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An effective method for estimation of rice (*Oryza sativa* L.) crown root numbers at the heading stage in saline-sodic soils of Northeast China

Un método eficaz para la estimación del número de raíces de la corona en el estado de espiga en arroz (*Oryza sativa* L.) en suelos salino-sódicos del noreste de China

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Abstract. Saline-sodic stress is a major abiotic constraint responsible for rice (*Oryza sativa* L.) yield reductions in Northeast China. The rice root system is crucial for yield development, and it is usually recognized as the key for improving future crop productivity. However, most of the saline-sodic soils in these areas contain high levels of soluble Na_2CO_3 and $NaHCO_3$, which results in a high pH (>8.5), clay dispersion, soil swelling, and overall poor soil physical properties. Isolation, washing and measurement of the rice crown roots is highly time-demanding in this kind of soil. Our aim was to explore whether differences in shoot characters could be reliable indicators, and a low cost and easy method for estimating crown root numbers in rice. In this study, 97 randomly selected field-grown rice hill plants, with 728 stem samples, of three genotypes at the heading stage were used in saline-sodic soils. Results gave a reliable estimation of values for crown root numbers as indicated by high positive correlations with number of stems (0.788***) and tillers (0.801***); diameter of long (0.545***), short (0.555***), and mean stems (0.586**), and hill (i.e., equal to 5 plants) circumference (0.796***) and mean hill diameter (0.366***) (P<0.001). The method proposed here was very useful in evaluating the crown root numbers through direct measurements under saline-sodic field conditions.

Keywords: Saline-sodic soil; *Oryza sativa* L.; Crown root; Sampling time; Estimation method.

Resumen. El estrés salino-sódico es una limitante abiótica importante responsable de las reducciones en rendimiento del arroz (*Oryza sativa* L.) en el Noreste de China. El sistema radical del arroz es crucial para el desarrollo del rendimiento, y usualmente es clave para determinar la futura productividad de la cosecha. Sin embargo, la mayoría de los suelos salino-sódicos en estas áreas contienen altos niveles de Na_2CO_3 y $NaHCO_3$ solubles, que resultan en un alto pH (>8,5), dispersión de arcilla, hinchamiento del suelo y propiedades físicas del suelo sobre todo bajas. El aislamiento, lavado y medición de las raíces de la corona de arroz es muy demandante de tiempo en esta clase se suelos. Nuestro propósito fue explorar si diferencias en características de la parte aérea podrían ser indicadores confiables, y un método fácil y de bajo costo para estimar el número de raíces de la corona en arroz. En este estudio, 97 grupos de 5 plantas de arroz que crecieron en el campo, con 728 muestras de tallo, de tres genotipos en el estado reproductivo se usaron en suelos salino-sódicos. Los resultados dieron una estimación confiable del número de raíces de la corona como se indicó por correlaciones altas positivas con el número de tallos (0,788***) y macollas (0,801***); diámetro de tallos largos (0,545***), cortos (0,555***) y promedio (0,586***), y circunferencia de 5 plantas (0,796***) y diámetro promedio de 5 plantas (0,366***) (P<0,001). El método aquí propuesto fue muy útil para evaluar el número de raíces de la corona a través de mediciones directas bajo condiciones de campo salino-sódicas.

Palabras clave: Suelo salino-sódico; *Oryza sativa* L.; Raíces de la corona; Tiempo de muestreo; Método de estimación.

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INTRODUCTION

Global demand for food is predicted to increase by 40% by 2030 (Foresight, 2011). Abiotic environmental stress is one of the major factors to determine yield reductions (Ashraf et al., 2008). Production in over 30% of irrigated crops and 7% of dryland agriculture worldwide is limited by salinity stress (Schroeder et al., 2013). This problem is particularly acute in Northeast China, where saline-sodic soils cover more than 6.2% (7.66 million ha) of the region (Lv et al., 2013). To respond to this set of challenges, we need to develop agricultural systems with significantly greater productivity and resilience that at the same time use limited natural resources more efficiently (Lynch & Brown, 2012).

Vigorous root development is important for plants to compete for nutrients and sustain crop yield (Ruffel et al., 2011). The mature rice root system is mainly made up of crown roots of various ages (Hochholdinger, Park, et al., 2004). The most common approach for isolating roots at the field is to extract a soil core and then separate roots from the surrounding soil over a sieve, either by hand or using some type of elutriation system (Benjamin & Nielsen, 2004). However, most of the saline-sodic soil in Northeast China contain high levels of soluble Na_2CO_3 and $NaHCO_3$, which results in a high pH (>8.5), clay dispersion, soil swelling, and overall poor soil physical properties. Obtaining rice crown root characters requires extracting and washing roots from the surrounding soil, which needs a large amount of time and labor. Moreover, a relatively large quantity of mineral grains and organic detritus will remain. It means the difficult task of separating roots from detritus. Such procedures are destructive and likely to underestimate the amount of root material in soil samples (Metcalfe et al., 2007).

Methods that can determine root numbers accurately, repeatedly and inexpensively are desperately needed. The objectives of this study were to use shoot characteristics to develop a statistically method in crown root numbers estimations, which would be of great help in evaluating crown root numbers which are hardly accessible through direct measurements in saline - sodic soils in Northeast China.

MATERIALS AND METHODS

Experiment site and soil properties. Field experiments were conducted in Da'an Sodic Land Experiment Station of the Chinese Academy of Sciences in 2012. The station is located in Baicheng District, Da'an City, Jilin Province of Northeast China (45° 35' 58" - 45° 36' 28" N, 123° 50' 27" - 123° 51' 31" E). The station is under a semi-humid and semi-arid monsoon climate. The local site has a mean annual temperature of 4.7 °C, an annual sunshine duration of 3014 h and an annual precipitation of 370-400 mm, 88% of which occurs from May to September. The annual average available accumulated temperature (≥10 °C) is 2935 °C.

Initially, a field survey was carried out in which several soil samples were collected and analyzed to select a representative soil sample. The samples were collected within a 0-20 cm soil depth, air-dried and then crushed to pass through a 2 mm sieve. The average values of electrical conductivity of saturated extract (ECe), sodium adsorption ratio (SAR), pH in saturated paste and ESP at a 0-20 cm soil depth were 5.11 dS/m, 97.81 $(mmol_c /L)^{0.5}$, 10.44 and 58.35%, respectively. The soil at this site is characterized as saline - sodic with a high soil pH value (Soil Survey Staff, 2010).

Cultivation methods. Three local elite rice genotypes, Dongdao-2, Dongdao-4, and Changbai-9, were grown in a paddy saline-sodic field. Rice seeds were sown on normal soil in a greenhouse on 20 April 2013, and the 40-day seedlings were manually transplanted at a density of 4 plants/hill on 30 May, 2013. Row width was 30 cm, and distance between plants within a row was 16.7 cm, resulting in an overall planting density of 20 hills/m^2. Rice was harvested on 26 September 2013. The N fertilizer was supplied as urea (N=46%), and compound chemical fertilizer (N: P_2O_5: K_2O = 18: 14: 14%) at a rate of 25 g N/m^2. They were split into the basal application seven days prior to transplanting (60% of the total N), side-dressing on June 9 (20% of the total N) and panicle initiation on July 3 (20%). Both P and K were applied as a compound chemical fertilizer at a rate of 19 g/m^2 as a basal application. Water was applied by horizontal irrigation as needed. Two days prior to sampling, the field was irrigated using irrigation cannon with 13 mm of water to soften the soil. This allowed to facilitate excavation of root crowns.

Analysis of samples. At the heading stage, 97 randomly selected rice hill plants (i.e., equal to 5 plants/hill) with 728 stem samples were evaluated. Rice roots were obtained using an auger (15 cm diameter, 40 cm long) with the plant base as the horizontal center of the soil cylinder. Because of the clay saline-sodic soil, most of the remaining soil particles were removed by soaking the root crown in water for three hours and vigorous rinsing at low pressure. The clean roots were visually scored for the following traits: stem (SN), tiller (TN) numbers, crown root number per stem (CRNS) and crown root numbers per hill (CRNH). In addition, long stem diameter (LSD), short stem diameter (SSD), mean stem diameter (MSD), the circumference of the hill (HC) and mean hill diameter (MHD) were measured using a high quality digital vernier caliper (Mitsutoyo caliper/021, Japan). The stem diameter was being estimated based on the number of stems and the circumference of the hill (Morita et al., 1989).

Statistical analysis. The relationship between CRN and shoot characteristics was described by the following linear equation:

$$Y = a + bX \quad (1)$$

Where Y was the dependent variable (CRN); X was the independent variable (shoot characteristics); a and b are regression coefficients. Trait values obtained by estimations and measurements were thereafter correlated. Significant correlations between traits with $R^2 < 0.5$ were considered weak, $0.5 < R^2 < 0.8$ moderate and $R^2 > 0.8$ strong (Trachsel et al., 2013). Variability among plants was assessed by using the coefficient of variation. The statistical analyses were performed using SPSS 19.0.

RESULTS

Relationships among shoot traits and crown root numbers. The properties of 97 hill rice plants and 728 stem samples used to determine the relationships of CRN-stem traits are presented in Table 1. Large phenotype variability was observed among samples; scores for the SN ranged from 1 to 28 and those for TN from 1 to 27. CRNH and CRNS ranged from 36 to 851 and 1to 106, respectively.

A theoretical analysis was made of the relationship among CRN with the shoot characteristics. Moderate-strong correlations were obtained for CRNH and LSD (0.545^{***}), SSD (0.555^{***}), and MSD (0.586^{***}) ($P<0.0001$) (Fig. 1 a-d). CRNS was strongly correlated with TN (0.801^{***}), moderately correlated with SN (0.788^{***}), and HC (0.796^{***}), and weakly correlated with MHD (0.366^{***}) ($P<0.0001$) (Fig. 2 a-c). In addition, TN, HC and SN gave better estimates of CRN than other shoot characters.

Validation of empirical relationships among shoot characters and crown root numbers. The measurements of crown root numbers for all selected genotypes were used to calibrate the values obtained by simulations. Results showed that the trait values were highly correlated and the measurements of the crown roots necessarily reflected the same patterns as the simulations.

A t-test was used to compare predicted CRN equivalents with actual CRN measurements. Mean CRNH predicted by CRNH-SN equation (412.38) was not significantly different ($P>0.05$) from mean actual CRN measurement (412.43) for 97 rice samples. Also, the mean CRNH of 412.43 predicted by CRNH-TN and 412.47 predicted by CRNH-HC regression equation was not significantly different ($P>0.05$) from mean actual CRNH measurements, which were both 412.43. The accuracy of the estimated method was highlighted by moderate to strong correlations found between simulated and measured CRNH values obtained by TN (0.788), SN (0.801) and HC (0.798) ($P<0.0001$) (Fig. 3 a-c), and weak correlations by HD (0.389) (Fig. 3). Also, there were moderate correlations between simulated and measured CRNS values obtained by LSD (0.557), SSD (0.617) and MSD (0.619) ($P<0.0001$) (Fig. 3 e-g). Therefore, CRN of rice planted in saline-sodic soils of Northeast China could be predicted from shoot characteristics. The regression analysis further showed that TN, SN and HC could be better input - parameters than others.

DISCUSSION

In this study, we evaluated the shoot characters and crown root numbers of 97 hill rice plants and 728 stem samples at the heading stage in a saline-sodic field. The crown root numbers per hill showed moderate-strong correlations with stem diameter ($P<0.0001$) (Fig. 1), while crown root numbers per stem showed strongly correlations with tiller number per hill than the stem numbers, the circumference of the hill, and the mean hill diameter ($P<0.0001$) (Fig. 2). However, there was no significant correlation among the crown root numbers and the other shoot characters ($P>0.05$). This may indicate that crown root numbers was mainly affected by tiller and stem numbers at the heading stage in the saline-sodic field.

Abe et al. (1998) had clearly indicated that crown roots were formed from the stem part of phytomers, which were structural units constructing tillers, and the CRN should be closely related with the number of tillers and/or phytomers. Nemoto et al. (1995) also thought that the mean diameter of tillers may be useful, as the diameter of the stem often affected the diameter of crown roots emerged from the stem. The results of the present study provide further evidence that not only the number of tillers and stems, but also the stem diameter of the either stem or hill, might be used for a quantitative analysis of the crown root numbers.

Our results were consistent with evidence presented previously. Abe et al. (1998) reported that in a pot experiment, the regression formula between CRN and TN was as follow: $Y=15.2X$, where Y was dependent variable (CRN) and X was the independent variable (TN). In our study, the regression formula was $Y = 162.426 + 20.43X$. The regressions of CRN to the TN for saline-sodic soils of Northeast China were different; this result indicated that the couple was not constant, because it was influenced by numerous factors such as soil types and sampling stage.

Current methods for characterizing root growth in saline-sodic stress were based on measurements of seedlings grown in artificial media (Lv et al., 2013a; 2013b). The artificial systems perhaps did not mimic highly heterogeneous, natural growth conditions as encountered in soils. Root growth in artificial media can differ from that of plants grown in heterogeneous soil environments (Chapman et al., 2012; Rich & Watt, 2013). Roots typically show large variability, especially when grown under natural environmental conditions. Root growth might be affected by seed reserves at the seedling stage, and constrained by the size of the volume/area of the growth media or container at late growth stages. Relative to undisturbed soil, differences in soil bulk density as a result of sieving and recompacting can equally affect elongation rate and trajec-

Table 1. Summary statistics for properties of 97 randomly selected rice hill plants with 728 stem samples of three genotypes collected at the heading stage in saline-sodic soils.
Tabla 1. Resumen estadístico de las propiedades de 97 grupos de 5 plantas de arroz seleccionadas al azar con 728 muestras de tallo de tres genotipos recolectados al espigar en suelos salinos y sódicos.

Statistic	SN	TN	LSD (mm)	SSD (mm)	MSD (mm)	HC (mm)	CRNH	CRNS
Minimum	1.00	1.00	2.71	2.31	2.49	2.40	36.00	1.00
Maximum	28.00	27.00	10.09	9.08	9.19	18.00	851.00	106.00
Mean	12.63	12.24	6.71	5.48	6.09	10.37	412.43	27.89
Median	13.00	11.00	6.83	5.52	6.22	11.30	415.00	23.00
Coefficient of variation (%)	57.06	58.53	18.30	20.48	18.11	37.46	44.32	70.51

Data were taken at the heading stage on Dongdao-2, Changbai-9 and Dongdao-4. Stem numbers (SN), tiller number (TN), long stem diameter (LSD), short stem diameter (SSD), mean stem diameter (MSD), hill circumference (HC), crown root numbers per hill (CRNH), and crown root numbers per stem (CRNS).

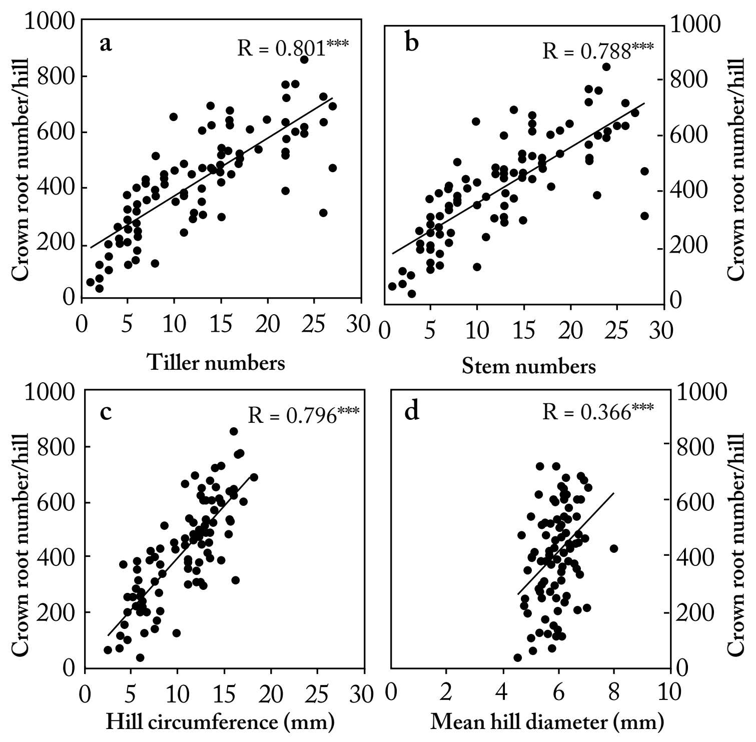

Fig. 1. Relationships of the rice shoot characteristics and the crown root numbers per hill at the heading stage in saline-sodic soils. Data were taken at the heading stage of Dongdao-2, Changbai-9 and Dongdao-4, n=97. Scatter plots of (a) crown root numbers per hill vs. stem numbers, (b) crown root numbers per hill vs. tiller numbers, (c) crown root numbers per hill vs. hill circumference, (d) crown root numbers per hill vs. mean hill diameter (P<0.0001).
Fig. 1. Relaciones de las características de brotes de arroz y los números de raíces de la corona por colina al espigar en suelos salino-sódicos. Los datos se obtuvieron al espigar en Dongdao-2, Changbai-9 y Dongdao-4, n=97. Gráficos de dispersión de (a) el número de raíces de la corona por grupo de 5 plantas, (b) el número de raíces de la corona por grupo de 5 plantas vs el número de macollas, (c) el número de raíces de la corona por grupo de 5 plantas vs la circunferencia del grupo de 5 plantas, (d) el número de raíces de la corona por grupo de 5 plantas vs el diámetro promedio del grupo de 5 plantas (P<0,0001).

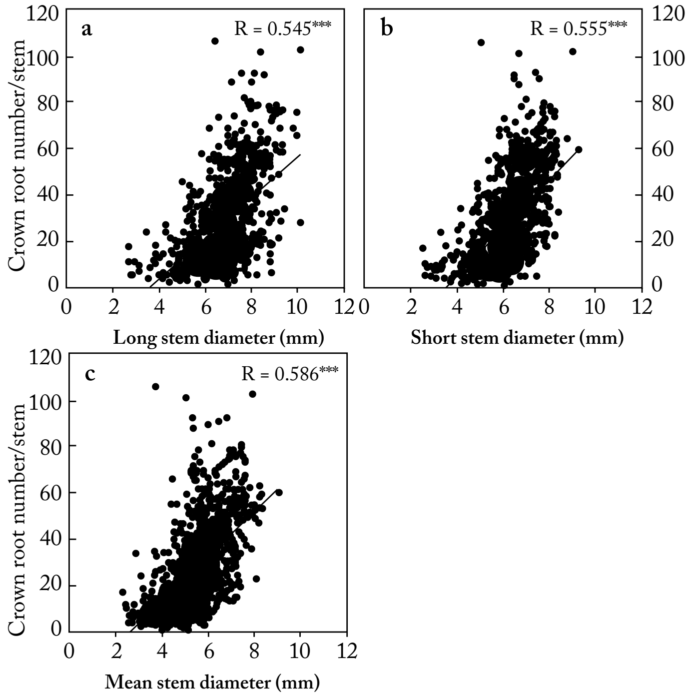

Fig. 2. Relationships of the rice shoot characteristics and the crown root numbers per stem at the heading stage in saline-sodic soils. Data were taken at the heading stage of Dongdao-2, Changbai-9 and Dongdao-4, n=728. Scatter plots of (a) crown root numbers per stem vs long stem diameter, (b) crown root numbers per stem vs short stem diameter, (c) crown root numbers per stem vs mean stem diameter (P<0.0001).

Fig. 2. Las relaciones de las características de brotes de arroz y los números de la raíz de la corona por tallo en la etapa epígrafe de suelos salino-sódicos. Los datos se obtuvieron al espigar en Dongdao-2, Changbai-9 y Dongdao-4, n=728. Gráficos de dispersión de (a) el número de raíces de la corona por tallo vs el diámetro mayor del tallo, (b) el número de raíces de la corona por tallo vs el diámetro menor del tallo, (c) el número de raíces de la corona por tallo vs el diámetro promedio del tallo (P<0,0001).

tory of growing roots (Trachsel et al., 2013). Moreover the root system is buffered from the atmospheric environment in a completely different way when grown in a small container compared to the field. Hence, there is a high risk for artifacts of root growth or root-shoot-interactions in such investigations, when aiming to estimate field - situations (Walter et al., 2009).

However, characterizing root growth in the field is challenging. The large time investment, and the resulting financial (i.e. personnel) cost, is the primary limiting factor for field sampling of rice roots. As a consequence, several researchers have tried to decrease the time invested in root sampling and analysis (Benjamin & Nielsen 2004; Metcalfe et al., 2007). Studies on growth of root systems in the field have been restricted by the lack of easy methods (Gregory et al., 2009; Huang et al., 2013). Most studies measure changes in root biomass and length, which requires roots to be extracted from soil, separated from the dead roots and roots of other species, and then quantified using image analysis, and/or dried to measure root dry weight. Other methods such as excavation and shovelomics have also been used (Zhu et al., 2010; Trachsel et al., 2011). The time needed for washing, together with the duration of root picking, represented most of the time spent per sample. The methods using a washing process of the soil samples may lead to the loss of a significant proportion of roots (Pearson & Jacobs, 1985), and measurement errors due to difficulties in adjusting the apparatus and preparing the root sample (Dusserre et al., 2009). These constraints have restricted studies on root systems in the field. Quantitative shoot characters in rice could detect short- and long-term changes of crown root numbers in response to stresses in saline-sodic soil with a high degree of accuracy, also with results directly obtained in the field.

In Northeast China, the saline-sodic soils contain high levels of soluble Na_2CO_3 and $NaHCO_3$ salts , the hydraulic conductivity at saturation is only 0.02 - 0.22 mm/d, which is in the low water-permeability range. Salinity and sodicity result in poor penetration of water, air and roots, low readily available water holding capacity. Depending on the poor physical and chemical properties of the saline-sodic soils, excavation and removing the soil surrounding the root crowns may require about 2 hours per hill. In our study. however,

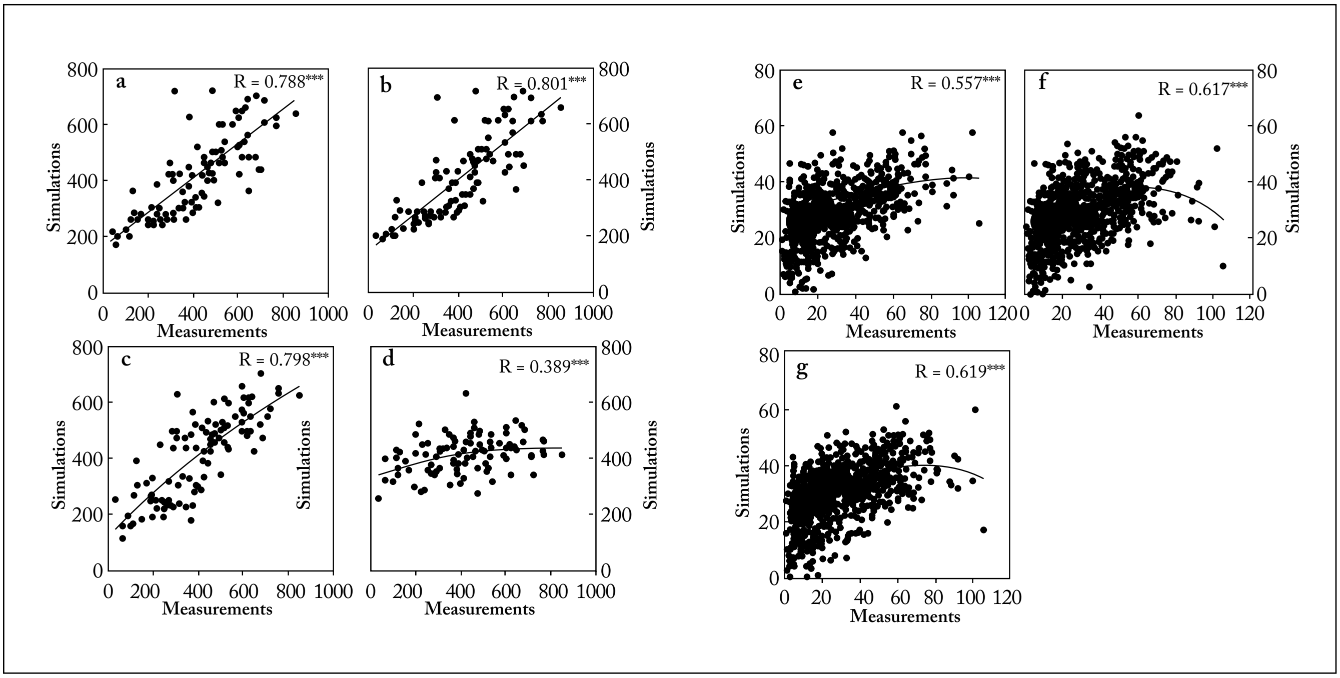

Fig. 3. Relationships of the estimations and measurements of rice crown root numbers at the heading stage in saline-sodic soils. Data were taken at the heading stage of Dongdao-2, Changbai-9 and Dongdao-4. Scatter plots of CRNS measurements vs. estimations (a) by stem numbers n=97, (b) by tiller numbers n=97, (c) by hill circumference n=97, (d) by hill diameter n=97, (e) by long stem diameter n=728, (f) by short stem diameter n=728, (g) by mean stem diameter n=728 (P<0.0001).

Fig. 3. Relaciones de las estimaciones y mediciones del número de raíces de la corona de arroz al espigar en suelos salinos y sódicos. Los datos se obtuvieron al espigar en Dongdao-2, Changbai-9 and Dongdao-4. Gráficos de dispersión de las mediciones de CRNS vs estimaciones (a) por el número de tallos n=97, (b9 el número de macollas n=97, (c) la circunferencia de 5 plantas n=97, (d) el diámetro de 5 plantas n=97, (e) el diámetro mayor del tallo n=728, (f) el diámetro menor del tallo n=728, (g) el diámetro promedio del tallo n=728 (P<0,0001).

estimations of the crown root number only required 5 min per sample which largely decreased the root sampling time investment. Based on these results, it appears feasible to measure rice shoot traits in other plant stages to improve the evaluated method. It might be possible to extrapolate trait values for other crown root characters, such as root volume and length. This would both reduce the time required for the evaluation of one sample as well as destroying the rice growth in the field. It should be noted that this study examined only three genotypes in the heading stage; the accuracy of measured trait values could be further increased by replicating the genotypes. However, this would entail increases in labor and land requirements, and several adaptations at different growth stages in future experiments. Moreover, it will be interesting to see how root angles measured on the root crown affect root distribution in the soil. We are currently investigating these topics.

In conclusion, our results showed that this low cost and easy method may provide a way of increasing the number of root samples processed per unit time; it is very useful in evaluating the crown root numbers which are hardly accessible through direct measurements in saline-sodic soils in Northeast China.

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