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

Sasanuma T[1,2], Takata K[3], Kawaguchi K[4], Oyanagi A[4]

[1] Kihara Institute for Biological Research, Yokohama City University, 244-0813 Yokohama, Japan. [2] Faculty of Agriculture, Yamagata University, 997-8555 Yamagata, Japan. [3] National Agricultural Research Center for Western Region, 721-8514 Hiroshima, Japan. [4] National Institute of Crop Science, 305-8518 Ibaraki, Japan

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, F_1 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 F_1 was intermediate between the parents. Microscopy of root cortical tissues revealed that configuraion 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.

INTRODUCTION

One of the most serious problems for wheat breeding in monsoon area like as Japan is water or moisture stress (Kubo et al., 2007). In spite of its importance, the breeding of water tolerant wheat has not well been improved. This is owing to the lacking of genetic resources in cultivated wheat and closely related wild species. As a breakthrough of it, we focus wild Triticeae species indigenous to Japan. Among them, *Elymus humidus* is the most notable candidate for the genetic resource because of its high level of adaptation to paddy field. The Japanese vernacular name of this species is "MIZUTA-KAMOJI", in which "MIZUTA" means paddy field and "KAMOJI" is a common name for Japanese wheatgrass. Thus the Japanese name of *E. humidus* totally means "wheatgrass of paddy field". As its Japanese name shows, *E. humidus* is mainly living around paddy fields and adapted to moist soil. Its taxonomical classification was confused but recent molecular study revealed it is a diverged species from other Japanese *Elymus* species (Sasanuma et al. 2002). In spite of its potential, its characteristics, for example genetic diversity, physiological traits and possibility to produce hybrids with wheat, have not been revealed.

Another necessity of this study is that *E. humidus* is a threatened species listed in the Red Data Book (Environment Agency of Japan, 2000). *E. humidus* is distributed only in Japan and a part of China (Osada, 1993). This species was not very rare till 1960's in Japan, but residential development and modernization of agriculture reduced the traditional paddy field, which was suitable habitat for *E. humidus*, and then made it endangered. The Red Data Book describes that the probability of extinction of this species after one hundred years is 100%. To avoid losing the valuable genetic resources unwittingly, we must start the research project to estimate and conserve the genetic diversity.

In this study, we tried to clarify its genetic diversity and water adaptation mechanisms. Furthermore, intergeneric crossing were performed to introduce its water tolerance to wheat. Based on the results, genetic and physiological characteristics of *E. humidus* and its application to wheat breeding are discussed.

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 *trn*F and *ndh*J was used. Primer sequences were forward: 5'-AATAAATCATACTCCATCTA-3': and reverse: 5'-GAGGACTGAAAATCCTCGTG-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 7min. The amplified products were electrophoresed in a 7.5% acrylamide gel and detected by silver staining.

Production of F_1 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 F_1 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 conduced 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 F_1 hybrid

In 2006 spring, we preliminarily crossed *E. humidus* with *T. aestivum* cv. Norin 61. From more than one hundred crossed florets, one F_1 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 F_1 plants regenerated from the immature embryos for each combination. It must be noted that the crossing succeeded only when using *E. humidus* as female.

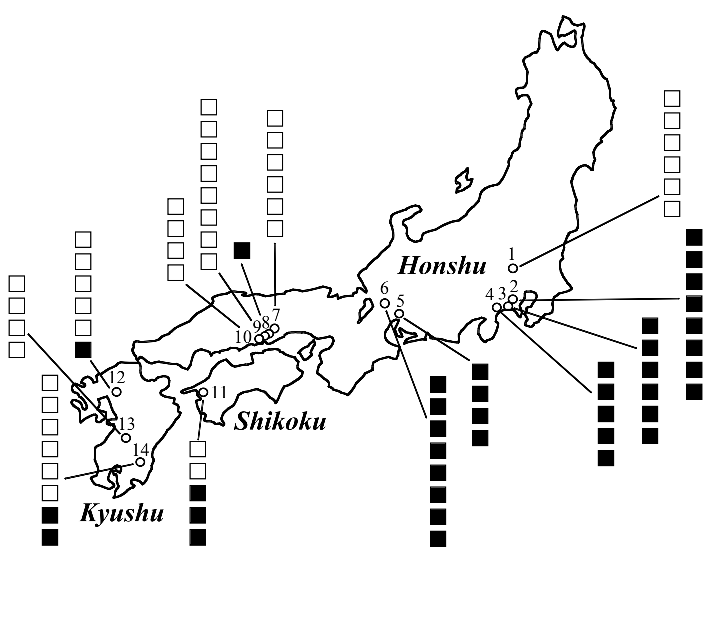

Fig. 1. Distribution map of two alleles of EcpSSR1 in Japan. White and black squares indicate alleles I and II, respectively.

Table 1. Crossing between *E. humidus* and wheat

Combination	No. of crossed florets	No. of obtained embryos
E. humidus × Shirogane-komugi	233	40
E. humidus × Kinuhime	262	26

Since all the F_1 plants are naturally sterile, chromosome doubling is required to obtain F_2 seeds. Although it has not yet succeeded, we can still retry because the F_1 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 F_1 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 F_1 seemed lower than *E. humidus* but much higher than wheat.

Table 2. Evaluation of water tolerance represented by shoot dry weight (g)

Species	Waterlogging	Control	Proportion
E. humidus	7.60	8.66	87.8%
F_1	5.09	7.17	71.0%
Wheat	0.40	1.45	27.6%

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 aerenchymas of *E. humidus* was more similar to wheat than rice in spite of its similarity in habitat with rice. The aerenchymas 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 F_1 plants of *E. humidus* with several wheat modern cultivars. There are still several problems to overcome, i.e., sterility of F_1 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 F_1 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.

ACKNOWLEDGEMENTS

This work was supported by the Sumitomo foundation and Grant-in-Aid for Exploratory Research (No. 19658007), Japan Society for the Promotion of Science.

REFERENCES

Environment Agency of Japan. 2000. Threatened wildlife of Japan – Red Data Book – 2nd ed. vol. 8 Vascular plants. Tokyo: Japan Wildlife Research Center.

Kubo K, Shimazaki Y, Kobayashi H, Oyanagi A. 2007. Specific variation in shoot growth and root traits under waterlogging conditions of the seedlings of tribe Triticeae including Mizutakamoji (*Agropyron humidum*). Plant Prod. Sci. 10: 91-98.

Muramatsu M. 1995. Japanese wheatgrass – Familiar and precious “Wild wheat” indigenous to Japan – minireview [II]). Kurashiki Natural History 14: 12-15 (in Japanese).

Osada M. 1993. Illustrated grasses of Japan, enlarged ed. Tokyo: Heibonsha Ltd. Publishers.

Sasanuma T, Endo TR, Ban T. 2002. Genetic diversity of three *Elymus* species indigenous to Japan and East Asia (*E. tsukushiensis*, *E. humidus* and *E. dahuricus*) detected by AFLP. Genes Genet. Syst. 77: 429-438.