Effects of a Reduction in Soil Moisture from One Month before Flowering through Ripening on Dry Matter Production and Ecophysiological Characteristics of Wheat Plants

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Abstract: We found in the previous study that the wheat plants grown under relatively low soil moisture conditions (D plot) could attain heavier dry matter than the plants watered on the basis of average local precipitation (W plot). The aim of this study was to make a detail analysis of the ecophysiological characteristics that cause the difference in dry matter production between the plants in the W and D plots under different soil moisture conditions. Soil matric potential at a depth of 30 cm was kept at about −4 kPa in the W plot. It decreased gradually after watering at about one month before heading and at heading, reaching about −80 kPa at heading stage and at the mid-ripening stage respectively, in the D plot. The plants in the D plot produced heavier dry matter and a better developed root system than the plants in the W plot. The higher net assimilation rate and larger leaf area, which accounted for the higher crop growth rate of the D plot, were due both to avoiding suppression of the photosynthetic rate and leaf expansion owing to water stress, and to maintaining high rates of leaf photosynthesis and a large leaf area during leaf senescence. A larger amount of nitrogen was accumulated at the flowering stage and the nitrogen content of leaves remained higher during senescence in plants in the D plot than those in the W plot. The activity of cytokinins in the xylem sap was higher in plants in the D plot. These characteristics might have contributed to the delay in the decline in the rate of photosynthesis and in leaf area during leaf senescence and seemed to be supported by the enhanced development of the root system under moisture-restricted conditions.

Key words: Cytokinin, Dry matter production, Leaf nitrogen content, Photosynthesis, Root system, Senescence, Soil moisture, Wheat.

We found in a previous study (Nakamura et al., 2003) that wheat plants produced heavier dry matter, without a reduction in the leaf area index, under the conditions where rain was excluded and no irrigation was provided for about one month before flowering, as compared with plants supplied with water in an amount that corresponds to an average year's precipitation or with plants grown under natural weather conditions. This result is quite different from the results obtained with summer crops, which suffer from water stress and markedly decreased leaf expansion and dry matter production when water is withheld for only a few weeks (Fujita et al., 2002, Hirasawa et al., 1994, 1998).

Extremely copious precipitation and/or bad drainage are major causes of damage to wheat plants in Japan. Therefore, almost all researches on the effects of wet soil on wheat plants focused on the damage in the soil with excess moisture. Yamazaki (1952) reported that the main damaging factor is not excessive soil moisture itself but, rather, a shortage of soil air that arises from the presence of too much water. However, if soil moisture is insufficient to cause the damage due to a shortage of soil air, there must be other reasons for the reduction in dry matter production and yield under wet conditions, such as the effects on plant development, morphology and physiological characteristics, which differ from the mechanisms of wet injury considered to date. Nakamura et al. (2003) considered that, in the ripening stage when the plants were grown with adequate moisture, the factor responsible for higher crop growth rate (CGR) in plants from which water had been withheld for about one month before flowering was the slower leaf senescence inducing the maintenance of high leaf photosynthetic rate and large leaf area index (LAI). However, factors that cause the higher CGR in the plants during the month before flowering, when the soil moisture and leaf xylem water potential were decreased to a greater extent under the conditions of decreased soil moisture, were not clarified sufficiently.
In this study, we reexamined the effects of reduction in soil moisture on dry matter production in wheat plants by identifying soil conditions and made a more detailed analysis of the ecophysiological characteristics that cause the differences in the dry matter productions of the plants growing under different soil moisture conditions. The soil moisture treatment was performed not only during one month before flowering but also during ripening in order to make the differences between the plants clearly. We also used plants grown in pots to confirm the plant response observed in the field.

Materials and Methods

1. Materials and treatments

Wheat plants (Triticum aestivum L. cv. Bandowase) were used for all experiments.

(1) Field experiment

Seeds were planted on November 15, 1996, at the University Farm on alluvial soil from the Tama River. The soil in the field from 0 to 34 cm below the surface was classified as loam to clay loam, that from 34 to 44 cm light clay, that from 44 to 71 cm sandy loam and that from 71 to 120 cm light clay. The level of the underground water at this site is expected to be far more than 1.5 m below the soil surface. The planting rate was 60 kg ha\(^{-1}\) with an inter-row space of 32.5 cm. Manure was applied at a rate of about 10 t ha\(^{-1}\). Chemical fertilizer was applied as basal dressing at a rate of 80, 80 and 80 kg ha\(^{-1}\) for N, P\(_2\)O\(_5\), and K\(_2\)O, respectively. Plants were grown under natural conditions before the start of the experiment. Heading started on April 13. Plants were harvested on June 22.

The experimental field, 114 m\(^2\) in area, was divided into four parts, and plastic boards that expanded 1 m below the soil were installed in order to isolate the parts hydraulically. Moisture treatments were arranged randomly with respect to the parts and each part was subdivided, to yield four replicates for each treatment. On March 15, about one month before heading, plants were watered with the equivalent of 35 mm of rain. Thereafter, they were grown under different soil moisture conditions (W and D plots). The plants in the W plot were watered at three-day intervals with the equivalent of 10, 12, 14 and 15 mm of rain in March, April, May and June, respectively (Fig. 1A). The amount of water supplied was calculated on the basis of the average precipitation for the past 30 years in Tokyo. Irrigation was withheld thereafter from the D plot, with the exception of watering equivalent to 70 and 90 mm of rain at the heading stage (April 17 and 18) and the mid-ripening stage (May 15 and 16), respectively (Fig. 1A), in order to avoid extreme reduction in soil moisture. The total amount of water supplied to the W and D plots was equal to 450 and 195 mm of rain, respectively. Natural rain was excluded with a roof that covered the experimental field when it rained.

(2) Pot experiment

Seeds were planted on November 18, 1998, in 1/2000 a Wagner pots (20 L in volume) filled with a mixture of Tama River alluvial soil and the Kanto diluvial soil (1 : 1, v/v) at a rate of three hills per pot and five plants per hill. Chemical fertilizer was applied as a basal dressing at a rate of 1, 1 and 1 g for N, P\(_2\)O\(_5\), and K\(_2\)O per pot, respectively. Ammonium sulphate was applied as top dressing on March 6, 1999, at a rate of 1 g for N per pot five days before the start of the soil moisture treatment. Plants were grown outside before treatments. Heading started on April 12.

For the soil moisture treatment, each pot was weighed daily or every other day from March 11 onwards and water was added as needed to keep the average soil moisture content of pots close to field capacity (W pots) or at approximately 80% of field capacity (D pots). The amount of water added per day to the D pots was 70% to 95% of that added to the W pots. No uniform distribution of moisture throughout the soil could be attained in the D pots. The soil moisture content differed by about 10% between the soil at the surface and that at the bottom in a pot. During the treatment, plants were grown in a greenhouse where they were arranged randomly.

2. Physical properties of the soil

The matric potential of the soil was measured with a tensiometer at high water potentials in the field. When the soil matric potential decreased to below -50 kPa, the potentials were estimated from the graphical correlation between electric resistance, measured with gypsum blocks, and matric potentials measured by a centrifugation method. The water potential of soil in pots was estimated from the graphical correlation between moisture content and water potential, as measured with an isopiestic psychrometer (Boyer and Knipling, 1965).

The distribution of gas, liquid and solids in the soil (three-phase distribution) was determined by estimating the actual volume of the solid and liquid phases of the soil (DIK-1120; Daiki Rika Kogyo Co., Tokyo, Japan). The concentrations of O\(_2\) and CO\(_2\) in soil air were measured with a gas chromatograph, which was equipped with a thermal conductivity detector (GC-8A; Shimadzu. Inc., Kyoto, Japan). A WG-100 column (GL Science Inc., Tokyo, Japan) was used to separate the gases. The soil air in pots was collected with a gas sampler (Rolston, 1986), that had been installed in the soil for 10 days. Active ferrous ion in soil was extracted with acetate buffer solution, and determined by the colorimetric method with 2, 2'-bipyridyl (Takai et al., 1958).

3. Growth and ecophysiological characteristics of wheat plants

In the field experiment, plants with the average
number of stems were sampled from a 50-cm length of a row for each replicate for measurements of LAI and dry weight of above-ground parts. Sampled plants were dried at 90°C in a ventilated oven after separation into leaf blade, leaf sheath plus stem, and spike. The leaf blade area of some plants from each replicate was measured with an area meter (AAM-8; Hayashi Denko Co., Tokyo, Japan). The specific leaf area was calculated from the leaf blade area and the weight. The entire leaf area for each replicate was calculated by multiplying the dry weight of all leaf blades by the specific leaf area. The sampling yield per unit area was determined for plants from a region of about 0.7m² for each replicate.

Root lengths at various depths were measured with a minirhizotron root-observation tube system as described previously (Hirasawa et al., 1995). Three observation tubes had been placed on the row in each plot at an installation angle of 60° from the soil surface since 1993. Cultivation around the tube was also done at the land preparation since then in order to make the soil conditions the same as in other places in the field. The lengths of roots at the surface of each observation tube were determined by the modified line-intersect method (Tennant, 1975).

The leaf xylem water potential and diffusive conductance were measured at midday on clear days. The leaf xylem water potential was measured with a pressure chamber (model 3005; Soil Moisture Equipment Inc., Santa Barbara, CA, USA) for a fully expanded and uppermost leaf of a main stem under direct sunlight, as described previously (Hirasawa and Ishihara, 1991). The diffusive conductance of the eighth leaf was measured in March and that of the ninth leaf was measured from April to June with a steady-state porometer (LI-1600; LI-COR Inc., Lincoln, NE, USA).

The leaf photosynthetic rate was measured in the field with a portable closed gas exchange-system (LI-6200; LI-COR Inc.). Measurements were made under artificial light (LA-100; Hayashi-Tokei Inc., Tokyo, Japan) with a light intensity higher than 2,000 μmol m⁻² s⁻¹ at the leaf surface. Measurements were repeated three times and the mean of the readings was taken as the measured value. The concentration of CO₂ in the air in the chamber ranged from approximately 360 to 310 μL L⁻¹ during the measurements. In the pot experiment, we used a portable open gas-exchange system (LI-6400; LI-COR Inc.). Measurements were made under a red/blue LED light (6400-02B; LI-COR Inc.) at 2,000 μmol m⁻² s⁻¹. The activity of the cytokinins in xylem exudates was determined essentially as described by Soejima et al. (1992). Forty stems were bundled and cut at a position that was about 20cm above soil level. The xylem exudates were collected with absorbent cotton, which was covered with a polyethylene bag during the collection period to prevent evaporation, from the tops of the decapitated stems for about 14 h during the night. The xylem exudates absorbed
by the cotton were extracted in 80% ethanol that contained 0.1% acetic acid and then each extract was evaporated to yield an aqueous residue. The residue was dissolved in water and loaded onto a Sep-pak C18 cartridge (Waters, Millipore Co., Milford, MA, USA). The cartridge was eluted with 40% methanol that contained 0.1% acetic acid. The eluate was evaporated to dryness and dissolved in 1.0 mL of water for the bioassay. The activity of cytokinins in the xylem sap was determined by the *Amaranthus* betacyanin bioassay (Biddington and Thomas, 1973), and expressed as "6-benzylaminopurine (BA) equivalents".

### Results

1. **Field experiment**

   (1) **Soil conditions and dry matter production**

   The soil matric potential at a depth of 30 cm in the W plot remained around −4 kPa during the treatment period (Fig. 1B). That in the D plot decreased to about −80 kPa gradually and watering at heading stage increased the soil matric potential to that of the W plot. In the ripening stage, it decreased again and recovered by watering. Even at a depth of 50 cm, remarkable reductions in matric potential in the D plot were observed during both the month before heading and ripening stage (Fig. 1C). Table 1 shows the three-phase distributions of the soil in the two plots. The percentage distribution of gaseous matter was larger and that of liquid was smaller in the soil in the D plot as compared to those in the W plot on April 15, immediately before watering. The differences were significant in the soil at a depth of 30 cm. The differences in the gas and liquid distributions were not significant on June 17, when the reduction in soil...
moisture was relatively small in the D plot. Ferrous ion was barely detectable in the soil at 30 cm in depth in both plots at the heading and harvest stages.

The leaf xylem water potential in the plants in the D plot changed lower than that of the plants in the W plot for about three weeks before heading and during the ripening stage (Fig. 2). The dry weight of the above-ground parts underwent a significantly larger change in the D plot than in the W plot (Fig. 3). At the heading stage, the plants in the D plot produced approximately 14% heavier dry matter than those in the W plot. At the late-ripening stage, the difference between the two plots was even larger, rising to approximately 18%. Figure 4 shows the vertical distribution of roots in the soil in the two plots. The length of roots in the D plot was greater than that in the W plot in the soil at a depth of 40 to 80 cm but there were no differences in the shallower layers as Nakamura et al. (2003) observed in the previous study. Any differences in root color could not be observed between the plants. Grain yield was 17% higher in the D plot than in the W plot (Table 2), perhaps because of the larger number of kernels and the greater kernel weight, although there were no significant differences in the mean number and weight of grains. The harvest index from each plot was similar and, thus, the difference in grain yield could be attributed to a difference in dry matter production.

(2) Ecophysiological characteristics during a month before flowering

During 38 days from March 15 to April 22, CGR in the D plot was significantly higher than that in the W plot (Fig. 5). It resulted from a higher net assimilation rate (NAR) and a higher mean LAI, although there were no significant differences in the rates and

| Plot | No. of spikes (m⁻²) | No. of kernels per spike (× 10⁴ m⁻²) | No. of kernels (g) | 1000 kernels weight (g) | Unit area sampling yield (g m⁻²) | Harvest index (%) |
|------|---------------------|-------------------------------------|--------------------|-------------------------|---------------------------------|------------------|
| W    | 684.6 ± 36.9 a**    | 26.2 ± 2.9 a                        | 17.8 ± 1.2 a       | 34.5 ± 1.8 a            | 608.6 ± 39.8 a                  | 41.6 ± 0.9 a     |
| D    | 687.2 ± 43.5 a      | 27.8 ± 1.8 a                        | 19.1 ± 0.9 a       | 38.5 ± 4.1 a            | 711.3 ± 67.2 b                  | 40.6 ± 3.9 a     |

* Weight at 12.5% moisture. ** Data are means±S.D. (n=4). Means followed by different letters are significantly different at the 5% level by Student’s t-test.

Fig. 5. Crop growth rate (CGR), net assimilation rate (NAR) and mean leaf area index (LAI) in plants in the W ( □ ) and D ( □ ) plots (field experiment). Vertical bars represent S.D. (n=4). *, ** Means are significantly different at the 5% and 1% level, respectively, by Student’s t-test.

Fig. 6. Changes in diffusive conductance during the daytime in plants in the W and D plots (field experiment). Measurements were made from 1200 to 1430 on a fine day. Vertical bars represent S.D. of six measurements. The measurements were made in the same parts of replicates. Numbers in parentheses represent the mean photosynthetic photon flux density (×10³ µmol m⁻² s⁻¹) during the measurements. The arrow indicates the date of heading.
Even though a number of repeated measurements were taken for diffusive conductance, the rate of photosynthesis and leaf nitrogen content in the field, replicates were not taken into consideration in these measurements because the difference was small between replicates in terms of growth and development.

Diffusive conductance in the early afternoon in the plants in the D plot was not lower than that in the W plot (Fig. 6). This means that there was no difference in the afternoon stomatal closure and, therefore, in the afternoon depression in photosynthesis even though the leaf xylem water potential in the plants in the D plot was lower than that in the W plot (Fig. 2).

The decline in the rates of photosynthesis in the seventh leaves as a result of leaf senescence was smaller in the plants in the D plot than in those in the W plot on April 11 (Fig. 7). There were no consistent differences in nitrogen content of upper younger leaves for the seventh and eighth leaves on March 19 and for the ninth and tenth leaves on April 11 (Fig. 8).

### Table 3. Amount of nitrogen accumulated in the above ground part and distributions of the nitrogen among the plant parts of field-grown plants at the flowering stage* (field experiment).

| Plot | Total amount of nitrogen (g m⁻²) | Distribution (%) |
|------|---------------------------------|------------------|
|      | Leaves       | Stem and          | Spike            |
| W    | 20.4 ± 0.3 a | 46.2 ± 0.2 a      | 13.8 ± 0.5 a     |
| D    | 22.4 ± 1.3 b | 46.1 ± 1.3 a      | 13.5 ± 0.7 a     |

*Data represent means±S.D. (n=4). Measurements were made on April 22. Means followed by different letters are significantly different at the 5% level by Student’s t-test.

### Table 4. Activities of cytokinins in xylem exudates of field-grown plants at the flowering and ripening stages* (field experiment).

| Stage    | Plot | Total volume of xylem exudate (mL) | Cytokinin concentration (nM BA eq.) | Cytokinin activity (pmol BA eq.) |
|----------|------|-----------------------------------|-----------------------------------|----------------------------------|
|          |      | (A)                               | (B)                               | (A×B)                            |
| Flowering| W    | 13.4 ± 2.6 a                      | 50.0 ± 8.5 a                      | 674.3 ± 181.6 a                  |
|          | D    | 13.2 ± 3.0 a                      | 80.2 ± 21.6 b                     | 1024.4 ± 114.3 b                 |
| Ripening | W    | 7.3 ± 1.1 a                       | 142.1 ± 65.8 a                    | 1064.5 ± 577.1 a                 |
|          | D    | 7.3 ± 1.3 a                       | 172.2 ± 110.4 a                   | 1226.2 ± 745.8 a                 |

*Xylem exudates were collected from 1900 on Apr.19 to 0900 on the next day and from 1900 on May 3 to 0900 on the next day at flowering and ripening, respectively. **Data represent means±S.D. (n=3-4). Means followed by different letters are significantly different at the 5% level by Student’s t-test.
meter for the seventh leaf was higher in the plants in the D plot than in those in the W plot on April 11.

Table 3 shows the amount of nitrogen accumulated in the whole above-ground parts of plants and nitrogen partitionings to organs at the flowering stage. It was approximately 10% higher in the D plot than in the W plot, but there was no significant difference in nitrogen partitioning to leaves between plots.

Even though the total volume of xylem exudate was similar in the two plots, the concentration of cytokinins in the xylem exudates was approximately 60% higher in the plants in the D plot than in those in the W plot at the flowering stage (Table 4). As a result, the activity of cytokinins transported from roots to shoots was also significantly higher in the D plot than in the W plot (Table 4).

(3) Ecophysiological characteristics during ripening

Also during the ripening stage, from April 22 to June 3, CGR in the D plot tended to be higher than that in the W plot (Fig. 5). It resulted from a significantly higher mean LAI, that is, the smaller reduction in LAI during ripening in the plants in the D plot. The NAR in the D plot was similar to that in the W plot although there must have been more mutual shading in the D plot because of the larger LAI. The maintenance of large NAR might also have contributed to the higher CGR in the D plot.

In general, NAR is governed by the structure of the canopy and the photosynthetic rate of the individual leaves that form the canopy. There was no difference in canopy structure between the two plots (data not shown), and the extinction coefficients of the upper layers of the canopy were 0.63 and 0.66 at the early ripening stage in the W and D plot, respectively. Therefore, it is likely that the maintenance of NAR in the D plot was caused by a higher rate of leaf photosynthesis. The rates of photosynthesis in all leaves declined after heading as a result of senescence (Fig. 7). In the D plot, however, the ninth and eighth leaves on May 6 and the tenth and ninth leaves on May 29 maintained higher rates of photosynthesis than those in the W plot.

Nitrogen content decreased in leaves at all positions during the ripening stage in both the W and D plots (Fig. 8). However, the contents were maintained

| Pot | Distributions of each phase (%) | Concentration of gases in soil air | Root/Shoot |
|-----|---------------------------------|-----------------------------------|------------|
|     | Gas | Liquid | Solid | O₂ | CO₂ | (× 10 mL⁻¹) | (mL L⁻¹) | |
| W   | 35.3 ± 2.4 a*** | 40.8 ± 1.3 a | 23.7 ± 1.1 a | 20.9 ± 0.03 a | 4.51 ± 0.28 a | 0.15 ± 0.01 a |
| D   | 45.6 ± 2.5 b | 31.1 ± 2.3 b | 23.3 ± 0.4 a | 21.0 ± 0.05 b | 3.19 ± 0.53 b | 0.18 ± 0.01 b |

* Taken from 10 cm in depth from soil surface in darkness 67 h after watering. ** Taken from the center of the pot at a depth of 10 cm from the soil surface 24 hours after watering. *** Data represent means±S.D. (n=3). Means followed by different letters are significantly different at the 5% level by Student's t-test.
higher in the plants in the D plot. The rate of photosynthesis correlated closely with leaf nitrogen content (Fig. 9). There was not a significant difference in the cytokinin contents and activity in the xylem sap at early ripening stage (Table 4).

2. Pot experiment

The soil water potential decreased to about -100 kPa at a depth of 10 cm in the D pots during the experiment whereas it remained at about -50 kPa in the W pots. The leaf xylem water potential in sunlight in the D pots was about 0.2 MPa lower than that in the W pots. The percent distribution of the gaseous phase in the soil was significantly larger in the D pots than in the W pots (Table 5). The concentration of CO$_2$ in the soil air was 5.98 ± 0.32 mL L$^{-1}$ and 3.34 ± 0.34 mL L$^{-1}$ in the W and D pots, respectively, 13 h after watering. The difference decreased within 24 h after watering but it remained 40% lower in the latter pots than in the former (Table 5). By contrast, the difference in terms of the concentration of O$_2$ in the soil air between the two types of pots was quite small, and the concentration in both types of pot was similar to that in the atmosphere (Table 5).

The dry weight of roots was 11% higher in the D pots, although there was no significant difference in mean total weight. As a result, the ratio of root weight to shoot weight was significantly larger in the D pots than in the W pots (Table 5). The rate of photosynthesis of the seventh leaf, which had just fully expanded at the start of the treatment, decreased as a result of leaf senescence in the two types of pot (Fig. 10A). The decline in the rate of photosynthesis was smaller in the plants in the D pots than in those in the W pots, reflecting the results of the experiment in the field. The nitrogen contents of the seventh leaves also maintained higher during senescence in the plants in the D pots (Fig. 10B). Although the exudation rate was lower in the plants in the D pots, the concentration of cytokinins in the xylem exudates was very much higher and the activity of cytokinins transported to each stem from roots was also higher in the plants in the D pots than it was in the W pots (data not shown).

Discussion

The plants in the D plot exhibited higher CGR and produced heavier dry matter than the plants in the W plot before flowering (Fig. 3). These results were identical to those obtained previously (Nakamura et al., 2003). Moreover, the CGR during the ripening stage was also higher in the plants in the D plot than those in the W plot (Fig. 5). As a result, the plants in the D plot produced heavier dry matter (Fig. 3) and grain (Table 2). The higher NAR and the higher LAI without reduction in NAR accounted for the higher CGR under the lower soil moisture conditions (Fig. 5) before and after flowering in the plants in the D plot even though they were grown under decreased soil moisture conditions (Fig. 1) and their leaf xylem water potential in daytime decreased (Fig. 2). The rate of reduction in the number of tillers was smaller in the plants in the D plot than in the plants in the W plot during one month before heading (data not shown). The former plants could avoid suppression of the photosynthetic rate and leaf expansion owing to water stress, and maintain high rates of leaf photosynthesis (Figs. 5, 7) and a large leaf area during leaf senescence (Fig. 5) throughout the period of soil moisture treatment. These facts caused the larger LAI without any reduction in NAR in the plants in the D plot during one month before flowering and during ripening (Fig. 5) although leaf water potential decreased.

The superior growth in the plants in the D plot increased grain yield and yield components (Table 2). A kernel tended to be heavier in the plants in the D plot even though the number of kernels was rather larger. This indicates that the superior growth in the plants in the D plot might be promoted during ripening.

In general, depletion of soil moisture has a significant effect on plant growth. Leaf expansion and stem elongation of soybean plants decreased significantly in the field before the soil matric potential at a depth of 30 cm decreases to -80 kPa (Hirasawa et al., 1994, 1998). The growth and the rate of photosynthesis of wheat plants also decreased when soil moisture decreased (Sen Gupta and Berkowitz, 1987; Kobata et al., 1992; Moustafa et al., 1996). In a growth chamber (air temperature, 25°C/18°C; relative humidity, 60%/80%; 12 h day/12 h night), the expansion of leaves of wheat seedlings decreased significantly in soil with a water potential of -50 to -80 kPa, which corresponds to the potential of the soil at a depth of 30 cm in the D plot in the field (Nakagami et al., unpublished). These results indicate that wheat plants are also susceptible to the reduction of soil moisture. However, in the plants in the D plot in the field, leaf expansion, stem elongation (data not shown) and diffusive conductance (Fig. 6) in the early afternoon were unaffected even when soil moisture and the leaf xylem water potential decreased (Fig. 2). The difference in the atmospheric vapor saturation deficit during the growing season might account, in part, for the remarkable difference in terms of the effects of a decline in soil moisture on crop growth between soybean and wheat plants. We can consider the reduction in soil moisture in the D plot was insufficient to decrease the rate of photosynthesis. Our preliminary examination showed that the rate of photosynthesis did not decrease until the leaf xylem water potential decrease to about -1.4 MPa (Nakagami et al., unpublished). The average leaf xylem water potential of the plants in the D plot did not fall below
A well-developed root system in the deeper layers of the soil allows plants to take up water efficiently from soil and contributes to the protection of plants from a water deficit when soil is moisture-deficient (Angus et al., 1983; Hirasawa et al., 1994; Hida et al., 1995; Inanaga et al., 1996; Yoshida and Hasegawa, 1982). The development of root system can be promoted under moisture-deficient conditions, even though it depends on the strength of the drought (Eghball and Maranville, 1993) and on both the species and the variety of plants (Matsuura et al., 1996; Hida et al., 1995; Huang et al., 1997). A well-developed root system has also been reported in wheat plants grown under lower soil-moisture conditions (Proffitt et al., 1985; Mian et al., 1993; Nakamura et al., 2003).

Excessive soil water damages plants. Yamazaki (1952) suggested that the main factor in such injury is a shortage of soil air rather than the excessive water itself. Under anaerobic conditions, such as flooding, root respiration might be inhibited and reducing compounds, such as ferrous ion and sulfide, might injure the roots. However, the soil still included a large gaseous phase, even in our W plot in the field (Table 1), and ferrous ion was barely detectable in both plots. The level of ground water was far lower in the field in this experiment than those in fields where obvious water injury occurs (Mori and Ogawa, 1967). We can conclude that the smaller dry matter production and yield in the W plot in the field resulted from the effects of soil moisture on plant development and did not result directly from the damage due to a shortage of soil air.

The same conclusion can be drawn from the results in the pot experiment. The better root development and delayed leaf senescence observed in the plants in the D plot in the field were also observed in the plants in the D plot. The gaseous phase appeared to be adequate for good performance of the plants even in the W pots (Noda and Ibaraki, 1961). The decrease, as compared to the D plots, in the concentration of O\textsubscript{2} in the soil air in the W pots was negligible. A slight decrease in the concentration of O\textsubscript{2} in the soil air does not inhibit root respiration (Palta and Nobel, 1989). The increase in the concentration of CO\textsubscript{2} in the soil air was also unremarkable, and no consistent effects of extremely high concentrations of CO\textsubscript{2} in soil air on the physiological activity of roots have been reported (Bouma et al., 1997; Qi et al., 1994). Thus, the earlier leaf senescence in the plants in the W pots does not seem to be caused by decline in physiological activities of roots owing to an oxygen deficit.

Water stress promotes leaf senescence (Nooden, 1988). However, in this study, the decrease in soil moisture suppressed rather than promoted leaf senescence (Figs. 7 and 10), perhaps because the plants in the D plot did not suffer from sufficiently severe water stress as mentioned above and also because they had a better-developed root system (Fig. 4 and Table 5). It has been reported that leaf senescence is delayed in plants with a well-developed root system (Jiang et al., 1988; Hirasawa et al., 1994, 1998; Kondo et al., 2000), and might be the cause of differences in both dry matter accumulation and yield between various cultivars and strains of rice (Jiang et al., 1988), sorghum (Amber et al., 1992), soybean (Ookawa et al., 1999b) and maize (Ma and Dwyer, 1998; Kondo et al., 2000) plants.

The maintenance of a high nitrogen content in leaves (Fig. 8 and 10) can explain, to some extent, the maintenance of a large LAI and also a high rate of photosynthesis during senescence in the plants in the D plot. Nitrogen is essential for the synthesis of photosynthetic enzymes and chlorophyll. There is a close correlation between nitrogen content and the level of ribulose-1,5-bisphosphate carboxylase/oxygenase (Rubisco), a key enzyme in photosynthesis (Makino et al., 1984). Leaf nitrogen content is governed by the uptake, distribution and remobilization of nitrogen. Plants in the D plot accumulated more nitrogen than those in the W plot, but no difference in nitrogen partitioning was observed (Table 3). Thus, in field-grown plants, the higher leaf nitrogen content of the plants in the D plot during senescence appears to have been caused by the accumulation of larger amounts of nitrogen. The amount of water supplied to the field at a time was far smaller in the W plot than in the D plot even though total amount of irrigated water was far larger in the former than the latter. Leaching of mineralized nitrogen from the surface of soil might not have been responsible for the decrease in the availability of nitrogen to the plants in the W plot in the field although this remains to be investigated. The soil water potential in the D plot was far higher than the critical value at which promotion of nitrogen mineralization by soil air-drying occurs (Toriyama et al., 1988).

There are reports on the high capacity for nitrogen accumulation of rice and maize plants with well-developed root systems (Ma and Dwyer, 1998; Kondo et al., 2000; Ookawa et al., 2003). The well-developed root system in the D plot might increase nitrogen absorption.

Maintenance of leaf nitrogen content might not always account for maintenance of the rate of photosynthesis during senescence. Significant degradation of photosynthetic components, with little decrease in leaf nitrogen content, has been demonstrated (Hikosaka, 1996; Ookawa et al., 1999a) and Makino et al. (1984) reported that the nitrogen content of the senescing leaves was not necessarily indicative of the level of photosynthetic activity. On the other hand, cytokinins prevent leaf
senescence by promoting the synthesis or suppressing the degradation of chlorophyll and photosynthetic enzymes (Badenoch-Jones et al., 1996; Lamattina et al., 1987). The activity of cytokinins in the xylem sap was higher in the plants in the D plots than in those in the W plots (Table 4). The activities of cytokinins in the xylem sap were high in slow-senescent rice (Soejima et al., 1992), sorghum (Amber et al., 1992), maize (Kondo et al., 2000) and wheat (Nakamura et al., 2003) plants. The activity of cytokinins in the xylem sap might also explain the higher rate of photosynthesis during leaf senescence in the plants in the D plots. The main site of cytokinin biosynthesis has been reported to be a root apex (Short and Torrey, 1972) and the roots of wheat plants form the branches under dry conditions (Morita and Okuda, 1994). Thus, the numbers of sites for the biosynthesis of cytokinins might increase under dry conditions.

In conclusion, we found that a limited decrease in soil moisture might promote development of the root system, and such development might mitigate the reduction in the leaf water potential and increase the absorption of nitrogen and the synthesis of cytokinins. These materials, transported from roots to leaves, would delay leaf senescence. As a consequence, the net assimilation rate and the leaf area of plants would remain high. The result would then be heavier dry matter and higher yield. Our analysis suggests that the development of an extensive root system is important for a higher yield via absorption of much nutrients and synthesis of plant hormones, as well as absorption of much water. The results of this study suggest that soil moisture during the wet season for about one month before heading known as “Natane-zuyu” through ripening in Japan might be one of the reasons for the low grain yield of wheat plants compared to the high-yielding European countries, like the difference in the cultivars and temperature during ripening stage. Although there are differences in responses to depletion of soil moisture among cultivars of wheat (Wada et al., 1994; Xu and Ishii, 1996), and the effects of precipitation on the growth of wheat plants depends on the texture of soil (Stephens and Lyons, 1998), effective drainage during the growth season might be necessary to achieve higher yield in many areas of Japan.

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