The Agriculturists 5(1&2): 35-43(2007) ISSN-1729-5211
A Scientific Journal of Krishi Foundation

Acetic Acid Effect on Seedling Growth of Some Anoxia-tolerant Land Race Rice (*Oryza sativa* L) Genotypes

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Received: 16 August 2007 Accepted: 31 October 2007

Abstract

An *in vitro* study was conducted to observe the effect of acetic acid in the process of seedling growth of five anoxia-tolerant land race rice genotypes (Tepi boro, Chaita boro, Bashful, Jahmir and Banajira) under hypoxic condition at two pH levels: pH 5 and pH 7. The acetic acid concentrations were 0, 4, 8 and 12 mM. Seedlings were grown in the test tubes containing 18 ml acetic acid-water solution (equivalent to 50 mm water column above the seed) under the two pH conditions at 30 °C for 7 days in dark. The nature and extent of the genotype responses varied with the concentrations of acetic acid and pH levels. Increased acetic acid concentration affected coleoptile length, mesocotyl length, 1st leaf length and root length significantly. The genotype Bashful was able to produce the longest 1st leaf length at pH 5. The 1st leaf growth of genotype Jahmir varied a little with the pH levels used in this study. Higher pH (pH 7) appeared to minimize acetic acid shock of the seedlings in the process of development.

Keywords: Land race rice, anoxia-tolerant rice, acetic acid, rice seedling

1. Introduction

Under direct seeded lowland rice culture (anaerobic seeding) some of the apparently anoxia-tolerant genotypes do not perform uniformly (Yamauchi and Biswas, 1996; Yamauchi *et al.*, 2000). This phenomenon could be attributed to accumulated volatile fatty acids (VFA) (Rao and Mekkelesen, 1977). Among the VFAs, acetic acid, propionic acid, butyric acid, and lactic acids are harmful to rice plants (Tsutsuki and Ponnamperuma, 1987). Many authors (Tanaka and Navasero, 1967; Chandrasekaran and Yoshida, 1973; Rao and Mikkelsen, 1977) have studied the effect of acetic acids and pH on the growth of the rice plant. Those studies were confined to root growth of aged seedlings. However, there was a few reports on the effect of acetic acid or organic acid on leaf growth in rice. Watanabe (1984) showed that acetic acid inhibited the germination and growth of 1st leaf. The genotypes they used were not considered as anoxia or hypoxia-tolerant. Cho and Ponnamperuma (1971) showed that the concentration of organic acids reached a maximum of 0 to 12 mM within 1 to 3 weeks after flooding and dropped to zero within 3 to 6 weeks. But in an intensive rice cropping area, to reduce the turn around time between the two crops, farmers may not wait until the disappearance or reduction of these aliphatic organic acids. Moreover, the distribution of added organic matter in the soil is heterogeneous, and the concentration of VFA remains high and the pH values remain low at the sites of the organic matter decomposition (1976). Thus pH of the growing media also plays an important role in seedling establishment and growth. Biswas *et al*. (2001ab) showed some interaction effects of VFA and pH on seedling growth in hypoxia. But the study was lacking information related to the land race genotypes from a country like Bangladesh holding the credit of extreme rice biodiversity. However, any information from a traditional genotype is considered quite worthy in the research and development of a rice variety or technology accommodative to certain stress condition. The present study therefore was conducted to investigate the influence of acetic acid and pH on the seedling growth of some anoxia/hypoxia-tolerant traditional rice genotypes under oxygen depleted environment.

2. Materials and Methods

The experiment was conducted at the Plant Physiology Division, Bangladesh Rice Research Institute, Gazipur, Bangladesh in 2006.

2.1. Treatments and experimental design

Five anoxia-tolerant land race rice genotypes viz. Tepi boro: (BRRI germplasm) accession No. 4526, Chaita boro: accession No. 1280, Bashful: accession No. 1471, Jahmir: accession No. 1706, Banajira: accession No. 007 was used in this study which was selected from the studies across the years (personal unpublished data).

Acid concentrations were decided following Biswas *et al.* (2001a). The four concentrations of acetic acid solution (growing media) were 0, 4, 8 and 12 mM. Two levels of pH namely 5 and 7 of the growing media were maintained. Factorial Randomized Complete Block Design with 3 replications was used for the experiment. The factors included 5 rice genotype (G), 4 levels of acid concentration (A), and 2 levels of pH (P). As the treatment combinations were 40, the replications were laid out over time (Gomez and Gomez, 1984).

2.2. Incubation of seeds

Prior to germination, the seeds were made disinfected by potting in 2% Sodium hypochlorite for 15 minutes. NaOH and HCl (1N each) were used to adjust the pH of the solutions to 7 or 5, used as growing media. Five sprouted (24 hour soaked and around 24 hour incubated in 30 °C) seeds were sown at the bottom of the test tubes (100 × 25-mm). Each test tube contained 18 ml of acetic acid solutions, equivalent to a 50 mm solution column above the seeds to maintain hypoxic condition. The test tubes (arranging in a rack) were wrapped in black cloth and kept in an incubator (Model: VWR Scientific Model 2015) at 30 °C for 7 days. The solutions were replaced with the fresh ones adjusting pH at every 2 days. Prior to the replacement, the adjusted pH was measured at every 2 days. In case of pH 5, the value increased, to some extent whereas in pH 7 it decreased to 5 at 0 mM acid concentration but for the other concentrations, the pH reached a value of 6.

2.3. Measurement

The data on the length of coleoptile, mesocotyl, 1st leaf, and root were recorded. The lengths of the parameters were measured from the first node to the tip of the respective organs. The acid concentration required for 50% length inhibition (LD_{50}) of 1st leaf length was used to estimate the toxicity level of acetic acid. This concept is borrowed from a widely used term in biological research, LD_{50}(Lethal dose 50%), defined as the dose that kills 50% of the organism in an experiment (Biswas *et al.*, 2002). LD_{50} was estimated as follows:

$Y = ax^2 \pm bx \pm c$ (when the relationship is quadratic)

When Y =50% of first leaf length as compared to the control treatment.

$x = LD_{50}$. a and b are the rate of curvilinear and linear co-efficient respectively, where c is the intercept.

The solution for x is given by...

$x = [-b \pm \sqrt{\{b^2-4a(c-Y)\}}]/ 2a$

Analysis of variance (ANOV), mean comparison through LSD, and regression were done to interpret the result.

3. Results and Discussion

3.1. ANOV of Seedling attributes

Acetic acid (A), genotype (G) and pH (P) interaction effect was significant for coleoptile, mesocotyl and root length at $p<0.01$ (Table 1). No similar interaction was observed for 1st leaf length. However, this attribute showed its significance at $p<0.01$ for A X G and A X P interaction and at $p<0.05$ for G X P interaction. The lowest CV (coefficient for variation) of 7.8% for coleoptile indicated minimum variation across the replications even under stress conditions. This is quite unique for this parameter observed in other studies too (Biswas, 1994). Mesocotyl appear to grow only under stressed conditions. The parameter showed a reasonable CV of 15.0%. In contrast, the CV for 1st leaf length and root length were 35.9% and 45%, respectively, meaning that these two parameters were more sensitive compared to the coleoptile and mesocotyl lengths.

3.2. Coleoptile

Coleoptile length varied from 46.30 to 53.93 mm at pH 5 and 46.29 to 57.77 mm at pH 7 at 0 mM acetic acid concentration (Table 2). Tepi boro maintained significantly its longest coleoptile length under both the pH conditions followed by Chaita boro at 0 mM acetic acid. Jahmir and Banajira had the similar but the shortest coleoptile length than the others. These are their inherent ability under hypoxic conditions as they did not experience any acetic acid-shock at 0 mM concentration. But at 4, 8 and 12 mM acetic acid concentration in addition to hypoxia the imposition of acetic acid stress was done to simulate lowland conditions. The genotypes were apparently tolerant to anoxia and they had the ability to produce elongated coleoptile under hypoxia. However, the variation they showed, was due to their genetic make up only. Increased acetic acid concentration reduced coleoptile length significantly. Accordingly, under pH 5, the coleoptile length of 53.93 mm for Tepi boro at 0 mM reduced to 38.27, 18.80, and 27.30 mm at 4, 8 and 12 mM, respectively. The corresponding values under pH 7 were 57.77, 55.00, 53.07, and 39.67 mm, respectively. Some of the discrepancies in pH 5 at 8 and 12 mM acid concentrations were not unlikely under the extreme stress conditions (Biswas, 1994 and Biswas *et al.*, 2001a). Tepi boro in 4 and 8 mM acid concentrations could not maintain its superior coleoptile length it attainted at pH 5. Chaita boro and Bashful performed better under the same condition. But at pH 7, all the genotypes performed almost equally up to 8 mM of acid concentration. pH had no effect at 0 mM. However, under different acid concentrations, increased pH level relaxed the coleoptile growth significantly.

Table 1. ANOV of seedling parameters as affected by acetic acid-concentrations, genotypes and pH levels.

SV	DF	F-Value			
		Coleoptile	Mesocotyl	1st leaf	Root
Rep (R)	2	1.79 ns	1.6 ns	<1	<1
Treatment	39	39.85**	25.06**	12.49**	26.50**
Acetic acid (A)	3	240.09**	37.40**	102.34**	184.65**
Genotype (G)	4	47.52**	161.65**	1.82**	20.04**
pH (P)	1	427.82**	7.64**	78.32**	123.06**
A×G	12	3.10**	5.78**	2.57**	7.93**
A×P	3	34.45**	35.15**	9.62**	19.10**
G×P	4	5.86**	1.10 ns	3.06*	16.55**
A×G×P	12	4.32**	2.63**	1.86 ns	4.80**
Error	78				
TOTAL	119				
CV%		7.8	15	35.9	45

Table 2. Coleoptile length (mm) as affected by acetic acid concentrations, Genotypes and pH levels.

Genotype (G)	pH (P) 5	pH (P) 7	Difference
Acetic acid (A) = 0 mM			
Tepiboro	53.93	57.77	-3.83
Chaitaboro	51.40	56.70	-5.30
Bashful	50.60	52.03	-1.43
Jahmir	43.40	46.29	-2.89
Banajira	46.30	46.53	-0.23
Acetic acid (A) = 4 mM			
Tepiboro	38.27	55.00	-16.73
Chaitaboro	37.93	51.80	-13.87
Bashful	41.90	50.53	-8.63
Jahmir	30.73	44.37	-13.63
Banajira	29.87	43.57	-13.70
Acetic acid (A) = 8 mM			
Tepiboro	18.80	53.07	-34.27
Chaitaboro	33.40	47.50	-14.10
Bashful	36.93	48.97	-12.03
Jahmir	21.47	38.03	-16.57
Banajira	21.47	38.03	-16.57
Acetic acid (A) = 12 mM			
Tepiboro	27.30	39.67	-12.37
Chaitaboro	25.20	36.87	-11.67
Bashful	23.70	40.00	-16.30
Jahmir	26.10	29.40	-3.30
Banajira	18.53	34.93	-16.40

Comparison LSD (5%)
2-AXGxP means 5.03

The enhanced coleoptile growth was observed in the present study due to hypoxic conditions. Coleoptile generally elongates under hypoxic conditions to the oxygenated layer of the soil to establish an O_2 transport system (Biswas and Yamauchi, 1997). The ability to elongate under O_2-stress could only be observed in some anoxia tolerant genotypes. The genotypes we used here were recognized as apparently anoxia tolerant in some previous studies. Coleoptile acts as a protecting attribute of the apical organ. Genotype difference was evident to some extent. The presence of minimum quantity of acid in this study affected coleoptile growth significantly. The coleoptile length showed by the genotypes from 29.87-41.90 mm under pH 5 is quite enough to establish seedling under lowland conditions. The minimum coleoptile length of 25 mm could establish seedling under lowland conditions (Biswas and Yamauchi, 1997). In the same pH level Jahmir and Banajira had coleoptile lengths less than 25 mm, which might affected seedling establishment at 8 mM acid concentration. At pH 7, coleoptile lengths for the genotypes in all concentrations were significantly longer.

3.3. Mesocotyl

At 0 mM acetic acid concentration mesocotyl length followed almost the same pattern as observed in case of coleoptile length. Tepi boro produced the longest mesocotyl length (8.67 mm in pH 5 and 6.23 mm in pH 7) compared to the others. Chaita boro and Bashful followed Tepi boro in both the pH levels. Jahmir had the poorest performance among the genotypes considered in this study. The increased pH level and acid concentration showed a little influence on the mesocotyl length (Table 3). Mesocotyl appeared to form only under stressed conditions of any kind. It is considered to be one of the attributes to push up the shoot apex under either upland or lowland conditions (Biswas, 1994).

Table 3. Mesocotyl length (mm) as affected by acetic acid concentrations, Genotypes and pH levels.

Genotype (G)	pH (P)		Difference
	5	7	
	Acetic acid (A) = 0 mM		
Tepiboro	8.67	6.23	2.43
Chaitaboro	6.00	4.90	1.10
Bashful	5.37	4.56	0.81
Jahmir	2.87	1.53	1.35
Banajira	5.80	3.43	2.37
	Acetic acid (A) = 4 mM		
Tepiboro	6.13	6.30	-0.17
Chaitaboro	3.37	4.30	-0.93
Bashful	4.23	4.70	-0.47
Jahmir	1.53	2.29	-0.76
Banajira	2.31	4.37	-2.05
	Acetic acid (A) = 8 mM		
Tepiboro	2.93	5.57	-2.63
Chaitaboro	3.06	3.77	-0.71
Bashful	4.26	4.90	-0.64
Jahmir	1.06	1.66	-0.60
Banajira	2.67	4.39	-1.72
	Acetic acid (A) = 12 mM		
Tepiboro	6.27	6.53	-0.27
Chaitaboro	2.77	3.51	-0.74
Bashful	3.31	4.20	-0.89
Jahmir	1.46	1.55	-0.09
Banajira	3.63	4.07	-1.43
P-MEAN	3.83	4.14	-0.30

Comparison LSD	(5%)
2-AXGXP means	0.98

3.4. First leaf length

There was a significant difference in respect of 1st leaf length growth among the genotypes in pH 5. But no such difference was observed in pH 7. Bashful produced the longest 1st leaf (11.55 mm) across the acid concentrations in pH 5 followed by Chaita boro and Jahmir (<10 mm). The longest coleoptile and mesocotyl producing genotype Tepi boro produced 5.48 mm long 1st leaf, which was the shortest compared to others in this pH level. But at pH 7, Tepi boro produced 16.07 mm long 1st leaf length, which was not different from the other genotypes at the same pH level. Bashful performed equally under both the pH levels. The other genotypes Tepi boro increased its 1st leaf length from 5.48 mm at pH 5 to 16.07 mm at pH 7, Chaita boro from 9.48 to 16.52 mm, Jahmir from 9.23 to 13.85 mm and Banajira from 6.28 to 15.19 mm (Table 4).

at 0 mm acetic acid concentration, 1st leaf length across the genotype was 18.69 mm at pH 5 and significantly increased to 22.78 at pH 7 (table 4). The 1st leaf growth was drastically reduced in presence of acetic acid (4 mm) at ph 5 and there was no significant difference among the concentrations from 4 mm to 12 mm. at pH 7, the reduction was not abrupt except in acetic acid concentration of 12 mm (5.59mm). Higher pH (ph 7) enhanced the leaf growth significantly from 0-8 mm concentration (table 5). The leaf growth was comparatively better at 8 mm in ph 7 than at 4 mm in ph 5. The concentrations required for 50% growth inhibition of 1st leaf length were used to estimate the toxicity level of organic acid. Recently some authors used this concept in case of similar studies (Biswas *et al.*, 2002). They designated ld_{50} as c_{50} in their articles. The quadratic dependence, r^2, and ld_{50} value of 1st leaf growth with respect to acetic acid concentrations were presented in table 6 (across the pH levels) and table 7 (across the genotypes). According to ld_{50}, tepi boro, chaita boro, bashful and banajira showed their similar response to acetic acid concentration ($ld_{50} \approx 4.0$). but jahmir had its ld_{50} value around 5 (table 6). It means jahmir might have some more tolerance to acetic acid concentration. The changing of pH from 5 to 7 relaxed the toxicity level (ld_{50}) from 3.036 to 9.345 (table 7).

Table 4. 1st leaf length (mm) as affected by Genotype and pH levels.

Genotype (G)	pH (P)		G-Mean	Difference
	5	7		
Tepiboro	5.48	16.07	10.77	-10.59
Chaitaboro	9.48	16.52	13.00	-7.04
Bashful	11.55	14.71	13.13	-3.16
Jahmir	9.23	13.85	11.54	-4.63
Banajira	6.28	15.19	10.73	-8.91

Comparison	LSD (5%)
2- GXP means	3.45

Table 5. 1st leaf length (mm) as affected by acetic acid and pH levels.

Acetic acid (A)	pH (P)		Mean	Difference
	5	7		
0	18.69	26.87	22.78	-8.19
4	5.83	18.32	12.07	-12.49
8	4.46	20.29	7.38	-5.84
12	4.64	5.59	5.12	-0.95

Comparison	LSD (5%)
2-AX P means	3.09

Table 6. LD_{50} of the genotypes used for study 2 (across the pH levels).

Genotype	Quadratic equation	R^2	LD_{50}
Tepi boro	Y = 0.609x2-14.823x+100.32	0.99	4.08
Chaita boro	Y = 0.563x2 -14.152x+101.54	0.99	4.42
Bashful	Y = 0.9641x2-14.875x+99.99	0.99	3.77
Jahmir	Y = 0.7688x2-16.29x+100.46	0.99	4.93
Banajira	Y = 0.5983x2-13.502x+98.74	0.99	4.27

Table 7. LD_{50} as affected by different pH levels (across the genotypes).

pH level	Quadratic equation	R^2	LD_{50} value.
5	Y = 1.0902x2-18.904x+97.34	0.96	3.04
7	Y = -0.357x2 – 1.467x+94.94	0.84	9.35

3.5. Root

Genotypes showed impeded root growth in hypoxia. With the exception for Bashful at pH 5 (10.43 mm) and pH 7 (18.98 mm) and for Tepi boro at pH 7 (20.27 mm), the root length varied from 4.90 to 9.53 mm. In addition to hypoxia, acetic acid even at the minimum concentration (4 mM) affected the root growth severely (Table 8). Increased pH level had a little effect on root growth in presence of higher concentrations of acetic acid. The restriction of root growth is one of the mechanisms attributed to seedling establishment. The root growth appeared to block seeds germination under poor oxygenated condition until the shoots have been produced, thereby, providing a mechanism to conserve energy during the early phase of seed germination (Cobb and Kennedy, 1987). Biswas and Yamauchi (1997) observed that coleoptile tip of the anoxia tolerant genotype could penetrate the reduced soil layer to reach the oxygenated layer of soil/water at 4 days after seeding to establish a snorkel mechanism to transport oxygen at the growing region (Kordan, 1974, 1976). The root growth was restricted from 0-6 days after seeding. In fact, this is an avoidance strategy to establish seedling through the initiation of 1st leaf. 1st leaf length plays an important role in seedling establishment under lowland conditions (Biswas and Yamauchi, 1997). The response of genotype towards acetic acid appeared to vary with the inherent potentials of the genotypes (Biswas *et al.*, 2001a). Under anoxic or hypoxic conditions, if the injury is due to the accumulation of toxic substances stress tolerance can conceivably be due to the prevention of the accumulation of toxic substances (strain avoidance) or tolerance to the accumulation of the toxic substances (strain tolerance) (Levitt, 1972). The toxic substance may be exogenous and endogenous in origin. In this study, we considered exogenous origin only.

The seedling parameters were remarkably affected by acetic acid at pH 5.. This might be due to the higher proportion of undissociated forms of this weak acid highly dependent on pH. Tanaka and Navasero (1967) observed that excised rice root absorbed undissociated forms of acids faster than dissociated forms, causing toxicity to the rice plants. They also observed a decrease of the respiratory rate by VFA at a low pH unlike at high pH. But of course that could be influenced by the ability of a genotype itself too as observed in this study.

Table 8. Root length (mm) as affected by acetic acid concentration, Genotype and pH levels.

Genotype (G)	pH (P)		G-Mean	Difference
	5	7		
Acetic acid (A) = 0mM				
Tepiboro	4.47	20.27	12.87	-14.80
Chaitaboro	4.90	6.77	5.83	-1.87
Bashful	10.43	18.98	14.71	-8.55
Jahmir	7.89	9.53	8.71	-1.65
Banajira	6.53	7.77	7.15	-1.23
Acetic acid (A) = 4 mM				
Tepiboro	0.27	15.13	7.70	-14.87
Chaitaboro	2.56	5.30	3.93	-2.74
Bashful	3.44	10.23	6.84	-6.79
Jahmir	0.17	3.30	1.73	-3.13
Banajira	0.00	3.43	1.73	-3.45
Acetic acid (A) = 8 mM				
Tepiboro	0.00 a	3.57 a	1.78	-3.57
Chaitaboro	0.57 a	0.93 a	0.75	-0.37
Bashful	0.15 a	2.00 a	1.07	-1.85
Jahmir	0.00 a	2.79 a	1.39	-2.79
Banajira	0.00 a	1.27 a	0.63	-1.27
Acetic acid (A) = 12 mM				
Tepiboro	0.00	0.00	0.00	0.00
Chaitaboro	0.07	0.20	0.13	-0.13
Bashful	0.00	0.17	0.08	-0.17
Jahmir	0.00	1.48	0.74	-1.48
Banajira	0.00	0.37	0.19	-0.37
P-MEAN	2.12	5.68	3.90	-3.55

Comparison	LSD (5%)
2- AXGXP means	2.85

4. Conclusions

From the present study, it may be concluded that the presence of minimum level of acetic acid (4 mM) could affect the seedling parameters in hypoxia at pH 5. The change of pH from 5 to 7 enhanced the growth of seedling parameters. Higher pH appears to mitigate the acetic acid influence in hypoxia. The genotype Bashful was able to produce the longest first leaf at pH 5. With respect to LD_{50} genotype Jahmir appears to have more tolerance to acetic acid than the others.

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