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Classification and Selection of Edible Cassava Lines Based on Cyanide Levels

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Abstract

Cassava contains naturally occurring, but potentially toxic compounds called cyanogenic glycosides, which release hydrogen cyanide (HCN) as a result of enzymatic hydrolysis following maceration of the plant tissue. The consumption of cassava can therefore be harmful to human health. Cassava and its products have drawn attention by international food safety regulators on a number of occasions. Although varieties are selected for their agronomic characteristics, their low levels of cyanide classify them for culinary use; those with higher levels are selected for industrial use. Hence an experiment was conducted to classify the 75 genotypes of cassava based on the cyanide levels. Sodium picrate method was used to determine the cyanide levels. Seventy five genotypes of Cassava were classified into low (0 to 50 ppm), medium (50-100ppm) and high (more than 100 ppm) cyanide groups. Twenty one edible genotypes showed a variation of potential cyanide between 24.3 ppm to 48.7 ppm classifying them as harmless. The accession MeAp-1 recorded highest cyanide content of 256.1 ppm which is fit for industrial use. The genotypes classified under low cyanide levels were for further evaluated for reducing sugars, non reducing sugars, starch and other agronomical characters to identify the best edible genotypes. The results revealed that Me AP-72 (PA-46) genotype registered highest values for reducing sugars (6.5%), non reducing sugars (5.2%), total sugars (11.7%) and least values for HCN content (24.3 ppm) and starch (17 %).

Key words: *Manihot esculenta*, cyanide levels, edible types

Introduction

Cassava contains naturally occurring, but potentially toxic compounds called cyanogenic glycosides, which release hydrogen cyanide (HCN) as a result of enzymatic hydrolysis following maceration of the plant tissue. These cyanogenic glycosides break down to release toxic hydrogen cyanide gas during digestion (Poulton, 1988). The consumption of cassava can therefore be harmful to human health. All cassava cultivars contain cyanogenic glucosides, however a wide variation in the concentration of cyanogens exists among different cultivars. Cassava plants are classified into three categories based on their potential cyanogenic content expressed in fresh pulp: a) harmless (less than 50 HCN $mg.kg^{-1}$); b) moderately poisonous (between 50-100 HCN $mg.kg^{-1}$), and c) dangerously poisonous (above 100 HCN $mg.\ kg^{-1}$) (Bourdoux *et al* 1982). As per this classification the recommended limit for safe intake of cyanide from cassava was established by the FAO/WHO as 10 HCN $mg.Kg^{-1}$ of body weight (WHO, 1991). Thus, the determination of cyanide in fresh cassava roots anticipates its potential toxic effect. The varieties grown at East and West Godavari districts of Andhra Pradesh are used for industrial purpose whereas varieties grown at high altitude and tribal areas and North coastal areas are used for edible purpose. Hence efforts have been made to collect the genotypes from the tribal areas of Srikakulam, viziyanagaram and Visakhapatnam districts of Andhra Pradesh. As cassava is a major tuber crop for consumption in tribal areas, there is a need to identify the edible genotypes based on their cyanide levels to avoid harmful health hazards. Therefore, an experiment was planned to classify the collected genotypes into harmless, moderately poisonous and dangerously poisonous, based on their cyanide levels and further evaluating the harmless category into agronomically superior edible genotypes.

Materials and Methods

The experiment was conducted from 2013 to February 2016 at the experimental farm of the, Horticultural Research station of Dr. Y.S.R. Horticultural University, Venkatramannagudem Andhra Pradesh. This location, at 16°83' N latitude, 81°50' E longitude, is 34 m above mean sea level. Nursery beds were prepared with dimensions of 500 × 100 × 15 cm in length, width and height. Well matured healthy and disease free stems of previous season of each genotype were used as planting material for the experiment. The bottom (5 to 10 cm) and top 1/3rd portion of the stems were discarded and the remaining part was cut into setts of 20 cm length. These setts were planted at 5 x 5 cm spacing on the raised nursery bed and watered regularly. The main field was thoroughly ploughed to a depth of 30 cm and brought to good tilth. The recommended synthetic fertilizers at the rate of $N:P_20_5:K_20$ at 60:60:60 $kg.ha^{-1}$ were applied. In which single-superphosphate was applied as basal dose whereas urea and muriate of potash were applied in three equal split doses at 30, 60 and 90 days after transplanting. Seventy five genotypes collected from Andhra Pradesh and other Cassava growing areas were used for the study and they were arranged in a randomized complete block design with 2 replications in 4.5 × 4.5 m plots. The young healthy, one week old sprouted settlings obtained from the nursery were planted at a spacing of 90 x 90 cm between and with-in rows and 5 cm depth. Plots were kept free from weeds by regular hand weeding. At the time of harvest tubers from five plants of each genotype in each replication were chosen for cyanide determination. The cyanide analysis was performed according to the procedure described by Bradbury *et al* 1999. The colorimetric reaction with alkaline picrate developed color gradient due to the variation of cyanide concentration, which was measured by spectrophotometer at 520 nm. A representative sample of the root was obtained by cutting 1-2 mm thick section across the root about half way along its length. After removal of the peel 100mg sectors were cut from the disc and six adjacent 100 mg sectors were analysed using the method described for the analysis. Those genotypes, whose cyanide levels under harmless category were further evaluated for 6 quantitative (plant height, stem girth, number of tubers, tuber length, tuber girth and total yield) and 5 qualitative characters (reducing sugars, non reducing sugars, total sugars, starch and HCN) to identify the sweet edible genotypes. Starch content was determined by using the method outlined by Mc Cready *et al*. (1950). Total sugars and reducing sugars were determined following the method described by Lane and Eyon (AOAC, 1965). Non reducing sugars in a sample were obtained by subtracting reducing sugar from total sugars. The data recorded for five plants in each replication were used for statistical analysis.

Results and Discussion

Based on the concentration of cyanide in the roots, the cultivars were classified as harmless (Table 1), moderately poisonous (Table 3) and dangerously poisonous (Table 2). The HCN ranged from 256.1 to 24.3 mg Kg^{-1} on FW basis. The dangerously poisonous group genotypes

Table 1. Cyanide level: HCN 0-50 mg kg^{-1} FW (Harmless genoytpes)

S.No	Entry	HCN (mg Kg^{-1}FW)	Cyanide level
1	Me AP 2	43.64	Low
2	Me AP 5	44.09	Low
3	Me AP 6	26.14	Low
4	Me AP 8	36.34	Low
5	Me AP 18	44.34	Low
6	Me AP 20	33.99	Low
7	Me AP 21	26.79	Low
8	Me AP 23	38.94	Low
9	Me AP 24	26.29	Low
10	Me AP 26	48.74	Low
11	Me AP 30	41.54	Low
12	Me AP 42	28.64	Low
13	Me AP 55	43.64	Low
14	Me AP 65	30.69	Low
15	Me AP 69	35.34	Low
16	Me AP 36	37.69	Low
17	Me AP 38	45.64	Low
18	Me AP 40	47.64	Low
19	Me AP 70	34.64	Low
20	Me AP 72	24.30	Low
21	Me AP 73	48.04	Low

Table 2. Cyanide level (HCN above 100 mg kg^{-1} FW) (Dangerously poisonous genotypes)

S.No	Entry	HCN (mg Kg^{-1}FW)	Cyanide level
1	Me AP 1	256.14	High
2	Me AP 12	110.69	High
3	Me AP R1	104.24	High
4	Me AP R2	155.04	High
5	Me AP 51	139.44	High
6	Me AP 54	130.24	High

consisted with higher cyanide levels ranging from 256.1 to 104.2 mg Kg^{-1} FW. Among these MeAp1 recorded maximum cyanide level. The moderately poisonous group consisted of genotypes with moderate levels ranging from 94.0 to 51.1 mg Kg^{-1} FW. The harmless group, which showed variations of cyanide levels from 48.7 to 24. 3 mg Kg^{-1} FW. The least cyanide level was recorded in MeAp72. Cassava tubers vary widely in their cyanogenic glycoside content, although most varieties contain 15 – 400 mg cyanide kg^{-1} fresh weight. Occasionally varieties of cassava tubers contain 1300 - 2000 mg HCN Kg^{-1} FW Padmaja (1995).

The classification of genotypes based on cyanide levels helps to distinguish them into "culinary purpose" and "industrial purpose" Bourdoux et al (1980). Embrapa (2003) classify the Roxinha cultivar as suitable for industrial use due to a moderate level of cyanide (100-150 mg HCN kg^{-1}) in raw pulp. The variations in cyanide concentration among different cultivars can be attributed to the genotypic variations of the cultivars either naturally present or introduced in the process of their evolution. It is interesting to note that although the cultivars were growing in the same ecological niche, their levels of cyanide are significantly different pointing to the fact that there is a close relationship between cultivar type and cyanide concentration.

For an adult, consumption of 50 to 100 mg HCN within 24 hours can completely block cellular respiration leading to death (Delange *et al*1994). Hence "harmless group" identified from this classification are safe for

Table 3. Moderately poisonous Genotypes (Cyanide level: HCN 50-100 mg kg^{-1} FW)

S.No	Entry	HCN(mg Kg^{-1}FW)	Cyanide level	S.No	Entry	HCN (mg Kg^{-1}FW)	Cyanide level
1	MeAP 3	69.64	Medium	26	MeAP 45	62.39	Medium
2	MeAP 4	51.84	Medium	27	MeAP 46	66.94	Medium
3	MeAP 7	60.94	Medium	28	MeAP 47	70.79	Medium
4	MeAP 9	76.69	Medium	29	MeAP 48	75.64	Medium
5	MeAP 10	87.34	Medium	30	MeAP 50	61.14	Medium
6	MeAP 11	51.14	Medium	31	MeAP 51	71.00	Medium
7	MeAP 13	63.19	Medium	32	MeAP 52	59.14	Medium
8	MeAP 14	51.59	Medium	33	MeAP 53	54.19	Medium
9	MeAP 15	67.29	Medium	34	MeAP 54	57.84	Medium
10	MeAP 16	67.34	Medium	35	MeAP 55	69.29	Medium
11	MeAP 17	55.64	Medium	36	MeAP 56	61.64	Medium
12	MeAP 19	76.69	Medium	37	MeAP 57	87.94	Medium
13	MeAP 25	74.69	Medium	38	MeAP 58	75.64	Medium
14	MeAP 27	73.34	Medium	39	MeAP 59	71.64	Medium
15	MeAP 28	81.14	Medium	40	MeAP 60	85.94	Medium
16	MeAP 29	66.64	Medium	41	MeAP 62	62.74	Medium
17	MeAP 31	94.06	Medium	42	MeAP 63	70.04	Medium
18	MeAP 32	55.99	Medium	43	MeAP 66	66.64	Medium
19	MeAP 33	61.84	Medium	44	MeAP 67	59.94	Medium
20	MeAP34	51.74	Medium	45	MeAP 68	89.99	Medium
21	MeAP35	66.69	Medium	46	MeAP 70	66.09	Medium
22	MeAP37	73.74	Medium	47	MeAP 71	53.64	Medium
23	MeAP39	57.79	Medium	48	MeAP 74	61.29	Medium
24	MeAP 43	52.64	Medium				
25	MeAP 44	69.89	Medium				

Table 4. Evaluation of edible Cassava genotypes

S.No	Edible Genotypes	Plant height (cm)	Stem girth (cm)	No. of tubers	Tuber length (cm)	Tuber girth (cm)	Red. sugars (%)	Non reducing sugars(%)	Total sugars (%)	Starch (%)	HCN (ppm)	Total yield (t ha^{-1})
1	MeAP8	349.9	10.5	10.9	47.5	19.3	2.2	2.4	4.6	26.9	38.9	38.6
2	MeAP21	315.1	10.3	8.2	40.2	17.0	2.7	3.1	5.8	23.3	29.0	20.1
3	MeAp23	292.8	9.9	9.4	34.6	18.8	2.8	3.2	6.0	27.8	39.4	23.9
4	MeAP24	289.5	10.4	9.2	34.1	17.9	1.9	3.5	5.5	24.4	28.3	20.1
5	MeAP18	324.3	9.2	9.0	34.8	18.6	2.9	2.9	5.7	22.6	41.5	25.9
6	MeAP20	291.0	9.4	10.7	33.5	18.8	2.8	3.0	5.8	27.8	36.2	26.8
7	MeAP72 (PA46)	253.1	7.3	7.2	24.0	16.0	6.5	5.2	11.7	17.3	24.3	22.0
8	S.Visakham	346.1	10.3	8.9	36.9	19.1	3.0	5.5	8.5	27.6	43.8	29.5
	CD (P=0.05)	57.6	1.3	1.7	6.3	NS	0.6	0.9	0.9	2.1	2.0	6.9
	CV%	10.6	8.2	11.1	10.1	9.6	11.0	14.0	8.0	4.9	3.2	15.4
	Sed	26.8	0.6	0.8	2.9	1.4	0.3	0.4	0.4	1.0	0.9	3.2

consumption. This group was further narrowed down to eight genotypes based on their consistency in expression of their cyanide levels and evaluated for their quantitative and qualitative characters with the released variety Sree visakham as a check.

Significant differences were observed between the treatments for the different characters studied (Table 3). Maximum numbers of tubers were observed in MeAp8 which was par with MeAP23, MeAP24 and MeAp20. Maximum amount of reducing sugars, non reducing sugars, total sugars and least amount of starch and cyanide levels were observed in MeAP72 which shows its sweet nature. Maximum yield was recorded in MeAP8.

Conclusion

By considering the quantitative as well as qualitative characters MeAP 72 was identified sweet cassava genotype. Although cyanide toxicity is the major concern when a cassava cultivar is recommended for use as food, the culinary quality of the fresh roots should also be taken into account as an essential parameter in the selection of cultivars for domestic use, involving complex factors (physical-chemical and sensory characteristics, cooking time, color, texture of the cooked pulp etc.).

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