Accumulation Dynamics of Starch and Its Granule Size Distribution of Cassava Genotypes at Different Growing Seasons

Anon Janket\textsuperscript{1}, Nimitr Vorasoot\textsuperscript{1}, Banyong Toomsan\textsuperscript{1}, Wanwipa Kaewpradit\textsuperscript{1}, Piyada Theerakulpisut\textsuperscript{2}, Carl Corley Holbrook\textsuperscript{3}, Craig K. Kvien\textsuperscript{4}, Sanun Jogloy\textsuperscript{1,5} and Poramate Banterng\textsuperscript{1,*}

\textsuperscript{1}Department of Agronomy, Faculty of Agriculture, Khon Kaen University, Khon Kaen 40002, Thailand; anon.agron@gmail.com (A.J.); nimitr1945@gmail.com (N.V.); banyang@kku.ac.th (B.T.); wanwka@gmail.com (W.K.); sjogloy@gmail.com (S.J.)
\textsuperscript{2}Department of Biology, Faculty of Science, Khon Kaen University, Khon Kaen 4002, Thailand; ptythe@kku.ac.th
\textsuperscript{3}Crop Genetics and Breeding Research Unit, USDA-ARS, Tifton, GA 31793, USA; corley.holbrook@ars.usda.gov
\textsuperscript{4}Department of Crop & Soil Sciences, University of Georgia, Tifton, GA 31793, USA; ckvien@uga.edu
\textsuperscript{5}Peanut, Jerusalem Artichoke and Cassava Improvement Research Group, Department of Agronomy, Faculty of Agriculture, Khon Kaen University, Khon Kaen 40002, Thailand
\textsuperscript{*}Correspondence: bporam@kku.ac.th; Tel.: +66-43-364-637

Received: 8 August 2020; Accepted: 25 August 2020; Published: 28 August 2020

Abstract: This study aims to evaluate seasonal variation on starch production of different cassava genotypes planted under irrigated condition. Three cassava genotypes (Kasetsart 50, Rayong 11 and CMR38-125-77) were evaluated in two different growing seasons, i.e., early rainy seasons (ERS) and post rainy seasons (PRS) for two years. A randomized complete block design with four replicates was employed in each growing season. The starch yield was more strongly associated with growing season (28.3%), whereas starch content, amylose content, amylose-amylopectin ratio were more associated with genotypes (19.9–30.7%). Planting cassava in the ERS had rapid growth rates of starch yield and granule size in early growth stages, whereas planting cassava in the PRS had rapid growth rates of these traits in the middle to late growth stages. Cassava planted in the PRS had higher starch yield than the ERS for most genotypes except for Rayong 11, which had no significant differences between growing seasons. The yield and content of starch and size of starch granule continuously increased from four to twelve months after planting (MAP) for most cassava genotypes. Unlike other genotypes, CMR38-125-77 in the ERS gave the highest starch yield and its granule at 10 MAP; however, at 12 MAP, it was still higher than other genotypes. CMR38-125-77 was a preferable genotype, since it had a faster rate of starch formation and more starch and amylose contents, as well as starch yield in both ERS and PRS.

Keywords: cassava breeding; $G \times E$ interaction; Manihot esculenta; seasonal variation; starch production rate

1. Introduction

As the world’s human population is growing rapidly and is expected to hit eight billion in the next decade [1], global energy security has become an urgent problem. Fuels from renewable sources, especially from biomass, have served the needs for the energy consumption of humanity, which are becoming increasingly important in the present and future times. Cassava (Manihot esculenta Crantz) is
a root crop that is considered one of the most important crops for this purpose as it is a starch-containing crop that can be used for ethanol production [2]. In tropical and sub-tropical regions, cassava also plays a particularly important role in agricultural economics as the crop can be used to produce a variety of agricultural products, livestock feed and human food [3].

Although the area for agriculture in Thailand is limited compared to other big countries, Thailand is the largest cassava exporter in the world, and cassava production in the country has rapidly increased in recent years in response to industrial demand [4]. Unfortunately, the average cassava yield in Thailand is still low compared to the yields of the best farmers and its yield potential in experimental plots. Hence, strategies to improve the production of cassava are required and should be developed rapidly to respond to the demand for cassava.

In Thailand, cassava is commonly cultivated in two different growing seasons, including the early rainy season (ERS) and the post rainy season (PRS), and it can be harvested from 6 until 24 months after planting (MAP) (normally at 12 MAP) depending on cultivation conditions [5,6]. The age in which the cassava should be harvested in order to obtain the highest storage root yield may not necessarily be the same as that to get the highest starch yields. Harvesting too early results in a reduction of starch content and its yield, whereas with the delayed harvest, the storage roots become woody and fibrous leads to the reduction in content and yield of starch [7]. Starch content generally increases with the age of the crop; however, there is still conflict in previous reports for the appropriate time for cassava harvesting to obtain maximum starch content. Santisopasri et al. [8] reported that the highest starch content in cassava was observed at 8 MAP. Conversely, Eban-Djedji et al. [9] reported the highest starch content of improved cassava varieties at 12 MAP. Genotypic differences were recorded not only in starch content, but also in the stage of the highest starch content. Earlier studies have also stated that starch yield declines when the cassava has surpassed maturity, and that differences exist among cassava genotypes in age to obtain maximum starch yield [8,9].

The fact that the starch translocation rate and its pattern differ among cassava genotypes, and therefore, their earliness, partially explains differences in harvesting times [10]. However, climatic factors during the growing season have an important effect on the starch accumulation of cassava planted under various environments [11–13]. Identification of cassava genotypes for specific environments and improvement of cassava varieties for starch yield, starch quality and wide adaptation to growing seasons are important factors to increase cassava starch yield and its qualities. Identification of cassava genotypes with high stability in yield and starch quality under diverse growing conditions is a challenging task for plant breeders. We recently showed that cultivation of cassava with supplemental irrigation in the PRS produced higher yield and content of starch at final harvest than did the crop planted in ERS [11]. We also suggested that high temperature, high solar radiation during the mid-growth stage (3–9 MAP) and short photoperiod at late growth stages were more favorable conditions for crop growth.

Whereas, in rainfed conditions, high air temperature during the growing period (28–30 °C) can reduce the growth rates of granule size, content and yield of starch in cassava compared to a crop planted in lower temperature (lower than 24 °C) [14,15]. Reduction in light intensity (32–78%) resulted in delaying the bulking of storage roots and reduction in starch yield growth rate [16]. Long photoperiod (14–20 h) promoted the growth of shoots but decreased storage root yield, whereas short photoperiod (8 h) increased storage root yield and decreased shoots [17]. Moreover, cassava planted under rainfed condition is typically interrupted by drought stress, resulting in depressed development of starch granule size and starch yield [8,18].

The research done so far is limited to the effects of climatic factors on starch accumulation and size distribution of starch granule at final harvest under full irrigation in a one-year experiment and at final harvest only, and almost studies in cassava have been focused under rainfed condition and simulation environments. The rates of starch accumulation and size of starch granule of cassava genotypes may respond differently across growing seasons and growth stages, and currently, the production of cassava under supplementary irrigation is one of the alternative methods to increase yield per unit area. The knowledge on the effects of climatic factors on starch accumulation rate (SAR) and its quality
under natural conditions in different growing seasons when the crop receives full irrigation is limited. Monitoring starch accumulation of cassava genotypes during different plant ages in different growing seasons should provide important evidence about the starch accumulation habit and adaptability of each genotype for specific planting seasons, and this information should be useful to identify suitable cassava genotypes for each growing season and to determine the optimum time to harvest for each cassava genotype for specific growing seasons. The superior genotypes also can be used as parents in cassava breeding programs. The purpose of this study was to study the SAR and starch granule sizes of cassava genotypes with different starch bulking periods in responses to different growing seasons at different plant ages under full irrigation.

2. Materials and Methods

2.1. Planting Details and Experimental Design

In each growing season, three cassava genotypes with different starch bulking times, i.e., CMR38-125-77 (starch bulking starting at the early growth stage), Kasetsart 50 (KL50; starch bulking starting at mid-stage) and Rayong 11 (RY11; starch bulking starting at the late growth stage), were laid out in a randomized complete block design (RCBD) with four replicates. The crops were planted in two different growing seasons at Khon Kaen University in Thailand (16°47′N, 102°81′E, 200 masl) consisting of the ERS (at May) and the PRS (at November) in 2015 and were planted again in 2016. The climate in this region is a tropical savanna climate following Köppen climate classification [19].

2.2. Crop Management

A hardpan was broken using a tractor ripper, and sunn hemp (Crotalaria juncea) was grown for green manures and plowed at flowering to improve the soil before planting the cassava. The experimental area was prepared by plowing three times, and then, ridges were made at 1 m spacing. The stem of cassava, at 9 months old, were cut into pieces 20 cm in length, and they were immersed into water containing thiamethoxam (Syngenta crop protection limited, Bangkok, Thailand) (3-(2-chloro-thiazol-5-ylmethyl)-5-methyl-(1,3,5)-oxadiazinan-4-ylidene-N-nitroamine 25% water dispersible granules) at a rate of 4 g per 20 L of water for 30 min to control mealybug (Phenacoccus manihoti). The stem cuttings were then planted vertically into the top of the soil ridges, and they were buried into the soils at 2/3 of the length. Plot size was 19 m long and 7 m wide with a spacing of 1 m × 1 m.

At one MAP, fertilization was practiced according to crop nutrient requirements proposed by Howeler [6] and the information from a soil analysis before planting. At two MAP, chemical fertilizers of ammonium sulfate ((NH₄)₂SO₄) (Chia tai company limited, Phranakhonsiayutthaya, Thailand) at the rate of 223.18 kg ha⁻¹ and potassium chloride (KCl; 60% K₂O) (Chia tai company limited) at the rate of 93.75 kg ha⁻¹ were applied to the plots according to the recommendations of the Department of Agriculture of Thailand. As phosphorus (P) content in the soil was sufficient, additional P was not applied. Weeds were controlled by spraying 3 L ha⁻¹ of alachlor (Kemfac limited, Samutprakan, Thailand) (2-chloro-2',6'-diethyl-N-(methoxymethyl) acetanilide 48%, w/v, emulsifiable concentrate) as a pre-emergent herbicide at planting. Additional weed control was obtained by manual weeding before canopy closure. Diseases and insects were controlled as necessary by following normal procedures for cassava cultivation.

The sets of tensiometers were used in order to maintain soil moisture content during the growing periods by installing at the depths of 20 and 40 cm in all growing seasons. The soil moisture recorded by tensiometer at 40 cm below the soil surface was maintained uniformly at values higher than −30 kPa, and additional water was supplied during the dry periods by mini sprinkler irrigation system.

2.3. Soil Properties and Meteorological Conditions

Soil samples were collected randomly before planting at 0–30 and 30–60 cm below the soil surface. After air-drying, the physical and chemical properties of the soil was determined, i.e., percentage of sand,
silt and clay, organic matter (OM), pH, electrical conductivity (EC), cation exchange capacity (CEC), total nitrogen (N), available P, exchangeable potassium (K) and total magnesium (Mg), calcium (Ca) and sulfur (S). From planting until harvest, daily maximum-minimum air temperatures, the intensity of solar radiation, relative air humidity and rainfall were also recorded by a weather station located in the experimental field (Watchdog 2700, Path computation element group, Meschede, Germany).

2.4. Determination of Content and Yield of Starch, Size of Starch Granule and Amylose Content

At 4, 6, 8 and 10 MAP plant data were recorded from six bordered plants in each sub-plot, whereas, at the final harvest (at 12 MAP), the data were measured from eighteen plants. Harvested plants were separated into petioles, leaves, stems, stumps and storage roots. Storage roots and stumps were washed. Thereafter, all plant parts (about 10%) of its fresh weight were then taken, chopped and oven-dried at 70 °C for 72 h to a constant weight.

For the preparation of starch samples, at least eight storage roots were sub-sampled immediately after harvesting. After washing and chopping, the samples of storage roots were then oven-dried at 50–55 °C using a tray drier (EQ-04SW, Leehwa industry company, Kyongbuk, Korea). The dried samples were then ground in an electric blender and sieved using 200 µm diameter sieves for determination of starch content and 106 µm diameter sieves for determination of granule size distribution. Starch content was measured by the polarimetric method using the sieved samples from unpeeled storage roots with a polarimeter (Polatronic MH8, Schmidt haensch, Berlin, Germany) and reported on a dry weight basis. Starch granule size was determined from the sieved samples from peeled storage roots by the laser diffraction method as described by Janket et al. [11]. Briefly, the starch was suspended in deionized distilled water and sonicated by the ultrasonic bath (50/60 Hz, Ney, Sacramento, CA, USA) for 30 min, and the then samples were read by laser diffraction (Mastersizer 2000, Malvern instruments limited, Malvern, UK). Amylose content was also evaluated [20].

Starch yield (tonne ha⁻¹) was obtained by multiplying the percentage of starch content and dry weight of storage root (tonne ha⁻¹). Starch accumulation rate (SAR) during each growth period was calculated using the following equation,

\[
\text{Starch accumulation rate (g m}^2\text{day}^{-1}) = \frac{(W2 - W1)}{(T2 - T1)}
\]

where W1 refers to starch yield per m² recorded at time T1; W2 refers to starch yield per m² recorded at time T2; T1 and T2 refer to the interval of observation time (days), respectively.

2.5. Statistical Analysis

A separate analysis of variance was performed for each year according to the RCBD. The error variances of each year’s data were tested for homogeneity of variance. Data with homogeneous error variances were subjected to analyze in a combined analysis of variance for two years [21]. A least significant difference (LSD) was employed for mean comparisons (p < 0.05), where significance was indicated. All calculation procedures were completed with the statistical software package MSTAT-C version 1.42 [22].

3. Results

3.1. Soil Properties and Weather Conditions

The soil used in this experiment is a Yasothon soil series (Yt), with averaged pH between 6.1–7.5 (Table 1). On average, at the depth 0–60 cm, the soil in this study was a loamy sand soil with low organic matter (0.38–0.48%). Based on nutrient requirements of cassava reported by Howeler [6], most soil chemical properties prior to planting, including total N (0.02–0.03%), exchangeable K (28.7–43.8 mg kg⁻¹), total Mg (30.7–48.0 mg kg⁻¹) and CEC (3.4–6.5 cmol kg⁻¹) were classified as low and very low. Whereas, exchangeable Ca (263–432 mg kg⁻¹), total S (4.2–50.0 mg kg⁻¹) and
EC (0.04–0.06 dS m$^{-2}$) were medium, and available P (48.4–84.3 mg kg$^{-1}$) were classified as high. All nutrients were added into the same range at an optimum concentration by applying soil fertilizers according to soil analysis before planting for all growing seasons. However, P was not applied as it was already at a high concentration in the soil.

**Table 1.** Soil physicochemical properties at pre-planting by averaging from two soil layers (0–30 cm and 30–60 cm) in the early rainy seasons (ERS) and post rainy seasons (PRS).

| Soil Physicochemical Properties | 2015/16 | 2016/17 |
|-------------------------------|---------|---------|
|                               | ERS     | PRS     | ERS     | PRS     |
| Sand (%)                      | 84.4    | 84.9    | 93.4    | 80.5    |
| Silt (%)                      | 7.4     | 9.5     | 5.5     | 9.4     |
| Clay (%)                      | 8.2     | 5.6     | 1.2     | 10.1    |
| Total Nitrogen (%)            | 0.02    | 0.02    | 0.03    | 0.03    |
| Available Phosphorus (mg kg$^{-1}$) | 84.3  | 48.4    | 79.8    | 63.6    |
| Exchangeable Potassium (mg kg$^{-1}$) | 43.8  | 34.6    | 28.7    | 29.6    |
| Exchangeable Calcium (mg kg$^{-1}$) | 432   | 263     | 362     | 412     |
| Magnesium (mg kg$^{-1}$)      | 30.7    | 33.9    | 48.0    | 41.2    |
| Sulfur (mg kg$^{-1}$)         | 50.0    | 6.4     | 21.3    | 4.2     |
| pH (1:1 H$_2$O)               | 7.5     | 6.1     | 7.4     | 7.2     |
| Electrical Conductivity (dS kg$^{-1}$) | 0.06 | 0.05    | 0.06    | 0.04    |
| Organic Matter (%)            | 0.43    | 0.38    | 0.48    | 0.42    |
| Cation Exchange Capacity (cmol kg$^{-1}$) | 4.0   | 6.5     | 3.4     | 4.7     |

The climatic factors during growth periods of the first-year experiment (2015/16) were similar to the second-year experiment (2016/17) for both ERS and PRS (Figure 1). Crops planted in the ERS experienced high air temperatures, high intensity of solar radiation, high relative air humidity and longer day length during the early growth period of approximately three months. Subsequently, the crops received lower air temperatures, lower intensity of solar radiation and shorter day length for long term during the stage of shoots and storage root development until high translocation of starch to storage roots (3–10 MAP). In contrast, relative air humidity during this period was high and decreased over time. The climatic factors during the late growth stage in the ERS were similar to the early stage of crop growth.

Crops planted in the PRS experienced lower air temperatures, lower intensity of solar radiation, lower relative air humidity and shorter day length during the early growth period of approximately four months. Thereafter, the crop experienced higher air temperatures, higher intensity of solar radiation and longer day length from 4 to 10 MAP, which is the stage of shoots and storage root development until high translocation of starch to storage roots. In contrast, relative air humidity during this period was low until 7 MAP; thereafter, it was high and decreased over time until harvest. The climatic factors during the late growth stage in the PRS were similar to the early stage of crop growth.
3.2. Combined Analysis of Variance

The variation between years (Y) and genotypes (G) was significantly different in terms of starch content and starch yield at final harvest but not for starch granule size, amylose content and amylose-amylopectin ratio (Table 2). Significant differences between Y and G were also recorded for starch accumulation rates for most growth periods. With the exception of granule size, there were statistically significant differences between growing seasons (S) in terms of starch content, starch yield, amylose content, the amylose-amylopectin ratio at final harvest and SAR for all growth periods. The interactions between years and growing seasons (Y × S), genotypes and years (G × Y) and secondary level interactions (Y × S × G) were not significantly different for most traits. However, significant interactions between genotypes and seasons (G × S) were observed for starch content, starch yield and SAR at 4–6 MAP, 6–8 MAP and 8–10 MAP.

Figure 1. Relative air humidity (%), day-length (h), rainfall (mm), solar radiation (MJ m$^{-2}$ day$^{-1}$), maximum and minimum air temperatures (°C) during the crop growth period of cassava. ERS = early rainy season, PRS = post rainy season.
Table 2. Mean squares from the combined analyses of starch content, starch yield, granule size, amylose, amylose-amylopectin ratio at final harvest and starch accumulation rate during 4–6, 6–8, 8–10 and 10–12 months after planting (MAP) for three cassava genotypes in early and post rainy seasons for two years.

| Source of Variance | df  | Starch Content (% | Starch Yield (Tonne ha\(^{-1}\)) | Granule Size [\(d\) (0.05)] | Amylose Content (%) | Amylose-Amylopectin Ratio |
|--------------------|-----|-------------------|-----------------------------------|-----------------------------|---------------------|--------------------------|
| Year (Y)           | 1   | 13.7 (2.2) *      | 52880 (9.0) **                   | 1.163 (5.7) **              | 2.88 (1.5) **       | 0.001 (1.5) **            |
| Growing season (S) | 1   | 49.7 (8.0) **     | 163950 (28.3) **                | 2.149 (10.5) **             | 28.43 (15.1) *      | 0.008 (15.1) **           |
| Y × S              | 1   | 132.1 (21.1) **   | 15210 (2.6) **                  | 1.030 (5.0) **              | 0.00 (0.0) **       | 0.000 (0.0) **            |
| Rep within Y and S | 12  | 2.3 (4.4)        | 3351 (6.9)                      | 0.302 (17.6)                | 3.40 (21.7)         | 0.001 (21.7)              |
| Genotypes (G)      | 2   | 95.9 (30.7) **    | 47060 (16.0) **                 | 0.965 (9.4) **              | 18.68 (19.9) **     | 0.004 (19.9) **           |
| G × Y              | 2   | 6.1 (2.0) *       | 4164 (1.4) **                   | 0.279 (2.7) **              | 0.00 (0.0) **       | -                        |
| G × S              | 2   | 32.8 (10.5) **    | 59430 (20.3) **                 | 0.624 (6.1) **              | 3.97 (4.2) **       | 0.001 (4.2) **            |
| Y × S × G          | 2   | 23.0 (7.4) **     | 4860 (1.7) **                   | 0.332 (3.2) **              | 0.00 (0.0) **       | 0.000 (0.0) **            |
| Pooled error       | 24  | 3.6 (13.8)        | 3397 (13.9)                     | 0.341 (39.8)                | 2.94 (37.5)         | 0.001 (37.5)              |

Growing season shared the largest variations for starch yield (28.3%), while starch content, amylose content and the amylose-amylopectin ratio were more associated with genotypes (30.7, 19.9 and 19.9%, respectively). The starch accumulation rates for all growth periods were mostly due to growing season (5.9–19.7%) and genotype (6.7–16.6%).

As the interactions between G and Y were not statistically significant for all traits, and the G × S interactions were significant in terms of starch content, starch yield at final harvest and starch accumulation rates for most growth periods, the data of two years were averaged and analyzed separately for each growing season.

3.3. Variation in Starch Content, SAR and Starch Yield

The starch content was slightly increased over the sampling period during 4–12 MAP. In the ERS, the highest values of starch content were observed for CMR38-125-77 at all sampling ages (77.3–83.2%), followed by RY11 (74.7–82.9%). The result showed that KU50 gave the lowest starch content (67.4–76.3%) at all sampling times, and the differences were large when compared to other genotypes in this growing season. Likewise, the genotypes that showed high starch content in ERS also had high starch content in PRS and a similar ranking of cassava genotypes was also observed, with the highest starch contents observed in CMR38-125-77 (78.0–84.0%), followed by RY11 (74.4–83.4%) and KU50 (75.0–81.9%), respectively. However, planting KU50 in the PRS seemed to result in more rapid accumulation of starch than did planting in the ERS, and the starch content of KU50 planted in PRS exhibited narrow differences when compared to other genotypes (Figure 2a–c).
Figure 2. Means of starch content (%) and starch yield (tonne ha\(^{-1}\)) of three cassava genotypes in early rainy seasons (ERS) and post rainy seasons (PRS). *, ** = significant differences between baseline and the respective time point at \(p < 0.05\) and significant at \(p < 0.01\) level, respectively (LSD test). All data are presented as mean ± standard error of the mean (\(n = 8\)). (a,d) = CMR38-125-77, (b,e) = Kasetsart 50 and (c,f) = Rayong 11.

Comparing between the genotypes, CMR38-125-77 and RY11 tended to give higher starch content during 6–10 MAP in the ERS than did the crop planted in the PRS. The starch content at 12 MAP was an exception as it showed no significant difference between growing seasons. Whereas, KU50 tended to give higher starch accumulation in PRS at all sampling ages, especially at 12 MAP as it showed a significant difference between growing seasons. However, KU50 still had a low performance for starch accumulation in terms of timing of starch bulking and starch content for both growing seasons when compared to other genotypes (Figure 2a–c).
Starch yield in both growing seasons was increased continuously through the growing periods, except CMR38-125-77 in ERS that exhibited a maximum at 10 MAP (14.230 tons ha\(^{-1}\)) and declined thereafter to 12 MAP (13.439 tons ha\(^{-1}\)). On average, planting cassava in the ERS had the highest SAR at early growth during 4–6 MAP (6.006 g m\(^{-2}\) day\(^{-1}\)), whereas planting cassava in the PRS had the highest SAR at the mid-stage during 6–8 MAP (6.977 g m\(^{-2}\) day\(^{-1}\)) (Table 3). Planting cassava in the PRS also had higher SAR at late growth stages during 8–10 MAP (5.442 g m\(^{-2}\) day\(^{-1}\)) and 10–12 MAP (5.112 g m\(^{-2}\) day\(^{-1}\)), while the crop planted in the ERS had lower SAR in these growth stages (4.671 g m\(^{-2}\) day\(^{-1}\) and 0.216 g m\(^{-2}\) day\(^{-1}\), respectively). The variation in SAR between genotypes in the ERS indicated that CMR38-125-77 gave the highest SAR during 4–10 MAP (Table 3), and it also produced the highest starch yield during these periods (Figure 2a). Although the starch yield of CMR38-125-77 was slightly declined at the final harvest (12 MAP), it was still higher than other genotypes. RY11 also gave the highest SAR during 6–10 MAP (Table 3), and it produced the second-highest starch yield for all growth stages, after CMR38-125-77 (Figure 2c). Whereas in this growing season, KU50 gave the lowest SAR for most growth stages (during 4–10 MAP) (Table 3) and produced the lowest starch yield for all growth stages (Figure 2b).

The variation in SAR between genotypes in the PRS indicated that CMR38-125-77 gave the highest SAR during 4–6 and 8–10 MAP (Table 3), and it also produced the highest starch yield during these periods (Figure 2a). In this growing season, KU50 also gave the highest SAR during 6–8 MAP (Table 3), but it produced the second-highest starch yield for all growth stages, after CMR38-125-77 (Figure 2c). Whereas, RY11 gave the lowest SAR for most growth stages (during 4–10 MAP), but it gave the highest SAR at 10–12 MAP (Table 3), however, RY11 produced the lowest starch yield for all growth stages in the PRS (Figure 2b).

Considering growing seasons, there was no significant difference among growing for starch yield during early growth periods for all genotypes, as it showed the differences between growing seasons in a narrow range. However, the differences between growing seasons for this parameter were observed at late growth stages. There were significant differences at approximately 8–12 MAP for most genotypes (Figure 2d–f). RY11 at 12 MAP was an exception as it showed no statistically significant difference between growing seasons (Figure 2f). CMR38-125-77 and KU50 showed the best performance for starch yield in the PRS, whereas KU50 showed the best performance in the ERS until 10 MAP, but it showed no statistically significant difference between growing seasons for this genotype at 12 MAP.

### Table 3. Starch accumulation rate during 4–6, 6–8, 8–10 and 10–12 months after planting (MAP) of three cassava genotypes grown in early rainy seasons (ERS) and post rainy seasons (PRS).

| Growing Season | Genotype      | Starch Accumulation Rate (g m\(^{-2}\) day\(^{-1}\)) |
|----------------|---------------|-------------------------------------------------|
|                | 4–6 MAP       | 6–8 MAP                           | 8–10 MAP   | 10–12 MAP  |
| ERS            | CMR38-125-77  | 7.175 a                          | 6.029 a     | 5.749 a   | –1.319     |
|                | Kasetsart 50  | 5.881 b                          | 2.699 b     | 2.504 b   | 1.292      |
|                | Rayong 11     | 4.961 b                          | 7.294 a     | 5.759 a   | 0.674      |
| Mean           |               | 6.006 A                         | 5.340 B     | 4.671 B   | 0.216 B    |
| F-test         |               | *                               | *           | *         | ns         |
| PRS            | CMR38-125-77  | 6.537 a                          | 6.751 b     | 8.938 a   | 3.601 b    |
|                | Kasetsart 50  | 5.360 b                          | 8.600 a     | 4.766 b   | 4.659 b    |
|                | Rayong 11     | 4.443 b                          | 5.579 b     | 2.624 c   | 7.076 a    |
| Mean           |               | 5.446 B                         | 6.977 A     | 5.442 A   | 5.112 A    |
| F-test         |               | *                               | *           | **        | *          |

Different lowercase letters with the same column indicate a significant difference between the genotypes for each growing season, and different capital letters indicate a significant difference between the growing seasons. ns, * = non significant and significant at \(p < 0.05\) and significant at \(p < 0.01\) level, respectively (LSD test).
3.4. Variation in Size of Starch Granule and Amylose Content

Three cassava genotypes planted under well-irrigated condition had granule sizes during 4–12 MAP ranging from 14.08 to 16.95 µm in the ERS and ranging from 14.39 to 17.00 µm in the PRS (Figure 3a–c), and sizes of the granules increased with time until final harvest for most growing seasons and genotypes. CMR38-125-77 and RY11 at 12 MAP in the ERS were an exception as the sizes of the granules in these genotypes slightly decreased. The results indicated that most cassava genotypes planted in the ERS gave bigger starch granules than when planted in the PRS at most sampling times except for CMR38-125-77 and RY11 at 12 MAP, which had slightly bigger starch granules when planted in the PRS (Figure 3a,c). Considering cassava genotypes in each growing season, RY11 gave the biggest starch granules followed by CMR38-125-77 and KU 50, respectively, for both ERS and PRS. However, there were only small differences between genotypes for the size of the granules in the ERS at 12 MAP. No significant difference between genotypes at the final harvest for both growing seasons was observed for amylose content (ranging 20.3–20.8% in ERS and 21.3–22.7% in PRS) and amylose and amylose content ratio (ranging 0.25–0.26 in ERS and 0.27–0.29% in PRS) (Table 4). Statistical significance between growing seasons was observed only in CMR38–125–77, which had significantly higher amylose content (22.7%) and amylose to amylopectin ratio (0.29) for the crop planted in the PRS.

Figure 3. Means of starch granule size of three cassava genotypes in the early rainy seasons (ERS) and post rainy seasons (PRS). *, ** = significant differences between baseline and the respective time point at $p < 0.05$ and significant at $p < 0.01$ level, respectively (LSD test). All data are presented as mean ± standard error of the mean ($n = 8$). (a) = CMR38-125-77, (b) = Kasetsart 50 and (c) = Rayong 11.
Table 4. Amylose, amylose-amylopectin ratio of starch isolated from storage roots of three cassava genotypes at final harvest in early rainy seasons (ERS) and post rainy seasons (PRS).

| Genotypes     | Amylose (%) | Amylose-Amylopectin Ratio |
|---------------|-------------|---------------------------|
|               | ERS         | PRS | F-Test       | ERS | PRS | F-Test       |
| CMR38-125-77  | 20.3        | 22.7 | *            | 0.26 | 0.29 | *            |
| Kasetsart 50  | 20.8        | 21.3 | ns           | 0.26 | 0.27 | ns           |
| Rayong 11     | 20.3        | 21.9 | ns           | 0.25 | 0.28 | ns           |
| Mean          | 20.5        | 22.0 |              | 0.26 | 0.28 |              |
| F-test        | ns          | ns  |              | ns  | ns  |              |

ns, * = non-significant and significant at p < 0.05 level, respectively (LSD test).

4. Discussion

The investigation on monitoring SAR, starch yield, starch content and size of starch granule for different cassava genotypes during different plant ages with varying growing seasons provided the information about starch accumulation habit and the adaptability of cassava genotypes in different growing seasons. The combined analysis revealed that there were significant differences for G for most crop traits, whereas Y and G were differences for starch content, starch yield and SAR. However, no significant difference between Y and G was found for granule size, amylose content and the amylose-amylopectin ratio. The interactions for Y × S, G × S and Y × S × G were significantly different for starch content only, but not for other traits except for G × S for starch yield and SAR at most growth periods. As the G × S interactions were significant for starch content, starch yield and SAR, this indicated differential responses of cassava genotypes in each growing season. This increased the difficulty in identifying a superior genotype for various growing seasons for these traits. Growing season shared the highest proportion of total variation for starch yield, while the content of starch and amylose, as well as the amylose-amylopectin ratio, were more associated by cassava genotype. The interactions for G × Y were not statistically significant and contributed to the low proportion of variation for all traits. Growing seasons contributed a larger proportion of variation than the variation contributed by Y for all traits, especially for starch yield. This indicated that a one-year experiment is acceptable for preliminary cassava testing using starch content and starch yield as a criterion to identify desirable genotypes. Highly significant effects of growing seasons for storage root growth rates and storage root yield under full irrigation has also been documented by Phonjaroen et al. [23], who observed that growing season and G × S interactions contributed to the greater proportion of the total variations for storage root growth rate at mid to late growth stages (from 6 to 8 MAP and 10 to 12 MAP) and storage root yield at final harvest.

Cassava planted in the ERS accumulated rapidly of starch yield in early growth during 4–6 MAP and slowed thereafter, whereas cassava planted in the PRS accumulated rapidly of starch yield at mid to late growth stages during 6–12 MAP. Interestingly, the rate of accumulation of starch during 10–12 MAP of the crop planted in the PRS (5.112 g m⁻² day⁻¹) was much higher than the crop planted in the ERS (0.216 g m⁻² day⁻¹). Differences in the peak times of starch accumulation between growing seasons would be due to changes in weather factors during crop growth for each growing season. Crops planted in the ERS experienced higher temperatures, higher solar radiation and longer day length during early growth stages, resulting in rapid shoot growth and starch accumulation in storage roots. Thereafter, however, the crops experienced lower air temperatures and lower the intensity of solar radiation during the mid-stage of growth, causing in decreased capacities of photosynthesis, biosynthesis of starch, as well as the distribution of starch from shoots to the storage roots [17,24,25]. An earlier report of Lebot [26] indicated that the expansion of fibrous roots is greatly decreased under low light intensity. The crops planted in the ERS may have poor ability to absorb nutrients and water from the soils during the mid-growth stage, which is a high demand growth stage. In the same way,
the biochemical processes, such as photosynthesis, starch biosynthesis and translocation are decreased under low-temperature conditions, thereby decreasing the growth of cassava [27].

Crops planted in the PRS experienced lower air temperatures, lower the intensity of solar radiation, lower air relative humidity and shorter day length during early stages of growth (during 1–3 MAP). During 4–10 MAP, the crop planted in PRS experienced higher temperatures, higher solar radiation and longer day length. Climatic factors during mid-growth phases indicated that the crops experienced optimum conditions from the stages of shoot development to storage root bulking, and it could accumulate higher starch in the shoots and translocate to storage roots. Many previous reports indicated that the optimal temperature to produce carbohydrates of cassava by photosynthesis and starch biosynthesis were computed to be 25–35 °C [28,29]. Fukai et al. [30] observed that cassava requires a high intensity of solar radiation for the production of total biomass and storage root dry weight. Likewise, the most favorable growing conditions of cassava to produce higher storage root yield were humid-warm climates with average net radiation of 22 MJ m$^{-2}$ day$^{-1}$, temperatures of between 25–29 °C and average relative air humidity of 70% [5,31]. Moreover, cassava planted in the PRS also experienced lower air temperatures, lower relative air humidity and shorter day length during late-growth phases. These growth phases are critical for cassava starch yield because cassava translocases photo-assimilates from shoots to storage roots [17,24]. Lower air temperature and shorter day-length reduced the rate of biomass production but increased the partition of photo-assimilates to storage roots [14,15,23]. Earlier reports also indicated that cassava storage root yield and starch yield are related to harvest index, a number of storage roots and size of storage root [32,33]. This may explain why crops planted in the PRS had much higher SAR and starch yield during late-growth phases than when planted in the ERS (Table 3). Similarity, Janket et al. [11] reported that cassava planted in the PRS had a higher content of starch, starch yield and starch granules than when planted in the ERS. These authors also described that high air temperature and high intensity of solar radiation during the mid-growth stage (3–9 mounts) and short day-length at late growth stages were more favorable conditions for crop growth.

For both growing seasons, the starch content was slightly increased over the sampling period during 4–12 MAP, and all cassava genotypes had their peak for starch accumulation from 4–6 MAP. After that, no marked peak or steady decrease in starch content related to increasing age was noted. However, differences between growing seasons for starch yield were observed at late growth stages for all genotypes. Most cassava genotypes had their peak of starch yield at 12 MAP except for CMR38-125-77 in the ERS which had the highest starch yield at 10 MAP. The decrease of starch yield of CMR38-125-77 at 12 MAP would be due to the regrowth after the return of the rainy season. Although all experimental plots were irrigated, cassava cv. CMR38-125-77, which is highly branching, produced new leaves during the maturity stage when it received high rainfall (Data not shown). The negative effect of rainfall during maturity stages to starch content and starch yield has been previously reported indicating that increased water availability allows more shoot growth by using accumulated photo-assimilates from the storage roots, consequent to decreases in both starch content and storage root dry weight [15,34].

The best harvest time was similar to that reported for storage root yields revealed in a previous experiment by Phoncharoen et al. [23], who reported that cassava planted in the PRS (November and December) had higher total dry weight than when planted in the ERS (June). These authors also reported that cassava cv. CMR38-125-77 when planted in the ERS had the highest storage growth rate at 6–8 MAP and declined at 10–12 MAP. However, this genotype is likely to be a good genotype, since it showed high performance in terms of storage root dry weights at the final harvest for both ERS and PRS. These reports conflict with Eban-Djedji et al. [9], who reported that starch yields decreased when the cassava has surpassed maturity, and that cassava genotypes had their peak starch yield between 8–17 MAP. This was similar to Santisopasri et al. [8], who reported that 12 MAP is the best harvest time for cassava.
When compared to the other genotypes CMR38-125-77 was preferable, since it had a faster rate of starch formation and the more starch yield at all sampling ages. RY11 and KU50 were the next best for all growing seasons. Even though starch yield in the ERS for CMR38-125-77 declined slightly at the final harvest, it was still higher than the other genotypes. The difference of starch yield is likely due to the canopy photosynthetic capacity and the speed of translocation of nutrients from shoots to storage roots during the vegetative growth stages [24]. Cassava genotypes with early bulking, fast rates of starch production and high starch yields are desirable. Satisfactory performances for CMR38-125-77 on chlorophyll fluorescence, storage root dry weight, growth rate, total dry weight, starch content and starch yield have been reported in the previous investigations conducted in different upper paddy fields in rotation with rice (off-season) [12,13,35], as well as experiments conducted in different growing seasons under full irrigation in Thailand [23]. A study reported by Santanoo et al. [36] also showed that although CMR38-125-77 had moderate photosynthesis, it had high-water use efficiency and efficient protective mechanisms, including high non-photochemical quenching and leaf drooping. These were useful traits for growth performance and canopy development for this cassava genotype. Genotypes with higher starch accumulation had high starch biosynthesis, high stem flow rate and high sugar transporter genes in the stems but low starch degradation in the storage roots [37]. Environmental factors during the growing period also impacted starch biosynthesis in the storage roots of cassava [8,18]. In our irrigated study, all cassava genotypes planted in the ERS gave a faster rate of starch granule formation than did the crop planted in the PRS at early growth periods, but they declined at the late growth period for CMR38-125-77 and RY11. Granule size of the crop planted in the PRS increased continuously throughout the growing periods from 4 to 12 MAP. All cassava genotypes exhibited similar sizes of starch granules for both growing seasons.

Genotype was the major contribution to amylose content, amylose-amylopectin ratio and granule sizes, which was consistent with earlier studies. Many researchers have reported that the granule size of starch is depended on changes in genetics and drought stress [38–40]. An earlier study of Janket et al. [11] also indicated the genotypes with high starch content also had high starch yield and larger granule size. CMR38-125-77 showed bigger granule of starch, and it had high performance in terms of starch content and starch yield. There were no statistically significant differences for amylose content and the amylose-amylopectin ratio at the final harvest for both growing seasons; however, a significant difference between planting dates was observed for CMR38–125–77, which had significantly higher values when planted in the PRS. In contrast, Teerawanichpan et al. [39] observed that growing seasons did not significantly affect amylose content. However, previous reports on other crops supported our observations. Earlier reports on indicated that season did cause a difference in amylose content in maize [41] and wheat [42]. The findings of this study will enable cassava breeders to choose superior genotypes parents for future cassava breeding and assist growers in choosing cassava genotypes and harvest times for different growing seasons. This could help to increase food, feedstuff and the potential of bioethanol production by increasing cassava starch yield.

5. Conclusions

Under the irrigated condition, the growing season plays an important role for differences in starch yield, but the contents of starch and amylose, as well as the amylose-amylopectin ratio, were more influenced by genotype. Planting cassava in the ERS had rapid accumulation rates of starch yield and size of starch granule in early growth stages, whereas planting cassava in the PRS had rapid growth rates of these traits during the middle to the late stages of growth. Planting cassava in the ERS resulted in bigger starch granule size than did planting in the PRS, especially during early to late growth stages. In most cassava genotypes, the content, yield and granule size of starch increased continuously thought the growing periods from four to twelve MAP. CMR38-125-77 in the ERS was an exception and should be harvested at 10 MAP to obtain the highest starch yield. CMR38-125-77 was the desirable genotype, due to a faster rate of starch formation and more contents of starch and amylose, as well as the higher
starch yield for both ERS and PRS. These findings provide information useful for choosing appropriate cassava genotypes and harvesting time for different planting seasons.

**Author Contributions:** Conceptualization, N.V., P.B., P.T. and S.J.; Data curation, A.J.; Formal analysis, A.J. and S.J.; Investigation, A.J.; Methodology, A.J., N.V., P.B. and S.J.; Supervision, S.J.; Writing—original draft, A.J.; Writing—review & editing, B.T., W.K., P.B., C.C.H., C.K.K. and S.J. All authors have read and agreed to the published version of the manuscript.

**Funding:** This research was funded by the Royal Golden Jubilee Program (grant no. PHD/0216/2560), the National Science and Technology Development Agency (NSTDA), Thailand.

**Acknowledgments:** Grateful acknowledgement is made to Khon Kaen University and Peanut, Jerusalem artichoke and Cassava Improvement Research Group, Khon Kaen University for providing facilities in conducting this experiment and training for manuscript preparation. Special thanks to the member of cassava team project and technicians of agronomy department for their help in field data collection and laboratory analyses.

**Conflicts of Interest:** The authors declare no conflict of interest.

**References**

1. Our World in Data. Available online: https://ourworldindata.org/world-population-growth (accessed on 2 January 2020).
2. Alves, A.A.C. Cassava botany and physiology. In Cassava: Biology, Production and Utilization; Hillocks, R.J., Thresh, J.M., Bellotti, A., Eds.; CABI Publishing: New York, NY, USA, 2002; pp. 67–89.
3. Maung Aye, T.; Howeler, R.H. Integrated crop management for cassava cultivation in Asia. In Achieving Sustainable Cultivation of Cassava Volume 1: Cultivation Techniques; Clair, H., Ed.; Burleigh Dodds Science Publishing Limited: Cambridge, UK, 2017; pp. 1–29.
4. Food and Agricultural Organization of the United Nations (FAO). Available online: http://fao.org/faostat/en/#home (accessed on 4 October 2019).
5. El-Sharkawy, M.A. Drought-tolerant cassava for Africa, Asia, and Latin America. Biol. Sci. 1993, 43, 441–451. [CrossRef]
6. Howeler, R.H. Cassava mineral nutrition and fertilization. In Cassava: Biology, Production and Utilization; Hillocks, R.J., Thresh, J.M., Bellotti, A., Eds.; CABI Publishing: New York, NY, USA, 2002; pp. 281–300.
7. Benesi, I.R.M.; Labuschagne, M.T.; Herselman, L.; Mahungu, N.M.; Saka, J.K. The effect of genotype, location and season on cassava starch extraction. Euphytica 2008, 160, 59–74. [CrossRef]
8. Santisopasri, V.; Kurotjanawong, K.; Chotineeranat, S.; Piyachomkwan, K.; Sriroth, K. Impact of water stress on yield and quality of cassava starch. Ind. Crops Prod. 2011, 33, 115–129. [CrossRef]
9. Ebah-Djedji, B.C.; Dje, K.M.; N’Zue, B.; Zohouri, G.P.; Amani, N.G. Effect of harvest period on starch yield and dry matter content from the tuberous roots of improved cassava (Manihot esculenta Crantz) varieties. Pak. J. Nutr. 2012, 11, 414–418. [CrossRef]
10. El-Sharkawy, M.A. Cassava biology and physiology. Plant Mol. Biol. 2004, 56, 481–501. [CrossRef] [PubMed]
11. Janket, A.; Vorasoot, N.; Toomsan, B.; Kaepradit, W.; Banterng, P.; Kemsala, T.; Therakulpisut, P.; Jogloy, S. Seasonal variation in starch accumulation and starch granule size in cassava genotypes in a tropical savanna climate. Agronomy 2018, 8, 297. [CrossRef]
12. Sawatraksa, N.; Banterng, P.; Jogloy, S. Chlorophyll fluorescence and biomass of four cassava genotypes grown under rain-fed upper paddy field conditions in the tropics. J. Agron. Crop Sci. 2018, 204, 554–565. [CrossRef]
13. Sawatraksa, N.; Banterng, P.; Jogloy, S.; Vorasoot, N.; Hoogenboom, G. Cassava growth analysis of production during the off-season of paddy rice. Crop Sci. 2019, 59, 1–12. [CrossRef]
14. Keating, B.A.; Evenson, J.P.; Fukai, S. Environmental effects on growth and development of cassava (Manihot esculenta Crantz) II. Crop growth rate and biomass yield. Field Crop. Res. 1982, 5, 283–292. [CrossRef]
15. Keating, B.A.; Evenson, J.P.; Fukai, S. Environment effects on growth and development of cassava (Manihot esculenta Crantz) III. Assimilate distribution and storage organ yield. Field Crop. Res. 1982, 5, 293–303. [CrossRef]
16. Aresta, R.B.; Fukai, S. Effects of solar radiation on growth of cassava Manihot esculenta Crantz II. Fibrous root length. Field Crop. Res. 1984, 9, 361–371. [CrossRef]
17. Boerboom, B.W. A model of dry matter distribution in cassava (Manihot esculenta Crantz). Neth. J. Agric. Sci. 1978, 26, 267–277.

18. Sriroth, K.; Piyachomkwan, K.; Santisopasri, V.; Oates, C.G. Environmental conditions during root development: Drought constraint on cassava starch quality. Euphytica 2001, 120, 95–101. [CrossRef]

19. Peel, M.C.; Finlayson, B.L.; McMahon, T.A. Updated world map of the Köppen-Geiger climate classification. Hydrol. Earth Syst. Sci. Discuss. 2007, 4, 439–473. [CrossRef]

20. Hoover, R.; Ratnayake, W.S. Current Protocols in Food Analytical Chemistry; John Wiley & Sons: New York, NY, USA, 2001.

21. Gomez, K.A.; Gomez, A.A. Statistical Procedures for Agricultural Research, 2nd ed.; John Wiley and Sons: New York, NY, USA, 1984.

22. Freed, R.D.; Nissen, O. MSTAT-C Version 1.42; Michigan State University: East Lansing, MI, USA, 1992.

23. Phoncharoen, P.; Banterng, P.; Jogloy, S.; Vorasoot, N.; Theerakulpisut, P.; Hoogenboom, G. Growth rates and yields of cassava at different planting dates in a tropical savanna climate. Sci. Agric. 2019, 76, 376–388. [CrossRef]

24. Veltkamp, H.J. Growth, total dry matter yield and its partitioning in cassava at different daylengths. Agric. Univ. Wagening. Pap. 1985, 85, 73–86.

25. Vongcharoen, K.; Santanoo, S.; Banterng, P.; Jogloy, S.; Vorasoot, N.; Theerakulpisut, P. Seasonal variation in diurnal photosynthesis and chlorophyll fluorescence of four genotypes of cassava in different growing seasons. Photosynthetica 2018, 56, 1398–1413. [CrossRef]

26. Lebot, V. Tropical Root and Tuber Crops: Cassava, Sweet Potato, Yams and Aroids; CABI: Wallingford, CT, USA, 2009; p. 406.

27. Rogers, D.J. Some botanical and ethnological considerations of Manihot esculenta. Econ. Bot. 1965, 19, 369–377. [CrossRef]

28. El-Sharkawy, M.A.; Cock, J.H.; Held, A.A. Photosynthetic responses of cassava cultivars (Manihot esculenta Crantz) from different habitats to temperature. Photosynth. Res. 1984, 5, 243–250. [CrossRef] [PubMed]

29. Saithong, T.; Rongsirikul, O.; Kalapanulak, S.; Chiewchankaset, P.; Siriwat, W.; Netrphan, S.; Suksangpanomrung, M.; Meechai, A.; Cheevadhanarak, S. Starch biosynthesis in cassava: A genome-based pathway reconstruction and its exploitation in data integration. BMC Syst. Biol. 2013, 7, 75. [CrossRef]

30. Fukai, S.; Alcoy, A.B.; Llamelo, A.B.; Patterson, R.D. Effects of solar radiation on growth of cassava (Manihot esculenta Crantz.). I. Canopy development and dry matter growth. Field Crop. Res. 1984, 9, 347–360. [CrossRef]

31. El-Sharkawy, M.A.; Cock, J.H.; Lynam, J.K.; Hernandez, A.D.P.; Cadavid, L.F.L. Relationships between biomass, root-yield and single-leaf photosynthesis in field-grown cassava. Field Crop. Res. 1990, 25, 183–201. [CrossRef]

32. Cock, J.H.; Franklin, D.; Sandoval, G.; Juri, P. The ideal cassava plant for maximum yield. Crop Sci. 1979, 19, 271–279. [CrossRef]

33. Ntawuruhunga, P.; Dixon, A.G. Quantitative variation and interrelationship between factors influencing cassava yield. J. Appl. Biosci. 2010, 26, 1594–1602.

34. Janket, A.; Vorasoot, N.; Toomsan, B.; Kaewpradit, W.; Jogloy, S.; Theerakulpisut, P.; Holbrook, C.C.; Kvien, C.K.; Banterng, P. Starch accumulation and granule size distribution of cassava cv. Rayong 9 grown under irrigated and rain-fed conditions. Photosynthetica 1984, 243–250. [CrossRef]

35. Nimlamai, T.; Banterng, P.; Jogloy, S.; Vorasoot, N.; Roytrakul, S.; Theerakulpisut, P. Seasonal variation in diurnal photosynthesis and chlorophyll fluorescence of four genotypes of cassava (Manihot esculenta Crantz) under irrigation conditions in a tropical savanna climate. Agronomy 2019, 9, 206. [CrossRef]

36. Santanoo, S.; Vongcharoen, K.; Banterng, P.; Vorasoot, N.; Jogloy, S.; Roytrakul, S.; Theerakulpisut, P. Starch accumulation and granule size distribution of cassava cv. Rayong 9 grown under irrigated and rainfed conditions. Field Crop. Res. 2020, 25, 109–126. [CrossRef]

37. Li, Y.Z.; Zhao, J.Y.; Wu, S.M.; Fan, X.W.; Luo, X.L.; Chen, B.S. Characters related to higher starch accumulation in cassava storage roots. Sci. Rep. 2016, 6, 18923. [CrossRef] [PubMed]
39. Teerawanichpan, P.; Lertpanyasampatha, M.; Netrphan, S.; Varavinit, S.; Boonseng, O.; Narangajavana, J. Influence of cassava storage root development and environmental conditions on starch granule size distribution. *Starch-Stärke* 2008, 60, 696–705. [CrossRef]

40. Vasconcelos, L.M.; Brito, A.C.; Carmo, C.D.; Oliveira, P.H.G.A.; Oliveira, E.J. Phenotypic diversity of starch granules in cassava germplasm. *Genet. Mol. Res.* 2017, 13, 16. [CrossRef]

41. Fergason, V.L.; Zuber, M.S. Influence of environment on amylose content of maize endosperm. *Crop Sci.* 1962, 2, 209–211. [CrossRef]

42. Liu, P.; Guo, W.; Jiang, Z.; Pu, H.; Feng, C.; Zhu, X.; Peng, Y.; Kuang, A.; Little, C.R. Effects of high temperature after anthesis on starch granules in grains of wheat (*Triticum aestivum* L.). *J. Agric. Sci.* 2011, 149, 159–169. [CrossRef]

© 2020 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (http://creativecommons.org/licenses/by/4.0/).