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Comparative Toxicity of Aliphatic and Aromatic Acids on Seedling Attributes of Anoxia-tolerant Rice (*Oryza sativa* L.) Genotypes Grown in Hypoxia

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Abstract: An understanding of the comparative effects of aliphatic and aromatic acid toxicity on the growth of seedling attributes of an anoxia tolerant rice genotype is essential to develop an appropriate direct seeded lowland rice. Haenuki, an anoxia tolerant rice genotype was used to compare the toxicity level of aliphatic and aromatic acids. Acetic, propionic, and butyric acid from aliphatic group and ferulic, p-coumaric, and p-hydroxybenzoic acid from aromatic group were used for the study. Sprouted seeds sown in sealed test tubes containing different acid solutions were allowed to grow for 7 days at 30 °C and pH 5-7 in the dark. The variation in organic acid concentrations for 50% growth inhibition (C_{50}) of seedling attributes was evident from the study. First leaf survival (%) was more tolerant as compared to 1st leaf length, plant height and root length for certain instances. C_{50} values indicate that ferulic acid and p-coumaric acids were more toxic than acetic, propionic, butyric and p-benzoic acids. Among the aliphatic acids, propionic acid was the most toxic, followed by butyric and acetic acid, whereas from the aromatic group, p-coumaric and ferulic acid showed the highest level of toxicity.

Key words: Aliphatic acid, aromatic acid, anoxia-tolerant rice genotype, direct seeding, seedling growth,

Introduction

The direct seeding of rice of the anoxia tolerant genotype could provide an improved method of seedling establishment under lowland conditions (Yamauchi *et al.*, 1993; Yamauchi *et al.*, 2000). The characteristic of these genotypes is vigorous performance of the seedling's organs under poorly aerated conditions (Biswas and Yamauchi, 1997; Yamauchi and Biswas, 1997). However, in practice, variation in performance among different locations is evident (Yamauchi and Biswas, 1996). This phenomenon could be attributed to lower carbon aliphatic acids (hereafter aliphatic acids only), aromatic acids and other harmful substances under lowland conditions (Rao and Mekkelesen, 1977; Chou and Lin, 1976; Chou and Chiou, 1979; Takijima, 1964a; Takijima, 1964b; and Takijima, 1964c). Among the aliphatic acids, formic, acetic, propionic, butyric and lactic acids were observed to cause injury to rice plants (Takijima, 1964a). Takijima (1963) mentioned that concentrations as low as 0.1-0.01mM aromatic acids might cause injury to the rice plant. Some of the predominant aromatic acids are p-coumaric, ferulic, p-hydroxybenzoic and vanillic acids (Kuwatsuka and Shindo, 1973). Despite the considerable studies on the effect of organic acids on the growth of rice, information related to the effect of these acids on the establishment of anoxia tolerant rice genotype in hypoxia is meager. Previously, we reported on the effects of some aliphatic acids and pH on a few anoxia tolerant rice genotypes (Biswas *et al.*, 2001). While this study has generated some information, specific data related to the comparative effect of aliphatic and aromatic acids regarding their influence on specific seedling organ, required to allow the development of genotype and cultural practices yielding a uniform seedling establishment under lowland conditions.

The objective of this study was to compare the extent of the effects of aliphatic and aromatic acids in the process of seedling growth of an anoxia-tolerant rice genotype under hypoxic conditions.

Materials and Methods

The experiment was conducted in the laboratory of Crop Science, Faculty of Agriculture, Yamagata University, Japan in 2000.

Plant materials: Haenuki, a japonica type rice (*Oryza sativa* L.) genotype, collected from Yamagata Prefectural Agriculture Station, Shonai branch, was used for the study. This genotype showed better seedling establishment under lowland conditions (0.08 mg L^{-1} dissolved oxygen, unpublished data) as compared to the anaerobic genotypes identified by Yamauchi *et al.* (1993). Henceforth we have designated this genotype as anoxia tolerant (Biswas *et al.*, 2001). Germination percentage and germination rate (Krishnasamy and Seshu, 1989) at 30 °C were 99.0% and 0.99 respectively.

Organic acids: For simplicity, acetic, propionic and butyric acids from the aliphatic group and ferulic, p-coumaric and p-hydroxybenzoic acids from the aromatic group were considered for these studies. Acid concentrations were decided on the basis of Biswas *et al.* (2001), Takijima, (1964a) and observation trials. The concentrations used for acetic acid were 0, 4, 8 and 12 mM. The concentrations for propionic acid were 0,1, 2, and 3 mM and for butyric acid were 0, 2, 4, and 6mM. The concentrations used for p-hydroxybenzoic acid were identical to those of propionic acid. For ferulic acid as well as p-coumaric acid, the concentrations were lower, 0, 0.1, 0.2, 0.3, 0.4 and 0.5mM and 0, 0.2, 0.4, 0.6 0.8 and 1.0 mM respectively.

NaOH and HCl were used to adjust the pH (7 or 5) of the organic acid solutions under consideration. Five sprouted seeds (surface sterilized in 2% NaOCl for 15 minutes), were sown in the test tubes (100X25mm^2). Each test tube contained 9ml of specified solution, equivalent to a 25mm sowing depth above the seeds, to maintain hypoxic conditions. The test tubes were then sealed with para film, wrapped in aluminum foil, and kept in an incubator at 30 °C. The seedlings were allowed to grow for 7 days. The solutions were replaced with fresh ones every 2 days. The study under each acid was considered as an individual experiment. The experiments were laid out in three - replicated completely randomized design.

Evaluation: First leaf survival (%), 1st leaf length, plant height and root length were observed. The concentrations required for 50% (designated as C_{50} in this article) growth inhibition of the seedling attributes (may be 1st leaf survival (%), or leaf/root length, or plant height) were used to estimate the toxicity level of the organic acids. We borrowed this concept from a widely used term in biological research, LD_{50} (lethal dose 50%), defined as the dose that kills 50% of the animals in an experiment. We observed the quadratic relationship between the seedling attributes and organic acids was better-fitted equation compared to the others like exponential or linear to estimate the C_{50}.

The C_{50} was estimated as follows: $Y = ax^2 \pm bx \pm c$...(I) where Y = 50% of 1st leaf survival (%) or 1st leaf length or plant height or root length as compared to the control treatment, x = C_{50}, a and b are the rate of curvilinear and linear coefficients respectively, while c is the intercept.

The solution for x is given by:

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

Results and Discussion

The quadratic relationship between seedling attributes (1st leaf survival percentage, 1st leaf length, plant height and root length) and acid concentrations were significant. Most of the coefficient of determination (R^2) for the equations were more than 90%. First leaf survival (%) was improved significantly for acetic and propionic acid from the aliphatic group in pH 7 at higher concentration. Though p-coumaric acid from the aromatic group showed some influence at pH 7 at mid level concentration, others showed little effect (Fig. 1). The pattern of 1st leaf length response with respect to pH for all acids was more or less similar to those of 1st leaf survival (%) (figure not shown). It was noted that pH 7 enhanced the plant height to some extent in acetic, propionic, and p-coumaric acid (Fig. 2). Prior to attaining the extreme concentration, ferulic and p-coumaric acid had enhanced root length at pH7. Root length was not affected by pH7 in butyric or p-hydroxy benzoic acid (Fig. 3).

The estimated C_{50} of different seedling attributes are presented in Table 1. Acetic acid had the highest C_{50} followed by butyric, p-benzoic, and propionic acid. Acetic acid showed a twofold increase of C_{50} for 1st leaf survival (%) at pH 7 as compared to pH 5. Similarly, C_{50} for plant height in acetic acid was almost 50% lower than that of the C_{50} of 1st leaf length. The other acids from aliphatic group showed the similar C_{50} values for 1st leaf length and plant height. The C_{50} of 7.19 for root length at pH 7 was reduced to 4.22 at pH 5 in acetic acid.

Among the organic acids, acetic acid is predominant, followed by propionic acid and butyric acid (Fujii *et al.*, 1972). Watanabe (1984) in his review, reported that the maximum accumulation of acetic, propionic and butyric acids could be 43.8, 1.3 and 4.8mM kg^{-1} soil respectively. Compared to that accumulated acetic acid, the estimated C_{50} for this acid was quite low. C_{50} in propionic and butyric acid for all seedling

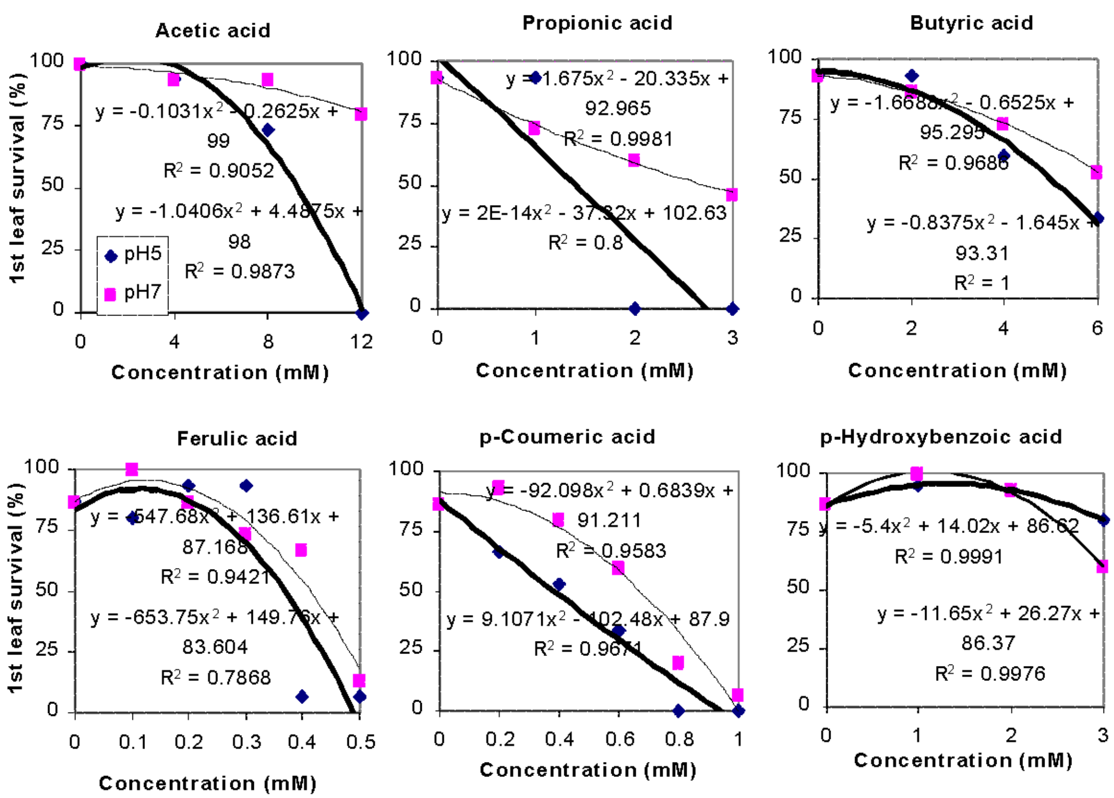

Fig. 1: 1st leaf survival (%) response of Haenuki to organic acids and pH

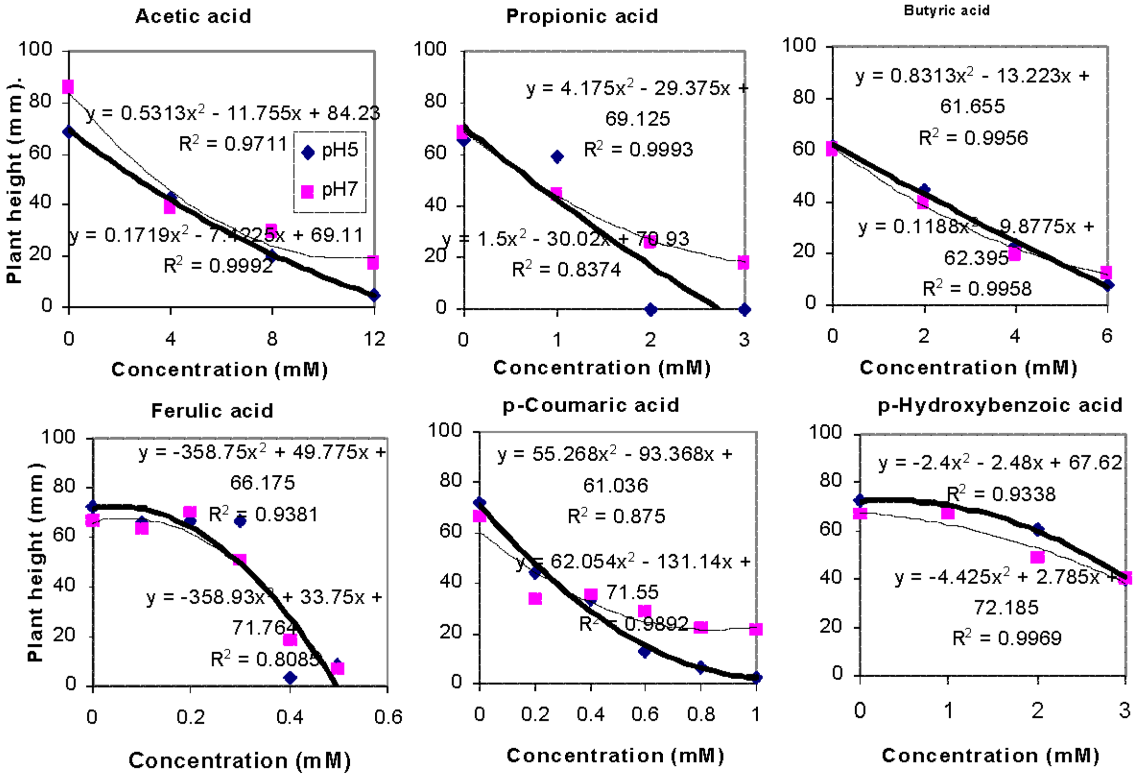

Fig. 2: Plant height (mm) response of Haenuki to organic acid and pH

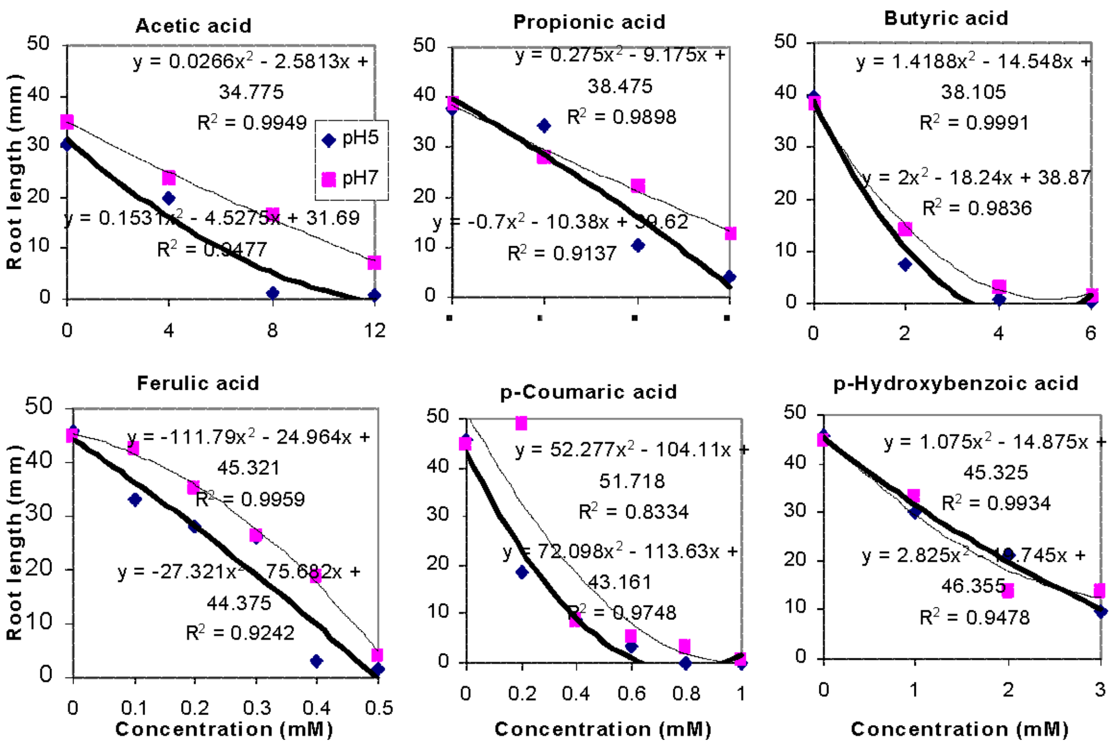

Fig. 3: Root length (mm) response of Haenuki to organic acids and pH.

attributes were approximately approached to those of the reported values (Table 1). Therefore, there is possibility of affecting seedling growth by aliphatic acids under lowland conditions.

The C_{50} for acetic acid was in the order of 1st leaf survival (%) > 1st leaf length > plant height. Except for the percent of 1st leaf survival at pH7, the seedling attributes exhibited reduced and similar C_{50} in propionic acid. Butyric acid had the lowest C_{50} for root length. That mean toxicity levels of certain acids might vary with seedling attributes. Aromatic acids showed lower C_{50} with the exception of p-hydroxybenzoic acid, which had a C_{50} comparable to that of butyric acid. pH showed little influence on C_{50} value of seedling attributes affected by aromatic acids. C_{50} values indicate that ferulic and p-coumaric acids were more toxic than acetic, propionic, butyric and p-benzoic acids. Takijima (1963) reported that aromatic acids are more toxic than the aliphatic ones. Among the aliphatic acids, propionic acid appears to be the most toxic, followed by butyric and acetic acids.

Compared to aliphatic acids, accumulation of aromatic acids in rice soils is low (Watanabe, 1984; Yamane and Sato, 1970; Tsutsuki, 1984; Tsutsuki and Ponnamperuma, 1987). Whitehead (1964) found p-hydroxybenzoic acid, vanilic acid, p-coumaric acid, and ferulic acid in rice soils, each at concentrations lower than 0.05mM. Tsutsuki and Ponnamperuma (1987) observed that p-hydroxybenzoic, ferulic acid and p-coumaric acid were accumulated as 0.136, 0.0742 and 0.0377mM kg^{-1} soil respectively, when rice straw was applied. Previous studies showed that the existence of 0.01mM-0.1mM of aromatic compounds could incur poor rice plant growth (Takijima, 1960; Chou and Lin, 1976; Chou and Chiou, 1979). However, compared to the accumulated concentrations our study showed that C_{50s} for all seedling attributes were reasonably high (0.20-4.41mM) for all aromatic acids under consideration. Even Takijima (1964a) observed that aromatic acid exhibited 50% growth inhibition of rice roots at concentrations of 0.6-3mM. Whether this variation in toxicity was due to growth stage or something else, was not clear. Lynch (1980) detected a small amount of aromatic compounds in an extract of the anaerobic decomposition of *Agropyron ripens* rhizomes, that showed growth-inhibiting activity and contained about 30mM aliphatic acids. From the study it appears that toxicity levels depend on the seedling attributes, pH and organic acid. Though aromatic acids seem to be more toxic than aliphatic ones, they might not affect seedling growth due to their low accumulation in the field.

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