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From lab to field: Open tools facilitating the translation of maize root traits

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ABSTRACT

Variation of root traits is associated with soil resource acquisition under abiotic stresses. However, plant breeding programs targeting beneficial root traits are hampered by lacking appropriate phenotyping tools. The availability of high-throughput root phenotyping technologies for seedlings as well as for mature plants grown in the field enabled us to investigate trait translation from the lab to the field. Here we used the established phenotyping techniques *Rhizoslides*, *Shovelomics*, and the image-based root phenotyping software, Digital Imaging of Root Traits (*DIRT)* to phenotype root traits of thirty seedlings and mature maize donor lines from the Drought Tolerance Maize for Africa (DTMA) project. The experiments were carried out in a controlled-environment and a field trial under well-watered and drought conditions. We found significant correlation between seedlings seminal and crown root number in the field ($r = 0.68$). Primary root branching of seedlings was negatively correlated with crown root branching of field-grown plants ($r = -0.54$). Our study showed indirect translation of traits between primary root length ($r = 0.29$) and primary root branching ($r = 0.20$) with plant performance in the field under drought. Our results suggest that root traits of maize seedlings are predictive for mature maize root traits and plant performance in the field. Our findings suggest that using open tools for high-throughput root phenotyping to screen in maize seedling could be beneficial for breeding programs and paves a cost-efficient way for plant improvement and breeding programs in developing countries.

1. Introduction

Global crop production must increase to sustain the projected world's population of 10 billion by 2050 (Lutz et al., 2017; Tilman et al., 2011). However, the necessary doubling of crop production worldwide is a challenge due to various climate change scenarios. In particular, global warming, precipitation changes, a higher frequency of extreme events and intensity of droughts are increasing (IPCC, 2014) and therefore, hamper the success of breeding programs. These effects are summarized by a 35 years' meta-analysis on maize yield in response to drought that showed a reduction in maize productivity by 40 % (Daryanto et al., 2016). In order to increase maize production in unfavorable environments, plant breeders have long been focusing on above ground physiological traits such as narrow leaf angle and appropriate leaf orientation, large stalk biomass and disease resistance (Hurni et al., 2015; Ku et al., 2010; Mazaheri et al., 2019). However, a major limitation for the improvement of water and nutrient acquisition efficiency is the complexity of the root system and its associated rhizosphere (York et al., 2013), which are both highly plastic and responsive to various environmental conditions (Bao et al., 2014; Lynch, 2018; Morris et al., 2017).

The early primary and seminal roots of the maize root system are

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Abbreviations: DTMA, Drought Tolerance Maize for Africa project; DAP, Days after planting; PL, Primary root length; SL, Seminal root length; SN, Seminal root number; LL, Lateral root length; LN, Lateral root number; LB, Lateral root branching; EA, Emergence angle; TA, Tip angle; HA, Hull area; SM, Shoot dry mass; CN, Crown root number; CA, Crown root angle; CB, Lateral root branching in crown root; CL, Lateral root length in crown root; BN, Brace root number; BA, Brace root angle; BB, Lateral root branching in brace root; BL, Lateral root length in brace root; AR, Projected root area; AD, Average root density; RA, Root top angle; DTDW, Total dry weight; DY, Yield

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embryonic roots that are responsible for nutrient and water acquisition and constitute a precursor to establish the post-embryonic root system at later developmental stages (Hochholdinger, 2009; Sanguineti et al., 1998). During the first few weeks of maize development, the post-embryonic roots become functional roots to the plant. For example, shoot-borne post-embryonic roots exhibit large phenotypic variation (Bayuelo-Jimenez et al., 2011; Burton et al., 2013) and respond to environmental changes like soil nutrient and water availability (Hufford et al., 2012; Lynch, 2013; York et al., 2015). Crown roots are a major sink for photosynthate compared to other root classes in the maize root system. An intermediate crown root number may be ideal, since fewer crown roots may not gain sufficient soil resources and increase a chance of lodging (Hetz et al., 1996). Large crown root lines (45 roots in high phosphorus field) had a shallow root system resulting in greater plant performance and yield compared to small crown root lines under low phosphorus (Sun et al., 2018). Gao and Lynch, 2016 showed that maize lines with reduced crown root number had greater total shoot mass and yield under drought, because less crown root number lines reduced metabolic cost, permitting plants to allocate more carbon to grow deeper roots into the subsoil strata and to gain more water (Gao and Lynch, 2016) and mobile nutrients like nitrogen (Saengwilai et al., 2014). Among different root types within the root system, lateral roots have been shown to efficiently optimize soil resource acquisition. Hydropatterning associates local soil water availability with lateral root formation (Giehl and von Wirén, 2018). Experiments confirmed that moderate soil drying accelerates lateral root elongation (Ito et al., 2006), while transient water deficit represses lateral root formation at the organ initiation stage (Orman-Ligeza et al., 2018). Maize with few but long lateral roots of crown root lines had 141 % less root respiration and 50 % greater shoot mass than those with many but short lateral roots under drought. Smaller lateral root number reduced the metabolic costs of growing axial roots allowing crown roots to grow deeper and gain more water from the deep soil profile (Zhan et al., 2015).

Despite these benefits, plant breeding programs targeting root traits are hampered by the lack of the appropriate phenotyping tools for plants grown in relevant agronomic settings. For plant breeders, the evaluation of plant seedling root traits is a more feasible option, because a large number of lines can be evaluated in a relatively short time period. Currently, *RootReader3D* (Clark et al., 2011), *GiaRoots* (Galkovskyi et al., 2012) and *Rhizoslides* (Le Marie et al., 2014) are methods to phenotype seedling root traits in controlled laboratory environments. The *Rhizoslide,* a soil-free phenotyping system, allows for a non-destructive observation of the variation of embryonic root traits (Le Marie et al., 2014). The soil-free environment is made of germination paper which constrains root growth to a 2D plane between a Plexiglas layer covered by polyethylene sheet to simulate a light-free soil environment. Root traits are observed through the transparent Plexiglas sheet. The images of the root are captured by using scanner and quantified with the software *RootNav* (Pound et al., 2013). Non-destructive root phenotyping for seedlings have also been performed in transparent gellan gum systems with 3-dimensional root image acquisition methods. Such 3D systems allow the observation of static root traits which are evaluated at a single time point, and dynamic root traits which are related to root growth and spatiotemporal changes in root characteristics (Clark et al., 2011; Symonova et al., 2015). Recently, the growth dynamics of maize have been recorded with X-Ray tomography to elucidate genetically pre-determined growth patterns in real soil (Jiang et al., 2019). However, these methods are limited for trait observations in young seedlings.

For field-grown plants, a manual phenotyping technique, *Shovelomics*, has been successfully implemented in maize (Trachsel et al., 2010; Zhan et al., 2019), bean (Burridge et al., 2016) and rice (Saengwilai et al., 2018). The scored traits include whorl number, diameter, number, angle, lateral root branching and lateral root length of brace and crown roots. Notably, the strength of the *Shovelomics* is its relevance to real agricultural production systems. To enhance the efficiency of root phenotyping, Digital Imaging of Root Traits (*DIRT*), a freely available web platform (Das et al., 2015), was developed for high-throughput computing. Previous studies showed high correlations between root traits from *Shovelomics* and *DIRT* algorithms (Bucksch et al., 2014; Kengkanna et al., 2019) and therefore confirmed the reliability of *DIRT*. However, field experiments are often expensive, tedious, and laborious. Most importantly, the negligible border rows and inappropriate experimental design are also confounding factors to the field experiment (Widdicombe and Thelen, 2002; Zavitkovski, 1980). Therefore, attempts to link seedling traits to growth performance and yield in the field have been made within the past century (Feret and Kreh, 1985).

Trait translation is a newly identified frontier for crop breeding (Zhu et al., 2011) and demands the development of methods that allow the association between seedling root traits in the lab with root traits in field-grown plants. Trait translation can be divided into two categories: direct and indirect. Direct trait translation refers to the relationship between the same root trait at different developmental stages such as number to number and angle to angle, while indirect trait translation refers to the relationships between one root trait with another root or shoot trait such as root number and grain yield. Only a limited amount of relationships between seedling roots and mature root system are known. The first documented relation might be the positive correlation between seminal root number and nodal root number at 35 days after planting in the field (Nass and Zuber, 1971). Seminal roots observed in hydroponic systems are associated with root pulling resistance which reduces root lodging in mature maize in the field (Sanguineti et al., 1998). Tuberosa et al. also showed the indirect association of seedling root traits including primary root diameter and seminal root weight; and grain yield under drought conditions (Tuberosa et al., 2002). In addition, direct basal root whorl number ($r^2 = 0.66$) and basal root number ($r^2 = 0.92$) translation between lab-grown seedling and field-grown bean were found in cowpea *Vigna unguiculata* subsp, *unguiculate* (Burridge et al., 2016). Thus, trait translation of seedling root traits could be feasible to predict root traits and plant growth performance in the field-grown maize and other plants in the breeding program.

In our study, we utilized the current high-throughput approaches for root phenotyping to reveal the association between laboratory and field phenotyping systems. We employed maize lines from the Drought Tolerant Maize for Africa project (DTMA). Our main goals were to (i) quantify the phenotypic variation of architectural root traits in maize seedlings (*Rhizoslide*) and root systems in field-grown maize (*Shovelomics* and *DIRT*) of thirty DTMA lines, (ii) compute correlations between architectural root traits in seedling maize (growing in the lab) and traits in field-grown maize, and (iii) to quantify translation from seedling maize root traits to traits in field-grown maize.

2. Material and methodology

2.1. Plant materials

Thirty DTMA donor lines were obtained from Nakhon Sawan Field Crops Research Center (NSFCRC), Thailand. The DTMA project was initiated in 2006 as a collaboration between the International Maize and Wheat Improvement Center (CIMMYT), International Institute of Tropical Agriculture (IITA) and several Sub-Saharan countries to mitigate regional heat and drought stress in maize production systems (CIMMYT, 2015, 2014). DTMA lines are assembled from maize inbred lines in CIMMYT, IITA, and the subtropical maize improvement program. The DTMA lines in the present study can be divided into 6 groups including CIMMYT Maize Line (CML), CIMMYT Line (CL), Drought Tolerant Population White grain (DTPW), Drought Tolerant Population Yellow grain (DTPY), La Posta Sequia C7 and the individuals from different origins (DTMA 34–40) (Monneveux et al., 2008; Wen et al., 2011). Detailed information for the 30 DTMA lines is provided in

Table 1
Summary of minimum, maximum, Z-score of minimum value, Z-score of maximum value, ratio of maximum and minimum values, mean square, standard deviation, *F* value and *P*-value, showing a large variation of root architectural traits in maize seedling (*Rhizoslide*) and vegetative stages (*Shovelomics* and *DIRT*) of 30 DTMA lines. *F* value and *P*-value indicate different significant levels as Spearman's principle correlation.

Traits	Description	Unit	Min	Max	Z-Min	Z-Max	Ratio	SD	*MS*	*F*	*P*-value
8 DAP maize seedling from the *Rhizoslide*											
PL	Primary root length	mm	85.713	248.28	2.13	6.17	2.90	40.21	6.42×10^3	3.42	< *0.001*
SL	Total Seminal root length	mm	10.47	140.05	−1.62	3.74	13.38	24.17	2.40×10^3	4.81	< *0.001*
LL	Total Lateral root length	mm	0	6.51	−1.93	2.24	6.51	1.56	9.64	3.74	< *0.001*
SN	Seminal root number	count	1.75	5.5	−1.44	1.88	3.14	1.13	5.22	4.58	< *0.001*
LN	Lateral root number	count	0	62.5	−1.36	2.83	62.50	14.91	888.40	3.16	< *0.001*
LB	Lateral root branching	root cm^{-1}	0	0.25	−1.63	1.94	0.25	0.07	279.50	9.64	< *0.001*
EA	Emergence angle	degree	4.70	36.03	−1.11	3.06	7.67	7.51	230.10	1.89	< *0.05*
TA	Tip angle	degree	6.38	32.43	−1.32	2.30	5.08	7.21	211.10	1.50	< *0.1*
HA	Hull area	mm^2	312.6	5.3×10^3	−1.23	2.92	16.95	1.2×10^3	5.37×10^6	2.69	< *0.001*
54 DAP vegetative maize from the field											
CN	Crown root number	count	22	54.67	−1.73	3.69	2.49	6.03	112.78	1.66	< *0.05*
CA	Crown root angle	degree	31.67	65	−2.42	1.42	2.05	8.67	228.16	3.28	< *0.001*
CB	Average lateral root branching in crown root	root cm^{-1}	1.33	4.33	−1.59	1.94	3.26	0.85	2.23	0.81	*NS*
CL	Average lateral root length in crown root	cm	0.50	6	−1.54	2.73	12.00	1.29	5.10	2.62	< *0.001*
CD	Crown root diameter	cm	0.18	0.84	−1.05	4.95	4.67	0.11	0.04	1.01	*NS*
BN	Brace root number	count	11.33	18.67	−1.82	2.35	1.65	1.76	9.36	1.22	*NS*
BA	Brace root angle	degree	26.67	78.33	−2.06	2.32	2.94	11.80	414	2.72	< *0.001*
BB	Average lateral root branching in brace root	root cm^{-1}	0	6	−2.16	2.56	6.00	1.27	2.96	1.41	*NS*
BL	Average lateral root length in brace root	cm	0	6	−1.60	3.02	6.00	1.30	3.26	1.45	*NS*
BD	Brace root diameter	cm	0.16	0.48	−1.77	2.81	2.29	0.07	7.20×10^{-3}	2.44	< *0.05*
AR	Projected root area	pixels	1.4×10^3	3.2×10^3	−2.05	1.60	8.78	493.73	7.31×10^5	1.13	*NS*
AD	Average root density	ratio	1.16	10.19	−0.59	5.20	5.92	1.56	7.50	0.93	*NS*
RA	Root top angle	Degree	10.17	60.28	−2.18	2.29	50.11	11.19	387	1.23	*NS*

Table 2
Summary of correlation analysis (correlation coefficients and significance levels) among root traits of DTMA maize seedling from the *Rhizoslide* experiment, and total dry weight and yield in the field experiment under drought. ***, **, * indicated significant level at $P < 0.001$, $P < 0.01$, $P < 0.05$. DTDW: Total dry mass under drought condition; DY: Yield under drought condition; PL: Primary root length; SL: Total seminal root length; LL: Total lateral root length; SN: Seminal root number; LN: Lateral root number; LB: Lateral root branching; EA: Emergence angle; TA: Tip angle; HA: Hull area.

DY	0.55***									
PL	0.0079	0.29**								
SL	0.19*	0.24*	0.51***							
LL	0.066	0.29**	0.48***	0.44***						
SN	−0.099	−0.011	0.19*	0.14	0.36***					
LN	−0.054	0.22*	0.67***	0.51***	0.53***	0.24*				
LB	−0.041	0.20*	0.44***	0.45***	0.51***	0.25**	0.92***			
EA	0.025	0.16	0.11	0.28**	0.087	−0.0055	0.24**	0.25**		
TA	0.13	−0.052	−0.17*	0.039	−0.071	0.082	0.0081	0.10	0.35***	
HA	0.08	0.35***	0.76***	0.57***	0.63***	0.21*	0.79***	0.62***	0.22*	0.0010
	DTDW	**DY**	**PL**	**SL**	**LL**	**SN**	**LN**	**LB**	**EA**	**TA**

Supplemental Tables 1 and 2.

2.2. *Rhizoslide system*

The experiment was performed at Mahidol University, Thailand (13°47′32.1″ N, 100°19′24.1″ E). The thirty DTMA maize lines were grown in a randomized design with 3 replications in the *Rhizoslide* system, designed as described in Le Marié et al. (2014) and mentioned earlier as root pouch (Hund et al., 2009). Maize seedlings were grown on germination paper (21.5 cm × 29.7 cm) in plastic sheets covered by black polypropylene sheets (Fig. 1). We drilled a regular grid of holes 3 cm apart into each A4 plastic sheet and then cut a V-notch on the top of the plastic sheet for aeration. A germination paper was soaked with 0.5 mM CaSO$_4$ and moved onto the perforated plastic sheet. Two maize seeds were placed under the V-notch on the germination paper and covered another plastic sheet. The system was covered with black polypropylene sheets, bound together with binder clips and transferred into a plastic box containing 0.5 mM CaSO$_4$ solution. The *Rhizoslides* were placed in the dark for seed germination. After 2 days, seedlings were thinned to one uniform seedling per rhizoslide and exposed to fluorescent light for 6 days under a photoperiod of 12/12 h with photosynthetic active radiation of 200 μmol m^{-2}s^{-1} at 28 ± 1 °C. After eight days, the germination papers covered with plastic sheets were scanned and root traits were quantified by *RootNav* image analysis program (Pound et al., 2013).

2.3. *Field experimental design and growth conditions*

The DTMA lines were planted at the Nakhon Sawan Field Crop Research Center, Thailand (15° 21′ N, 100° 30′ E, and 87 m ASL) during November 2014 to March 2015. The soil properties include clay loam and pH at 7.81. At seed sowing, fertilizer containing N (15 %), P (15 %) and K (15 %) was applied at the rate of 312.5 kg ha^{-1}. Additionally, N fertilizer was applied at 28 days after planting (DAP) and 45 DAP at the rate of 32.8 kg ha^{-1} and 57.5 kg ha^{-1}, respectively to ensure optimum nutrient levels for maize production. The maize lines were planted under well-watered and drought conditions. The experiments were arranged in a randomized complete block design with 4 replications. Each plot consisted of 4 rows of 5.00 m. length with row spacing of 0.75 m. and 0.20 m. between plants resulting in a planting density of 5.20 plants m^{-2}. Both treatments received 30 mm of irrigation weekly until the imposition of drought. Drought was imposed by withholding irrigation 2 weeks prior to anthesis stage (Banzinger et al., 2000). Chlorophyll content was measured using Chlorophyll meter SPAD 502

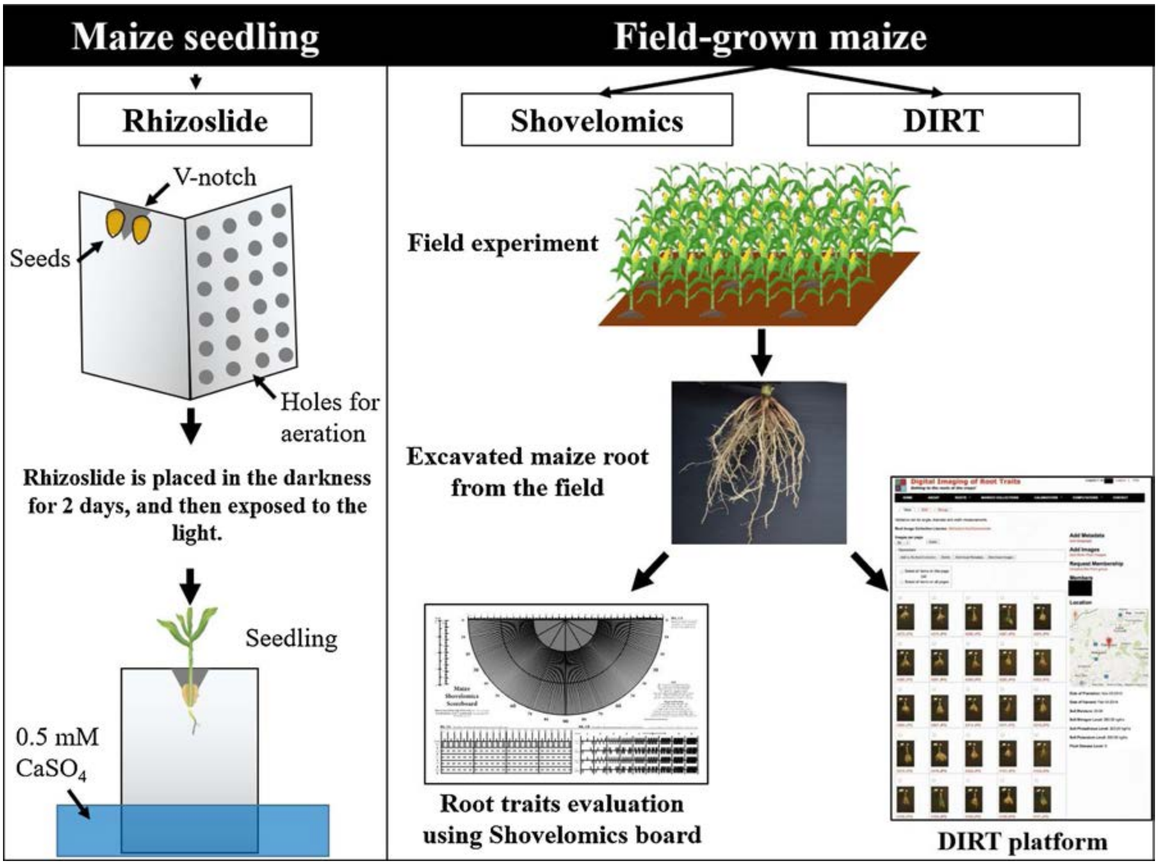

Fig. 1. Schematic of plant root phenotyping techniques employed in this study. Maize seedling root architectural traits were studied using the Rhizoslide system (Le Marie et al., 2014). Field-grown maize root architectural traits were studied using *Shovelomics* (Trachsel et al., 2010) and Digital Imaging of Root Traits (*DIRT*; http://dirt.iplantcollaborative.org) (Das et al., 2015).

plus (Konica Minolta, Europe) on the fourth fully expanded leaves of five representative plants per plot. Stomatal conductance measured by SC-1 Leaf porometer (Decagon Devices, Inc.) and leaf temperature measured by IR50i infrared thermometer (IRtek) were recorded on the fourth fully expanded leaves of three representative plants per plot. The measurements were conducted between 9am and 11am. At 54 days after planting (DAP), plants were excavated and processed according to *Shovelomics* (Trachsel et al., 2010). Fresh shoots were weighed and then dried at 80 °C for 48 h for dry mass measurement. Root traits were evaluated according to *Shovelomics* (Trachsel et al., 2010). Root images were taken using a Nikon D40X DSLR camera (Nikon Corporation, Japan), whereas root architectural traits were further analyzed using the *DIRT* imaging analysis platform (Das et al., 2015). Ears from the two middle rows (50 plants) were harvested at physiological maturity stage. Kernels were shelled and weighed as grain weight in kg per plot. Grain yield in kg per hectare was estimated at 15 % moisture content.

2.4. *Shovelomics*

Three representative plants of each plot were excavated with standard shovels. In total, nine plants per line in each treatment were selected for sampling. The selection was made based on plant height, presence of bordering plants, and general appearance that represented individuals in the plot. The selected plants were cut by pruning shears at the shoot and the root after the excavation. The approximated size of the excavated root system was 30 cm diameter and 30 cm depth. Soil was initially removed from the roots by carefully shaking. The remaining soil was removed by soaking the roots in diluted commercial detergent for approximately 15 min followed by vigorously rinsing at low pressure with tap water. Root architectural traits were evaluated with the *Shovelomics* protocol (Trachsel et al., 2010). The stem, brace root, and crown root diameters were measured with a standard Vernier caliper. The numbers of above-ground whorls occupied by brace roots (BW), the numbers of brace roots (BN), the number of below-ground whorls occupied with crown roots (CW) and the number of crown roots (CN) were manually counted. The *Shovelomics* scoring board was used to evaluate the angles of brace roots (BA), the branching of brace roots (BB), the length of lateral root of brace roots (BL), the angles of crown roots (CA), the branching of crown roots (CB) and the length of lateral roots on crown root s (CL).

2.5. *Digital imaging of root traits (DIRT)*

Digital imaging of root traits (*DIRT*) is an automated high-throughput platform for root phenotyping. *DIRT* is an online and open platform (Das et al., 2015) to compute root architecture from digital images. To acquire the root system images, the washed roots were placed on a black fabric background with a circular scale marker with 2 inch in diameter and a plant identification tag. A standard color root image was obtained with a Nikon D40X DSLR camera (Nikon Corporation, Japan) mounted on a tripod. Root images were uploaded onto the *DIRT* platform version 1.1 and computed with the current software version in April 2018. For computation, the color image is converted to a grayscale image, and passed into an adaptive thresholding algorithm to distinguish foreground and background. The resulting binary image contains the root with the scale marker and identification tag as separated objects in the foreground. In the next step, the root crown images were used to compute the root-width profile, root density, root angle and root tip paths (RTPs). RTPs allow the calculation of root length from an emerging point to an identified tip, lateral root length, root branching frequency, and root diameter. The 2 in. (5.08 cm) circular scale was used as a reference to transform pixels to millimeters (Bucksch et al., 2014).

2.6. Statistical analysis

Statistical analysis was performed using R version 3.14 (RS team, 2018). Spearman correlation coefficients were calculated to assess the relationship among root traits. Linear regression analysis and Random sample consensus (RANSAC) regression were performed to find the best fit linear mathematical model between 2 sets of the observed data including inliers and outliers (Fischler and Bolles, 1981). We computed Linear and RANSAC correlations using scikit-learn v. 0.22.2 (Pedregosa et al., 2011) and python 3.7. To generate the plots we also used Numpy 1.19 for array operations (Oliphant, 2007), statsmodels 0.11.1 (Seabold and Perktold, 2010) to compute p-values for the RANSAC regression and matplotlib 3.2.1 (Hunter, 2007) to visualize and save the regression graphs. The full dataset and code are available in the Supplementary Materials. A two-way ANOVA was applied for comparisons between well-watered and drought treatments. The protected LSD post-hoc test ($a = 0.05$) was used for multiple comparison tests. Principal component analysis (PCA) was employed to explain multiple correlations among root traits and plant performance.

3. Results

3.1. Phenotypic variation among 30 inbred lines

Root architectural traits of thirty DTMA maize lines were studied among different systems in maize seedlings and field-grown 54-day-old plants (Fig. 1). DTMA lines showed considerable phenotypic variation of root traits, except for emergence and tip angles in maize seedlings. The ratio of maximum and minimum values showed the greatest variation for hull area and the lowest variation for lateral root branching in seedling root traits. Traits with a 10-fold or greater variation ($p < 0.001$) included total seminal root length, total lateral root length, lateral root number and hull area. Interestingly, lateral root number had the highest variation with the maximum value 62.5 times greater than the minimum value.

Shovelomics and *DIRT* phenotyping system revealed that crown root number, crown root angle, brace root angle, average lateral root length in crown roots, and projected root area was significantly different among DTMA lines in field-grown maize (Table 1). Brace root angle was between 26–78 degrees, whereas crown root angle varied from 32 to 65 degrees. Crown root number seemed to be less variable compared to other root architectural traits among DTMA lines, varying between 22–55 roots.

3.2. Correlations among maize seedling and field-grown maize root traits

DTMA lines, grown in the *Rhizoslide* system, showed correlations among root architectural traits in the 8-day-old seedlings (Table 2). The length of the primary root correlated positively with seminal root length ($r = 0.51$) and number ($r = 0.19$). Lateral roots on the primary root had high correlations with primary root length in terms of branching ($r = 0.44$) and length ($r = 0.48$). Seminal roots, which grow from the scutellar node after primary root, showed significant associations with lateral roots on the primary root which is emerged from a different origin. Furthermore, seminal root number was correlated with lateral root number ($r = 0.24$), and seminal root length was correlated with lateral root length ($r = 0.44$). Hull area correlated with most root traits except tip angle, suggesting that hull area is a complex trait associated with other root architectural traits. Principal component analysis (PCA) for seedling root traits identified two components that explained 53.3 % of the total variation in seedling traits (Fig. 2A). Primary root length, seminal number and lateral root branching were grouped together and mostly contributed to PC1. Primary root length and its lateral root density were major contributors to PC1. However, PC2 associated with the emergence and tip angles wasindependent from other seedling root traits.

In field-grown plants, correlation analysis from *Shovelomics* revealed significant correlations among brace root traits (Table 3). For axial roots, brace root number had a strong correlation with brace root angle. In the lateral root system of the brace roots, there was a highly significant correlation between lateral root length and lateral root branching. Interestingly, lateral root length in brace root was negatively associated with crown root number which had a different origin site. There was no correlation within the crown root system in field-grown plants. From the *DIRT* data, root top angle, the angle between the outer most part of the root system and the horizontal plane, was negatively correlated with lateral root length on brace roots. PCA of root traits in field-grown plants showed that the first two components accounted for 40.1 % of the total variation. We found that lateral root length and branching of brace roots, and crown root angle, strongly contributed to PC1 (Fig. 2B). Brace and crown root numbers and their lateral root length mainly contributed to PC2.

3.3. Translation of root traits from the lab to the field

Root traits of DTMA lines during seedling and field-grown maize were previously described (Table 4). Here, seedling root traits grown in a laboratory were correlated with field-grown maize root traits from the *Shovelomics* and *DIRT* phenotyping platform (Fig. 3). The seminal root number of maize seedlings had a strong correlation with crown root number of field-grown plants under well-watered condition at $r = 0.68$ ($p < 0.001$). The seminal root number was significantly correlated with D90, which is the fraction of accumuated width of the root system over the depth at 90 %, of field grown maize from the *DIRT* phenotyping platform ($r = 0.72$, $p < 0.01$). Moreover, lateral root branching on the primary root had a strong correlation with lateral root branching on crown roots at $r = -0.54$ ($p < 0.001$). We also found a positive correlation between brace root angle and emergence angle at $r = 0.58$ ($p < 0.01$).

3.4. Root traits in Rhizoslide relevant to drought adaptation in the field

Drought drastically affected overall growth performance in the field. Plant physiological traits, such as chlorophyll content was reduced by 51 % while leaf temperature was increased by 16 %. In addition, shoot mass and yield were reduced by 15.37 % and 92.63 %, respectively, under drought (Fig. 4).

We found significant correlations between root traits in seedlings and plant growth performance of field-grown maize under drought, which we refer to as indirect root trait translation (Table 3). Primary root length was positively correlated with grain yield under drought at $r = 0.29$. There were relationships between seminal root length with total dry weight, and yield under drought at $r = 0.19$ and $r = 0.24$, respectively. Lateral root length was also positively correlated with yield under drought ($r = 0.29$). Interestingly, lateral root number ($r = 0.22$) and branching on the primary root ($r = 0.20$) were associated with yield. There was no root trait correlation with plant performance for seminal root number, emergence angle and tip angle under drought. PCA revealed that most of seedling root traits were grouped together in PC1 which contributed 38.8 % of total variation (Fig. 2). Seedling root traits contributing mainly to PC1 were root length and number, shaping the root system. Total dry mass and yield under drought mainly contributed to PC2 which was 14.5 % of the total variation. Emergence and tip angles were also grouped with those plant performance traits under drought. In addition, angles were positively associated with plant performance under drought.

In *Shovelomics* phenotyping systems, root angle showed significant correlations with plant performance. Brace root angle had a positive

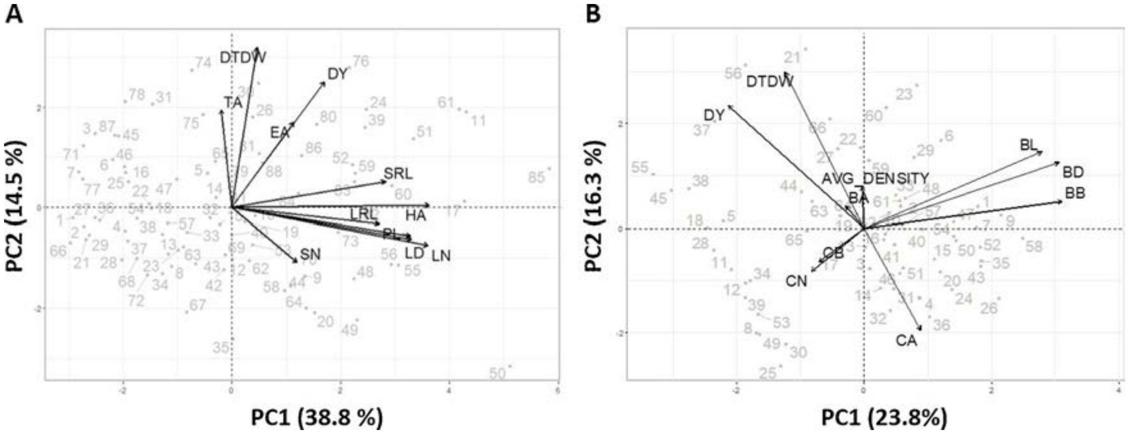

Fig. 2. Biplots of principal component 1 and 2 of root architectural traits, root mass and yield in DTMA seedlings (A) and vegetative stage maize (B). The x and y axes are component 1 and 2, respectively. Axis labels include the percentage of variation explained by each of these two components.

Table 3
Cross-Correlation (correlation coefficients and significance levels) between manually measured root traits of 54 DAP maize at vegetative stage of 30 DTMA inbred lines from the field experiment in 2015. Moreover, maize root traits from the field were significantly correlated with total dry weight and yield in the field experiment under drought. ***, **, * indicated significant level at $P < 0.001$, $P < 0.01$, $P < 0.05$ as Spearman's principle correlation. All traits varied significantly within each population. DTDW: Total dry mass under drought condition; DY: Yield under drought condition; CN: Crown root number; CA: Crown root angle; CB: Lateral root branching on crown root; CL: Lateral root length on crown root; CD: Crown root diameter; BN: Brace root number; BA: Brace root angle; BB: Lateral root branching on brace root; BL: Lateral root length on brace root; BD: Brace root diameter; AR: Projected root area; AD: Average root density; RA: Root top angle.

DY	0.55***													
CN	0.039	−0.038												
CA	−0.24*	−0.18*	0.11											
CB	−0.0067	−0.065	0.058	0.13										
CL	−0.10	−0.12	0.076	0.06	0.091									
CD	−0.013	−0.058	0.14	0.032	0.059	0.019								
BN	0.17	0.039	−0.043	−0.07	0.03	−0.11	0.13							
BA	0.28**	0.12	−0.12	−0.0032	−0.055	−0.027	0.062	0.57***						
BB	0.011	−0.17	−0.16	0.19	−0.057	−0.002	0.015	0.26*	0.23*					
BL	0.11	−0.18	−0.21*	−0.039	−0.087	−0.15	−0.058	0.22*	0.16	0.53***				
BD	0.12	−0.12	−0.15	0.042	−0.15	0.042	−0.08	0.38***	0.27*	0.67***	0.60***			
AR	−0.16	−0.10	−0.11	−0.032	−0.12	−0.044	0.13	0.12	0.041	0.12	0.043	0.014		
AD	0.069	0.18*	−0.057	0.14	−0.067	−0.014	−0.11	0.029	0.071	0.037	−0.014	0.084	−0.06	
RA	−0.019	0.037	0.11	0.17	−0.085	0.12	0.028	−0.14	−0.043	−0.12	−0.19*	−0.16	−0.061	0.038
	DTDW	**DY**	**CN**	**CA**	**CB**	**CL**	**CD**	**BN**	**BA**	**BB**	**BL**	**BD**	**AR**	**AD**

correlation with total dry mass under drought condition. Conversely, crown root angle was negatively correlated with total dry mass and yield under drought. In *DIRT*, average root density positively correlated with yield under drought. PCA and Pearson correlation analysis support this observation by showing sppositing vector directions between crown root angle and brace root angles, total dry weight and yield under drought.

4. Discussion

Phenotypic variation of root traits influences maize adaptation to drought (Lynch, 2019). In recent years, several phenotyping platforms have been developed to facilitate physiological studies and plant breeding programs. However, it is still unclear how much root traits measured in young seedlings can actually translate directly and indirectly to traits in the field. In our study, we demonstrated that root architectural traits in seedlings have a predictive potential for root and shoot traits in field-grown maize. In addition, root traits such as primary root length, seminal root number and lateral root branching were found to be associated with drought tolerance among maize from the Drought Tolerant Maize for Africa (DTMA) project.

DTMA seedlings had a large variation of primary root length. The primary root emerges first from the seed and is associated with water limitation condition (Voetberg and Sharp, 1991). The maximum primary root length in DTMA lines was 10 % longer than the maximum primary root length in Thai maize genotypes (Salungyu et al., 2018). In *Arabidopsis*, primary root function is crucial for seedling establishment under drying soil to ensure that plants gain enough water supply before shoot emergence (Van Der Weele et al., 2000). It has been shown that the maize primary root apex continuously elongates at the well-watered rate under drought conditions (Sharp et al., 2004). Therefore, primary root length may associate with water acquisition. In the field, DTMA lines had less lateral root branching on nodal roots compared to previous studies (Bayuelo-Jimenez et al., 2011; Zhu et al., 2005), and the steepest nodal root angle of DTMA lines was steeper than previously reported nodal root angles (Trachsel et al., 2013). Reduced lateral root branching and steep root angle of DTMA lines supports the suggested Steep, Cheap, and Deep ideotype for nitrogen and water acquisition in maize (Lynch, 2013). Maize with reduced lateral root branching tends to reduce inter-root competition with other laterals in term of soil resource in the rhizosphere (York et al., 2013) and internal carbon within the root system. The reduction of lateral root branching has been demonstrated to reduce metabolic cost for lateral root initiation, respiration, and maintenance, permitting plants to allocate more carbon to axial roots and grow deeper into the deep soil profile (Zhan et al., 2015). Similarly, to explore the deep soil profile, maize with steep root angles may increase the possibility to uptake more nitrogen and water (Ho et al., 2005; Mace et al., 2012). In maize, nodal root angle was found to correlate with grain yield under drought (Ali et al., 2015). Among the *Poaceae* family, steep rice nodal root angle was also

Table 4
Trait translation of root architectural traits observed in this study and reported in previous studies. Positive and negative correlations between traits are represented by + and – respectively. SM: Shoot mass; Low N: N limitation condition; Low P: P limitation condition; D: Drought condition.

Root traits	Direct trait translation	Indirect trait translation	
	Root traits	Root traits	Physiological/performance traits
Primary root			
Length			Yield under D, + (This study)
Branching	Crown root branching, - (This study)		Water uptake, + (Ahmed et al., 2016
Diameter		Nodal root angle under D, + (Ali et al., 2015)	Yield under D, + (Ali et al., 2015)
Seminal roots			
Length			SM under low P, + (Zhu et al., 2006)
Number	Crown root number, + (This study)		SM under low P, + (Zhu et al., 2006)
Angle	Nodal root angle, + (Ali et al., 2015)		Yield under D, + (Ali et al., 2015)
Lateral roots			
Length			
Emergence angle			Yield under D, + (This study)
Tip angle			Yield under D, + (This study)
Seedling hull area/ root surface area			Yield under D, + (This study)
Brace roots			
Whorl number		Root lodging resistance, + (Ku et al., 2012)	Central spike length, - (Hardy et al., 2007)
Number		Root lodging resistance, + (Bruce et al., 2001)	Tassel branching, + (Hardy et al., 2007)
Angle	Seminal root angle, + (Ali et al., 2015)		Yield under D, + (Ali et al., 2015)
Crown roots			
Diameter		Root lodging resistance, + (Stamp and Kiel, 1992)	
Whorl number		Root lodging resistance, + (Ku et al., 2012)	
Number	Seminal root number, + (This study)	Root lodging resistance, + (Bruce et al., 2001)	SM and yield under low P, + (Sun et al., 2018)
		Rooting depth under low N and D, - (Gao and Lynch, 2016; Saengwilai et al., 2014; Sebastian et al., 2016) Rooting depth under low P, - (Sun et al., 2018)	SM and yield under low N and D, - (Gao and Lynch, 2016; Saengwilai et al., 2014; Sebastian et al., 2016)
Angle	Seminal root angle, + (Ali et al., 2015)	Rooting depth under low N, + (Trachsel et al., 2013)	Yield under low N and D, + (Ali et al., 2015; Trachsel et al., 2013)
Branching	Primary root branching, - (This study)	Rooting depth under low N and D, -(Jia et al., 2018; Zhan et al., 2015; Zhan and Lynch, 2015)	SM and yield under low N and D, - (Hazman and Brown, 2018; Zhan et al., 2015; Zhan and Lynch, 2015)
		Rooting depth under low P, + (Jia et al., 2018)	SM and yield under low P, +(Jia et al., 2018; Postma et al., 2014)
Lateral root length		Rooting depth under low P, low N and D, + (Jia et al., 2018; Zhan et al., 2015; Zhan and Lynch, 2015)	SM and yield under low N and D, + (Zhan et al., 2015; Zhan and Lynch, 2015) SM and yield under low P, - (Jia et al., 2018)

correlated with low nodal root number which might be associated with drought tolerance ability as shown in previous maize studies (Saengwilai et al., 2018; Zhan et al., 2015). The long primary root, reduced lateral root branching on crown roots and steep nodal root angle of DTMA lines contributed to the deep soil exploration which might be target traits for maize growing under water limited conditions.

Direct trait correlation in axial roots between seedling seminal roots and crown roots in field-grown maize, particularly root number, was found in this study. These results are consistent with those reported by Salvi et al. (2016), in which the authors reported a positive correlation between seminal root number of maize plants grown in a paper roll system and crown root number of plants grown in a pot system (Salvi et al., 2016). In other monocotaledonous species such as wheat, the relationship between seminal roots of seedlings and nodal roots in the field has been shown to be significant only in young seedlings (Watt et al., 2013). The reason for the observed correlation is possibly due to tillering nature of the species, which may complicate the interactions. Maize does not typically form tillers, and thus the relationship between the two root classes are relatively simple. Molecular genetics studies in maize revealed that QTLs associated with seminal and crown root

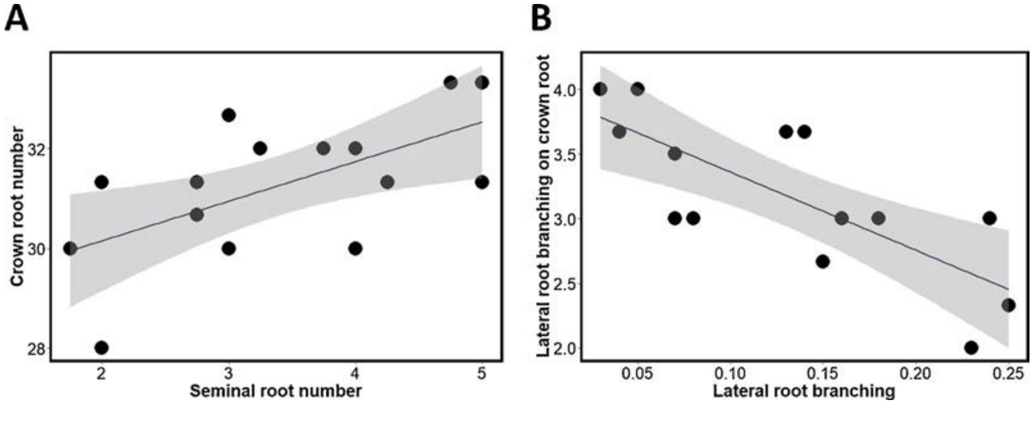

Fig. 3. Correlation analysis between root traits from 7 DAP seedling and 54 DAP field-grown maize. (A) Seminal root number from seedling correlated with crown root number in field-grown maize at $r = 0.68$ ($p < 0.001$). (B) Lateral root branching on primary root (branch cm^{-1} of primary root) in seedlings was correlated with lateral root branching on crown root number (branch cm^{-1} of crown root) in field-grown maize at $r = -0.54$ ($p < 0.001$). Data shown excluded outliers using RANSAC. Confidence interval at 95 % was also shown in the chart.

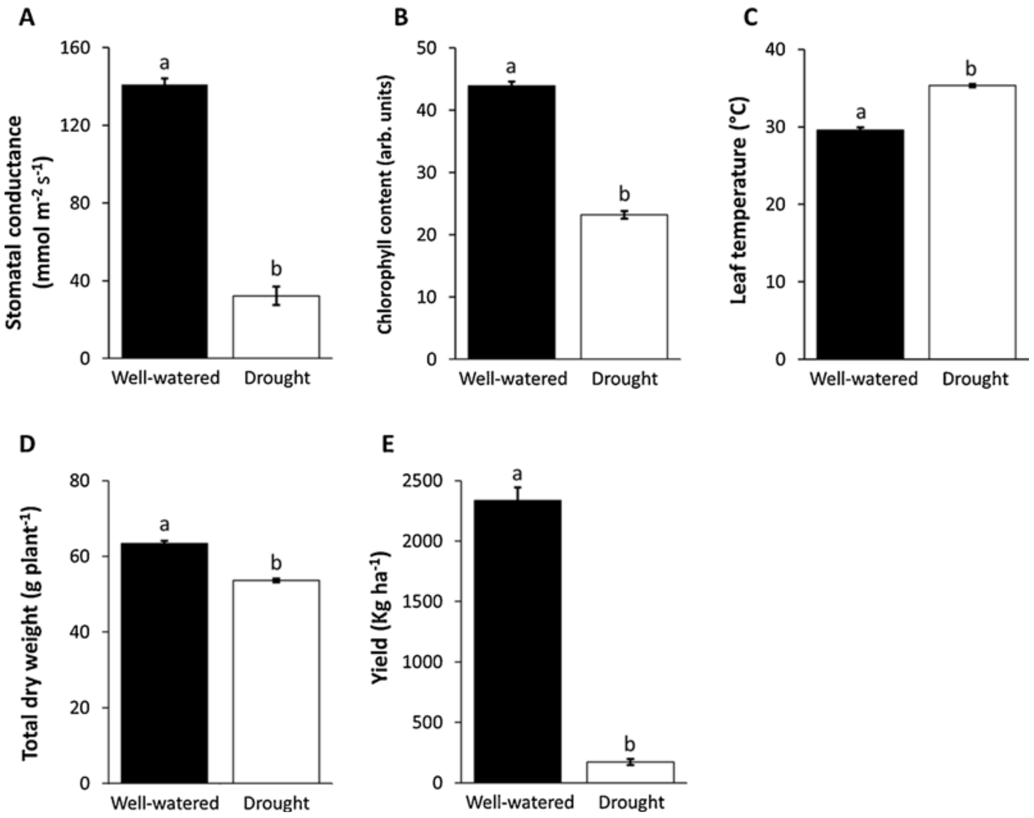

Fig. 4. Physiological traits including (A) Plant stomatal conductance measuring at 1 week after tasseling (mmol m^{-2} s^{-1}), (B) chlorophyll content measuring 1 week after tasseling (arb. units), (C) leaf temperature measuring 1 week after tasseling (°C), (D) total dry weight measuring at physiological maturity in the field (g $plant^{-1}$) and (E) yield collecting at physiological maturity in the field (kg ha^{-1}) were measured with three replicates ± SE. Means of all genotypes with the different letters are significantly different ($P < 0.001$).

number were co-localized at the same region on chromosome 2 (Li et al., 2015). Additionally, the mutation of *RTCS (ROOTLESS CONCERNING SEMINAL AND CROWN ROOTS)* gene inhibits the formation of seminal and crown roots (Hetz et al., 1996). The proteomic analysis also supported that seminal root and crown root initiation might be controlled by the same gene through the auxin triggers transcriptional response (Hochholdinger et al., 2018). Interestingly, the *RTCS* gene in *Setaria viridis* and *Setaria italic* grass was inhibited under drought conditions (Sebastian et al., 2016). Hence, seminal root number may be a predictor for the number of crown roots in the field-grown maize. Since soil resources and water are heterogeneously distributed, seminal root number should be numerous in order to uptake both mobile and immobile nutrients that are more available in the topsoil after fertilization at the early planting season (Lynch, 2013). However, under nitrogen and water limitation, seminal root number selection should be targeted at the intermediate seminal root number, which will provide low to medium crown root number for mature maize. Many studies have been shown that low crown root number benefits nitrogen and water acquisition under sub-optimal conditions (Gao and Lynch, 2016; Saengwilai et al., 2014). However, under phosphorus deficiency, seminal root and crown root number should be targeted at maize lines with large root number. Since large seminal root number was found to be positively correlated with shoot dry weight under low phosphorus (Zhu et al., 2006), consequently large crown root number which was shown to increase topsoil exploration where phosphorus and potassium are relatively higher compared to the subsoil (Sun et al., 2018).

Another trait that correlated directly between seedling stage and field grown roots was lateral root branching. We found that high lateral root branching on primary roots of maize seedlings was correlated with low lateral root branching on crown roots and vice versa. The negative correlation between lateral root branching on primary and crown roots may be -explained by internal resource competition to optimise metabolic carbon use within the root system. Since metabolic resources from the photosynthesis process are limited, plants need to balance internal resources. The geometric simulation model *SimRoot* in common bean revealed the association of greater lateral root branching and a decrease in their axial basal root whorl number (Rangarajan et al., 2018). In our present study, increased lateral root branching on the primary root may decrease lateral root branching on crown root, because the plant might try to optimize the limiting internal metabolic cost within the root system. However, the plant also needs to balance the products of photosynthesis (metabolic carbon) and soil resource acquisition (nutrients and water) (York et al., 2013). Increased lateral root branching on the primary root during seedling stage has been hypothesized to benefit the acquisition of mobile and immobile nutrients which are more concentrated in the topsoil profile at the early planting season in the high-input agricultural field (Lynch, 2013). Later in development, the primary root and its laterals lose their function, while crown roots become the major root class (Hochholdinger et al., 2004). Reduced lateral root branching on the crown root has been reported to reduce the metabolic cost of soil exploration permitting 34 % longer axial root which may increase nitrogen and water uptake (Zhan et al., 2015; Zhan and Lynch, 2015). Therefore, this transferability of lateral root branching on the primary root could be used to predict lateral root branching on crown roots. It is noteworthy that phenotyping of lateral root traits from field grown plants can be challenging due to the destructive nature of root excavating process. We suggest that at least 3 plants per plot should be phenotyped and rigorous statistical algorithms should be employed for data analysis.

Several indirect correlations were found among the DTMA lines. For example, there was a positive correlation between seedling root traits including primary root length and seminal root length, and total dry weight and yield under drought. These root traits have been reported to be associated with water acquisition and may be involved in deep soil exploration (Comas et al., 2013; Lynch, 2013; Sharp et al., 2004). Correlations between root length and physiological traits have been found in many crop species. In wheat (*Triticum aestivum* L) seedlings, 2 week-old plants' seminal root length was substantially correlated with the thousand-grain weight of field-grown wheat (Bai et al., 2013; Xie et al., 2017). In rice, total root length was significantly related to greater water use efficiency and shoot dry mass under drought conditions (Owusu-Nketia et al., 2018). In maize, the primary root maintained its elongation rate under well-watered conditions and indicated an association with rate under water limited conditions (Voetberg and Sharp, 1991). These relationships between root length and plant performance under drought conditions could be described as a drought avoidance strategy in which roots in drying soil grow longer roots in order to seek for wet soil (Kano-Nakata et al., 2011).

We found that the emergence angle of lateral roots was associated with plant growth performance under drought. Our PCA analysis revealed that the angle of lateral root emergence at the primary root was grouped with yield under drought. This grouping indicates an indirect trait translation between seedling root and performance traits. A small change in lateral root traits significantly affects the metabolic cost for soil exploration (Rangarajan et al., 2018). Intensive studies on lateral root angle in *Arabidopsis* demonstrated that lateral roots are able to alter their growth angle toward environmental factors such as gravity, nutrient availability and water (Guyomarc'h et al., 2012; Huang et al., 2018; Kiss et al., 2002; Malamy, 2005; Mullen and Hangarter, 2003). It has been shown that plants reorient their root angle to increase nutrient uptake at the minimum cost (Lynch and Brown, 2001). Our finding suggests that maize might adjust their lateral root angle to be steeper in order to capture more soil resource from the steep soil strata where water is abundant, resulting in early plant establishment and greater yield in the mature stage.

Hull area, the smallest area surrounding axial and lateral roots, reflects the root surface area (Nguyen and Stangoulis, 2019). It has been shown that root surface area was associated with soil nutrient and water uptake (Tachibana and Ohta, 1983). Large root surface area allows plants to gain more nitrogen in the form of nitrite (Ansari et al., 1995). Our finding demonstrated that hull area was largely influenced by the number and length of the roots. Since the nutrient source in the seed endosperm is limited, plants establish root before shoot in order to access water and nutrients in the soil (Berger et al., 2006). Increased hull area could be essential for the early root establishment. As we showed in the present study, hull area was correlated with yield performance under drought conditions. Similarly, in wheat (*Triticum aestivum* L.), total root surface area of seedling roots was highly correlated with grain yield (Bai et al., 2013). It is noteworthy that a large hull area might be associated with greater metabolic cost for root construction and maintenance, especially during the vegetative stage. Additionally, since plants need to invest more carbon for shoot traits, hull area is needed to be optimized in order to establish an appropriate root system during early development and translocate carbon budget to shoot and other more important root class during the vegetative stage.

Molecular plant breeding techniques have increasingly evolved in the past century. These methods allow plant breeders to identify QTL and genes associated with some root traits including primary root length and seminal root number (Salvi et al., 2016; Sanchez et al., 2018). Additionally, genomic selection, which employs next generation sequencing, allows plant breeders to demonstrate genetic dissection of complex root traits such as total root length. For example, genomic estimated breeding value of total root length was proposed to predict root length within a breeding population (Pace et al., 2015). Therefore, knowledge about root trait translation could facilitate plant breeders to investigate root traits that are impossible to measure in the field such as root emergence angle and hull area. To accelerate plant root phenotyping performance, researchers could integrate molecular plant breeding techniques and our root trait translation concept to overcome a bottleneck on plant root study in order to improve crop production and secure future food security.

Our initial associations between phenotypes observed in the lab and the field demonstrates the feasibility of our approach. We see our initial translation between one lab experiment and one field experiment as an enabling research to foster further investigations that characterize commonalities and differences of root traits among environments, locations and seasons. Further investigations are in particular needed to ensure wide adoption by plant improvement and breeding programs around the world. Certainly, the use of open tools in the present study are an attractive cost-efficient aspect to plant researchers and breeders world-wide and gives the possibility to influence and collaborate on the development of phenotyping technology. Therefore, we recommend a global open data sharing and collaboration on open software development to benefit plant improvement around the world.

CRediT authorship contribution statement

Jirawat Salungyu: Data curation, Formal analysis, Investigation, Methodology, Visualization, Writing - original draft, Writing - review & editing. **Suriphat Thaitad:** Resources, Investigation. **Alexander Bucksch:** Formal analysis, Software, Funding acquisition, Writing - review & editing. **Jitrana Kengkanna:** Investigation, Writing - original draft. **Patompong Johns Saengwilai:** Supervision, Conceptualization, Methodology, Funding acquisition, Resources, Writing - review & editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.fcr.2020.107872.

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