Wet Injury of Wheat in Upland Field Converted from Paddy Field in Japan

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Abstract

Wheat and barley are very important crops used as staple food and feed. Growth delay or injury of wheat and barley under waterlogged soils is one of agricultural constraints to be solved. The common symptoms are germination failure, leaf color degradation, and wilting. Since there are different types of waterlogging conditions, the mechanisms of injury also varied. However, root sensitivity to low-oxygen atmosphere in soil is critical. Rice plant has been widely cultivated in Japan. In recent years the agricultural policy enhanced rice-wheat-soybean rotation using paddy fields for economic reasons. Crop production using paddy fields is thought to be an important key for sustainable agriculture in eastern Asia but wet injury of wheat usually occurs in the upland fields converted from paddy fields because of the poor drainage. Under a particular case, a phenomenon called ground subsidence due to the earthquake induced wet injury in this year. The most popular measure to mitigate the waterlogging damage on farm level is the field management to facilitate drainage. In addition, we have great hopes for establishing tolerant varieties; however, such commercial one is not yet obtained by traditional breeding. We are now engaged on an innovative research project about root aerenchyma which is thought to facilitate gas exchange between root and aerial parts of plants. We hypothesized its modification could change the sensitivity of root to low-oxygen. The project plan was; i) to investigate anatomical and physiological basis of aerenchyma formation in the recipient wheat cv. Bobwhite. ii) to establish transgenic wheat lines using several candidate genes for root aerenchyma formation. iii) to analyze function of the genes using the transgenic Bobwhite lines.

Keywords: aerenchyma, barley, subsidence, waterlogging, wheat

Introduction

The main production areas of wheat in Japan are in Hokkaido, Kanto and Kyushu district. Except in Hokkaido, they have relatively high precipitation. It is over one thousand mm a year. Sowing and harvest of wheat often coincide with the two rain periods, we call them "*Akisame*" (autumnal rain in September and November) and "*Baiu*" (rainy season in June). Moreover, wheat is widely grown in upland fields converted from rice paddy fields in Japan. The main reason to cultivate wheat instead of rice is due to decline of the amount of rice consumption. Full efficient utilization of rice paddy fields is one of the main policies of the Japanese government. Average of wheat acreage in recent 10 years is 210 thousand ha and the over half of the acreage is the rice fields. Oyanagi (2008) reported that about 20% of the wheat acreage is affected by waterlogging.

Wet injury usually occurs in the converted fields because of the poor drainage and it causes severe damage on the grain yields as well as their quality. One of the causes of the wet injury is shortage of oxygen in the low layer, where the wheat roots develope and obtain oxygen to grow. The most effective way to prevent wet injury is the field management for well drainage, for example, ditch making and raised bed farming, etc., however, the economic cost for such field management is relatively high in the farm level. Therefore, we develop new lines of wheat having high tolerance to wet soil.

To overcome the agricultural constraint, understanding of the mechanisms of wet injury and tolerance in wheat is essential. In our study, first, we characterized wheat growth under wet conditions in farmer's fields and in our experimental paddy fields. The results showed that i) there were high correlations between yield and soil moisture in the farm level and ii) wheat growth suffered from wet stress from the early development under the condition of paddy field. Second, we focused on root aerenchyma. Aerenchyma is thought to be enhancing gas exchange between subterranean part (root) and aerial part (shoot) of plant tissues. Therefore, root aerenchyma is one of the important traits to contribute wet tolerance of plants. To investigate the basic nature of root aerenchyma formation during early development of wheat, we examined when and where root aerenchyma were formed in the wheat seminal root under waterlogging condition using a plant pot system.Third, we recently studied the strategy to improve wheat using modern biotechnology.

Materials and Methods

Field experiment

Wheat (*Triticum aestivum* L.) cultivars. Bobwhite (Mexican cultivar), Norin 61 (Japanese cultivar), Iskra and NS-302 (Yugoslavian cultivars) were used in this experiment. The latter two were selected as candidates for high tolerant cultivars (Dr Sato, unpublished data). Norin 61 is the major cultivar in Japan except in Hokkaido region. Bobwhite is often used as a host for establishing fertile transgenic by means of particle bombardment in wheat. Experimental fields were located at Yawara, Tsukuba-mirai city, Ibaraki, Japan. Five are area each of contiguous two upland fields converted from paddy fields were used. One was used under non-tilling cultivation as control plot and the other was used after soil puddling as wet treatment plot. Wheat seeds were sown with 70 cm rows on 30th October, 2009 and 10th November, 2010. Fertilizers (6, 9 and 6 kg/10a each as N, P_2O_5 and K_2O) was applied at ten days before sowing. Growth characters were monitored in 3 weeks interval. The soil water contents were measured by TDR-method (Time Domain Feflectometry) using a HydroSense CD620 display and CS620 sensor with a 12-cm probe (Campbell Scientific Australia Pty. Ltd., Thuringowa Central, Australia) in the top 12 cm of the soil. The soil's redox potentials at 10cm depth were recorded using an Eh meter (PRN-41, Fujiwara Scientific Co.LTD., Tokyo, Japan) with a reference electrode.

Pot experiment for aerenchyma study

Wheat cv. Bobwhite line SH 98 26 was used. Three seeds were sown at a 3-cm depth in each of 27 soil-filled pots (Fujiwara Scientific, Tokyo, Japan; 1/5000a deep type Wagner pot, 30 cm height x 15.9 cm inner diameter). The wheat plants were grown in a greenhouse maintained at a temperature of approximately 20°C day/night, with natural light. Water treatments were imposed to 5 d old seedlings: (i) control, with a well-drained; (ii) T-15 treatment, the water level was maintained at 15 cm below the soil surface, and (iii) T+3 treatment, the water level was maintained at 3 cm above the soil surface. The O_2 concentrations were recorded at a 14 cm depth in the soil using an OXY-10 O_2-sensor (PreSens Precision Sensing GmbH, Regensburg, Germany). Transverse cross-sections were obtained from primary seminal roots using a D.S.K. Microslicer (DTK-1000, Dosaka EM Co. Ltd., Kyoto, Japan). The sections were examined and photographed with a light microscope equipped with a camera (BX51 microscope and DP72 camera, Olympus Co. Ltd., Tokyo, Japan) at 10 magnification.

Results and Discussion

Growth of 4 cultivars of wheat in the experimental fields

The soil conditions in the experimental fields were measured. Ground water levels were almost less than 75 cm below the soil surface during the entire experiment period. Soil water contents were higher in puddling plot than that in control plot until April 19. Redox potentials in the soil were constantly over +500 mV and showed no significant difference between the plots. The plant height and dry weight were significantly affected by puddling (wet) treatment (Figures 1 and 2 show the results of 2009 – 2010 season). Similar tendency was observed in the repeated testing in the next cropping season of wheat. Puddling/Control ratio in dry weight was very low (20%) at young (on 17 February) and was gradually recovered (80%) at ripening (on 13 May). These results indicated that wheat suffered from wet stress since their early stage of development and suggested that high tolerance to wet conditions at the seedling stage is important for wheat production in paddy fields in Japan.

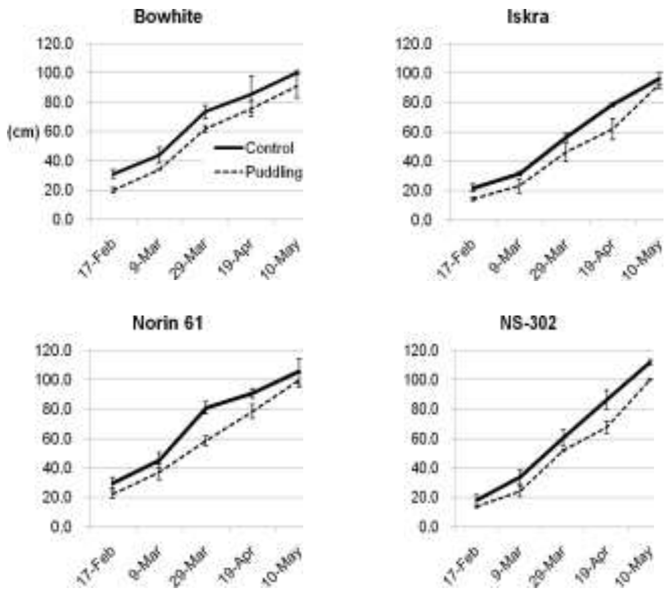


Figure 1. Changes in the plant height of 4 cultivars of wheat under control condition (solid line) and puddling (wet) condition (dashed line) in 2009 – 2010 season.

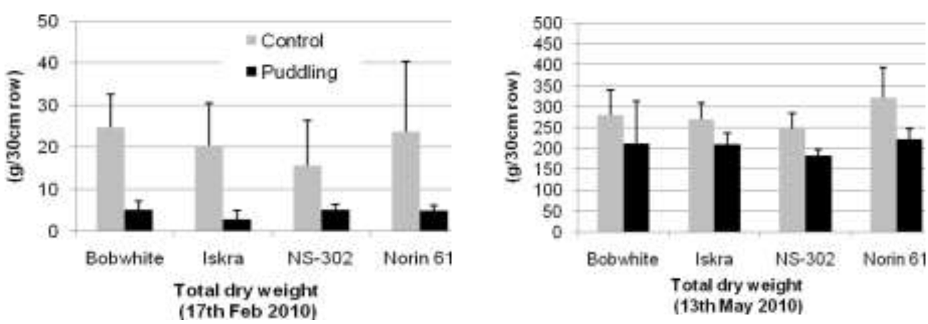
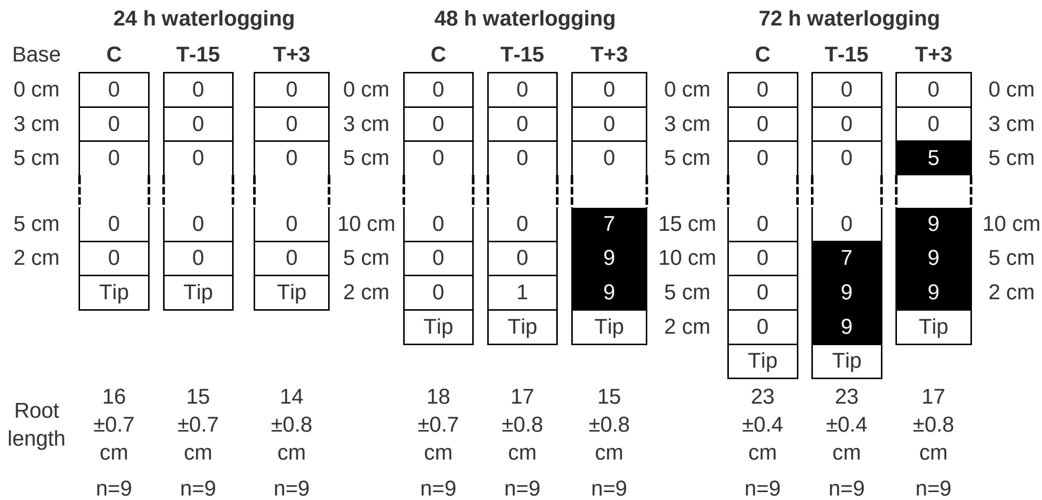


Figure 2. Dry weight of wheat plants (aerial part only) under control condition (gray bar) and puddling (wet) condition (black bar) in 2009 -2010 season.

Growth and root aerenchyma formation of wheat in pot system

The soil conditions in the plant pot were measured by TDR-method. Under the control, T-15, and T+3, the soil water contents were 12-14; 23-27; and 74-82%, respectively. The O_2 concentrations after 72 h in T-15 soil decreased from the control soil 18.7 to 10.1%, whereas that in T+3 soil decreased to 4.9%. While the redox potential in the control soil remained around +400 mV, the values in the T-15 and T+3 soils were about +360 and +290 mV, respectively, after 72 h treatment. These results showed that the soil conditions in T-15 and T+3 became hypoxic in order of water depth during waterlogging treatment.

The length of primary seminal roots and the position of aerenchyma were schematically drawn in Figure 3. The root length was reduced by 25% in the T+3 plants. The reduction of seminal root dry mass was 12.5% in T-15 and 31% in T+3 plants, respectively. For the aerial parts, the plants did not show significant difference (data not shown). Wheat primary seminal roots showed no aerenchyma in our control condition. Aerenchyma was appeared after 48 h under T+3 and after 72 h under T-15 waterlogging conditions, respectively, at 2 to 5 cm behind the root tip. The aerenchyma in T+3 plants extended to 2-10 cm behind the tip at 72 h waterlogging.



Numbers of root sections with developing aerenchyma are indicated at the sampling position. Roots (n=9) were tested. Black background indicates aerenchyma formation in more than half number of the nine sections.

Figure 3. Aerenchyma formation in the seminal root of wheat seedlings under waterlogging conditions.

Our results provide basic information on the aerenchyma formation process in seminal roots of wheat. To our knowledge, this is the first report to describe the time of the aerenchyma formation in wheat seminal roots under hypoxic conditions (Haque *et al.* 2010). The development nature of aerenchyma, such as the formation timing, might be similar in part among several upland crops. Root aerenchyma can be seen within 48 h after the initiation of hypoxia in the seminal (our study) and adventitious roots (Malik *et al.* 2003) in wheat. In maize, aerenchyma formation in the seminal (Gunawardena *et al.* 2001) and adventitious roots (Drew *et al.* 1981) is induced by hypoxia within a few days. Thus, wheat requires several days to form aerenchyma by waterlogging stress, while rice plant constitutively forms well developed aerenchyma in their roots. Such different ability to form aerenchyma among plant species may be one of the important keys to determine their adaptive nature to water.

Wheat plants grown in our pot system can be used for analyzing the molecular and physiological mechanisms of aerenchyma formation. Proteome analysis using the wheat seminal root showed that 10 candidates of proteins would be associated with the metabolism during root development under hypoxia (Haque *et al.* 2011). Elucidation of the protein function during aerenchyma formation is a next step of this study.

Future direction

Wheat cv. Bobwhite line SH 98 26 is known to be one of the most useful recipients for producing transgenic wheat lines (Pellegrineschi *et al.* 2002). Therefore, the information obtained from this study will be useful not only for understanding the basic nature of aerenchyma formation, but also for efforts to improve aerenchyma formation, including the creation of transgenic plants. In fact, the transgene approach in our experiment showed the ability satisfactorily on the investigation of seed dormancy of wheat and the related gene (Nakamura *et al.* 2011). Introducing genes can be widely selected from various plant species, including rice and teosinte, etc., which have high capacities to form aerenchyma (Nakazono *et al.* 2011 in this session, Mano and Omori 2009).

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