

# Ecotypic variation of *Imperata cylindrica* populations in Taiwan: II. Physiological and biochemical evidence

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**ABSTRACT.** Cogon grass [*Imperata cylindrica* (*I. cylindrica*) L. Beauv. var. *major*], one of the top ten weeds in the world, is one genus and one species in Taiwan. In the field, the alcohol dehydrogenase (ADH) activity, proline, and sodium content in tissues of *I. cylindrica* showed variation between the wetland (Chuwei) ecotype and the other two non-wetland ecotypes (Neihu and Sarlun). Moreover, in the greenhouse, flooding and salt treatment on the vegetative shoot of *I. cylindrica* showed that the Chuwei ecotype has higher survivability to flood and salt. A three-month flooding treatment led to a differential increase of ADH activity in leaf tissues of plantlet of Chuwei and Sarlun ecotypes. In addition, a four-day salt treatment led to a significant accumulation of proline in leaf tissues of Chuwei ecotype plantlets, yet to a significant amount of sodium accumulation in root and stem tissues of the Chuwei ecotype following an eight-day (short-term) and two-month (long-term) treatment. These physiological and biochemical differences revealed the ecotypic variation among *I. cylindrica* ecotypes, and the wetland ecotype in Chuwei is physiologically distinct.

**Keywords:** Alcohol dehydrogenase; *Imperata cylindrica*; Proline; Sodium.

## INTRODUCTION

*Imperata cylindrica* (L.) Beauv. var. *major* (Nees) Hubb., a top-ten weed (Holm et al., 1977), is only one genus of one species and is widely distributed in Taiwan (Hsu, 1975). A special population was found in the Chuwei Mangrove Forest in the estuary area located at the river mouth of the Tamshui River in northern Taiwan. It was also found as a non-endemic species invading Florida and neighboring states in both dry and wetlands of the United States (King and Grace, 2000). Cheng and Chou (1997a) examined the leaves of *I. cylindrica* in the Chuwei population with a scanning electron microscope (SEM). They found the lower stem surfaced with wax instead of trichomes and the stele empty instead of solid. The phenotype of the Chuwei plant remained unchanged after transplanting into the greenhouse. The molecular polymorphism of *Imperata* populations was investigated by use of Random Amplified Polymorphic DNA (RAPD) (Cheng and Chou, 1997b) and restriction fragment length polymorphism (RFLP) on the ribosomal DNA (rDNA) (Chiang et al., 1998; Chou and Tsai, 1999). The Chuwei

population appeared to be a distinct ecotype. However, little physiological or biochemical evidence of variation among ecotypes appeared.

Under low oxygen conditions, plants increase ADH activity to survive. Hageman and Fisher (1960) first found that anaerobiosis induced ADH activity in root of maize seedlings. Freeling (1973) analyzed the protein level of ADH in seedlings of maize under 5-72 h anaerobiosis and found that they increased dramatically. Furthermore, Schwarte (1969) demonstrated that the induction of ADH1 in maize seedlings correlated to the plants' tolerance to anaerobiosis. The induction of ADH activity is regulated on a transcriptional level. In 1984, Dennis and his coworkers cloned the anaerobiosis response region (ARE), a promoter of the ADH1 gene. Later, Walker and his coworkers sequenced the ARE (Walker et al., 1987). ADH isozymes of maize (Freeling, 1973), tomato (Tanksley and Johns, 1981), and *Echinochloa* (Fox et al., 1988) were induced in anaerobiosis. Smits et al. (1990) reported that the number of ADH isozymes in hydrophytes correlated with their alcohol content and that the polymorphism of isozymes provided natural selection superiority. The induction of *Adh* gene expression is also regulated on a translational level (Bailey-Serres, 1999). Maize *Adh1* mRNA is selectively translated under low oxygen conditions (Fennoy and Bailey-Serres, 1995; Fennoy et al., 1998). In Arabidopsis, RopGap4 regulates ADH activity under low oxygen conditions (Baxter-Burrell et al., 2002).

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Under high salt conditions, special organic compounds like proline, glycinebetaine, choline, glycerol, and sorbitol accumulate in tobacco (Binzel et al., 1987), barley (Stewart and Michelle, 1983), spinach (Coughlan and Wyn Jones, 1980), and eggplant (Jain et al., 1987). Proline accumulates with increased salinity, and the accumulation is regulated by abscisic acid (Stewart, 1980; Stewart and Voeberg, 1985). Proline plays many roles in stress physiology. For example, Stewart and Lee (1974) indicated that the accumulation of proline in plants was correlated with salt tolerance. They also pointed out that high proline levels would protect many N metabolism-related enzymes from harm. A large amount of proline would inhibit 1-aminocyclopropane-1-carboxylate (ACC) from converting to ethylene, thus protects the plant from ethylene damage (Chrominski et al., 1988; Chrominski et al., 1989). Furthermore, proline could be an osmo-protectant, maintaining an osmotic potential balance (Jain et al., 1987). In addition to accumulating proline, plants also regulate sodium ion homeostasis in response to salt stress. A Salt-Overly-Sensitive 1 (SOS1) protein acts as a  $\text{Na}^+/\text{K}^+$  antiporter to confer salt tolerance in Arabidopsis (Wu et al., 1996; Shi et al., 2002; Shi et al., 2003).

We examined the variation of salt and flooding stress responses of *I. cylindrica* wetland and non-wetland ecotypes on both a biochemical and physiological level. Both field and greenhouse studies of three ecotypes showed that ADH activity, proline content in leaf tissues, and sodium content in tissues were differentially up-regulated in the Chuwei ecotype, which is flood and salt tolerant. Here we provide a new model to study flooding and salt stress physiology in plants. Our findings support the previous discovery of the variation among ecotypes on a molecular level (Cheng and Chou, 1997a), and we found that the Chuwei ecotype is physiologically distinct.

## MATERIAL AND METHODS

### Plant material and sampling sites

*Imperata cylindrica* (L.) Beauv. var. *major* (Nees) Hubb, Cogon grass, was sampled from Chuwei mangrove salt-marsh wetland (wetland site) (Hwang and Chen, 1995). The area had been periodically flooding, and the grass grown at this site had been designated as a salt-tolerant ecotype. The other two representative sites designated as control sites were Sarlun (sandy beach) and Neihu (inland-park), no flooding (non-wetland) sites. Plant leaves were harvested from each site every two weeks. Plant samples from the Chuwei sites were collected on both neap tide days (low tide and no flooding; during August and September in 1995) and spring tide days (high tide and flooding; during the same period of time). During the harvesting, each leaf sample was washed with de-ionized water, prepared and excised by sterilized scissors, stored in Ziplock bags in an ice bucket with dry ice to keep it fresh, and brought back to the lab immediately before use. Water content of plant leaves harvested from each site was

assayed. Plants rhizomes collected from the field were washed by sterilized water and cultured in pots ( $60 \times 20 \times 20 \text{ cm}^2$ ) in greenhouse for two weeks before being transplanted to Kimura's culture solution (Ma et al., 2001) to grow plantlets and vegetative shoots. The culture solution was aerated with an air pump (NS, Model 8200, Taiwan) for 24 h. The culture solution was replaced every week. After two weeks, 14-day-old plantlets of the Chuwei ecotype (designated PC14), Sarlun ecotype (PS14), and Neihu ecotype (PN14) were used as the study material. After two months, 90-day-old plantlet, Chuwei ecotype (designated PC90), Sarlun ecotype (PS90), and Neihu ecotype (PN90), were obtained. Greenhouse temperature was controlled at 25 to 30°C, and these plantlets were harvested after flooding and salt treatment.

### Soil water content and salinity assay

Soils from three different habitats were harvested from each site every month in 1995. Fresh weight (W1) and dry weight (W2) of soil (after drying in oven at 100°C for 24 h) was measured in order to calculate soil water content ( $[(W1-W2) / W1 \times 100 \text{ \%}]$ ). Electrical conductivity (MS/CM) of soil was determined by use of an electrical conductivity meter (Jenco, Model 1010, Taiwan) (Kalra and Maynard, 1991). Soil salinity (M) was calculated by converting soil electrical conductivity into soil salinity in consideration of soil water content with NaCl as the standard.

### Flooding and salt treatment

A flooding experiment was conducted in water culture in a greenhouse of the Institute of Botany, Academia Sinica, Taipei, Taiwan. Flooding was achieved by growing plantlets in culture solution without air pumping. A culture solution aerated with an air pump was used as the control. For the flooding treatment, PN14, PS14 and PC14 were flooded for three months. Dissolved oxygen (DO) concentration was measured by use of an  $\text{O}_2$  meter (Consort, Model Z521, Taiwan). A salt treatment experiment was also conducted in water culture. In the salt treatment group, NaCl was added to water to make salt water with salinities of 1%, 2%, and 3% (w/v). PN90, PS90 and PC90 were cultured for four days, eight days (short term) and two months (long term) in the salt water. For the control group, NaCl was not added, and the culture solution was sodium-free.

### ADH activity assay

Half a gram of fresh leaves frozen by liquid nitrogen were ground and homogenized in 5 mL extraction buffer [0.1 M Tris-HCl, pH 8.0, 0.01 M beta-mercaptoethanol, 1 mM dithiothreitol, 0.02 mM phenylmethylsulfonyl fluoride (PMSF)(Sigma)] with 5 grams sea sand added. The homogenates were centrifuged at 5510 rpm Centrifuge (Sigma, Model 2K15, Taiwan) at 4°C for 10 min, and the supernatant was obtained as ADH crude extract. All extraction steps were performed at 4°C, and the extracts

were kept on ice. ADH enzyme activity was assayed spectrophotometrically at a wavelength of 340 nm by use of a spectrometer (Hitachi, Model U-2000, Taiwan) at 30°C by the method previously described (Irish and Schwartz, 1987). The reaction mixture contained 0.1 M Tris-HCl, pH 8.5, 0.06 M ethanol, 0.002 M semicarbazide-HCl, and 0.003 M beta-nicotinamide adenine dinucleotide ( $\beta$ -NAD). Data were the average of four replicates. Enzyme activity was calculated based on a standard curve of pure NADH and expressed as the initial rate of reduction of NAD per gram fresh weight. One unit (u) of enzyme activity was defined as the amount that reduced 1  $\mu$ mole of NAD per min per gram of fresh weight. The optical density (absorbance expressed as A value) of the base line at wavelength 340 nm was measured with ten replicates for each data set.

### Proline content assay

L-proline content was measured by the method previously described (Bates et al., 1973). Half a gram of fresh leaf tissues were chopped into pieces, and frozen in liquid nitrogen. Five ml extraction buffer [3% (w/v) 5-sulfosalicylic acid] was added to 5 grams of sea sand for grinding and homogenization. The homogenate was centrifuged at 5380 rpm (Sigma, Model 2K15) for 10 min. The supernatant was obtained as proline crude extract. The reaction mixture contained 2 ml acid-ninhydrin solution containing 0.14 M ninhydrin, 60% (v/v) acetic acid, 2.4 M phosphoric acid, and 2 ml proline crude extract. The reaction was performed at 100°C for one h, and samples were then put into a refrigerator at -20°C to stop the reaction immediately. Four ml methanol was added into the samples and vortexed. The solution was then fractionated into two layers, the upper methanol layer and the lower water layer. Three ml of the upper methanol layer solution was transferred into a cuvette. Proline content was analyzed spectrophotometrically at wavelength 520 nm using a spectrophotometer (Beckman, Model DU-50) with twenty replicates for each proline content assay and quantified based on a standard curve of pure proline.

### Sodium content assay

Plant material was dried in an oven at 110°C for 24 h and chopped into pieces. Half a gram of leaf, 0.1 gram root and 0.1 gram stem, were dry-ashed individually by a furnace (NEY Box Furnace, Model 6-1350A) at 470°C for 16 h, then wet-ashed with 7 ml 4.7 M HCl distilled water and 7 ml 8 M HNO<sub>3</sub>. The mixture was heated on a digestion block at 80°C for 2 h in the hood until the solution turned colorless. The final remains were diluted to 50 ml with distilled water prior to analysis. Afterwards, 20 ml of 3 M HCl were added. Each sample was filtered by a filter paper (Whatman no. 42). Sodium content was measured flame-photometrically using a flame photometer (Siba, Model 410) by the method described by Kalra and Maynard (1991) and quantified based on a standard curve of pure Na<sup>+</sup>.

### Statistical analysis

Data were analyzed by Duncan's multiple range test in an SAS statistical package (Academia Sinica, Taiwan) and by a Student's Pairwise T-test in an SPSS statistical package (Microsoft, Taiwan).

## RESULTS AND DISCUSSION

### Chuwei soil has much higher water content and salinity than other dry habitats

Since a soil physical property reflects the environmental conditions of each sampling site, soil chemistry was taken into consideration for comparison. To determine the water content in the soil of three habitats, soil samples were collected at monthly intervals from August to October in 1995 (Supplemental Table I). Our results showed that the water content of soil from Chuwei mangrove forest sampling sites (24.7 % on average, ranged between 17.3 and 33.3 %) was much greater than that of soil from other sites (Table 1). The soil water content was 2.4 times that of Neihsu (10.27% on average, ranged between 2.6 and 17.4 %), and 5.87 times that of Sarlun (4.21% on average, ranged between 1.9 and 6.4%). We concluded that soil from the Chuwei mangrove forest sampling sites is much wetter than that from other sites. On the other hand, soil oxidation-reduction potential from the topsoil (5-10 cm from ground zero) was measured by use of an oxidation-reduction potential meter (Jenco, Model 62, Taiwan). Chuwei soil showed low oxidation-reduction potential (between -88 and 175 mV) compared to other sites (between 19 and 117 mV), suggesting a low oxygen status (Hseu and Chen, 2000). In addition, chemical properties

**Supplemental Table I.** Average ADH activity in leaves of *I. cylindrica* from Chuwei before and after flooding. Sampling was performed from August to September in 1995. ADH activity on neap-tide (low tide) days was shown in regular font. ADH activity on spring-tide (high tide) days was shown in bold. ND, not determined.

Sampling date	Before flooding	After flooding
	ADH (u)	ADH (u)
Aug. 3	210.86±10.5	ND
Aug. 5	209.21±12.7	ND
<b>Aug. 13</b>	<b>222.11±15.6</b>	<b>273.70±25.6<sup>1</sup></b>
Aug. 21	250.19± 4.6	ND
<b>Sep. 11</b>	<b>270.06±9.6</b>	<b>330.37±21.3<sup>1</sup></b>
Sep. 20	290.44±13.1	ND
<b>Sep. 28</b>	<b>280.80±17.0</b>	<b>298.63±11.8</b>

<sup>1</sup>Values showed significant difference in Student's t-test at the 5% level. S.E. was at the 5% level.

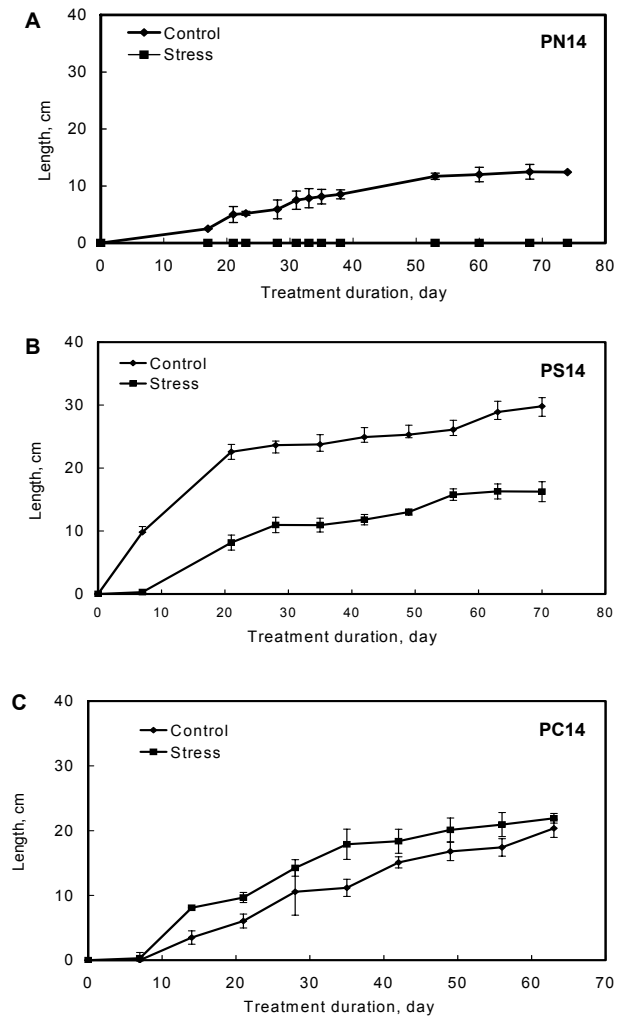
of soil from three different sampling sites were compared. Soil samples from three habitats were collected at monthly intervals from August to October in 1995. Data showed that the salinity of soil from Chuwei sampling sites (1.01 M on average, ranging between 0.5 and 1.4 M) was much greater than that from others (Table 1). The soil salinity was 5.6 times that of Neihu (0.18 M on average, ranging between 0.1 and 0.4 M), and 10.1 times that of Sarlun (0.10 M on average, ranging between 0.05 and 0.2 M). It is concluded that the soil from Chuwei wetland sampling sites is much saltier than that from other sites. Therefore, Chuwei soil is under both low oxygen and high salt conditions.

### The Chuwei ecotype showed higher survivability to flood and salt

To test the variation in growth of three ecotypes, PN14, PS14 and PC14 were grown under flooding conditions for three months. Leaf length of PN14, PS14 and PC14 were measured at weekly intervals, and growth of plantlet was expressed as the increase in shoot (leaf) length. Results showed that plantlets from the Chuwei sampling site could survive anaerobic conditions and were flood tolerant (Figure 1C). PN14 did not grow throughout the whole culturing (Figure 1A) while PS14 grew well but slower than control (Figure 1B). PC14 grew well and with an enhanced growth rate. After three months, the height (shoot length) of PN14, PS14 and PC14 was measured. PN14 showed a 100% growth inhibition while PS14 showed 43%. However, PC14 showed no growth inhibition at all. The result showed that the Chuwei ecotype had a higher survivability to flood.

To test the variation in growth of three ecotypes, PN14, PS14 and PC14 were grown under salt conditions for two months. Leaf lengths of PN14, PS14 and PC14 were measured at weekly intervals, and growth of plantlet was indexed by the increase of leaf length. Results showed that the plantlets of Chuwei wetland ecotypes could survive in 1% salt water and were salt tolerant. PN14 stopped growing after 41 days at 1% salinity. After 31 days, it stopped growing at 2% salinity (Figure 2A). PS14 stopped growing at 1% salinity after 45 days, but did not grow at 2% salinity (Figure 2B). Nevertheless, PC14 grew well, kept growing at 1% salinity even after two months, and stopped growing at 2% salinity after 50

days (Figure 2C). Our result showed that Chuwei ecotype showed higher survivability to salt. The salt tolerance of the Chuwei ecotype (1%) was even stronger than *Kandelia candel* (tolerant to 0.8% salt), a dominant mangrove forest species in the Chuwei salt-marsh wetland (Huang and Chen, 1995).

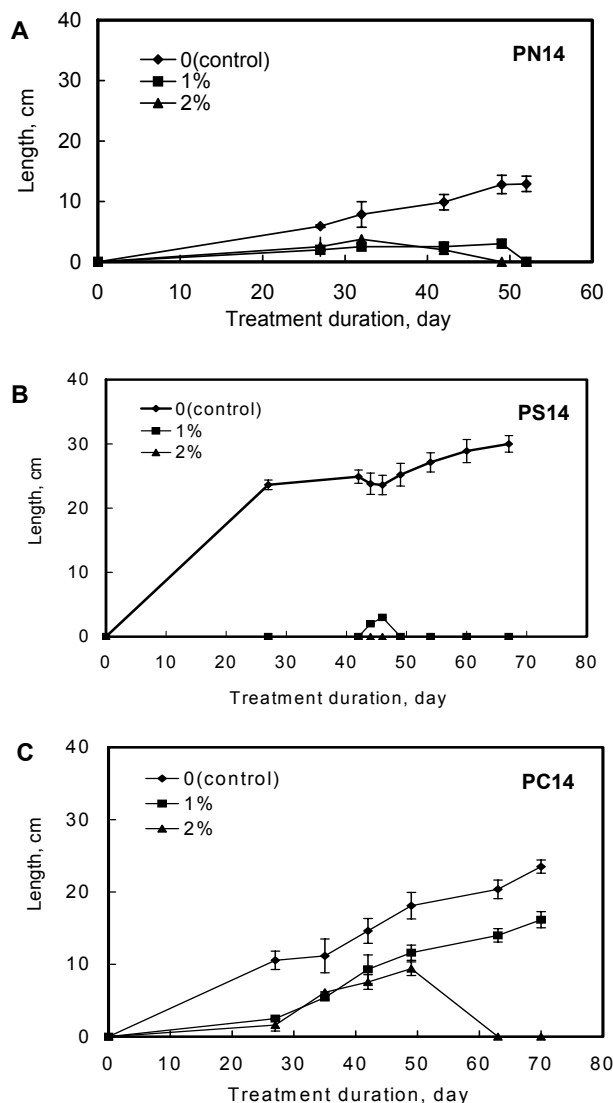


**Figure 1.** Variation of flood survivability of plantlet under flooding conditions for three months. Growth rate was expressed as shoot length of plantlet PN14 (A), PS14 (B), and PC14 (C) versus time duration. Error bars were S.E. at the 5% level.

**Table 1.** Variation of soil water content, salinity, and average ADH activity and proline content in leaves of three *I. cylindrica* ecotypes in the field. Values were means of the samples collected between July and October in 1995. Values followed by the same letter showed no significant difference in Duncan's multiple-range test at the 5% level. S.E. was at the 5% level.

Sampling site	Soil water (%)	Soil salinity (M)	Leaf metabolites	
			ADH (u)	Proline ( $\mu\text{g gfw}^{-1}$ )
Neihu	10.27 $\pm$ 0.8 <sup>b</sup>	0.18 $\pm$ 0.01 <sup>b</sup>	123.41 $\pm$ 6.7 <sup>c</sup>	17.64 $\pm$ 1.8 <sup>b</sup>
Sarlun	4.21 $\pm$ 0.2 <sup>b</sup>	0.10 $\pm$ 0.00 <sup>c</sup>	158.57 $\pm$ 7.8 <sup>b</sup>	31.68 $\pm$ 1.1 <sup>b</sup>
Chuwei	24.7 $\pm$ 4.0 <sup>a</sup>	1.01 $\pm$ 0.04 <sup>a</sup>	302.97 $\pm$ 7.6 <sup>a</sup>	120.55 $\pm$ 8.9 <sup>a</sup>

In the United States, *Imperata cylindrica* was found in both dryland and wetland in Florida (King and Grace, 2000), and was found with flood-tolerant potential (King and Grace, 2000). It appears that the flood-tolerance potential of *Imperata cylindrica* was noted worldwide. Since we found that flooding led to the increase of the growth rate of the *I. cylindrica* Chuwei ecotype (Figure 1C) and also found that during spring-tide day the seedlings emerged from the soil, flooding stress appears to be an important limiting factor for *I. cylindrica* Chuwei ecotype to survive. This induced-shoot-growth phenomenon has also been characterized in other species (i.e. rice and *Rumex palustris*) (Vriezen et al., 2003; Voesenek et al., 2003). In addition to maize, rice and *Echinochloa*, our discovery revealed *Imperata* as another monocot model for the study of low oxygen stress physiology in plants.



**Figure 2.** Variation of salt survivability of plantlet under salt conditions for two months. Growth rate was expressed as shoot length of plantlet PN14(A), PS14(B), and PC14(C) versus time duration. Error bars were S.E. at the 5% level.

**ADH activity in leaves of Chuwei ecotype was much higher in the field and up regulated after a three-month flooding treatment**

To compare the ADH activity in leaves of *I. cylindrica* in the field, leaf samples were collected every two weeks from August to October in 1995 and January to June in 1998 (Table 1, Supplemental Table I). Overall, ADH activity in leaf of Chuwei ecotype was 302.97 u on average, 2.45 times that of Neihu (123.41 u on average) and 1.91 times that of Sarlun (158.57 u on average). Results showed that the average level of ADH activity in leaves of *I. cylindrica* Chuwei ecotype was much greater than that of leaves from other sites.

In order to test if increase of ADH activity in leaves of the Chuwei ecotype was due to flooding, a three-month flooding treatment on plants was performed. Leaves of PN14, PS14 and PC14 were harvested for activity study after three months flooding treatment. Data were means of four individuals. Results showed that ADH activity in leaves of Chuwei plantlets was differentially up regulated with decreased dissolved oxygen (D.O.) in comparison with Sarlun plantlets (Figure 3). Because PN14 did not grow, there were not enough leaf tissues to be analyzed.

Evidence of ADH activity assay from both the field and the greenhouse suggested that the *I. cylindrica* Chuwei ecotype had undergone alcoholic fermentation in anaerobiosis. It is commonly observed that ADH activity increases under anaerobiosis in plants, i.e., in maize (Andrews et al., 1993; Wignarajan and Greenway, 1976), soybean (Sachs et al., 1990), *Echinochloa* (Cobb and Kennedy, 1987), and rice (John and Greenway, 1976). Most of the plants expressed ADH in roots (Andrews et al., 1993). However, it was reported that rice ADH activity was detected in leaves (Cobb and Kennedy, 1987). In our study, ADH was also expressed in leaves of *I. cylindrica*, which is consistent with the study of rice. However, we did not test the ADH activity in other tissues. It may be that tissue specific expression of ADH activity exists among different kinds of plants. Differences in the mechanism of hypoxia tolerance between roots and shoots in *Arabidopsis* is possible (Ellis et al., 1999).

Based on a current model, plants can be categorized into either carbohydrate-conserving or carbohydrate-consuming type based on their responses to hypoxia (Fukao and Bailey-Serres, 2004). In the former type, low ADH activity and restricted growth were observed. In the latter type, high ADH activity and rapid shoot growth were observed. The Chuwei ecotype appeared to be of the carbohydrate-consuming type.

**Proline accumulation in leaves of the Chuwei ecotype was much higher and up regulated significantly after a four-day salt treatment**

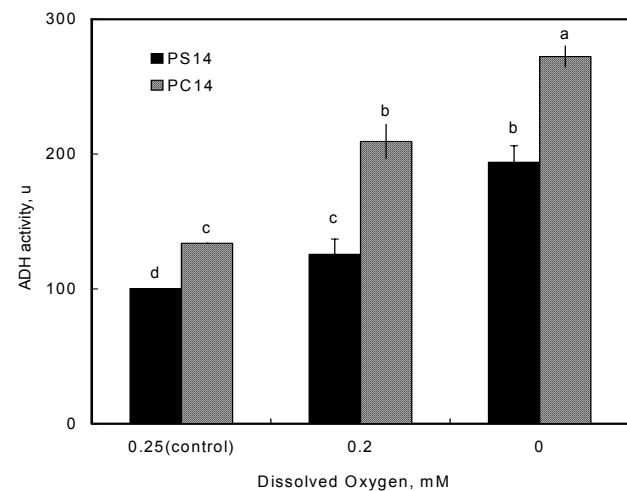
To compare proline content in leaves of *I. cylindrica* in the field, samples were collected at monthly intervals from August to December in 1995. For proline content

analysis, leaves of 20 individuals were sampled within a day. Results showed that the amount of proline in leaves of the *I. cylindrica* Chuwei ecotype ( $120.55 \mu\text{g gfw}^{-1}$  on average) was much greater than that of leaves from others. The proline content was 6.83 times that of Neihu ( $17.64 \mu\text{g gfw}^{-1}$  on average), and 7.4 times that of Sarlun ( $31.68 \mu\text{g gfw}^{-1}$  on average) (Table 1). In order to test if proline content in leaves of plantlets increased differentially in response to salt, a salt treatment was performed for 4 days to see the short-term effect on proline accumulation. Leaves of PN90, PS90 and PC90 were harvested for study after an eight-day salt treatment. Samples were collected at four-day intervals. Data were average of nine individuals. After four days, the proline content in leaves of PC90 was  $2.06 \mu\text{g gfw}^{-1}$ , 47.87 times control at 2% salinity, and  $3.12 \mu\text{g gfw}^{-1}$ , 72.43 times control at 3% salinity (Figure 4). Our results showed proline accumulated significantly in Chuwei plantlet (PC90) (Figure 4).

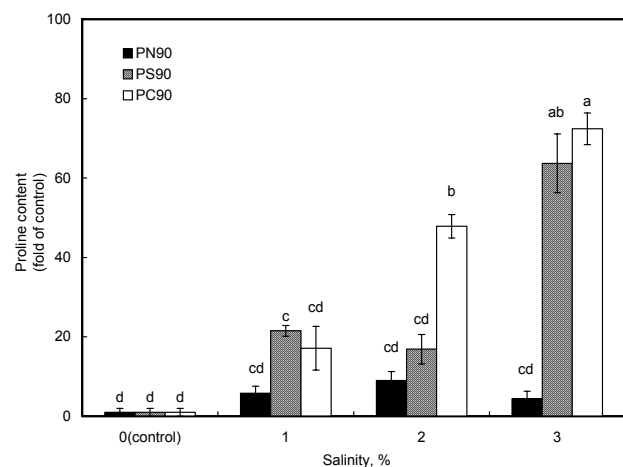
### Sodium accumulation in roots and stems of Chuwei ecotype was much higher in the field and up regulated significantly after a short and long-term salt treatment

To compare sodium content of *I. cylindrica* in the field, samples were collected at monthly intervals from August to December in 1995. For sodium content analysis, sampling was performed with 10 replicates within a day. Results showed that the sodium content in roots was  $215.79 \text{ mg gdw}^{-1}$  on average, 11.64 times that of Neihu ( $18.54 \text{ mg gdw}^{-1}$ ), and 6.81 times that of Sarlun ( $31.68 \text{ mg gdw}^{-1}$ ). The sodium content in stems was  $178.23 \text{ mg gdw}^{-1}$  on average, 5.49 times that of Neihu ( $32.49 \text{ mg gdw}^{-1}$ ), and 4.46 times that of Sarlun ( $39.93 \text{ mg gdw}^{-1}$ ). However, sodium content in leaves of *I. cylindrica*, showed no difference (Table 2). Therefore, sodium content in roots and stems of the Chuwei ecotype was much higher.

Short-term (eight-day) salt treatment was performed to see its effect on sodium accumulation. To determine sodium content of plantlet in response to short term salt treatment, root, stem, and leaves of PN90, PS90 and PC90 were harvested for study after an eight-day salt treatment, and data were means of seven individuals. Results showed that sodium accumulated in roots and stems of plantlets. Sodium accumulated with increased



**Figure 3.** Variation of increase of ADH activity in leaf of plantlet of Chuwei and Sarlun ecotypes after a long-term flooding treatment. ADH activity was measured after three months treatment. Because PN14 did not grow, there were no leaves to be analyzed. The dissolved oxygen (D.O.) concentration of control (aeration) was 0.25 mM. Bars having different letters are significantly different,  $p=0.05$ , ANOVA, with Duncan's multiple range test. Error bars were S.E. at the 5% level.



**Figure 4.** Variation of accumulation of proline in leaves of plantlet of three ecotypes after a four-day salt treatment. Bars having different letters are significantly different,  $p=0.05$ , ANOVA, with Duncan's multiple range test. Error bars were S.E. at the 5% level.

**Table 2.** Variation of sodium content in roots, stems and leaves of three *I. cylindrica* ecotypes in the field. Values were means of the samples collected between July and October in 1995. Values followed by the same letter showed no significant difference in Duncan's multiple-range test at the 5% level. S.E. was at the 5% level.

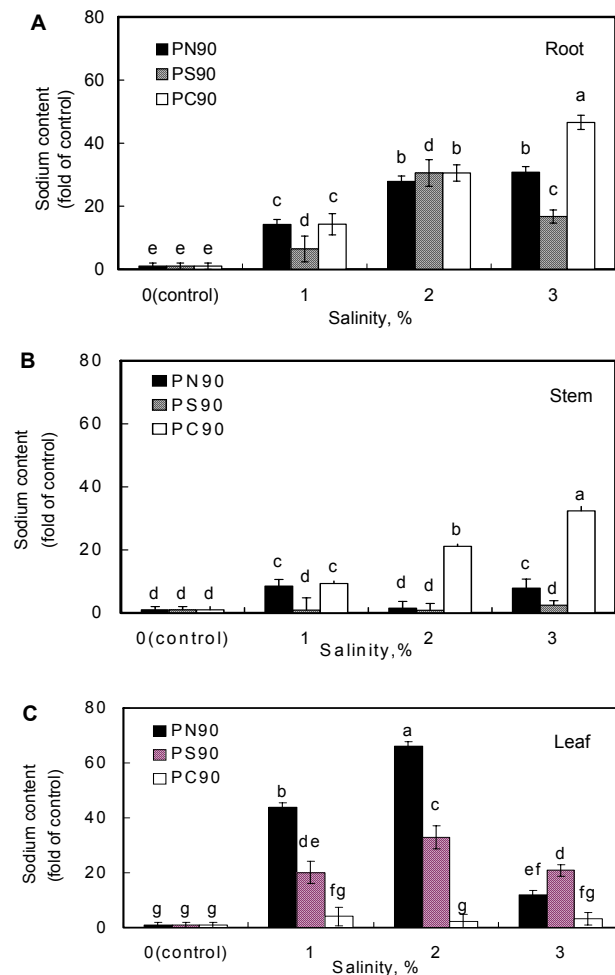
Sampling site	Plant sodium content ( $\text{mg gdw}^{-1}$ )		
	Root	Stem	Leaf
Neihu	$18.54^b \pm 2.3$	$32.49^b \pm 3.1$	$23.01^a \pm 0.9$
Sarlun	$31.68^b \pm 6.0$	$39.93^b \pm 3.8$	$23.39^a \pm 1.0$
Chuwei	$215.79^a \pm 9.2$	$178.23^a \pm 29.5$	$21.68^a \pm 1.0$

salinity with the Chuwei plantlet (PC90) appearing to be the highest (Figure 5A, B). As the salt concentration was 2%, the sodium content in the stem of PC90 (229.74 mg gdw<sup>-1</sup>) increased 22.46 fold. On the other hand, as the salt concentration was 3%, the sodium content increased 45.57 fold in the root of PC90 (350.47 mg gdw<sup>-1</sup>) and 31.37 fold in the stem of PC90 (213.16 mg gdw<sup>-1</sup>). The accumulation of sodium was also found in leaves with increased salinity, but PC90, with extremely low sodium content (Figure 5C), was an exception.

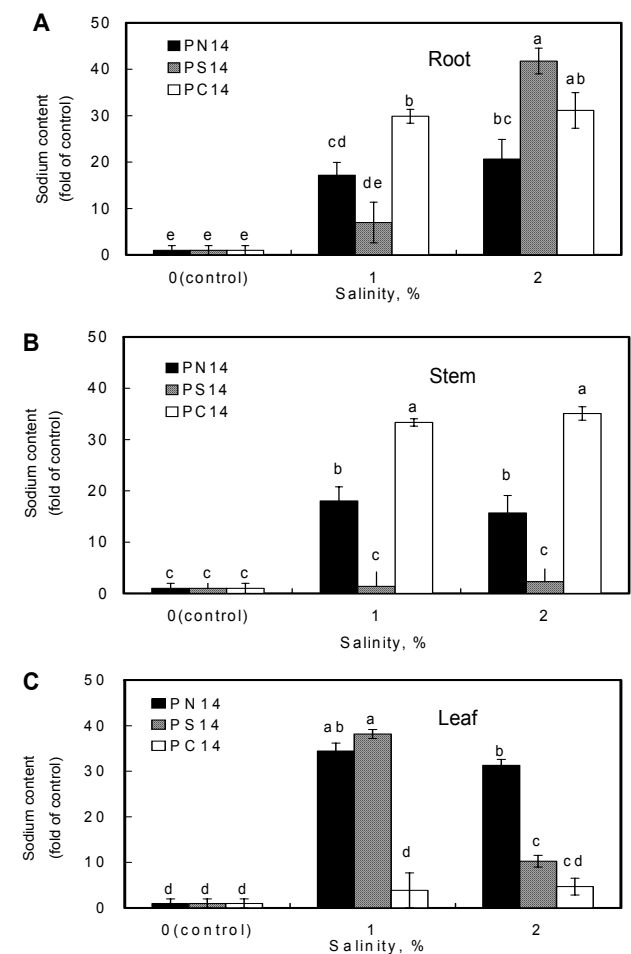
To determine the sodium content of plantlets in response to long-term (two months) salt treatment, root, stem and leaves of PN14, PS14 and PC14 were harvested for study after a two-month salt treatment, and data were means of seven individuals. Results showed that sodium accumulated in roots and stems of plantlets. It increased with increased salinity, and plantlets of the Chuwei ecotype (PC14) appeared to undergo the highest increases (Figure 6A; Figure 6B). With salt concentration

at 1%, the sodium content increased 28.87 fold in roots of PC14 (224.77 mg gdw<sup>-1</sup>), and 32.33 fold in stems of PC14 (221.94 mg gdw<sup>-1</sup>). With salt concentration at 2%, the sodium content increased 30.14 fold in roots of PC14 (234.32 mg gdw<sup>-1</sup>), and 34.08 fold in stems of PC14 (234.31 mg gdw<sup>-1</sup>). The accumulation of sodium was also found in leaves with increased salinity, but PC14 was an exception. Here, the sodium content was extremely low (Figure 6C). Therefore, we found sodium accumulated significantly in roots and stems, but not in leaves, of the Chuwei ecotype after a short-term and a long-term salt treatment.

Halophytes were categorized into two types according to their responses to salt: the regulation type and the accumulation type (Hellebust, 1976). The salt levels in the regulation type plants were often low. This type of plant secretes salt. The regulation type usually consists of mangrove plants and can be classified into two classes. The first class is salt-exclusion species. These species allow



**Figure 5.** Variation of accumulation of sodium in roots (A), stems (B) and leaves (C) of plantlet of three ecotypes after an eight-day salt treatment. Bars having different letters are significantly different,  $p=0.05$ , ANOVA, with Duncan's multiple range test. Error bars were S.E. at the 5% level.



**Figure 6.** Variation of accumulation of sodium in roots (A), stems (B) and leaves (C) of plantlet of three ecotypes after a two-month salt treatment. Bars having different letters are significantly different,  $p=0.05$ , ANOVA, with Duncan's multiple range test. Error bars were S.E. at the 5% level.

salt to stay in roots, but a little to stay in other organs. The second class is salt-secretion species (Teas, 1979). These species secrete salt out of their leaves. Therefore, the regulation type plants accumulate very little salt in their bodies. By contrast, the accumulation type plants accumulate high levels of salt in their bodies, gathering salt in their cell vacuoles until concentrations become very high. Halophyte grown in salinized water accumulates salt, mainly sodium for osmoregulation. It was reported that halophytes accumulated 90% sodium in their stems and 80% in leaves (Flowers et al., 1977). However, glycophytes do not accumulate salt. They avoid salt entering their leaves by allowing it in their roots. For example, *Atriplex halimus* fed with  $\text{Na}^{22}$  will transport  $\text{Na}^+$  to leaves (Matsushita and Matoh, 1992). Flowers et al. (1986) also pointed out that the  $\text{Na}^+/\text{K}^+$  ratio was higher in Dicotyleden than in Monocotyleden. Wu et al. (1996) used  $\text{Rb}^{86}$  as a tracer to determine its absorbance in Arabidopsis. They found that salt-sensitive mutant did not accumulate  $\text{K}^+$ , which demonstrated that it is necessary for glycophytes to absorb salt in response to salt stress. It appears that the *I. cylindrica* Chuwei ecotype can be classified as an accumulation type.

Flowers et al. (1977) indicated that halophytes accumulate sodium mainly in leaves. However, the *I. cylindrica* Chuwei ecotype acted in the opposite way. It accumulated sodium, instead, in roots and stems. Our result differs from Flower's finding. Maybe the *I. cylindrica* Chuwei ecotype transported its sodium from leaves to roots and stems in order to regulate the osmotic potential against salt stress. Maybe plants transported sodium to roots and stems in order to protect leaves from harm. It has been reported that halophytes transport sodium from leaves to roots and stems. For example, Matsushita and Matoh (1992) used stable isotope  $^{22}\text{Na}$  as a tracer to define its transportation route. In halophyte *Phragmites communis* Trinius, they found that sodium was transported to roots, and Na-dependent ATP-ase activity was detected. Our results in Figures 5 and 6 suggest the same manner. However, whether the Chuwei ecotype utilizes the same mechanism remains to be studied.

In conclusion, this study detected variation among *Imperata* ecotypes on a physiological and biochemical level. We confirmed the wetland (Chuwei) ecotype to be physiologically distinct. These results supported the assertion of variation among ecotypes on a molecular level by a previous study (Cheng and Chou, 1997a). These findings may help us design a better strategy to control the weed, *Imperata cylindrica*.

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## 白茅族群的生態變異—II. 生理及生化的證據

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白茅 (*Imperata cylindrica* var. *major*) 是一種常見的禾本科雜草，在臺灣各地都有分布，且為一屬一種。尤其它生長在淡水紅樹林沼澤地。紅樹林環境相當特殊，不僅是鹽分地，又有週期性的淹水。作者分析野外環境下各地區白茅之酒精去氫酵素活性、脯胺酸與鈉含量。結果顯示在野外環境下竹圍白茅之酒精去氫酵素活性、脯胺酸與鈉含量比其它非紅樹林地區（沙崙及內湖）之白茅來得高。此外，將野外採得之白茅植於盆栽中並置於溫室內利用水耕實驗以缺氧及不同濃度之鹽分處理，以觀察並測試不同地區之白茅幼苗對缺氧及鹽分處理之生理反應。在缺氧處理下竹圍白茅可以忍受 0 ppm 的缺氧逆境。其葉中之酒精去氫酵素活性比其它地區之白茅來得高。在鹽分處理下竹圍白茅可以忍受 1% 的鹽分逆境。其葉中之脯胺酸及根、莖中累積之鈉含量比其它地區之白茅來得高。這些生理及生化之分析顯示白茅生態型間之差異，以及竹圍生態型之獨特性。

**關鍵詞：**白茅；酒精去氫酵素；脯胺酸；鈉。