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THE CYANOGENIC POTENTIAL OF ROOTS AND LEAVES OF NINETY NINE CASSAVA CULTIVARS

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ABSTRACT

Studies on cyanogenic potential (CP) of roots and leaves of Indonesian cassava germplasm are still inadequate in relation to food toxicity and on human health. The CP of leaves of 99 cassava cultivars was analyzed using picrate paper kits. Effort to reduce CP of cassava leaves by boiling them was also conducted. The results showed that roots and leaves had low and moderate level of CP. There was a significant correlation between the CP of leaves (Y) and roots (X) of 45 cassava cultivars with regression equation Y = 36.214 + 1.3085 X (r = 0.5228). The CP content was high in the young leaves (241 ppm) and low in the older ones (99 ppm). The proximal portion of the roots had the highest CP content (300 ppm), and that in the distal end was the lowest (56 ppm). The root part close to cortex had highest CP content (550 ppm), whereas the central part was the lowest (35 ppm). Boiling cassava leaves for 20 minutes significantly reduced the CP up to 75%, indicating that for safety, cassava should be completely processed or cooked. This study implied that CP content should be considered in cassava breeding programs. Forty two of 99 cassava cultivars have CP below 50 ppm which is safe for consumption.

[*Keywords*: Cyanogenic potential, cassava, linamarin]

INTRODUCTION

The increasing price of wheat flour has forced the Indonesian people to look into the potential of cassava (*Manihot esculenta* Crantz) substitution for wheat as raw materials of most food, breads, cakes, and others. This condition has generated the re-emerging of various traditional cassava cakes and supplement for rice as the main staple food of the people. More than 15 traditional cassava cakes have been widely sold in the markets (Darjanto and Murjati, 1980).

Cassava leaves have been used as a vegetable in many countries, i.e., African countries, the Philippines, and Indonesia. They can significantly improve the nutrition of people, and are a good source of protein, calcium, and vitamin.

Indonesia produces 14 million tons of cassava (fresh root) per year from 1.2 million ha areas (Widodo and Sumarno, 1990). About 64% is consumed directly as food (fresh root or dried root locally called *gaplek*). A large amount is used locally and exported to the European Community as feed and smaller amount is processed to flour, tapioca, starch, and other products (Damardjati *et al*., 1993).

Young leaves of cassava have long been consumed as green vegetable in various restaurants. Aside from this extensive consumption, however, problems remain on the cyanide content of roots and leaves that is harmful to humans. Several food toxicity cases of cassava have been reported in a number of African countries (Osuntokun, 1973; Delange and Ahluwalia, 1983; Cliff, 1994), and also in Indonesia (Kompas February 18, 1999). Endemic diseases such as ataxic neuropathy and konzo, associated with cassava consumption have also been reported from Nigeria, Tanzania, Zaire, Mozambique, and Central African Republic (Osuntokun, 1973; Delange and Ahluwalia, 1983; Cliff, 1994).

There are three different forms of cyanogens present in cassava leaves and roots, i.e., linamarin, acetonecyanohydrin (lotaustralin), and free HCN. The linamarin and lotaustralin undergo a sequential enzymatic breakdown, and the final form is a toxic free cyanide. The total of these three forms is called cyanogenic potential (CP).

The cyanide contents of cassava leaves and roots varied according to ages and parts of the roots, and ranged from 189 to 2466 ppm (Cooke, 1978; Fukuba *et al*.,1984; Morales and Villegas, 1994; Ekanayake and Bokanga, 1994), while the lethal dose for humans is 1 mg kg^{-1} bodyweight (Jones *et al.,* 1993). The older leaves contain lower cyanide compared to the younger ones. The leaves located at lower part of the plant may have different CP content from those located at the upper one. The root segments close to stem end may have different CP content from the distal end, and part of the root close to the cortex may also have different CP content from the central part of root (Bolhuis, 1954; De Bruijn, 1973).

Information on the CP content of Indonesian cassava germplasm collection, particularly on leaves,

is still lacking. This information is important to avoid toxicity hazard for consumers and to understand which parts of plant are more toxic, so that program on reducing the risk of CP in cassava can be implemented effectively. The purpose of this paper is to study the CP of leaves of 99 cassava cultivars, and several traditional cakes made of cassava product. Selection of 99 cassava cultivars out of 150 germplasm collections was due to priorities and workload of the sampling and analysis.

MATERIALS AND METHODS

Cassava Cultivars

The analysis of HCN in cassava roots and leaves reported in this paper is part of the ongoing germplasm program of Indonesia to investigate the potential toxicity of cassava in our germplasm collection. The CP content of roots of 179 cultivars has been reported by Hidayat *et al*. (2000). The study was continued to analyse CP content of 99 new cultivars, particularly on the leaves. The cultivars were obtained from the Indonesian Germplasm Collection at Muara Experimental Station, Bogor, West Java. Most popular cultivars were included in the study, and others were selected by excluding those with very similar characteristics.

Correlation Analyses of Cyanide Potential of Roots and Leaves

Two 8-month-old plants were sampled from 45 cultivars. Due to the limitation of germplasm collection, harvest schedule, and workload, only 45 selected cultivars were permitted to be harvested for root-leaf CP correlation analyses. Two representative roots and leaves (third leaf from the top) were taken from each plant. The roots were peeled and processed according to protocol A described by Bradbury *et al.* (1999). This sampling technique has been studied earlier by several workers (Cooke, 1978; Bradbury *et al.,* 1999). Finally, all slices were mixed well and subsampled as a single sample per cultivar. Correlation between CP of roots and leaves was studied using Statistica-V5 for Windows. The significance of the slope was tested by comparing the t-table using a one-tailed test.

To study the variation of CP within the roots, the roots were peeled and cut transversely into seven blocks, from the proximal part (stem end) to the distal end of the root, then each block was cut diagonally into six parts (Fig. 1). Variation of CP from cortex through the central part of root was studied by cutting the roots in a ring form into four parts (Fig. 2). Each part was mixed and analyzed for CP content individually.

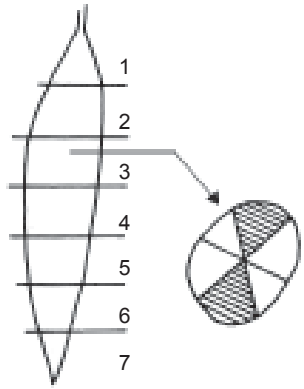

Fig. 1. Cross section of the root. Tuber was devided into seven blocks and each block was cut into six part.

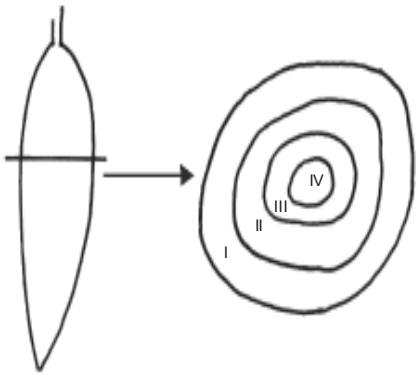

Fig. 2. Longitudinal section of the root. Tuber was cut in the middle; cross section was devided into four parts.

Analyses of CP Variation in Leaves

Ninety nine cassava cultivars were used for the study of the variation of CP in the leaves and its cluster analysis. Two 8-month-old plants were sampled from each cultivar. Two representative leaves (third leaf from the top) were taken from each plant. Ten parts were sampled to know the variation of CP from younger to older leaves. The youngest leaves were taken from the top and the oldest ones were from the lowest part (Fig. 3). Each part was analyzed individually.

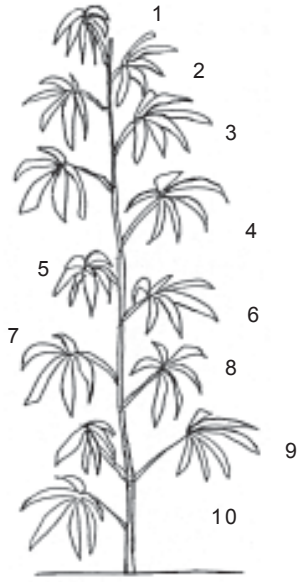

Fig. 3. Position of 10 sampled leaves (duplicate) in the plants.

Effect of Leaf Boiling on the CP Content

Effect of boiling time on the CP of leaves was studied by using three cultivars, i.e., BIC 32 represented high CP, BIC 28 for medium CP, and BIC 14 for low CP. Each cultivar was divided into three subsamples and analyzed individually. About 100 g samples were soaked in boiled water for 5, 10, and 15 minutes, and then the samples were taken, drained, and analyzed.

Analysis of Cyanogenic Potential

The rapid picrate paper test of Bradbury *et al*. (1999) was used to analyze the CP. The picrate paper was prepared by dipping a sheet of Whatman-3MM filter paper in a picrate solution. The paper was then air dried and cut into 3 cm x 1 cm strips, and put on 5 cm x 1 cm clear plastic strips to keep the paper clear of the liquid.

Sample (roots, leaves, or cassava cakes) was sliced in about 1-mm size, and 100-mg weight and placed on top of a 21-mm diameter Whatman-3MM filter paper in a flat-bottomed plastic vial. Water (0.5 ml) was added and a yellow picrate paper was immediately inserted in the vial. The vial was closed immediately with a screw lid and allowed to stand at 30^{o}C for 24 hours. The picrate paper was separated from the plastic strip, and the color of the filter paper was eluted by placing it in 5 ml water for about 30 minutes. The absorbance of the solution was measured at 510 nm against a blank that contained a yellow solution produced from a picrate paper not exposed to HCN. The measurement of brownish-yellow color of the picrate paper was accomplished by using Hitachi-2100 spectrophotometer at a wavelength of 510 nm.

Cyanogenic potential of cassava cakes and boiled leaves were determined using the same method. However, because cassava cakes and boiled leaves did not contain linamarase, two drops of linamarase solution prepared by the method of Rezaul and Bradbury (1999) were added before the addition of 0.5 ml water. Linamarase breaks down the cyanide complexes into simple HCN form, which reacts with pricrate paper to form a brownish-yellow solution. The measurement of brownish-yellow color of the picrate paper was accomplished spectrophotometrically. Cyanogen content (ppm = $\mu g\ HCN^{-1}g^{-1}$ fresh samples) is 396 x absorbance.

Cluster analysis was implemented using Statistica-V5 for Windows. Partitioning methods were chosen rather than hierarchical one because the number of clusters were pre-determined (five clusters). Cyanogenic potential of the samples was clustered into five categories, e.g., very high, high, medium, low, and very low, based on the lowest variation within the cluster and the highest variation between the cluster.

RESULTS AND DISCUSSION

Cyanogenic Potential of Cassava Leaves

Cluster analysis of leaf CP of 99 cassava cultivars is shown in Table 1. There is a great variation of CP containing in the leaves amongst cultivars. The highest CP was found in CM14-14 cultivar (779 ppm) and the lowest was (9 ppm) in Sikabu-kabu cultivar .

Fourty eight percent of leaves tested contain CP below 85 ppm. Concentration of CP in the leaves was three times higher than that in the roots. This was smaller than that reported by Fukuba *et al*. (1984) where cassava leaves contained CP 2-27 times higher than in the roots. Our previous studies found that the CP of roots of 179 cassava cultivars varied from 12 to 258 ppm with an average of 82 ppm (Hidayat *et al*., 2000). The F-test shows that the CP content of cassava roots and its cluster were not significantly different.

There are observable differences in the leaf color among cultivars tested, i.e., green, dark green, and slightly purple. There is no correlation between leaf color and its CP content indicating that leaf color cannot be used for predicting the CP of cassava

Tabel 1. Cluster analysis of cyanogenic potential of leaves of 99 cassava cultivars.

Category	CP content (ppm)		SD	Cultivar/CP content (ppm)
	Range	Mean		
Very high	491-779	645.3	116.4	CM 14-14/779, G63/591, GM 3/566
High	335-490	429.5	39.7	BIC 11/490, CRM 33.19.2/478, No. 77.19.2/470, SL 75/468, Mlk 29/438, Mlk 10/427, No. 72.2.10/423, BIC 147/413, BIC 317/400, BIC 162/391, BIC 133/387, Bogor/369.
Medium	216-334	283.0	35.4	Sidoli/334, BIC 366/333, 18/327, 302/312, 116/307, 11/303, 3/297, 135/286, 27/284, 57/275, 224/263, 216/262, 207/247, 126/238, 172/231, 178/230.
Low	94-215	152.1	38.0	BIC 190/215, BIC 17/206, BIC 86/205, BIC 8/200, BIC 153/186, BIC 22/185, BIC 348/171, Manalagi/154, Rawi/148, Rostopi/147, SM 1875.2/137, SM 803.2/137, SM 809.1/129, BIC 90/126, BIC 43/124, BIC197/122, BIC 230/119, BIC 155/119, BIC 19/112, BIC 5/103, BIC 205/94,
Very low	9-93	29.8	17.4	Sikabu-kabu/9, Baturaja/11, CMR 33.10.11/12, BIC 141/12, BIC 163/12, BIC 173/13, BIC 13/16, BIC 83/16, BIC109/16, BIC 14/17, BIC 214/17, BIC 69/17, BIC 150/18, BIC 164/19, BIC 106/19, BIC 183/20, BIC 228/22, BIC151/22, Sigaronggang/22, Basiran/22, Urang/23, Valenca/23, Klenteng/23, BIC300/23, BIC 80/22, BIC 105/25, BIC157/25, BIC 28/29, BIC 15/31, BIC 95/32, BIC 142/32, BIC108/34, BIC 103/34, BIC 249/34, BIC 298/34, BIC 339/36, BIC 70/40, BIC 16/41, BIC 21/44, BIC 4/46, BIC 7/46, BIC 219/50, BIC 179/51, BIC 69/55, BIC 82/69, BIC 140/83, BIC 190/84.

leaves. The higher the CP content of leaf, the smaller the cluster member, and the higher the standard deviation of the data. The same observation was done in the CP content of the roots (Hidayat *et al*., 2000).

For food safety, the Indonesian Ministry of Industry and Trade has the safety level for cyanide content of cassava chip at 40 ppm (Widowati *et al.,* 1992), whereas the World Health Organization (FAO/WHO, 1991) established the critical level at 10 ppm. On the basis of the cyanide content, Bolhuis (1954) and Coursey (1973) classified cassava into three groups: (1) the sweet or non-toxic level with CP content less than 50 ppm; (2) intermediate level or moderately poisonous containing 50-100 ppm; and (3) bitter, toxic or very poisonous containing above 100 ppm. According to the these safety levels, 41 cassava cultivars studied are considered safe, because the CP is below 50 ppm. Another 6 cultivars are intermediate, and 52 cultivars are very poisonous. These 47 cultivars are definitely safe for further development.

The correlation between CP in the leaves and in the roots of 45 cassava cultivars is presented in Fig. 4 and Table 2. This information is important to predict the CP content of roots and its particular leaves, and the dynamic behavior of CP in the plant parts.

There is a significant correlation between CP in the leaves and CP in the roots of 45 cultivars studied, with correlation equation of leaf-CP_{ppm} = 36.214 + 1.309 root-CP_{ppm} (r = 0.5228). This result supports the study by Cooke (1978) with 108 population, Ekanayake and Bokanga (1994) with 4 populations, and Morales and Villegas (1994) who reported that there is a significant correlation between linamarase activity in young leaves and in the roots. However, some researchers did not find any correlation in some cultivars (Bokanga, 1994; Ekanayake and Bokanga, 1994). These differences suggest that the correlation may depend on varieties and areas where the study is conducted. This significant correlation could have a practical use in the recognition of bitter or mild cultivars using leaf analysis, without harvesting the roots.

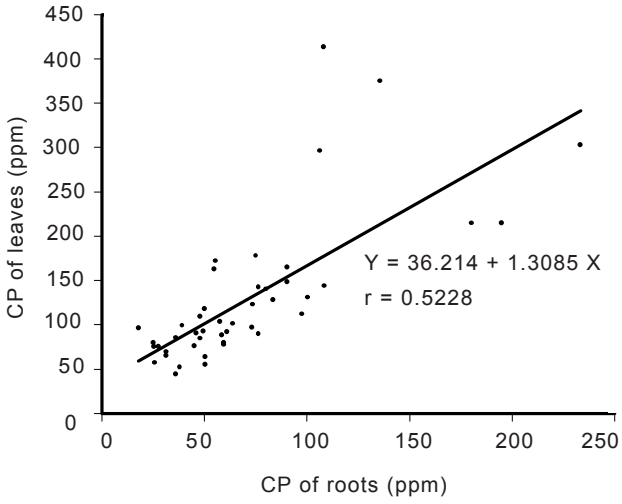

Fig. 4. Correlation between cyanogenic potential (CP) of roots and leaves of 45 cassava cultivars.

The CP of leaves of our cassava germplasm collection is much lower compared to the study by Fukuba *et al.* (1984), who reported that CP of cassava leaves varies from 189 to 2466 ppm. In our study, there are 63 cultivars which contain CP of the leaves below 189 ppm. Although there is no comprehensive reports on the consumer preference of particular cassava cultivars in Indonesia, based on this study and considering the long-term effect of poisonous cyanide on human health, it is recommended that 41 cassava cultivars containing CP less than 50 ppm in the leaves could be safely planted. The spread of the high CP cultivars, however, must be carefully controlled. Consumption of these cultivars, especially without processing may still result in considerable cyanide exposure. Therefore, strict adherence to efficient processing methods is needed if large amounts of roots or leaves from the high CP cultivars are consumed. Breeding programs should therefore be continued to take cyanide levels into consideration.

The CP of leaves varies according to the age or position of the leaf the plant. The CP was highest in the young uppermost leaves (241 ppm), and it decreased to 99 ppm in the older leaves (the lowest part). For high leaf-CP cultivars, the decrease in CP is 40%, and for medium and low leaf-CP cultivars are 26% and 35%, respectively (Fig. 5). The results support the works of Morales and Villegas (1994), that linamarase activity is higher in the young leaves than that in the old ones.

Table 2. Cyanogenic potential of roots and leaves of 45 cassava cultivars[1].

Local name/origin	CP content (ppm)	
	Root	Leaf
Adira II/Lianera	233.2	302.8
L 212/Yogyakarta	194.8	214.8
Ampera/Bogor	180.2	214.4
Adira II/Mangi/Ambon	135.4	374.9
SM-1803.2	108.5	143.8
Perelek/Bogor	108.1	413.1
Adira I/Adira II	106.1	295.9
JF-12/W1548/W 1705	100.2	130.7
Adira IV/ Pop Muara	97.4	112.0
G 168/Gading-7	90.3	165.0
BIC 224	90.3	148.6
Pop 1435	83.6	128.1
Pop 1435	80.0	140.3
SM 1809.1	76.4	142.6
L 163/Yogyakarta	76.4	89.9
Sao Pedro Petro (SPP)/Brazil	75.2	177.7
W 1167/Muara	73.3	122.5
L 144/Yogyakarta	72.9	97.1
Maleka/East Java	63.8	101.1
Gading/Ambon	61.0	92.3
Gading-7/Muara	59.4	80.1
U.247/Seleksi Bogor	59.4	77.2
A 29/Ambon	58.6	88.4
BIC 29	57.4	103.5
Ambon/Ambon	55.4	171.7
Si Pulutgadung/Padang	54.6	162.5
Pop Gading/Ambon-3	50.3	63.9
Pop Ambon	50.3	55.5
Gading/Lianera	49.9	117.9
Adira I/Adira II	49.5	92.5
Pop Adira II	47.9	109.3
Seleksi Bogor	47.9	84.2
Seleksi Bogor	45.9	90.5
Sigaronggang	45.1	76.1
Mantri/Pekalongan	39.2	98.9
Gading/Ambon	38.0	52.2
Pop Kla5-2	36.0	85.2
Seleksi Bogor	36.0	44.2
Criolinca/Brazil	31.3	69.1
G-186/Pop Gading	31.3	65.3
Markonah/Bogor	27.7	75.6
Valenca/Brazil	25.7	56.8
Mangi/Ambon	25.3	75.5
T.p86/Seleksi Bogor	24.9	79.8
H7-3/Pop Gading-3	17.8	96.5
Average	70.5	128.4

[1]Leaves and roots were harvested from 8-month-old plants.

Cyanogenic Potential of Cassava Roots

The CP of roots is highest (300 ppm) in the root closest to proximal (stem end) and the lowest (56 ppm) is in the distal end (Fig. 6). Along the proximal to the distal end, the CP decreases sharply. Iwatsuki *et*

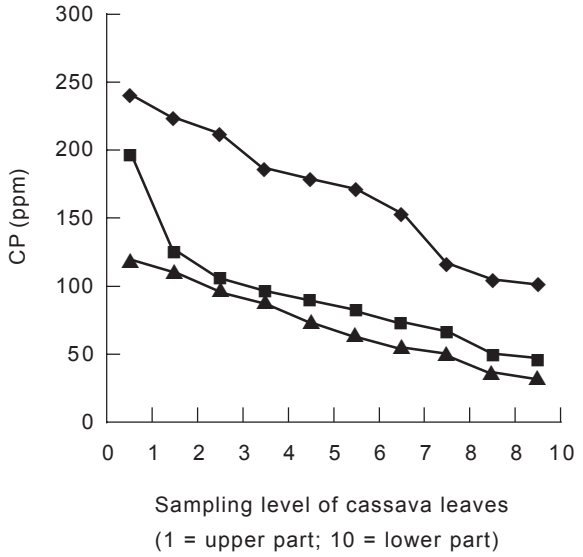

Fig. 5. Cyanogenic potential of leaves at different position of three cassava cultivars: BIC 395 (high CP), BIC 370 (medium), and BIC 14 (low).

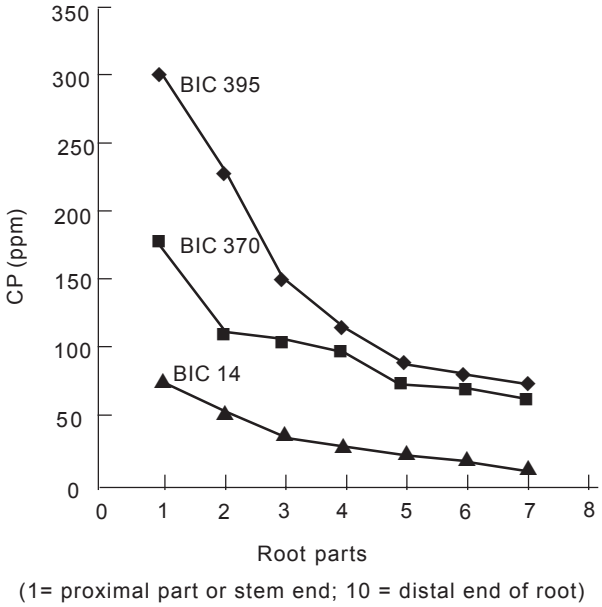

Fig. 6. Cyanogenic potential in various parts of root of three cassava cultivars: BIC 395 (high CP), BIC 370 (medium), BIC 14 (low) (B).

al. (1984) reported similar findings. The decrease is sharply in the three sectors closest to the proximal (stem), and after the three sectors the CP concentration begins leveling off. The CP in the proximal portion (10 to 70 ppm) was higher than that in the distal portion (20-120 ppm). The systematic decrease in CP of the roots is important in relation to determine the sampling method for CP analysis, and also for other purposes related to CP concentration.

The CP of roots is highest in the root part closer to cortex (550 ppm), and it decreases to 35 ppm in the central part of root (Fig. 7). The decrease is sharply in the two sectors closest to the cortex, and after two sectors the CP concentration begins leveling off. These results supported previous studies by Cooke (1978) and Iwatsuki *et al*. (1984) that there were both radial and longitudinal gradients in CP content of cassava roots. Fukuba and Mendosa (1984) also reported that CP content was higher in the cortex (200-300 ppm) than in the parenchymal tissues (78-150 ppm). The results suggest that it is safe to remove or exclude the cortex portion before cooking the roots. This figure is important when we wish to take a representative sample for CP analysis of roots. Bradbury *et al.* (1999) suggested sampling 10-mm thickness of roots in the central parts of the roots and took diagonal part of the cross vertical section (Fig. 1). Using this sample technique, the average CP content of roots of 179 cultivars was 82 ppm (Hidayat *et al*., 2000), which varies between high (134-84 ppm),

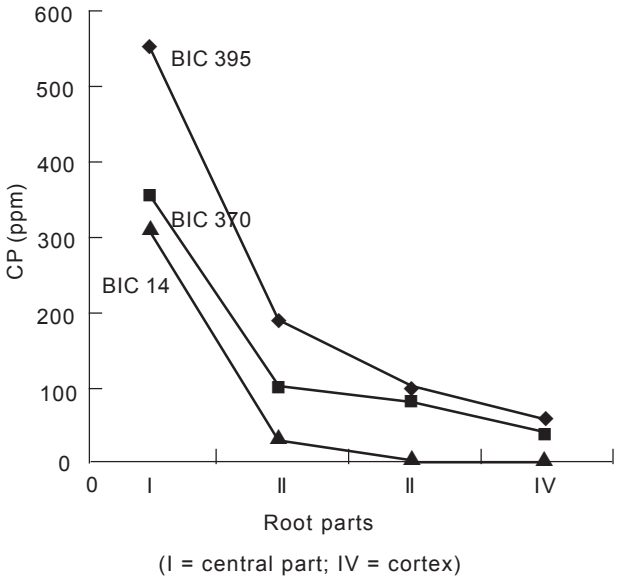

Fig. 7. Cyanogenic potential of various longitudinal parts of root of three cassava cultivars: BIC395 (high CP), BIC370 (medium), BIC14 (low) (B).

medium (83-55 ppm), low (54-36 ppm), and very low (35-9 ppm). However, Djazuli and Bradbury (1999) reported that the average CP content of 27 selected samples taken from centers of cassava production in East Java, Central Java, West Java, and Lampung was

19 ppm (SD 14). It indicates that selected cultivars from the four provinces are classified as very low CP concentration among the 179 cultivars.

Effect of Leaf Boiling on the CP Content

Boiling of cassava leaves significantly reduced the CP from 415 ppm to 150 ppm (three times lower) (Fig. 8). This means that the longer the boiling time the safer the cassava leaf for consumption. However, if the CP in the leaves is too high, e.g., 700 ppm, boiling for 15 minutes will theoretically reduce it to 210 ppm, which is still unsafe for consumption.

Fukuba *et al*. (1984) reported that boiling cassava leaves up to 1 hour reduced CP content as much as 85%. According to Jones *et al.* (1993), the lethal dose (LD) of cyanide is 1 mg kg^{-1} bodyweight, which means that 50 mg cyanide will cause death to human with 50 kg bodyweight. Therefore, for cassava leaves containing CP 100 ppm, the lethal dose is 500 g fresh leaves. People usually consume about 100 g boiled cassava leaves per serving. Based on this calculation, fresh cassava leaves containing higher than 500 ppm CP, will be unsafe for consumption without boiling or steaming.

Our result identified that 47-121 cassava cultivars containing 9-215 mg CN^- equivalent, are suitable for consumption, but the others are potentially dangerous (Table 1). However, consuming cassava leaves for a long period of time is not recommended because accumulation of cyanide metabolism product in the body may cause goiter and cretinism (Fukuba *et al*., 1984). There are 15 out of 99 cultivars that contain CP more than 343 ppm CN^-. Steaming the leaves is not recommended. When cassava is to be boiled for 15 minutes, varieties containing CP more than 500 ppm should not be used. Out of 99 cultivars, only three cultivars contain CP more than 500 ppm. However, this finding cannot be ignored. Although a small amount of CP will not cause acute poisoning symptoms, endemic goiter and cretinism have been observed as chronic effects in several countries.

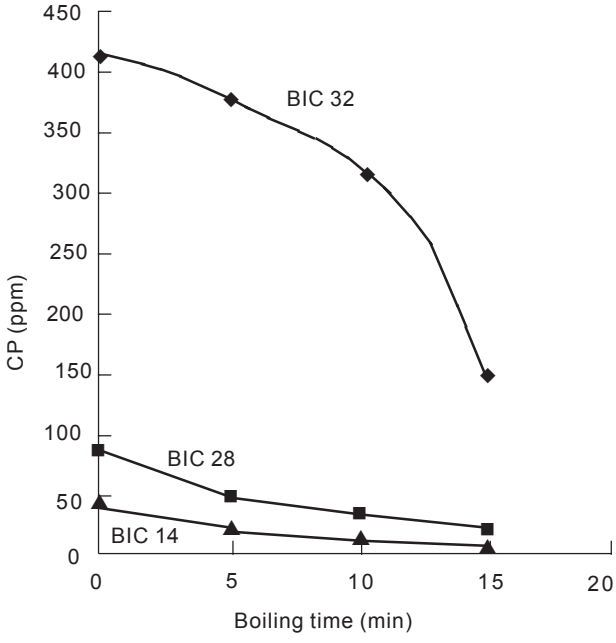

Fig. 8. Effect of boiling time on cyanogenic potential (CP) in leaves of three cassava cultivars: BIC32 (high CP), BIC28 (medium CP), BIC14 (low CP).

CONCLUSION

Cyanogenic potential of roots and leaves of cassava cultivars of Indonesian germplasm have low and moderate levels of CP. There was a significant correlation between CP in the leaves (Y) and in roots (X) of 45 cassava cultivars with regression equation $Y = 36.214 + 1.3085\ X$ ($r = 0.5228$). The CP was highest in the young leaves (241 ppm) and lower in older leaves (99 ppm).

The proximal root had the highest CP (300 ppm), and the distal end of the root was the lowest (56 ppm). The root part closest to the cortex had higher CP of 550 ppm, whereas in the central part of the root was 35 ppm.

Boiling cassava leaves up to 20 minutes significantly reduced CP up to 75%, indicating that cassava should be completely processed or cooked to prevent toxicity to human health. This study implies that CP content should be considered in breeding programs. Forty two of 99 cassava cultivars have CP in the leaves below 50 ppm which is safe for consumption. Therefore, these cultivars should be cultivated widely.

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