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RESEARCH ARTICLE

Image-based root phenotyping links root architecture to micronutrient concentration in cassava

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Societal Impact Statement

Micronutrient deficiency or "hidden hunger" is estimated to affect two billion people worldwide and increasing the micronutrient concentration of food could play an important role in tackling this global challenge. Using a combination of imaging techniques and atomic absorption spectroscopy, we describe a link between root phenotype and micronutrient concentration in cassava, which could enable new phenotypic selection strategies for breeding. This approach could be used with existing breeding infrastructure to enhance the micronutrient concentration of cassava and hence, benefit the health of people, particularly in low-income countries where cassava is consumed as a staple crop.

Summary

- Cassava storage roots are a staple food in low-income countries of South-East Asia and sub-Saharan Africa, where growth stunting is prevalent as a consequence of micronutrient deficiencies. We aim to link phenotypes of field-grown cassava roots to micronutrient concentration in the edible storage roots as a simple way to improve phenotypic selection for nutritional value in cassava.
- We used existing and newly developed imaging techniques to quantify root phenotypes of the cassava root architecture over time and used flame atomic absorption spectroscopy to measure micronutrient concentration in storage roots. Both together allow the association of root phenotypes with micronutrient concentration in mature cassava roots.
- We show that early and late bulking genotypes in cassava exhibit distinct foraging behaviors that are associated with micronutrient concentration in the edible storage root. Our observations suggest that late bulking cassava is a key to provide sufficient micronutrients in the edible storage root.
- The association between root phenotype and micronutrient concentration with imaging techniques allows phenotypic selection for enhanced micronutrient concentration. Therefore, implementing image-based phenotyping into cassava breeding programs in sub-Saharan Africa and South-East Asia could be an essential element to resolve micronutrient deficiencies that puts individuals at a higher risk of growth stunting.

KEYWORDS
cassava breeding, growth stunting, malnutrition, micronutrient concentration, root phenotyping

1 | INTRODUCTION

Growth stunting is one of the most common effects of micronutrient deficiencies in humans. The World Health Organization (WHO) and the United Nations Children's Fund (UNICEF) define children as being stunted if their height-for-age is more than two standard deviations below the WHO Child Growth Standards median (WHO & UNICEF, 2009). Consequences of growth stunting are associated with impairments such as increased morbidity and mortality, reduced physical abilities, neurodevelopmental and economic capacity, and an elevated risk of metabolic disease in adulthood (Prendergast & Humphrey, 2014). World-wide approximately two billion people suffer from one or more micronutrient deficiencies (Tulchinsky, 2010). One of the major reasons for these deficiencies is that the consumption of local staple crops does not cover the daily nutritional needs of humans (Bain et al., 2013). According to a study of the National Statistical Office of Thailand and UNICEF (NSO & UNICEF, 2016), about 16% of all Thai children under the age of 5 years are growth stunted. Lower birth weight and height are known indicators of growth stunting associated with deficiencies in the micronutrients calcium and zinc (Branca & Ferrari, 2002). Cassava is a staple root crop in Thailand (Nandagopal & Syeda, 2018) and previous studies reported that micronutrient concentration in cassava correlates with bioavailability of micronutrients to the human body (Dilworth, Brown, Brown, & Asemota, 2013). In response, we asked if higher micronutrient concentrations of calcium and zinc are achievable by integrating high-throughput root phenotyping into breeding and selection pipelines for cassava. We performed image-based root phenotyping (Bucksch et al., 2014) on Thai cassava genotypes (Kengkanna et al., 2019). The results suggest that calcium and zinc concentrations are associated with highly heritable root phenotypes that relate to the foraging behavior of cassava genotypes. In the future, it might be possible to develop selection strategies based on root phenotypes for breeding programs that integrate image-based root phenotyping. We believe that phenotypic selection of cassava varieties with enhanced calcium and zinc concentration may help to mitigate malnutrition in low-income countries.

Plant physiology explains why the accumulation of calcium and zinc in the edible storage root is also crucial for the plant. In a plant, calcium is critical for the growth of structural elements like cell walls, and its deficiency can lead to poor root development or leaf necrosis and curling (Hepler, 2005). Zinc is involved in the ability to regulate the permeability of calcium into the roots system (Cakmak & Marschner, 1988) and is therefore associated with calcium availability in the root system. This zinc-calcium association enables us to measure three root traits to link zinc and calcium concentration to root phenotypes in cassava:

(i) We quantify biomass accumulation throughout the development of the cassava root system. In that way, we quantify the amount of soil-root surface contact as a proxy for calcium uptake potential.
(ii) We use shape descriptors to distinguish foraging strategies quantitatively. These shape descriptors summarize the architecture of the root system in one mathematical construct to quantify architectural changes during root development.
(iii) We estimate the genotype-specific distribution of storage root volumes within the cassava root system. The volume distribution throughout the root system development relates to the amount of zinc and calcium to the root system phenotype.

2 | MATERIALS AND METHODS

2.1 | Plant material

We used six Thai cassava genotypes in our trials: Rayong 5 (R5), Rayong 9 (R9), Rayong 11 (R11), Huay Bong 60 (HB60), Huay Bong 80 (HB80), and Kasetsart (KU50). Figure 1 illustrates the genetic relationships between the genotypes and the certification year of each genotype. An important relationship in the context of this paper is that HB60 and HB80 are both crosses of R5 and KU50. Additionally, R5 is one of the parents of R11—the other being OMR 29-20-118. Although OMR 29-20-118 is not a genotype used in our study, we note that it was crossed with CMR 31-19-23 to breed the R11 genotype. SI Figure 1 shows a complete pedigree of cassava varieties certified and released in Thailand.

2.2 | Growth conditions and harvesting protocol

2.2.1 | Trial 1

Stem cuttings of the five cassava genotypes R5, R9, R11, HB60, and KU50 were planted in a split-plot design with five replications in clay loamat Bang Len, Nakhon Pathom province, Thailand (14°01′39.1″N 100°08′24.3″E) from October 2016 to January 2017. The plot area was approximately 125 m^2. The experiment followed a daily irrigation scheme and per plant fertilization of 20 g of fertilizer containing 16% of nitrogen, phosphorus, and potassium each (16-16-16). The in-row planting distance between all plants was 1m and we harvested the plants at 3MAP (Months After Planting).

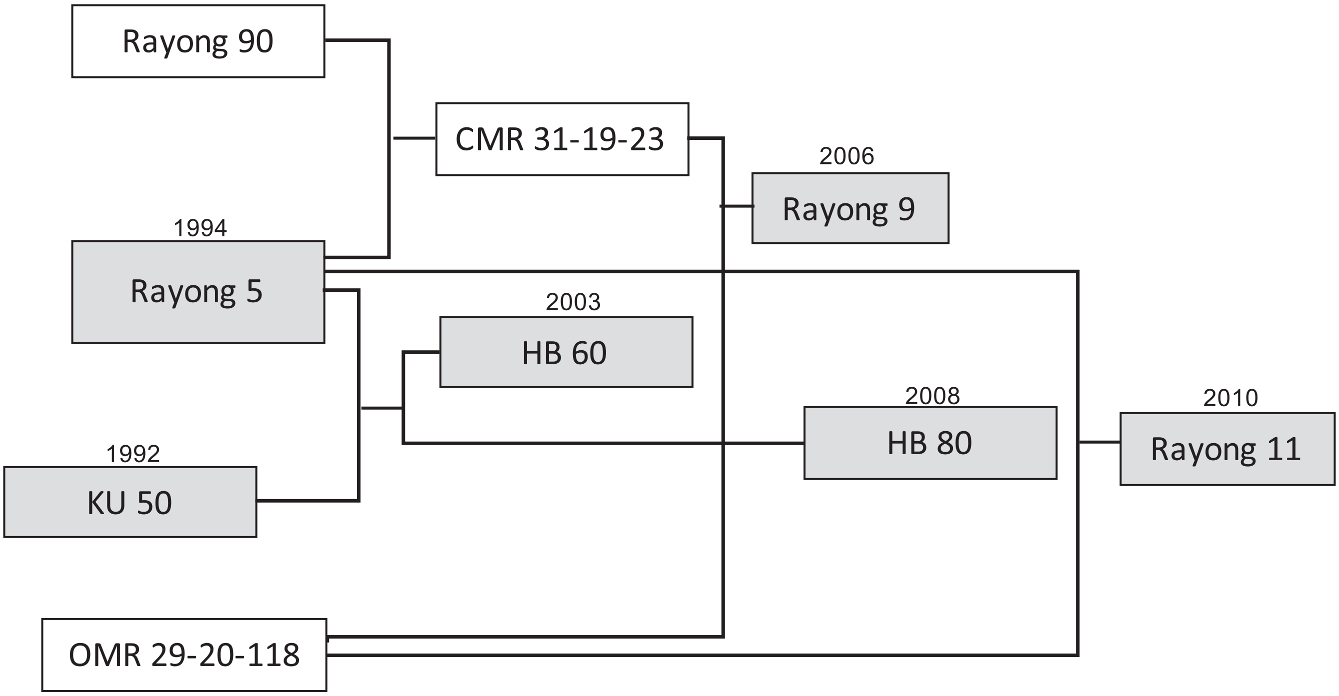

FIGURE 1 Pedigree of relevant Thai cassava genotypes. Of note, R5 is a parent of late bulking R11 and early bulking HB80. The early bulking variety KU50 is the second parent of HB60 and HB80

2.2.2 | Trial 2

We harvested the five cassava genotypes and HB80 at Rayong Field Crops Research Center (Rayong, Thailand) with an in-row distance of 1m in sandy loam. The experiment was arranged in a randomized complete block design with 100 plants per genotype. We harvested the two growth stages 5 MAP (October 2015-February 2016) and maturity (December 2015-December 2016). Under rainfed conditions, we applied 625 kg/ha of fertilizer containing 15% of nitrogen, 17% of phosphorous, and 18% of potassium (15-17-18) to the field. We harvested the roots at 5 MAP and 12 MAP. The total amount of rainfall during 5 MAP and 12 MAP was 768.5 and 1658.0 mm, respectively.

2.2.3 | Trial 3

For the individual storage root analysis of the five cassava genotypes and HB80, we used data from a 5 MAP and a maturity trial between 9 and 11 MAP (April 2015 to February 2016). Again, we used a 15-17-18 fertilizer regime under rainfed conditions at the Rayong Field Crops Research Center. The total amount of rainfall during the 9-11 MAP was 1656.3–1742.7 mm.

2.2.4 | Trial 4

Storage roots to measure the micronutrient concentration were available for five cassava genotypes (R5, R9, R11, HB60, and KU50) at the Rayong Field Crops Research Center. We used mature samples (14 MAP during November 2016-January 2018) grown under rainfed field conditions using a 15-17-18 fertilizer regime. The total amount of rainfall during 14 MAP was 1771.6 mm.

Harvesting of cassava roots followed the validated protocol published in (Kengkanna et al., 2019) in all of our experiments.

2.3 | Imaging and trait computation

Digital Imaging of Root Traits (DIRT) (Das et al., 2015) is an automated, high-throughput and image-based phenotyping platform for roots. DIRT analyses images of excavated root crowns that are collected with the DIRT imaging protocol (Bucksch et al., 2014). We photographed every harvested cassava root and uploaded the 2D image to the DIRT platform hosted at Cyverse (Goff et al., 2011; Merchant et al., 2016). In this protocol, harvested roots are imaged against a dark, diffuse reflecting background with a white circle of 2 inches (50.8mm) in diameter. The white circle was placed in the image to allow conversion from pixel space to units. First, "side-view" pictures of the entire root system architecture were taken for each sample. Next, the storage roots were separated from the adventitious root system and photographed with a digital camera. The raw trait data are available in Dataset S1 and imaging data of all trials are available in Dataset S2.

We developed new algorithms that are not available on DIRT to perform the trait calculations for individual storage roots. Figure 2 shows an example image of segmented storage roots. The algorithm to compute the segmentation and subsequent trait measurements

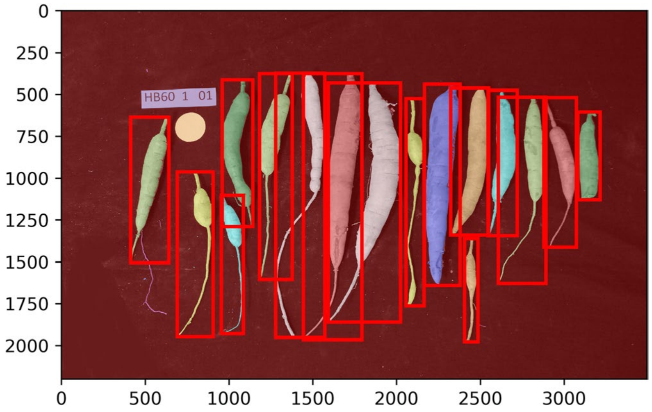

FIGURE 2 Segmentation of the storage root image (see Supporting Information Code S1 and Dataset S1 and S2). After binarizing the image, we used connected component labeling to identify each root on the black background. We applied the medial axis transform to each storage root and derived length and diameter at each location of the medial axis. The units of x- and y-ax are pixel

uses the routines developed for DIRT to extract a binary mask where the foreground is the storage roots, separated from a black background. Subsequently, the algorithm performs connected component labeling to identify the individual storage roots in the images using SciKit-Learn (Van der Walt et al., 2014). Each connected component corresponds to a single storage root for which the algorithm computes the medial axis. Each pixel of the medial axis corresponds to a local storage root diameter, and we trace the length of the storage root along the most centered path on the medial axis within the storage root outline. From these length and diameter calculations, the storage root algorithm computes the storage root volume. Estimates for mean diameter, length, and volume of the storage roots for each genotype were calculated at the 5 MAP time point and at the 12 MAP to characterize changes in storage root morphology. The code of the new algorithm is available in the file Code S1.

2.4 | Micronutrient concentration analysis

We analyzed the micronutrient concentration per storage root for the five Thai cassava varieties R5, R9, R11, HB60, and KU50. We sampled the 14-month-old storage roots from plants grown at the Rayong Field Crops Research Center. For each cassava genotype, four storage roots from three different plants compromised a total of 12 storage roots samples per genotype. Next, the storage roots were washed with tap water, peeled and rinsed with deionized water. Slices were cut from the distal, central, and proximal sides of the storage root and then manually mixed. For each sample, we chopped 500 g into smaller pieces, placed them in plastic containers and dried them for 48 hr at 60°C. As a next step, we ground the dried samples into flour using a non-contamination grinder mill (IKA, Germany) equipped with a high-grade stainless-steel beater. The flour samples were packaged into labeled plastic bags and stored at room temperature (Ceballos & Stangoulis, 2008). We placed 0.5 g of flour from each sample in test tubes and added 5 ml of a 2:1 solution of HNO3:HClO$_4$. The samples were then digested in a digestion block at 100°C with the Aqua regia method. Yellow fume rising from the test tube signaled to increase the temperature to 150°C until the fume disappeared. Next, the temperature was increased to 200°C for 15 min to obtain a clear solution. We then retrieved the samples from the digestion block and cooled them to room temperature. After cooling, we added 1 ml of HCl to each test tube. The new mixture was again heated to 150°C for 15 min until the solution appeared clear. We filtered 25 ml of the clear solution into a volumetric flask using a No. 42 Whatman filter paper. As a last step, flame atomic absorption spectroscopy (FAAS Varian Spectra AA 55B) measured the concentration of calcium and zinc in each of the samples.

2.5 | Broad sense heritability

Estimates of broad-sense heritability, H^2_{mean}, characterizes the effectiveness and repeatability of breeding strategies for desired traits. H^2_{mean} is estimated for quantitative traits by performing an analysis of the mean trait values among genotypes. Analyzing multiple genotypes allows to omit the hybridization and population development steps typically required for determining H^2_{mean}. Using the method described in (Falconer, 1989), high H^2_{mean} values imply that variation in the measured phenotype is small within a genotype. In the following, V denotes the variance of the subscripts, where p denotes the phenotype, g denotes the genotype, and e denotes the environment.

$$Vp = Vg + \frac{Ve}{\text{Number of replications}}$$

$$Vg = \frac{\text{Genotype Mean Square (GMS)} - \text{Error Mean Square (EMS)}}{\text{Number of replications}}$$

$$Ve = \text{Error Mean Square (EMS)}.$$

$$\text{EMS} = \frac{\text{standard deviation of all trait values}}{\sqrt{\text{number of replications}}}$$

$$\text{GMS} = \sqrt{\text{average of all trait values}}$$

2.6 | Statistical analysis

We generated all graphs using Python 3.7 and matplotlib 3.2.1 (Hunter, 2007) supported by the imported libraries NumPy 1.16 and SciPy 1.2.1 (Oliphant, 2007). Python scripts to generate the figures from the provided raw data are available in Code S1.

3 | RESULTS

We used the digital root phenotyping software DIRT (Das et al., 2015) for six Thai cassava genotypes (KU50, R9, R5, R11, HB60, and HB80) at the developmental stages 3, 5, and 12 MAP to compute proxies of overall biomass and root architecture from digital images. We developed a new algorithm to calculate distributions of individual storage root volumes (see Material and Methods). The image data and samples shared plants from existing breeding trials due to cassava's long growth period to maturity of 9–12 months (Howeler & Kawano, 1988). Genotype HB80 was not available in Trial 1 and genotype HB60 was not available in Trial 4. However, both genotypes have the same parents, which allow to draw interchangeable conclusions for the extracted root traits (see SI Figure 1). The genotypes represent typical Thai genotypes and include the well-studied genotypes KU50 and R5 shown in Figure 3 (Charles, Sriroth, Sriroth, & Huang, 2005).

The projected root system area was used as a proxy to quantify the overall biomass accumulation of the root system (Table S1) including the non-bulking adventitious root system (Figure 4a). Genotype R5 is distinguished from KU50, R11, HB60, and HB80 in the measured root area at 5 MAP. R9, which showed no significant difference in root area at 3 MAP, resulted in the smallest increase at all measured time points. R5 has an insignificantly larger mean root area than KU50 at 3 MAP. The mean root area of KU50 is indistinguishable from R11 and HB60 at 3 MAP. Between 5 and 12 MAP, we observed the most substantial increase in root area for R11 and KU50.

Within these extremes, we found that the projected root system area relates to the maturity stage of the storage roots. Therefore, we hypothesize that KU50 forms mature storage roots early in its development. In contrast, R5 reaches storage root maturity much later in development (Santisopasri et al., 2001). Our quantitative observations, combined with the known zinc-calcium association in roots, suggest that the timing of storage root formation results in different calcium and zinc assimilation and, consequently, in different root shapes.

The difference in absolute root area accumulation motivated us to compute differences of width accumulation in relation to the rooting depth of cassava plants. Width accumulation at depth is a trait to test for differences in foraging strategy that consequently result in different root system shapes. Here, we used the D-value (Das et al., 2015) at 10% (D10, Table S2) and 80% (D80, Table S3) depth of the storage root system (Figure 4 b and c). From our analysis, it is evident that R5 and KU50 have an inversely related relationship between the 3 and 5 MAP. During the 3-5 MAP period, KU50 accumulates the majority of its width throughout the entire root system. In contrast, width accumulation in the R5 root system predominantly occurs between 5 and 12 MAP at the deepest root parts (D80). We already observed that the early-bulking type KU50 and the late bulking genotype R5 develop mature storage roots at different times during development. Hence, we expect drastic differences in foraging that leads to distinguishable width accumulation at different depths.

In Trial 3, we harvested storage roots at two critical time points and compared early and late bulking varieties. To reveal the hypothesized differences in the storage root development at depth, we chose early harvesting at 5 MAP and storage root maturity at 9-11 MAP dependent on the genotype. We used the newly developed imaging pipeline (see Methods and Materials) to estimate individual storage root volume from existing images at 5 MAP and at maturity. From the collected images, we computed diameters along the centerline of the storage roots and determined the length of each storage root (see Methods and Materials). Diameters and lengths allowed us to reconstruct the distribution of storage root volumes per genotype (Figure 5a,b) from over 50 storage roots per genotype. Comparison of all genotypes showed a distinct accumulation of storage root volume which allowed us to catagorize these varieties as early bulking (HB60, HB80, KU50) and late bulking (R5, R9, R11) (Figure 5a-c). R5 is a parent of late bulking R11 and early bulking HB60 and HB80 (see Material and Methods). However, the second parent of HB60 and HB80 is the early bulking genotype KU50, which explains, in part, the observed difference in bulking and storage root volume.

At 5 MAP, we observed the largest mean volume for R11, followed by KU50, R5, R9, HB60, and HB80. This observation is similar to the observation made for the root system area trait shown in Figure 4a. The mean volume for R11 was not significantly different than the

FIGURE 3 Photographs of KU50 (top row) and R5 (bottom row) genotypes illustrate differences in the storage roots between early and late-bulking genotypes. The photographs represent a typical root system architecture and a typical distribution of individual storage roots at 12MAP. Differences between early (KU50) and late (R5) bulking genotypes are visible as uniformity in storage root development

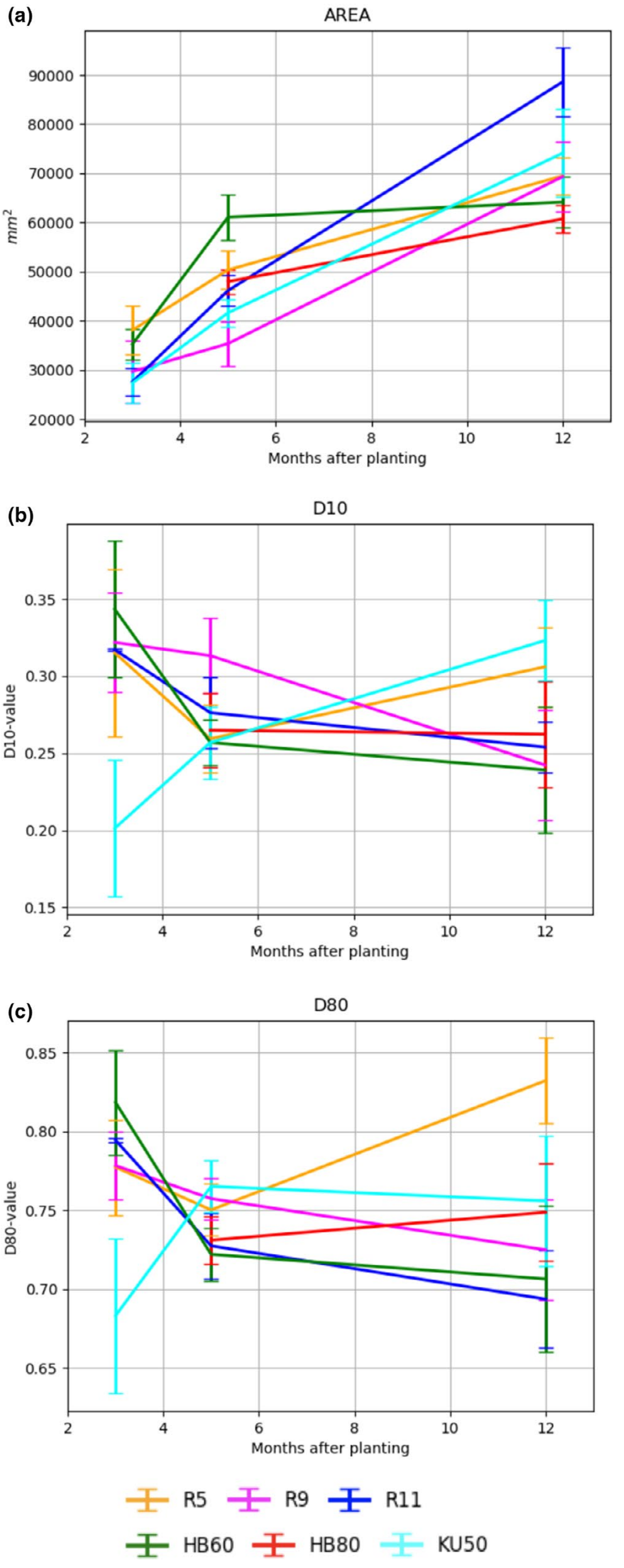

FIGURE 4 Analysis of cassava root development: (a) Accumulation of root system biomass differs between early and late-bulking genotypes. AREA trait measurements of five cassava genotypes compared at 3 MAP, 5 MAP, and 12 MAP. The AREA trait computed with DIRT (Das et al., 2015) is a proxy for root system biomass and reports as the number of pixels belonging to each root sample in a standardized image. (b) Comparison of root system development between early and late-bulking genotypes. Percentage of width accumulation at 10% depth (defined as "D10") calculated for five different cassava genotypes at 3 MAP, 5 MAP, and 12 MAP. (c) shows the same comparison, yet at 80% depth (defined as "D80"). The error bars in (a-c) represent the standard error of the mean at the particular MAP

value for KU50, but was considerably higher than the estimates for all other genotypes (see Table S4). We also observed that the storage root volume of KU50 is indistinguishable from R11, R9, or R5 at 5 MAP. However, from the individual tuber analysis, we know that KU50 has a significantly larger storage root volume than HB60 and HB80 at 5 MAP. At the same point in development, the mean volume of R5's storage roots were substantially larger compared to HB60 (Figure 5c). Yet, R5's volume was indistinguishable from the KU50 or R9.

The surprising result of our study emerges at maturity. Genotype R5 resulted in the by far highest mean storage root volume compared to all other genotypes in our study. In contrast, the KU50 mean storage root volume did not significantly differ from R9 and R11. Our observation suggests that the R5 has a prolonged development with active storage root formation in the soil. We could not anticipate this observation with the 2D root area because many of the formed storage roots in R5 are occluded and number of roots is not evaluated by the area trait. However, our interpretation is also supported visually by Figure 3, which compares a higher morphological variability and deeper rooting of the extremal genotypes R5 and KU50. In this comparison, KU50 exhibits a more dominant lateral root system with less variation in the storage root morphology. In addition, we showed already that D-values and individual mean storage root volume can differentiate late and early bulking genotypes. Therefore, we would expect R5 to have the largest concentration of zinc and calcium accumulated in the storage roots because of its prolonged development in different soil volumes.

As expected from our hypothesis, the measured calcium and zinc concentrations in Trial 4 show the same quantitative distinction of early and late bulking genotypes than the computation of the root architectural traits (Figure 6). We found broad sense heritability above 0.7 for the calcium and zinc concentration traits (see Table S5). Furthermore, our study found that the whole root descriptors D10 and D80 are highly heritable traits to measure foraging behavior (Broad sense heritability >0.7, see Table S5).

4 | DISCUSSION

In contrast to a plethora of previous studies (Chipeta, Shanahan, Shanahan, Melis, Sibiya, & Benesi, 2016; Okechukwu & Dixon, 2009; Okogbenin & Fregene, 2002; Olasanmi et al., 2014, 2017), we suggest that the late-bulking cassava genotypes are the key genotypes to target for nutritional food security. Our results suggest that the increased soil-root contact and foraging strategy of late bulking varieties is the driver of increased calcium and zinc concentrations in the

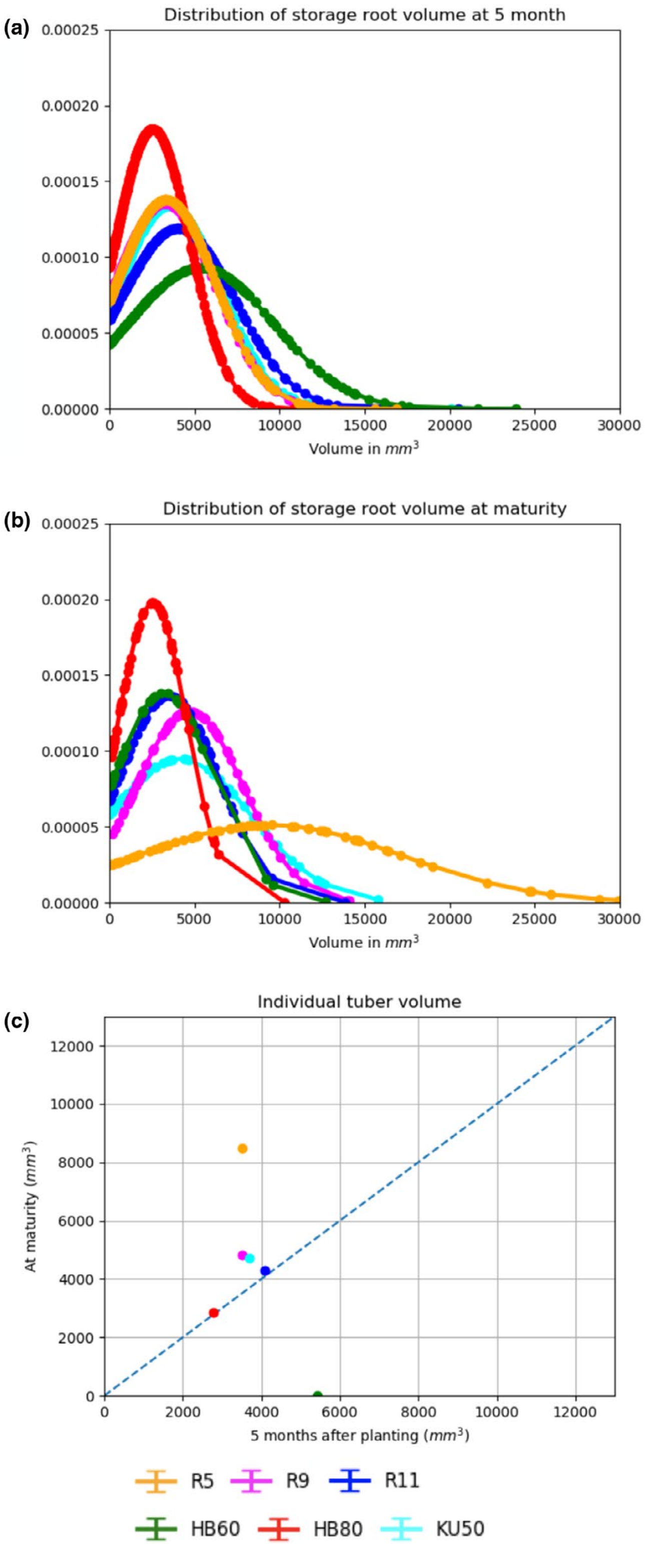

FIGURE 5 (a) Distribution of storage root volumes within the root system of each genotype at 5-month after planting. Dots represent the individual samples used to reconstruct the normalized volume distribution. (b) Distribution of storage root volumes within the root system of each genotype at maturity. Dots represent the individual samples used to reconstruct the volume distribution. (c) Tuber volume computation with the new algorithm (see Material and Methods) highlights different bulking characteristics between early and late-bulking cassava genotypes. Mean tuber volume calculated for each genotype at the 5 MAP and maturity. Storage root data at maturity were not available for HB60. The line f(x) = x is the constant rate line that is the minimum limit for mean trait value points in the plot

storage roots. Previous studies potentially paid less attention to the nutritional value because of their focus on producing faster harvesting cycles through early bulking varieties.

The presented results are limited by the availability of cassava plants from several ongoing breeding trials. As a consequence, the individual plants used to measure root phenotypes are distinct from the individuals that used to measure the micronutrient concentration. Yet, the presented results are still consistent because the micronutrient concentration measured in mature plants is from the same field as the plants collected for phenotyping.

Confidence in the suggested association of observed root architecture traits with micronutrient concentration stems from the characteristics of our root phenotyping, which defines traits for selection based on physiological functions. The statistical analysis of the entire root system and the individual storage root traits demonstrates that late-bulking genotypes yield higher calcium and zinc concentration. Our observation is noteworthy because the experiments excluded lower calcium concentration in the soil as a known trigger for earlier storage root formation (Yao et al., 2013) by being grown in the same environment.

Presumably, the higher micronutrient concentrations in late bulking genotypes resulted from an increased time to acquire soil nutrients compared to early-bulking genotypes. We speculate that the prolonged contact with the soil during active volume development allows for increased uptake of the abundant calcium and zinc nutrients. As a quantitative indicator, we measured a distinct width accumulation of the overall root system. Additional support for our hypothesis stems from similar relationships that are known for phosphorous uptake in *Arabidopsis thaliana* (Nord & Lynch, 2008). As a result, the storage roots of the late bulking genotypes (R5, R9, R11) have increased calcium and zinc concentration as a consequence of longer and distinctly distributed soil exploration compared to early bulking genotypes (KU50, HB60). Our results also find support in previous observations of mineral profiles in the cassava genotypes KU50 and R5 (Charles et al., 2005). The reported mineral profiles show an almost twofold higher calcium concentration for R5 compared to KU50. Therefore, our data analysis took the next step in suggesting the link between foraging behavior and bulking time. This link also supports the plant physiological basis of higher calcium and zinc concentrations for human nutrition as a consequence of improved calcium permeability resulting from abundant zinc in the soil.

The quantitative association of micronutrients and root architecture implies that breeding for root architecture is very likely to improve micronutrient concentration. Therefore, our root phenotyping protocol (Kengkanna et al., 2019) using freely available imaging methods like DIRT (Das et al., 2015) is a useful and cost efficient tool for initiatives that aim to improve nutritional value (Sayre et al., 2011) of

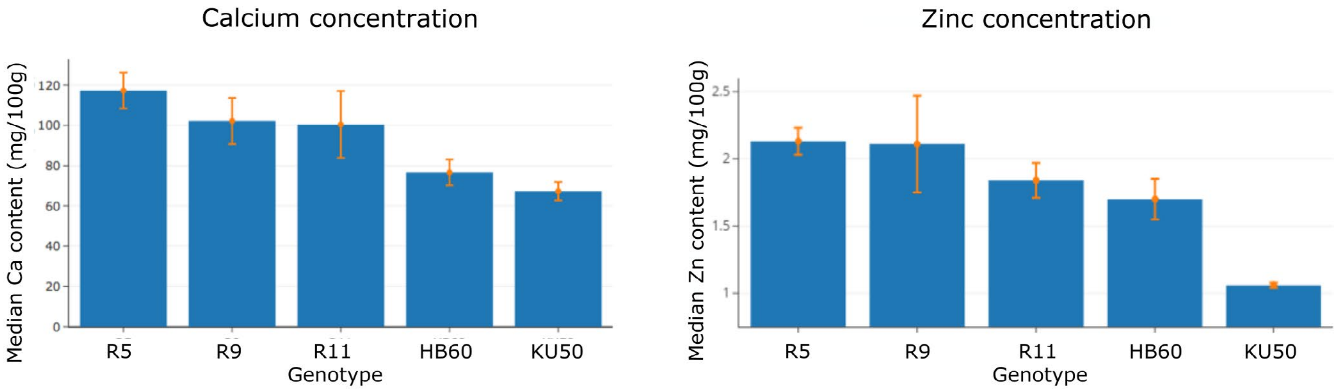

FIGURE 6 Zinc and calcium concentration of storage roots increases with longer duration of storage root formation. (left) Median calcium concentration in mg/100 g calculated for five cassava genotypes. (right) Median zinc concentration in mg/100 g calculated for five cassava genotypes error bars represent the standard error of the median. See Table S6 for actual values. HB80 was not available in ongoing trials

cassava. On a societal scale, the chance to breed for higher calcium and zinc concentrations using phenotypic selection of root traits is hope for about two billion people that consume cassava root daily. In particular, sub-Saharan Africa and Southeast Asia consume cassava root because of its remarkable resistance to drought (Costa & Delgado, 2019). The association between human growth retardation and zinc deficiency (Prasad, 2008) is known. It is also no surprise that calcium deficiency can cause growth stunting, as it is essential for bone formation during development and skeletal and developmental diseases such as rickets (Bueno & Czepielewski, 2008). In our study, we associated the uptake of the major mineral targets zinc and calcium into the edible root crop with root phenotypes. Both mineral concentrations in cassava are associated with bioavailability to humans of 12%–15% in processed and unprocessed form (Dilworth et al., 2013; Narayanan et al., 2019).

At present, Thai farmers select cassava varieties based on factors such as the amount of rainfall, tolerance to biotic and abiotic stresses, starch quality, market demand, etc. Early bulking cassava can be used as an alternative root crop grown in paddy fields (Sawatraksa et al., 2019) because it can increase net revenue, whereas some late bulking varieties such as R9 and R11 produce starch with better ethanol gain for the biofuel industry. Our observationof enhanced micronutrient concentration in late bulking cassava varieties provides a novel aspect that could be implemented into cassava breeding programs in Thailand, South-East Asia, and sub-Saharan Africa, and might be an important contribution to a future solution to nutrient deficiencies (Ghislain, Muzhingi, Muzhingi, & Low, 2019; Graham, Senadhira, Senadhira, Beebe, Iglesias, & Monasterio, 1999). However, further experiments are needed to elaborate if the underlying genes regulating root architecture are complementary to already identified genes to enhance the micronutrient concentration in cassava (Narayanan et al., 2019). It also remains as an additional complexity to determine the influence of the soil type on the observed micronutrient concentration in storage roots. Therefore, it will certainly take more than a decade to develop varieties with regular storage root volume that market directly to the consumer. However, we envision that the presented first observation will inspire new research for staple root crops like cassava, taro, or yam to dampen the humanitarian challenge of hidden hunger in the poorest regions of the world (Von Grebmer et al., 2014).

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AUTHOR CONTRIBUTIONS

N.B performed research, analyzed data, wrote the manuscript. J.K. collected data, contributed to writing the manuscript, and performed micronutrient analysis. P.S. contributed to writing the manuscript, data analysis, data interpretation, and oversaw the experimental design. A.B. designed and programmed imaging algorithms, conceived the research, wrote the manuscript, and performed data analysis.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

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