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Studies of the relationship between rice stem composition and lodging resistance

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Abstract

Plant height and lodging resistance can affect rice yield significantly, but these traits have always conflicted in crop cultivation and breeding. The current study aimed to establish a rapid and accurate plant type evaluation mechanism to provide a basis for breeding tall but lodging-resistant super rice varieties. A comprehensive approach integrating plant anatomy and histochemistry was used to investigate variations in flexural strength (a material property, defined as the stress in a material just before it yields in a flexure test) of the rice stem and the lodging index of 15 rice accessions at different growth stages to understand trends in these parameters and the potential factors influencing them. Rice stem anatomical structure was observed and the lignin content the cell wall was determined at different developmental stages. Three rice lodging evaluation models were established using correlation analysis, multivariate regression and artificial radial basis function (RBF) neural network analysis, and the results were compared to identify the most suitable model for predicting optimal rice plant types. Among the three evaluation methods, the mean residual and relative prediction errors were lowest using the RBF network, indicating that it was highly accurate and robust and could be used to establish a mathematical model of the morphological characteristics and lodging resistance of rice to identify optimal varieties.

Introduction

To breed super rice, Yuan Longping, an academic at the Chinese Academy of Sciences, proposed developing long-stalked rice varieties to increase biological yield. An ideal rice plant type that exhibited no decrease in the number of spikes per m^2, high harvest index and enhanced lodging resistance was evaluated to transform the rice plant from having a dwarf and semi-high stature to high and even super-high stature of over 160–170 cm that would effectively improve the air and light conditions in a paddy field, increase leaf area index, biological yield and grain yield (Wu 2000). However, increasing plant height and lodging resistance have always been in conflict in crop cultivation and breeding because the two traits seem to be mutually exclusive. Thus, developing rice varieties that are tall but resistant to lodging has only been a dream for breeders, but it remains one of the key goals in achieving the objectives of super rice breeding. Many factors affect plant lodging and various plant-type evaluation methods have been proposed from different perspectives (Yoshida, 1972; Donald and Hamblin, 1976; Yagi 1983; Tang *et al.*, 1989, 2004; Tian and Yang, 2005). Currently, there is an urgent need for a simple and accurate mechanism to evaluate rice plant types and predict the ideal ideotype, especially with the aim of breeding long-stalked lodging-resistant varieties.

The most common methods for constructing mathematical models of plant lodging resistance have been correlation analysis, path analysis and grey relational analysis (GRA) (Zhang *et al.*, 1999, 2010; Wan and Ma 2003; Guan and Shen, 2004; Ma *et al.*, 2004; Lang *et al.*, 2011; Yang *et al.*, 2012; Wang *et al.*, 2015*a*). Both correlation analysis and path analysis are based on linear regression, but in the case of a small sample size, it would be inappropriate to establish a linear relationship based simply on correlation coefficients. The core of GRA is the calculation of the relational grade, which treats each sample with equal weight and leads to poor objectivity (Wang *et al.*, 2005). Moreover, the classification effectiveness and non-linear fitting of these methods also need to be improved. In contrast, the artificial neural network method has been applied widely due to its capacity for strong pattern recognition and powerful non-linear fitting (Tang, 2003; Wang and Zhang, 2008; Ma *et al.*, 2009; Wang *et al.*, 2010; Khan 2013) with the following distinctive features: (1) it has a strong fault tolerance and makes judgments by learning from imperfect data and graphics; (2) it can be used to approximate a non-linear system of any degree of complexity; and (3) it has good artificial intelligence including self-learning, self-adaptation and association, which provide unique advantages in pattern

classification and non-linear fitting. Because of these distinctive properties, the neural network method is able to simulate complex ecological processes and behaviours that are impossible for many traditional models, and it has been applied extensively in various fields (Lek and Guégan, 1999). The most applied neural networks are the radial basis function (RBF) and back propagation.

In the current study, plant morphological, anatomical and histochemical methods were used to investigate the relationship between lodging resistance and stem-wall thickness, length of the third internode, plant height, stem lignin content, stem vascular bundle number and the large vascular bundle ratio using rice lines with varying degrees of lodging resistance and height. The relationship between stem structure and lodging index was investigated using the RBF neural network and multivariate regression analyses, and the optimal plant type was predicted for each of the morphological types. The current study aimed to establish a rapid and accurate mechanism for evaluating lodging and plant type and to provide a basis for breeding tall, lodging-resistant super rice varieties.

Materials and methods

Materials

Fifteen rice lines obtained from the Institute of New Rice Germplasm, Henan Normal University, China (35°19′ N, 113° 54′ E, 118 m a.s.l.) were used: 14040, XF5, F121, X3, 14052, 14078, 14074, 14053, 14085, 14076, 14088, 14030, 121-1, 131-1 and 14012. The lines were planted in three experimental fields (Field 1, 113°54′ E, 35°19′ N, 118 m a.s.l., Field 2, 113°56′ E, 35°4′ N, 81.65 m a.s.l. and Field 3, 114°34′ E, 35°9′ N, 65.32 m a.s.l.) at Henan Normal University on 20 May in both 2015 and 2016, at a density of 67 500 plants/ha. The soil at the fields were all Chao soil (National Soil Census Office, 1998) with pH of 8.3 containing 15–20 g/kg organic matter, 430–560 mg nitrogen (N)/kg, available phosphorus (P) 9.1–11.0 mg/kg, exchangeable potassium (K) 145–156 mg/kg and no previous crop was grown in any of them. Cultivation and management practices were the same for each year, experimental field and treatment. Fertilizer was applied as follows, in both years: compound fertilizer (N:P:K at 15:15:15) was broadcast before ploughing with a urea topdressing broadcast on 10 June and 18 July. Alternate wetting and drying irrigation was used throughout the growing season as follows (growth stages (GS) are according to Zadoks *et al.*, 1974): during seedling growth and tillering (GS 13–29), 3 days drying (i.e. with no irrigation) and 5 days flooded (to a depth of 2 cm); during stem elongation (GS 30–39), 3 days drying and 9 days flooded (4 cm deep); during booting, inflorescence emergence and anthesis (GS 40–69), 2 days drying and 9 days flooded (5 cm deep); during milk development (GS 70–75), 3 days drying and 7 days flooded (5 cm deep); during late milk and dough development (GS 76–90), 5 days drying and 2 days flooded (5 cm deep). Insecticides were sprayed on 14 July and 25 August each year. Manual weeding was carried out twice, on 3 July and 3 August in both years. Each line was assigned to a plot 5 m × 65 cm under a randomized block design and three replicates with five rows per plot surrounded by three guard rows. Samples (20 plants/line) were collected randomly at the inflorescence emergence stage (GS 50), milk development stage (GS 70), dough development stage (GS 80) and ripening stage (GS 90). The third internode, counting from the base of the stem, served as the material used for analysis.

Analysis of anatomical and morphological characteristics

Sections of the central portion of the third internode (20 μm thick) were cut by hand and incubated for 2 min in 2% phloroglucinol solution according to the Wiesner reaction method and mounted using 50% hydrochloric acid (Strivastava, 1966). The sections were analysed under an optical microscope equipped with a digital camera (Nikon DS-Fi1c, Japan) to determine the stem radius, stem wall thickness and number of large and small vascular bundles, based on which the value of the stem wall radius was calculated and statistical analysis performed. The area of the vascular bundles was determined under a fluorescence microscope (Zeiss Axioskop 40, Germany).

Determination of the flexural strength and lodging-resistance index of rice stems

Ten representative non-lodged plants were sampled at GS 50, GS 70, GS 80 and GS 90 to analyse flexural strength (a material property, defined as the stress in a material just before it yields in a flexure test), height of the centre of gravity and fresh weight of the stem. All the measurement protocols were modified from Wei *et al.* (2008).

The stem flexural strength was determined using a plant stem strength tester (SY-S03, Zhengzhou, China) consisting of a sensor rod positioned perpendicular to the surface of a table. The third internode, used as the test material, was unsheathed and placed on the table perpendicular to the sensor rod, and strength values were recorded after exerting compression. The procedure was done five times in total, and the average taken as the flexural strength value of the material. The distance (in cm) between the bottom and the balance point of the stem (including spikes, leaves and sheaths) was measured three times and the mean distance used as the height of the sample stem's centre of gravity. To determine the fresh weight of the stem, the roots were removed and the above-ground portion of the stem washed clean; the water was absorbed with filter paper. Then, the stems were weighed three times and the mean considered as the stem fresh weight of the sample (g). The stem lodging index = (height of the centre of gravity × fresh weight)/flexural strength (Chen *et al.*, 2011).

Determination of lignin content

Samples were collected from non-lodged plants (ten plants/plot) 0, 10, 20, 30 and 40 days after formation of the third internode. The third internodes without their sheaths were frozen in liquid nitrogen and stored at −20°C. According to Lin *et al.* (1996), 0.5 g of each sample was weighed accurately and placed in a mortar; 95% ethanol was added; and the mixture was homogenized and then transferred to a centrifuge tube for centrifugation at 2600 ***g*** for 5 min. The pellet was washed twice with 95% ethanol and twice with 1:2 ethanol/*n*-hexane (V/V); it was then air-dried, dissolved in 25% bromoacetyl acetic acid and incubated for 30 min in a 70°C water bath. Then, 0.9 ml of NaOH (2 mol/l), 5 ml of glacial acetic acid and 0.1 ml of hydroxylamine hydrochloride (7.5 mol/l) were added, and the volume was ultimately fixed with glacial acetic acid at 15 ml. The absorbance of the supernatant at 280 nm was measured, and the value per gram of fresh sample was used to represent the lignin content (Chen *et al.*, 2011).

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Table 1. Analysis of variance of the studied traits for the 15 rice lines grown at three fields for 2 years

Source of variation	D.F.	Cortical thickness (cm)	No. vascular bundles	Length of 3rd internode (cm)	Proportion of large vascular bundles	Plant height (cm)	Area of vascular bundles (cm^2)	Lignin content (%)
Years	1	NS	NS	NS	NS	NS	NS	NS
Fields	2	NS	NS	NS	NS	NS	NS	NS
Lines	14	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
Y × F	2	NS	NS	NS	NS	NS	NS	NS
Y × L	14	NS	NS	NS	NS	NS	NS	NS
F × L	28	NS	NS	NS	NS	NS	NS	NS
Y × F × L	28	NS	NS	NS	NS	NS	NS	NS

Data analysis

The test for significant differences and the linear regression analysis were performed using SPSS17.0 software. Data were standardized using the range standardization method and an artificial neural network was constructed using MATLAB.

Results

The current study was carried out for 2 years at three experimental fields at Henan Normal University in 2015 and 2016. Statistical analysis of the results found no significant differences between sites or seasons (Table 1); therefore, only results from experimental Field 1 (35°19′ N, 113°54′ E, 118 m a.s.l.) in 2016 are presented.

Variations in the flexural strength and lodging index of rice stems

The flexural strength and lodging index of rice stems varied with developmental stage and rice accession (Table 2).

The flexural strength of the third internode tended to increase gradually from GS 50 to GS 90, but it did not change significantly from GS 80 to GS 90. Stem lodging indices for 14053 and 131-1 were significantly ($P < 0.05$) higher than those of the other lines and were followed by those of 121-1, 14085, X3 and 14052. Stem lodging indices for 14076 and F121 were not significantly different from those of 14040, XF5 and 14088, and the indices for 14074, 14030, 14078 and 14012 were significantly ($P < 0.05$) lower than those of the other lines. Within the same line, the stem lodging index varied among different GS, and there was an interaction between GS and line; e.g., X3 and 14053 had the highest lodging indices at the mid grain-filling stage, whereas 131-1 and 121-1 had the highest lodging indices at the late grain-filling stage.

Under normal conditions for growth and development, rice is most susceptible to lodging from the mid to late grain-filling stage. The results of the current study showed that the flexural strength of rice stems did not change much during this period, whereas the lodging index changed significantly. This is probably

Table 2. The flexural strength and lodging indices of rice stems at different developmental stages

Rice lines	Stem flexural strength				Stem lodging index			
	GS 50	GS 70	GS 80	GS 90	GS 50	GS 70	GS 80	GS 90
14012	24 ± 1.3	25 ± 1.3	27 ± 1.1	27 ± 1.2	0.6 ± 0.05	0.7 ± 0.10	0.8 ± 0.09	0.8 ± 0.09
14078	24 ± 1.2	24 ± 1.4	28 ± 1.1	26 ± 1.2	0.6 ± 0.06	0.6 ± 0.05	0.7 ± 0.11	0.6 ± 0.07
14074	18 ± 1.5	19 ± 1.5	21 ± 1.8	20 ± 2.0	0.6 ± 0.06	0.7 ± 0.12	0.9 ± 0.17	0.7 ± 0.11
14030	18 ± 1.1	19 ± 1.1	20 ± 1.3	20 ± 1.5	0.7 ± 0.09	0.7 ± 0.11	0.80.14	0.8 ± 0.08
14088	18 ± 1.3	18 ± 1.1	19 ± 1.3	20 ± 1.4	1.0 ± 0.12	1.0 ± 0.12	1.2 ± 0.23	1.1 ± 0.19
XF5	17.2 ± 0.90	17.7 ± 0.99	19 ± 1.3	18 ± 1.4	1.0 ± 0.11	1.1 ± 0.11	1.2 ± 0.11	1.2 ± 0.14
14040	17 ± 1.2	17 ± 1.1	18 ± 1.4	17 ± 1.5	1.0 ± 0.29	1.0 ± 0.22	1.1 ± 0.22	1.1 ± 0.13
F121	15 ± 1.1	16 ± 1.0	16 ± 1.6	17 ± 1.3	1.2 ± 0.24	1.2 ± 0.16	1.4 ± 0.14	1.3 ± 0.18
14076	14 ± 1.4	15 ± 1.1	16 ± 1.5	16 ± 1.0	1.2 ± 0.29	1.2 ± 0.11	1.3 ± 0.26	1.2 ± 0.09
14052	14 ± 1.2	14 ± 1.0	16 ± 1.1	16 ± 1.1	1.2 ± 0.31	1.3 ± 0.08	1.3 ± 0.23	1.3 ± 0.24
X_3	14 ± 1.0	14 ± 1.0	15 ± 1.2	15 ± 1.2	1.2 ± 0.17	1.2 ± 0.12	1.4 ± 0.17	1.3 ± 0.23
14085	12.2 ± 0.88	12.3 ± 0.98	14 ± 1.0	14 ± 1.4	1.2 ± 0.18	1.3 ± 0.17	1.5 ± 0.15	1.4 ± 0.18
121-1	9.3 ± 0.63	8.7 ± 0.92	14 ± 1.1	13 ± 1.3	1.2 ± 0.66	1.2 ± 0.16	1.4 ± 0.11	1.3 ± 0.19
131-1	8.6 ± 0.96	10 ± 1.0	12.6 ± 0.89	12 ± 1.6	1.4 ± 0.24	1.4 ± 0.12	1.4 ± 0.22	1.4 ± 0.07
14053	7.8 ± 0.59	8.2 ± 0.72	12 ± 1.0	12 ± 1.4	1.4 ± 0.22	1.4 ± 0.11	1.5 ± 0.32	768 ± 0.17

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because the material in the base of the rice stem is reallocated during this period, leading to changes in the centre of gravity and the construction of tissue in the stalk, which ultimately causes the lodging index to change. Furthermore, the new starch that accumulates in the grain from flag leaf photosynthates also increases panicle weight.

Correlation between tissue building and the lodging index of rice stems

In different rice lines, the lodging index of the stem is associated not only with flexural strength but also with plant height, height of the centre of gravity and stem fresh weight. Therefore, the mechanism of rice lodging was further investigated by analysing the structural characteristics of stem tissues at different developmental stages. The relationship between those structural characteristics and the stem lodging index is shown in Table 3.

The rice stem lodging index (Y) was highly significantly ($P < 0.01$) and negatively correlated with lignin content (X_7) and stem wall thickness (X_1) and significantly ($P < 0.05$) negatively correlated with the area of vascular bundles (X_6) and proportion of large vascular bundles per unit area (X_4). However, it was highly significantly ($P < 0.01$) but positively correlated with plant height (X_5), length of the third internode (X_3) and number of vascular bundles (X_2), indicating that the length of the third internode, stem wall thickness and number of vascular bundles were the major structural factors affecting the rice lodging index. Therefore, a moderate increase in stem thickness and decreasing the length of the third internode and number of vascular bundles can decrease the lodging index. Stem wall thickness (X_1) was highly significantly ($P < 0.01$) and positively correlated with lignin content (X_7), indicating that the level of lignification increased with the relative thickness of the stem wall. The number of vascular bundles (X_2) was significantly ($P < 0.05$) and negatively correlated with the area of the vascular bundles (X_6) and with the proportion of large vascular bundles per unit area (X_4). Plant height (X_5) was significantly ($P < 0.01$) and positively correlated with the length of the third internode (X_3); the tallest line, 131-1, which also had the longest third internode, had the smallest lodging resistance. However, 14078, one of the lines with the lowest lodging index, was taller than 180 cm but had rather a good lodging resistance because the length of its third internode was between that of XF5 (dwarf) and 131-1 (tall), and it had a thicker stem wall and larger vascular area (Figs 1*a* and *b*) dominated by large bundles. Lines with a high lodging index, represented by 14085, had a significantly ($P < 0.01$) higher number of predominantly small vascular bundles as well as a smaller vascular area than the other lines and exhibited traits such as a thinner stem and poorer compression resistance (Figs 1*e* and *f*). The proportion of vascular bundles in 14088, which had a moderate lodging index, was not significantly different from that of 14012, which had the lowest lodging index, but the area of vascular bundles was higher in 14088 than in 14012 (Figs 1*c* and *d*).

Radial basis function neural network established between stem structure and stem lodging index

Choosing the influential factors

Based on the correlation coefficients between lodging index and the other variables, the following were chosen as the influencing factors for a model using the RBF neural network method: stem wall thickness (X_1), number of vascular bundles (X_2), length of the third internode (X_3), proportion of large vascular bundles per unit area (X_4), plant height (X_5) and area of vascular bundles (X_6).

Prediction by radial basis function neural network

The RBF neural network model to predict lodging resistance was constructed in the Matlab environment. The input and output vectors of the training samples were established by assigning X_1–X_6 as the neurons of the input layer, and the lodging resistance coefficient (Y) was assigned as the neuron of the output layer.

Determination of hidden layer basis function centres and variances

The fuzzy K-means clustering algorithm was used to determine the centre of each basis function and its corresponding variance.

Table 4 shows that six cluster centres were identified through the training and the spreading coefficient was also determined for each basis function.

Determination of the weight from the hidden layer unit to the output unit

The weight from the hidden layer to the output layer was revised using the gradient-descent algorithm to minimize the error

Table 3. Correlation coefficients between the structural characteristics of rice stem tissue and rice stem lodging index

	Y	X_1	X_2	X_3	X_4	X_5	X_6	X_7
Y	1							
X_1	−0.95**	1						
X_2	0.92**	0.77**	1					
X_3	0.95**	0.72**	0.60**	1				
X_4	−0.85*	−0.25	−0.90*	−0.71*	1			
X_5	0.92**	0.89**	0.83**	0.94**	−0.72*	1		
X_6	−0.89*	0.35*	−0.93*	−0.45*	0.88*	0.78*	1	
X_7	−0.91**	0.96**	0.83**	0.81**	0.91	0.82**	0.91**	1

Y, lodging index; X_1, cortical thickness (cm); X_2, number of vascular bundles; X_3, length of the third internode (cm); X_4, proportion of large vascular bundles; X_5, plant height (cm); X_6, area of vascular bundles (cm^2); X_7, lignin content (%).
*$P \leqslant 0.05$; **$P \leqslant 0.01$.

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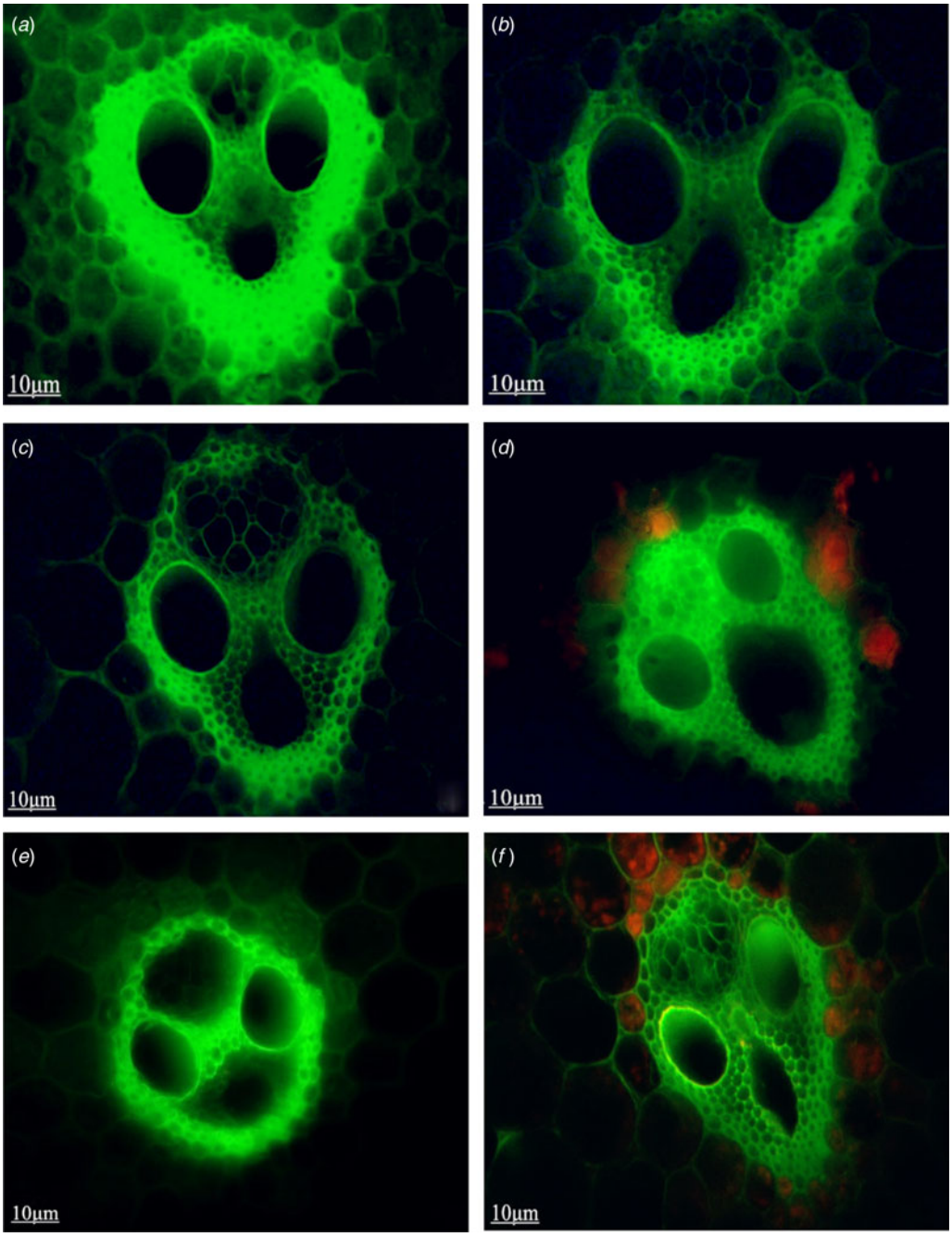


Fig. 1. Cross-section of rice stem vascular bundles. The structure and area of vascular bundles are shown. 14078 (*a*, *b*) has the largest area of vascular bundles followed by 14088 (*c*, *d*) and then 14085 (*e*, *f*) with the smallest.

function. The weight vector after training was $[-8.4101, -3.0024, 14.5441, -5.0603, -5.6558, 7.8235]^{T}$.

Multivariate regression analysis of stem structure and stem lodging index

Multivariate linear regression (MLR) was performed on the factors described above to screen the major factors affecting the stem lodging index, giving rise to the final fitting equation:

$$Y = -108.37 + 9.44X_1 - 1.11X_2 - 7.18X_5 (R^2 = 0.997)$$

in which three factors, i.e., the stem wall thickness, number of vascular bundles and plant height, were ultimately included in the equation with a determination coefficient (R^2) of 0.997. *F*- and *t*-tests showed that the regression equation and regression coefficients were statistically significant ($P < 0.05$). Above all, the fitting was ideal.

Comparison of the predictive ability of three mathematical models in evaluating the rice lodging index

Based on the correlation data, comparative analysis of the means of the prediction results regarding the built-up area was

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Table 4. Hidden layer basis function centre and variance

C_1	C_2	C_3	C_4	C_5	C_6
0.32	0.23	0.47	0.53	0.35	0.18
0.23	0.81	0.11	0.95	0.35	0.67
0.13	0.24	0.31	0.61	0.56	0.61
0.16	0.33	0.12	0.58	0.61	0.76
0.24	0.18	0.62	0.89	0.48	0.17
0.10	0.39	0.24	0.80	0.46	0.62
σ_1	σ_2	σ_3	σ_4	σ_5	σ_6
1.18	1.52	0.92	1.29	0.72	1.45

performed (Table 5), and the prediction and actual curves were also compared (Fig. 2). Figure 2 shows that the predicted curve of the RBF network and the actual curve essentially overlapped; the prediction curve of the MLR network overlapped the actual curve in the area of samples 1–5, deviated from it slightly in the area of samples 12–13 and deviated obviously in the area of samples 7–12. Table 6 shows that the mean residual and relative errors of the RBF network prediction were the lowest at 0.04 and 7.07%, respectively, whereas those of the MLR were obviously higher than those of the RBF network at 0.11 and 11.27%, respectively; approximately twice those of the RBF neural network with poorer prediction accuracy. With the same data support, the overall prediction effectiveness of RBF was better than that of MLR. The residual, which is the difference between the predicted value and the actual value of the model, is capable of portraying the accuracy of the model prediction precisely and visually. Table 5 shows that the RBF network residuals were the lowest for all the samples, whereas those of the MLR were the highest for all the samples, of which the residuals of samples 2, 6, 10 and 11 were obviously higher, and highest for samples 2 and 6, than those of the other samples, indicating that the lodging index values of these samples might also be affected by other factors. The residual data indicated that RBF exhibited better predictive ability. In addition to the overall accuracy and the residuals, another key point in comparing the predictive ability of different models is to analyse the prediction accuracy on the mutation value of the models. Figure 2 shows that the curve of the change in the rice lodging index exhibited two distinct inflection points at samples 8 and 11, generating three periods. In the period from samples 1 to 6, the lodging index increased slowly but then increased sharply in the period from samples 8 to 11. It then increased slowly again, indicating that the stem structure exerted a greater impact on lodging between samples 8 and 11. In summary, the RBF neural network demonstrated significant advantages in prediction accuracy and was suitable for complex non-linear interactions among variables.

Identifying the optimal rice plant type from using the radial basis function neural network

By comparing the predictive accuracies of the two models, the RBF neural network was found to have better prediction ability, so it was used to determine the lodging index of different types of rice stems and identify the optimal plant type.

Table 7 shows the parameters of the optimal rice plant types with respect to lodging resistance found in the current study.

Discussion

Lodging causes poor grain filling and yield loss, reduces grain quality, and reduces mechanical harvesting efficiency (Acreche and Slafer, 2011; Berry and Spink, 2012). Much research has been carried out on the external form, internal structure, chemical composition and mechanical strength of cereal crops and how this affects lodging (Esechie, 1985; Luo *et al.*, 2007; Kong *et al.*, 2013; Novacek *et al.*, 2013; Tian *et al.*, 2015).

The morphology of plants and internodes strongly affects the plant's ability to resist lodging. Pickett *et al.* (1969) reported

Table 5. Comparison of the predictive ability between RBF neural network and multivariate regression analysis

		Predicted value		Residual		Relative error (%)	
Serial number	Actual value of lodging index	RBF	MLR	RBF	MLR	RBF	MLR
1	0.42	0.39	0.35	0.03	0.07	7.14	16.67
2	0.89	0.82	0.75	0.08	0.14	8.43	15.73
3	0.39	0.36	0.47	0.03	0.08	8.46	19.49
4	0.88	0.85	0.93	0.03	0.05	2.95	5.68
5	0.39	0.36	0.33	0.03	0.06	6.67	15.38
6	1.00	0.94	0.89	0.07	0.10	6.50	10.70
7	0.20	0.19	0.23	0.01	0.03	5.00	15.00
8	0.28	0.26	0.29	0.02	0.01	6.43	3.57
9	0.20	0.19	0.18	0.01	0.02	5.50	9.00
10	0.78	0.69	0.86	0.09	0.08	11.79	10.26
11	0.73	0.66	0.77	0.07	0.04	9.04	5.48
12	0.20	0.18	0.24	0.02	0.04	11.00	17.50
13	0.10	0.10	0.11	0.00	0.00	3.00	2.00

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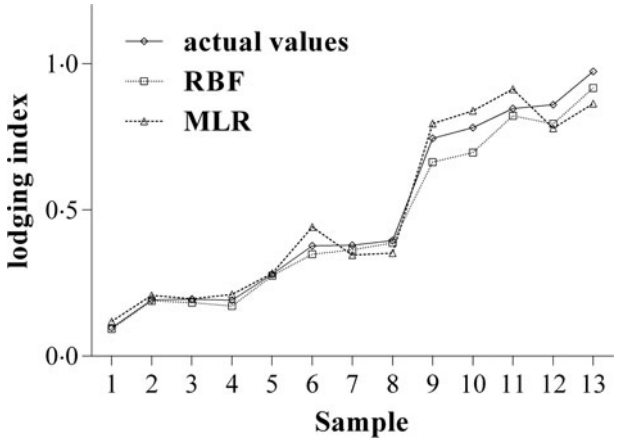


Fig. 2. Comparison of the prediction curve and the actual curve.

Table 6. Comparison of prediction results from different models

Model	Residual mean	Average relative error
RBF	0.04 ± 0.011	7 ± 1.2
MLR	0.11 ± 0.023	17 ± 2.8

that the resistance of maize to stalk lodging was correlated with plant height, stalk diameter and length of internodes below the ear. Stalk lodging in maize was positively correlated with basal internode length (Esechie, 1985), but negatively correlated with basal internode diameter (Martin and Russell, 1984; Novacek *et al.*, 2013). The lodging coefficient of the stem in foxtail millet was associated with specific morphological properties of the culm, such as greater culm diameter, culm wall thickness (Tian *et al.*, 2015). Wang *et al.* (2006, 2012) reported that the most important anatomical and chemical properties for selection of lodging-resistant wheat cultivars were the ratio of culm wall thickness to the outer radius. A negative correlation between plant height and lodging resistance has been reported in some studies (Crook and Ennos, 1994; Navabi *et al.*, 2006). In the present study, the rice stem lodging index was highly positively correlated with plant height, length of the third internode, and negatively correlated with stem wall thickness, basically consistent with the results of previous studies.

The mechanical strength of the stem, closely related to stalk lodging, depends not only on the thickness and length of the stem, but also on the anatomical structure (vascular bundle, mechanical tissue, etc.) and chemical composition of the stem (Luo *et al.*, 2007; Tian *et al.*, 2015). The unit number of large vascular bundles and proportion of sclerenchyma tissue in the culm had a positive correlation with the stem's mechanical strength in wheat (Wang *et al.*, 2006). The present study demonstrated that the rice stem lodging index was highly negatively correlated with the area of vascular bundles and proportion of large vascular bundles per unit area but positively correlated with a number of vascular bundles. This indicates that similar to the findings of other cereal crops research, the proportion of large vascular bundles per unit area was a more important trait affecting stem-based lodging. After comparing the diameter and area of the vascular bundles of wheat, barley and oats, Pinthus (1974) pointed out that the diameter and area of the vascular bundle in lodging-resistant varieties were significantly larger than those in non-resistant varieties. Zhang *et al.* (2016) found in rice that a decline in the area of vascular bundles resulted in lower stem strength and a higher lodging index. However, there are different reports on the relationship between the number of vascular bundles and lodging resistance. Duan *et al.* (2004) reported that the number of vascular bundles in rice stem was significantly positively correlated with its mechanical strength. Luo (2005) also emphasized that the number of large and small vascular bundles in rice stems was co-developed: lodging resistance was stronger when the number of vascular bundles was higher. Therefore, the number of vascular bundles could also be used as a characteristic for breeding lodging resistant rice cultivars.

The mechanical strength of rice stem depends primarily on the cell walls of mechanical tissue (i.e. sclerenchyma cells under the

Table 7. The optimal plant type of rice as predicted by RBF

Sample	Stem wall thickness (cm)	Number of vascular bundles	Large vascular bundle proportion	Plant height (cm)	Inverted three stem length (cm)	Lodging index
50	0.3 ± 0.04	13 ± 2.9	0.7 ± 0.02	114 ± 1.1	17 ± 1.2	0.9 ± 0.12
56	0.3 ± 0.03	13 ± 2.4	0.8 ± 0.02	122 ± 2.7	20 ± 1.5	0.7 ± 0.13
60	0.4 ± 0.04	13 ± 1.5	0.8 ± 0.02	126 ± 2.6	17 ± 2.8	0.5 ± 0.12
48	0.3 ± 0.03	12 ± 1.6	0.7 ± 0.03	131 ± 2.9	10 ± 2.1	0.9 ± 0.13
54	0.4 ± 0.08	13 ± 2.7	0.7 ± 0.02	137 ± 1.1	18 ± 2.0	0.7 ± 0.11
52	0.4 ± 0.03	13 ± 1.1	0.7 ± 0.02	141 ± 2.7	18 ± 1.2	0.9 ± 0.16
49	0.3 ± 0.08	13 ± 2.9	0.7 ± 0.01	145 ± 2.5	19 ± 1.7	1.4 ± 0.14
53	0.4 ± 0.08	13 ± 1.6	0.7 ± 0.01	152 ± 1.7	22 ± 2.3	1.5 ± 0.13
59	0.5 ± 0.06	13 ± 1.2	0.8 ± 0.01	155 ± 1.1	18 ± 2.9	0.7 ± 0.19
55	0.6 ± 0.06	13 ± 1.4	0.8 ± 0.03	161 ± 2.2	18 ± 2.4	0.6 ± 0.10
57	0.5 ± 0.08	13 ± 1.8	0.7 ± 0.03	171 ± 2.7	23 ± 3.0	1.3 ± 0.16
51	0.4 ± 0.01	13 ± 2.1	0.7 ± 0.01	175 ± 1.7	17 ± 1.3	1.4 ± 0.14
58	0.4 ± 0.03	13 ± 1.1	0.7 ± 0.02	181 ± 2.5	19 ± 2.2	0.7 ± 0.12

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epidermis and vascular bundle sheath cells) in the internodes. Lignin, the main component of secondary cell walls, is important not only to plant growth and development but also for plant mechanical support (Ma 2009). A significant correlation was found between lignin accumulation and the mechanical strength of arabidopsis (*Arabidopsis thaliana* L.) shoots (Jones *et al.*, 2001). In wheat, it was also found that lignin accumulation of culms in varieties susceptible to lodging was lower than in lodging-resistant varieties (Berry *et al.*, 2003; Chen *et al.*, 2011; Kong *et al.*, 2013). A similar pattern was found in common buckwheat (Wang *et al.*, 2015*b*) and in the present study.

In rice, the stem's mechanical strength increases gradually as the plant grows and matures, and the flexural strength increases correspondingly (Luo *et al.*, 2007). The present study showed that from the mid grain-filling stage onwards, the flexural strength of rice no longer changed significantly, which differs from the findings in wheat (Chen *et al.*, 2011). Although there was no significant change in the flexural strength of rice stems in the mid grain-filling stage, changes in the structure of the rice stem tissue resulting from the reallocation of matter in the stem and in the degree of lignification, as well as the new starch accumulated from the flag leaf photosynthates, led to changes in the lodging index.

Analysis of the correlation between rice stem structure and lodging index revealed that the two factors have a rather complex relationship with various interactions among the independent variables, so a neural network approach is needed to identify the macroscopic structural factors that affect lodging index of the stalk. There were significant differences in flexural strength and lodging index between rice lines with different plant heights. Therefore, it is possible to identify the variables that have the highest impact on rice lodging by analysing the structural properties of different lines at different developmental stages and to mathematically model the relationship between these variables and lodging index.

In the current study, factors affecting lodging were identified by analysing the correlation between morphological characteristics of the stems and lodging index values of different rice lines, and optimal rice plant types were predicted through three evaluation methods, including correlation analysis, multivariate regression analysis and artificial neural networks. A comparison of the three methods showed that the prediction accuracy of artificial neural networks was approximately twice as high as that of multivariate regression analysis. Through a vertical comparison of the changes in lodging index among the lines, it was found that choosing lines with significant variation in lodging index and examining the structural differences in their stems can provide the appropriate samples for further quantitative analysis of changes in the structural construction in the stem wall and the lignification process of the vascular bundles. Ultimately, based on the predictions using the established RBF neural network, optimal rice plant types were identified, and based on their morphological structures, it was found that the tall rice varieties with strong lodging resistance have stem wall thicknesses of 4.5–6.0 cm and third internode lengths of 17.5–19.5 cm, which is between that of the super-tall, lodging-susceptible and conventional dwarf varieties. Additionally, the proportion of large vascular bundles should be 0.73–0.75 to achieve the breeding objectives of increased plant height and lodging resistance. Radial basis function modelling of the changes in flexural strength and lodging index during rice development provides a new approach for studies evaluating the mechanism of lodging resistance in rice as well as theoretical references for rice breeding.

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