RESEARCH ARTICLE

SABRAO Journal
of Breeding and Genetics
52 (2) 109-126, 2020

STARCH ACCUMULATION OF CASSAVA GENOTYPES GROWN IN PADDY FIELDS DURING OFF-SEASON

T. NIMLAMAI[1], P. BANTERNG[1,2*], S. JOGLOY[1,2] and N. VORASOOT[1]

[1] Department of Agronomy, Faculty of Agriculture, Khon Kaen University, Khon Kaen, 40002, Thailand
[2] Plant Breeding Research Center for Sustainable Agriculture, Khon Kaen University, Khon Kaen, 40002, Thailand
*Corresponding author email: bporam@kku.ac.th
Email addresses of coauthors: tanapon_koza@hotmail.com, sjogloy@gmail.com, nvorasoot1@gmail.com

SUMMARY

Studies on the starch production rates and starch yields of cassava genotypes provide valuable information for planting in upper paddy fields during the off-season of rice. The objective of this study was to evaluate the starch production of different cassava genotypes grown in different upper paddy fields during the off-season of rice. Four cassava genotypes (Kasetsart 50, Rayong 9, Rayong 11, and CMR38−125-77) were planted in four different upper paddy fields during the off-season of rice in Thailand. A randomized complete block design with four replications was used for each experimental site. The number of storage roots, storage root dry weight, starch content, starch production rate, starch yield, area under curve (AUC) for starch yields, and weather data were recorded. The results revealed no statistically significant difference for genotype × environment in starch content at 120, 150, and 180 days after planting (DAP). Early storage root formation, rapid starch production rate, and high starch yield accumulation were relevant traits for cassava to become a top yielder. The CMR38-125-77 genotype produced significantly greater starch contents at 120 DAP and starch yields at 120 and 180 DAP than the others. This genotype showed outstanding AUCs for starch yield, starch production rate, starch yield at 150 DAP, number of storage roots at 150 DAP, number of storage roots at 180 DAP, and weight per storage root at 120 DAP, leading to the opportunity of the CMR38−125-77 as a preferred genotype and a parental source for cassava production during the off-season of rice.

Keywords: Area under curve, dry weight, starch content, starch production rate, storage root, yield

Key findings: Planting suitable cassava genotypes under paddy field conditions during the off-season of rice is an alternative solution to improving cassava production. Crop performances with respect to early storage root formation, rapid starch production rate, and high starch yield accumulation were relevant traits for

cassava to be a top yielder for this cropping system. The genotype CMR38-125-77 was identified as the satisfactory genetic resource for planting cassava in paddy fields during the off-season of rice.

Manuscript received: November 13, 2019; Decision on manuscript: May 2, 2020; Accepted: May 3, 2020.

Communicating Editor: Dr. Naqib Ullah Khan

INTRODUCTION

Cassava (*Manihot esculenta* Crantz) is commonly utilized worldwide as foods, medicine, feed, biopolymers, and biofuels. It is generally grown in tropical and subtropical regions (between 30 °N and 30 °S) with a total cultivated area of over 26 million hectares (El-Sharkawy, 1993; Anyanwu *et al*., 2015; FAO, 2017). This crop can be grown under rain-fed conditions with the total amount of rainfall between 600 mm to 1000 mm (De Tafur *et al*., 1997; Pellet and El-Sharkawy, 1997 El-Sharkawy, 2004; El-Sharkawy, 2007; El-Sharkawy and De Tafur, 2010). Southeast Asian countries are now growing cassava to support the world market, and the global cassava demand has been increasing due to a rising world population (FAO, 2017). Searching for alternative growing areas and increasing productivity can help improve cassava production.

The cultivation of cassava in Southeast Asian countries during the off-season of rice in some upper paddy fields that have proper rainfall and soil texture for root growth is an option to increase cassava production and land use efficiency (Polthanee *et al*., 2014; Sawatraksa *et al*., 2018; Sawatraksa *et al*., 2019). This process also provides more aggregate income to farmers and generates wealth with sustainability for agriculture in the region. Thailand, a member of the Association of Southeast Asian Nations, produces a large amount of cassava for the world market. A cassava crop is mostly grown under upland conditions for a duration of 8–12 months, and the total amount of fresh storage root production for the whole country is approximately 30.9 million tons in 2017 (Department of Agriculture, 2008; FAO, 2017). In Thailand, paddy rice farming is commonly conducted during the rainy season (August to early December), and some farmers leave their land fallow for 6–8 months due to rainfall shortage and limited supplementary irrigation. The residual soil moisture at the end of rice growing season in some places is sufficient for growth of cassava. The crop duration for planting cassava throughout the off-season of rice, however, is shorter than the common cassava growth period. The identification of the production feasibility and the suitable cassava genotypes that can produce reasonable starch yields within a short period, therefore, could help support this cropping system. The selection for early storage root formation and high starch yield for this particular ecology also challenges plant breeders.

Polthanee *et al*. (2014) reported the performances of different cassava genotypes (Rayong 7, Rayong 11, Rayong 72, Kasetsart 50, and Huay Bong 80) grown in a single location in Thailand. Kasetsart 50, Rayong 9, Rayong 11, and CMR38-125-77 grown under upper paddy field conditions during the off-season of rice in Thailand have been evaluated on the basis of chlorophyll fluorescence, biomass, and starch content at final harvest (Sawatraksa *et al*., 2018), as well as growth rate, net assimilation rate, biomass, and harvest index (Sawatraksa *et al*., 2019). However, information on starch production rates, starch content, and starch yield for different cassava genotypes grown in upper paddy fields during the off-season of rice is important and still limited. Monitoring the starch production of different cassava genotypes at early growth stages under different growing environments enhances the better understanding in terms of the adaptability of each genotype. This information allows us to identify the genetic resources that possess rapid starch production rate and high starch accumulation and is involved in designing valuable recommendations to improve cassava production in Southeast Asian countries. The objective of this study was to evaluate the starch production dynamics of different cassava genotypes grown in different upper paddy fields during the off-season of rice.

MATERIALS AND METHODS

Experimental detail

The performances of the four cassava genotypes with different forking patterns (Kasetsart 50, Rayong 9, Rayong 11, and CMR38-125-77) were evaluated under upper paddy field conditions during off-season of rice across four different environments in Thailand. Kasetsart 50, Rayong 9, and Rayong 11 are now popular in Thailand as recommended commercial genotypes. CMR38-125-77 (an elite genotype) is a progeny of Rayong 5 and Kasetsart 50. Experiments were conducted in the Ban Kho district (16.5° N, 102.7° E; 173 m asl; sandy loam soil), Kham Pom district (16.2° N, 102.6° E; 171 m asl; loamy sand soil), and Kham Thao district (17.2° N, 104.7° E; 147 m asl; sandy loam soil) during December 2016 to June 2017. During December 2017 to June 2018, an experiment was conducted in Khok Kung District (16.6° N, 102.1° E; 202 m asl; sandy loam soil). For each experimental site, a randomized complete block design with four replications was assigned. The individual plot size was 60 m^2 with spacing between plots of 1 m. Land preparation was conducted by following the normal procedures for cassava experiments, and soil ridges were created. The stems of cassava at 12 months after planting were cut into sticks 20 cm in length and soaked in 25% thiamethoxam with the water rate of 4 g per 20 l of water for 5−10 min to remove aphids that had attached to the pieces. The sticks were inserted in the soil ridge and buried to 2/3 of their lengths. A spacing of 1 m between rows and 1 m between plants within the row was assigned. Irrigation was done immediately following planting to facilitate uniform germination. Then, the plants were grown under rainfed condition. Manual weed control was conducted, and a chemical fertilizer, 15–7–18 ($N–P_2O_5–K_2O$), was applied at a rate of 312.5 kg ha^{-1} at 30 and 60 days after planting (DAP).

Data collection

Storage roots were harvested from the four cassava plants per plot at 120, 150, and 180 DAP. The total numbers of storage roots were recorded. The storage roots were subsampled for approximately 10% of total storage root fresh weight of each plot, and these samples were then oven dried at 80 °C (BF 720, INDER GmbH [Headquarters], Tuttlingen, Germany) to a constant dry weight. The value of dry weight per storage root unit for each individual plot was calculated on the basis of total fresh weight, sampled dry weight, and total number of storage roots.

The remaining fresh storage roots in each plot were oven dried at 50 °C–55 °C to a constant weight by using a tray drier (EQ-04SW, Leehwa Industry Company, Kyongbuk, Korea). The dried samples were ground in an electronic blender (Standard EM−11, Sharp Thai Company Limited, Bangkok, Thailand) and then sieved with 200 µm diameter sieve. The ground samples were used for measuring starch contents via the polarimetric method (Janket *et al*., 2018; Janket *et al*., 2020).

A total of 5 g of the ground sample was transferred to a 200 mL glass flask. Then, 50 mL of hydrochloric acid (RCI labscan limited, Bangkok, Thailand) (0.31 N) was added. The flask was plugged and shaken until the sample was uniformly suspended, and 50 mL of hydrochloric acid was added. The flask was immersed in a boiling water bath (WNE-22, Memmert, Schwabach, Germany), shaken vigorously and steadily to avoid the coagulation of the sample, and kept in the bath for a 15 min. Then, 60 mL of cold water was added immediately to obtain a temperature of 20 °C. After cooling, 20 mL of 4% sodium phosphotungstate (Sigma-Aldrich Company Limited, St. Louis, MO, USA) was added, and the samples were shaken for approximately 30 s. The sample was diluted to 200 mL with distilled water, mixed, and then filtered by using number 1 filter paper. The first 25 mL of the solution was discarded, and the remaining filtrate was transferred into a 200 mm tube and subjected to total rotary power measurement with a polarimeter (Polatronic MH8, Schmidt Haensch, Berlin, Germany).

A total of 12.5 g of the ground samples was transferred to a 250 mL glass flask, and 200 mL of distilled water was added. The flask was shaken vigorously every 10 min for a total of 1 h (six times) to disperse the sample. The solution was diluted to 250 mL with distilled water, mixed, allowed to stand, and then filtered through a number 42 filter papers. A total of 100 mL of the filtrate was transferred into a 200 mL glass flask, and 4.2 mL of 25% hydrochloric acid was then added to the filtrate. Then, the sample was shaken vigorously. The flask was then immersed in a boiling water bath for 15 min, and the processes for rotary power determination were continued as above. Starch content (%) was calculated with the following equation:

$$\text{Starch content: } \frac{2{,}000 \times (P - P') \times 100 \times L}{[a]^{20}\,{}^{o}D \times (100\text{-}M)}$$

Where P is total rotator power in degrees, P′ is the rotator power in degrees given by water-soluble substances, $[a]^{20}$ ^{o}D is the specified optical rotation of pure starch (cassava starch = 180^{o}), M is starch moisture (%), and L is the standard tube length (200 mm is 1). Starch yields for the four tested cassava genotypes at 120, 150, and 180 DAP were calculated on the basis of starch content and storage root dry weight. In addition, the meteorological data for the four environmental sites were recorded. These data consisted of maximum temperature, minimum temperature, and total rainfall amount.

Statistical analysis

Analysis of variance (ANOVA) for each environmental site and combined analysis across all four environments were done for all crop traits. Mean comparisons were performed by using the Least Significant Difference Test. Regressions were performed between times of observation, and starch yields (simple linear function) and starch production rates were recorded by using the slope of linear regression, as well as the values of the area under curve (AUC) for the simple linear regression were calculated for all cassava genotypes.

ANOVA, mean comparisons, and regression analysis were performed by following the procedure of Gomez and Gomez (1984) by using Statistix 10 software program (Statistix, 2013). Stability analysis was conducted by using a graphical approach for analyzing the genotype main effect plus genotype by environment interaction (G + GE) or GGE biplot software (Yan, 2001; Yan and Hunt, 2003). The GGE biplot was constructed from the first two components (PC1 and PC2) that were derived by subjecting environment-centered crop data to singular value decomposition.

RESULTS

Nonsignificant difference was found for the number of storage root for 120 DAP at Ban Kho, Kham Pom, and Kham Thao (Table 1), but not for storage root dry weight. The highest values were recorded for the CMR38-125-77 genotype. A significant difference for starch content was found at Kham Pom, and CMR38-125-77 and Rayong 9 genotypes gave the highest values. High starch yield values with statistical significances were observed for CMR38-125-77 at Ban Kho; CMR38-125-77 and Kasetsart 50 at Kham Pom; and CMR38-125-77, Kasetsart 50, and Rayong 9 at Kham Thao.

Significant differences were observed among the four cassava genotypes in terms of the number of storage roots at 150 DAP at Ban Kho, Kham Pom, and Kham Thao (Table 2). Nonsignificant differences were recorded for the performances among the genotypes in terms of storage root dry weight and starch content. High values of starch yield with statistical significance were observed for CMR38-125-77 and Kasetsart 50 at Ban Kho, CMR38-125-77 and Rayong 9 at Kham Pom, and for CMR38-125-77 at Kham Thao and Khok Kung.

At 180 DAP (Table 3), no statistically significant differences for number of storage root, storage root dry weight, starch content, and starch yield among the four cassava genotypes at Ban Kho, but not for Kham Pom, were found. The results revealed significantly different performances in terms of storage root

Table 1. Number of storage root, storage root dry weight (g root^{-1}), starch content (%), and starch yield (kg plant^{-1}) at 120 days after planting (DAP) for the four different cassava genotypes grown at the four environmental sites.

Environments	Traits	Genotypes				F
		Kasetsart 50	Rayong 9	Rayong 11	CMR38-125-77	
Ban Kho in 2016–2017	Number of storage root	1.4	1.5	1.3	1.4	ns
	Storage root dry weight (g root^{-1})	42.1 ab	24.1 b	23.0 b	62.2 a	**
	Starch content (%)	72.1	68.9	72.5	74.3	ns
	Starch yield (kg plant^{-1})	0.04 b	0.02 bc	0.02 c	0.07 a	*
Kham Pom in 2016–2017	Number of storage root	2.4	2.8	1.9	2.1	ns
	Storage root dry weight (g root^{-1})	34.7 b	25.8 c	14.1 d	45.7 a	**
	Starch content (%)	73.0 b	73.2 ab	71.7 b	75.7 a	*
	Starch yield (kg plant^{-1})	0.06 ab	0.05 b	0.02 c	0.07 a	**
Kham Thao in 2016–2017	Number of storage root	1.9	2.0	2.0	2.0	ns
	Storage root dry weight (g root^{-1})	30.0 ab	28.0 b	8.0c	42.2 a	**
	Starch content (%)	68.2	70.3	66.3	71.8	ns
	Starch yield (kg plant^{-1})	0.04 a	0.04 a	0.01 b	0.05 a	**
Khok Kung in 2017–2018	Number of storage root	8.6	9.8	5.7	9.4	ns
	Storage root dry weight (g root^{-1})	3.6	2.4	1.8	6.3	ns
	Starch content (%)	72.8	75.2	72.6	75.8	ns
	Starch yield (kg plant^{-1})	0.02	0.02	0.01	0.04	ns

ns, *, and ** = nonsignificant in statistics, significant at $P < 0.05$, and significant at $P < 0.01$, respectively. Values within a row followed by the same letter are not significantly different. All means were compared via the Least Significant Difference Test at the 0.05 level of significance.

Table 2. Number of storage root, storage root dry weight (g $root^{-1}$), starch content (%), and starch yield (kg $plant^{-1}$) at 150 days after planting (DAP) for the four different cassava genotypes grown under the four environmental sites.

Environments	Traits	Genotypes				F
		Kasetsart 50	Rayong 9	Rayong 11	CMR38-125-77	
Ban Kho in 2016–2017	Number of storage root	3.5 b	2.9 b	3.0 b	5.0 a	**
	Storage root dry weight (g $root^{-1}$)	69.9	64.5	61.7	58.6	ns
	Starch content (%)	75.8	77.1	78.0	77.3	ns
	Starch yield (kg $plant^{-1}$)	0.19 ab	0.14 b	0.14 b	0.22 a	*
Kham Pom in 2016–2017	Number of storage root	2.2 bc	4.4 a	1.6 c	2.9 b	**
	Storage root dry weight (g $root^{-1}$)	49.7	40.9	33.4	53.9	ns
	Starch content (%)	74.9	75.2	77.2	77.5	ns
	Starch yield (kg $plant^{-1}$)	0.09 b	0.13 a	0.04 c	0.12 a	**
Kham Thao in 2016–2017	Number of storage root	3.7 bc	3.3c	5.6 a	4.7 ab	**
	Storage root dry weight (g $root^{-1}$)	71.5	70.7	59.4	106.4	ns
	Starch content (%)	76.1	75.3	75.1	76.2	ns
	Starch yield (kg $plant^{-1}$)	0.20 b	0.17 b	0.25 b	0.37 a	**
Khok Kung in 2017–2018	Number of storage root	9.9	11.1	7.4	10.4	ns
	Storage root dry weight (g $root^{-1}$)	13.5	12.1	20.0	24.3	ns
	Starch content (%)	75.7	76.9	75.8	77.5	ns
	Starch yield (kg $plant^{-1}$)	0.10 b	0.10 b	0.10 b	0.20 a	**

ns, *, and ** = nonsignificant in statistics, significant at $P < 0.05$, and significant at $P < 0.01$, respectively. Values within a row followed by the same letter are not significantly different. All means were compared via the Least Significant Difference Test at the 0.05 level of significance.

Table 3. Number of storage root, storage root dry weight (g $root^{-1}$), starch content (%), and starch yield (kg $plant^{-1}$) at 180 days after planting (DAP) for the four different cassava genotypes grown under the four environmental sites.

Environments	Traits	Genotypes				F
		Kasetsart 50	Rayong 9	Rayong 11	CMR38-125-77	
Ban Kho in 2016–2017	Number of storage root	6.6	6.1	6.9	7.1	ns
	Storage root dry weight (g $root^{-1}$)	117.7	94.2	98.5	93.7	ns
	Starch content (%)	78.8	80.0	80.6	79.6	ns
	Starch yield (kg $plant^{-1}$)	0.61	0.46	0.54	0.51	ns
Kham Pom in 2016–2017	Number of storage root	10.8 bc	17.0 a	9.3 c	14.1 ab	**
	Storage root dry weight (g $root^{-1}$)	60.5 a	34.2 b	54.8 a	55.4 a	*
	Starch content (%)	77.0 b	78.9 a	78.3 ab	79.1 a	*
	Starch yield (kg $plant^{-1}$)	0.46 b	0.45 b	0.40 c	0.60 a	**
Kham Thao in 2016–2017	Number of storage root	6.6	6.1	6.9	7.1	ns
	Storage root dry weight (g $root^{-1}$)	113.4 b	128.9 ab	105.3 b	153.2 a	*
	Starch content (%)	79.8	80.4	79.7	90.0	ns
	Starch yield (kg $plant^{-1}$)	0.60 b	0.63 b	0.58 b	0.88 a	**
Khok Kung in 2017–2018	Number of storage root	9.6	10.6	7.9	10.3	ns
	Storage root dry weight (g $root^{-1}$)	19.4	18.6	22.7	27.9	ns
	Starch content (%)	79.7	79.9	81.0	79.7	ns
	Starch yield (kg $plant^{-1}$)	0.15 b	0.16 b	0.13 b	0.22 a	**

ns, *, and ** = nonsignificant in statistics, significant at $P < 0.05$, and significant at $P < 0.01$, respectively. Values within a row followed by the same letter are not significantly different. All means were compared via the Least Significant Difference Test at the 0.05 level of significance.

dry weight and starch yield for Kham Thao and starch yield for Khok Kung. CMR38-125-77 was an outstanding genotype based on starch yield for Kham Pom, Kham Thao, and Khok Kung.

CMR38-125-77 gave the highest values for starch production rate with a statistically significant difference for Kham Pom, Kham Thao, and Khok Kung (Table 4). The calculated AUC values were used to explain the performance of each cassava genotype based on the quantitative summary of starch yield over time, and CMR38-125-77 also had significantly higher AUC values for all four environmental sites.

The results from combined ANOVA indicated that environment had the largest proportion of variation when compared with genotype and genotype × environment, and it had a significant effect on almost all crop traits, except for starch content, at 150 DAP (Table 5). Genotype had no significant effect on starch content at 150 and 180 DAP, the number of storage root at 120 DAP, and storage root dry weight at 180 DAP. Statistically significant differences for the responses of four cassava genotypes to environmental sites (genotype × environment) were also observed on the basis of the AUC values of starch yield, starch production rate, starch yield at 150 DAP, number of storage root at 150 and 180 DAP, and storage root dry weight at 120 DAP.

CMR38-125-77 had significantly higher starch content at 120 DAP and starch yield at 120 and 180 DAP than the other tested genotypes (Table 6). Significant differences were observed among the four environmental sites in terms of starch content and starch yield at 120 and 180 DAP. Kham Thao and Kham Pom provided the lowest values for starch content at 120 and 180 DAP, respectively. The lowest value of storage root dry weight at 180 DAP accounted for the smallest total amount of starch yield at Khok Kung. The Kham Thao environment had the smallest value of starch content at 120 DAP. However, this environmental site provided the highest storage root dry weight and starch content at 180 DAP.

The statistical significance of genotype × environment in AUC of starch yield, starch production rate, starch yield at 150 DAP, number of storage root at 150 DAP, number of storage root at 180 DAP, and weight per storage root at 120 DAP (Table 5) demonstrated that the cassava genotypes showed differential responses in each environmental site. GGEbiplot is an effective tool for determining the effect of genotype and genotype × environment interaction. Therefore, GGEbiplot was acceptable for assessing the stability of each tested genotype for this study. The results in Figure 1 show that the genotype on the right side of double-arrow line had higher-than-average performance. The genotype with high mean performance and high stability was identified by using the ideal position (the center of the concentric circle). This position was defined by a projection on the mean-environment axis that the genotype on right side of the double arrow line and longest vector possessed above-average mean yield and by a zero projection on the perpendicular line (Yan, 2001; Yan and Hunt, 2003; Klomsa-ard *et al*., 2013; Banterng and Joralee, 2015).

Table 4. Starch production rate (kg day^{-1}) and area under curve (AUC) for starch yield (kg day unit) for the four different cassava genotypes grown under the four environmental sites.

Environments	Traits	Genotypes				F
		Kasetsart 50	Rayong 9	Rayong 11	CMR38-125-77	
Ban Kho in 2016–2017	Starch production rate (kg day^{-1})	0.28	0.20	0.27	0.30	ns
	AUC for starch yields (kg day unit)	0.55 bc	0.42 c	0.47 ab	0.62 a	*
Kham Pom in 2016–2017	Starch production rate (kg day^{-1})	0.19 b	0.18 b	0.18 b	0.25 a	**
	AUC for starch yields (kg day unit)	0.40 b	0.41 b	0.30 c	0.51 a	**
Kham Thao in 2016–2017	Starch production rate (kg day^{-1})	0.27 b	0.29 b	0.28 b	0.42 a	**
	AUC for starch yields (kg day unit)	0.57 b	0.55 b	0.56 b	0.87 a	**
Khok Kung in 2017–2018	Starch production rate (kg day^{-1})	0.19 b	0.18 b	0.18 b	0.25 a	**
	AUC for starch yields (kg day unit)	0.40 b	0.41 b	0.30 c	0.51 a	**

ns, *, and ** = nonsignificant in statistics, significant at $P < 0.05$, and significant at $P < 0.01$, respectively. Values within a row followed by the same letter are not significantly different. All means were compared via the Least Significant Difference Test at the 0.05 level of significance.

Table 5. Percentages of sum squares to total sum of squares form combined analysis of variance for crop traits of the four different cassava genotypes in four environments.

Traits	Source of variation				
	Environment (E)	Replication/E	Genotype (G)	G × E	Pooled error
AUC for starch yield (kg day unit)	40.7 **	16.4	29.3 **	8.1 **	5.6
Starch production rate (kg day^{-1})	42.1 **	21.3	19.5 **	6.7 *	10.4
Starch content at 120 DAP (%)	24.8 **	16.0	12.8 *	8.4 ns	37.9
Starch content at 150 DAP (%)	7.8 ns	11.2	9.7 ns	12.5 ns	58.9
Starch content at 180 DAP (%)	22.2 *	20.2	8.4 ns	9.2 ns	40.1
Starch yield at 120 DAP (kg plant^{-1})	22.4 **	12.4	43.7 **	5.3 ns	16.2
Starch yield at 150 DAP (kg plant^{-1})	43.9 **	10.9	19.8 **	13.5 **	12.0
Starch yield at 180 DAP (kg plant^{-1})	65.8 **	9.8	5.8 **	5.8 ns	12.8
Number of storage root at 120 DAP	72.2 **	3.6	2.2 ns	4.1 ns	16.0
Number of storage root at 150 DAP	73.6 **	4.2	2.7 *	8.5 **	11.0
Number of storage root at 180 DAP	53.7 **	11.8	6.7 **	15.1 **	12.8
Storage root dry weight at 120 DAP (g root^{-1})	45.8 **	8.7	26.6 **	9.2 **	9.7
Storage root dry weight at 150 DAP (g root^{-1})	61.2 **	6.9	5.1 *	7.6 ns	19.2
Storage root dry weight at 180 DAP (g root^{-1})	77.1 **	6.1	1.4 ns	5.0 ns	10.4

AUC = area under curve; DAP = days after planting; ns, *, and ** = nonsignificant in statistics, significant at $P < 0.05$, and significant at $P < 0.01$, respectively.

Table 6. Means for number of storage root at 120 days after planting (DAP), storage root dry weight at 150 and 180 DAP (g root^{-1}), starch contents at 120, 150, and 180 DAP (%), and starch yield at 120 and 180 DAP (kg plant^{-1}) for the four different cassava genotypes over four environmental sites.

Factors	Crop traits							
	Number of storage root at 120 DAP	Storage root dry weight at 150 DAP (g root^{-1})	Storage root dry weight at 180 DAP (g root^{-1})	Starch content at 120 DAP (%)	Starch content at 150 DAP (%)	Starch content at 180 DAP (%)	Starch yield at 120 DAP (kg plant^{-1})	Starch yield at 180 DAP (kg plant^{-1})
Genotypes								
Kasetsart 50	3.6	51.1 ab	77.8	71.5 b	75.6	78.8	0.01 c	0.5 b
Rayong 9	4.0	46.9 b	69.0	71.9 b	76.1	79.8	0.03 b	0.4 b
Rayong 11	2.7	43.6 b	70.3	70.8 b	76.5	79.9	0.04 b	0.4 b
CMR38-125-77	0.4	60.8 a	82.5	74.4 a	77.1	79.9	0.06 a	0.6 a
F	ns	**	ns	*	ns	ns	**	*
Environments								
Ban Kho	1.4 b	63.7 a	101.0 b	71.9 a	77.1	79.8 a	0.04 b	0.5 b
Kham Pom	2.3 b	44.3 b	51.2 c	73.4 a	76.2	78.3 b	0.05 a	0.5 b
Kham Thao	2.0 b	77.0 a	125.2 a	69.2 b	75.7	80.2 a	0.03 bc	0.7 a
Khok Kung	8.4 a	17.5 c	22.1 d	74.1 a	76.5	80.1 a	0.02 c	0.2 c
F	**	*	**	**	ns	*	**	**

ns, *, and ** = nonsignificant in statistics, significant at $P < 0.05$, and significant at $P < 0.01$, respectively. Values within a column followed by the same letter are not significantly different. All means were compared via the Least Significant Difference Test at the 0.05 level of significance.

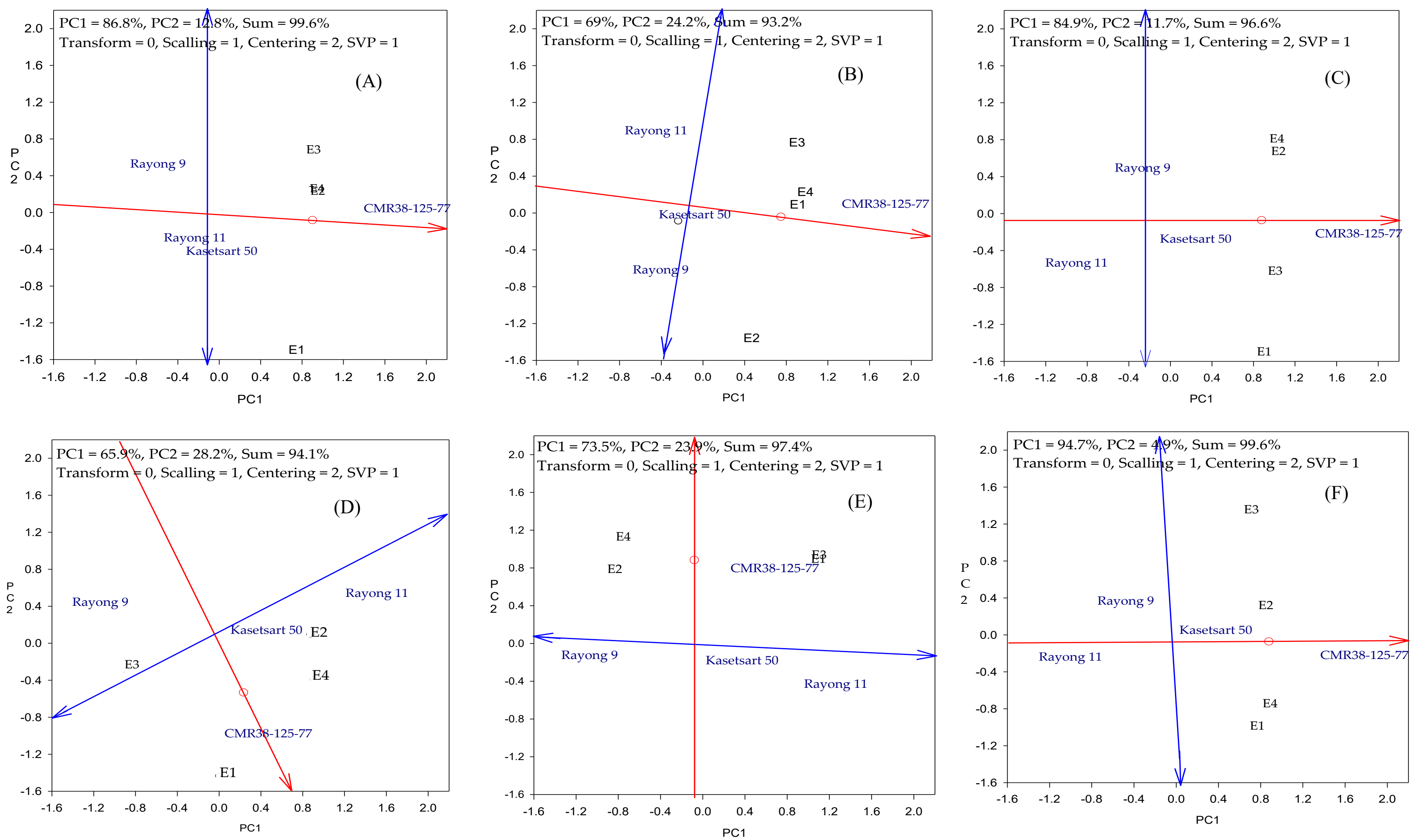

Figure 1. Area under curve for starch yield (A), starch production rate (B), starch yield at 150 days after planting (DAP) (C), number of storage root at 150 DAP (D), number of storage root at 180 DAP (E), and weight per storage root at 120 DAP (F) over four environments (E1, Ban Kho 2016-2017; E2, Kham Pom 2016-2017; E3, Kham Thao 2016-2017; E4, Khok Kung 2017-2018). Stability analysis was determined using a graphical approach of the genotype main effect plus genotype by environment interaction (G+GE) or GGEbiplot software (PC1 and PC2 are first and second principle component, respectively).

The CMR38-125-77 genotype was identified as a superior genotype with respect to the AUC of starch yield, starch production rate, starch yield at 150 DAP, the number of storage root at 150 DAP, the number of storage root at 180 DAP, and weight per storage root at 120 DAP over four environments.

DISCUSSION

Determination among the four environments based on number of storage root and storage root dry weight (g $root^{-1}$), although Khok Kung had more number of storage root, but lower total amount of rainfall and number of rainy days (19 mm and 6 days, respectively) during three months after planting (Figure 2) might cause this environment produced smaller size and lower dry weight of storage root than the other environments. The total amount of rainfall and number of rainy days for Kham Thao, Ban Kho, and Kham Pom were 110 mm and 13 days, 28 mm and 19 days, and 23 mm and 10 days, respectively.

The results showed the outstanding performance of the CMR38-125-77 genotype with respect to storage root dry weight, starch content, and starch yield at 120 DAP at all four experimental sites. Therefore, it is likely to be an early storage root formation genotype. However, the criteria in terms of rapid starch production rate and high starch yield accumulation are also crucial for a better understanding of crop adaptability to determine the superior cassava genotype for planting under upper paddy field conditions during the off-season of rice. Compared with the other tested genotypes, CMR38−125-77 also was a preferable genotype given its faster rate of starch formation and higher total amount of starch yield accumulation (higher AUC values).

An experiment conducted in different upper paddy fields during the off-season of rice in Thailand recorded the satisfactory performances of the CMR38-125-77 genotype in terms of chlorophyll fluorescence, storage root fresh weight, storage root dry weight, total dry weight, and starch yield at final harvest as compared with those of Kasetsart 50, Rayong 9, and Rayong 11 (Sawatraksa *et al*., 2018; Sawatraksa *et al*., 2019). In experiments regarding six different planting dates with supplementary irrigation under upland conditions at Khon Kaen Province, Thailand, the CMR38-125-77 genotype also showed higher growth rate, storage root yield, and starch content at final harvest (Phoncharoen *et al*., 2019a, b). Janket *et al*. (2018) documented that CMR38-125-77 produces a greater starch content and starch yield at final harvest than Kasetsart 50 and Rayong 11 when grown in an upland area with well irrigation in Khon Kaen Province, Thailand. In addition, Wongnoi *et al*. (2020) found that the CMR38-125-77 genotype has preferable performances in terms of physiology, growth, and yield for planting under upland conditions in a dry environment during the high storage root accumulation stage.

The nonsignificant results and low proportion of variability for genotype × environment in starch content at 120, 150, and 180 DAP indicate that a small number of tested environments is acceptable for cassava yield trials based on starch content. This involves less time and resources in multilocation trials and allows the possibility of using starch content at various growing stages as a criterion to identify desirable genotypes for different environments.

A low proportion of variation for other cassava characteristics has been reported. Sawatraksa *et al*. (2018) indicated no statistically significant difference and a small variation in genotype × environment for the chlorophyll fluorescence of four cassava genotypes grown under different upper paddy field conditions, indicating the similar responses and high stability of this crop trait. A low proportion of variation with nonsignificant genotype × environment interaction for leaf growth rate during 120–180 DAP for cassava grown after rice harvest in different upper paddy fields has also been reported (Sawatraksa *et al*., 2019). In addition, these crop traits have been suggested as additional criteria for multilocation trails to obtain a further understanding of the adaptation of each cassava genotype and improve the efficiency of varietal selection for upper paddy fields given that chlorophyll fluorescence and leaf growth rate for 120 to 180 DAP were associated with the final storage root yield. In our study, however, a poor relationship between starch content and final storage root yield was observed (data not shown).

The other studies reported that starch biosynthesis in cassava is commonly controlled by several genes, which include adenosine diphosphate glucose (ADPG) pyrophosphorylase, granule-bound starch synthase, starch synthase, starch branching enzyme, debranching enzyme, and glucan water dikinase; in addition, these genes and quantitative trait loci play a very important role in improving starch content in cassava through biotechnology, i.e., transgenic breeding and molecular marker assisted selection (Lopez *et al*., 2004; Li *et al*., 2016; Tappibana *et al*., 2019).

In addition to the genetic background of each genotype, environmental factors during the growing period evidently affect starch biosynthesis in the storage root of cassava. Janket *et al*. (2018) conducted experiments with different planting dates under upland conditions in Thailand and demonstrated that the planting dates of 5 October and 15 December with higher temperature and solar radiation during summer and rainy seasons (at the time of the stem and leaf development stage until high translocation of carbohydrate to storage roots or approximately 90–270 DAP) produced a greater starch content and starch yield. By contrast, in our study, the maximum and minimum temperatures during the growing period between the four experimental sites were not considerably different (Figure 2) and may not account for the variation in starch content.

Our results revealed that the highest total amount of rainfall during May to June in Kham Pom was associated with low starch content at 180 DAP. The negative correlation between starch content and the total amount of rainfall prior to harvest in Thailand has been reported by Howeler (2007). The increased availability of water allows more aboveground growth (Akhtar *et al*., 2018; Akhtar *et al*., 2019a) through the translocation of photoassimilates from the storage roots to the top and reductions in starch content and storage root dry weight (Ceballos *et al*., 2007).

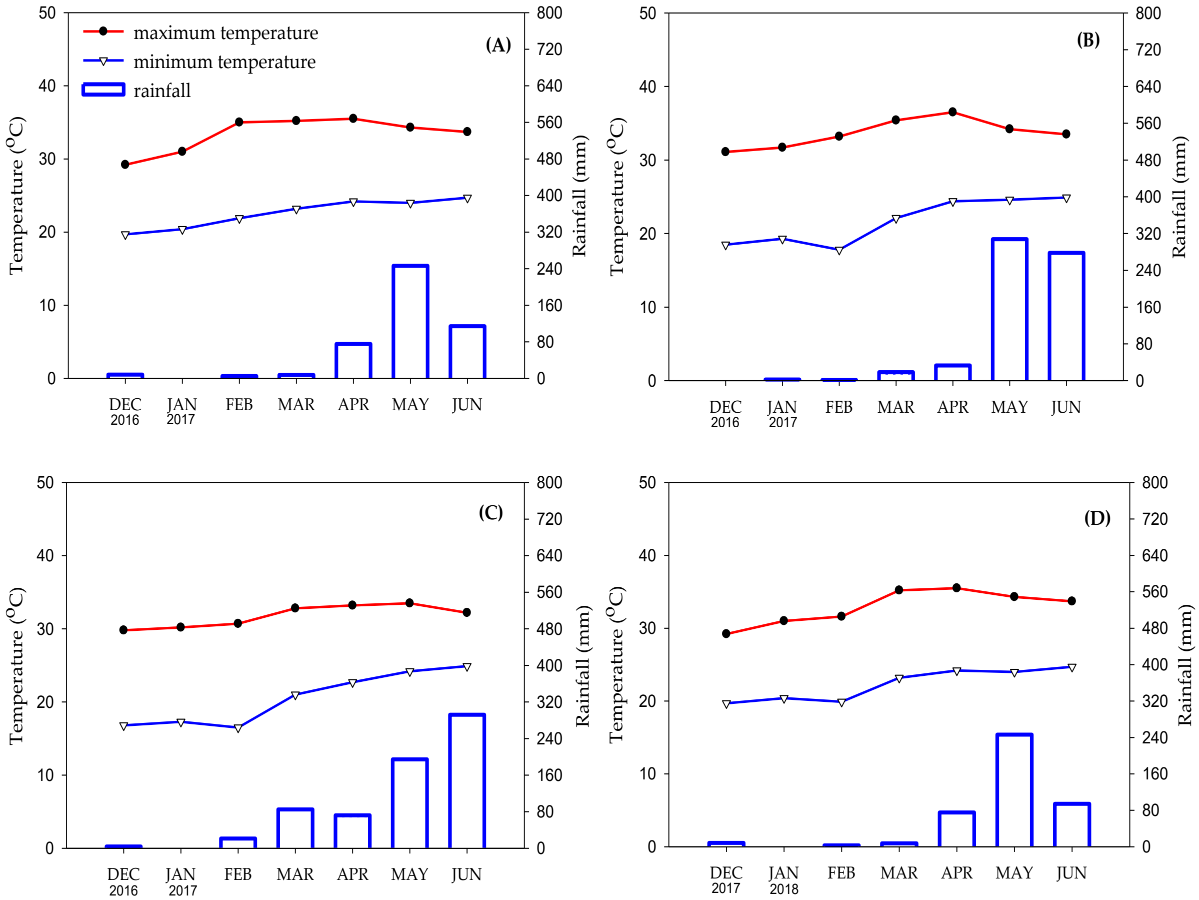

Figure 2. Rainfall (mm), maximum temperature (°C), and minimum temperature (°C) at Ban Kho during 2016–2017 (A), Kham Pom during 2016–2017 (B), Kham Thao during 2016-2017 (C), and Khok Kung 2017–2018 (D).

For cassava grown under extremely different rainfall regimes and upland conditions in Nakorn Ratchasrima Province, Thailand, Santisopasri *et al*. (2001) reported that the planting date with limited amount of rainfall (158 mm) at the early crop growing period causes remarkably lower starch content in storage roots at 6 months after planting as compared with another planting dates with a total rainfall of 970 mm. Based on this present study, the appropriate rainfall levels in Kham root and starch yields than cassava grown under upland conditions. Presently, however, some of farmers Thao are related to high storage root dry weight and starch yield at 180 DAP, indicating suitability for cassava production in upper paddy field during the off-season of rice (Sawatraksa *et al*., 2019).

Given that the growing cassava during the off-season of rice in upper paddy fields involves a crop duration (about 6–7 months) that is shorter than the common growing condition in upland areas (about 10 to 12 months and/or longer), cassava grown in upper paddy fields has lower storage in Thailand are interested in planting cassava in upper paddy fields after rice harvest because this approach can help increase land use efficiency and household income. Cassava genotypes with early storage root formation, fast starch production rate, and high starch yield is a desirable genetic resource.

The results of this study pointed out that early storage root formation, fast starch production rate, and high AUC of starch yield are relevant traits for increased final starch yield and indicated that CMR38-125-77 was outstanding genotype for cassava production during the off-season of rice in upper paddy fields. Although the CMR38-125-77 genotype showed potential as a genetic resource for planting in upper paddy field ecology, the exploration of other genetic resources with better crop growth rate, early storage root formation, rapid starch production rate, and high starch yield still challenges cassava breeders.

The off-season of rice in Thailand covers dry and hot seasons and involves drought occurrence. The introgression of additional genes related to the drought-tolerant characteristic is also an interesting issue for cassava breeding programs. Furthermore, planting cassava in this particular ecology could also impact nutrient balance and soil fertility. Improving soil properties by applying optimal fertilizer with the combined application of organic fertilizer would also be an alternative strategy for sustainable agriculture (Akhtar *et al*., 2019b). Therefore, studies on nutrient balance and suitable management practices for the environmentally friendly support of cassava and rice with environmental friendliness and the impact of this cropping system on economic value and environment are also interesting as further research topics.

CONCLUSION

No statistical significance for genotype × environment in starch content at 120, 150, and 180 DAP was found. Environment provided the largest proportion of variation compared with genotype and genotype × environment and had significant effect to almost all crop traits (except for starch content at 150 DAP). More suitable total amount and better distribution of rainfall in Kham Thao accounted for the higher storage root dry weight, starch content, and starch yield at 180 DAP at this environment than at the other tested environments. The genotype CMR38-125-77 had significantly higher starch content at 120 DAP and starch yield at 120 and 180 DAP than the other tested genotypes. It was also a superior genotype given its AUC of starch yield, starch production rate, starch yield at 150 DAP, number of storage roots at 150 DAP, number of storage roots at 180 DAP, and weight per storage root at 120 DAP. Therefore, CMR38-125-77 has potential as genetic resource for planting cassava under upper paddy field conditions during the off-season of rice.

ACKNOWLEDGEMENTS

This study was supported by Khon Kaen University, Thailand and the National Science and Technology Development Agency (NSTDA), Thailand. Assistance for conducting the field research was provided by the Plant Breeding Research Center for Sustainable Agriculture, Khon Kaen University, Thailand.

REFERENCES

Akhtar K, Wang W, Khan A, Ren G, Afridi MZ, Feng Y, Yang G (2018). Wheat straw mulching with fertilizer nitrogen: An approach for

improving soil water storage and maize crop productivity. *Plant Soil and Environ.* 64: 330–337.
Akhtar K, Wang W, Khan A, Ren G, Afridi MZ, Feng Y, Yang G (2019a). Wheat straw mulching offset soil moisture deficient for improving physiological and growth performance of summer sown soybean. *Agric. Water Manag.* 211: 16–25.
Akhtar K, Wang W, Ren G, Khan A, Feng Y, Yang G, Wang H (2019b). Integrated use of straw mulch with nitrogen fertilizer improves soil functionality and soybean production. *Environ. Int.* 132: 105092.
Anyanwu CN, Ibeto CN, Ezeoha SL, Ogbuagu NJ (2015). Sustainability of cassava (*Manihot esculenta* Crantz) as industrial feedstock, energy and food crop in Nigeria. *Renew. Energ.* 81: 745–752.
Banterng P, Joralee A (2015). Evaluation of black glutinous rice genotypes for stability of gamma oryzanol and yield in tropical environments. *Turk. J. Field Crops.* 20(2): 142–149.
Ceballos H, Pérez JC, Calle F, Jaramillo G, Lenis JI, Morante N, López J (2007). A new evaluation scheme for cassava breeding at CIAT. In *Cassava Research and Development in Asia: Exploring New Opportunities for an Ancient Crop.* Proceedings of the 7th Regional Workshop, Bangkok, Thailand, 28 October–1 November 2002; Howeler, R.H, Ed.; Centro Internacional de Agricultura Tropical (CIAT), Cassava Office for Asia: Bangkok, Thailand, pp. 125–135.
De Tafur SM, El-Sharkawy MA, Calle F (1997). Photosynthesis and yield performance of cassava in seasonally dry and semiarid environments. *Photosynthetica.* 33(2): 249–257.
Department of Agriculture (2008). Good Agricultural Practices for Cassava, National Bureau of Agricultural Commodity and Food Standards Ministry of Agriculture and Cooperatives: Bangkok, Thailand.
El-Sharkawy MA (1993). Drought-tolerant cassava for Africa, Asia, and Latin America: breeding projects work to stabilize productivity without increasing pressures on limited natural resources. *Bio. Sci.* 43(7): 441–451.
El-Sharkawy MA (2004). Cassava biology and physiology. *Plant Mol. Biol.* 56(4): 481–501.
El-Sharkawy MA (2007). Physiological characteristics of cassava tolerance to prolonged drought in the tropics: Implications for breeding cultivars adapted to seasonally dry and semiarid environments. *Braz. J. Plant Physiol.* 19(4): 257–286.
El-Sharkawy MA, De Tafur SM (2010). Comparative photosynthesis, growth, productivity, and nutrient use efficiency among tall– and short–stemmed rain–fed cassava cultivars. *Photosynthetica.* 48(2): 173–188.
FAO. (2017). Statistics databases: Production; FAO, Rome. http://www.fao.org/faostat/en/#data/QC (accessed 15 January 2019).
Gomez KA, Gomez AA (1984). Statistical Procedures for Agricultural Research; John Wiley and Sons: New York, NY, USA.
Howeler RH (2007). Agronomic practices for sustainable cassava production in Asia. In *Cassava Research and Development in Asia: Exploring New Opportunities for an Ancient Crop.* Proceedings of the 7th Regional Workshop, Bangkok, Thailand, 28 October–1 November 2002; Howeler, R.H, Ed.; Centro Internacional de Agricultura Tropical (CIAT), Cassava Office for Asia: Bangkok, Thailand, pp. 288–314.
Janket A, Vorasoot N, Toomsan B, Kaewpradit W, Banterng P, Kesmala T, Theerakulpisut P, Jogloy S (2018). Seasonal variation

in starch accumulation and starch granule size in cassava genotypes in a tropical savanna climate. *Agronomy.* 8: 297.

Janket A, Vorasoot N, Toomsan B, Kaewpradit W, Jogloy S, Theerakulpisut P, Holbrook CC, Kvien CK, Banterng P (2020). Starch accumulation and granule size distribution of cassava cv. Rayong 9 grown under irrigated and rainfed conditions using different growing seasons. *Agronomy*.10:412.

Klomsa-ard P, Jaisil P, Patanothai A (2013). Performance and stability for yield and component traits of elite sugarcane genotypes across production environments in Thailand. *Sugar Tech.* 15(4): 354–364.

Li YZ, Zhao JY, Wu SM, Fan XW, Luo XL, Chen BS (2016). Characters related to higher starch accumulation in cassava storage roots. *Sci. Rep.* 6: 19823.

Lopez C, Jorge V, Pie´gu B, Mba C, Cortes D, Restrepo S, Soto M, Laudie´ M, Berger C, Cooke R, Delseny M, Tohme J, Verdier V (2004). A unigene catalogue of 5700 expressed genes in cassava. *Plant Mol. Biol.* 56(4): 541–554.

Pellet D, El-Sharkawy MA (1997). Cassava varietal response to fertilization: growth dynamics and implications for cropping sustainability. *Exp. Agric.* 33: 353–365.

Phoncharoen P, Banterng P, Vorasoot N, Jogloy S, Theerakulpisut P, Hoogenboom G (2019a). The impact of seasonal environments in a tropical savanna climate on forking, leaf area index, and biomass of cassava genotypes. *Agronomy.* 9: 19.

Phoncharoen P, Banterng P, Vorasoot N, Jogloy S, Theerakulpisut P, Hoogenboom G (2019b). Growth rates and yields of cassava at different planting dates in a tropical savanna climate. *Sci. Agri.* 76(5): 376–388.

Polthanee A, Janthajam C, Promkhambut A (2014). Growth, yield and starch of cassava following rainfed lowland rice in northeast Thailand. *Int. J. Agric. Res.* 9(6): 319–324.

Santisopasri V, Kurotjanawong K, Chotineeranat S, Piyachomkwan K, Sriroth K (2001). Impact of water stress on yield and quality of cassava starch. *Ind. Crops Prod.* 13(2): 115–129.

Sawatraksa N, Banterng P, Jogloy S, Vorasoot N, Hoogenboom G (2018). Chlorophyll fluorescence and biomass of four cassava genotypes grown under rain-fed upper paddy field conditions in the tropics. *J. Agro. Crop Sci.* 204(6): 554–565.

Sawatraksa N, Banterng P, Jogloy S, Vorasoot N, Hoogenboom G (2019). Cassava growth analysis of production during the off–season of paddy rice. *Crop Sci.* 59(2): 760–771.

Statistix version 10 (2013). Analytical software; Analytical Software: Tallahassee, FL, USA.

Tappibana P, Smith DR, Triwitayakorn K, Baoa J (2019). Recent understanding of starch biosynthesis in cassava for quality improvement: A review. *Trends Food Sci. Tech.* 83: 167–180.

Wongnoi S, Banterng P, Vorasoot N, Jogloy S, Theerakulpisut P (2020). Physiology, growth and yield of different cassava genotypes planted in upland with dry environment during high storage root accumulation stage. *Agronomy.* 10: 576.

Yan W (2001). GGEbiplot–a windows application for graphical analysis of multienvironment trail data and other types of two–way data. *Agron. J.* 93(5): 1111–1118.

Yan W, Hunt LA (2003). Biplot analysis of multi-environment trial data. In *Quantitative genetics. Genomics and plant breeding.* Kang, M.S., Ed.; CAB Int., Wallingford, UK. pp. 289–303.