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THE EFFECT OF DROUGHT STRESS ON WETTABILITY OF BARLEY LEAF SURFACE*

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A b s t r a c t. The aim of the study was to determine the changes of plant surface wettability caused by environmental drought stress. Two Polish cultivars of spring barley (*Hordeum vulgare*) Poldek and Stratus were tested. The low soil moisture was stabilised at pF 3.5 while the control soil moisture at pF 2.2. The wettability was determined by water contact angle. The measurements were performed on fresh leaves before and after washing in chloroform. Generally, drought led to hydrophobisation of the surface of plant leaves. The use of chloroform caused greater changes in contact angle values for stressed than for control plants, but this reaction was variety-specific and leaf-age dependent.

K e y w o r d s : contact angle, surface plant waxes, barley

INTRODUCTION

The surface of majority of higher plants leaves is covered with a thin layer of wax called the cuticle. This layer consists mainly of cutin and cutan (Nip *et al.* 1986) and water-soluble lipids. Cutin consists of ω-hydroxy fatty acids of chain length C16-18 (Holloway, 1994). The plant lipids are a complex mixture of compounds commonly known as aliphatic waxes (Barthlott *et al.* 1998). The composition and quantity of epicuticular wax is unique and characteristic for particular species. Thanks to their specific structure and properties, epicuticular waxes pre-

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vent the excessive evaporation of water vapour, determine the adhesion of microorganisms on the surface of leaves and reflect sunlight (Koch *et al.* 2006).

Abiotic environmental stresses affect the morphology and quantity of secreted wax (Baker, 1974). It was also discovered that the size and spatial distribution of the wax crystals on the surface of the leaf can be significantly modified by the environmental conditions in which a plant is grown (Hull *et al.* 1979). Limited availability of soil water and low humidity are key factors in the increased production of wax (Koch *et al.* 2006) and therefore specific wetting behaviour of plant leaves.

Wetting of the leaf surface is governed by the same physicochemical factors which control the wetting of any solid surfaces and is complicated as it depends upon the solvent as well as the leaf surface nature. These are primarily the nature of chemical group exposed on the surface and the surface roughness (Holloway, 1969). The wettability of different surfaces is described by water contact angle (CA, θ). The CA is the angle, conventionally measured through the liquid, where a liquid/vapour interface meets a solid surface and the theoretical basis for description of this phenomenon is Young equation (Young, 1805). There is limited literature data focused on the influence of stress conditions on physicochemical properties of plant leaves surface (Koch *et al*. 2006).

The aim of the study was to determine the changes of plant surface wettability (contact angle) caused by drought stress environment of two spring barley cultivars – Poldek and Stratus.

MATERIAL AND METHODS

Grains of Stratus and Poldek varieties were obtained from a local seed material distributor in Lublin. They were sown on the soil in polypropylene cylinders (with height of 40cm and diameter of 10 cm) to receive 5 plants in each cylinder. The soil was obtain from experimental field form Institute of Soil Science and Plant Cultivations in Grabów. The soil was built from sand (85.01 %), silt (9.59 %) and clay (5.4%). The soil was mixed with liquid nutrients (Starck 2007). Plants were cultivated in a growth chamber in two irrigation options: control and drought. The soil moisture for control was stabilised at pF (3.5) which corresponds to 10% (w/w) by adding the appropriate quantity of distilled water. After the plants developed 3 leaves (about 3 weeks from germination) in the cylinders with the stressed plants the moisture was decreased to pF (2.2) which corresponds to 6-8% (w/w). These drought conditions were then kept for 10 days. Next the plants were cut down. The leaves were segregated according to the order of their appearance (1^{st}, 2^{nd}, 3^{rd} and 4^{th}). Two samples were taken from each fresh leaf for each cultivar and variant. One fresh and a second one, washed with chloroform by the use of cotton robe, were fixed on a glass plate with double sided adhesive

tape. Chloroform was applied to remove surface waxes. The wettability of the upper leaf surfaces was determined by the measurement of static contact angle (CA) of a water droplet (5 μl, about 0.5 mm diameter) using a microscope (DSA100, KRUSS) equipped with a goniometer and a CCD camera.
The contact angle can be determined by Young's equation:

$$(\gamma_s = \gamma_{Sl} + \gamma_L \cos\theta)$$

which combines the cosine of the contact angle of a liquid (cosθ), interfacial free energy of a solid/liquid (γ_{SL}), the surface tension of the liquid (γ_L) and free energy of the solid surface (γ_S). When water molecules are strongly attracted to solid substrate, the CA is less than 90^o and the surface is hydrophilic. If the water molecules are not strongly attracted to solid surfaces and it takes the shape of a droplet, the CA is higher than 90^o and the solid surface is hydrophobic. The measurements were done in 3 replicates. Statistical analysis of the results obtained, that included one-way ANOVA and Tukey's HDS tests, was made using the STATISTICA 10.0 software.

RESULTS AND DISCUSSION

The surface of Poldek and Stratus leaves had the hydrophobic character (contact angle higher than 90^o). Exemplary photographs of water drop on the barley leaves surface are presented in Figure 1.

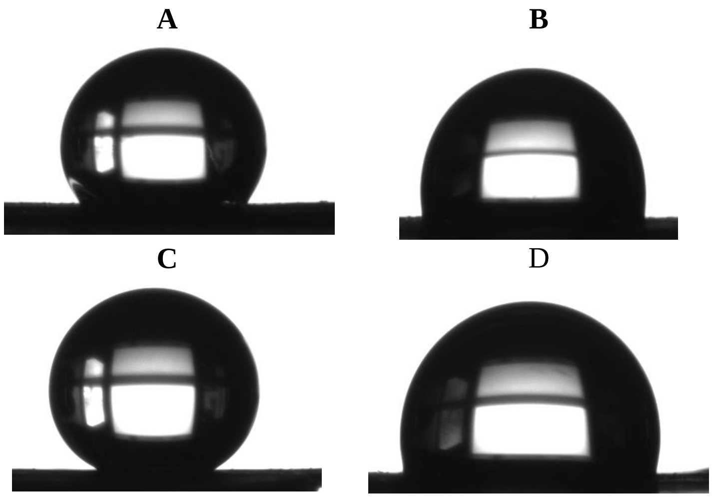

Fig. 1. Water drop on Poldek and Stratus leaves at drought and control conditions. A – Poldek, drought, fresh leaves, B – Poldek, control, fresh leaves, C – Stratus, drought, fresh leaves, D – Stratus, control, fresh leaves

The results of water contact angle measurements are presented in Figure 2.

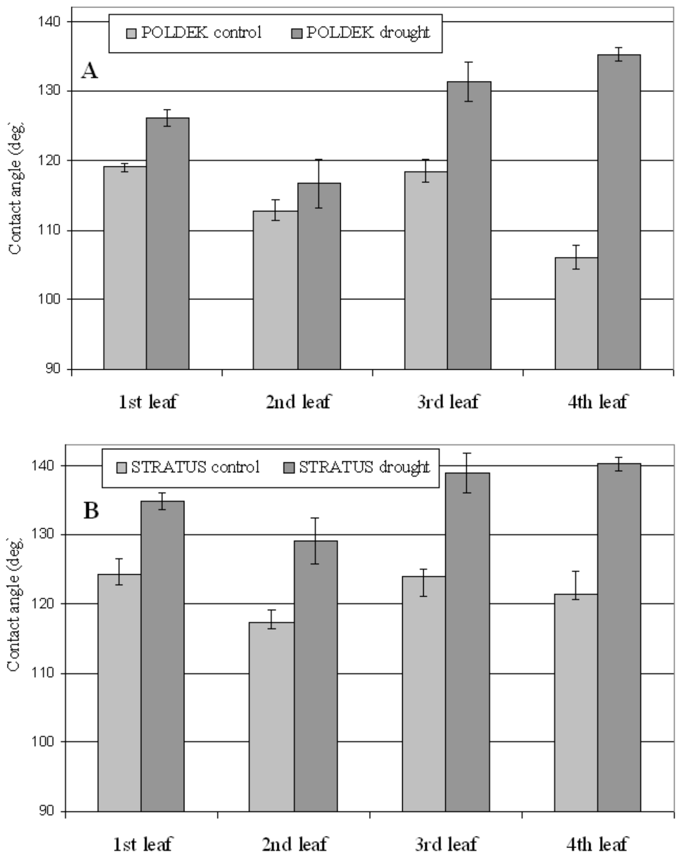

Fig. 2. Contact angle values for particular fresh leaves of two cultivars A – Poldek and B – Stratus in control and drought conditions, average value from 3 replicates, vertical pillars show ± standard deviation

The wettability of a plant leaf is complicated as it depends upon the solvent as well as the leaf surface nature. The results of CA reflected the changes in chemical and spatial composition of the leaf surface (Zhu *et al.* 2014). Generally, the CA obtained for the leaves of stressed plants were higher than for the control plants for both varieties (Fig. 2). The contact angle for Poldek plants was from 106.1º to 120.5º ± 1.5 for the control and from 116.7º to 135.2º ± 2.1 for the stress conditions. Statistical analysis revealed significant differences between the CA values for stress and control plants ($F(1;22) = 11.567$; $p = 0.003$). The CA for STRATUS plants was from 125.8º to 140.3º ± 1.5 for stressed plants and from 117.2º to 125.1º ± 2.1 for control plants. Statistical analysis revealed significant differences between the CA values for stress and control plants ($F(1; 22) = 49.70$, $p = 0.00$). The value of CA was variety-specific ($F(1;46) = 10.88$, $p = 0.002$) for the analysed barley cultivars. That was compatible with the literature data where the chemical composition and structure of wax layer is unique for particular species (Post-Bettenmiller 1996). The cereal plants leaves are hydrophobic, for instant for barley leaves: advancing contact angle of dry leaves is 129º and receiving contact angle 115º (Wisniewska *et al.* 2003). Moreover, contact angle of different plant leaves surface ranges from 120º for *Eucalyptus globulus* to 29º for *Vicia faba* (Hietala *et al.* 1997).

Taking into account the fact that the plant leaves surface is very heterogenic, it is not surprising that the CA obtained for particular leaves was differentiated (Fig. 2). This was strongly marked in Poldek cultivars where $F (3;8)$ was 78.39 and $p = 0.00$. This observed heterogeneity of barley surface properties is due to the extremely complex structure and chemistry of the leaf surface. In STRATUS such significant differences were not observed, which suggests that the surface of its leaves is more homogeneous. Moreover, it should be noted that for stressed plants there was a trend for the younger leaves to be affected by drought much more than the older ones, which was indicated by higher CA values. These results confirm that the physicochemical plant reaction against drought is to increase the secretion of epicuticular wax of younger leaves, which is reflected in very high water repellence (Zhu *et al.* 2014). The CA for control plant in both cultivars did not show such a dependence on leaf age. The plants did not have to respond to the environmental stress, so their leaves' surface was more or less the same.

Washing with chloroform caused a high decrease in the hydrophobicity of plant leaves surface (Fig. 3). The reason of such changes is the removal of cuticular waxes. The chemical composition of these waxes is well-known and the surface waxes extracted from leaves consist essentially of n-alkanes: $C_{21} – C_{35}$ (n-Nonacosane, n-hentriacontane, n-tritriacontane), fatty acids $C_{16} – C_{32}$ acid (hexadecanoic and octadecanoic acids), aldehydes $C_{24} – C_{26}$ (hexacosanol), primary alcohols, $C_{22} – C_{26}$ (1-hexacosanol), esters of $C_{34} – C_{62}$ esters (esters of hexadecanoic and octadecanoic acids) (Baker 1982, Post-Bettenmiller 1996). Moreover, at the molecular level, epicuticular waxes consist of three structurally distinct fractions with different degrees of organisation: a crystalline fraction, a solid amorphous fraction and a liquid amorphous phase (Barthlott *et al.* 1998).

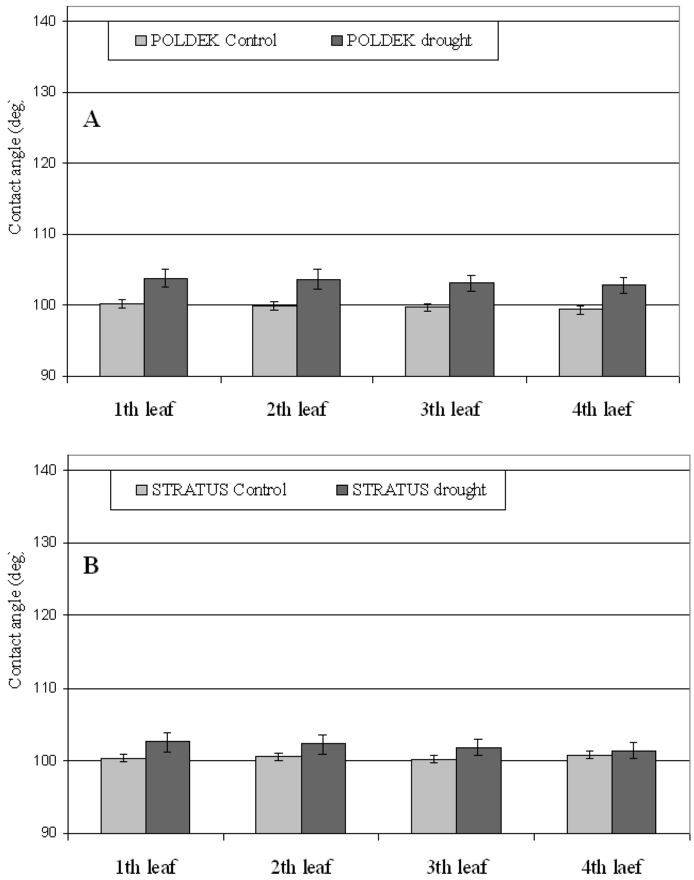

Fig. 3. Contact angle value of leaves washed with chloroform for particular variants and cultivars: A – Poldek and B – Stratus, average value from 3 replicates, vertical pillars show ± standard deviation

Washing with chloroform causes the dissolution of long-chain carbohydrates and similar compounds which are components of epicuticular waxes (Deas *et al.* 1974). After washing with chloroform, the CA values for both cultivars and variants decreased by up to 20% (Poldek, control) and 31% (Poldek, drought), 24% (Stratus, control) and 34% (Stratus, drought). The decrease of CA for all the cultivars and the leaves age was higher for the stressed than for the control plants. It confirmed the higher secretion of plant epicuticular waxes at the drought stress conditions. After the chloroform treatment the CA values of stressed plants were still higher than those for the control. It suggests that the limited water availability modifies not only the wax outer layer but also their intercellular layer which consists of pectin cellulose strongly connected with cutine and cutane (Wisniewska *el al*. 2003)

The washing with chloroform reduced the specific cultivars differences ($F(1;46) = 0.27$, $p = 0.60$). The CA for both varieties was stabilised at the value of 101.3° ±.3.

CONCLUSIONS

1. The surface of leaves of two barley cultivars Poldek and Stratus had hydrophobic character. The contact angle was higher than 90°.
2. Drought stress influenced the CA values of plant leaves. The surface of stressed plants leaves became more hydrophobic and the contact angle became higher related to control.
3. The use of chloroform caused changes in wettability for stressed and for control plants. The contact angle value decreased more for stress plants than for control plants.

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WPŁYW STRESU SUSZY NA ZWILŻALNOŚĆ POWIERZCHNI LIŚCI JĘCZMIENIA

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S t r e s z c z e n i e . Celem badań było określenie zmian zwilżalności powierzchni liści jęczmienia spowodowane stresem suszy. Badano dwie polskie odmiany jęczmienia jarego (*Hordeum vulgare*) – Poldek i Stratus. Warunki niskiej wilgotności gleby ustalono przy pF 3.5, natomiast warunki kontrolne przy pF 2.2. Jako miarę zwilżalności powierzchni zastosowano statyczny kąt zwilżania wodą. Pomiary wykonano na żywych liściach przed i po ekstrakcji wosków za pomocą chloroformu. Stres suszy prowadził do dalszej hydrofobizacji powierzchni liści badanych roślin. Zastosowanie chloroformu spowodowało zmiany wartości kąta zwilżania dla roślin poddanych stresowi oraz dla roślin kontrolnych. Reakcja roślin na warunki stresu środowiskowego różniła się w zależności od odmiany jęczmienia oraz wieku liści.

S ł o w a k l u c z o w e : kąt zwilżania, powierzchniowe woski roślinne, jęczmień