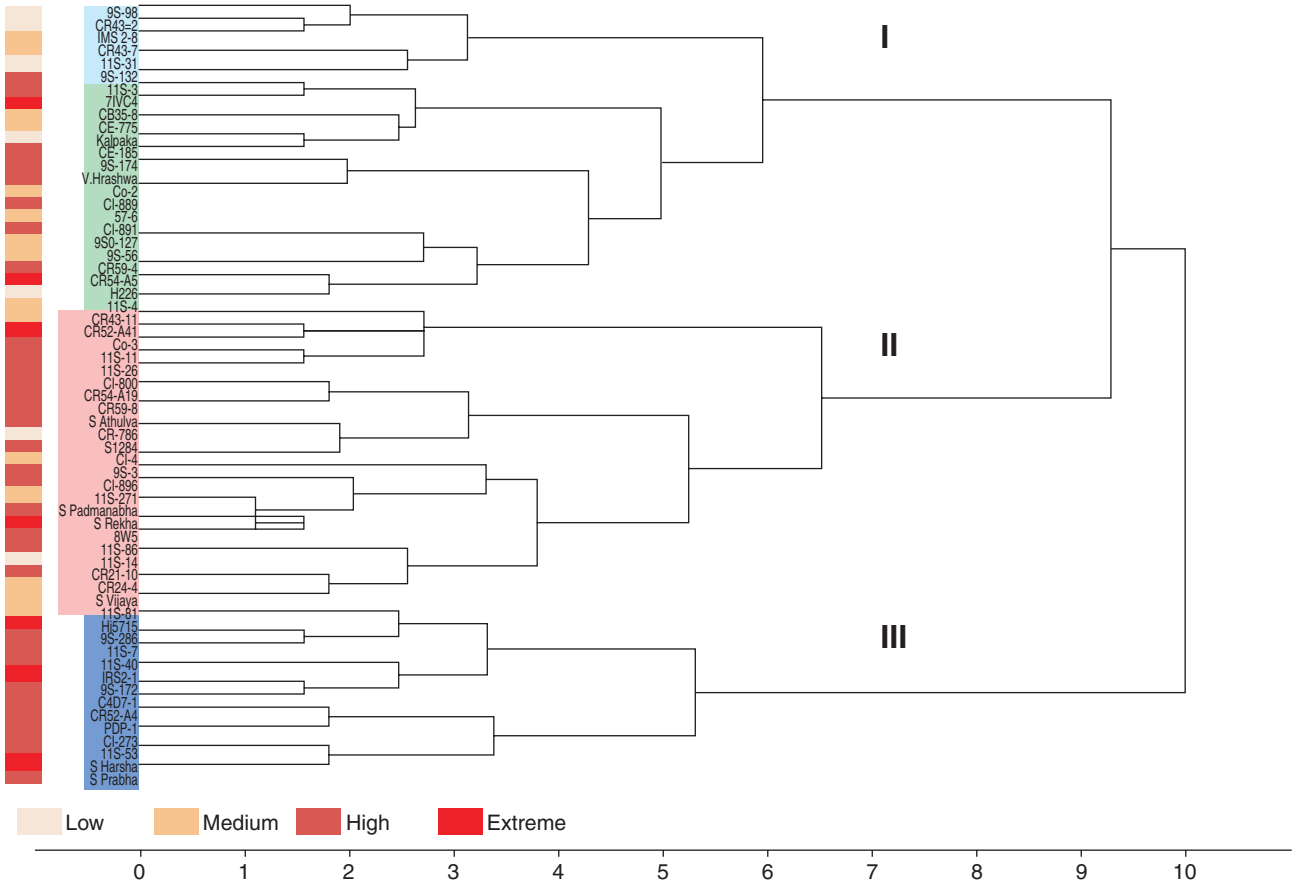

Fig 3 Dendrogram of cassava genotypes based on the chemotypic profile of roots under PPD by ward's method using squared Euclidean distance. Colored boxes indicate the cassava genotype with specific PPD characteristics.

a greater extent in deteriorating cassava roots (Bayoumi *et al.* 2010). Secondary metabolites (carotenoids, phenolics, flavonoids, and anthocyanins) are found in many species of the plant kingdom and are well recognized as potential antioxidants. Physiological deterioration of cassava roots resulted in altered enzyme activities, which generate phenols and leucoanthocyanins (Rickard 1981). Accumulation of secondary metabolites like hydroxycoumarins, e.g. scopoletin and its glucoside scopolin may help in quenching reactive oxygen species. Oxidation and polymerization of these coumarins may give rise to the blue/black discolouration that is typical of PPD. In vitro, scopoletin and hydrogen peroxide give a dark bluish colour in a peroxidase-mediated reaction (Edwards *et al.* 1997).

There were nine polymorphic bands and five monomorphic bands and genotypes were grouped into three major groups by hierarchical clustering analysis of chemotypic profile (Fig 3). A dendrogram was constructed using Ward's method using squared Euclidean distance as it was intuitive and straight forward way of defining the similarity of objects. There was some degree of overlapping in I and II main groups, however there was a clear separation of genotypes having high and severe PPD in group III. The visual symptoms of deterioration during the advanced stages of PPD especially in the high and severe categories overlapped and assigning the scores to individual roots of genotypes were increasingly difficult. Furthermore, the genotypes in the high and severe PPD category showed similar symptoms of root deterioration and nearly identical chemical profile. The classification of genotypes based on chemical profile matched with their visual scoring to a great extent. It is interesting to note that there were no clear correlation between hydroxyl-coumarin accumulation and PPD intensity of cassava (Wheatley and Schwabe 1985, Buschmann *et al.* 2000 and Oirschot *et al.* 2000). In the present study, we found similarity in chemical profile and PPD intensity when the symptoms are at advanced stage. Genotypes like 11S-8, HI5/15, 92-286, 11S-7, 11S-40, IRS2-1, 9S-172, C4D7-1, CR54-A4, PDP-1, CI-273, 11S-53, Sree Harsha and Sree Prabha were grouped together (Cluster III) with similar chemical profile and PPD symptoms. The differentiation of cassava roots at the metabolites level corresponding to visual symptoms and chemoprifle of PPD offer a rapid screen tool and can be incorporated into cassava breeding programmes on PPD tolerance.

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