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Simple estimation of green area rate using image analysis and quantitative traits related to plant architecture and biomass in rice seedling

Kwang-O. Jong · Kwang-Myong Han · Son-Il Kwak · Yu-Jin Jang · Chol-Ho · Kwang -Phil Kim

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Abstract Available methods using image analysis (IA) are expensive and evaluated only partally in rice plants in controlled and field conditions. The objectives of this study were to: (i) estimate green area rate per seedling (GAR) in various rice cultivars and lines using new IA software, and (ii) elucidate relationship between GAR and some quantitative traits in seedlings grown at nursery. After taken randomly seedlings grown for 45 days at nursery, digital image of individual seedling was taken using a digital camera. Digital images were processed with IA software using k-means algorithm. After obtained R (red), G (green) and B (blue) values of each pixel from seedling image, these values were transformed into HSB color system. Pixel counts were obtained from HSB data, and GAR was estimated as rate of pixel counts of green area per seedling and pixel counts of input image. Results indicated that GAR was quantitative index and phenotyping trait that can be measured simply by non-destructive method using IA software without any significant alteration of plant morphology. GARs were significantly different among genotypes. GAR was significantly and positively correlated with number of tillers per seedling (NTS), leaf number per seedling (LNS), seedling dry mass (SDM) and aboveground total dry mass (TDM), i. e., aerial biomass in seedling and wasn't significantly correlated with seedling height. Significant level for linear regression of GAR and SDM, TDM related to seedling was shown with high determination coefficients of 0.796–0.8762. GAR had a stronger relationship with SDM and TDM related to biomass than NTS and LNS.

Keywords Correlation coefficient · Growth · Image-based phenotype · Linear regression model · Nursery · Phenotyping

K.-O. Jong (✉) · K.-M. Han · Chol-Ho · K. -PhilKim
Department of Genetics, Faculty of Life Science, Kim Il Sung University, Ryongnam Dong, Taesong District, Pyongyang, Democratic People's Republic of Korea
e-mail: life5@ryongnamsan.edu.kp

S.-I. Kwak · Y.-J. Jang
Faculty of Information Science, Kim Il Sung University, Ryongnam Dong, Taesong District, Pyongyang, Democratic People's Republic of Korea

1 Introduction

Data for quantitative traits such as nitrogen content in plant, biomass, leaf area index (LAI), the number of tillers and plant height in the experimental fields, are widely collected to compare the characteristics of cultivars or lines, mutants and transgenic plants, i.e. differences in growth and development (Lubis et al. 2003), fertilization response (Nakano et al. 2008), response to biotic or abiotic stress (Sarangi et al. 2015), and soil fertility conditions (Jearakongman et al. 2003) in crops. Producing high quality seedlings requires periodically collecting seedling growth data to control growth and development of seedlings at nursery. Moreover, it is indispensable to measure many seedlings with different genetic backgrounds to collect data for quantitative traits related to their growth and development (Abdelkahalik et al. 2005).

Morphological traits provide a feasible way to assess plant growth, development and impacts of biotic or abiotic stress and yield (Fahlgren et al. 2015). In the conventional methods for analyzing the whole-plant phenotype in rice, plant architecture (PA) is measured with a ruler or just by counting the the number of leaves and tillers that are well known parameters to indicate the growth and development (Ishizuka et al. 2005). Hand-held measuring of quantitative seedling traits under field conditions may introduce fluctuation into measurements. Moreover, since the conventional measurement of quantitative traits such as plant height, length and width of leaf blade, the number of tillers and leaves in rice is often time-consuming and labor-intensive work (Morishima et al. 1967; Abdelkahalik et al. 2005), the establishment of more efficient methods for data collection from the experimental fields would facilitate the precise statistical analyses, model building/validation, database construction, and selection of lines for breeding of high-yielding rice cultivars (Shibayama et al. 2011).

Attempts have been made to improve the method using image analysis (IA) in rice for estimation of stratified leaf area (Oka and Hinata 1986), characterization of plant architecture (PA) (Suzuki et al. 2011), comparison of PA between new and old cultivars (Oka and Hinata 1989), kinetic measurement of growth (Ishizuka et al. 2005), and evaluation of the degree of sprout leaf bending (Zheng et al. 2008).

Some IA methods evaluated only part of the rice plant in controlled and field environments (Suzuki et al. 2011; Duan et al. 2015; Gong et al. 2018). In addition, many IA methods for phenotyping are used on the complex camera apparatus (Ishizuka et al. 2005), 3D scanalyzer system (Hairmansis et al. 2014), multispectral and hyperspectral remote sensing (Huang et al. 2012; Golzarian et al. 2017), thermal infrared imaging (Jones et al. 2009), laser imaging (Paulus et al. 2014), fluorescence imaging (Harbinson et al. 2012), 3D imaging (Klose et al. 2009) and nuclear magnetic resonance imaging (Hillnhütter et al. 2012), positron emission tomography (Poorter et al. 2012) and computed tomography (Karunakaran et al. 2004). Since these approaches are expensive and complex, these seem to be difficult to apply low-cost and simple methods to many plants and populations in controlled and field environments.

In general, the phenotyping of quantitative traits in crops is labor intensive and expensive (Polania et al. 2017). Thus, the development of highly accurate and efficient morphological data acquisition and processing approaches is of great significance for plant phenotyping, plant physiology, and further plant breeding (Tardieu et al. 2017).

In order to compare the early growth and development in hybrid plants, mutants and transgenic plants with the high-yielding potential, quantitative traits such as plant height, number of tillers, number of leaves and plant dry mass have been used (Abdelkahalik et al. 2005). Leaves are the primary structure for photosynthesis (Yan et al. 2012). Ideotypes with high-yielding potential should exhibit a large leaf area in rice (Hu et al. 2013). Thus, to investigate a set of plants grown at nursery, it is necessary to establish the measurement method that not only reflects some quantitative traits but also is easy to apply to elevated seedling number. Although the study on quantitative evaluation of PA using P-type Fourier descriptors in seedling (Suzuki et al. 2011) was performed, no data to elucidate the relationship between green area rate per seedling (GAR) and quantitative traits in various genotypes have been conduced; GAR might show any relationship with some quantitative PA and biomass traits in seedlings grown at nursery.

The objectives of this study were to: (i) estimate GAR in various rice cultivars and lines using IA software, and (ii) elucidate the relationship between GAR and some quantitative PA and biomass traits of seedlings grown at nursery.

2 Material and methods

2.1 Material

In present experiment, we used four *japonica* rice (*Oryza sativa* L. ssp. *japonica*) cultivars: cv. Pyongyang 53, cv. Phyongbuk 18, cv. Sohae 8, cv. Sohaechal 16, two *japonica* inbred lines: 5-39-1 and 10-21-1, and an *indica* rice (*O. sativa* L. ssp. *indica*) cultivar: cv. Tongsung 1. The former four cultivars and the last cultivar are the leading cultivars in the western regions of Democratic People's Republic of Korea (DPR Korea). Seeds of genotypes were provided by the Rice Research Institute, the Academy of Agricultural Sciences (AAS) of DPR Korea.

2.2 Site description

The experiment for growth of rice seedling was carried out in trial field (lat 39° 01′10″N, long 125° 44′44″E, alt 30 m a.s.l.) of life science faculty of Kim Il Sung University in 2019.

2.3 Experimentation and data collection from nursery in paddy field

A randomized split-plot design (a plot size of 1.0 m × 1.5 m with three replicates) was used in the present experiment. The seeds were sown manually in each plot at a rate of 9 g of seeds m^{-2} on April 6, 2019. Chemical fertilizers of N, P_2O_5 and K_2O were applied at a concentration of 30 g m^{-2} each as a basal manure and 30 g m^{-2} each as an additional manure. Plots were regularly hand-weeded and pesticides were used to prevent pest damage. Plant height and tillering in rice are crucial factors determining rice PA and influencing grain production. Hence we measured some quantitative traits involving the seedling height (SH) and number of tillers per seedling (NTS). After taken randomly for each genotypes (except the border seedling at plots) grown for 45 days at nursery after sowing, seedlings were washed with water, and then SH, NTS and LNS (leaf number per seedling) were hand-held measured, respectively.

Seedling dry mass (SDM) was weighed after 48 h in an oven at 80 °C to a constant mass, and after cutted root in seedling dried in the oven, aboveground total dry mass (TDM), i. e., aerial biomass was determined.

2.4 Image capture and image analysis procedure

After taken randomly ten seedlings, photograph of individual seedling profile was taken using the digital camera (SONY, Japan, 5.1 Mega Pixels) at a height of 25 cm on white background (Fig. 1a).

The Original side-view digital images of photographs taken from seedling were processed with the IA software (Golden Field 1.0) developed by authors using k-means algorithm (Hartigan and Wong 1979) (Fig. 1b). After obtained R (red), G (green) and B (blue) values of each pixel from the corresponding side view RGB images, these values were transformed into HSB color system. The pixel counts were be obtained from the HSB data.

GAS (green part area including leaves and stem except root in seedling) was estimated from the side-view digital image of seedling. It was the projection of a seedling body including leaves and stem except root to two-dimensional (2D) plane.

GAR, relative index, was calculated as the rate of pixel counts of GAS and total pixel counts of input image.

$$GAR(\%) = (\text{pixel counts of GAS / total pixel counts}) \times 100$$

2.5 Data analysis

Statistical analyses were performed using the SPSS 21.0 (SPSS Inc. Chicago, IL, USA). Means were compared based on the Fisher's least significant difference (LSD)

Fig. 1 Image acquisition from rice seedling using digital camera (**a**) and processing of image using IA software (**b**) (online color)

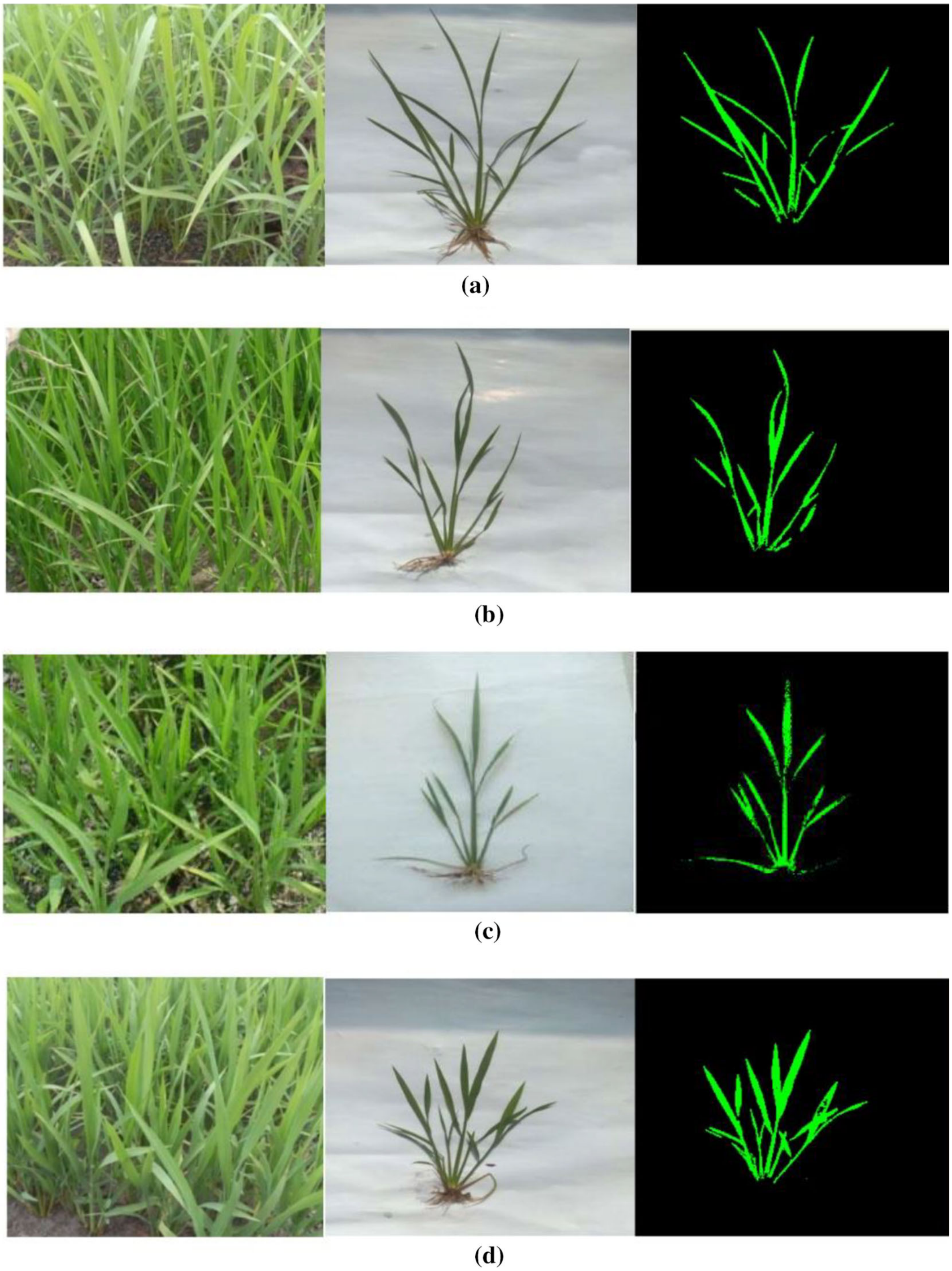

Fig. 2 Seedlings grown during 45 days after sowing at nursery in paddy field (left), Original side-view digital images of 45 days old seedlings after sowing of accessions (middle), and corresponding side view RGB images of processed with IA software (right) (online color). **a** *Japonica* rice cv. Pyongyang 53, **b** *Japonica* rice cv. Phyongbuk 18, **c** *Japonica* rice cv. Sohae 8, **d** *Japonica* rice cv. Sohaechal 16, **e** *Indica* rice cv. Tongsung 1, **f** *Japonica* rice line 5–39-1, **g** *Japonica* rice line 10–21-1

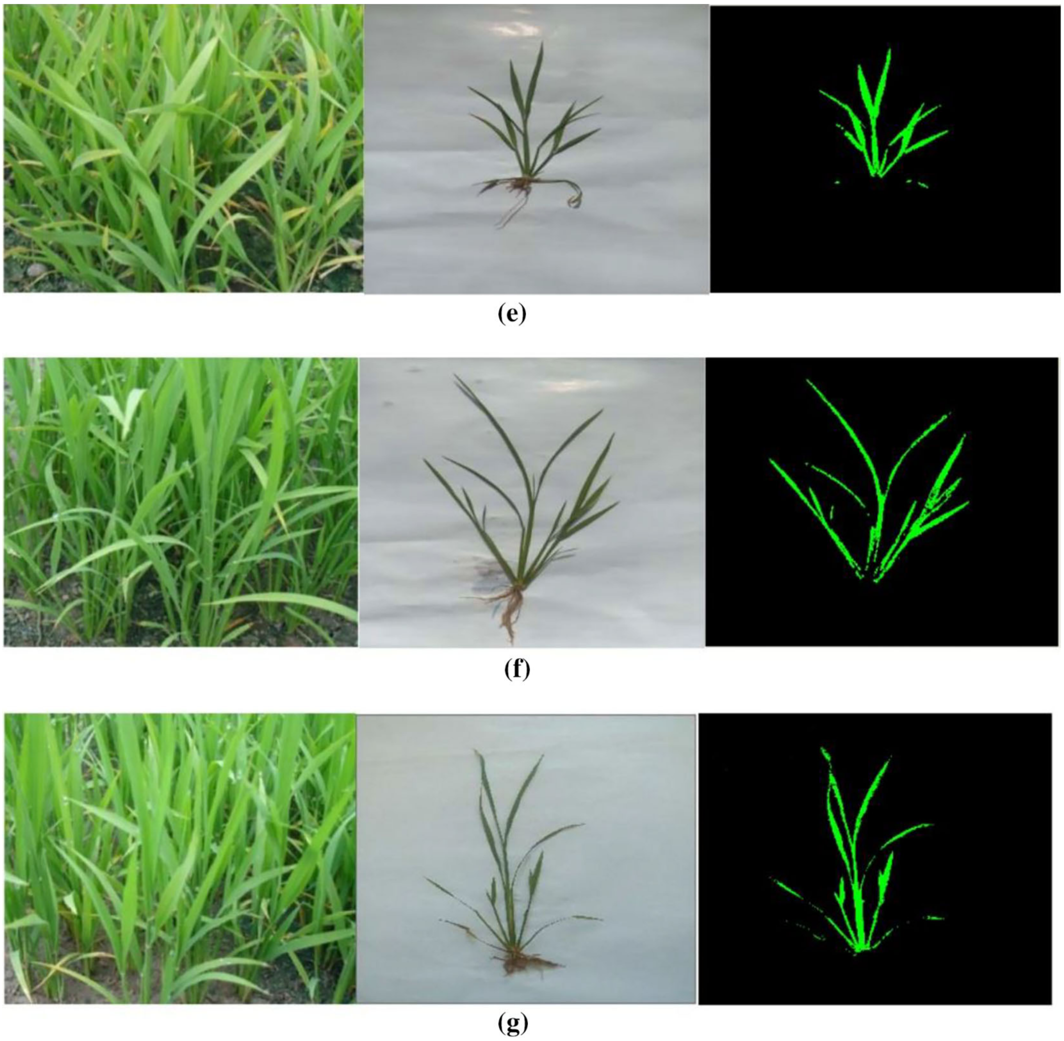

Fig. 2 continued

test at the 0.05 level. Pearson correlation analysis with two-tailed test was used to determine whether a significant relationship existed between GAR and some quantitative PA and biomass traits in seedling. The regression model was built in SPSS using curve estimation with the least square method.

3 Results

3.1 Differences in some quantitative traits at nursery

Various genotypes were visually discriminated by PA in seedling (right of Fig. 2). Some quantitative traits such as SH, NTS and LNS were significantly different among genotypes (Table 1). In addition, TDM and SDM were also significantly different among genotypes. TDM and SDM of cv. Pyongbuk 18 were largest as 0.34 g and 0.52 g among genotypes, respectively. TDM of line 10-21-1 was least as 0.20 g among genotypes, and SDM of cv. Sohae 8 was least as 0.24 g among genotypes.

3.2 GAR at nursery

Leaves of plants did not overlap in 45 days old seedlings. When the original side-view digital images (middle of Fig. 2) were processed with IA software, plant body including leaves and stem except the root in

Table 1 Some quantitative traits in seedlings grown for 45 days after sowing at nursery

Cultivars and lines	SH (cm)	NTS	LNS	TDM (g)	SDM (g)
cv. Pyongyang 53	26.4 ± 2.9[a]*	4.0 ± 0.1[a,*]	7.5 ± 0.2[a,*]	0.33 ± 0.06[a,*]	0.42 ± 0.06[a,*]
cv. Phyongbuk 18	25.1 ± 2.1[a]*	4.0 ± 0.1 [a,*]	7.7 ± 0.3[b,*]	0.34 ± 0.12[b,*]	0.52 ± 0.16[b,*]
cv. Sohae 8	23.9 ± 0.8[a,b,*]	2.4 ± 0.7 [c]	6.4 ± 0.5[c,*]	0.20 ± 0.05[c,*]	0.24 ± 0.05[c,*]
cv. Sohaechal 16	20.7 ± 1.4[b]*	3.6 ± 0.5[a,b,*]	7.2 ± 0.2[d,*]	0.25 ± 0.06[c,*]	0.31 ± 0.0[d,*]
cv.Tongsung 1	16.3 ± 1.4[c]*	3.2 ± 0.8[b,*]	6.9 ± 0.6[e,*]	0.25 ± 0.09[c,*]	0.32 ± 0.0[e,*]
line 5–39-1	23.8 ± 2.4[a,b]*	2.3 ± 1.1[c,*]	6.7 ± 0.6[f,*]	0.22 ± 0.09[d,*]	0.27 ± 0.1[f,*]
line 10–21-1	26.1 ± 3.1[a]*	1.7 ± 1.0[d,*]	6.1 ± 0.5[g,*]	0.20 ± 0.08[c,*]	0.26 ± 0.13[g,*]

Values are means ± standard errors with the results of statistical analysis (n = 10)

SH seedling height, *NTS* number of tillers per seedling, *LNS* leaf number per seedling, *TDM* aboveground total dry mass, *SDM* seedling dry mass

*Means in each column followed by the same letters are not significantly different at $p < 0.05$ level by the Fisher's LSD test

seedling was changed the corresponding side view RGB images (right of Fig. 2). GARs were significantly different among genotypes (Table 2). Especially, GAR of cv. Pyongyang 53 was largest as 3.62 among genotypes, whereas one of cv. Sohae 8 was least as 2.12.

3.3 Relationship between GAR and some quantitative PA and biomass traits

GAR was significantly and positively correlated with NTS, LNS, SDM and TDM ($r = 0.83$, $p < 0.05$), and was not significantly correlated with SH (Table 3). Correlation coefficient (r) between GAR and TDM was highest as 0.94 ($p < 0.01$).

Positive correlation among GAR and some quantitative traits was established with a high level of significance (Figs. 3, 4). The linear regression models showed the relationships between GAR, NTS, LNS with the determination coefficient (R^2) of 0.6958 and 0.7789. However, GAR had poor linear relationship with SH ($R^2 = 0.0365$). Significant level for the linear regression models of GAR and SDM, TDM were shown with the high determination coefficients of 0.796 and 0.8762. GAR had a stronger relationships with SDM and TDM than NTS and LNS.

4 Discussion

4.1 Differences in GAR and some quantitative PA and biomass traits among genotypes

In previous studies using IA, PA in rice seedling has been not expressed comprehensively (Zheng et al. 2008; Suzuki et al. 2011). 3D PA is spatial arrangement of the aboveground parts in plants and canopies (Yan et al. 2012). Architecture of leaves and stem is essential for elevating the efficiency of the light harvest in plant (Hayashi 1972). Thus, PA is one of the most important types of phenotyping traits (Wang et al. 2019).

SH, LNS, seedling biomass, leaf area per seedling are well known parameters to indicate the growth and development of seedling (Sarangi et al. 2015).

SH, NTS, LNS, TDM and SDM were significantly different among genotypes (Table 1).

GAR in this study is the relative value and image-based phenotyping trait that can be measured simply by the non-destructive method using IA software without any significant alteration of plant morphology (Table 1; Fig. 2). GAR is conceptually different with LAI (total one-side area of leaf tissue per unit ground surface area (Watson 1947)). Although LAI is a major index of photosynthetic production in crops, the measurement requires equipments such as laser leaf area meter, plant canopy analyzer and scanner, and data collection for the determination of LAI is time-consuming and labor-intesive (Shibayama et al. 2011; Hu et al. 2013). However, estimation of GAR using IA software can be proposed as one of the simple, non-destructive, and repeatable assessment methods that don't introduce principally fluctuation into measurement.

4.2 Relationships between GAR and some quantitative PA and biomass traits

The elucidation of relationship among quantitative traits in crop may facilitate the selection of the most

Table 2 Green are per seedling (GAR) among the assessed rice genotypes

Cultivars and lines	Pixell counts of GAS	GARs (%)
cv. Pyongyang 53	3 059 ± 182	3.62 ± 0.02[a,*]
cv. Phyongbuk 18	3 025 ± 238	3.58 ± 0.28[b,*]
cv. Sohae 8	1 791 ± 191	2.12 ± 0.23[f]
cv. Sohaechal 16	2 619 ± 168	3.10 ± 0.20[c,*]
cv. Tongsung 1	2 391 ± 320	2.83 ± 0.03[d,*]
line 5–39-1	2 231 ± 196	2.64 ± 0.28[e,*]
line 10–21-1	2 256 ± 196	2.67 ± 0.21[e,*]

Totall pixel counts = 84,500

Values are means ± standard errors with the results of statistical analysis (n = 10)

*Means in each column followed by the same letters are not significantly different at $p < 0.05$ level by the Fisher's LSD test

GAS green part area including leaves and stem except root in seedling, *GAR* green area rate per seedling

Table 3 Phenotypic correlation coefficients (r) among Green are per seedling (GAR) and some quantitative PA and biomass traits in seedlings

Traits	SH	NTS	LNS	TDM	SDM	GAR
SH		− 0.12	− 0.01	0.18	0.21	0.19
NTS			0.97**	0.90**	0.82*	0.83*
LNS				0.94**	0.89**	088**
TDM					0.97**	0.94**
SDM						0.89**
GAR						

Results marked with *, **are significant at the 0.05 and 0.01 probability levels, respectively

SH seedling height, *NTS* number of tillers per seedling, *LNS* leaf number per seedling, *TDM* aboveground total dry mass, *SDM* seedling dry mass, *GAR* green area rate per seedling

important characteristic in breeding programs (Sabesan et al. 2009; Polania et al. 2017). However, no data to elucidate the relationship between image-based phenotype and quantitative traits have been conduced in rice.

Since the measurement for quantitative PA traits takes much time and labor (Morishima et al. 1967; Abdelkahalik et al. 2005), PA in rice has been estimated visually and empirically in the pratical breeding (Yang and Hwa 2008). By using IA, some researchers have been investigated PA of rice seedling grown at pot (Oka and Hinata 1988; Suzuki et al. 2011). However, because the distribution of leaves and tillers in plant is affected by the environmental conditions such as temperature (Huang et al. 2001), nitrogen supply (Amin et al. 2002), photoperiod (Goto 2003), especially plant stand density (Ariyaratna et al. 2011; Zand and Shakiba 2013), PA of seedling grown at nursery may be different with that grown at pot.

Tillering in rice is not only one of the key factors relating to PA but also a central subject concerning grain yield (Zhao et al. 2006; Liang et al. 2014).

In practice, the evaluation of PA in cultivars and growing conditions has been carried out by the experience and impression (Yang and Hwa 2008).

Although the present method had a shortcoming that the processing image was 2D projection of spatially arranged leaves and tillers, PA in seedling was well characterized (Fig. 2) than results of computer IA in rice (Oka and Hinata 1988, 1989; Suzuki et al. 2011).

Based on the estimation of GAR using IA software, we elucidated the relationship between it and some quantitative PA and biomass traits in seedling grown at nursery. GAR was significantly and positively correlated with NTS, LNS, SDM and TDM, and wasn't significantly correlated with SH (Table 3). In addition, The linear regression model could be used to describe the relationship between GAR and NTS, LNS, and SDM, TDM (Figs. 3, 4). The results showed that GAR increased linearly with increasing SDM and TDM. Based on the relations, it seems to be possible to present the biomass properties of seedling from GAR.

This survey approach using IA seems to be not only simpler, faster and more repeatable but also to be time- and labor-saving than the other methods (Oka and Hinata 1988; van Hees and Mead 2000; Suzuki et al. 2011).

In general, the condition and management of the nursery define the quality of rice seedlings, and producing high-quality seedlings mostly depends on the management of nurseries (Lampayan et al. 2015). Thus, GAR estimated using IA software seems to be an index that can carry out assessment of not only the condition and management of the nursery but also the quality of growth and development of seedling.

Based on the concept of ideotype (Peng et al. 2008), in the future study we intend to elucidate relatiobship between GAR and some quantitative PA and biomass traits from the seedling stage to the mature stage, and

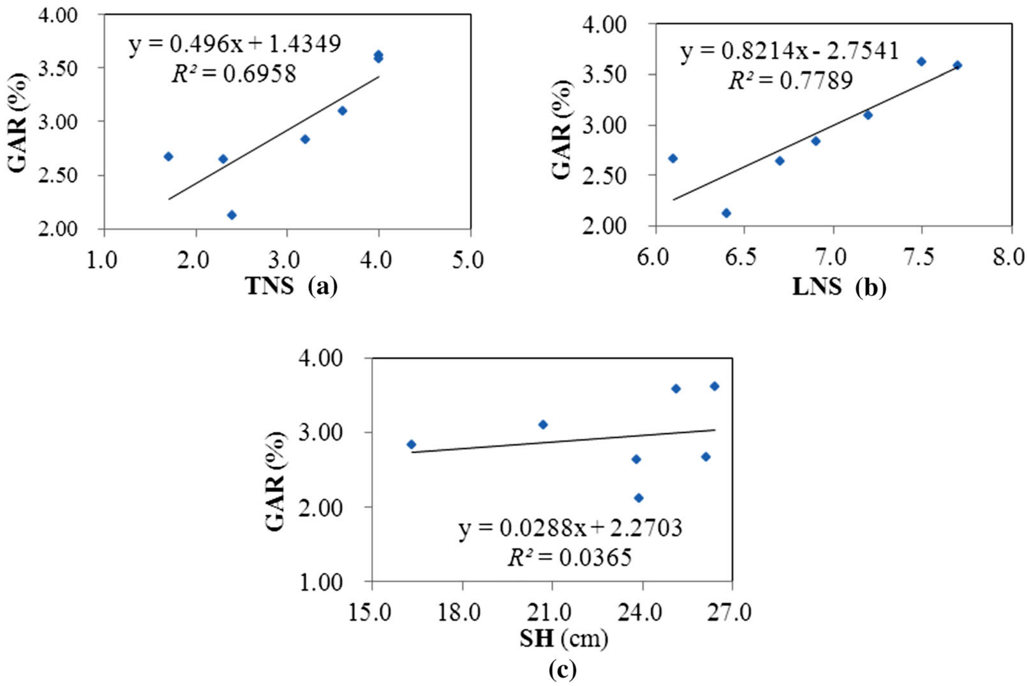

Fig. 3 Relationships between the green area rate per sedling (GAR) and number of tillers per seedlings (NTS) (**a**), leaf number per seedling (LNS) (**b**) and seedling height (SH) (**c**)

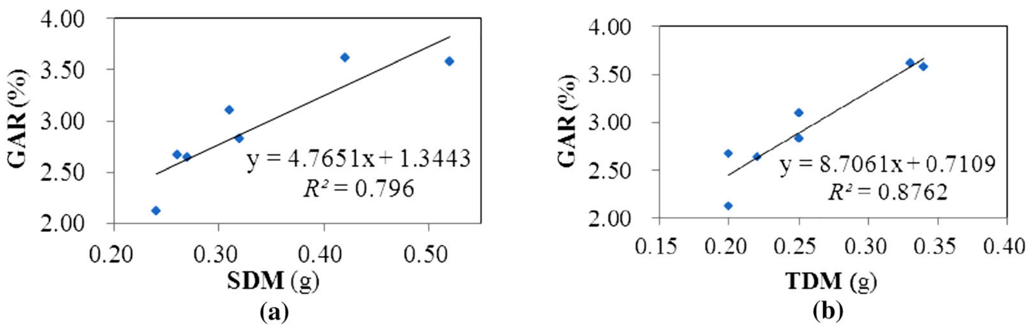

Fig. 4 Relationships between green area rate per seedling (GAR) and seedling dry mass (SDM) (**a**) and aboveground total dry mass (TDM) (**b**)

finally to evaluate yield components from GAR in cultivars with high-yielding potential.

We intend to make available the present method without charge to anyone in the world who is interested in image-based phenotyping in rice.

The results from this study suggested that GAR estimated using IA software from digital image of seedling grown at nursery is the image-based phenotype, i.e., phenotyping trait. GAR was significantly and positively correlated with NTS and LNS, and SDM and TDM to related biomass, respectively. The linear regression model showed the relationship between SDM, TDM, NTS and LNS with GAR in seedling. It seems to be possible to present the biomass and PA properties of seedling from GAR. The assessment on GAR estimated using IA software seems to be the new method for the quantitative selection index of seedling at nursery.

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Author Contributions Kwang-O Jong conceived and designed this study and wrote the manuscript, Kwang-Myong Han and Kwang-Phil Kim conducted data gathering, Son-Il Kwak and Yu-Jin Jang developed IA software (Golden Field 1.0) and performed IA and Chol Ho performed statistical analyses. All authors read and commented on the manuscript.

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Data availability Seeds of rice cultivars used in this study were provided by the Rice Research Institute (RRI), the Academy of Agricultural Sciences (AAS) of DPR Korea.**Code availability**. Authors have not been used any code.

Declarations

Conflict of interest All authors declare that they have no conflict of interest.

Ethical approval This manuscript has not been published before, and is not under consideration for publication anywhere else. *Required Statements* Rice researchers can use the results in this study for the survey of rice seedling at nursery.

Informed consent All co-authors have been participated equally in this research. This manuscript publication has been approved by all co-authors.

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