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Research article

Potential yield and cyanogenic glucoside content of cassava root and pasting properties of starch and flour from cassava Hanatee var. and breeding lines grown under rain-fed condition

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Abstract

The study evaluated the yield and cyanogenic glucoside content of cassava root and assessed the pasting properties of starch and flour of Hanatee (HNT), the cooking variety grown mostly in irrigated areas, and of four cassava breeding lines grown in a rain-fed location in the major cassava-growing area in Thailand. The study was carried out at 12 months after planting and compared the results with those of two control industrial varieties. The Hanatee variety had significantly lower root cyanogenic potential, root yield and root starch content compared to the two control industrial varieties. All cassava breeding lines had levels of cyanogenic glucoside content in the roots as low as the HNT variety. The breeding line LC-52-HB60xHNT-5 was as good as the Kasetsart 50 variety for its high root yield (21.9 t/ha) and high starch production (23.2%), but it had the advantage of lower cyanogenic potential in the root (110 mg/kg) than that of KU50. In addition, the flour of HNT and all breeding lines with alpha-amylase activity had significantly higher pasting viscosity values (range of trough viscosity was 79–163 RVU and range of final viscosity was 31–47 RVU) than those of the two industrial varieties (range of trough viscosity was 25–40 RVU and range of final viscosity was 12–17 RVU). Therefore, the breeding line LC-52-HB60xHNT-5 grown under rain-fed conditions was suggested as suitable for flour production when greater shear stability is required. Moreover, variations in the starch and flour pasting properties of these cassava varieties and breeding lines showed the possibility for using these criteria for screening germplasm or breeding lines in a future cassava breeding program for the food industry.

Introduction

Cassava (*Manihot esculenta* Crantz) is a perennial plant with C3-C4 intermediate photosynthetic characteristics which make it proficient with a higher carbon dioxide exchange rate than the C3 plants such as rice and wheat (El-Sharkawy and Cock, 1987). The cassava dry root yield was also reported to increase up to 100% under elevated CO_2 conditions (Rosenthal et al., 2012). Therefore, cassava is suitable to be promoted as a reserve food crop in the future in increased CO_2 situations. Nowadays, cassava is one of the major staple crops for people in many countries in tropical and subtropical areas especially in Sub-Saharan Africa due to its ability for drought

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adaptation and potential for food security due in part to its positive response to global elevated carbon dioxide levels (Rosenthal and Ort, 2012). Not only are the cassava roots a good source of carbohydrate and carotenoid in some yellow-fleshed varieties, but the leaves also have high levels of proteins, vitamins and minerals. These nutritional values have made cassava suitable bio-fortification programs (Montagnac et al., 2009). Moreover, cassava is an important material for many industries using starch, pellets, chips and animal feed (Balagopalan, 2002).

However, cassava produces cyanogenic glucosides mostly in the form of linamarin, which is a compound consisting of glucose and acetone cyanohydrin which can be catalyzed by enzymes into hydrocyanic acid (HCN) (Vetter, 2000). Consumption of cassava with a high cyanogenic glucoside content, without proper processing, could cause acute or chronic intoxication such as a paralytic disease known as Konzo (Bhattacharya and Flora., 2009; Adamolekun, 2011). Moreover, the continuous intake of cyanide in people who have iodine deficiency can cause goiter and cretinism resulting in retarded physical and neural growth in children (Nhassico et al., 2008). Cassava root is considered safe for consumption if the cyanogenic glucoside equivalent to HCN is lower than 50 mg/kg, whereas a cyanogenic potential in the range 50–100 mg/kg was suggested to be moderately toxic for consumption and a value over 100 mg/kg was highly toxic for consumption (Balagopalan, 2002). For food safety, The World Health Organization (WHO) restricted that the standard of cyanogenic glucoside equivalent to HCN in cassava-derived products must not exceed 10 mg/kg (Food and Agriculture Organization and World Health Organization, 1991). In cassava germplasm and in breeding lines in many countries, especially where cassava is popularly grown for consumption, the distribution of cyanogenic glucoside content is mostly right-skewed with the mode being less than 50 mg/kg (Bokanga, 1994). However, in Thai commercial varieties and their breeding lines, the range of cyanogenic glucoside in the root is 13–272 mg/kg with a mean value of approximately 110 mg/kg (Kongsil et al., 2016). Thus, breeding cassava for low cyanogenic potential in the root is an important objective to promote cassava for food security in the current climate change era and to generate greater cassava genetic diversity for cooking varieties, particularly, in Thailand.

Hanatee (HNT) is a popular cassava variety for cooking by boiling in Thailand due to its low cyanogenic glucoside content and mealy flesh texture after boiling. However, the area of Hanatee plantation in Thailand is approximately 148 ha (Department of Agriculture Extension, 2017) because it is mostly grown in irrigated areas which is very small compared to the 1.48 million ha of total cassava plantations in Thailand which are mostly grown under rain-fed conditions (Office of Agricultural Economics, 2017). Growing the HNT variety for consumption in rain-fed areas would be a challenge because the cyanogenic glucoside content in the cassava roots would increase under drought conditions, while the root yield has been reported to decrease (Cardoso et al., 2005; Srihawong et al., 2015; Chaengsee et al., 2018). The Hanatee variety grown in irrigated plantations in Pathum Thani province has a reported root yield of approximately 67 t/ha, while under rain-fed conditions in Nakorn Rachasima province the root yield is approximately 28 t/ha (unpublished data from final report of author's breeding program submitted to NSTDA, Thailand). Cassava flour and starch are alternative forms of cassava products for consumption providing the level of cyanogenic glucoside is reduced to safe levels. In particular, cassava flour can be used as a substitute for cassava root as it has the same components as the whole root, except for a lower moisture content (Hongbété et al., 2009). Chotineeranat (2006) reported that the production of flour from the Thai industrial variety, Kasetsart 50 (KU50), in addition to adding soaking in the process, could reduce the cyanogenic glucoside content in the flour product to a level safe for food. Thus, there have been suggestions to compare the potential for flour production of Hanatee and breeding lines to those of the industrial varieties. Charoenkul et al. (2011) investigated the physicochemical properties of starches and flours from the HNT and Rayong2 varieties together with those of 10 breeding lines with low cyanogenic potential and found no correlations between the starch or flour pasting properties and the texture quality of cooked cassava; furthermore, the flour had lower paste viscosity than that of the starch. Therefore, the Hanatee variety grown under rain-fed conditions together with HNT developed and non-HNT developed breeding lines were evaluated and compared with two control industrial varieties, namely Kasetsart 50 (KU50) and Huay Bong 80 (HB80), with regard to the root yield, starch content and cyanogenic potential in the root, and the pasting properties of starch and flour in order to identify promising breeding lines having a relatively high root yield and low cyanogenic potential for flour production. In addition, the differences in the pasting property profiles of flour and starch from these varieties and breeding lines could have potential as breeding line screening criteria in cassava breeding programs for the food industry.

Materials and Methods

Plant materials

The Hanatee variety together with four cassava breeding lines from a breeding program for low cyanogenic and high root yield potential were grown in a field at the Tapioca Development Institute, Dan Khun Thot district in Nakhon Ratchasima province, Thailand with the controls being two Thai industrial varieties—Kasetsart 50 (KU50) and Huaybong 80 (HB80)—having high root yields but also high cyanogenic glucoside content in the root. Four breeding lines—LC-52-R2xHNT-12 (cross of Rayong 2 variety (R2) × Hanatee variety (HNT)), LC-52-HB60xHNT-5 and LC-52- HB60xHNT-29 (crosses of Huaybong 60 variety (HB60) × Hanatee variety (HNT)), and LC-52-R3xHB60-5 (cross of Rayong 3 (R3) × Huaybong 60 variety (HB60))—were previously selected from a single-row trial to a preliminary yield trial due to their low cyanogenic glucoside content in the root and their root yield. These seven varieties/breeding lines were grown in May 2016 using a randomized complete block design with four replications. The number of plants for data collection in each experimental unit was 8 × 3 plants in a standard yield trial plot size of 10 × 5 plants by leaving an edge row of plants in each plot unit as a border buffer. The plant layout in this experiment was 1 m × 1 m. This experiment was conducted as a rain-fed yield trial. The soil texture at the experimental site was a loamy sand (85% sand, 6% silt, 9% clay) with 0.33% organic matter. The soil contained 0.04% total N, 6 mg/kg P, 98 mg/kg K, 505 mg/kg Ca, 29 mg/kg Mg with electrical conductivity of 0.49 dS/m at pH 6.7. The fertilizer application consisted of 15-15-15 (N-P-K, respectively) at 312.5 kg/ha at 1 mth after planting.

Plants were harvested at 12 mth after planting and the leaf, stem, stalk and root fresh weights of 24 plants were measured per experimental unit. The harvest index (HI) was defined as the fresh root weight divided by the total cassava fresh biomass. Roots were randomly sampled within the pool of roots within the experimental unit for starch content using the Reimann scale and were also sampled for the pasting properties of the flour and starch.

Cassava starch pasting property analyses

Starch extraction

Fresh cassava roots were washed before being peeled and the upper and lower edges were cut off. The peeled cassava flesh was chopped into small pieces before being crushed in water at a ratio of 1:2 of cassava to water. The crushed paste was separated from the water by passing through a cloth and 90 μm screen respectively. The paste was washed in water before being oven-dried at 50°C for 24 hr. Then, the dried starch was milled and screened through a 90 μm screen before further analysis.

Starch viscosity analysis

A starch sample (3 g at 14% moisture content) was weighed to make a slurry in 25 mL of water for analysis of the starch viscosity using a Rapid Viscosity Analyzer (RVA 4500, Perten Instrument, PerkinElmer Company, USA) following the method of Newport Scientific (1995). Viscosity was defined using Rapid Visco Units (RVUs). The temperature change rate was 12°C/min and the stirring rate was 960 rounds/min in the first 10 s followed by 160 rounds/min until the end of analysis. The starch slurry was heated at 50°C for 1 min. The temperature change rate was 12°C/min from 50°C to 95°C and was kept at 95°C for 2.5 min. After that, temperature was lowered to 50°C at a rate of 12°C/min and then was kept at 50°C for 2 min. The viscosity at every stage and temperature was logged and analyzed using the Thermocline program for Windows (Perten Instrument, PerkinElmer Company, USA).

Cassava flour pasting property analysis

Flour preparation

Fresh cassava roots were washed before being peeled and the upper and lower edges were cut off. The cassava flesh was sliced into chips and dried in the hot-air oven at 50°C until thoroughly dry. Each dried sample was crushed and screened through a 90 μm screen.

Flour viscosity analysis

The cassava flour viscosity was analyzed as explained earlier under the starch viscosity analysis. A flour sample (9.2% by weight) in both water and 15 mM silver nitrate ($AgNO_3$) was used for alpha-amylase activity inhibition as explained in Collado and Corke (1999) using Equations 1–2:

$$\Delta P = (PV2 - PV1) / PV1 \quad (1)$$

$$\Delta F = (FV2 - FV1) / FV1 \quad (2)$$

where PV1 is the peak viscosity determined in water, PV2 is the peak viscosity determined in $AgNO_3$ solution, FV1 is the final viscosity determined in water and FV2 is the final viscosity determined in $AgNO_3$ solution.

Analysis of cyanogenic glucoside content equivalent to HCN in root

Cyanogenic glucoside was measured as the equivalent value of hydrogen cyanide using linamarin as the standard following the method of Bradbury et al. (1999). A fresh root sample (100 mg) was crushed in a 15 mL plastic test tube with 500 μL of phosphate buffer at pH 8 and capped with picrate paper for hydrocyanic acid absorption. The reaction tube was incubated at 30°C for 24 hr. The picrate paper was removed from the tube and submerged in 5 mL water for 30 min. The absorbance of the solution at 510 nm wavelength was analyzed using the colorimetric method in a spectrophotometer.

Statistical analysis

The Statistical Tool for Agricultural Research STAR software (version 2.0.1; Copyright International Rice Research Institute; Los Baños, the Philippines) was used for analysis of variance in a randomized complete block design for all traits together with correlation analysis among the traits using Pearson's correlation coefficient (r). Significance was tested in all analyses at $p < 0.05$.

Results and Discussion

Root yield, starch content and cyanogenic potential in root of cassava

For the HNT variety in a rain-fed plantation, the root yield, harvest index (HI) and root starch content were the major factors of interest. The fresh root yield of HNT was significantly lower than for KU50 and HB80 which are popular industrial varieties (Table 1). However, two breeding lines developed from HNT and HB60, namely LC-52-HB60xHNT-5 and LC-52-HB60xHNT-29, had root yields statistically comparable to both KU50 and HB80, while two other industrial breeding lines developed from HNT (LC-52-R2xHNT-12 and LC-52-R3xHB60-5), had root yields statistically similar to HB80, but not to KU50. These results indicated that regarding root productivity, the Hanatee variety itself was not suitable for directly adoption under rain-fed conditions. On the other hand, some breeding lines from HNT and the industrial varieties such as HB60 (which might have a heterotic compatible genotype with HNT) could be developed and grown under rain-fed conditions with comparable root productivity to the popular industrial varieties. The Hanatee variety had a plant type distributed equally above ground and underground with an HI of 0.45 which was statistically similar to KU50 and HB80, but it did not perform well regarding total biomass production in the rain-fed trial. The Hanatee variety and the two breeding lines, LC-52-HB60xHNT-29 and LC-52-R3xHB60-5, had significantly lower starch contents than the two control industrial varieties which were lower than 20% which is considered low for starch productivity for industrial purposes (Sanni et al., 2005). However, the breeding line LC-52-HB60xHNT-5 had a starch content that was relatively the same as KU50. Interestingly, the breeding line LC-52-R2xHNT-12 had a significantly higher starch content than HB80 which is regarded as a high starch variety (Kittipadakul et al., 2012). For food safety, the cyanogenic potential in the root of the HNT variety was measured and compared with other varieties and breeding lines (Table 1). Though the cyanogenic glucoside contents in the root of HNT and all four breeding lines were significantly lower than those in the two control industrial varieties, the cyanogenic glucoside contents of HNT and the breeding lines were considered as having a moderate toxicity level for consumption because the levels of HCN were above 50 mg/kg, but lower than 100 mg/kg (Balagopalan, 2002), except for LC-52-HB60xHNT-5 which had a higher HCN level of 110.3 mg/kg. These results corresponded with the results of Chaengsee et al. (2018) who reported on the combined analysis of HNT grown in three rain-fed locations in Thailand which had significantly lower root yield, starch content and cyanogenic glucoside content in the root than did the industrial control varieties.

Pasting properties of cassava starch

The viscous paste of cooked cassava starch is a unique characteristic. Therefore, cassava starch is mostly preferred for application as a thickening agent, binding agent and adhesive in various products (Tan et al., 2017). The Hanatee variety had significantly lower peak viscosity and breakdown values than the two control varieties, while; LC-52-HB60xHNT-5 and LC-52-HB60xHNT-29 had peak viscosity, trough viscosity and breakdown values as high as the two control varieties (Table 2). Upon cooking, starch paste can be shear-thinned and this reduces the paste viscosity. Typically, cooked paste of cassava starch has low resistance to mechanical shearing under high temperature during the holding period of viscosity testing which leaches amylose due to starch granule disruption (Ragaee and Abdel-Aal, 2006), as indicated by a lower trough viscosity upon prolonged cooking during RVA analysis which indicated less stability of the starch paste of HNT when exposed to heat treatment and mechanical stirring. This drawback sometimes requires the modification of cassava starch for certain applications. After cooking, the paste is usually cooled down and this can cause a change in the viscosity profile of the starch. In starch molecules, in particular the linear glucan amylose can re-associate and form an ordered structure, which induces an increase in the viscosity of the cold paste (a process called retrogradation) as shown in the final viscosity (Ragaee and Abdel-Aal, 2006). All cassava lines and varieties in the current experiment had similar final paste viscosities. The lower final viscosity of the cold paste than the peak viscosity of cooked paste and the low setback value implied that cassava starch had a low tendency for retrogradation. The peak time reflects the rate

Table 1 Fresh weight of root, harvest index, root starch content and cyanogenic glucoside content of seven cassava varieties/lines

Factor	Root fresh weight (t/ha)	Harvest index	Root starch content (%)	Root HCN equivalent content (mg/kg)
Variety				
LC-52-R2xHNT-12	17.4 ± 1.2^{bc}	0.32 ± 0.01^{c}	30.0 ± 0.3^{a}	78.8 ± 5.9^{b}
LC-52-HB60xHNT-5	21.9 ± 0.8^{ab}	0.54 ± 0.01^{a}	23.3 ± 0.6^{c}	110.3 ± 17.1^{b}
LC-52-HB60xHNT-29	20.8 ± 0.9^{abc}	0.38 ± 0.01^{bc}	16.9 ± 1.1^{d}	63.0 ± 8.5^{b}
LC-52-R3xHB60-5	17.8 ± 1.1^{bc}	0.52 ± 0.03^{a}	17.5 ± 0.3^{d}	52.3 ± 8.0^{b}
HNT	12.1 ± 1.6^{c}	0.45 ± 0.02^{abc}	16.5 ± 1.6^{d}	70.6 ± 12.0^{b}
KU50	26.1 ± 2.4^{a}	0.46 ± 0.03^{ab}	24.4 ± 0.5^{bc}	199.3 ± 20.0^{a}
HB80	21.6 ± 0.8^{ab}	0.46 ± 0.02^{ab}	26.9 ± 1.0^{b}	204.6 ± 30.0^{a}

Mean ± SD values with different superscript letters within each column denote significant differences ($p < 0.05$) between varieties

Table 2 Starch pasting property values from 9.2% starch measured using rapid viscosity analysis of seven cassava varieties/lines

Factor	Peak viscosity (RVU)	Trough viscosity (RVU)	Breakdown (RVU)	Final viscosity (RVU)	Set back (RVU)	Peak time (min)	Pasting temperature (°C)
Variety							
LC-52-R2xHNT-12	410 ± 4^{b}	155 ± 3^{cd}	255 ± 4^{c}	238 ± 5	83 ± 6	3.8 ± 0.1^{abc}	69.7 ± 0.1^{d}
LC-52-HB60xHNT-5	457 ± 10^{a}	164 ± 3^{ab}	293 ± 9^{ab}	246 ± 5	81 ± 4	3.9 ± 0.0^{ab}	70.2 ± 0.2^{cd}
LC-52-HB60xHNT-29	490 ± 10^{a}	166 ± 1^{a}	324 ± 9^{a}	247 ± 5	81 ± 4	3.6 ± 0.0^{c}	70.5 ± 0.2^{bc}
LC-52-R3xHB60-5	410 ± 12^{b}	146 ± 2^{d}	265 ± 10^{bc}	229 ± 4	83 ± 2	3.9 ± 0.0^{ab}	68.9 ± 0.1^{e}
HNT	389 ± 9^{b}	150 ± 2^{bcd}	238 ± 9^{bc}	248 ± 2	98 ± 1	4.1 ± 0.1^{a}	71.8 ± 0.3^{a}
KU50	467 ± 7^{a}	160 ± 2^{abc}	306 ± 7^{a}	245 ± 1	85 ± 2	3.8 ± 0.0^{bc}	71.1 ± 0.2^{ab}
HB80	460 ± 7^{a}	158 ± 3^{abc}	302 ± 7^{a}	245 ± 3	87 ± 2	3.8 ± 0.0^{ab}	70.3 ± 0.2^{cd}

RVA = Rapid Viscosity Analyzer; RVU = rapid viscosity unit

Mean ± SD values with different superscript letters within each column denote significant differences ($p < 0.05$) between varieties

of absorption and swelling of starch granules (Ragaee and Abdel-Aal, 2006). For a given pasting temperature, a longer pasting time reflects the delay of gelatinization of granules which could be attributable to the restriction of water entering the granule due to some granule components (Ragaee and Abdel-Aal, 2006). Hanatee starch had a high peak time and pasting temperature, while starch of HB80 and all the breeding lines except LC-52-HB60xHNT-29 had the same peak time as the starch of HNT, but lower pasting temperatures than the starch of HNT. In conclusion, regarding the starch pasting properties of the HNT variety, the HNT starch granules tended to have a low rate of water absorption and granule swelling and consequently low resistance to shearing under high temperature. Therefore, starch of HNT might not be suitable for industrial purposes compared with the two control industrial varieties. Tan et al. (2017) reported that the cassava SC5 variety at age 9 mth had the lowest pasting temperature and the highest pasting viscosity and the pasting temperature of cassava tended to increase in the dry season and decrease in the rainy season. However, the cassava in the current study was harvested at age 12 mth when it was in the dormancy growth stage regarding cassava developmental stages as defined by Alves (2002), while cassava at age 9 mth as in Tan et al. (2017) was in the root carbohydrate accumulation stage. For this reason, the harvesting time of the HNT variety under rain-fed conditions should be further optimized to achieve an appropriate pasting viscosity. Among the starch pasting properties, there were significant, positive correlations between the root yield and peak (r = 0.482), trough (r = 0.434) and breakdown viscosity (r = 0.452) of starch and a negative correlation between the root yield and peak time (r = -0.396). These trends could be due to the KU50 and HB80 which were used as control varieties in this experiment being high yield and high HI varieties which had high levels of starch peak viscosity and trough viscosity and low peak times. However, the Hanatee variety had low starch viscosity and a low root yield and HI. Remarkably, the two breeding lines crosses between HB60 and HNT had high root yields comparable to the two control varieties and similar high starch paste viscosity.

Pasting properties and amylase activity of cassava flour

For flour viscosity in water in which the alpha-amylase activity was still active (Table 3 and Fig. 1), the flour paste in water had a lower paste viscosity than in the presence of $AgNO_3$ (Table 4). The amylogram of flour paste in water (Fig. 1) shows that the overall trends of flour pasting viscosity for varieties and breeding lines could be classified into three groups: the pattern of HNT flour, the pattern of flour of the two control commercial varieties and the pattern of flour for all four breeding lines. When flour was cooked with $AgNO_3$, an alpha-amylase enzyme inhibitor, starch was not hydrolyzed and there was still a viscous paste. The differences in peak paste viscosity (ΔP) and final paste viscosity (F) in Table 5 then implied the presence of this indigenous enzyme in flour samples (Collado and Corke, 1999). The alpha-amylase activities interpreted from ΔP and ΔF were not significantly different among the tested varieties and lines even though the mean values of these parameters differed as the standard deviations were high (Table 5). However, alpha-amylase activity in this study was only a relative property which was reported by Collado and Corke (1999) to have high positive correlation with alpha-amylase activity determined using the colorimetric method (Collado and Corke, 1999; Charoenkul et al., 2011) in which the specificity and sensitivity should be higher than from using the relative method. The Hanatee flour with alpha-amylase activity also had a higher peak time and pasting temperature than KU50 and HB80 and most of the breeding lines. Therefore, compared to KU50 and HB80, the Hanatee variety and the two breeding lines, LC-52-R2xHNT-12 and LC-52-HB60xHNT-29 had high potential for utilization in flour production due to the higher viscosity for high stability of flour paste at high temperature and high mechanical stirring and a high ability for retrogradation. Chotineeranat (2006) suggested the cyanogenic glucoside reduction in the production process of KU50 flour enhanced its food safety. However, due to the flour pasting properties identified in the current study, the use of low cyanogenic glucoside breeding lines having equivalent root yield and starch content to industrial varieties was more advantageous regarding the higher flour pasting properties and lower cyanogenic potential. There were negative correlations between the root yield and most of the flour viscosity properties (r with trough viscosity = -0.395, r with final viscosity = -0.410, r with set back = -0.441), but positive correlations between the root yield and amylase activity (r = 0.428) and breakdown viscosity (r = 0.625). Charoenkul et al. (2011) reported that paste viscosity and setback were positively correlated to the starch content and negatively correlated to the alpha-amylase activity, while; in the current experiment, the starch content was negatively correlated with the flour paste viscosity in water (r with trough viscosity = -0.300, r with final viscosity = -0.258) and positively correlated to the alpha-amylase activity (r = 0.331).

Table 3 Flour pasting property values from 9.2% flour in water measured using rapid viscosity analysis of seven cassava varieties/lines

Factor	Peak viscosity (RVU)	Trough viscosity (RVU)	Breakdown (RVU)	Final viscosity (RVU)	Set back (RVU)	Peak time (min)	Pasting temperature (°C)
Variety							
LC-52-R2xHNT-12	196 ± 19	101 ± 20^{a}	95 ± 6^{bc}	141 ± 25^{a}	40 ± 5^{a}	4.6 ± 0.3^{bc}	72.0 ± 0.2^{d}
LC-52-HB60xHNT-5	187 ± 22	91 ± 24^{ab}	97 ± 6^{bc}	122 ± 30^{ab}	31 ± 6^{ab}	4.4 ± 0.3^{bc}	72.8 ± 0.2^{cd}
LC-52-HB60xHNT-29	209 ± 22	120 ± 25^{a}	89 ± 5^{cd}	154 ± 30^{a}	34 ± 6^{a}	4.8 ± 0.3^{ab}	73.0 ± 0.1^{cd}
LC-52-R3xHB60-5	165 ± 18	79 ± 14^{abc}	86 ± 5^{cd}	113 ± 19^{ab}	34 ± 5^{a}	4.6 ± 0.2^{bc}	72.3 ± 0.3^{d}
HNT	207 ± 15	163 ± 14^{a}	44 ± 8^{d}	210 ± 16^{a}	47 ± 3^{a}	6.1 ± 0.1^{a}	76.1 ± 0.5^{a}
KU50	163 ± 7	25 ± 4^{c}	138 ± 6^{a}	37 ± 5^{c}	12 ± 1^{c}	3.9 ± 0.1^{c}	74.2 ± 0.2^{b}
HB80	160 ± 15	40 ± 7^{bc}	120 ± 11^{ab}	57 ± 10^{bc}	17 ± 2^{bc}	4.1 ± 0.1^{bc}	73.6 ± 0.4^{bc}

RVA = Rapid Viscosity Analyzer; RVU = rapid viscosity unit.

Mean ± SD values with different superscript letters within each column denote significant differences ($p < 0.05$) between varieties

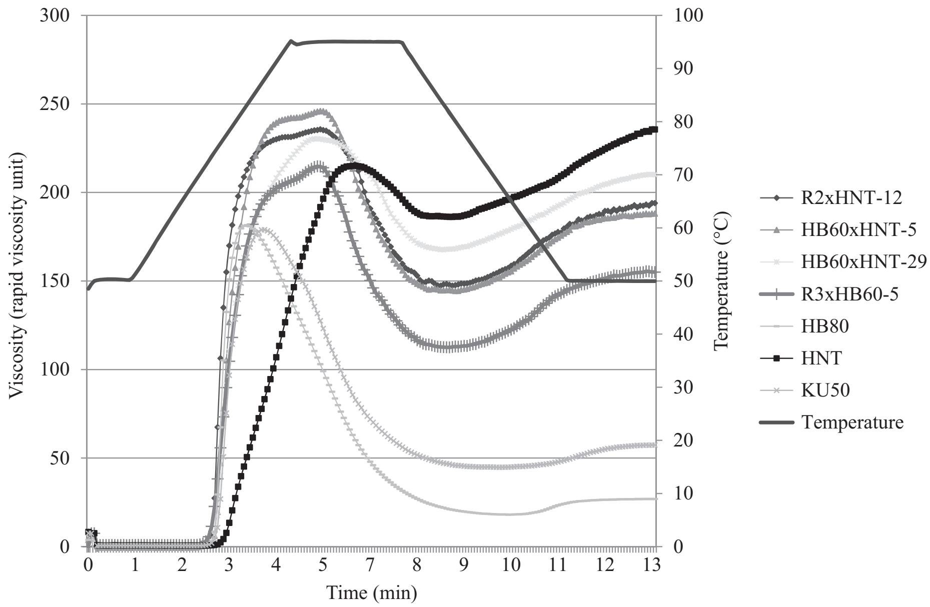

Fig. 1 Amylogram of flour pasting property values from 9.2% flour in water measured using rapid viscosity analysis of seven cassava varieties/lines

Table 4 Flour pasting property values from 9.2% flour in $AgNO_3$ measured using rapid viscosity analysis of seven cassava varieties/lines under two fertilizer applications

Factor	Peak viscosity (RVU)	Trough viscosity (RVU)	Breakdown (RVU)	Final viscosity (RVU)	Set back (RVU)	Peak time (min)	Pasting temperature (°C)
Variety							
LC-52-R2xHNT-12	305 ± 6[a]	132 ± 3[a]	173 ± 5[a]	195 ± 4[a]	63 ± 1[a]	4.4 ± 0.1	71.9 ± 0.3[b]
LC-52-HB60xHNT-5	323 ± 11[a]	140 ± 6[a]	183 ± 5[a]	205 ± 7[a]	65 ± 2[a]	4.5 ± 0.1	72.8 ± 0.4[ab]
LC-52-HB60xHNT-29	311 ± 9[a]	138 ± 6[a]	173 ± 5[a]	199 ± 8 [a]	61 ± 2[ab]	4.4 ± 0.2	72.8 ± 0.2[ab]
LC-52-R3xHB60-5	246 ± 11[c]	114 ± 5[b]	132 ± 6[b]	167 ± 7[b]	53 ± 2[b]	4.6 ± 0.1	72.2 ± 0.4[b]
HNT	247 ± 10[bc]	115 ± 5[ab]	132 ± 6[b]	176 ± 7[ab]	62 ± 3[ab]	4.4 ± 0.1	74.6 ± 0.5[a]
KU50	307 ± 10[a]	132 ± 4[a]	175 ± 7[a]	199 ± 6[a]	66 ± 2[a]	4.5 ± 0.1	73.4 ± 0.2[a]
HB80	303 ± 8[ab]	135 ± 4[a]	169 ± 5[a]	200 ± 6[a]	66 ± 3[a]	4.4 ± 0.1	73.1 ± 0.4[ab]

RVA = Rapid Viscosity Analyzer; RVU = rapid viscosity unit.
Mean ± SD values with different superscript letters within each column denote significant differences ($p < 0.05$) between varieties

Table 5 Alpha-amylase activity in the peak viscosity (ΔP) and alpha-amylase activity in the final viscosity (ΔF) of seven cassava varieties/lines

Factor	Peak viscosity in $AgNO_3$ (RVU)	Peak viscosity in Water (RVU)	ΔP	Final viscosity in $AgNO_3$ (RVU)	Final viscosity in Water (RVU)	ΔF
Variety						
LC-52-R2xHNT-12	305 ± 6[a]	196 ± 19	0.73 ± 0.79	195 ± 4[a]	141 ± 25[a]	1.34 ± 2.57
LC-52-HB60xHNT-5	323 ± 11[a]	187 ± 22	0.99 ± 0.93	205 ± 7[a]	122 ± 30[ab]	4.49 ± 6.97
LC-52-HB60xHNT-29	311 ± 9[a]	209 ± 22	0.73 ± 0.93	199 ± 8[a]	154 ± 30[a]	3.71 ± 7.64
LC-52-R3xHB60-5	246 ± 11[c]	165 ± 18	0.65 ± 0.67	167 ± 7[b]	113 ± 19[ab]	1.29 ± 2.39
HNT	247 ± 10[bc]	207 ± 15	0.35 ± 0.44	176 ± 7[ab]	210 ± 16[a]	-0.52 ± 0.34
KU50	307 ± 10[a]	163 ± 7	0.93 ± 0.41	199 ± 6[a]	37 ± 5[c]	5.06 ± 2.14
HB80	303 ± 8[ab]	160 ± 15	1.04 ± 0.67	200 ± 6[a]	57 ± 10[bc]	3.67 ± 2.92

RVA = Rapid Viscosity Analyzer; RVU = rapid viscosity unit.
Mean ± SD values with different superscript letters within each column denote significant differences ($p < 0.05$) between varieties

However, in cassava flour without alpha-amylase activity due to the inhibition of $AgNO_3$ (Table 4), the pasting properties of flour in $AgNO_3$ were higher than those in water in which alpha-amylase was available. The reason for the low pasting properties of flour in water was the hydrolysis of starch granules by alpha-amylase activity. However, the pasting properties of flour in $AgNO_3$ were still lower than those of starch (Table 2) due to the interaction of lipids and proteins in the flour, containing both starch and non-starch components, which inhibited starch swelling in the flour that in turn resulted in lower pasting properties than in the pasting starch samples (Charoenkul et al., 2011). The Hanatee variety and LC-52-R3xHB60-5 had low values for most of the flour viscosity properties, but HNT had a significantly higher pasting temperature than flour from LC-52-R3xHB60-5. The starch content was positively correlated with the flour paste viscosity values (r with peak viscosity = 0.419, r with trough viscosity = 0.296, r with breakdown value = 0.456, r with final viscosity = 0.353, r with set back = 0.403) in $AgNO_3$ which was a similar outcome to that of Ragaee and Abdel-Aal (2006), who reported that a high starch content in the flour of cereal was related to the high viscosity of the flour. The root yield was also positively correlated with most flour viscosity properties (r with peak viscosity = 0.425, r with trough = 0.305, r with breakdown = 0.456) However, the root yield was negatively correlated with the pasting temperature (r = -0.304).

In summary, under rain-fed conditions, the Hanatee variety, though having a low root cyanogenic glucoside content, had a low root yield and root starch content compared with industrial varieties such as KU50 and HB80. Moreover, the paste viscosity values of HNT starch and flour in $AgNO_3$ were significantly lower than those of KU50 and HB80. However, considering flour production, the flour of HNT with alpha-amylase activity had a significantly higher pasting viscosity than KU50 and HB80. In addition, these patterns also occurred in flour with alpha-amylase activity for all breeding lines in this research. Considering the individual characteristics, the breeding line LC-52-R2xHNT-12 had a remarkably high starch content, but it had low root yield and starch pasting viscosity values. Although the breeding line LC-52-R3xHB60-5 had low root cyanogenic potential, several characteristics were not suitable for either direct consumption or industrial purposes compared with the other breeding lines due to its low root yield, low starch content and low starch pasting viscosity. The breeding lines LC-52-HB60xHNT-5 and LC-52-HB60xHNT-29 had root yields as high as for KU50 and HB80 as well as starch paste viscosity values as high as for these two industrial varieties. However, the breeding line LC-52-HB60xHNT-29 had a low root starch content. Therefore, breeding line LC-52-HB60xHNT-5 was suggested for low cyanogenic flour production with a comparable starch yield to industrial varieties such as KU50. Moreover, the variations in paste viscosity characteristics of these breeding lines and the HNT variety will be useful as screening criteria for the selection of parents for recurrent selection breeding for further lower cyanogenic potential, higher root, high starch yield and high starch and flour pasting viscosity in a cassava breeding program for good cooking quality to develop a more diverse cassava population for food security in the climate-affected era.

Conflict of Interest

The authors declare that there are no conflicts of interest.

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