Short report

Comparison of root growth angles of wheat cultivars grown in a hydrogel polymer medium

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Received on March 19, 2015; Accepted on September 22, 2015

Abstract: A simple method for evaluating wheat (*Triticum aestivum* L.) root system characteristics at an early growth stage is needed. We used a hydrogel polymer medium to visualize the growth characteristics of seminal roots. The method is straightforward, and the medium is inexpensive and can easily be adjusted to better visualize the rooting characteristics. We found that: a) the optimum preparation conditions involve the addition of distilled water at about 98ºC to the hydrogel, which leads to low root penetration resistance and enough oxygen to avoid hypoxic damage; b) 97.8% of seeds germinated and the roots of all plants that germinated penetrated the hydrogel medium. c) the hydrogel medium is well suited for observing the first opposite seminal (adventitious) roots in wheat plants, making it possible to discriminate differences in growth angles among cultivars. This strategy can be used to identify deep-rooting cultivars. This method could be used for evaluation of genetic resources.

Keywords: first opposite seminal (adventitious) roots, hydrogel polymer culture medium, root growth angle, SkyGel, wheat

Abbreviations: AT, ambient temperature, CR, chiller room, CW, cold water, HW, hot water

Introduction

The roots of crop plants are essential for the absorption of nutrients and water from the soil. In Japan, wheat in both dry and wet areas suffers problems related to soil water stress. The root system of a crop plays an important role in both growth and yield. In Abashiri city, Hokkaido, Ito et al. (2009) and Hayashi et al. (2004, 2005) found that stable yield is generally due to high subsoil root density. Similarly, Wong and Asseng (2007) showed that crop yield in a Mediterranean environment was highly dependent on the accessibility of soil water, which was governed by rooting depth. We consider improvements in the root system to be essential for the stability of high-productivity crops. However, the development of cultivars capable of adapting to stress, such as high temperatures and water scarcity, has been slow. Oyanagi et al. (1993b) examined wheat cultivars that exhibited a strong relationship between root depth in an upland field and the growth angle of the seminal root. They found that the growth angle of the seminal root in agar medium was larger in northern cultivars than in southern cultivars (Oyanagi et al. 1993a), suggesting that cultivars with shallow root systems have been selectively bred in southern Japan. Despite the significance of these findings, very few attempts have been made to produce a deep-rooting cultivar, likely because a simple and easy method for doing so has not yet been developed. In 2014, Mebiol Inc. started selling a product called SkyGel, a film that can be used as a water retention medium for plants and can be used for plant establishment and garden stock cultivation. Since high concentrations of this polymer form a gel when exposed to water, it has been considered well suited for use as a culture medium. In this study, we used this hydrogel polymer medium instead of an agar medium to culture seeds of several wheat cultivars, and observed their rates of germination and characteristics of seminal root growth. In particular, we examined the growth angle of first opposite seminal roots of several wheat cultivars from Honshu and Hokkaido, as these cultivars are

Ito H, Kaneko M, Nakamura T, Nakazawa Y, Yoshida H 2016 Comparison of root growth angles of wheat cultivars grown in a hydrogel polymer medium. Plant Root 10:4-10. doi:10.3117/plantroot.10.4

considered to exhibit the greatest difference in seminal root growth angle. We found that culturing on the hydrogel polymer medium was a simple and effective method for observing the growth angle of first opposite seminal roots of wheat cultivars.

Materials and Methods

Preparing hydrogel polymer media and agar medium

First, 2.75 g of a water retention medium for plants (SkyGel, Mebiol Inc., Japan) was placed in a 500 mL container (7.0 cm x 7.0 cm x 8.2 cm; Daiso, Japan)(Fig.1). To this, 450 mL of distilled water at ambient temperature (AT) was added. The containers were then incubated for 24 h at AT (approx. 20°C) or at 6.2°C in a chiller room (CR) to allow the hydrogel polymer to absorb water and form a gel. Hydrogel polymer was also prepared using 450 mL of hot water (HW), which was distilled and had been heated to approximately 98°C, and the mixture was then left to cool to AT or placed in a CR. The hydrogel media produced under these four sets of conditions were referred to as either "0.6% (v/w) CW/AT hydrogel polymer culture medium" (cold water (CW), approx. 7°C, was added to the hydrogel and incubated at AT); "CW/CR hydrogel polymer culture medium" (CW was added to the hydrogel and incubated in the CR); "HW/AT hydrogel polymer culture medium" (HW was added to the hydrogel and incubated at AT) or "HW/CR hydrogel polymer culture medium" (HW was added to the hydrogel and incubated in a CR). After producing the hydrogel polymer culture media, they were incubated at one of two temperatures (18°C or 30°C) for 24 h. We examined them for the presence of bubbles to assess their transparency after 24 h of incubation. For comparison, 450 mL of a 0.2% (w/v) water agar culture medium at 80°C was poured into a 500 mL container and cooled at AT. After 24 h, wheat seeds that had been placed onto the agar medium at 18°C and left in darkness were observed.

Just after preparing each medium, a PreSens Microx TX3 oxygen meter was used to measure the oxygen concentration of 10 samples each of HW/AT hydrogel polymer culture medium, CW/AT hydrogel polymer culture medium, and agar medium. In addition, penetration tests of the hydrogel polymer culture media and agar media were performed using a Tensipresser TTP-50BX2-2006 texture analyzer (Taketomo Electric Inc., Tokyo).

Observation of seminal root growth in hydrogel polymer culture medium and agar medium

We used wheat cultivar 'Kitahonami'. Nine seeds

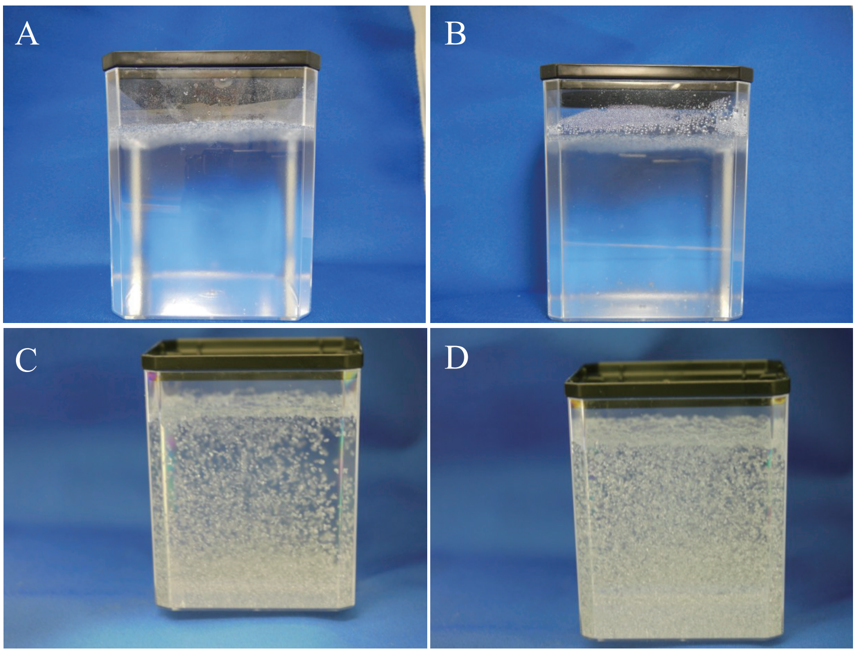

Fig. 1. Effect of different preparation conditions on the occurrence of bubbles in 0.6% (w/v) hydrogel culture media. A, B: HW/AT hydrogel culture medium; HW (approx. 98ºC) added to gel, which was allowed to stand at AT (20ºC). C, D: CW/CR hydrogel culture medium; CW (approx. 7ºC) added to gel, which was allowed to stand at CR (6.2ºC). A, C: 18ºC incubation. B, D: 30ºC incubation.

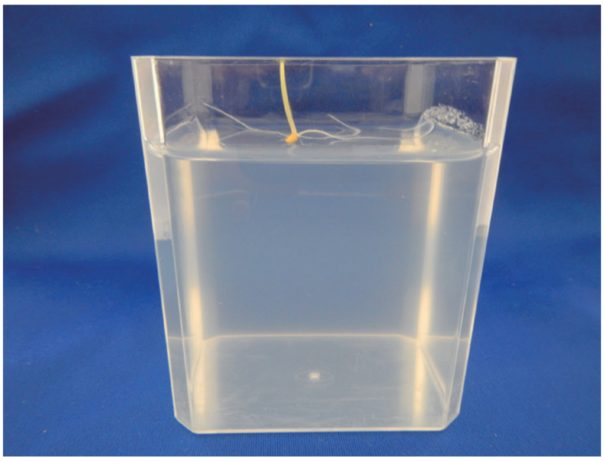

Fig. 2. Germination and creeping growth of wheat cultivar 'Yumechikara' cultured on 0.2% (w/v) agar medium on 7 days after seeding.

each were placed evenly on high-transparency HW/AT hydrogel polymer culture medium and agar medium. The number of germinated seeds was recorded on day 5 of incubation at 18°C in darkness, with 10 replicates for each medium. For comparison, wheat cultivar 'Kitahonami' seeds were placed on filter paper in a petri dish (90 × 15 mm) containing 4 mL of distilled water. The petri dishes were incubated in darkness at 18°C, with nine seeds per treatment and ten replications. Seeds that germinated were counted on day 5.

Roots were observed on day 5. In this study, the growth of roots was categorized as either penetrating the medium or showing a creeping pattern on the surface of the medium, as shown in Fig. 2.

Measurements of root growth angle and root length in wheat cultivars

Containers containing high-transparency HW/AT hydrogel culture medium were prepared and seeded with wheat seeds in the middle of each container, with all of the seeds set such that they were oriented in the same direction. Seeds were placed ventral side up. Only seeds from the eastern Hokkaido winter wheat cultivars 'Kitahonami', 'Yumechikara', 'Hokushin' and 'Chihokukomugi', and the Honshu cultivars 'Minaminokomugi' and 'Shiroganekomugi' were used for these experiments. Seeds were incubated in darkness at 18°C and the growth angle of the seminal roots was measured daily from the fourth to seventh day after seeding. The seminal roots of wheat are composed of a primary seminal root, a pair of first opposite seminal roots on opposite sides of the primary seminal root, a second pair of opposite seminal roots and, rarely, a seminal root originating from the ventral side. In this study,

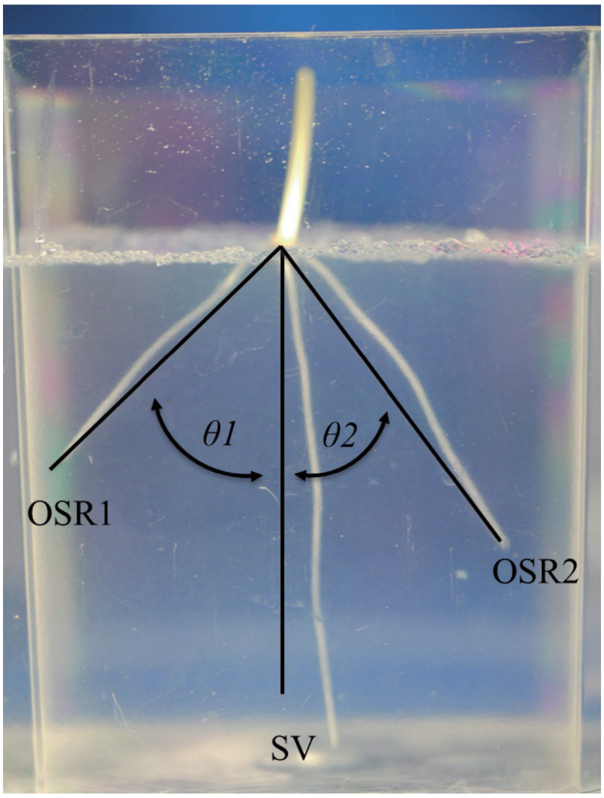

Fig. 3. Measurements of growth angles for the first opposite seminal (adventitious) roots (OSR) of wheat. Mean value (θ) of the two angles (θ1 and θ2) was taken as a representative angle value of one seedling

measurements of lengths and angles of first opposite seminal roots were performed using digital images, which were taken from the embryo side so that these are parallel to a plane including first opposite seminal roots (Fig. 3). The growth angle of the first opposite seminal roots was defined as the angles formed by the intersection of lines SV, a line drawn vertically from the seed, and OSR1 or OSR2, a line drawn from the seed to the root tip. The lengths of the first opposite seminal roots were represented as the length of the lines OSR1 and OSR2. The growth angle and length of the first opposite seminal roots was measured in 10 specimens of each cultivar until the seventh day after seeding. Mean value of the two angles of opposite seminal roots was taken as a representative angle value of one seedling. When the roots of a plant reached the wall surface of the container, we ended observations.

Results

Comparison of properties of hydrogels prepared at different temperatures

The transparency of hydrogel polymer media

Table 1. Comparison of germination rate, root characteristics and medium properties of 0.2% (w/v) agar medium and 0.6% (w/v) hydrogel polymer medium (HW/AT)

		Petri dish	Agar medium	Hydrogel polymer medium
Dissolved oxygen	μmol L^{-1}	—	195.4	117.3
Breaking strength	kPa		10.3	2.0
Germination rate	%	100	86.0	97.8
Penetration growth	%	—	0	100
Creeping growth	%	—	86	0

produced under three different conditions (i.e., CW/AT, CW/CR and HW/AT) was compared. There were no bubbles in the CW/CR or HW/AT media, but there were in the CW/AT medium. However, when the CW/CR medium was incubated in darkness at 30°C, it developed bubbles after 24 h (Fig. 1D). As the bubbles got progressively larger, they interfered with root observations. On the other hand, the HW/AT medium maintained high transparency and remained stable and bubble-free, even when it was used in an incubator set at 30°C (Fig. 1B).

The water potential of the hydrogel media was -2.5 kPa. The dissolved oxygen of the HW/AT hydrogel culture medium was approx. 117 μmol L^{-1}, while that of the CW/AT hydrogel culture medium was approx. 248 μmol L^{-1}. Both media were swollen and soft, with a breaking strength of approx. 6.2 kPa (Table 1).

Root growth of wheat in hydrogel polymer culture media and agar media

The germination rate, root characteristics and medium properties are compared for agar medium and HW/AT hydrogel polymer medium in Table 1. The germination rate on the agar medium and in HW/AT hydrogel medium were respectively 86.0% and 97.8% of that on petri dishes containing moistened filter paper. Figure 2 shows the appearance of roots undergoing creeping growth. In agar medium, 86% of individual roots showed creeping growth, but all roots in HW/AT hydrogel medium showed penetration growth. The amount of dissolved oxygen was higher in agar medium than HW/AT medium. The agar medium tended to be harder than HW/AT hydrogel medium.

Based on the observations mentioned above, while penetration root growth was not observed in hard agar medium, neither showed creeping growth on soft HW/AT hydrogel medium. Furthermore, the germination rate in HW/AT hydrogel culture medium tended to be higher than in agar medium.

Cultivar differences in root gravitropic response

Trends in growth angle of seminal roots from the fourth to seventh day after seeding of six cultivars of winter wheat are shown in Fig. 4. Only the primary seminal root and the first seminal roots on opposite sides of it were observed in all cultivars. The primary seminal root of all specimens grew vertically downward and no marked difference was observed among them. Conversely, although there was no marked difference between cultivars in the growth angle of the first opposite seminal root on the fourth day after seeding, over time, there was an increasing difference, with the greatest difference observed on the seventh day (Table 2). The first opposite seminal roots of 'Yumechikara' (Fig. 5A, Table 2) grew at the most acute angle (approx. 40°), followed by 'Chihokukomugi', 'Kitahonami', 'Hokushin', 'Shiroganekomugi', and 'Minaminokomugi' (Fig. 5B, Table 2) with increasingly obtuse angles, indicating that the first opposite seminal roots of the four Hokkaido cultivars grew at more acute angles than the two Honshu cultivars.

The lengths of the first opposite seminal roots from day 4 to day 7 of the six cultivars are shown in Fig. 6. 'Yumechikara' roots elongated most, to

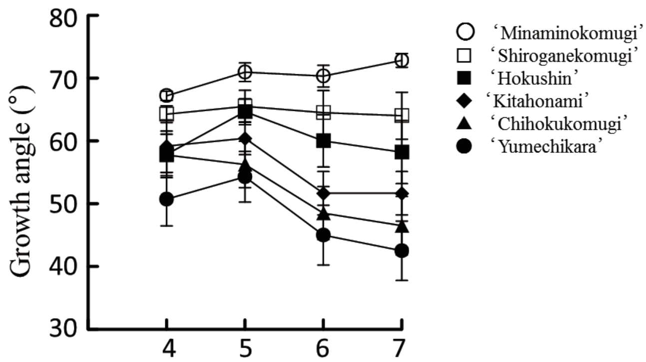

Fig. 4. Growth angle of first opposite seminal roots in different wheat cultivars cultured on 0.6% (w/v) hydrogel medium when seeded on the surface of the medium. Values are means with standard errors.

Table 2. Differences in growth angle of the first opposite seminal roots in different wheat cultivars cultured on 0.6% (w/v) hydrogel medium

Cultivar	Days after seeding			
	4	5	6	7
Minaminokomugi	67.2a*	70.3a	70.3a	72.8a
Shiroganekomugi	64.3a	65.5ab	64.5a	64.0ab
Hokushin	57.9ab	63.4ab	60.0abc	58.2abc
Kitahonami	59.2ab	60.4ab	51.7bc	52.5bc
Chihokukomugi	57.8ab	56.3b	48.5c	46.5cd
Yumechikara	50.7b	55.9b	47.8c	40.4cd

*Mean angles followed by the same letter in different cultivars were not significantly different within the same day ($P < 0.05$, Tukey's multiple range test).

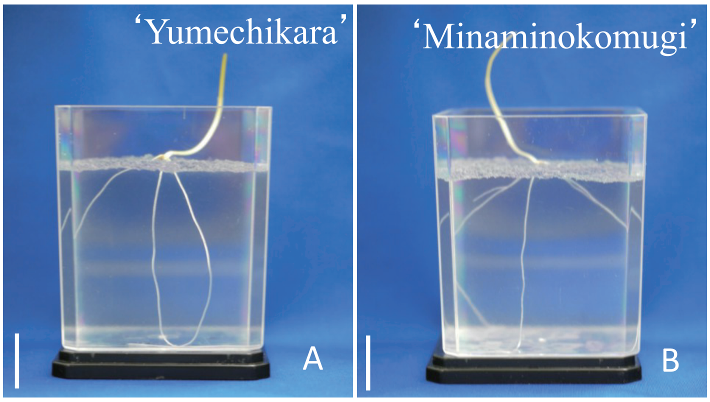

Fig. 5. Lateral views of seminal roots of the wheat cultivars 'Yumechikara' and 'Minaminokomugi' on 7 days after seeding on the surface of the medium. Bars = 1 cm.

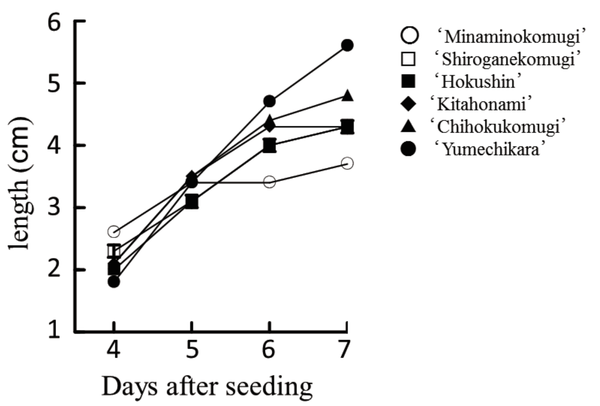

Fig. 6. Growth in length of first opposite seminal roots of different wheat cultivars cultured on 0.6% (w/v) hydrogel medium when seeded on the surface of the medium. Values are means with standard errors.

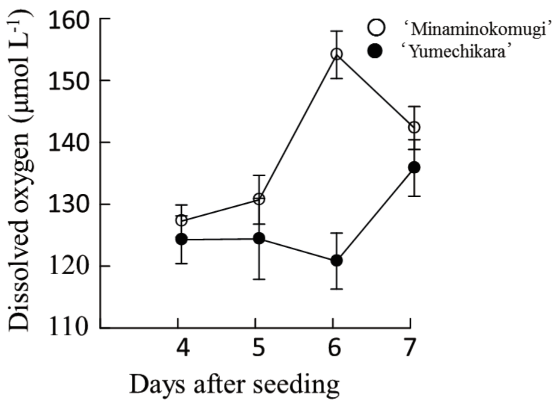

Fig. 7. Dissolved oxygen of 0.6% (w/v) hydrogel medium seeded with different wheat cultivars on the surface of the medium. Values are means with standard errors.

about 5.5 cm. In contrast, 'Minaminokomugi' seminal roots only elongated to 3.5 cm and their growth had decelerated by day 5.

Concurrently, the amount of dissolved oxygen in the medium from day 4 to day 7 ranged from 121.1 μmol L^{-1} to 154.4 μmol L^{-1} (Fig. 7).

Discussion

Shallow root systems are associated with low yield in wheat (Ito et al. 2009, 2014). The root depth index in the field is closely related to the growth angle of seminal roots obtained in agar medium (Oyanagi 1993). To observe the growth angle of wheat seminal roots, Oyanagi et al. (1993a), who used agar medium, stimulated the germination of seeds in dishes and then transplanted them onto the medium before observing them. The first stage in this study was to create a method of observing the growth angle of wheat seminal roots. In attempts to observe the growth angle of seminal roots using agar medium, although 86% of individuals germinated, all of the roots showed creeping growth over the medium (Fig. 2, Table 1).

Transparent media such as agar, gelatin or gellan gum can be used for observation of root growth. Although a gel medium can provide a three-dimensional growth environment for roots, it has mechanical and structural properties resembling poor soil (Clark et al. 1999). In this study, the wheat roots examined did not directly enter agar medium (Fig. 2, Table 1), so we had to find another medium to observe roots that penetrate the medium in which they are growing. Using such methods to study roots has the advantages of being inexpensive and having a minor impact on the environment or other living organisms.

SkyGel also is sold at a lower price than other likely alternatives (42 USD kg^{-1}). Initially, roots were not visible in the hydrogel culture medium, which was made with distilled water and stocked, because bubbles formed. However, we found that greater transparency could be achieved with HW/AT hydrogel medium than with CW/AT or CW/CR hydrogel media (Fig. 1). In addition, higher transparency could be maintained in the HW/AT hydrogel medium than the CW/CR hydrogel medium. Dissolved oxygen was low in the HW/AT hydrogel medium, but high in the CW/AT and CW/CR hydrogel media. A negligible oxygen concentration causes anoxia, which inhibits various aspects of plant growth, including respiration and ion uptake (Colmer and Greenway 2011). In the present study, roots grew through day 7 after seeding, showing no signs of ceasing to grow. The roots of ‘Yumechikara’, which elongated at the most acute angle up to day 7, grew steadily (Fig. 6). In contrast, the roots of ‘Minaminokomugi’, which elongated at the most obtuse angle, had reached the container wall by day 5, when they were about 3.5 cm long. Considering the size of the container used in this study, the roots that grow in the horizontal direction should be half the length of the roots that grow in the vertical direction. A dissolved oxygen concentration of 120 μmol L^{-1} to 154.38 μmol L^{-1} at the lowest level observed (Fig. 7), is unlikely to cause injury due to low oxygen.

Our hydrogel medium had a low resistance to penetration (Table 1). This softness is comparable to that of topsoil in a crop field. As Figs. 1 and 6 indicate, the roots did not grow along the gel surface on the hydrogel medium. Based on these findings, the HW/AT hydrogel medium was used to examine germination rates, as this medium had the fewest bubbles and was most stable under storage at different temperatures. Of the total seeds, 97.8% germinated and the first opposite seminal roots of all germinated seeds penetrated the HW/AT hydrogel medium (Table 1). In addition, the roots that grew exhibited positive gravitropism. The HW/AT hydrogel medium had high water content, and was swollen and softer than the agar medium (Table 1). Although the dissolved oxygen concentration of the hydrogel medium was lower than the agar medium, it contained sufficient oxygen for germination and growth in the very earliest stages, without being saturated. These results indicate that the hydrogel medium used in this study is appropriate for wheat and better facilitates observation of the first opposite seminal root growth than agar medium.

To observe the growth angle of wheat seminal roots, Oyanagi et al. (1993a), who used agar medium, stimulated the germination of seeds in dishes and then transplanted them onto a medium before observing them. Oyanagi et al. (1993a) reported primary seminal root growth angles that ranged from 4° to 64° (with a method analogous to the method of the present study, 26° to 96°) among 134 cultivars of spring and winter wheat. Because the primary seminal roots showed strong positive gravitropism, the growth angle measured to the tip of the primary seminal root showed no difference between cultivars. Therefore, we investigated the growth angle of the first opposite seminal roots. The greatest difference in growth angles among cultivars was observed seven days after seeding between ‘Minaminokomugi’ and ‘Yumechikara’, which respectively had a first pair of opposite seminal roots that grew at approximately 73° and 40° (Fig. 4 and Table 2). Thus, our results resemble those of Oyanagi et al. (1993a), although the difference between ‘Minaminokomugi’ and ‘Yumechikara’ was approx. 30°, less than the difference of approx. 80° that they reported. This difference may be due to the difference in number of cultivars used in the study.

Our findings showed that hydrogel polymer medium is effective for observing the growth angle of the first opposite seminal roots of wheat. In eastern Hokkaido, shallow root systems are associated with low wheat yields (Ito et al. 2009, 2014). Observation of the first opposite seminal roots in wheat plants indicates that it is possible to discriminate differences in growth angles among cultivars. The growth angle of the primary seminal roots of the seedlings is closely related to the vertical distribution of the roots at the stem-elongation stage (Oyanagi et al. 1993b). These types of studies should provide important information useful in developing strategies for selecting an optimal rooting depth for different wheat cultivars. However, because environmental differences were also observed, future research must consider the precision of forecasting, which would require comparing actual root system distributions in the crop field.

Acknowledgements

We thank the Hokkaido Research Organization, Agriculture Research Department, Central Agricultural Experiment Station for providing the seeds used in the present study.

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