Adaptive Diversity of *Echinochloa* Species to Osmotic Stress

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**ABSTRACT.** Three *Echinochloa* species inhabit various crop fields with different soil moisture conditions. Therefore, a growth pouch test was conducted to investigate adaptive diversity of six *Echinochloa* species, three from Korea and three from USA, to osmotic stress by assessing shoot and root growths. *Echinochloa crus-galli* var. *praticola* showed the greatest tolerance to osmotic stress in both root (GR$_{50}$=1316.3 g PEG L$^{-1}$) and shoot (GR$_{50}$=212.2 g PEG L$^{-1}$) growths, while Korean *E. oryzicola* was most sensitive to osmotic stress in both root (GR$_{50}$=116 g PEG L$^{-1}$) and shoot (GR$_{50}$=126.2 g PEG L$^{-1}$) growths. Root to shoot (R/S) ratio of *Echinochloa crus-galli* var. *praticola* increased with increasing osmotic stress, while that of Korean *E. oryzicola* decreased, suggesting that R/S ratio is closely related to osmotic stress tolerance in *Echinochloa* species. Our results clearly demonstrate that *E. crus-galli* var. *praticola* maintains high R/S ratio even under high osmotic stress, which enables this species to well adapt to dry upland condition. In contrast, while *E. oryzicola* fails to maintain sufficiently high R/S ratio, resulting in poor adaptability to dry upland condition.

**Key words:** Adaptive diversity, Barnyardgrass, *Echinochloa crus-galli*, *Echinochloa oryzicola*, Growth pouch test, Late watergrass, Osmotic stress

Introduction

*Echinochloa* genus is composed of around 50 species, which are distributed around the world (Holm et al., 1977). Although some of *Echinochloa* species used to be cultivated as a food crop, most of them are now regarded as problematic weeds because of their high competitiveness against crop under various environmental conditions, causing inevitable crop yield losses (Moon et al., 2010, 2014). Several studies related with environmental stresses such as flooding (Kim, 1993; Im, 2016), salt (Aslam et al., 1987; Yamamoto et al., 2003; Kim et al., 2004; Nguyen et al., 2005), and drought (Hamim et al., 2016) were supporting that two *Echinochloa* species inhabit various field conditions from paddy field to dry upland (Yamasue et al., 1989). In *E. crus-galli*, *E. crus-galli* var. *praticola* inhabits upland field only, while *E. crus-galli* var. *crus-galli* inhabits areas from dry land to paddy field. These various habitats of three *Echinochloa* species imply that adaptive diversity to abiotic stress exists in *Echinochloa* species.

The three *Echinochloa* species inhabiting Korea have close morphological similarity except for several traits particularly related to seed such as seed awn and seed size. In ploidy level, *E. oryzicola* is tetraploid (2n=4X=36), while *E. crus-galli* is hexaploid (2n=6X=54) revealed by flow cytometric analysis and chromosome counting (Nah et al., 2015a). It is known that *Echinochloa crus-galli* is an allohexaploid produced by natural hybridization between tetraploid *E. oryzicola* and unknown diploid *Echinochloa* species and then subsequent chromosome doubling (Yabuno, 1966). *Echinochloa crus-galli* was then further developed into new biological sub-species with various water-condition adaptabilities. Therefore, it can be hypothesized that *Echinochloa* species have expanded their...
habitats from flooded condition to dry upland condition during evolution processes including natural hybridization and chromosome doubling, enabling *Echinochloa crus-galli* to have more diverse environmental adaptability than *E. oryzicola*. Eco-physiologically Korean *Echinochloa* species, *E. oryzicola*, *E. crus-galli* var. *crus-galli*, and *E. crus-galli* var. *praticola*, have their favorite habitats depending on soil water conditions. Seed germination study showed that under deep water flooding condition, *E. oryzicola* could germinate better than *E. crus-galli* (Kim, 1993), suggesting that adaptive diversity to flooding condition exists in *Echinochloa* species and *E. oryzicola* better adapts to flooded condition than *E. crus-galli*. However, it is unclear how *Echinochloa* species respond to osmotic stress. No studies have been conducted about the diverse response and adaptability of *Echinochloa* spp. to dry soil condition.

Therefore, this study was conducted to investigate the growth response and adaptability of *Echinochloa* spp. to dry soil condition by using growth pouch method (Zhang et al., 2015). Dry soil condition was artificially provided using polyethylene glycol (PEG), which has often been used to give different levels of osmotic stress (O’Donnell et al., 2013).

**Materials & Methods**

**Plant materials**

Six *Echinochloa* species were used in this experiment. Three of them were collected in Korea such as *Echinochloa crus-galli* var. *praticola* (ECHPT-KO), *E. crus-galli* (ECHCG-KO), and *E. oryzicola* (ECHOR-KO), and the other three species were collected in California, USA such as *E. crus-galli* (ECHCG-US), *E. oryzoides* (ECHOZ-US), and *E. oryzicola* (ECHOR-US).

**Growth pouch test under osmotic stress**

Seeds of *Echinochloa* species were incubated in petri dishes in a plant growth chamber maintained at 30/20°C of temperature (day/night). When seeds were germinated, the germinated seeds were transplanted into 7-mL PEG-containing (without nutrient solution) growth pouches and incubated in the plant growth chamber with 16 hours of photoperiod. Dark condition was maintained for the roots by placing the growth pouches in a top-open paper box. The schematic illustration of the growth pouch method is shown in Fig. 1. The PEG-6000 was used to mimic dry soil conditions and a range of PEG concentrations were given; 0 g, 40 g, 80 g, 120 g, and 160 g PEG L⁻¹. Deionised water was added to the growth pouches every day to compensate for evaporation. At 6 days after PEG treatment (DAT), root and shoot lengths were measured. The experiment consisted of four replicates of a split-split plot design with 5 concentrations of PEG as the main plot, which was then split with 6 *Echinochloa* species as subplots.

**Statistical analysis**

All measurements were initially subjected to analysis of variance (ANOVA). Non-linear regression analysis was conducted by fitting the log-logistic model (Streibig, 1980) to the root and shoot lengths observed at 6 days after sowing. All statistical analyses were conducted using Genstat (Genstat Committee, 2002).

**Results and Discussion**

**Root growth response of *Echinochloa* species to osmotic stress**

To clearly investigate root growth response of *Echinochloa* to osmotic stress by avoiding the effects on seed germination,
pre-germinated seeds were exposed to PEG solutions at a range of PEG concentrations. All tested *Echinochloa* species showed various responses to PEG in their root growths up to 6 DAT (Fig. 2). Among them, *Echinochloa crus-galli* var. *praticola* (ECHPT-KO) was the least influenced by PEG treatment in its root growth, while the other five *Echinochloa* species showed significant decreases in their root growths (Fig. 2). At no PEG treatment, two Korean *Echinochloa crus-galli* species (ECHCG-KO and ECHOR-KO) showed the smallest roots with 4.52 cm and 4.94 cm of root lengths at 6 DAT, respectively, while Korean *E. oryzicola* (ECHOR-KO) showed the longest root, 9.70 cm, followed by *E. oryzoides* (ECHOZ-US), *E. crus-galli* (ECHCG-US), and *E. oryzicola* (ECHOR-US) from the USA with root lengths of 8.12 cm, 7.01 cm, and 6.58 cm, respectively. With increasing PEG concentration, the root growth of *Echinochloa* species was significantly affected except for ECHPT-KO. ANOVA revealed that osmotic stress by PEG significantly inhibited root growths of 2 Korean *Echinochloa* species, ECHCG-KO and ECHOR-KO, and 3 American *Echinochloa* species, ECHCG-US, ECHOZ-US, and ECHOR-US, while osmotic stress did not significantly inhibit the root growth of ECHPT-KO (Table 1). This result suggests that *Echinochloa crus-galli* var. *praticola* is much more tolerant to osmotic stress in its root growth than the other tested *Echinochloa* species.

Non-linear regression by fitting the log-logistic model to the root lengths at a range of PEG concentrations at 6 DAT could estimate the maximum root growth at no PEG treatment and the GR50 values, the PEG concentrations required for 50% root growth inhibition for each *Echinochloa* species (Table 2 and Fig. 3). The GR50 value for root length at 6 DAT was the least in ECHOR-KO with 116 g PEG L$^{-1}$, followed by ECHCG-US, ECHOZ-US, ECHOR-US, and ECHCG-KO with 130.3 g, 144.8 g, 153.2 g, and 156.1 g PEG L$^{-1}$, respectively. Exceptionally ECHPT-KO showed extremely high GR50 value with over 1300 g PEG L$^{-1}$, almost 10 times greater than those of

### Table 1. Summary of analysis of variance (ANOVA).

<table>
<thead>
<tr>
<th><em>Echinochloa</em> spp.</th>
<th>Root length</th>
<th>Shoot length</th>
<th>R/S ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>ECHPT-KO</td>
<td>1.59NS</td>
<td>8.16***</td>
<td>2.66NS</td>
</tr>
<tr>
<td>ECHCG-KO</td>
<td>5.23**</td>
<td>9.09***</td>
<td>1.27NS</td>
</tr>
<tr>
<td>ECHOR-KO</td>
<td>28.17***</td>
<td>60.87***</td>
<td>2.12**</td>
</tr>
<tr>
<td>ECHCG-US</td>
<td>43.58***</td>
<td>9.16***</td>
<td>0.69NS</td>
</tr>
<tr>
<td>ECHOZ-US</td>
<td>12.29***</td>
<td>12.71**</td>
<td>0.68NS</td>
</tr>
<tr>
<td>ECHOR-US</td>
<td>13.7***</td>
<td>16.59***</td>
<td>0.68NS</td>
</tr>
</tbody>
</table>

NS indicates no significance at P=0.05. ** and *** indicate significance at P=0.01 and P=0.001, respectively.

### Table 2. Parameter estimates for the log-logistic model fitted to root lengths of *Echinochloa* species grown under a range of PEG concentration.

<table>
<thead>
<tr>
<th><em>Echinochloa</em> spp.</th>
<th>$Y_0$</th>
<th>GR50</th>
<th>B</th>
</tr>
</thead>
<tbody>
<tr>
<td>ECHPT-KO</td>
<td>4.88</td>
<td>1361</td>
<td>1.53</td>
</tr>
<tr>
<td>ECHCG-KO</td>
<td>4.92</td>
<td>156</td>
<td>2.42</td>
</tr>
<tr>
<td>ECHOR-KO</td>
<td>9.09</td>
<td>116</td>
<td>4.81</td>
</tr>
<tr>
<td>ECHCG-US</td>
<td>7.91</td>
<td>130</td>
<td>5.21</td>
</tr>
<tr>
<td>ECHOZ-US</td>
<td>8.53</td>
<td>144</td>
<td>2.14</td>
</tr>
<tr>
<td>ECHOR-US</td>
<td>6.85</td>
<td>153</td>
<td>3.31</td>
</tr>
</tbody>
</table>
the other *Echinochloa* species. However, the estimation for ECHPT-KO was not accurate due to lack of observed values at higher concentrations than 160 g PEG L\(^{-1}\), so an additional test with high concentrations of PEG is required in order to estimate more accurate GR\(_{50}\) value. Nonetheless, our results clearly indicate that *E. crus-galli var. pratapica* (ECHPT-KO) is the most tolerant to osmotic stress, while *E. oryzicola* (ECHOR-KO) is the most sensitive to osmotic stress in root growth response.

### Shoot growth response of *Echinochloa* species to osmotic stress

After placing germinated seeds of *Echinochloa* species, shoot growth was also daily recorded up to 6 DAT (Fig. 4). All *Echinochloa* species significantly responded to increasing PEG concentrations with no exception. At no PEG treatment, *E. crus-galli var. pratapica* (ECHPT-KO) was the smallest with 3.72 cm plant height, while American *E. oryzicola* (ECHOR-US) was the tallest with 9.38 cm plant height. The other species were around 5-6 cm tall. At PEG treatment, Korean *E. oryzicola* (ECHOR-KO) was most significantly affected in its shoot growth by PEG, while *E. crus-galli var. pratapica* (ECHPT-KO) was least significantly affected by PEG (Fig. 4 and Table 1). At 120 g PEG L\(^{-1}\), ECHPT-KO showed the least growth reduction of 24%, followed by ECHCG-US, ECHOZ-US, ECHOR-KO, ECHCG-KO, and ECHOR-US with 31%, 37%, 40%, 46%, and 51% growth reduction, respectively (Fig. 4). At 160 g PEG L\(^{-1}\), ECHPT-KO still maintained good shoot growth, while ECHOR-KO showed significant growth inhibition, resulting in the greatest shoot growth reduction of 84%. In overall, our results indicate that *E. crus-galli var. pratapica* is the most tolerant to osmotic stress in shoot growth, while *E. oryzicola* is the most sensitive to osmotic stress.

Non-linear regression analysis revealed that *Echinochloa* shoot growth responding to PEG concentration is well described by the log-logistic model, resulting in estimation of GR\(_{50}\) values, the PEG concentration causing 50% shoot growth reduction (Table 3 and Fig. 5). The GR\(_{50}\) value for shoot length at 6 DAT was the least in ECHOR-KO with 126.2 g PEG L\(^{-1}\), followed by ECHCG-KO, ECHOR-US, and ECHCG-US with 128.8 g, 134.6 g, and 168.3 g PEG L\(^{-1}\), respectively. ECHPT-KO showed the greatest GR\(_{50}\) value of 212.2 g PEG L\(^{-1}\), and ECHCG-US also showed a similar GR\(_{50}\) value of 205.1 g PEG L\(^{-1}\). Similarly to root growth response, these results also suggest that *E. crus-galli var. pratapica* (ECHPT-KO) is the most tolerant to osmotic stress, while *E. oryzicola* (ECHOR-KO) is the most sensitive to osmotic stress in shoot growth response.

![Fig. 4. Shoot lengths of 3 Korean *Echinochloa* species (*E. crus-galli var. pratapica* (ECHPT-KO), *E. crus-galli var. crus-galli* (ECHCG-KO), and *E. oryzicola* (ECHOR-KO)) and 3 American *Echinochloa* species (*E. crus-galli* (ECHGC-US), *E. oryzoides* (ECHOZ-US), and *E. oryzicola* (ECHOR-US)) grown in the growth pouch containing a range of PEG concentrations.](image)

![Fig. 5. Responses of 3 Korean *Echinochloa* species (*E. crus-galli var. pratapica* (a), *E. crus-galli var. crus-galli* (b), and *E. oryzicola* (c)) and 3 American *Echinochloa* species (*E. crus-galli* (d), *E. oryzoides* (e), and *E. oryzicola* (f)) in their root growths to PEG solution. Shoot lengths were measured at 6 days after placing germinated seeds on the paper wick of the growth pouch.](image)

### Table 3. Parameter estimates for the log-logistic model fitted to shoot lengths of *Echinochloa* species grown under a range of PEG concentration.

<table>
<thead>
<tr>
<th><em>Echinochloa</em> spp.</th>
<th>Parameter estimates</th>
<th>R(^2)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Y(_0)</td>
<td>GR(_{50})</td>
</tr>
<tr>
<td>ECHPT-KO</td>
<td>3.80 (0.232)</td>
<td>212.2 (52.20)</td>
</tr>
<tr>
<td>ECHCG-KO</td>
<td>5.55 (0.413)</td>
<td>128.8 (17.77)</td>
</tr>
<tr>
<td>ECHOR-KO</td>
<td>6.12 (0.212)</td>
<td>126.2 (3.66)</td>
</tr>
<tr>
<td>ECHCG-US</td>
<td>4.98 (0.239)</td>
<td>205.1 (33.23)</td>
</tr>
<tr>
<td>ECHOZ-US</td>
<td>5.42 (0.302)</td>
<td>168.3 (20.70)</td>
</tr>
<tr>
<td>ECHOR-US</td>
<td>9.50 (0.557)</td>
<td>134.6 (20.99)</td>
</tr>
</tbody>
</table>
under flooded paddy condition at around 5 cm water depth, while Californian rice cultivation is mainly by airplane-direct-sowing under deep flooded paddy condition at deeper than 10 cm water depth, suggesting that continuous deep water rice culture in California may have selected submergence tolerant and fast growing E. oryzicola in California.

Response of *Echinochloa* species in their R/S ratio to osmotic stress.

Plant shoot is a sink of water, while root is a source of water. Under sufficient water supply condition, it is better for plant to maintain low root and shoot (R/S) ratio. However, if water is limited, plant instantly alters its strategy, so minimizing shoot growth to avoid or minimize water loss from leaves but maintaining or promoting root growth to maximize water uptake from soil. Therefore, under osmotic stress, increasing R/S ratio is a common phenomenon often observed in osmotic stress tolerant plant.

At no PEG treatment, wide variation of R/S ratio exists among *Echinochloa* species. ECHOR-KO showed the greatest R/S ratio of 1.68, followed by ECHOZ-US, ECHCG-US, ECHPT-KO, ECHCG-KO, and ECHOR-US with R/S ratios of 1.57, 1.34, 1.23, 0.88, and 0.70, respectively (Fig. 6). ANOVA showed that PEG treatment significantly affected R/S ratios of ECHOR-KO and ECHCG-US, while no significantly affected those of the other *Echinochloa* species. The R/S ratio of *E. crus-galli* var. *praticola* (ECHPT-KO) was even smaller than that of Korean *E. oryzicola* (ECHOR-KO). However, with increasing PEG concentration, there is a clear trend of increase in R/S ratio of *E. crus-galli* var. *praticola*, while Korean *E. oryzicola* showed opposite trend of decreasing R/S ratio although its R/S ratio was greatest at no PEG treatment (Fig. 6). In the case of American *E. oryzicola* (ECH-US), its R/S ratio was smallest and showed no significant change with increasing PEG concentration. Although it is difficult to explain variation in adaptability of *Echinochloa* species only using R/S ratio, our finding supports that *E. crus-galli* var. *praticola* increases R/S ratio with increasing osmotic stress, while *E. oryzicola* decreases R/S ratio or shows smallest R/S ratio. This difference in R/S ratio between *E. crus-galli* var. *praticola* and *E. oryzicola* may explain why these two *Echinochloa* species have contrasting adaptability to osmotic stress by differently responding to osmotic stress in their root and shoot growths.

![Fig. 6. Root and shoot (R/S) ratios of 3 Korean *Echinochloa* species (*E. crus-galli* var. *praticola* (ECHPT-KO), *E. crus-galli* var. *crus-galli* (ECHCG-KO), and *E. oryzicola* (ECHOR-KO)) and 3 American *Echinochloa* species (*E. crus-galli* (ECHCG-US), *E. oryzoides* (ECHUS-US), and *E. oryzicola* (ECHOR-US)) grown in the growth pouch containing a range of PEG concentrations. Root and shoot ratios were estimated by dividing root lengths by shoot lengths measured at 6 days after placing germinated seeds on the paper wick of the growth pouch.](image)
our findings in this study, which revealed more diverse adaptability in *E. crus-galli* than *E. oryzicola*. Different responses of *Echinochloa* species to osmotic stress indicate different tolerances to osmotic stress among *Echinochloa* species, playing an important role in determining favorite habitats of *Echinochloa* species. To uncover mechanism of osmotic stress tolerance which determines such an adaptive diversity in *Echinochloa* species, molecular approaches are required by using genetic information from previous genomic studies (Nah et al., 2015b; Yang et al., 2013). Our report is the first result to reveal the reason why *E. crus-galli var. pratensis* is predominantly found in dry upland fields, while *E. oryzicola* is not found in dry upland fields but only in flooded paddy fields. This study can be a basis to explain diverse adaptability of *Echinochloa* spp. in relation to soil moisture.

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