Characterization of *Elymus humidus* as a candidate for genetic resource of wheat water tolerance

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ABSTRACT

*Elymus humidus* is one of the wild Triticeae species indigenous to Japan, which is well adapted to moist soil. In order to reveal genetic diversity of this species, SSR analysis was conducted using 79 plants collected from 14 natural populations in Japan. The results suggested that *E. humidus* contains genetic variation within a species and generally diverges between the eastern and western Japan. For the purpose of breeding utilization, F1 hybrids of *E. humidus* with wheat cultivars were produced. The level of water tolerance of *E. humidus* is much higher than wheat and that of F1 was intermediate between the parents. Microscopy of root cortical tissues revealed that configuration of aerenchymas of *E. humidus* is more similar to wheat than rice in spite of its habitat. In conclusion, we confirmed in this study the potential of *E. humidus* as a genetic resource of water tolerance for wheat.

MATERIALS AND METHODS

Genetic diversity analysis

Living plants of *E. humidus* were collected from 14 natural populations that cover all the distribution area in Japan. From each population, one to eight plants were collected. Total DNA was extracted from leaves or stems. Genetic diversity of collected materials was analyzed using a chloroplast SSR marker named EcpSSR1 located on IGS region between *trnF* and *ndhJ* was used. Primer sequences were forward: 5′-AATAAATCATACCTCCATCTA-3′; and reverse: 5′-GAGGACTGAAATCCCTCGTG-3′. PCR condition was 30 cycles of 94°C for 1 min, 55°C for 1 min and 72°C for 2 min, preceded by 94°C for 5 min and followed by 72°C for 7 min. The amplified products were electrophoresed in a 7.5% acrylamide gel and detected by silver staining.

Production of F1 between *E. humidus* and wheat

Intergeneric crossing was performed between *E. humidus* as female and *Triticum aestivum*. The accession of *E. humidus* was provided by Dr. R. Miura, Kyoto University, Japan, that was collected in Kyoto Prefecture and maintained by selfing. Wheat cultivars used were “Norin 61” in 2006 and “Shirogane-komugi” and “Kinuhime” in 2007. Embryo rescue was practiced around 14 days after pollination. Immature embryos were cultured on 1/2 MS medium at 20°C with 16 h day length. Regenerated plants were transferred clay pots and grown in greenhouse.

Evaluation of water tolerance

Three F1 plants and 3 *E. humidus* plants (the accession provided by Dr. R. Miura, Kyoto University) were transplanted to the pots with the soil from a paddy field in Tsukuba on October 2007. Other 3 plants were...
transplanted in the pots with upland soil made by Kureha Corporation. Three wheat seeds cv. Norin 61 were sowed in the pots with the same soils. They grew at the greenhouse of 24°C and under natural day length. Flooding treatments were conducted in the pot with paddy soil. The water level was set over 5 cm from the soil surface. On the other hand, appropriate watering was done in the control pots with the upland soil. Shoot and root of the plants were observed in each plant after 2 weeks from the beginning of the treatment.

Furthermore, to observe root tissues under water condition, seedlings of *E. humidus* were planted in plastic pots with soil and grown in a greenhouse (22.5°C, Natural day length). Pots were soaked in water at soil surface level after emergence of the first leaf. Roots that run off the bottom of pots into water were harvested and 5 mm long segments were cut from the regions 0 to 5 (Sub-apical zone), 5 to 10, 10 to 15, and 15 to 20 cm from the tip. The sections were examined under a microscope (Axiophoto, Carl Zeiss, Germany).

**RESULTS AND DISCUSSION**

**Genetic diversity**

A total of 79 plants from 14 populations were investigated in the diversity analysis. The EcpSSR1 amplified two types of fragment, named alleles I and II. The distribution of each allele was generally divided in the central Japan (Fig. 1). The allele I was mainly found in the eastern Japan, whereas the allele II was found more in the western Japan, although there are some exceptions. Interestingly, three populations in Kyushu and Shikoku islands (population number 11, 12 and 14) had both types in a population. From these results, we considered that the original center of the distribution of *E. humidus* was Kyushu and then this species expanded its distribution to Honshu along with human agricultural activity. In fact, some researchers considered that the distribution of *E. humidus* expanded along with the spread of milk vetch agriculture because *E. humidus* is often found with milk vetch on fallow fields (Muramatsu, 1995). The population 11 on Shikoku Island also includes both alleles, but this area has similar climate with Kyushu and has connected with the Kyushu Island in the glacial period. We supposed that introduction from Kyushu to Honshu initially occurred with a few individuals and separately between the Western and Eastern Japan, and secondary introduction should produce the exceptional genotype within an area.

**Production of F₁ hybrid**

In 2006 spring, we preliminarily crossed *E. humidus* with *T. aestivum* cv. Norin 61. From more than one hundred crossed florets, one F₁ plant was obtained through embryo rescue. In 2007 spring, more number of interspecific crosses using two different wheat cultivars were carried out. The wheat varieties used were cultivars “Shirogane-komugi” and “Kinuhime”, both of which are Japanese modern cultivar. More than 200 emasculated florets of *E. humidus* were crossed with wheat pollen for each combination (Table 1). The success rate to gain embryos was 17.2% for *E. humidus* × Shirogane-komugi and 9.9% for *E. humidus* × Kinuhime. The difference in the rate between the combinations was statistically significant at 5% level by chi square test. Throughout embryo rescue, four and one F₁ plants regenerated from the immature embryos for each combination. It must be noted that the crossing succeeded only when using *E.
Since all the F1 plants are naturally sterile, chromosome doubling is required to obtain F2 seeds. Although it has not yet succeeded, we can still retry because the F1 plants possess perenniality inherited from E. humidus and are maintained for future study.

**Evaluation of water tolerance**

The level of water tolerance of E. humidus and its F1 with bread wheat (T. aestivum cv. Norin 61) were evaluated in the wet endurance test (Table 2). The growth of E. humidus under waterlogging condition was not remarkably different from the control. On the other hand, wheat showed an obvious weakness against waterlogging. The level of water tolerance of F1 seemed lower than E. humidus but much higher than wheat.

<table>
<thead>
<tr>
<th>Species</th>
<th>Waterlogging</th>
<th>Control</th>
<th>Proportion</th>
</tr>
</thead>
<tbody>
<tr>
<td>E. humidus</td>
<td>7.60</td>
<td>8.66</td>
<td>87.8%</td>
</tr>
<tr>
<td>F1</td>
<td>5.09</td>
<td>7.17</td>
<td>71.0%</td>
</tr>
<tr>
<td>Wheat</td>
<td>0.40</td>
<td>1.45</td>
<td>27.6%</td>
</tr>
</tbody>
</table>

To elucidate the physiological water tolerant mechanism of E. humidus, its root cortical tissues were observed. E. humidus formed clear aerechymas in adventitious roots (data not shown). The configuration of aerechymas of E. humidus was more similar to wheat than rice in spite of its similarity in habitat with rice. The aerechymas of rice were large and regularly arranged, where as those of wheat and E. humidus was small and seemed more irregular.

**CONCLUSION AND PROSPECT**

In this study, we revealed the genetic diversity of E. humidus in Japan. Even though the level is low, genetic variation clearly exists in this species, and geographic separation was also suggested. This result demonstrates that each population of E. humidus is different genetic resource. Our field survey from 2002 strongly warned us that natural populations of this species are endangered in many places due to residential development and wetland reclamation. We must conserve this precious threatened species including its genetic diversity and transfer them to the future generation.

As a result of this study, we obtained F1 plants of E. humidus with several wheat modern cultivars. There are still several problems to overcome, i.e., sterility of F1 and cytoplasmic incompatibility of pollen of E. humidus with wheat. For the purpose of producing practical cultivars, several steps such as backcrossing and development of addition lines are necessary.

The water adaptation mechanism of E. humidus is still unknown, but the microscopy of the root cortical tissues suggests the different mechanism from rice. The level of water tolerance of F1 hybrid is not as high as E. humidus but is higher than wheat. This result provided us a confidence for the usefulness of E. humidus as a genetic resource of water tolerance.

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**REFERENCES**


