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Effects of Simulated Acid Rain on Pollination of Rice Plants[1]

Ming-Hwi Yao,[2] Shu Chen[3], Jin-Chuan Tsai[2] and Lien-Sheng Liang[4]

Abstract: This study was conducted to determine the effect of simulated acid rain of pH 3.0, 4.0 and 5.6 on pollen grain germination and yield in rice (*Oryza sativa L.*) plants. Results indicated that direct exposure to acid rain of pH 3.0 and pH 4.0 acid rain reduced pollen grain germination by up to 30% and 10%, respectively. These alternations closely paralleled to decreasing in seed setting, number of spikelets per panicle and grain yield. When exposed to pH 5.6 acid rain, rate of pollen grain germination decreased, but the effect was not significant. Germination rate of pollen grown on agar medium at pH 4.0 was 54% of that grown on pH 5.6. At pH 3.0 the pollen graims almost did not germinate. The two rice varieties (Tainung 67 and Taichung sen 10) had similar pollen grain germination rate both *in vivo* and *in vitro* in response to acid rain.

Key words: Acid rain, Rice (*Oryza sativa L.*), Pollen grain germination, Yield components.

INTRODUCTION

Nitrogen and sulfur oxides emitted into the atmosphere may react with atmospheric moisture to form acidic solutions. Natural rain water, in equilibrium with atmospheric CO_2 has an approximate pH value of 5.6. As a result of increased pollutant levels, the annual average pH value of precipitation in Taiwan is 4.86, with individual rainfalls as low as 3.8 [3]. The oxidation and hydration of oxides of sulfur and nitrogen from anthropagenic sources attributed to the increased acidity in precipitation.

The effects of the deposition of acidic substances on plant growth and development are not well understood. The acidity of simulated rain may increase, have no effect upon, or decrease crop yield[8,12]. Assessing the effects of general atmospheric pollutants and particular acid rain on agricultural and forest ecosystems will be difficult until the physiological and developmental mechanisms involed in plant response to atmospheric deposition are better understood.

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1. Contribution No. 1835 from Taiwan Agricultural Research Institute.
2. Respectively, Assistant, Associate Researcher, and Head, Department of Agricultrual Engineering, TARI, Wufeng, Taichung, Taiwan, ROC.
3. Assistant, Department of National Plant Gentic Resources Center, TARI, Wufeng, Taichung, Taiwan, ROC.

Acid conditions are known to have adverse effect *in vitro* on germination and growth of pollen from several plants[1,3,16]. Forsline et al. (1983) reported that simulated rain pH below of 2.75 inhibited *in vitro* pollen germination in apple. In studying the effects of acid rain on pollen germination *in vivo*, Cox (1984) observed that pH 3.6 simulated rain applied before pollination to the stigma of evening primrose decreased subsequent pollen germination. Wertheim and Craker (1988) noted that germination of corn pollens was reduced on silks treated with simulated acid rain. Both of these observations suggest that acid precipitation falling on exposed stigma may hinder pollen-silk interaction and /or the development of pollen tube required for fertilization of an egg cell. This reduced the number of fertilization and resulted in decreasing of seeds.

The objectives of these experiments were to determine if acid rain event on the flower would affect seed setting and grain yield in rice and if any yield reduction was related to the quantities of pollen used in the pollination process.

MATERIALS AND METHODS

Acid treatments:

The pH value of the simulated rain was adjusted to one of the three levels (pH 5.6, 4.0 or 3.0) with sulfuric and nitric acids at a constant sulfate/nitrate ratio of 2.5: 1 on an equivalent basis. Acid solution in a PVC supply canister was pressurized with hand and forced through a small orifice of a commercial stainless steel rain nozzle. Where it impinged on the tip of a J-shaped pin and was broken into particles with mean stain diameter of 1.0 mm. Spraying was carefully done to wet the panicles surface evenly and completely.

Under normal weather conditions in Tawian, most rice varieties begin anthesis at about 0900 and end at about 1100 hour. On rainy days, pollination may occur without the opening of the lemma and palea. For this reason, exposure were conducted at 0800 (before flowering) and 1000 (flowering) hour, to determine the indirect and direct exposure of pollen to simulated acid rain applied on the processes of sexual reproduction of rice. At the beginning of anthesis, simulated rain was delivered to the plants five times a week until the conclusion of the flowering.

Greenhouse study:

Rice seeds ("Tainung 67" and "Taichung sen 10") were sown in plastic pots (3 seeds a pot, pot size: 1/5,000 ha) filled with 2L of loamy soil. Seedlings were thinned to one plant per pot 7 days after sowing. The experimental plants were allocated in a greenhouse that served as an ambient rain shelter for the delivery of simulated acid rain.

In vivo pollen grain germination experiment:

A subsample of five spikelets was removed from the top three primary branches, placed in 3: 1 v/v basis solution of absolute ethylalcohol and acetic acid fixative. Stigmas from sample spikelets were excised and placed on a glass slide, then stained with acetocamine dye. Using an optical microscope, the total number of pollen grains on stigmas and number of germinated pollen grains were counted. Pollen viability was computed as the ratio of germinated pollen grains to total pollen grains on a stigma.

Field study

Field study was conducted to determine the effects of simulated acid rain on yield and yield components of rice plants when treated at the anthesis. Two rice cultivars (Tainung 67-japonica and Taichung sen 10-indica) selected to represent a diversity of agronomic characters were used in the experiment designed to identify the damage by simulated acid rain treatment. The experiment was conducted on the experimental farm in the first and second crop seasons 1994. Seeds were sown using wetbed technique and one month-old seedlings were transplanted to a plot (5.0* 2.0m^2) with 1 plant/hill. Each plot contained seven 5-m rows of rice plants spaced 0.2-m apart. The layout was a randomized complete block design (RCBD) with 3 replications. Fertilizer was applied at the rate of 60-40-40 kg N-P_2O_5-K_2O per hectare before transplanting. Weeds were controlled with occasional hand weeding. Insecticides were applied as often as necessary.

In vitro pollen grain germination experiment:

The preparation of the agar medium followed the method of Kariga (1989). 1% agar and 20% sucrose were dissolved in heated distilled water (pH adjustment, pH 5.6, 4.0 and 3.0) and the solution was cooled to about 60°C with stirring in a waterbath. 20ppm $K_2B_4O_7$ were added immediately into the agar and sucrose medium. The prepared medium was poured into petri dishes (4.5 cm in diameter) and the surplus was removed in order to make a thin layer. The germinated pollen grains were counted under a microscope about 20 min after shedding of pollen grains on the medium. Any pollen grains with a tube more than a half of the grain diameter were regarded as germinated. In each experiment, 200-300 pollen grains were counted.

RESULTS

Pollen grain germination was reduced on stigma under direct exposure to the simulated rain of pH 3.0 as compared with germination on stigma under direct exposure to the simulated rain of pH 4.0 and pH 5.6 (Table 1). However, there were no significant differences of indirect exposure to acid rain on all the germinating characters of pollen.

Germination rate of the two cultivars on agar medium at pH 4.0 were both near 54% of that grown on medium at pH 5.6 (Table 2). At pH 3.0, the pollen grains almost did not germinate.

For direct exposure, the different pH treatments did not significantiy affect the number of panicle per hill and 1,000 grain weight for both cultivars. The average percentages of seed setting of panicles which was directly exposed to pH 3.0 rain 6 times were inhibited were 35.2% in the first crop (Table 3). and 39.6% in the second crop (Table 4). of Tainung 67 in 1994. Similar results were found in Taichung sen 10 (Table 3 and 4). In addition, the seed setting of first crop for Tainung 67 and Taichung sen 10 at pH 5.6 was significantly lower than the no rain treatment (control), however, two cultivars treated with pH 5.6 rain was not significantly lower than control, but, average lower was found in second crop, indicating that pollination process is unable to resist wetting. The yield of both cultivars at the pH 3.0 treatment was significantly less than average yield of the other three treatment. In 4.0

treatment, yield of Taichung sen 10 tended to be less than the pH 5.6 or no rain treatment, whereas the pH 4.0 yield for Tainung 67 was not significant. However, the yield components of two in which panicles were cultivars indirectly exposed to different acid rain treatments were unaffected.

Table 1. Means of pollination characteristics of Tainung 67 and Taichung sen 10 rice cultivars by simulated acid rain at anthesis stage

		Direct			Indirect		
	no rain	5.6	4.0	3.0	5.6	4.0	3.0
Tainung 67							
pollen grains/stigma (no.)	98.6a	73.3b	76.3b	73.5b	101.7a	95.6a	91.5a*
germinated pollen grains/stigma(no.)	88.6a	59.3b	58.3b	45.9c	88.1a	81.9a	80.3a
In vivo pollen grain germination(%)	89.9a	80.9ab	76.4b	62.4c	86.6a	85.7a	87.8a
spikelets with 0-10 germinated pollen grains/stigma (%)	0.0d	3.7c	15.4b	34.6a	0.0d	0.0d	0.0d
spikelets with >10 germinated pollen grains/stigma (%)	100.0a	96.3b	84.6c	55.4d	100.0a	100.0a	100.0a
unfilled bloomed spikelets (%)	11.6a	18.8ab	7.2b	41.6c	9.8a	12.3a	14.8ab
Taichnung sen 10							
pollen grains/stigma (no.)	84.6a	77.4a	58.6b	65.3b	82.0a	83.2a	79.6a
germinated pollen grains/stigma(no.)	76.4a	67.4b	7.8c	49.5c	77.2a	19.1a	72.1a
In vivo pollen grain germination(%)	90.3a	87.1ab	81.6b	75.8c	94.1a	95.0a	90.6a
spikelets with 0-10 germinated pollen grains/stigma (%)	0.0c	0.0c	8.7b	24.2a	0.0c	0.0c	0.0c
spikelets with >10 germinated pollen grains/stigma (%)	100.0a	100.0a	91.3b	75.8c	100.0a	100.0a	100.0a
unfilled bloomed spikelets (%)	9.6a	10.3a	17.8b	30.8c	9.2a	9.5a	10.6a

* Means in each row with the same letter are not signficantly different at 5% level (Duncan's New Multiple Range Test)

Table 2. Means of pollen grain germination rate (%) of Tainung 67 and Taichung sen 10 rice cultivars under different pH of culture medium *in vitro*.

	Medium pH		
	5.6	4.0	3.0
Tainung 67	92.5a	51.3b	5.6c*
Taichnung sen 10	90.6a	48.6b	3.7c

* Means in each row with the same letter are not signficantly different at 5% level (Duncan's New Multiple Range Test)

Table 3. Yield and yield components of Tainung 67 and Taichung sen 10 in simulated acid rain treatments in the first crop of 1994.

		Direct			Indirect		
	no rain	5.6	4.0	3.0	5.6	4.0	3.0
Tainung 67							
panicle/hill (no.)	9.6a	9.7a	9.0a	9.3a	9.5a	9.0a	8.9a*
spikelet/panicle (no.)	107.6a	97.4a	86.7b	82.3c	104.3a	101.2a	97.4a
seed setting (%)	95.2a	83.2b	76.6c	64.8d	95.6a	90.6a	94.1a
1,000 grain wt (g)	24.6a	25.1a	23.6a	21.9a	24.1a	24.3a	22.6a
yield (g/hill)	29.7a	28.4a	26.5a	27.1b	30.6a	29.1a	27.8a
Taichung sen 10							
panicle/hill (no.)	11.6a	10.7a	11.2a	10.6a	12.0a	11.4a	11.6a
spikelet/panicle (no.)	100.6a	94.3a	80.9b	73.4c	98.1a	99.8a	90.1ab
seed setting (%)	89.6a	82.0b	69.4c	59.6d	87.5a	90.6a	90.1a
1,000 grain wt (g)	23.6a	22.1a	21.8a	21.8a	23.0a	23.6a	22.7a
yield (g/hill)	30.1a	28.6a	25.3ab	19.4b	30.1a	29.6a	25.7ab

* Means in each row with the same letter are not significantly different at 5% level (Duncan's New Multiple Range Test)

Table 4. Yield and yield component of Tainung 67 and Taichung sen 10 in simulated acid rain treatments in the second crop of 1994.

		Direct			Indirect		
	no rain	5.6	4.0	3.0	5.6	4.0	3.0
Tainung 67							
panicle/hill (no.)	9.1a	8.8a	9.0a	9.1a	8.8a	8.6a	8.9a*
spikelet/panicle (no.)	98.2a	99.8a	94.0a	91.1a	97.5a	100.2a	98.2a
seed setting (%)	92.8a	84.3ab	79.1b	60.4c	93.7a	89.4a	83.5a
1,000 grain wt (g)	25.2a	24.6a	24.7a	22.5a	23.8a	22.5a	24.6a
yield (g/hill)	25.4a	23.6a	22.7a	16.4b	26.1a	25.0a	20.6a
Taichung sen 10							
panicle/hill (no.)	11.2a	10.5a	9.5a	10.2a	10.8a	11.6a	11.0a
spikelet/panicle (no.)	94.6a	93.0a	79.6b	67.1c	93.2a	91.3a	89.4a
seed setting (%)	87.9a	84.5a	74.4b	66.3c	84.4a	82.9a	81.7a
1,000 grain wt (g)	22.5a	23.1a	21.6a	21.7a	22.1a	21.8a	23.2a
yield (g/hill)	27.9a	25.4ab	22.6b	19.5c	28.2a	27.6a	23.0a

* Means in each row with the same letter are not significantly different at 5% level (Duncan's New Multiple Range Test)

DISCUSSION

Acid deposition can negatively affect crops reproduction in many ways. For example, acid deposition can alter physilogical processes, such as photosynthesis; cause visible injury to leaves, such as chlorosis, necrosis, and premature leaf fall. The effects of acid rain on photosynthesis and leaves may be indirectly responsible for the reduction of seed production. Reproduction in rice and most other plants requires the germination and growth of pollen on stigmatic tissue. Any environmental stress that limits the ability of the male

gametophyte to germinate and grow on the stigma could reduce seed production and could alter the gene pool, especially in natural ecosystems with changes in gametophytic competition[13].

The effects would be more severe if acid rain occurred during pollen grain germination stage. Acid rain of pH 4.0 or less would seriously inhibit germination as well as growth of the pollen tube. Several authors have reported that rain of similar acidity (pH values 3.0-4.0) have inhibitory effects on specific phases of plant growth and fruit setting in tomato[11], adversely affected fruit production in term of individual fruit weight, fruit set and fruit appearance in apple[14]. Results of the study demonstrated that in rice, pollen grain germination was reduced when stigma and pollen grains directly exposed to simulated acid rains of pH 4.0 and 3.0 as compared to a "non-rain" control. At pH 5.6, pollen grain germination was decreased, but not significant. In addition, acid solution had a negative influence on the total number and percentage of germinated pollen grains *in vivo* (Table 1). The deleterious effects on pollen shedding and germination indicate that lack of germination is partly due to a decrease in the number of pollen grains, particularly fertile pollen grains. In pH 3.0 treatment, more than 30% of spikelets had less than 10 germinated pollen grains, It is noted that at least 10 germinated pollen grains per stigma are necessary for successful fertilization[7].

The reasons for reduced pollen grain germination on rice directly exposed to acid rain may be due to chemical or physical effects of acid rain on the stigmatic surface, creating an environmental inhospitality to pollen, or to a direct effect of rain residue on either the pollen grain or elongation pollen tube. However, when pollen grains reach the stigmatic surface, they imbibe water and stigmatic exudation and release enzymes[15]. The presence of toxic compounds or abnormally high acidity may affect their ability to germinate.

Acid rain of pH 3.0 almost totally inhibited pollen grain germination in agar medium culture (Table 2). whereas *in vivo* pollen grain germination (Table 1). and seed settings of field studies (Table 3 and 4) at pH 3.0 were produced a lower 30-40% compared with pH 5.6 or no rain treatments difference. For this result, simulated acidic rain at pH 3.0 may be sufficiently acidic to cause direct damage to and consequent leakage of basic solutes from stigmatic cells[5], such leakage might then buffer surface acidity, may explain the difference of pollen grain germination between *in vivo* and *in vitro* treatments. Karnoaky and Stairs (1974) suggested, based on his work *in vitro* and on experiments with *Populus tremuloides* Michx. (trembling aspen) seed set, that pollen grain germination and tube elongation may be more sensitive when fumigated *in vitro* than when fumigated *in vivo*. He was also proposed that the difference might be due to the greater pH buffering capacity of stigmatic tissues as compared to agar media.

The 10% decrease in seed setting caused by applying pH 5.6 simulated rain after pollination as compared to the no-rain and indirect exposure treatments could be due to wash away of ungerminated but still viable pollen from the stigma. Wet, damp, or humid conditions have been observed to inhibit successful pollen function[4], and even simulated rain without adding pollutants may have similar effect.

The adverse effects of single episodes of simulated acid rain reported here and elsewhere [3,17] demonstrate the potential for acute effects of atmospheric deposition on flowering and sexual reproductive processes should be considered when studying the influence of atmospheric pollutants on plant growth, development, and yield in agricultural and forest ecosystems.

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人工模擬酸雨對水稻授粉之影響[1]

姚銘輝[2] 陳述[3] 蔡金川[2] 梁連勝[4]

摘 要

本試驗主要探討水稻開花時人工模擬酸雨對花粉發芽的影響，並以田間資料探討影響產量減少的原因。結果顯示，水稻在開花時，直接噴施 pH 3.0及 pH 4.0的酸雨時花粉發芽率分別受抑制30%及10%，另發芽率降低將使穀粒結實率、每穗小花數及產量下降。噴施 pH 5.6酸雨則發芽率略爲下降，但未達顯著水準。若花粉未直接受酸雨噴施，則花粉發芽率及產量皆不影響。另將花粉置於不同 pH 值培養基培養，則酸性抑制花粉發芽的效果更爲明顯。兩個水稻品種(臺農67號及臺中秈10號)的花粉發芽率對酸雨的反應相似。

關鍵詞：酸雨、水稻、花粉發芽、產量構成因素。

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1. 臺灣省農業試驗所 研究報告第 1835 號。
2. 本所農工系助理、副研究員。臺灣省 臺中縣 霧峰鄉。
3. 本所作物種原室助理。臺灣省 臺中縣 霧峰鄉。
4. 本所農工系主任。臺灣省 臺中縣 霧峰鄉。