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A Convenient Method for Determining the Effect of Abscisic Acid on the Stomatal Aperture of Rice (*Oryza Sativa* L.) by Feeding Solution to the Leaf Tip

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Abstract : We established a convenient method for determining the effect of abscisic acid (ABA) solution on the stomatal aperture in wilting-sensitive plants such as rice (*Oryza staiva* L.). Water or ABA solution in a small vial was fed into a leaf tip under field conditions. Stomatal conductance was decreased by concentrations of ABA from 10^{-3} to 10^{-8} mol L^{-1} three hours after application. The amount of solution fed into the plant depended on the transpiration rate of the leaf. It was shown by the infiltration method that the stomata of an ABA-fed leaf closed first at the leaf edge and progressively toward the midrib. This heterogeneous stomatal closing may cause variance of stomatal conductance in the mid portion of the leaf in some cases. However if we can measure the stomatal conductance of portions of the leaf nearer to the tip, it may be possible to detect the stomatal response sooner and with less variation. We conclude that the assay method helps to define stomatal response to growth substances such as ABA of plants which suffer drought or any stress.
Key words : Abscisic acid, *Oryza sativa* L., Stomatal conductance, Wilting.

アブシジン酸に対するイネ葉身の気孔伝導度反応の検出法 : 小葉田亨・原　慎一（島根大学農学部）

要　旨 : 水ストレスを受けた植物体で作られるアブシジン酸（ABA）などの物質が気孔開閉におよぼす影響を知るための簡単な検査法を確立しようとした．葉片の浸漬や散布のような葉面を湿潤させる方法ではポロメータ法による気孔伝導度測定はできず，また気孔の開くような光条件の基でイネ葉身は切断するとすぐに萎凋するのでさし葉への吸収法は難しく，葉鞘への一定量の注入は組織が薄く困難であった．そこで生育したままのイネの葉身先端を切除して小型のバイアルにつけ，試験液を吸収させポロメータで測定する方法を試みた．その結果，葉身切除により始めの15分は葉身中央部の気孔伝導度は低下するもののその後は無切除の葉身とほぼ同じになった．バイアルからの液の吸収量は蒸散速度の増加にともない増えた．蒸留水と 10^{-3} から 10^{-8} mol L^{-1} までの ABA 溶液を吸収させると気孔伝導度は濃度に応じて低下した．ただし，はっきりした効果が現れるのには3時間以上かかり，葉身によって吸収開始後の気孔伝導度に変動が認められた．これは浸潤法で気孔閉鎖部位を見るとバイアルを付けた葉身先端から中央に向かい徐々に気孔が閉じていくこと，同一部位では葉身中央に比べ縁の方が閉鎖が早く，バイアルから遠いほどわずかな測定部位の変化が値を変動させるためと推定された．従って，よりバイアルに近い部位を測定すれば，短い時間で気孔開閉を少ない変動で測定できると考えられる．以上から，本方法を用いれば比較的簡単に栽培条件に近い環境でイネの気孔伝導度への各種溶液の影響を調べることができると結論された．
キーワード : アブシジン酸，萎凋，イネ，気孔伝導度，蒸散，水ポテンシャル．

It is known that abscisic acid (ABA) solution or xylem sap exuded from the roots of a plant under drought conditions reduces stomatal conductance in various plant species[2]. To distinguish whether the solution or xylem sap directly affects the stomatal conductance it is essential that the solution or the xylem sap is assayed to leaves of well watered plants and the stomatal responses is observed[5]. There are several methods of applying solution to the plant ; immersing leaves in solution, spraying the solution on leaves, suction of solution from the base of a cut leaf by the transpirational stream or injection with a syringe et al.[1,5,6]. However, these methods seem to be rejected or difficult for the following reasons. Immersing leaves in solution poses the problem of measuring the stomatal conductance because porometory as a standard method of measuring stomatal conductance is impossible for leaf surface wetted by the solution. Moreover, direct measurements with a microscope by using of the sump method is difficult in *Gramineae* such as rice, since magnitude of change in stomata aperture is very small[3]. Spraying solution on leaves wastes a good deal of solution and porometory is impossible just after spraying because the leaf sur-

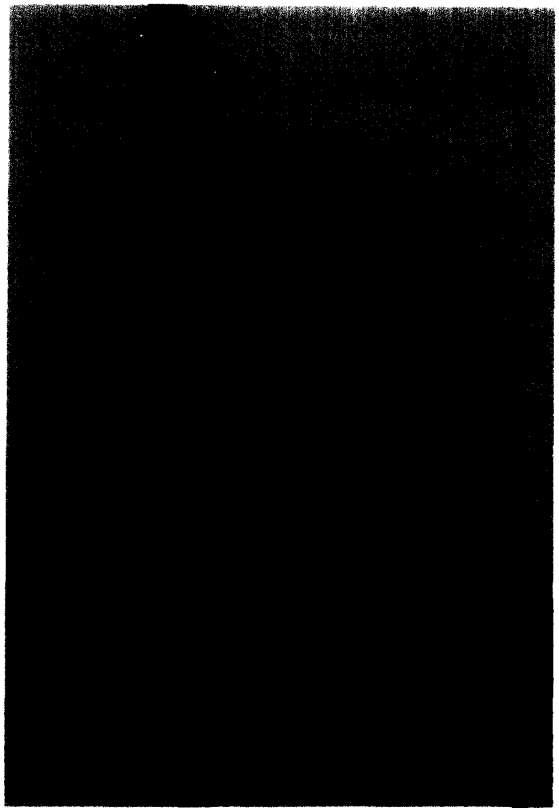


Fig. 1. A vial attached to a rice leaf. The leaf tip cut in distilled water was immersed in 1 ml distilled water or abscisic acid (ABA) solution in the vial, which was closed and held by a rubber stopper. The vial was held on a steel pipe.

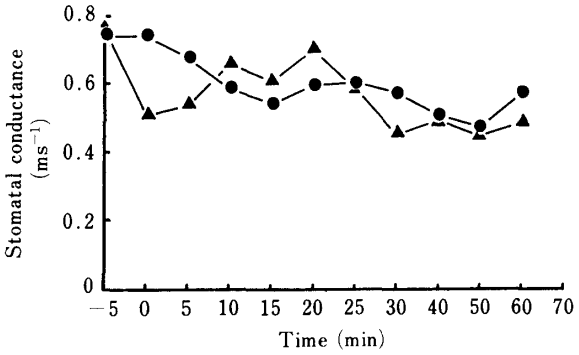


Fig. 2. Stomatal conductance at various times in non treated (●) and treated rice leaf (▲) in which the leaf tip was cut and immersed in distilled water. Each set of data was gathered from a single leaf.

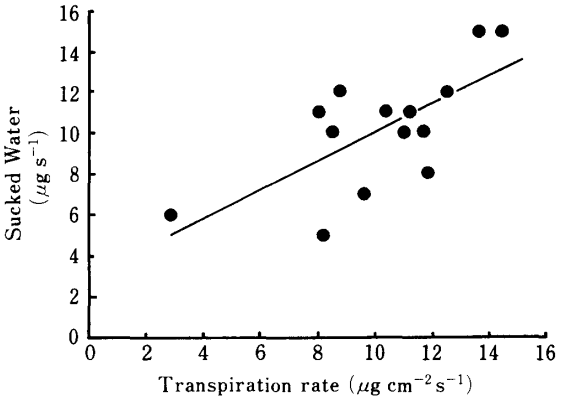
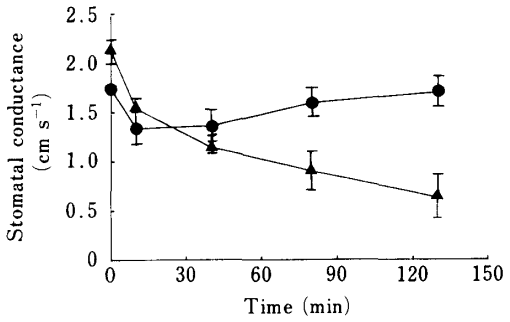


Fig. 3. Relationship between intake rate of the distilled water from the vial by a leaf (S) and transpiration rate (Tr) from the same leaf during diurnal trend.
$S = 3.04 + 0.70Tr$, $r^2 = 0.468$ ($P < 0.01$)



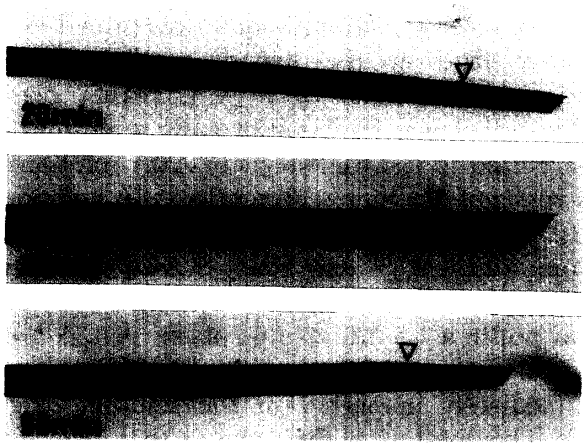


Fig. 4. Stomatal conductance and stomatal aperture by infiltration method at various times after the treatments in controlled (●) and treated (▲) leaf by distilled water and 10^{-3} mol L^{-1} ABA solution, respectively. Data indicates means ± standard error of three observations. ▽ in the photograph indicates the top edge of the vial on the treated leaf.

face is wet. Stomata may reflect responses to condensed solution if porometory is postponed after drying of the leaf surface. Suction of solution from the base of a cut leaf fits the assay to detect the effect of solution because the solution is sucked by whole leaves and less is wasted, and porometory or measurements of transpiration rate can be done[5]. However rice leaves seem to be very sensitive to water shortage after leaf cutting; the leaf blade wilted and the stomata closed within a few minutes after the leaf has been cut from a plant under natural evaporative conditions, even if the base of the leaf was immediately immersed in distilled water. Injection of solution in leaves wasted solution because it sometimes leaked from the stem or leaf sheath, since rice has a thin leaf sheath and stem wall. This method is difficult for rice leaves unless a special technique for injection is developed.

Our objective was to obtain a more convenient and sensitive method for measuring the effect of the test solution on the stomatal aperture in plant species such as rice, which are very sensitive to water shortage under high evaporative conditions. A method of feeding solutions to leaf tip was tried as the bioassay for stomata regulators such as abscisic acid.

Materials and Methods

Rice cultivar "Nipponbare" was grown in a paddy field at the experimental farm of Shimane University or in 4 L plastic pots. Plants at flower initiation or early grain filling were used to test the effects of ABA solutions. A series of graduated strengths ABA solution (10^{-3}, 10^{-4}, 10^{-6} and 10^{-8} mol L^{-1}) was made from distilled water and S-ABA powder (Kyowa Hakko co.). One mL of each solution was put in a 1.5 mL volume plastic vial. A leaf tip was cut in distilled water with scissors and immediately immersed in the solution (Fig. 1.). This method is the same as that used for N^{15} leaf feeding in wheat[7]. The stomatal aperture on the abaxial side of the center of the leaf was monitored with a steady state porometer (LI-1600, Li-co co.). We used a mixed solution of iso-butanol and ethylene glycol for the infiltration method[3] to find the location of stomatal closure in a leaf blade. A leaf blade which had been fed with ABA solution for several hours was cut from the plant, immediately immersed in the infiltration solution (solution number II) and photographed within one minute. All measurements were done in sunny outdoor conditions.

Results

When about 1 cm of the leaf tip was cut and immersed in distilled water, stomatal conductance was reduced by 35% for a period of ten minutes after the treatment, but recovered to the same level as that of a non-treated control leaf within fifteen minutes (Fig. 2). The amount of solution taken up by a leaf from the vial increased depending on the transpiration rate (Fig. 3). We calculated that about three percent of the transpired water was taken up from the solution in the vial.

When 10^{-3} mol L^{-1} ABA solution was applied, stomatal conductance started to decrease noticeably after thirteen minutes and was reduced by 70% after 2h compared with a control leaf given only distilled water (Fig. 4). It was observed that the stomata of the ABA treated leaf started to close from the leaf tip thirteen minutes after the treatment and then proceeded to the center of the leaf. Stomata at the edge of the leaf closed earling than those on the mid-portion of the leaf which were the same distance from the leaf tip.

Stomatal conductance decreased depending on the ABA content of the solution, although the conductance at the start of the treatment differed slightly between the leaves which were tested and variations in hourly trends of stomatal conductance were observed, such as in the case of 10^{-6} mol L^{-1} (Fig. 5). When stomatal conductance was indicated as a relative value of the rate of the start of the treatment, we found that stomatal apertures were inhibited even by small amounts of ABA 3 hours after application (Figs. 5 and 6). Decrease of stomatal conductance in the control leaf was due to reduction of solar radiation.

Discussion

When we tried to apply the solution from the base of cut rice leaves in an outdoor plot or a growth cabinet such as wheat[5], the leaves wilted and stomata closed within about 15 minutes after cutting (data are not provided). It was difficult to inject a constant amount of solution into a leaf sheath with a syringe with

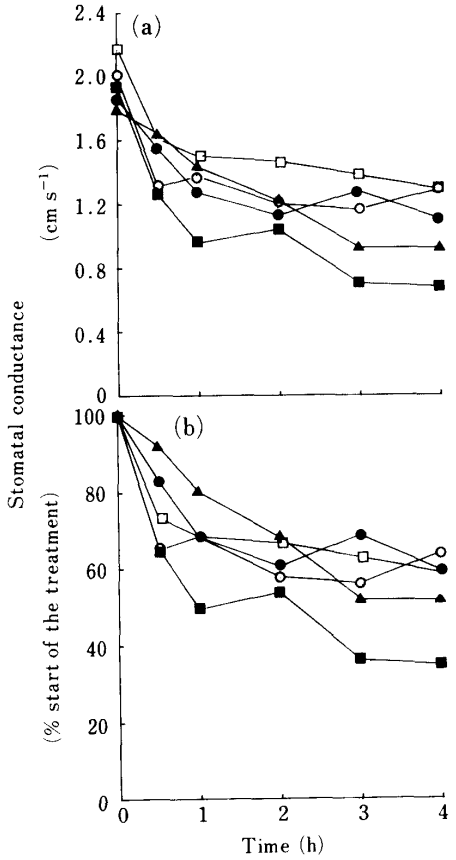


Fig. 5. Absolute (a) and relative (% start of the treatment) (b) value of stomatal conductance at various times in controlled (●), 10^{-3} (■), 10^{-4} (▲), 10^{-6} (○) and 10^{-8} (□) mol L^{-1} ABA treated leaf. Data indicate means of three replicates. Error bar are not shown for clarity of presentation.

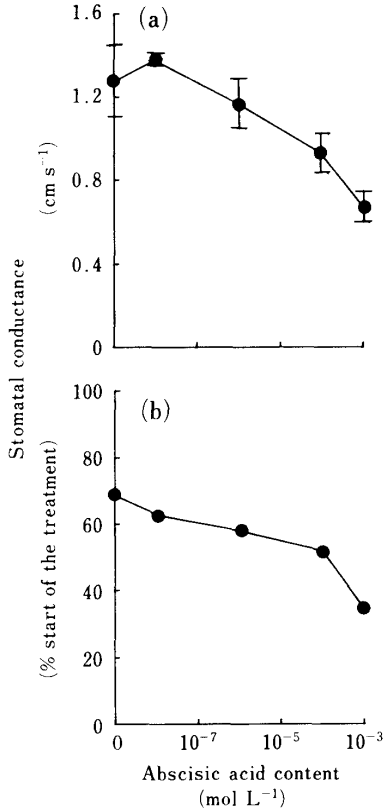


Fig. 6. Effect of ABA on stomatal conductance of absolute (a) and relative (% start of the treatment) (b) in leaves 3 hours after the feeding from a leaf tip. Values are means ± standard error of three observations in (a).

a needle with 0.4 mm diameter because there was a high flow resistance to the injection and the solutions sometimes leaked from the side of the needle. However, it is possible to apply ABA solutions to rice leaves and observe changes of stomatal conductance during long intervals under high evaporative, natural light conditions using this method.

Cutting the leaf tip seems to reduce stomatal conductance at first, but the stomatal aperture recovers completely ten minutes later. We suggest that ABA solution moves depending on the transpiration stream and on differences in water potential between portions of a leaf resulting from transpiration. Stomata at the edge of the leaf may close earlier than those on the mid-portion because ABA solution moves easily from the vial to the edge depending on a higher leaf water potential gradient between the vial and the edge than the vial and the mid-portion. And the amount of water taken up by a leaf from the vial increased depending on the transpiration rate. These results suggested that the transpiration rate from leaves affects the amount of solution fed from the vial[7]. High transpiration rate may accelerate suction of the solution and cause high responses of stomatal conductance. Thus the effect of solutions in stomatal conductance has to be compared with control leaves fed water or sap from non-treated plants under the same environmental conditions as used in other assay methods[5,6]. The method may give more reliable results if the assay is done under stable evaporative demand such as a growth chamber having a constant high photon flux density over 1000 μmol cm^2 s^{-1} which is needed for stomatal opening of rice[4] and controlling vapour deficit.

Stomata appear to respond to very low amounts of ABA, since stomata closed after contact with the solution even at low concentration (10^{-8} mol L^{-1}) and in solution in

which only a small percentage of the transpiration rate was ABA solution taken up from the tip. Variation in stomatal conductance was observed after treatments in some cases. Stomata closing may not be uniform in the middle of the leaf where stomatal conductance was measured several hours later, since the effect of ABA solution appears first at the edge and progresses to the midrib of the leaf. A small change in the portion of the leaf measured with a porometer may cause the variation. In addition we need to measure stomatal conductance at least three hours after the start of the treatment to establish the effects of the solutions when the middle portion of the leaf is measured. More hours may be needed for stomata closure of a whole leaf although complete stomata closure of the whole leaf need not only be a gauge for the effect of the solutions. However if we can measure the stomatal conductance of those portions of the leaf nearer to the tip, it may be possible to detect the stomatal response sooner, and with greater accuracy and less variation.

We conclude that the assay method helps to define stomatal response to xylem sap or inhibitor solution such as abscisic acid of plants which suffer drought or any stress using natural standing crops under outdoor or high transpirational conditions.

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References

1. Austin R.B., I.E. Henson and S.A. Quarrie 1982. Abscisic acid and drought resistance in wheat, millet, and rice. In Drought Resistance in Crops with Emphasis on Rice. IRRI, Los Baños. 171—180.
2. Davies, W.J. and J. Zhang, 1991. Root signals and the regulation of growth and development of plants in drying soil. Ann. Rev. Plant Physiol. Plant Mol. Biol. 42 : 55—76.
3. Ishihara, K., T. Nishihara and T. Ogura 1971. The relationship between environmental factors and behavior of stomata in the rice plant. 1. On the measurement of the stomatal aperture. Proc. Crop Sci. Soc. Jpn. 40 : 491—496.
4. ———— and K. Saito 1987. Diurnal courses of photosynthesis, transpiration, and diffusive conductance in the single-leaf of the rice plants grown in the paddy field under submerged condition. Jpn. J. Crop Sci. 56 : 8—17.
5. Munns, R. and R.W. King 1988. Abscisic acid is not the only stomatal inhibitor in the transpiration stream of wheat plants. Plant Physiol. 88 : 703—708.
6. Oritani, T. 1990. Chapter 5 Physiological function of growth regulating substances. 4. Abscisic acid. In Matsuo, T. et al. eds., Compiled Monograph of Rice. Nobunkyo, Tokyo. 137—143.
7. Palta, J.A., I. Fillery, E.L., Mathews and N.C. Turner 1991. Leaf feeding of [^{15} N] urea for labeling for wheat with nitrogen. Aust J. Plant Physiol. 18 : 627—636.