Effect of Rainfall Exposure on Leaf Wettability in Near-Isogenic Barley Lines with Different Leaf Wax Content

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Abstract
Water retention, contact angle of water droplets, and amount of epicuticular wax on leaf surfaces were examined in two lines of near-isogenic barley with normal and glossy leaves. In addition, the effect of rainfall on the wettability of leaf surfaces was examined. Mean water retention of leaves from the 1st to 5th node was 5.6 mg/cm² in normal leaf lines and 12.8 mg/cm² in glossy leaf lines. The mean contact angle of water drops was 125° in normal leaf and 93° in glossy leaf. The mean epicuticular wax content was 17.3 μg/cm² in normal leaf and 9.3 μg/cm² in glossy leaf. In glossy leaves, contact angles decreased with increasing duration of rainfall treatment. After treatment for 5 days, the angle was about 70% of that without rainfall treatment. Reduction of epicuticular wax amount by rainfall exposure occurred in all leaf positions of both barley lines. The reduction rates in the normal leaf line were 10–20%. In the glossy leaf line, the reduction rates were 15% in the 1st leaves and about 50% in the 2nd–3rd leaves.

Key words: Barley leaf, Droplet contact angle, Epicuticular wax, Rainfall exposure, Water retention.

1. Introduction
Rainfall may have a modifying influence on plant growth and development. Some modifications can be attributed directly to wetting of the leaf. Leaf wetting by rainfall causes leaching of certain substances (Tukey, 1970) and injury by long term exposure.

It has been reported that the wetting of plants by rainfall causes a decrease of dry matter accumulation (Kimura and Tanakamaru, 1980) and sugar content (Kimura and Ariyoshi, 1994), and results in growth inhibition (Kimura et al., 1982; Kimura and Ariyoshi, 1983), leaf wilting, and injury after rainfall exposure (Kimura, 1984a; 1984b; 1994).

Furthermore, exposure to rainfall not only causes weak effects of submergence on plant, but also has a sever wilting phenomenon than submergence on plant leaves and successive leaf injury (Kimura, 1986). The effect of shoot wetting by rainfall on growth mainly depends on the wetting duration rather than the amount of rainfall (Kimura, 1987).

These findings suggest that leaf wetting by rainfall greatly influences physiological and developmental responses. It is considered that the specific wetting effects are associated with the characteristics of the leaf surface.

Water retention on the leaf surfaces of plants is associated with contact angles of water droplets (Ito and Kadota, 1974; Haines et al., 1985), and the contact angle of water drops increases with decreasing degree of leaf wettability.

The experiments described herein were designed to investigate wettability of leaves of two near-isogenic lines (normal and glossy) in barley, and the effect of rainfall treatment on the wettability of leaves.

2. Materials and Methods

2.1 Plant materials
Two barley (Hordeum vulgare L.) lines, “Goseshikoku-sai-I” (Normal leaf line) and “Goseshikoku-sai-1-hen” (Glossy leaf line), used in this study were obtained from the Barley Germplasm Center of the Research Institute for
Bioresources, Okayama University. The latter is a mutant of the former and has different glossiness in leaves. These plants are near-isogenic lines of each other.

Plants were cultured on a medium of vermiculite in a clay pot and grown for 2 months after germination in a glasshouse. Leaves of these plants were used as experimental materials.

2.2 Artificial rainfall treatment

Plants were exposed to artificial rainfall (mist treatment) in a growth chamber at 20°C under a 12hr-light/12hr-dark cycle. The rainfall treatment was applied at the rate of 5mm/h of deionized water by using an overhead mist apparatus. The average diameter of water drops was 0.24mm and the number density of water drops was 40/cm²/sec. The light source in the chamber was a “Yoko lamp” (Toshiba Electric Co.) with an intensity of 61W/m² (PPFD 177 μmol/m²/sec). Control plants were grown in a second chamber under the same conditions without artificial rainfall, expect that relative humidity was 95% with artificial rainfall and 80% in the control.

2.3 Water retention

After leaves (lamina) of barley were detached, the fresh weight was measured. The leaves were placed on a vinyl-coated wirenet with an inclination of 15 degrees, and misted for one hour on the adaxial surface. After the treatment, leaves were placed vertically and fallen water drops were removed. Thereafter, wetted leaves were weighed. Water retention was measured from the difference between the fresh weight of leaves and the weight of wetted leaves, and was expressed as mg water/cm² leaf area.

2.4 Contact angle of water droplet

Contact angles of water droplet on leaves formed by a 2μl droplet of deionized water from micro pipette were measured within 1 minute after the application of each water droplet. Five measurements were made on each adaxial leaf surface by a goniometer and their mean values were expressed as contact angles on the leaf surface.

2.5 Amount of epicuticular wax on leaf surface

12 leaves from the 1st (apical) to the 3rd node in 4 plants were removed and their leaf areas were measured. Leaves were held vertically and epicuticular wax was extracted from the adaxial surfaces by delivering 5ml/leaf of chloroform from a burette. Afterwards, the solvent was concentrated to 20ml at 40°C and centrifuged at 3000rpm for 5min. The chloroform layer was combined with two washes of the precipitate with 2ml chloroform. These combined chloroform solutions were evaporated to dryness, and the residue was weighed as the total epicuticular wax (Mayeux and Jordan, 1987). Amount of epicuticular wax was calculated as the weight of wax per unit area (g/cm²).

3. Results

3.1 Leaf wettability

(1) Water retention

Water retention on the expanded leaves from the 1st (apical) to 5th (bottom) node was examined in two barley lines with normal and glossy leaves. Water retention after misted for one hour by deionized water showed significant differences between normal and glossy leaves (Fig. 1). Retention on glossy leaves was greater than that on normal leaves. However, in relation to leaf position, similar tendencies were exhibited in both barley lines. Leaf water retention was smaller in young leaves than in older leaves. Retention was the smallest on the 2nd leaves. Mean retention of five leaves was 5.6mg/cm² in normal leaf lines and 12.8mg/cm² in glossy leaf lines.

(2) Contact angle of water droplet

Contact angles of water droplets placed on adaxial leaf surfaces were measured in normal and glossy leaf lines (Fig. 2). The mean contact angle for leaf positions from 1st to 5th was 125° in the
normal leaf line and 93° in the glossy leaf line. The contact angles of the normal leaf lines were nearly the same in all leaf positions from the 1st to the 5th, but considerable differences of the angle in the glossy leaf line were observed between younger and older leaves; from 103 to 110 degrees in younger leaves in apical 1st to 3rd positions and about 70 degrees in the older leaves in 4 or 5th leaf positions.

(3) Epicuticular wax on leaf surface

The amount of epicuticular wax in normal and glossy leaf lines of barley are shown in Table 1. Leaves from the 1st (apical) through the 3rd node were removed and assayed for epicuticular wax. Each value is the average of 4 replicates. The average wax content was 17.3 μg/cm² in the normal leaf line and 9.3 μg/cm² in the glossy leaf line. Thus, wax content of glossy leaves was about half that of normal leaves. Differences in wettability of leaves of two lines may be due to differences in the amount of epicuticular wax.

<table>
<thead>
<tr>
<th>Lines</th>
<th>epicuticular wax (μg/cm²)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal leaf line</td>
<td>17.3 ± 1.4*</td>
</tr>
<tr>
<td>Glossy leaf line</td>
<td>9.3 ± 0.7*</td>
</tr>
</tbody>
</table>

*Mean ± standard error. Number of samples used is 4 in each lots.

3.2 Changes of leaf wettability by artificial rainfall

(1) Changes of water retention

Leaves (from the 1st through 3rd node) of normal and glossy leaf lines were exposed to artificial rainfall for 1, 5 and 24 hours. Immediately after the treatment, water retention was examined (Fig. 3). No significant differences in water retention of normal leaves were found among the treatment durations of 1, 5 and 24 hours. In glossy leaves, considerable differences in water retention were found between rainfall duration of 1 hour and 5 hours. However, there were no significant difference in water retention between 5 and 24 hours treatments for glossy leaves.

(2) Changes of contact angle of water droplet

Plants were exposed to artificial rainfall for 1, 2, 3 and 5 days. One hour after rainfall, contact angles of water droplets placed on adaxial leaf surfaces were measured in both of normal and glossy leaf lines (Fig. 4). In the normal leaf line, rainfall treatment for 5 days had no influence on contact angles. In the glossy leaf line, contact angles decreased with increasing duration of rainfall. After 5 days treatment, the angle was about 70% of the non-rainfall treated controls.

(3) Changes in epicuticular wax on leaf surface

Changes in epicuticular wax by artificial rainfall for 3 days were examined (Fig. 5). Glossy leaves had considerably less wax than normal leaves. The amount of wax in both barley lines decreased with
Fig. 4. Effect of rainfall treatment on contact angle of water droplet in the leaves of barley. NL: Normal leaf line. GL: Glossy leaf line. Leaves from 2nd node was examined. ○, □: Control, ●, ■: Rainfall treatment. Number of samples used is 6 in each lots. Vertical bars indicate standard error of the means.

Fig. 5. Effect of rainfall treatment on epicuticular wax in the leaves of barley. NL: Normal leaf line. GL: Glossy leaf line. C: Control. R: Rainfall treatment.

increasing age of leaves. By the rainfall treatment, reduction of wax amount occurred in all leaf positions of both barley lines. Reduction rates in the normal leaf line were 10–20%. In the glossy leaf line, reduction rates were 15% in the 1st leaves and about 50% in the 2nd–3rd leaves.

4. Discussion

In the present experiment, it was shown that leaves of glossy line has smaller amount of epicuticular wax, heavier water retention and smaller contact angle of water droplets than leaves of normal lines, so they thought to have higher wettabilty than normal lines. In both lines, leaf wettabilty was low in younger leaves and high in older leaves. Leaves exposed to rainfall reduced epicuticular wax on leaf surface and became more wettable.

Similar tendencies were obtained as previous study (Ito and Kadota, 1974) on relation between water retention and contact angle of water droplets in barley leaves. Water retention in normal leaves was smaller than that in glossy leaves. The contact angle of water droplets on leaves of a glossy leaf line was smaller than that of normal leaf lines. There was little difference in the contact angle of water droplets between leaf positions in normal leaf lines, but considerable differences in the angles of glossy leaves were observed between younger and older leaves.

Leaf wettability differs according to amount, physical structure and chemical properties of epicuticular wax (Holloway, 1969; Johnson et al., 1983; Haines et al., 1985; Berg, 1985; Leece, 1987).

In this experiment, the amount of epicuticular wax in glossy leaves was almost half of normal leaves. Rainfall exposure caused an increase of water retention and a decrease of contact angle of water droplets in the glossy leaf line. Epicuticular waxes were lost from leaves of both lines, especially in older leaves of glossy lines where wax amounts were reduced by about 50%.

Wax reduction due to rainfall has been observed in some plants and such wax reduction is caused by the effects of physical and chemical erosion (Baker and Hunt, 1986; Mayeux and Jordan, 1987). In the current experiment, the reason for wax reduction was not clear, but it was deduced that the degree of wax reduction was closely related with leaf wettability.

The results of our previous work and of the above experiments suggest that we need to examine various physiological phenomenon and leaf damage from the viewpoint of rainfall wetting, especially in terms of wax amount and wettability of leaf surfaces.

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References

ワークス量の異なる同質遺伝子系統オオムギ葉の
濡れ特性におよぼす降雨処理の影響

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要約

葉面の濡れやすさの異なる2種のオオムギ品種を用いて、葉面の濡れ状態（葉面の水滴付着量、水滴の接触角）及び濡れ特性と葉面ワークスの関係について検討を行った。さらに降雨処理による濡れ特性の変化についても検討した。供試した材料として、同質遺伝子系統で、葉面の濡れやすさを除いて同じ遺伝的性質をもっていたオオムギ「五穀四石塚1号」（正常葉系統）と「五穀四石塚1号変」（濡れ葉系統）の2系統を使用した。

第1葉（最上位葉）から第5葉（下位葉）の平均水滴付着量は、正常葉系統では、5.6 mg/cm²、濡れ葉系統では12.6 mg/cm²であった。葉面の水滴接触角度は、正常葉系統で125°、濡れ葉系統93°であった。また葉面ワークス
ス量は、正常葉で17.3 μg/cm²、濡れ葉では9.3 μg/cm²であった。

葉位別に水滴付着量、接触角度を検討した結果、両系統とも第2葉位がもっとも濡れにくく、下位葉ほど付着量がむしろ上位葉を表す傾向がみられた。水滴接触角は正常葉系統では第2葉位葉が異常を示したが、濡れ葉では下位葉の角度は著しく上位葉と比較して低い値であった。

両系統とも5時間の降雨処理により水滴付着量は増加する傾向がみられたが、正常葉系統では増加が少なく、濡れ葉系統では約40%の増加がみられた。また5日間の降雨処理により、正常葉系統の接触角度はほとんど影響されなかったが、濡れ葉系統では約30%の減少がみられた。また降雨処理により両系統ともワックス量の減少がみられた。正常葉系統では10〜20%, 濡れ葉系統では第1葉で15%, 第2〜3葉で約50%の減少がみられた。

以上のことから濡れ葉系統は正常葉系統と比較してワックス量が少なく、雨水付着量が多く、水滴接触角度が小さく、濡れやすい性質をもっていることが明らかになった。また両系統とも活動の盛んな若い葉は濡れにくく、葉齢の進んだ葉は濡れやすくなる傾向がみられた。さらに長時間降雨を受けると、葉が濡れやすくなることを明らかにした。

キーワード：オオムギ葉、降雨処理、水滴接触角度、水滴付着量、葉面ワックス