Sterility in Rice (Oryza Sativa L.) Subject to Drought during the Booting Stage Occurs not because of Lack of Assimilate or of Water Deficit in the Shoot but because of Dehydration of the Root Zone

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Abstract: In rice plants a drought at the booting stage is the event most damaging to grain yield because it drastically increases sterility. Our objective was to establish what physiological processes caused sterility during drought. We applied several soil-drying treatments to pot-grown plants of three rice cultivars (one lowland and two upland) for 10 days during the booting phase. Flag leaf elongation rate (LER), the rate of dry matter production (DMP) and leaf water potential (LWP) decreased and sterility increased in all cultivars as the intensity of soil drying increased. Increasing sterility was closely correlated with reduced DMP in all cultivars. It has been assumed previously that suppressed assimilate supply causes sterility. However, when DMP was strongly suppressed by shading under well-watered conditions, sterility was unaffected. Moreover, when part of the root system was partially dried at the booting stage, sterility significantly increased and leaf diffusive conductance decreased when compared with the results for well-watered plants, although there was no significant difference in LWP between the treatments.

We suggest that sterility in rice plants subjected to soil drying during the booting stage occurs not by reduced assimilation or because of water deficits in the shoot, but by some chemical signal from roots to shoots.

Key words: Booting stage, Degeneration of spikelet, Dehydration, Drought, Oryza sativa L., Sterility.

Much evidence exists to support the conclusion that a drought at the booting stage of a rice crop (Oryza sativa L.) drastically inhibits fertility, causing reduced grain yield\(^1\),\(^2\),\(^3\),\(^4\),\(^5\). The booting stage is one of the most sensitive to water stress\(^1\),\(^2\),\(^3\),\(^4\). It has been suggested that soil desiccation causes chromosome aberrations in the pollen mother cell which in turn inhibits the development of pollen\(^3\). However it is not known what physiological processes dominantly cause reductions of fertility (sterility) in rice plants which have been subjected to soil desiccation during the booting stage, because all kinds of physiological processes are affected by soil drying: dehydration of the plant organs may be accelerated, assimilate supply to growing organs suppressed, partition of assimilate in the plant changed and production of some growth substances stimulated during drought\(^7\),\(^8\),\(^9\). Our objective was to establish what physiological processes were the chief cause of sterility in rice plants subjected to drought during the booting stage.
Materials and methods

Experiment 1.

Plant material

The lowland improved rice cultivar Nipponbare, the upland improved Tachiminori and the upland traditional Senshou were used for a pot experiment. Fifty seeds were sown in an eight liter plastic pot based on an improvement of the method by Satake\textsuperscript{15}). The pot contained eight liters of sandy soil (Masatuchizumi) mixed with compost. 5 g of ammonium sulfate, 12.5 g of superphosphate and 3.8 g of potassium chloride were mixed into the soil at sowing and 2.5 g ammonium sulfate was added at 30 days after sowing and at heading. All tillers were cut, and only the main stem was allowed to remain and grow. After germination under wet soil conditions in a glasshouse, all pots were carried outdoors and a flooded condition was maintained except for about 10 days of the booting stage.

Treatments

When most of the auricle of the flag leaf was about 10 cm below the auricle of the second leaf, all pots were moved into an artificial light growth cabinet (Koitco). A temperature of 30/25°C was maintained day and night, daylength was 12 hours and light intensity was 0.2 kW m\textsuperscript{-2} falling on the leaf canopy. Humidity was not controlled. A five series of soil water status conditions was set by changing the irrigation timing. At the start of the treatments, flooded water in all pots was sucked out with a vacuum pump. Abundant water was immediately added to a pot to maintain flood conditions as a control. Watering of the second pot started 2 days after the end of the irrigation (desiccated 1) and a reduced amount of water was added every evening to maintain the intended level of soil desiccation. For the desiccated pots 2, 3 and 4 the watering started from 3, 4 and 5 days after the end of irrigation, respectively. Since every day the evapotranspiration rate from the wet pot changed little during the experiment, the atmospheric demand of air in the growth cabinet seemed to be maintained at a constant level. All treatments were stopped 10 or 13 days later. After treatment all pots were moved to a glasshouse and maintained under flooded conditions until full ripening of the grain.

Measurements

Each pot was weighed every evening to determine the soil water content. Soil water content was indicated as percentage of field capacity \((0.297 \text{ H}_2\text{O} \text{g dry soil g}^{-1} \text{ at field capacity} = 100\% )\). Field capacity was determined from gravitational soil water content 24 h after irrigation in the pot covered by an aluminum sheet. The shoots were harvested at beginning and end of the treatments to calculate the matter production rate. Five shoots per treatment were cut and dried on an oven at 80°C for 48 hours, then weighed. Ear development stage was calculated from the distance between the auricles of the flag and penultimate leaves (DAP)\textsuperscript{8}). It is suggested that meiosis starts at DAP = -10 cm and ends at DAP = +10, and is most active in whole ears at DAP = 0. The position of the auricles of the second leaf at the beginning and end of the treatment was marked on the flag leaf with a marker pen. At full ripening, DAP at the beginning and end of the treatments was calculated from the distances from the marks to an auricle on the flag leaf. After grain maturity, plant ears were harvested and the number of fertilized grains and degeneration of the spikelet were recorded by eye\textsuperscript{9}). Fertility and survival of the spikelets was indicated as a percentage of fertilized spikelet to total spikelet numbers and as a percentage of total spikelets to initiated spikelet numbers (= 100 — a percentage of degeneration of the spikelet), respectively.

Leaf water potential (LWP) of the second fully expanded leaf during periods of light and darkness was measured using the pressure chamber method\textsuperscript{6}) on the last day of the treatments. Three leaves from each treatment were used for the measurements.

Experiment 2

Plant materials

The improved lowland rice cultivar Nipponbare was used. A mixture soil of sandy soil (66\%) and seedling soil (33\%) (Green soil) was packed into eight liter pots. Then 2.5 (N) or 5.0 (2N) g of ammonium sulfate was added at the panicle formation stage. Other conditions were as for Experiment 1.

Treatments

Three different soil-drying treatments and three shading treatments were applied to four
or five pots per treatment in a non-temperature controlled vinyl-covered house for about 10 days of the booting stage (from about -10 cm DAP). Shade treatments were begun at the same time as the soil drying. Radiation in shaded treatments 1 and 2 was reduced by 42% and 72.3% by using two types of shade cloth, respectively. Pots which had received two times more nitrogen at the panicle formation stage (2N) were also added to the shaded treatments. It is suggested that heavy nitrogen application accelerates sterility under shading\(^{17}\). We tried to produce a condition which accelerates sterility under adequate soil water conditions.

**Measurements**

The fundamental method of measurement was the same as in Experiment 1 except that LWP was measured just before dawn.

**Experiment 3**

**Plant materials**

The improved lowland cultivar Nipponbare was sown on seed beds for rice and transplanted to larger volume pots under flooded conditions at the four leaf stage. Only the main stem was allowed to grow and all tillers were removed. At the eight leaf stage 25 plants were transplanted to an eight liter plastic pot in which a four liter volume plastic pot was housed in the center. 3.5 Kg of mixed soil (sandy soil 33% and seedling soil (Green soil) 66%) was packed in the both parts of the pot. The same amount of roots were divided between the inside and outside portions of the pot. The plan of fertilizer application has been previously described in Experiment 1.

**Treatments**

Plants can maintain high leaf water potential when part of the root system was partially dried\(^{24}\). We tried to see whether sterility occurs in that plant. At the booting stage all pots were divided into two groups containing four or five pots in a vinyl shade house. In the first group, wet conditions were maintained in both the inside and outside portions of the pot for about 10 days of the booting stage (from about -10 cm DAP), and in the second group, well watered conditions were maintained in the outside portion only; no water was applied to the inside portion of the pot. After the treatments, all pots were maintained under flooded conditions.

**Measurements**

The methods of measurement were the same as in Experiment 1 except that midday LWP and abaxial water vapour conductance in the second fully expanded leaf were measured with a pressure chamber and a steady state porometer (Lico co., LI-1600), either every day or at intervals of several days.

**Results**

1. **Effects of soil desiccation on fertility and survival of the spikelets**

   **Leaf elongation rate of the flag leaf, dry matter increase of the shoot, fertility and survival of the spikelet**

   Mean percentage for soil water content (field capacity = 100%) during the soil desiccation treatments decreased from 100% to 30% with reduced water application in all cultivars (Fig. 1-a). The leaf elongation rate of the flag leaf (LER) for all three cultivars decreased in response to soil desiccation. The LER of the upland cultivars, Tachimimori and Senshou, was more severely inhibited than for the lowland cultivar Nipponbare, but the relative suppression in LER was the same (the value relative to the maximum LER was 80% at SWC=50% and 20% at SWC=30%) because the leaf length of both the upland cultivars was longer than that of the lowland cultivar. The dry matter increase of the shoots for all three cultivars decreased in response to soil desiccation, but for Nipponbare this was smaller than for the other cultivars; this applied both to the absolute rate and to the rate relative to the maximum (Fig. 1-b).

   There were no clear relationships between fertility or survival of the spikelets and DAP resulting from each soil desiccation treatment (Fig. 2). Changes of DAP during the soil desiccation treatments applied to these pots where soil drying was severe were smaller than in those where soil drying was moderate, because LER was inhibited more by the severe drying conditions (Fig. 1-a). Thus there are no considerable differences in sensitivity of fertility or survival of the spikelets to soil desiccations at various stages of DAP with the same soil drying conditions. We used the averages of all data concerning fertility and survival of the spikelets in each soil desiccation treatment to indicate the effects of drought.

   The fertility of Nipponbare growing in
desiccated soil was maintained at a relatively higher level than that of Senshou and, more noticeably, Tachiminori (Fig. 1–c). Survival of the spikelets decreased for all three cultivars, but for Nipponbare it was relatively higher in moderately desiccated conditions (Fig. 1–d).

The LWP of the plants for light and dark periods on the last day of the treatments reflected the degree of soil desiccation applied to each cultivar (Fig. 1–e). There was little difference between the cultivars in the response of LWP to soil drying.

(2) Relationships between fertility or survival of the spikelets and soil water content, water status of the leaves and dry matter increase of the shoot

There were clear differences between three cultivars regarding relationships between fertility or survival of the spikelet and soil water content (Fig. 1-c and d). There were similar differences in the relationships between fertility or survival of the spikelets and mean values for LWP in periods of light and darkness on the last day of treatment (data are not provided). However there was a close relationship between the decrease of fertility or survival of the spikelets and the rate of dry matter increase in shoots during the treatments regardless of which cultivar was used (Fig. 3). These results indicated that sterility or degeneration of the spikelet in all three cultivars occurs not directly due to the intensity of soil drying or to shoot water deficit but due to inhibition of assimilation or another factor which causes synchronous decrease in assimilation.

2. Comparison of fertility and survival of the spikelet between the soil desiccated and shaded plants during the booting stage

Mean soil water content (percentage of field capacity) during the treatments decreased to 69% and less than 60% in desiccated pots 1 and 2, respectively. The mean LWP measured before dawn of the top fully expanded leaves during the treatments in the flooded control pot and desiccated pots 1 and 2 was $-0.12 \pm 0.01$, $-0.30 \pm 0.01$ and $-0.43 \pm 0.02$ MPa, (means \(\pm se\) for 10 days of treatments) and the midday LWP on the last day of the treatments was $-0.20$, $-0.71$ and $-1.52$ MPa, respectively.

Dry matter increase in the shoots was sup-
pressed from 60 mg plant$^{-1}$ day$^{-1}$ in the flooded control pot to 30 mg in desiccated pot 1 or 10 mg in desiccated pot 2. Shaded treatments reduced the dry matter increase of the shoots to near zero. The dry matter increase in the flooded control pot with nitrogen rich treatment (2N) was smaller than for the normal control (Fig. 4). Fertilization decreased significantly under dry-soil conditions but did not decrease at all under shaded treatments. Under shaded conditions survival of the spikelets deceased slightly depending on the amount of shade.

3. Effect of partial root drying on the fertility and survival of spikelets during the booting stage

The soil water of the inside small pot in the partial soil drying treatments decreased to 14. 9% of the field capacity at the last day of the treatments. Soil water content in the outside portion of all pots and in the flooded control pot was maintained at near 100%. There was little difference in midday leaf water potential between the flooded control and the partially dried soil of the inside pot during the treatment period (Fig. 5 and Table 1). However leaf water vapour conductance in the plants receiving the desiccation treatment decreased

Fig. 2. Percentage of (a) fertile grain and (b) survival of the spikelets as affected by soil desiccation during the booting stage in rice (cultivar Senshō). Stage of flower development at the end of the treatments is indicated by distance between auricles of the flag and penultimate leaves (DAP). When DAP is 0 the majority of the spikelets is in a stage of meiosis$^9$. Meiosis starts from −10 cm and ends at 10 cm DAP. The lateral bars are the ranges of DAP during the treatments indicated by the means of all observations in each treatment. Means of soil water content (SWC, %) during the treatments and leaf water potential (LWP) during dark and light periods on the final day of the treatments are indicated.

Fig. 3. Relationships between dry matter increase of the shoots ($\Delta$T) during the soil desiccation treatments and (a) decrease of percentage of fertile spikelet (F) and (b) of survival of the spikelet (= 100 − percentage of degenerated spikelet) (S) in three rice cultivars. See Figure 1 for the treatments and Figure 2 for symbols.

F = −107.27 + 3.50$\Delta$T ($r^2 = 0.854$, 0.001 > P)
S = −34.90 + 1.08$\Delta$T ($r^2 = 0.688$, 0.001 > P)
Fig. 4. Percentage of (a) fertile grain and (b) of survival of the spikelet (= 100 – percentage of degenerated spikelet) and dry matter increase of the shoots under soil desiccations (●) or under shading (▲) for well watered plants during booting stage in Nipponbare. Twice the amount of nitrogen was added to part of the shaded pots at panicle formation stage (■). Values are means ± standard error of four or five replicates containing 30–35 plants in each replicate.

Fig. 5. Changes of leaf water potential and stomatal conductance of the second leaf from the flag leaf on the control (■) and on the partially drying part of the root system (●) for Nipponbare during the booting stage. Measurements were done at midday. Values are means ± standard error of three observations.

significantly more than in the flooded control during the latter half of the treatment (Fig. 5). Dry matter production and LER of the same plants decreased slightly (Table 1). Sterility and degeneration of the spikelets increased significantly, from 6.8% to 11.6%, and from 17.9% to 23.8%, respectively.

Discussion

We can assume that three dominant mechanisms inhibit fertility or survival of the spikelets in rice plant in response to soil drying during the booting stage. The first mechanism concerns direct effects of dehydration on floral development. In this experiment we also observed decrease of LWP in response to soil drying. Pollen injury occurred in plants subject to low leaf water potential during the booting stage. However when a root system was divided into pots of wet and dry soil, LWP of the leaves did not decrease but sterility increased. Moreover it was known that the water potential of the floral organs at the booting stage in rice decreases less under soil drying conditions than does the leaf water potential. It is suggested that the floral organ is protected from dehydration by a covering of leaves or through vascular connection in high evaporative conditions. The water potential of the floral organ as well as of the leaves of the partially droughted plants may also decrease scarcely. We suggest that sterility occurs even if the plant water status of the shoot, probably of the floral organs too, is maintained at a high level during the booting stage.

The second mechanism is a deficiency of assimilate supply to the developing floral organ. Assimilation of rice decreases in response to soil desiccation. A close relationship was found between decrease of fertility and dry matter production of the shoots being
Table 1. Midday leaf water potential of the second leaf from the flag leaf, dry matter increases of the shoot, leaf elongation rates of the flag leaf of the plants subject to partially drying part of the root system during the booting stage, percentage of sterile and degenerated flowers in the treated plants at maturity.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Leaf water potential (MPa)</th>
<th>Dry matter increase of shoot (g plant⁻¹)</th>
<th>Leaf elongation rate (cm day⁻¹)</th>
<th>Percentage of fertile grain</th>
<th>Percentage of fruitful flower</th>
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<tr>
<td>Control</td>
<td>-0.42 ± 0.04</td>
<td>0.66 ± 0.17</td>
<td>2.18 ± 0.8</td>
<td>6.8 ± 0.7</td>
<td>17.9 ± 0.7</td>
</tr>
<tr>
<td>Partially drying</td>
<td>-0.51 ± 0.05</td>
<td>0.41 ± 0.13</td>
<td>1.98 ± 0.8</td>
<td>11.6 ± 1.6</td>
<td>23.8 ± 0.8</td>
</tr>
<tr>
<td>F (&lt;0.05)</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
<td>*</td>
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</table>

1. Values of leaf water potential and others are means ± standard error of 11 days and 4 or 5 replicates, respectively.
2. * and ns significant at the 0.05 and no significant, respectively in t test.

subjected to soil drying during the booting stage regardless of the very different cultivars. It has been suggested that deficiency of assimilate supply in corn plants induced reproductive failure near anthesis. However, when the plants were grown in heavily shaded conditions and dry matter production was drastically reduced, sterility of the rice plants did not occur. It is known that shading and drought do not inhibit translocation of carbohydrate to growth organs such as young leaves or grain. The growing flower may dominate in carbohydrate consumption under stressed conditions. Thus we suggest that reduction of assimilate supply to the flower does not seem to be a dominant cause of sterility; and that sterility occurs as a consequence of the same factors that reduce dry matter production. Degeneration of the spikelets seemed to be affected only a little by reduction of assimilate supply, because the degeneration increased slightly under shaded conditions.

The third mechanism is inhibition of floral development by any chemical substances produced from the plant organs. Partially drying part of the root system caused stomatal closure and sterility, even though leaf water potential was maintained at a high level. It is known that stomata close under partial root drying, and abscisic acid (ABA) content in root xylem increases under soil-drying conditions. ABA produced in plant organs which lack turgor appears to inhibit pollen development in wheat. Our results suggest that root drying and changing of root activity inhibits fertilization and survival of the spikelets without apparent water deficit of the rice shoot. This regulator seems to inhibit both dry matter production of the shoot through closing stomata etc., and fertility and survival of the spikelets. The reason why the three different cultivars had the same relationship between decrease of fertility or survival of the spikelets and dry matter production of the shoot (Fig. 3) may be that the reduction of dry matter production reflects the sensitivity of all physiological responses, including the root activity in each cultivar, to soil desiccation. The upland cultivars (Tachiminori and Sen-shou) did not have any higher desiccation tolerance in fertility and matter production than the lowland cultivar (Nipponbare). Drought avoidance, by growing deep root systems into deep, wet soil may be important to maintain fertility of upland rice under soil-drying conditions. It is possible that responses of dry matter production are a good indicator of fertilization under drought conditions, although there may not be a direct causation between them.

However, increased spikelet ABA content does not regulate the induction of spikelet sterility when wheat was grown using a split root system to dry half the roots while the remainder were kept watered. We can not establish why there is a difference in the effect of partial root drying for rice and wheat, but it is possible that there is a difference in sensitivity of response of fertility to concentration of a regulator such as ABA between the two crops.

We conclude that neither lack of assimilate
We conclude that neither lack of assimilate nor water deficit of the shoot is a dominant factor in causing sterility and degeneration of spikelets in rice which has been subject to soil drying during the booting stage. We suggest that sterility is caused by some chemical substance produced from the root in dry soils.

References


* In Japanese.
** In Japanese with English summary.