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Influence of Nutrients and Nutrient Use Efficient Genotypes on Tuber and Leaf Cyanogen and Tuber Starch in Cassava

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Abstract

The present study reports the variation in tuber quality parameters like starch, cyanogenic glucosides (CN) and leaf CN content among four nutrient use efficient (NUE) cassava genotypes viz., Sree Pavithra, CI-905, CI-906 and 7 III E3-5. The impact of secondary nutrient Mg and micronutrients viz., Zn and B on the above parameters in cassava variety Sree Visakham (H-1687) towards the later period of maturity of cassava also was studied. Among the genotypes, Sree Pavithra had the least and 7 III E3-5 had the highest tuber CN. A decreasing trend in tuber CN from 7-9 months after planting (MAP) was recorded in the genotypes. The leaf CN was very high compared to tuber CN. The highest leaf CN was in 7 III E3-5 as in the case of tuber CN. The genotype CI-906 had the lowest leaf CN followed by Sree Pavithra. But the leaf CN increased at 9 MAP compared to 7 and 8 MAP. There was a positive correlation between tuber and leaf CN. In the case of tuber starch, there was a significant difference among genotypes with Sree Pavithra on par with CI-906, having the highest, and 7 III E3-5 on par with CI-905 having lower starch contents. However, there was a significant increase during 8-8.5 MAP.

As regards to the influence of secondary and micronutrients, compared to PoP, different combinations of these nutrients resulted in significantly low tuber CN which in turn were on par. The stage of plant growth did not impact on tuber CN. Leaf CN was considerably lowered due to the application of secondary and micronutrients. Compared to PoP, the leaf CN was highest under three nutrient combination of Mg, Zn and B as well as Zn along with Mg. A sharp decline in leaf CN was also noticed from7 to 9 MAP. Significant positive correlation was seen between tuber and leaf CN. In the case of tuber starch, application of Mg along with Zn resulted in significantly the highest tuber starch whereas application of Mg alone, resulted the lowest. Significant negative correlation was seen between tuber starch and tuber CN and between tuber starch and leaf CN.

Key words: Nutrient efficient, cassava, leaf cyanogen, tuber starch

Introduction

Tropical tuber crops like cassava, sweet potato, yams, aroids and minor tuber crops like Chinese potato, arrowroot under the root and tuber crops deserve special attention due its role in the subsistence of millions of people in the developing and under developed countries of the world. These crops are important for the food, nutrition and employment security of mankind and play a crucial role by way of providing energy in the form of starch or carbohydrate primarily. In addition, they supply various micronutrients in the form of minerals and some growth promoters such as vitamins, hormones and anti-oxidants required for human health. Moreover, they are important as it can provide job opportunities to many people engaged in preparing many value added food and industrial products.

Cassava (*Manihot esculenta* Crantz) is considered as the most important crop among the tropical tuber crops due to its many unique attributes like better biological efficiency manifested as high tuber yield to the tune of

20-100 t ha^{-1}, ability to withstand marginal soil and environmental conditions, tolerance to pests and diseases and the quality starch which can be processed to many value added domestic and industrial products like food, feed, ethanol, gum, gels, hardboards, films and even biodegradable plastics. Considering the quality attributes of cassava, the two important components are starch and cyanogenic glucosides (CN). If starch is a nutritional factor, CN responsible for the bitterness in cassava tubers is regarded as an anti-nutritional factor.

Cassava is cultivated for the edible starchy tubers which accounts to 25-30% on fresh weight basis (FWB) and is the richest source of starch among tropical tuber crops. CN content in cassava tubers can range from 1 to 2,000 mg kg^{-1} (Cardoso et al., 2005; CIAT, 2007). Cultivars with $<$ 100 mg kg^{-1} hydrogen cyanide are called sweet while those with $>$100 mg kg^{-1} are called bitter (Wheatley et al., 1993). Apart from this, the leaves of cassava plant also contains high amount of CN (1000-2000 mg kg^{-1}) (Gomez and Valdivleso, 1985) which in turn hinders its use as a protein rich leafy vegetable as well as a feed in fresh form.

As it is clearly known worldwide from different researches on plant nutrition, it is established that, cassava responds well to manures and fertilizers (Susan John et al., 2005). There are many reports for and against plant nutrition especially with major nutrients like N, P, K affecting the quality of cassava tubers. Susan John et al., (2019b) reported the adverse effect of imbalanced use of N fertilizers in enhancing CN content in cassava tubers and favourable impact of K in increasing the starch and reducing the CN content of tubers. Other than primary nutrients, secondary and micronutrients are found inevitable in crop production. In this line, the research work conducted at ICAR-CTCRI on the continuous use of secondary nutrient Mg and micronutrients Zn and B since 2005 under a long term fertilizer experiment (LTFE) revealed Zn application can enhance the starch content and reduce the CN content in cassava tubers (Susan John et al., 2018, 2019a,b) though no significant effect on starch content was noticed during these years.

Nutrient use efficient (NUE) cultivars which can use the unavailable fixed form of soil nutrients through bringing them in soil solution for plant uptake with its unique root architecture and finally incorporating them in their plant biomass recently became important in plant nutrition (Blair, 1993). According to Hawkesford et al., (2014), by planting NUE cultivars, the use of external inputs in the form of inorganic fertilizers can be reduced. There are several reports indicating genotypes (Burns et al., 2012), planting season (Bokanga et al., 1994), soil type (Bokanga et al., 1994; Bradbury et al., 2013) in affecting the CN content of the crop. The starch quality and quantity was also found genotype and environment dependent (Ceballos et al., 2004; Benesi et al., 2003). In the case of both CN in tuber and leaf and tuber starch contents, maturity of the crop is always found as a key determinant.

Considering all these facts, results are presented from the existing experiments under LTFE and NUE cassava genotypes to infer on the effect of secondary and micronutrients and NUE genotypes on CN in leaf and tuber and starch in tuber towards the maturity of the crop during the 7 to 9 MAP. Correlation has also been worked out among these biochemical parameters.

Materials and Methods

The present investigation on the effect of secondary and micronutrients and the effect of NUE genotypes on biochemical parameters viz., tuber and leaf CN and tuber starch was carried out in two existing activities ongoing at ICAR-CTCRI viz., Long term fertilizer cum manurial experiment (LTFE) in cassava variety Sree Visakham and screening NUE cassava genotypes viz., Sree Pavithra, CI-905, CI-906 and 7 III E3-5 for low input management respectively.

As regards to the study on the influence of secondary and micronutrients, the existing activity on LTFE is ongoing for the last 43 years at ICAR-CTCRI since 1977. The present third phase of the activity initiated in 2005, originally had 20 treatments replicated thrice in RBD. The cassava variety used is a hybrid H 1687 (Sree Visakham). The experiment is in progress at Block V of ICAR-CTCRI farm where the soil type is coarse sandy clay loam (Kandi Ustult) having pH ranging from 4.5-5.0, high organic carbon ($>$0.75%), low, very high and medium available N, P, K with values as 75-150, 50-150 and 100-200 kg ha^{-1} respectively. As regards to the secondary nutrients viz., Mg, Zn and B, Mg is very low to the tune of $<$0.5 meq $100g^{-1}$, Zn as very high (2-5 ppm) and B is less than 0.5 ppm. From these 20 treatments, we have recorded observations from treatments as enumerated below for the present study:

T1: Boron alone (B), T2: Zinc+ Boron (Zn + B), T3: Boron+ Magnesium+ Zinc (B + Mg + Zn) T4: Zinc alone (Zn), T5: Boron +Magnesium (B + Mg), T6: Zinc + Magnesium (Zn+ Mg) T7: Mg (Magnesium alone) and T8: Package of Practices (PoP) where N, P and K were applied @ 100:50:100 kg ha^{-1} along with 12.5 t ha^{-1} farm yard manure (FYM).

In the case of the first seven treatments, they were applied along with the existing PoP of cassava. Moreover, PoP kept as one of the treatments was the control.

Based on soil test value of Mg, Zn and B as 0.788 meq $100g^{-1}$, 5.6 and 0.312 ppm, $MgSO_4$, $ZnSO_4$ and borax were applied @ 5, 2.5, 7.5 kg ha^{-1} (Susan John et al., 2010, KAU, 2011) during 3-4 MAP at an interval of 10 days between applications in the case of two nutrients and three nutrients combinations. Here, tuber and leaf samples were collected at fortnightly intervals four times during 7 and 9 MAP from two replications for the analysis of tuber and leaf CN and tuber starch.

In the case of NUE cassava genotypes, the four NUE genotypes tested in the experiment were the K efficient released cassava variety, Sree Pavithra, and three NPK use efficient genotypes viz., 7 III E3-5, CI-905 and CI-906 presently in pipeline for release. These four genotypes require only 25% of the recommended dose of PoP for getting yield on par with PoP thereby 75% of the NPK can be saved (Susan John et al., 2019b). This was also an ongoing experiment planted with the afore mentioned four NPK use efficient genotypes with 25% of the recommended dose of NPK near to the LTFE experimental site having soil of almost the same physico-chemical characteristics. Here, tuber and leaf samples were collected from two replications six times at weekly intervals during 7 to 9 MAP.

Analysis of the biochemical parameters were made in fresh samples of tubers and leaves immediately after sampling and processing. Tuber and leaf CN was determined following the methodology of Indira and Sinha, (1969). As regards to starch, the rapid titrimetric method standardized at ICAR-CTCRI by Moorthy and Padmaja, (2002) was followed .

The data generated from these two independent experiments on parameters viz., leaf and tuber CN and tuber starch were subjected to statistical analysis for analysis of variance (ANOVA). Correlation among the three parameters was done using R environment version 4.0 (R Version 4.0, 2020).

Results and Discussion

The results interpreted based on the statistical analysis of the data on the effect of secondary and micronutrients alone, two nutrient and three nutrient combination as well as the effect of NUE genotypes on tuber starch and leaf and tuber HCN over a period of time towards the later period of crop maturity is discussed below. The high starch content measured here can be attributed to the estimation made in fresh tuber samples directly from field.

1. Effect of secondary and micronutrients

a. Tuber starch

Statistical analysis of the data indicated significant difference among treatments as well as intervals on starch content (Table 1). Among the eight treatments tested, the starch content was significantly highest under the

Table 1. Effect of secondary and micronutrients on tuber starch during 7-9 MAP in cassava variety H-1687

Treat-ment	Treatments(T)	Tuber starch content (% FWB) at different intervals (I)				
		7.5 MAP	8 MAP	8.5 MAP	9 MAP	Mean (T)
1	PoP+B	41.03	37.5	33.38	28.49	35.10^{bc}
2	PoP+Zn+B	28.98	30.96	33.62	33.59	31.78^{d}
3	PoP+B+Mg+Zn	28.87	31.81	32.62	34.94	32.06^{d}
4	PoP+Zn	28.32	35.54	37.82	41.34	35.75^{b}
5	PoP+B+Mg	37.71	37.50	36.15	35.61	36.74^{b}
6	PoP+Zn+Mg	34.63	40.92	42.41	43.37	40.33^{a}
7	PoP+Mg	26.06	29.82	31.92	36.03	30.95^{d}
8	PoP	33.46	33.38	32.15	31.48	32.62^{cd}
Mean (I)		32.38^{b}	34.68^{a}	35.01^{a}	35.60^{a}	

treatment Zn+Mg (40.33% on FWB). This was followed by B+Mg (36.74%) and was on par with Zn alone (35.75%) and B alone treatments (35.10%). Treatments viz., Zn+B, B+Mg+Zn and Mg alone resulted in significantly low tuber starch to the tune of 31.78, 32.06 and 30.95% respectively and was on par with PoP (32.62%) (Table 1).

Susan John et al., (2005, 2018, 2019a) reported increase in tuber starch contents with combined application of Zn along with Mg. As regards to the different intervals, there was significant effect with significantly the lowest during first sampling followed by an increase during all the intervals which in turn was on par (Table 1). Santisopasri et al., (2001), reported the need to harvest the crop when there is optimum maturity for maximum starch yield.

b. Tuber cyanogen

Statistical analysis of the data presented in Fig. 1a revealed significant difference among treatments on tuber CN. The highest tuber CN was recorded in plants under PoP to the tune of 95.07 ppm and all other treatments were on par. However, tuber CN was lowest under Mg (40.99 ppm) followed by Zn+Mg (43.08 ppm), Zn (44.36 ppm), B+Mg+Zn (44.68 ppm), B (45.00 ppm), Zn+B (50.95 ppm) and B+Mg (62.20 ppm). However, compared to PoP, there is substantial reduction in tuber HCN due to the application of secondary and micronutrients alone and in different combinations (Fig.1a). Susan John et al., (2005, 2018, 2019a) reported no significant variation among different treatments comprising of various combination of nutrients in affecting tuber CN.

As regards to the different intervals of sampling, no significant difference was noticed. However, there was a decrease till 8.5 MAP and afterwards, it increased slightly (Fig. 1b). Though there was no significant difference among growth stages, the slight decrease in tuber CN seen towards later period can be attributed to the findings of Bokanga et al., (1994), Gitebo et al., (2009) and Splittstoesser and Tunya (1992). It is seen that, except B and PoP, tuber CN drastically decreased in all treatments over the growth period. At ICAR-CTCRI, the studies conducted on cyanide potential of cassava tubers indicated the highest tuber CN at 3MAP and there is decline towards tuber bulking with minimal during 6-7MAP (CTCRI, 1990).

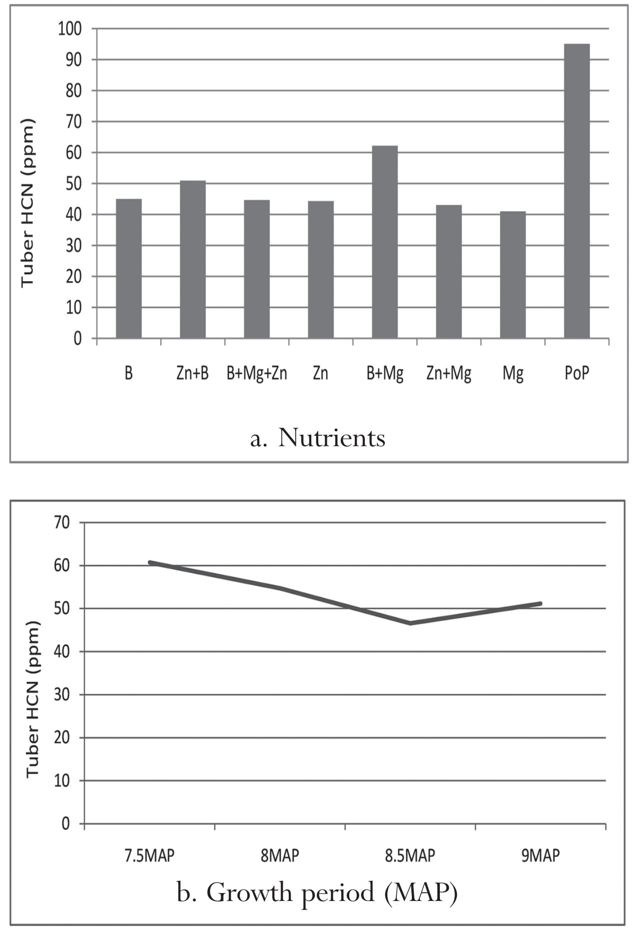
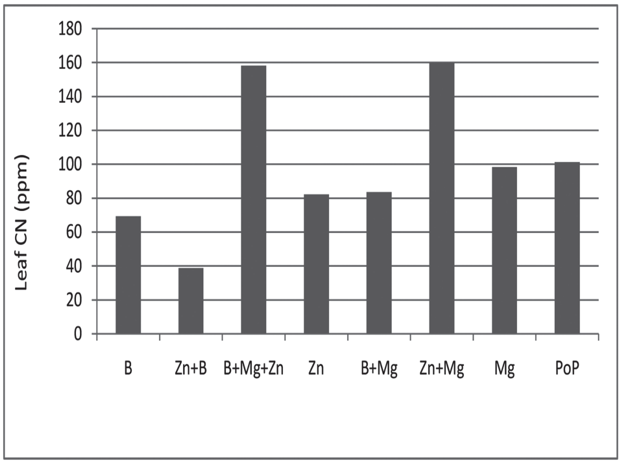


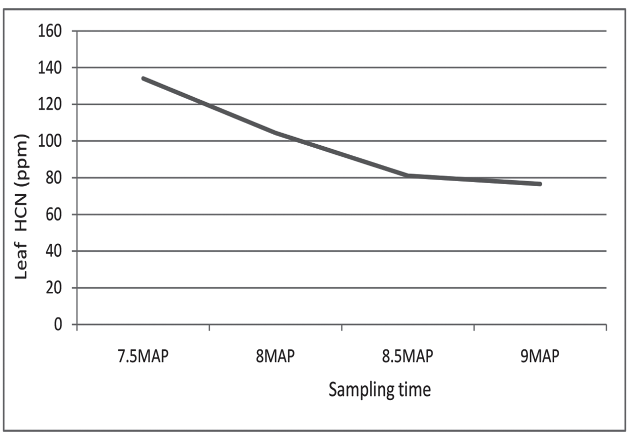
Fig. 1. Tuber CN as affected by different nutrients during the growth period of the crop

Leaf cyanogen

The differences in leaf CN content were statistically significant among treatments (Fig.2a) and intervals (Fig.2b). Among the treatments, application of Zn+Mg resulted in significantly highest leaf CN (159.76 ppm) and this was on par with B+Mg+Zn (158.15 ppm). Application of Zn+B resulted in significantly the lowest leaf CN (38.90 ppm) which was on par with application of B (69.43 ppm) and B+Mg (83.58 ppm) (Fig.2a). Susan John et al., (2018, 2019a) reported significant reduction in tuber CN with Zn application. But not much studies have been undertaken in leaf CN. In the present study it was seen that, compared to tuber CN, the leaf CN was always higher. Gomez and Valdivleso (1985) already reported that, the content of leaf CN is higher than that of tuber CN. As regards to different growth period, 7.5 MAP had significantly the highest leaf CN (134.05 ppm). But this was on par with 8 MAP (104.31



a. Nutrients



b. Growth period (MAP)

Fig. 2. Leaf CN as affected by different nutrients during the growth period of the crop

ppm). Leaf CN was the least (76.51 ppm) during 9 MAP which was on par with 8.5 MAP (81 ppm) (Fig. 2b). This is in agreement with the reports of Elizabeth et al., (2012) that, there is reduction in leaf CN towards maturity.

d. Correlation among tuber CN, leaf CN and tuber starch

Correlation studies made using the R environment version 4.0 indicated significant negative correlation between tuber starch and tuber CN ($r = -0.36$, p (0.05) = 0.0038), tuber starch and leaf CN ($r = -0.37$, p (0.05) = 0.0025) and significant positive correlation between tuber CN and leaf CN ($r = 0.35$, p (0.05) = 0.0047).

2. Effect of nutrient use efficient genotypes

Variation on tuber CN, leaf CN and tuber starch among the four NUE genotypes during 7-9 MAP towards maturity of the crop is presented below:

a. Tuber cyanogen

The data on tuber CN statistically analysed is presented in Table 2. It is seen that, there is significant interaction effect of genotypes and growth period on tuber CN content. Among the genotypes, 7 III E3-5 had the highest and Sree Pavithra had the lowest tuber CN which in turn was on par with CI-906. An evaluation of 38 cassava cultivars for tuber CN at ICAR-CTCRI indicated a range from 27-1100 mg kg^{-1} (CTCRI, 1984). As regards to the different intervals, highest CN was recorded during 7.5 MAP which in turn was on par with 7 MAP. The least tuber CN was recorded during 9 MAP which was on par with 8 MAP (Table 2). The differences in tuber CN content at different sampling during 7-9 MAP indicated a decreasing trend during the later stages of maturity (9 MAP) compared to 7 MAP.

The variation found in different cassava genotypes with respect to the tuber CN is in agreement with the report of Cardoso et al., (2005) that there is variation among

Table 2. Variation in tuber CN content of NUE genotypes at 7-9 MAP

Genotypes (G)	Tuber CN (ppm) at different intervals (I)						
	7 MAP	7.25 MAP	7.5 MAP	8 MAP	8.5 MAP	9 MAP	Mean (G)
Sree Pavithra	41.78	91.93	53.68	29.89	28.61	17.04	43.82[c]
CI-905	110.3	141.4	68.79	45.00	31.82	35.04	72.06[b]
7 lll E3-5	154.3	127.9	70.24	127.3	163.00	73.78	119.42[a]
CI-906	117.00	75.22	28.61	51.11	42.75	27.64	57.06b[c]
Mean (I)	105.84[a]	109.13[a]	55.33[bc]	63.32[b]	66.54[b]	38.37[c]	

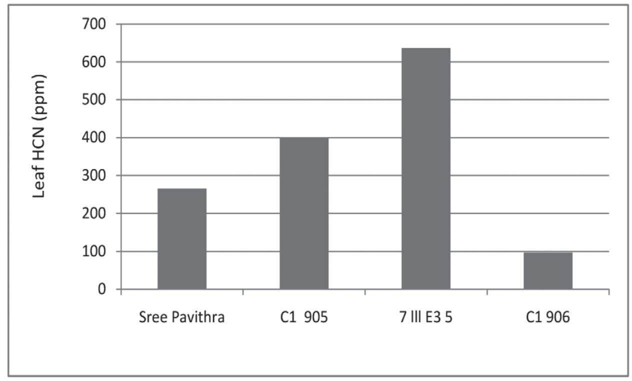
genotypes in the case of tuber CN. In the present study, the decrease in tuber cyanogen observed towards the harvesting, is in agreement with the findings of Bokanga et al., (1994), Gitebo et al., (2009) Splittstoesser and Tunya (1992) and Hidayat et al., (2002) who in turn reported that cyanide potential of roots drops as plant ages. But studies conducted at ICAR-CTCRI indicated no significant difference in the rate of increase in linamarin at different intervals among cultivars (CTCRI, 1997)

b. Leaf cyanogen

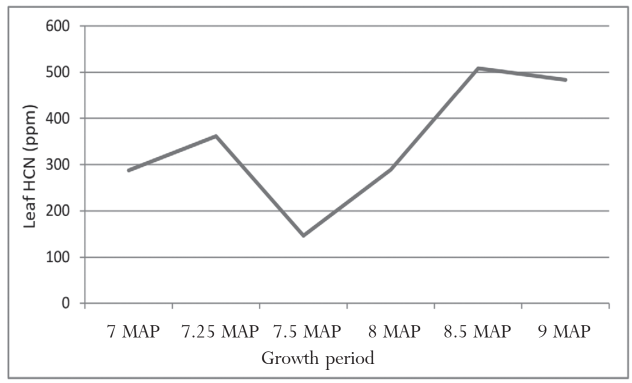
Compared to tuber CN, the leaf CN content is very high in all genotypes. Gomez and Valdivleso (1985) reported that the content of leaf CN is higher than that of tuber CN in cassava. As regards to the different genotypes, almost the same trend as in the case of tuber CN was followed with highest in 7 III E3-5 followed by CI-905, Sree Pavithra and the least in CI-906 (Fig. 3a). The leaf CN ranged from 25.71 ppm in genotype CI-906 to 992.63 ppm in 7 III E3-5. There is not any definite trend observed in the leaf CN during 7-9MAP in all these genotypes. The mean leaf CN of Sree Pavithra, CI-905, 7 III E3-5 and CI-906 respectively were 265.3, 398.59, 637.09 and 96.64 ppm. Cassava leaves contain high CN, mostly in the range of 1000 to 2000 mg kg^{-1} on dry weight basis (Gomez and Valdivleso, 1985). All cassava genotypes had the lowest leaf CN during the 7.5 MAP. The highest leaf CN was recorded during the 8.5 MAP except in the case of CI-906 which had the highest at 7.25 MAP (Fig. 3b). Since the tuber and leaf CN are interrelated and as the partitioning of CN to roots is reduced towards maturity, there may be concomitant increase in leaf CN to balance both. Studies at ICAR-CTCRI revealed that differences in tuber cyanide potential may not be related to rate of transport of linamarin from leaves to tuber (CTCRI, 1997)

As per Fig. 3a, the leaf CN was highest in 7 III E3-5 and lowest in CI-906. Sree Pavithra and CI-905 were on par with respect to leaf CN. The variation found in different genotypes with respect to the leaf CN as well as the declining trend noticed towards the maturity of the crop is in agreement with the report of Elizabeth et al., (2012).

Correlation worked out between tuber CN and leaf CN of the four NUE genotypes revealed significant positive



a. NUE genotypes



b. Growth period

Fig.3. Variation in leaf CN of NUE genotypes during 7-9 MAP

correlation (r = 0.396, p-value (0.05) = 0.00539) between the two confirming the direct relation between leaf and tuber CN. Hidayat et al., (2002) in 99 varieties of cassava showed that there is a significant positive correlation between cyanide potential of roots and leaves.

c. Tuber starch

The starch content of the NUE genotypes varied from 19.14% to 45.45% on FWB over the growth period. As regards to the different genotypes, Sree Pavithra had the starch content ranging from 31.28 to 45.45 % with a mean value of 38.67 %. In the case of CI-905, it ranged from 19.14 to 39.13 % with a mean value of 29.45 %. Starch content of 7 111 E3-5 ranged from 23.31 to 37.5 % with a mean value as 31.42 % and in CI-906, it was in the range of 33.33 to 43.68 % with a mean value of 36.35% (Table 3).

The variation in starch content in the different genotypes is in conformity with the findings of Ceballos et al., (2004) and Benesi et al. (2003). Among the genotypes, Sree Pavithra had the highest starch content on par with

Table 3. Variation in starch content in tubers of cassava genotypes during 7-9 MAP

Genotypes (G)	Tuber starch (%) on FWB at different samplings (I)						
	7 MAP	7.25 MAP	7.5 MAP	8 MAP	8.5 MAP	9 MAP	Mean (G)
Sree Pavithra	34.91	44.15	41.10	38.30	41.47	32.06	38.67[a]
CI-905	20.28	23.09	36.53	31.09	34.56	31.14	29.45[b]
7 lll E3-5	31.70	33.67	30.36	36.61	26.85	29.35	31.42[b]
CI-906	33.52	39.52	35.32	36.89	37.30	33.65	36.04[a]
Mean (I)	30.10[c]	35.11[ab]	35.83[a]	35.72[a]	35.05[ab]	31.55[bc]	

CI-906. Genotypes CI-905 and 7 III E3-5 were on par with respect to tuber starch. During a time span of three months from 7-9 MAP, though a drastic increase was occurred during the four samplings after the first sampling, in the last sampling at 9MAP, there observed a sharp drop on par with that at 7MAP (Table 3). At ICAR-CTCRI, a study on the changes in tuber starch from 6-11 MAP in eight varieties of cassava showed differences among varieties in tuber starch and there is increase in starch from 6-10 MAP and then declined at 11MAP (CTCRI, 1968).

Among the four genotypes, Sree Pavithra had the highest starch content (38.67 %) in tubers followed by CI-906 (36.35 %), 7 III E3-5 (31.42 %) and CI-905 (29.45 %). Though a decrease in starch content was observed during 9 MAP this was not uniform in the case of all genotypes. However, all the genotypes at 9 MAP had very low tuber starch content to the tune of 32.06, 31.14, 29.35 and 33.97 % respectively. As regards to the interaction effect of genotypes and sampling intervals on tuber starch, Sree Pavithra and CI-906 had the maximum starch content during 7.25 MAP, CI-905 during 7.5 MAP and 7 III E3-5 during 8 MAP. Sree Pavithra had low starch content on 9 MAP, 7 III E3-5 during 8.5 MAP but CI-905 and CI-906 during 7 MAP. These findings are in adherence to the reports of Santisopasri et al., (2001); EI-Sharkawy (2006) and Egesi et al., (2007) that there is a decrease in the tuber starch content towards the maturity of the crop from 8-10 MAP.

Conclusion

The primary aim of this investigation carried out in two ongoing research programmes at ICAR-CTCRI was to see the influence of secondary and micronutrients and nutrient use efficient genotypes on tuber starch, tuber CN and leaf CN at periodic intervals towards the later stages of maturity as well as to establish the correlation between these biochemical attributes. Though there are reports available on the effects of primary nutrients on these parameters, there are not much information as regards to secondary nutrient Mg and micronutrients Zn and B either alone or in different combinations.

As regards to the effect of secondary and micronutrients, there was favourable effect of these nutrients alone and in combination on tuber and leaf CN and tuber starch. Though all nutrients alone and in combination were on par in affecting tuber CN, tuber starch was enhanced significantly under Zn along with Mg application whereas leaf CN increased under Zn along with B. Though tuber maturity had no significant effect in the case of tuber CN, leaf CN showed a significant reduction and tuber starch showed a significant increase under these nutrients. These results can definitely be useful while secondary and micronutrient applications are practiced in cassava for specific purposes like human consumption, animal feed and starch for industrial purposes.

Though the variation among cassava genotypes for biochemical attributes as mentioned above are already reported, these information were very limited on nutrient use efficient genotypes. The study established that, Sree Pavithra followed by CI-906 had low tuber CN and high starch. In this case, when tuber CN was reduced drastically towards the later period of crop maturity, the leaf CN increased significantly whereas the tuber starch fluctuated significantly. These information could really be useful in selecting NUE genotypes for specific purposes like culinary and industrial as well as in fixing the date of harvest.

Both the studies clearly established the significant positive correlation between tuber CN and leaf CN in addition to the significant negative correlation established between tuber starch and tuber CN and tuber starch with leaf

CN. Compared to the NUE genotypes, the cassava hybrid variety H-1687 showed comparatively low CN both in tuber and leaf during all intervals of sampling. Hence, the information derived from this investigation can definitely be of academic as well as strategic research interest on the use of nutrients and NUE genotypes for domestic and industrial purposes.

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