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Thigmotropic responses of *Oryza sativa* L. to external rubbing stimulation

Benliang Zhao[1,2], Lili Teng[1], Jia-en Zhang[1,2,*], Huimin Xiang[1,2], Meijuan Li[1] and Kaiming Liang[3]

[1] *Institute of Tropical and Subtropical Ecology, South China Agricultural University*, Guangzhou, 510642, China

[2] *Key Laboratory of Agro-Environment in the Tropics, Ministry of Agriculture of the People's Republic of China, Guangdong Engineering Research Center for Modern Eco-agriculture and Circular Agriculture*, Guangzhou, 510642, China

[3] *The Rice Research Institute of Guangdong Academy of Agricultural Sciences*, Guangzhou 510640, China

***Corresponding author**: jeanzh@scau.edu.cn

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Abstract: Our aim was to study the morphological and physiological responses of rice to rubbing stimulation. Rice was subjected to rubbing 30 times/day (R30), 60 times/day (R60), 90 times/day (R90) and 0 times/day (control) for 35 days. The height, elongation rates and second internode length were significantly decreased by the three treatments, whereas stem width increased significantly. The tiller number and chlorophyll contents of the top third and top fifth of leaves increased significantly after R30 and R60. In R90, the aboveground biomass was significantly decreased and dead leaf biomass was increased. In R30 and R60, the transpiration rates were 16% and 13% higher than in the control, whereas photosynthetic rates increased 25% and 23%, respectively. Root biomass was significantly increased in R30, and root/aboveground was enhanced in R90. Stomatal conductance and root triphenyltetrazolium chloride-deoxidizing ability was significantly increased by the three treatments. The SOD activities in all treatments and the control were similar after stimulation. POD and CAT activities increased significantly in R30 and R60, and malondialdehyde increased by 42% in R90. Membrane permeability in R30 and R60 decreased 26% and 15%, respectively. The calcium content and soluble protein content increased in R30, whereas the magnesium content decreased. The nitrogen content increased significantly in R30 and R60. The silicon content in the whole plant and the size of stomata increased significantly in the three treatments. Thus, rubbing stimulation had complex effects on rice growth.

Key words: thigmomorphogenesis; rice; mechanical stimulation; nutrients; enzyme activity

INTRODUCTION

Mechanical stimulation is a widespread physical factor in the living environment of a plant and includes wind, animal and plant rubbing, the impacts of rainfall and hail on stems and leaves, soil particle hindrance, shaking, vibration, and other factors [1,2]. Mechanical stimulation has dual effects on plant growth and development. Low-intensity mechanical stimulation promotes the division and growth of plant cells, whereas high-intensity mechanical stimulation impairs plant growth and development [3]; thus, plant growth and development are regulated by mechanical stimulation among others.

Thigmomorphogenesis is often used to describe morphological changes in the process of mechanical stimulation-induced growth of plants without specialized induction cells [4]. Plants subjected to mechanical stimuli such as wind, touching or rubbing usually have short, thick stems and distribute more of their biomass to the roots [1,2]. Mechanical stimulation can significantly change plant morphology. The height of *Liquidambar styraciflua* L. was reduced by 80% compared with the control after shaking for 30 s every day for 27 days [5]. The leaves of *Nicotiana tabacum*, when bent 40 times/day, had shorter and thicker stems [1]. *Arabidopsis* plants exposed to repetitive touch stimulation showed a delayed flowering stage and decreased inflorescence elongation compared with the unstimulated control [2,6]. Mechanical stimuli promoted branching in *Arabidopsis thaliana* and increased the number of stoloniferous branches of the perennial herb *Potentilla reptans* L [7,8].

Mechanical stimuli can affect plant cell characteristics, plant resistance, enzyme activity, endogenous hormones and other physiological and biochemical indicators. The cold resistance of *Lycopersicon esculentum* Mill. cv. Caruso was enhanced after applying brushing stimulation to plants twice a day and the formation of this resistance might be related to an increase in soluble sugar content [9]. Wind-induced mechanical stimulation of bean can improve the pest-resistance potential [10]. Mechanical stimulation applied to *Cucumis sativus* leaves can improve resistance to pathogens [11]. Mechanical vibration stimulation can promote the growth of the *Actinidia chinensis* callus [12]. Mechanical stimulation by stroking in *Glycine max* (L) Merr. can promote 4-aminobutyric acid synthesis and inhibit hypocotyl elongation [13].

Changes in Ca^{2+} are commonly involved in the physiological responses of plants to mechanical stimulation. Wind stimulation can induce a cytoplasmic Ca^{2+} increase in transgenic seedlings of *Nicotiana plumbaginifolia* [14]. In the mesophyll tissue of *N. plumbaginifolia*, the cytoplasmic Ca^{2+} concentration of cells increased significantly under rotation stimulation [15].

However, few studies have been performed on the response of rice to mechanical stimuli. Field investigations have shown that mechanical stimulation associated with biological activity significantly affect rice growth and development, morphology and physiology. In Asia, farmers raised ducks in paddy fields, and the diverse activities of ducks directly stimulated the rice, and involved touching the stems, rubbing leaves, trampling the rhizosphere and shaking the aboveground [16,17]. These stimulating effects significantly changed the morphological traits of rice in paddy fields, including decreased plant height, thicker and harder rice stems, enhanced lodging resistance and improved tillering and leaf area expansion [18,19]. Thus, the anatomical structure of the rice culm internodes was significantly impacted by the ducks' activities [20]. Moreover, after raising crabs (*Eriocheir sinensis*), crayfish (*Macrobrachium nipponensis*) and fish (*Aristichthys nobilis*, *Carassius auratus gibelio*) in paddy fields, the leaf area index and the leaf area of the rice canopy were increased significantly. Plant height, the aerenchyma of the rice and the stem thickness were also improved significantly [21].

We previously observed that the plant height, biomass and root-zone methane oxidative capacity of rice plants all changed after stimulation by vibration [22]. The biological activities in paddy fields are closely related to the surface friction between animals and plants, and the gentle rubbing caused by this biological behavior could affect rice growth. However, until now, information about the response of rice to continuous rubbing has been limited. Therefore, the objective of this study was to investigate the changes in rice morphology with different intensities of rubbing and to identify related responses in the physiological traits of rice. This study will contribute to further understanding plant responses as affected by changes in physical factors and adaptation processes.

MATERIALS AND METHODS

Plant material and pot experimental conditions

Rice seeds (*Oryza sativa* L. 'Shengbasimiao') were sterilized in a beaker for 30 min using 0.01% $KMnO_4$. The sterilized seeds were washed with purified water 3 to 5 times to remove the residual $KMnO_4$. The seeds were immersed in pre-aerated purified water for 24 h in darkness before germination in a sterile Petri dish in an incubator under 16-h day (27°C) and 8-h night (25°C) conditions for 6 days. The resultant seedlings were transplanted to pots, which were placed in a greenhouse with temperatures between 25°C and 37°C and moisture maintained between 45% and 60%. The soils used in the pots were sampled from the surfaces (10 cm) of paddy fields of the South China Agricultural University Farm, China (23°14'N, 113°37'E). The collected soil was air-dried and passed through a 5-mm sieve before transfer to a plastic bucket with an inner diameter of 35 cm and a height of 28 cm. The water level in the bucket was maintained between 2 and 4 cm. The pots were irrigated with deionized water for 3 days before the application of nitrogen ($CO(NH_2)_2$, 0.35 g/kg), phosphate ($Ca(H_2PO_4)\cdot H_2O$, 0.25 g/kg) and potassium (KCl, 0.25 g/kg). Seedlings cultivated for 15 days to a height of 20±1 cm were selected and transplanted into pots for the experiment. Five seedlings were planted in a plastic bucket, and gentle rubbing stimulation was initiated after 10 days.

Trial design and rubbing parameters

Three rubbing treatments were designed: rubbing stimulations 30 times/day (R30), 60 times/day (R60) and 90 times/day (R90). For the control, no rubbing stimulation was applied (0 times/day). Rubbing was performed using a feather duster each day from 9 am to 10 am. The aboveground part was rubbed upward to the maximum height during growth using a feather duster at a position 2 cm above the water surface. Two feather dusters were used together to rub one plant from opposite sides at the same height. In each rubbing, the supporting poles inside the feather dusters were kept at a distance of 1 cm from the stem. All treatments and the control were laid out in a randomized design with 3 replications, and the gentle rubbing stimulation continued for 35 days.

Determination of rice morphological characteristics

At 7, 14, 21, 28 and 35 days, shoot height was determined using a ruler, and tiller number was recorded. The elongation rate was calculated from equation 1 (Eq. 1).

$$R(\text{cm/ day}) = \frac{H_f - H_i}{t} \qquad \text{(Eq. 1)}$$

where R is the height increase rate, H_f is the final height (cm) at measurement time, H_i is the initial height (cm) and t = 7, 14, 21, 28, and 35 days. After 35 days of stimulation, the internode length was recorded, and the stem width was determined using a vernier caliper. The fresh weights of the shoot, root and dead leaf were determined using an electronic balance after 35 days of stimulation. For the determination of root biomass, the entire soil contents were removed from the pots and immersed in deionized water. The rice roots were carefully washed to remove any adhering soil. The dead leaves in the treatments and the control were collected using tweezers. Roots and dead leaves were cleaned with deionized water 3-5 times and dried using filter paper. The root:aboveground ratio was calculated based on the fresh weight at 35 days.

Root activity, enzyme activity, malondialdehyde (MDA) and membrane permeability assay

Roots were washed with deionized water, and the root tips were cut into small pieces (0.5 cm in length). Root activity was determined using the triphenyltetrazolium chloride (TTC) method after 35 days of stimulation based on the amount of deoxidized triphenylformazan per root and expressed as μg/(g·h) [23]. Fresh top first leaves in the fully expanded state collected after 35 days of stimulation (0.10 g) were homogenized in liquid nitrogen in 50 mmol/L phosphate buffer (pH 7.8) containing 1% (w/v) polyvinylpyrrolidone (PVP). After mixing for 10 s on an oscillator (xw-80A, Shanghai Jingke Ltd.), the homogenate was centrifuged at 15,000×g for 20 min at 4°C. The supernatant was used for enzyme activity assay determinations of superoxide dismutase (SOD), peroxidase (POD) and catalase (CAT). SOD activity was determined based on the inhibition of the reduction of nitroblue tetrazolium (NBT) [24] and expressed as U/g FW. POD activity determination was based on guaiacol oxidation at 470 nm by H_2O_2. POD activity was expressed as U/g FW [25]. CAT activity was determined based on H_2O_2 consumption. The enzyme activity was expressed as U/g FW [26]. Fresh leaves (0.10 g) were homogenized with liquid nitrogen in 50 mmol/L of phosphate buffer. After mixing for 10 s, the homogenate was centrifuged for 20 min at 4°C at 15,000×g. One mL of supernatant was used for the determination of MDA [27]. The MDA content was expressed as μmol MDA/g FW. Leaf membrane permeability was determined according to the published method [28].

Assay of chlorophyll content, soluble sugar content and soluble protein

The top third of the fully expanded leaves, fifth leaves and seventh leaves in the treatments and the control were extracted with 96% (v/v) ethanol after 35 days of stimulation, and the chlorophyll content (mg/g FW) was determined on a spectrophotometer (UV-1750, Shimadzu, Japan) [29]. Fresh top first leaves in the fully expanded state collected after 35 days of stimulation (0.1 g) were added to a tube containing 10 mL of deionized water. The mixture was boiled for 30 min and then filtered into a 25-mL flask. To measure the

soluble sugar content, 0.5 mL of extract was placed in a 20-mL tube, and 5 mL of anthrone in ethyl acetate (2%), 1.5 mL of deionized water and 5 mL of sulfuric acid were added. The mixture was mixed on an oscillator and placed in a 99°C water bath for 1 min. After cooling, the absorbance of the mixture was recorded at 595 nm; the soluble sugar content was expressed as mg/kg FW. The soluble protein was measured using the Coomassie Plus protein assay [30]. Leaves (0.1 g) were homogenized in liquid nitrogen by grinding fresh leaves with pre-cooling buffer (0.05 M PBS, pH 7.0). The soluble protein was expressed as mg/kg FW.

Measurements of photosynthetic gas exchange

Photosynthetic gas exchange parameters were obtained from 9 am to 10 am with a photosynthesis system (CIRAS-1, PP Systems International Ltd., UK) after 35 days of stimulation. The leaf photosynthesis rate, transpiration rate and stomatal conductance were measured from the top first fully expanded leaves in the control and treatments. During measurement, the leaf temperature was 25±2°C.

Determination of nutrient contents

After 35 days of stimulation, rice plants in 3 replicates in the control and treatments were removed from the bucket. The collected plants were washed with purified water 3-5 times and dried in an oven. The dried plants were ground to determine the content of nutrients [31]. In addition, fresh top first leaves in the fully expanded state were sampled in 3 replicates from the control and treatments. The leaf blade and sheath of each sampled leaf were carefully separated using scissors. Dried leaves were ground for the determination of nitrogen, calcium and magnesium content [31]. The N content was determined using micro-Kjeldahl digestion, distillation and titration through an automatic Kjeldahl apparatus (VELP Scientific Inc., Italy). For determinations of Ca and Mg, dried leaves were homogenized with mortars and ashed at 525°C for 1 h in digestion crucibles. The Ca and Mg contents were determined using atomic absorption spectroscopy. The contents of N, Ca and Mg in the leaves were then calculated based on the weight and measured concentration. To determine silicon content, whole plants or leaf samples (0.10 g) were placed in plastic tubes and mixed with 3 mL of 50% NaOH before treatment in an autoclave at 121°C for 20 min. The Si content in whole plants, leaf blades and sheaths was determined using the modified high-temperature alkaline fusion method [32].

Analysis of the leaf sheath structure by scanning electron microscopy

After 35 days of rubbing stimulation, a small part of the leaf sheath (0.5 cm width × 0.5 cm length) was selected randomly for measurements of cell area (μm^2), stoma quantity, stoma area (μm^2) and vascular bundle area (μm^2). The sheaths were rinsed in 2.5% (w/v) glutaraldehyde for 4 h at 4°C. Each sheath was washed with phosphate buffer solution three times, followed by dehydration in 30%, 50%, 70%, 90% and 100% ethanol solutions and immersion in methylbutyl acetate. After drying to a critical point under vacuum, the sheaths were adhered to a copper platform before gold plating with an ion-sputtering apparatus. The sheath samples were analyzed using scanning electron microscopy (SEM; XL-30-ESEM, Philips, Amsterdam, the Netherlands). The cell area (μm^2), stroma area (μm^2) and vascular bundle area (μm^2) were calculated from the width (μm) and length (μm) determined using the standard scale provided by SEM.

Statistical analysis

Data were processed using OriginPro (OriginLab Corporation, USA). One-way ANOVA was performed in SPSS 13.0. Multiple comparisons were performed using the Duncan method.

RESULTS

Change in morphological traits under rubbing stimulation

The heights in treatments during weeks 1 and 2 were similar to those of the control (Fig. 1A). From weeks 3 to 5, all treatments reduced shoot height significantly compared with the control. No significant differences in shoot height were observed among R30, R60 and R90 at 5 weeks (Fig. 1A). The elongation rates in R30, R60 and R90 were significantly lower than in the control at weeks 3, 4 and 5 but similar at weeks 1 and 2.

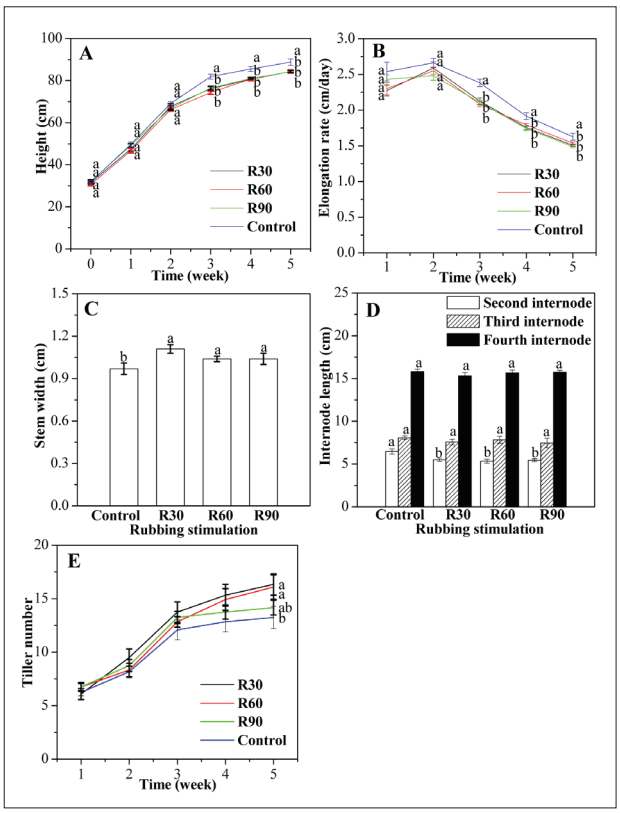

Fig. 1. Changes in height (**A**), elongation rate (**B**), stem width (**C**), internode length (**D**) and tiller number (**E**) of rice (*Oryza sativa* L.) under rubbing stimulation of the aboveground part for 35 days. R30 – rubbing 30 times/day; R60 – rubbing 60 times/day; R90 – rubbing 90 times/day; Control – rubbing 0 times/day. Different letters indicate significant differences between treatments and control at P<0.05 according to the Duncan method. Stem width and internode length was measured after 35 days.

The elongation rates peaked at week 2 and decreased steadily at week 3 to 5 (Fig. 1B). Moreover, rubbing the aerial part influenced the stem width (Fig. 1C). The stem widths in R30, R60 and R90 were significantly higher than that of the control. Internode length was also significantly affected by rubbing (Fig. 1D). The length of the second internode decreased under continuous rubbing. R30, R60 and R90 decreased the length of the second internode by 14.99%, 17.61% and 15.76%, respectively, compared with the control. No significant changes in the lengths of the third and fourth internodes were observed between the three treatments and the control. Rubbing also induced a change in the tiller number per hill (Fig. 1E). After 35 days, the tiller numbers in R30 and R60 increased significantly and were 23.25% and 21.36% higher than that of the control, respectively.

Change in biomass traits under rubbing stimulation

The R90 treatment decreased the aboveground biomass significantly, whereas the aboveground biomasses in R30 and R60 were similar to that of the control (Fig. 2A). The root fresh biomass in R30 was significantly higher than that in the control but was not significantly different from those in R60 and R90 (Fig. 2A). The ratio of root:aboveground biomass was improved in R90 compared with the control (Fig. 2B). Because the aboveground biomass decreased and the root biomass was not affected, rubbing stimulation led to an increase in the root:aboveground ratio in R90. An increase in the biomass of dead leaves was observed in R90, indicating that intensive rubbing had a negative effect on growth (Fig. 2C). Dead leaf biomasses in R30 and R60 were not significantly different from that in the control.

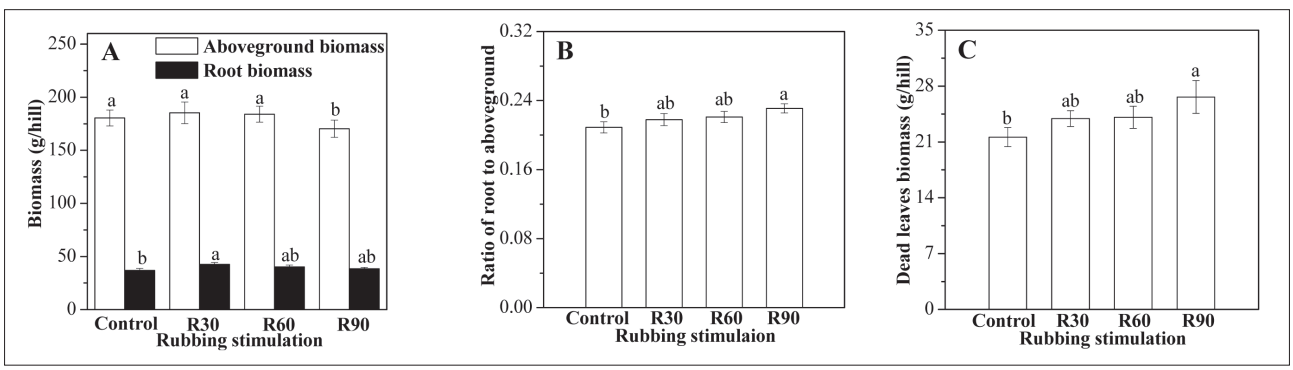

Fig. 2. Changes in biomass (**A**), root:aboveground ratio, (**B**) and dead leaf biomass (**C**) of rice (*Oryza sativa* L.) under rubbing stimulation of the aboveground part for 35 days. R30 – rubbing 30 times/day; R60 – rubbing 60 times/day; R90 – rubbing 90 times/day; Control – rubbing 0 times/day. Different letters indicate significant differences between treatments and control at P<0.05 according to the Duncan method.

Changes in root activity, enzyme activities, MDA and membrane permeability under rubbing stimulation

The activities of SOD in all treatments and the control were similar after rubbing stimulation (Fig. 3A). The POD and CAT activities in R30 and R60 were significantly higher than those in the control (Fig. 3B, 3C). No significant differences in these two enzymes' activities were observed among R30, R60 and R90. The MDA content of leaves in R30 and R60 was similar to that in the control, whereas the MDA content in R90 was significantly enhanced and increased by 42.22% (Fig. 3D). The membrane permeability of leaves in R30 and R60 was significantly decreased, by 25.64% and 14.96%, respectively, compared to that of the control (Fig. 3E). Furthermore, the membrane permeability of the leaves was distinctly higher in R60 than in R30. Compared with the control, the root activity in the treatments increased significantly after stimulation (Fig. 3F). The root activity in R30, R60 and R90 was 19.41%, 19.07% and 12.98% higher than that in the control, respectively, but no differences were observed between treatments.

Changes in chlorophyll, nutrients, soluble protein and soluble sugar under rubbing stimulation

The chlorophyll content of third leaves in R30, R60 and R90 was 1.94%, 1.94% and 3.6% higher than that of the control, respectively (Fig. 4A). In addition, the chlorophyll contents of the fifth leaves in all treatments were significantly enhanced. For the seventh leaves, the chlorophyll contents in R30 and R60 were similar to the control but were significantly lower in R90.

The N contents in R30 and R60 were significantly enhanced to 25.12% and 9.18%, respectively, higher

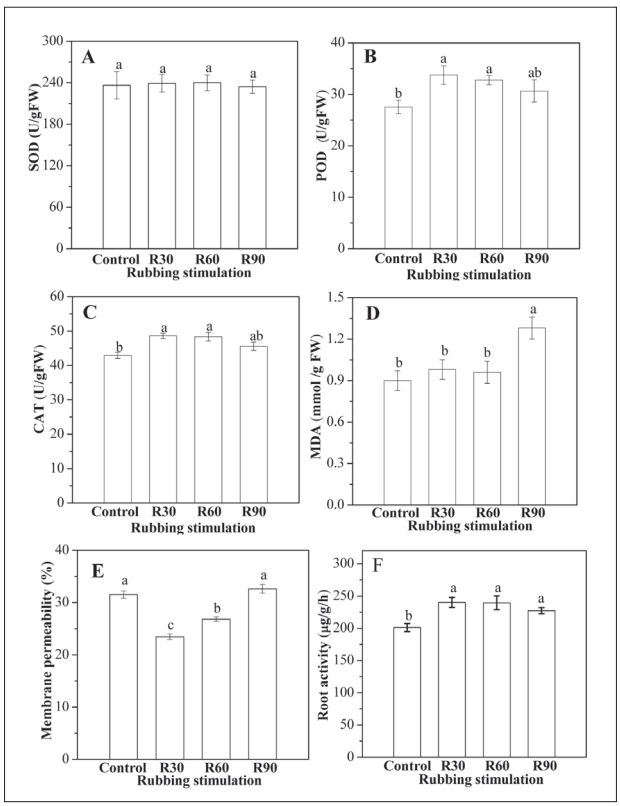

Fig. 3. Changes in SOD (**A**), POD (**B**), CAT (**C**), MDA (**D**), membrane permeability (E) and root activity (**F**) of rice (*Oryza sativa* L.) under rubbing stimulation of the aboveground part for 35 days. R30 – rubbing 30 times/day; R60 – rubbing 60 times/day; R90 – rubbing 90 times/day; Control – rubbing 0 times/day. Different letters indicate significant differences between treatments and control at P<0.05 according to the Duncan method.

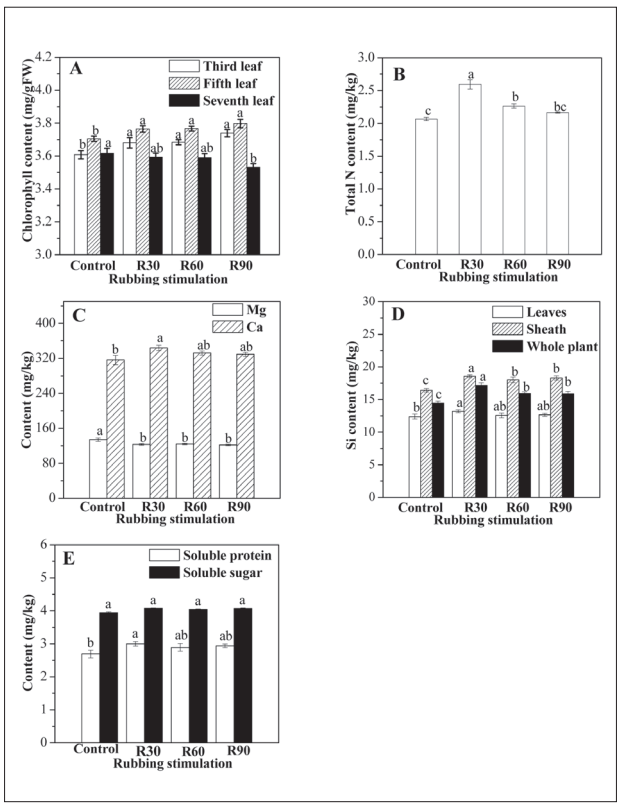

Fig. 4. Changes in the contents of chlorophyll (**A**), total N (**B**), Ca and Mg (**C**), Si (**D**), soluble sugar and soluble protein (**E**) of rice (*Oryza sativa* L.) under rubbing stimulation of the aboveground part for 35 days. R30 – rubbing 30 times/day; R60 – rubbing 60 times/day; R90 – rubbing 90 times/day; Control – rubbing 0 times/day. Different letters indicate significant differences between treatments and control at P<0.05 according to the Duncan method.

than the control (Fig. 4B). The N content in R60 was similar to that in R90, but significantly lower than that in R30. The Ca content in R30 was significantly enhanced and was 8.72% higher than that in the control, whereas the Ca contents in R60 and R90 remained unchanged compared with the control (Fig. 4C). The Mg contents of leaves in R30, R60 and R90 all decreased significantly compared with those in the control (Fig. 4C). The Si content of leaves in R30 was 6.54% higher than that of the control (Fig. 4D). The Si content of sheaths in response to rubbing stimulus increased in all three treatments compared with the control; the increases in R30, R60, and R90 were 13.23%, 9.82% and 11.59%, respectively. The Si content of the whole plant in R30, R60 and R90 was 18.98%, 10.26% and 9.91%, respectively, higher than that in the control.

No significant differences in soluble sugars in leaves were observed in response to the three treatments compared to the control (Fig. 4E). The soluble protein in R30 significantly increased after rubbing stimulation, whereas the soluble protein levels were similar in R60, R90 and the control.

Changes in photosynthesis, transpiration and stomatal conductance under rubbing stimulation

The photosynthesis rate increased significantly in R30 and R60 compared with the control, by 24.84% and 23.32%, respectively, but R90 was not significantly different from the control (Fig. 5A). The transpiration rate in R30 and R60 was 15.52% and 13.06%, respectively, higher than that of the control. Transpiration rate in R90 remained unchanged compared with that in the control (Fig. 5B). Compared with the control, the stomatal conductance rate in R30, R60 and R90 was increased by 25.40%, 22.21% and 13.65%, respectively (Fig. 5C).

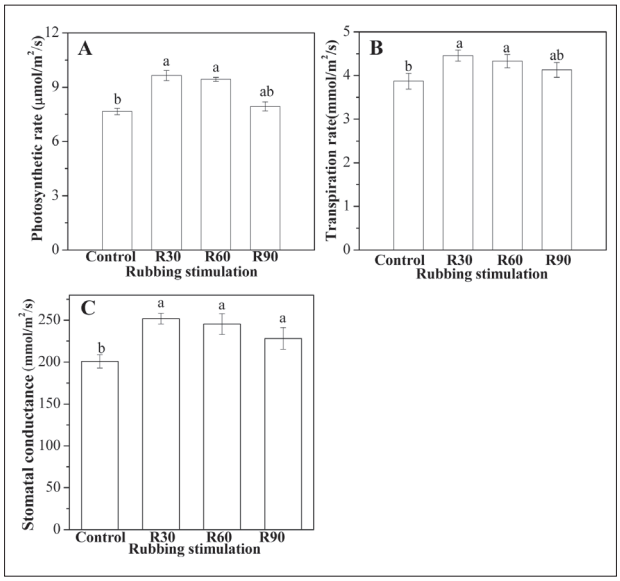

Fig. 5. Changes in photosynthetic rate (**A**), transpiration rate (B) and stomatal conductance (**C**) of rice (*Oryza sativa* L.) under rubbing stimulation of the aboveground part for 35 days. R30 – rubbing 30 times/day; R60 – rubbing 60 times/day; R90 – rubbing 90 times/day; Control – rubbing 0 times/day. Different letters indicate significant differences between treatments and control at P<0.05 according to the Duncan method.

Changes in sheath microstructure under rubbing stimulation

The cell area, stoma quantity and vascular bundle area were not significantly different among R30, R60, R90 and the control (Table 1). The size of stomata in R30, R60 and R90 increased 19.06%, 20.40% and 15.61%, respectively, compared with the control.

DISCUSSION

Stem elongation inhibition is a classical plant response to stimuli, including touching, rubbing and flexing.

Table 1. Change in microstructure of rice sheath under rubbing stimulation on the aboveground part for 35 days.

Traits	Rubbing stimulation*			
	Control	R30	R60	R90
Cell area (μm²)	1438.39±150.61a	1435.95±107.91a	1507.48±86.46a	1404.73±99.59a
Stoma quantity	9.50±0.71a	11.75±0.70a	10.25±0.84a	9.50±0.53a
Stoma area (μm²)	180.65±11.77b	215.09±15.51a	217.51±5.55a	208.85±8.99a
vascular bundle area (μm²)	1222.73±212.62a	1159.51±102.07a	1258.42±170.82a	1123.16±139.71a

*R30 – rubbing 30 times/day; R60 – rubbing 60 times/day; R90 – rubbing 90 times/day; Control – rubbing 0 times/day. Different letters indicate significant differences between treatments and control at P<0.05 according to the Duncan method.

Rubbing can be considered an environmental factor. Elongation rates also slowed down after rubbing on the aerial part in this trial. A similar phenomenon was reported in the response of *Maytenus ilicifolia* to mechanical stimulus, in which stem bending induced a reduction in height increment among its seedlings [33]. Rubbing on the aerial part increased the stem width. Similarly, trampling stimulation can decrease the vertical height and increase the basal diameter of dryland shrub species [34].

The tiller number can change under external stimuli. Inoculation with the beneficial fungus *Trichoderma* sp. SL2 was reported to increase the tiller number [35]. The growth of internodes is regulated by cell division in the intercalary meristem and extension of individual cells between nodes [36]. Here, rubbing stimulation reduced the second internode of the stem and increased the stem width. The reduction of internodes was attributed to both reduced elongation of epidermal and cortical cells and a reduced number of cells in the vascular and pith tissues [37]. The enhancement of stem width was helpful in improving lodging resistance. Rubbing applied to the fourth internode of *Solanum lycopersicum* inhibited the elongation of the internode in the rubbed area and the neighboring internodes [38].

The allocation of total-plant biomass between aboveground and the root was closely related to disturbance intensity. The aboveground biomass decreased in R90, indicating excessive disturbance of growth. The dead leaf biomass also increased in R90. R90 was a negative environmental stress for aboveground elongation and biomass accumulation. However, the root biomass was enhanced in R30, as more biomass was allocated to roots during rubbing stimulation. Mechanical stimuli can alter the auxin distribution and regulate root development through the mechanical and chemical feedback of a plant [39]. The root:aboveground ratio was altered significantly in R90, suggesting that R90 greatly influenced the morphological traits. Partial mechanical stimulation increased the total biomass, root:shoot ratio and number of ramets of *Leymus secalinus* [40]. The biomass of *Hedysarum laeve* was negatively affected by mechanical perturbation [41]. Brushing significantly decreased the total biomass of *Plantago major* leaves [42]. The response of plants to stimuli is a gradually adapted physiological state. When the stress is in the threshold range, plants can adapt, and plant assimilation and growth can be promoted. However, beyond the threshold range, stress destroys plant tissue and cells and impairs plant growth [43]. Root activity is an important physiological index for evaluating the state of plant growth. Considering the increase in root biomass and root activity in R30, rubbing with some intensity was possibly beneficial for root metabolism. Thus, root activity can be an indicator that partly reflects the adaptive responses of rice to aboveground disturbances. Interestingly, R90 was beneficial to root activity but impaired whole-plant biomass accumulation by increasing the dead leaf biomass. We assume that nutrient transport from rice roots to aboveground might be hindered by disturbance from rubbing. Similarly, stem bending as a mechanical stimulus led to an increase in the root biomass of *Maytenus ilicifolia* seedlings [33].

Environmental stresses or disturbances can induce the production of reactive oxygen species (ROS) and damage plant tissues. SOD, CAT and POD are closely related with ROS produced by external stimulation. In this work, rubbing stimulation induced increases in CAT and POD of leaves in R30 and R60 to protect normal metabolism. However, SOD activity did not exhibit a similar increase. A similar phenomenon was reported in the response of rice under the chemical stress of 1-octyl-3-methylimidazolium chloride ionic liquid, in which the CAT of leaves was enhanced, but SOD was not significantly changed [44]. The enzyme-responsive thresholds for SOD, CAT and POD under stress were not synchronous. We deduced that the POD and CAT of leaves responds more sensitively to rubbing stimulation. The SOD, CAT and POD activities of leaves in R90 were not different from those in the control and the normal metabolism in R90 was possibly impaired by stimulation.

Higher antioxidant enzyme activities are associated with lower MDA contents, which helps alleviate membrane damage due to ROS under environmental stress [45]. The MDA differences among R30, R60 and the control were not significant, which was in agreement with the changes in POD and CAT activity levels. Lipid peroxidation in tissues is closely related to oxidative damage of plants under environmental stress, which leads to severe electrolyte leakage from plant

cells due to changes in membrane permeability. The MDA content in R90 increased significantly; however, no significant changes in SOD, POD and CAT activities were observed, suggesting that rice leaf tissue was damaged. The soluble protein in R30 significantly increased after rubbing stimulation. Plants adapt to stress by increasing their soluble protein levels [46].

The top third, fifth and seventh of leaves are responsible for approximately 80% of net whole-plant photosynthesis in the growth period of grain filling [47]. All treatments improved the chlorophyll contents of the third leaves, which contributed to less leaf senescence and higher rice yield. The increase in the chlorophyll content in the fifth leaves could be related to changes in nutrient transport. Mechanical stimulation reduced the chlorophyll content of the aquatic macrophyte *Elodea nuttallii* by about 40% [48]. The significant decrease in the chlorophyll content of seventh leaves in R90 indicated that excessive stimuli damaged the photosynthesis process. R30 and R60 effectively enhanced the N contents in rice. Rubbing stimulation on the aerial part improved the capacity of N uptake. The rubbing stimulus led to changes in the Ca contents of leaves. Ca is a sensor for mechanical perturbations and mechanically stimulating stems caused an increase in the Ca of leaves in R30. In this trial, rubbing retarded Mg accumulation in plant tissue. Rubbing may have influenced nutrient translocation, leading to changes in the utilization of mineral elements.

Si can help alleviate damage from biotic stresses and improve both the mechanical properties and the regeneration of the cell walls of rice [49]. The increases in Si content in the sheath and whole plant suggested that the rice was more resistant to environmental stresses after continuous rubbing. Si accumulation in rice was greatly influenced by rubbing.

Photosynthesis is sensitive to diverse environmental disturbances. In this trial, rubbing had a positive effect on the photosynthesis rate of rice, which was enhanced in R30 and R60. The transpiration rate can be used to assess the growth of plants under wind stimulation [50, 51]. In contrast to wind stress, rubbing was beneficial for the transport and allocation of nutrients in tissues due to improvements in the transpiration rate. Briefly, rubbing simulated the photosynthesis and the transpiration rate, suggesting a potential means of improving yield. The size of stomata was enhanced in all treatments, which may have been the main reason for the increased stomatal conductance.

CONCLUSIONS

Low-intensity rubbing stimulation promoted the growth and development of rice by improving the tiller number, chlorophyll content, transpiration rate and nutrient contents. Low-intensity rubbing stimulation also enhanced the resistance of rice plants by improving enzyme levels and soluble protein content. High-intensity rubbing stimulation led to leaf damage and accelerated leaf senescence. Low-intensity rubbing stimulation enhanced photosynthesis, transpiration, stomatal conductance and the stoma area. In practice, low-intensity rubbing stimulation can be achieved by regulating the density of duck, fish, crab and crayfish in a paddy field. In summary, appropriate mechanical stimulation is helpful for improving rice growth and resistance to environmental stress.

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