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Research article

Effects of simulated acid rain on morphological traits of Thai rice cultivars

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Abstract

Increasing air pollution is associated with acid precipitation (especially acid rain and acid fog), a serious environmental issue that affects plant growth and development. This study investigated the effects of simulated acid rain (pH adjusted to 2.5, 3.5, 4.5, 5.5 and 6.5 as a control using 5:1 volume per volume mix of H_2SO_4 and HNO_3) on the growth and development of 10 rice cultivars during the vegetative growth stage under greenhouse conditions. The results showed that simulated acid rain at pH 2.5–3.5 reduced the chlorophyll (SPAD) index values at both 4 and 8 d after spraying. However, increasing the acidity of the simulated acid rain promoted the plant height, number of leaves and root-to-shoot ratio compared with the control, indicating that simulated acid rain promoted rather than inhibited some characteristics of rice. Simulated acid rain at pH 2.5 produced white-to-tan spots on the abaxial surfaces of the rice leaves, with the frequency of these necrotic spots depending on the rice cultivar. The visual scoring effects of simulated acid rain at pH 2.5 revealed that CMJ, KDML105, TTCP and HPYTD were moderately affected cultivars, RD27 and KTH17 were tolerant cultivars and RD57, RD31, MLL and KKN were highly tolerant cultivars.The results indicated that rice is moderately tolerant to simulated acid rain.

Introduction

The Acid Deposition Monitoring Network in East Asia (EANET) initiated regular activities in 2001 involving 10 East Asian countries, including Thailand. Based on Acid Deposition Monitoring Network in East Asia (2011), the five-year average pH of rainwater for all EANET sites is in the range 4.4–6.2, with 26 (62%) of the 42 sites having pH values lower than 5.0 (proposed as the threshold value for acid rain). Furthermore, in terms of acid rain causes, sulfuric acid and nitric acid predominated, produced from anthropogenic emissions of

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sulfur dioxide (SO_2) and nitrogen oxides (NO_x), respectively. The EANET network predicted the contribution of sulfur and nitrogen emissions to acid rainwater would increase in East Asia and for Thailand, the pH value was in the range 4.0–7.8, with an average of 6.1. Panyakapo and Onchang (2008) reported data from 319 rain events that showed 72 events were in the acidic range (pH < 5.6).

The effect of simulated acid rain has been studied across plants species worldwide, including tree species, soybean, wheat, rape, tomato, cassava and rice, as well as weed species (Yao et al., 1996; Fan and Li, 1999; Fan and Wang, 2000; Zabawi et al., 2008; Kacharava et al., 2013; Wang et al., 2013; Chen et al., 2014; Hu et al., 2014; Tong and Zhang, 2014; Ramlall et al., 2015). The physiological and biochemical processes of plants exposed to simulated acid rain were investigated by Shaukat and Khan (2008). These studies observed that simulated acid rain (pH 3.0 and 4.0) caused white-to-tan spots on the abaxial and adaxial surfaces of tomato leaves. Santos et al. (2006) reported the effect of simulated acid rain on seedlings of *Spondias dulcis* Forst. F., *Mimosa artemisiana.* Heringer and Paula and *Gallesia integrifolia* (Spreng) and identified necrotic spots on leaf blades and the epidermis in all species, with *S. dulcis* displaying epicuticular wax erosion and rupture of the epidermis. The abaxial surface of *M. artemisiana* was colonized by a mass of fungal hyphae and stomatal outer ledge rupture occurred, while some epidermal cells of *G. integrifolia* showed symptoms similar to plasmolysis. Wyrwicka and Skłodowska (2006) reported bleaching of cucumber leaves exposed to simulated acid rain. Yao et al. (1996) investigated the effect of simulated acid rain on pollen grain germination and yield of the rice Tainung 67 and Taichungsen 10 cultivars, with reduced pollen grain germination by up to 30% and 10%, respectively. Studies of simulated acid rain effects on biochemical processes revealed that high acidity rainwater caused changes in the metabolism of the oxidative system (Kacharava et al., 2013). The proteomic analysis of differences in tolerance to acid rain of two broad-leaf tree species revealed that the differences expressed by proteins involved in metabolism, photosynthesis, signal transduction and transcription were substantial (Hu et al., 2014). Chen et al. (2014) noted that there were changes in the proteins involved in photosynthesis, starch synthesis and translation, however, the responses of plants to simulated acid rain were different depending on the plant species. Tong and Zhang (2014) investigated five tree species subjected to simulated acid rain and reported there were both inhibitory and promotional effects on growth and the rate of photosynthesis. However, plants exposed to simulated acid rain of pH 2.5–5.5 were not killed. Wang et al. (2008) reported that rice tended to be acid-resistant. However, the effects of simulated acid rain may include reduced yield potential of the plant. Yao et al. (1996) suggested that responses of rice plants to simulated acid rain included decreased values for the seed setting rate, number of spikelets per panicle and the grain yield. Consequently, the objectives of the current study were to investigate the effects of simulated acid rain on the morphological traits of rice during the vegetative stage and the responses of rice cultivars to different levels of simulated acid rain.

Materials and Methods

Preparation of simulated acid rain

The simulated acid rain (SAR) was prepared using a solution of H_2SO_4 and HNO_3 in the ratio of 5:1 volume per volume based on chemical equivalents (Wang et al., 2008) that was then diluted to pH level of 2.5, 3.5, 4.5 and 5.5; a neutral solution (pH 6.5) was used as the control group (CK). The pH level was measured using a pH meter series of pH 6+ (EuTech Instruments).

Plant materials and treatments

The seeds of 10 cultivars (6 natives and 4 improved) of cultivated Thai rice (*Oryza sativa* L.) were collected from various regions in Thailand. The cultivars were given codes: RD21, Khao Dawk Mali 105 (KDML105), Khao Tah Haeng (KTH17), Cew Mae Jan (CMJ), RD57, RD31, Tubtim Chumphrae (TTCP), Haum Phaya Tongdum (HPYTD), Khao Kam Noi (KKN) and Mali Lueay (MLL) with other information on each cultivar summarized in Table 1. The rice seedlings of these cultivars were planted using the rapid generation advance cultural methods as described in detail by Vergara et al. (1982), with three plants per replication of each cultivar. The study was carried out using a split-plot design, with the main plots having five different pH levels and the sub-plots containing the 10 Thai rice cultivars. The simulated acid rain was sprayed during 0900–1000 hours with about 30 mL per replicate beginning at 25 d after transplanting and continuing for 7 d, which is comparable to rainfall of 27 mL/m^2/hr. The minimum, average and maximum temperatures in the greenhouse were 31.9°C, 34.7°C and 38.6°C, respectively. The minimum, average and maximum relative humidity values were 35.4%, 45.5% and 63.9%, respectively. The pH

Table 1 Information on 10 rice cultivars studied

Cultivar	Abbreviation	Photoperiod	Glutinous/ non-glutinous rice	Improved cultivar/landrace
RD21	RD21	Insensitive	Non-glutinous rice	Improved cultivar
Khao Dawk Mali 105	KDML105	Sensitive	Non-glutinous rice	Improved cultivar
Khao Tah Haeng	KTH17	Sensitive	Non-glutinous rice	Landrace
Cew Mae Jan	CMJ	Sensitive	Glutinous rice	Landrace
RD57	RD57	Insensitive	Non-glutinous rice	Improved cultivar
RD31	RD31	Insensitive	Non-glutinous rice	Improved cultivar
Tubtim Chumphrae	TTCP	Insensitive	Non-glutinous rice	Improved cultivar
Haum Phaya Tongdum	HPYTD	Sensitive	Non-glutinous rice	Landrace
Khao Kam Noi	KKN	Sensitive	Glutinous rice	Landrace
Mali Lueay	MLL	Sensitive	Non-glutinous rice	Landrace

of water was recorded before and after spraying every day. The morphological characteristic data were collected including epidermal hairs of leaves, leaf injury and root lengths at 8 d after treatment (DAT). The leaf characters (leaf length, leaf width, number of leaves) and plant height were recorded at 0, 4 and 8 DAT. Leaf greenness was measured using a SPAD-502 chlorophyll meter at three points of a fully expanded leaf (tip, middle and base of the leaf) at 4 and 8 DAT with simulated acid rain. The root and leaf fresh weights were collected at 8 DAT (33 d after sowing), the dry weights of roots and leaves were measured after drying in a hot air oven at 65°C continuously for 3 d. Leaf injury was scored as a necrotic percentage on fully expanded leaves, using three leaves per plant and three plants per replication. The data were combined to determine the average for each cultivar. Leaf necrosis was divided into five severity levels: 80% necrotic spots per leaf area was scored as a highly sensitive cultivar; 60–79% was scored as a sensitive cultivar; 40–59% was scored as a moderately sensitive cultivar; 20–39% was scored as a tolerant cultivar; and 0–19% was scored as a highly tolerant cultivar.

Statistical analysis

Data were analyzed using one-way analysis of variance followed by Duncan's multiple-range test. The tests were considered significant different at $p < 0.05$. The analysis was done using IRRIstat version 7.2.

Results

Effect of different pH levels of simulated acid rain on morphological traits of rice

The results were divided into three sections: the effects of different pH levels of simulated acid rain on morphological traits of rice; the response of the 10 Thai rice cultivars exposed to simulated acid rain and the level of leaf injury of rice exposed to different pH levels of simulated acid rain at a rate of about 27 $mL/m^2/hr$. The results showed no interaction between the pH levels of simulated acid rain and the Thai rice cultivars tested.

There were significant differences for simulated acid rain at different pH levels on the 12 morphological traits of rice for most traits except leaf length, leaf width and plant height at 0 DAT. The roots-to-shoot ratio on a fresh weight basis and the root fresh weight were not significantly different among cultivars but the effect of simulated acid rain on shoot fresh weight was significant compared to the control (Fig. 1), with the highest values being 0.749 g and 0.770 g for pH 4.5 and 2.5, respectively. However, simulated acid rain at pH levels of 6.5, 5.5 and 3.5 did not produce significant differences in the shoot fresh weight. The shoot and root dry weights of rice exposed to simulated acid rain at pH 4.5 and 2.5 were significantly higher than for the control (pH 6.5). These results indicated that the simulated acid rain at pH 4.5 and 2.5 stimulated growth in the shoot fresh weight and the shoot and root dry weights of rice. The results for the root-to-shoot ratio (both fresh and dry weights) revealed that the root-to-shoot fresh weight ratio was highest at pH 6.5–5.5, indicating that the increased acidity of the simulated acid rain reduced the root-to-shoot ratio on

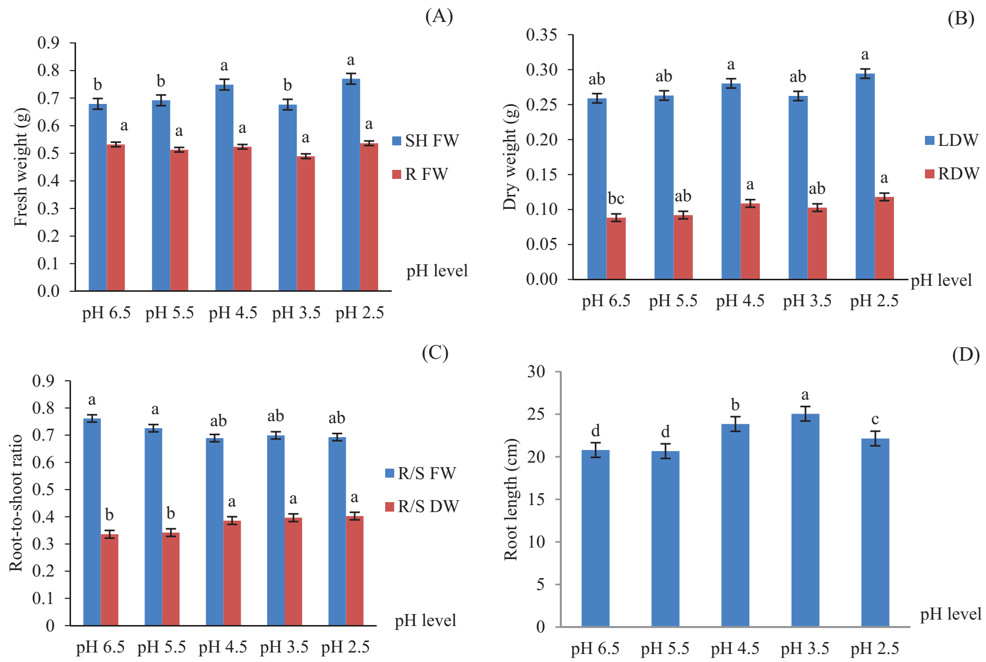

Fig. 1 Effect on 10 Thai rice cultivars of simulated acid rain at various pH levels after continuous exposure for 7 d: (A) shoot and root fresh weights (SH FW and R FW, respectively); (B) root and shoot dry weights (RDW and LDW, respectively); (C) root-to-shoot ratio of fresh and dry weights (R/S FW and R/S DW, respectively); (D) root length (RL); where values represent mean; error bars represent SE; different lowercase letters above bars indicate significant ($p < 0.05$) difference among pH levels.

a fresh weight basis. However, the increased acidity of the simulated acid rain increased the ratio on a dry weight basis at pH 4.5–2.5. The results revealed that the greatest root length was for pH 3.5, indicating that this level stimulated root growth, while for pH 2.5 it was lower. However, all acidity levels of simulated acid rain stimulated the root growth of rice compared to the control (pH 6.5). These results suggested that the effect of simulated acid rain was to change the rate of growth and development of different parts of the rice plant. The simulated acid rain was sprayed on the top of plant, which reduced the upper plant part and promoted the lower plant part.

The dynamic effects of simulated acid rain on rice leaf characteristics and plant height (Fig. 2) were recorded at 0 DAT, 3 DAT and 8 DAT. The results revealed that the leaf lengths of plants exposed to simulated acid rain at pH 5.5 and pH 4.5 were reduced at 3 DAT compared with the control, while the leaf length at 8 DAT was reduced at pH 5.5. However, the effect of simulated acid rain at pH 4.5, pH 3.5 and pH 2.5 at 8 DAT on leaf length were not different compared to the control. The effect of simulated acid rain on leaf width revealed that pH 4.5 and pH 2.5 stimulated leaf width at 3 DAT. However, the effect of simulated acid rain was not significant across the treatments at 8 DAT. The results indicated that the rice leaf width was not significantly affected by the different levels of simulated acid rain.

The number of leaves of rice exposed to different levels of simulated acid rain increased at pH 4.5 and pH 2.5, at both 3 DAT and 8 DAT. Plant height was stimulated by the effect of simulated acid rain at pH 4.5 and pH 2.5, at both 3 DAT and 8 DAT. SPAD indices were investigated based on measurement at three points on the first full leaf blade, using three plants per replications. Lin et al. (2010) suggested that the SPAD value was dependent on the rice cultivar, growth stage, leaf position and measurement point on the leaf blade. The current results showed a decline in the SPAD index at 4 DAT for pH 4.5–2.5, while the SPAD index at 8 DAT declined when exposed to pH 3.5–2.5. The results indicated that simulated acid rain reduced the chlorophyll concentration in the rice leaves.

Response of 10 Thai rice cultivars exposed to simulated acid rain

The effects of simulated acid rain on the root and shoot fresh weights, root and shoot dry weights, root-to-shoot ratio and root length of 10 Thai rice cultivars were significantly different for most traits except for the root length of the rice cultivars (Fig. 3). The shoot and root fresh weights were measured at final harvest (33 d after sowing) and the results indicated that treatment effects on shoot and root fresh weights were significantly different, with the rice cultivars RD27, CMJ, KTH17, MLL, TTCP and HPYTD having the highest shoot fresh weights and the RD27, CMJ and HPYTD cultivars having the highest root fresh weights. There were significant differences in shoot and root dry weights, with the RD27, CMJ, KDKL105, KTH17, MLL, TTCP, KKN and HPYTD cultivars having the highest shoot dry weights, while the root dry weights of the RD27, CMJ, KDKL105, MLL, KKN and HPYTD cultivars were the highest. The root-to-shoot ratios (fresh and dry weights) in the rice cultivars were significantly different, with RD27 being higher than the other cultivars for the root-to-shoot ratio on a fresh weight basis, whereas on a dry weight basis CMJ, MLL and HPYTD were the highest cultivars. These results suggested that the RD27, CMJ, MLL and HPYTD cultivars were high biomass accumulators when exposed to simulated acid rain. However, the effect of simulated acid rain on the root length of the rice cultivars was not significant.

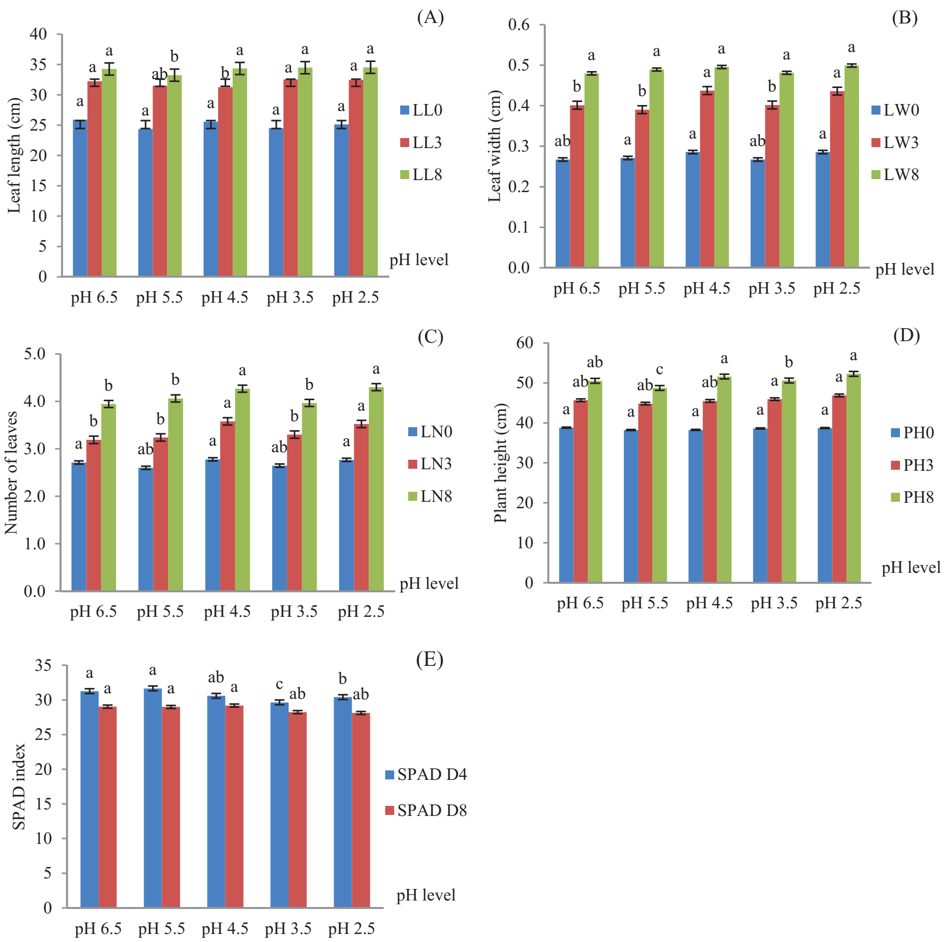

Fig. 2 Effect on 10 Thai rice cultivars of simulated acid rain at various pH levels after continuous exposure for 7 d: (A) leaf length; (B) leaf width; (C) number of leaves; (D) plant height; (E) SPAD index at 4 d and 8 d after treatment (D4 and D8, respectively), where values represent mean; error bars represent SE; different lowercase letters above bars indicate significant ($p < 0.05$) difference among pH levels; LL = leaf length; LW = leaf width; LN = number of leaves; PH = plant height; 0, 3 and 8 = days after treatment

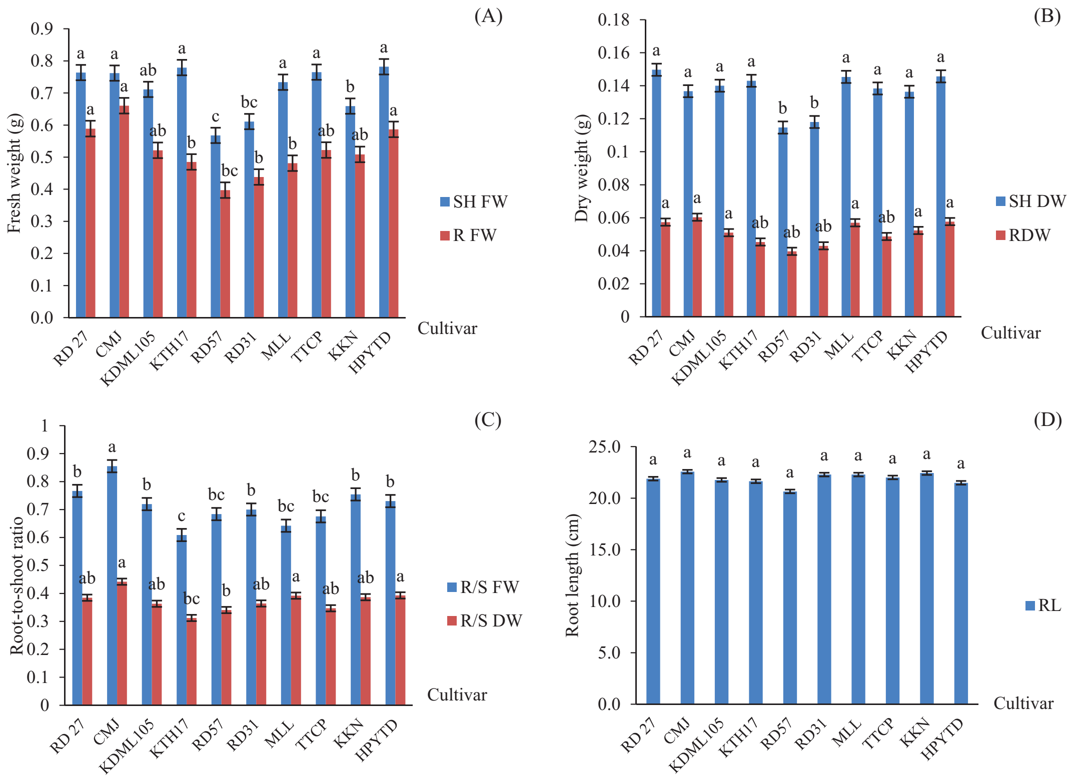

Fig. 3 Effect on 10 Thai rice cultivars of simulated acid rain after continuous exposure for 7 d: (A) shoot and root fresh weight (SH FW and R FW, respectively) (B) root and shoot dry weight (RDW and SH DW, respectively); (C) root-to-shoot ratio of fresh and dry weight (R/S FW and R/S DW, respectively); (D) root length (RL); values represent mean; errorbars represent SE; different lowercase letters above bars indicate significant ($p < 0.05$) difference among cultivars

The dynamic effects were investigated of simulated acid rain on leaf length, leaf width, number of leaves, plant height and SPAD index of the rice cultivars exposed to simulated acid rain continuously for 7 d. The leaf length, leaf width, number of leaves and plant height were measured at 0 DAT, 3 DAT and 8 DAT and the SPAD indices were collected at 4 DAT and 8 DAT (Fig. 4). The results showed significant differences for all traits and times of measurement. However, the differences across cultivars at 0 DAT for all traits should be attributed to genotype and not as a treatment effect. Therefore, the percentage increases in these traits were used to compare rice cultivars and their responses to simulated acid rain at 8 DAT for each morphological trait. The results revealed that the leaf length at 0 DAT was high in the RD27, KDML105, KTH17, MLL, TTCP and HPYTD cultivars; however leaf length at 3 DAT was high in the KTH17 and MLL cultivars. The leaf length at 8 DAT was highest for the RD27 and KTH17 cultivars. The increasing percentage in leaf length for each cultivar was low in the RD31 (31.4%), TTCP (30.5%), KKN (28.35%) and HPYTD (32.5%) cultivars, indicating that the responses of these cultivars to simulated acid rain were high. The leaf widths at 0 DAT and 3 DAT were the highest for the KKN cultivar, while at 8 DAT the width was the greatest for the CMJ and KNN cultivars. The percentage increase in the leaf width was low for the RD27 (65%), KDML105 (65.2%) and RD57 (61.3%) cultivars. The results revealed that at 0 DAT the number of leaves was the highest for the RD27, KDML105, RD57 and KKN cultivars, while at 3 DAT, it was highest for the RD27 cultivar. The RD27, KDML105, RD37 and TTCP cultivars had the greatest leaf widths at 8 DAT, whereas the percentage increase was low for the RD57 (40.9%) and KKN (35.4%) cultivars. The plant height was the highest at 0 DAT for the KTH17, MLL and HPYTD cultivars, while at 3 DAT and 8 DAT, the highest were the KTH17 and MLL cultivars. The percentage increase was the lowest in the KTH17 (29.4%), MLL (25%) and HPYTD (21.6%) cultivars. The effect of simulated acid rain reduced the SPAD values, indicating reduced levels of chlorophyll concentration. At 4 DAT, the highest SPAD value was for the RD31 cultivar and the highest percentage reductions in SPAD value were obtained in the CMJ (-6.6%), RD31 (-6.7%), MLL (-6.6%), TTCP (-6.9%), KKN (-10.8%) and HPYTD (-6.5%) cultivars. These results suggested that these cultivars were susceptible to simulated acid rain as there was a reduction in the leaf chlorophyll concentration.

Leaf injury of rice exposed to simulated acid rain at different pH levels

The effects of simulated acid rain on rice plants at vegetative stage (Fig. 5A) and leaf characteristics were scored for leaf injury (necrotic effect) and epidermal leaf hairs. The epidermal hair length and number of epidermal hairs per unit area were investigated using 3× spectro-microscopy with three replications (Table 2). The leaf samples were taken randomly from three plants for each cultivar × treatment combination. The results revealed that the RD27, CMJ, KDML105, KTH17, TTCP and HPYTD cultivars were scored as leaf pubescent (P) cultivars (Fig. 5B) and the RD31, RD57, MLL and KKN cultivars were scored as non-pubescent cultivars (Fig. 5C). For the pubescent cultivars, the average length epidermal hair length of HPYTD was the greatest (0.29 mm) and KTH17 had the shortest (0.20 mm). The number of epidermal hairs per unit area (31) was highest for the KDML105 cultivar and the lowest (11) for the CMJ cultivar. Leaf injury was scored as a necrotic percentage and the effects of simulated acid rain at pH 2.5 on leaf necrosis symptoms are shown in Figs. 5D and 5E. The CMJ, KDML105, TTCP and HPYTD cultivars were scored as moderate (40–59% leaf necrosis), while the RD27 and KTH17 cultivars were scored as tolerant (20–39% leaf necrosis). The highly tolerant (0–19% leaf necrosis) cultivars were RD57, RD31, MLL and KKN. The results indicated that rice plants could be considered a moderately-tolerant-to-tolerant regarding exposure to simulated acid rain.

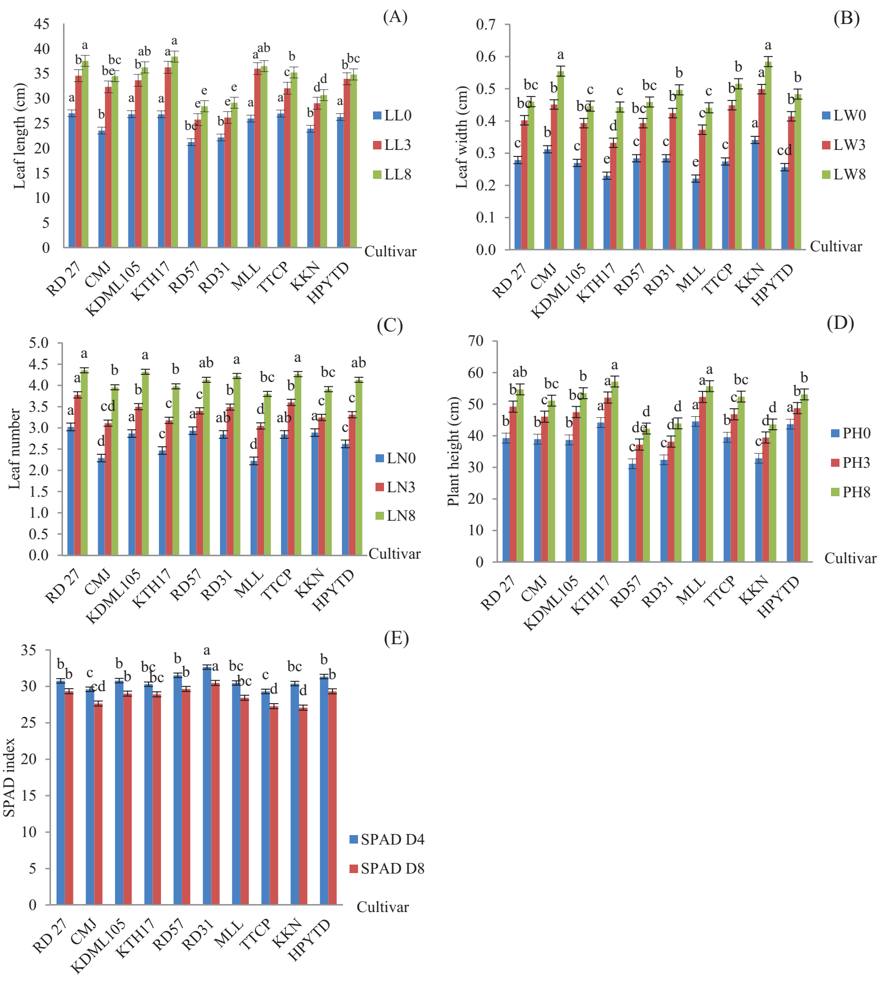

Fig. 4 Effect on 10 Thai rice cultivars of simulated acid rain after continuous exposure for 7 d: (A) leaf length; (B) leaf width; (C) number of leaves ; (D) plant height; (E) SPAD index at 4 d and 8 d after treatment (D4 and D8, respectively); values represent mean; error bars represent SE; different lowercase letters above bars indicate significant ($p < 0.05$) difference among cultivars; LL = leaf length; LW = leaf width; LN = number of leaves; PH = plant height; 0, 3 and 8 = days after treatment

Table 2 Leaf injury, leaf epidermal hairs, epidermal hair length and number of epidermal hairs per leaf area for 10 Thai rice cultivars affected by simulated acid rain

Cultivar	Leaf injury[1]	Epidermal hairs on leaf	Leaf epidermal hair length (mm)*	Number of leaf epidermal hairs per unit area*
RD 27	T	EH	(0.118–0.324)±0.227	(20–38)±29.3
CMJ	M	EH	(0.129–0.453)±0.258	(2–16)±11
KDML105	M	EH	(0.129–0.370)±0.217	(25–36)±31
KTH17	T	EH	(0.126–0.322)±0.201	(2–8)±5
RD57	HT	No EH	-	0
RD31	HT	No EH	-	0
MLL	HT	No EH	-	0
TTCP	M	EH	(0.102–0.219)±0.156	(12–23)±17
KKN	HT	No EH	-	0
HPYTD	M	EH	(0.162–0.461)±0.290	(20–23)±21.6

HT = highly tolerance; T = tolerance; M = moderate; EH = epidermal hairs; Non EH = no epidermal hairs

[1] = leaf injury affected by simulated acid rain at pH 2.5;

* = data investigated in a 3× field view using spectro-microscopy with three replications and (unit area = 5.6 mm^2) with values presented as mean ± SD

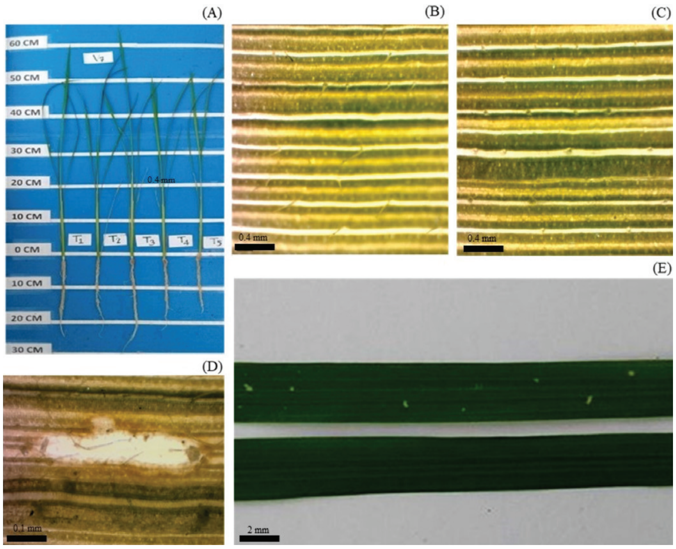

Fig. 5 Effects of simulated acid rain on rice plants at vegetative stage: (A) of MLL cultivar (T1–T5, for treatments of pH 6.5, 5.5, 4.5, 3.5 and 2.5, respectively); (B) leaf pubescence of KDML 105; (C) non-pubescent rice leaf of RD31; (D) close-up of necrotic leaf damage of KDML 105 under 4× spectro-microscopy; (E) visual symptoms of rice exposed to simulated acid rain at pH 2.5 (upper) *vs* control (lower)

Discussion

The effects of simulated acid rain on rice has been investigated for various traits such as physiological traits, biochemical processes and treatment by genotype interaction (Wang et al., 2008; Chen et al., 2014; Satoh et al., 2014; Ramlall et al., 2015). The results of such investigations showed that the effect of simulated acid rain varied depending on the plant species, plant stage, the pH of the acid rain and the environmental conditions (Tong and Zhang, 2014). Furthermore, plants could only utilize a comprehensive range of nutrients and growth normally in a suitable pH range. The current study investigated the effect of different pH levels of simulated acid rain on the morphological traits of 10 Thai rice cultivars during the vegetative stage. The spraying method of simulated acid rain was assumed to mimic rain events, resulting in the simulated acid rain falling on the plants and draining down into the pots. However, the average pH of the ground water (7.40–7.70) did not change in the tray of each treatment after simulated acid rain spraying. It could be expected that a real acid rain events might cause a change in the pH of ground water that could be related to the pH and soil properties. The current results suggested that increasing the acidity of simulated acid rain reduced the root-to-shoot ratio on a fresh weight basis and SPAD indices, while it stimulated the root-to-shoot ratio on a dry weight basis, the root and shoot fresh weights, root and shoot dry weights, root length, number of leaves, plant height and leaf weight compared with pH 6.5 as the control treatment. However, the leaf length was reduced at pH 5.5 but was not affected by simulated acid rain at higher levels of acidity. The current results indicated that leaf greenness (as indicated by the SPAD index), which is correlated with the chlorophyll content, was directly affected by the simulated acid rain, which might have reduced the rate of photosynthesis and the accumulation process of fresh weight in the roots and shoots of the rice cultivars. These results indicated that different morphological traits of rice responded differently to simulated acid rain. Zhao et al. (2013) considered that the morphological parameters of a plant might be affected by acidic conditions. Another study reported that rice seed germination, seedling root length, the root-to-shoot ratio and the number of crown roots per seedling decreased following exposure to simulated acid rain at pH 2.5 (Sreesaeng et al., 2021)

The number of epidermal hairs of leaf has been related to an increased rate of necrotic spots on rice leaves. Visual observation confirmed that the water droplets of simulated acid rain might stick to epidermal leaf hairs causing damage, whereas it might flow off leaves having no epidermal hairs leaf. Thus, water droplet adhesion of simulated acid rain could be correlated with the leaf surface properties of plants. Wang et al. (2014) reported that leaf roughness, surface free energy and the trichrome on abaxial and adaxial surfaces were related to the rate of water adhesion. The presence of a trichrome or wax crystals may explain the lower retention of water droplets on leaves and the reduced leaf water retention on young leaves compared with older leaves. Simulated acid rain caused epidermal and mesophyll cell alterations followed by plasmolysis of the guard cells and cuticle rupture at necrotic spots (Anna-Santos et al., 2006).

The results of the current study revealed that simulated acid rain promoted rice growth and development rather than inhibiting it. This was in agreement with Fan and Wang (2000) who suggested that seedling growth in hardwood species was stimulated at pH 3.5–5.5 and Imran et al. (2014) revealed that plant height and branch numbers of mash (*Vigna mungo* L.) were stimulated by acidic water. Fan and Li (1999) suggested that simulated acid rain stimulated the seedling growth of five broadleaved species at pH 3.5–5.5. However, the effect of simulated acid rain at pH < 2 inhibited seedling growth of plants (Fan and Li, 1999; Fan and Wang, 2000). Santos et al. (2006) and Ramlall et al. (2015) found that necrotic spots on the leaf blades of tree species occurred at pH 3.0, while Tong and Zhang (2014) reported that simulated acid rain at pH 2.5–5.5 did not affect the final mortality of five tree species and the effect of simulated acid rain on the physiological traits of plants was unclear in terms of economic yield losses. However, the stress of simulated acid rain on a plant's health might cause economic yield losses at different levels, depending on the species and the environmental conditions, because the effect of simulated acid rain on biochemical processes in plants has been reported at different levels. Kacharava et al. (2013) revealed that simulated acid rain increased the levels of ascorbic acid, carotenoids, anthocyanins, proline and soluble phenols, and altered the antioxidative system that might affect a plant's resistance to stress. Ruuhola et al. (2009) suggested that peroxidase activity plays an important role in the quenching of the oxidative stress in birches. Gabara et al. (2003) indicated that simulated acid rain altered chloroplasts and mitochondria and at the end of their experiment, there was disruption of the structure of these organelles accompanied by changes in the activities of catalase, ascorbate peroxidase and superoxide dismutase, including CuZnSOD, glutathione peroxidase and glutathione transferase. However, the combined pollution of rare earth elements and acid rain has become an emerging environmental issue; for example, the deleterious effects of combined pollution were stronger than those of lanthanum (La^{3+}) or acid rain pollution alone (Sun et al., 2013). Wen et al. (2011) reported that the growth and photosynthesis of soybean seedlings were clearly inhibited. The combined treatment of 81.6 lM lanthanum chloride ($LaCl_3$) and acid rain at pH 3.5 produced toxic effects on the net photosynthetic rate, stomatic conductance, intercellular CO_2 concentration, Hill reaction activity, apparent quantum yield and carboxylation efficiency in rice and that the higher acid load decreased the soil microbial activities (Wang et al., 2014). Liao et al. (2005) revealed that the complex toxic effects of Cd^{2+}, Zn^{2+} and acid rain adversely affected the growth of kidney beans, especially with higher amounts of cadmium (Cd^{2+}) and zinc (Zn^{2+}) and a higher level of acidity in the acid rain. Velikova et al. (2014) revealed that simulated acid rain caused an increase in peroxidase and decreased catalase activities during the first hours after treatment. Their report suggested that the single and combined effects of simulated acid rain and other stresses affected the physiological and biochemical properties of plants. Therefore, the effect of simulated acid rain on the physiological and biochemical properties of rice deserves investigation. Future studies could investigate the effect of simulated acid rain on the yield and yield components of Thai rice cultivars. This information could be used to inform breeding perspectives of rice to adapt to global warming and climate change.

The effects of simulated acid rain might affect the morphological characteristics and yield of rice, with the expected increasing rates of sulfur oxide and nitrogen oxide from natural and human sources. Increasing rates of pollutants may increase the frequency and acidity of acid rain in many area of the world, specifically in industrial or developed countries. Although the reported frequency of acid rain events in Thailand has been low, the cross border transmission of these pollutant might influence future levels of acid rain in Southeast Asia and rice planted in the rainy season may be affected. Therefore, the study of the effect of simulated acid rain on the morphological traits of rice might be used to improve cultural practices in rice growing to avoid this situation as well using this information obtained to produce or improve rice cultivars that are resistant to acid rain in the future.

Conflict of Interest

The authors declare that there are no conflicts of interest.

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