Studies on Silicification of Epidermal Tissues of Grasses as Investigated by Soft X-ray Image Analysis

I. On the method to detect and calculate frequency of silica bodies in bulliform cells*

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Received April 30, 1983

It has been reported by TAKEOKA and SHIMIZU⁸⁾ that epidermal tissues of the lemma of the rice plant were transformed in a manner similar to that of the foliage leaf at a time prior to their gross morphological change under the influence of spikelet proliferation. In that paper, it was shown that structural changes of epidermal tissues were of considerable significance for the development and adaptation of the rice crop to the surrounding environment. We therefore considered it necessary to study rice epidermal tissues from the following two points of view: (1) structural changes in the epidermal surface and (2) tissue silicification. These points of view are important because the surface structure of plants is influenced directly by the external environment. One such structural feature is the silicification of epidermal tissues; this includes the formation of silica bodies in bulliform cells of rice leaves, especially in relation to transpirational regulation¹⁵⁾ and to increasing blast disease resistance^{3,5)}.

The present studies were undertaken to elucidate the pattern of silicification of cells and tissues of grasses using the Soft X-ray irradiation combined with Image Analysis Method (SIAM). Several methods employed to detect silica bodies and silicified tissues in the rice plant have already been reported. These include, for example,

staining the tissues with safranin followed by treatment with carbolic acid⁷⁾; the spondogram method¹⁾; spondogram-hydrogen carbon treatment method⁴⁾; electronmicroprobe X-ray analysis method⁶⁾, and a histochemical reaction for silicon with hydrofluoric acid^{9,10,11)}. Those methods, however, require much time and many efforts to prepare the specimens, and in addition, do not allow one to observe extensive areas of tissues or organs. The method, SIAM, is based on a fact that amorphous silica gel, $\text{SiO}_2 \cdot n\text{H}_2\text{O}$ ²⁾ absorbs X-ray energy more markedly than epidermal tissues do, causing more clear transparency on the developed X-ray film. Thus, the objectives of this paper are two-fold: to report a method which was developed by the authors to detect the presence of silica bodies in bulliform cells, and to calculate the areas and distribution for such silica bodies over wide areas of plant specimens with convenience.

Materials and Methods

Fifth leaves of paddy rice plant cultivar 'Tan-ginbozu', and flag leaves of rice cultivars 'Akabo', 'Iwaga', and 'Eiko' were the experimental materials. Plants were grown in 1/5,000a WAGNER pots filled with paddy soil under usual manual condition. The fifth leaves and flag leaves were sampled at the stage of sixth leaf and panicle emergence, respectively. Those of cultivars were selected, based on an analysis of micro-

* An outline of this paper was presented at the 173 rd meeting of the Crop Science Society of Japan, April, 1982.

photographs of more than one hundred of rice cultivars planted. The present soft X-ray method is composed of two processes, here designated as Process I and Process II. The former involves the preparation of specimens for the purpose of microphotographs of silica bodies, and the latter involves an analysis of the microphotographs in order to obtain various kinds of information on silica bodies through the use of an image analyzer. Microphotographs obtained by soft X-ray irradiation rely on differences X-ray absorption, as mentioned above, due to the different material densities causing various degrees of blacking on the X-ray film, thus forming photographic contrasts. Most of the silicon accumulated in the rice plant is in the form of amorphous silica gell, $\text{SiO}_2 \cdot n\text{H}_2\text{O}^{2)}$, which, in our preliminary experiments, showed high X-ray density that made clear negative shadows in the developed film. Details for both processes were as follows; Process I: Leaves were fixed by FAA (solution of formalin, acetic acid, and ethyl alcohol) for five days, then were washed, pressed and dried. The dried specimens were irradiated on enclosed soft X-ray film by the microsource of X-rays, CMR, emitted by the SOFTEX machine (Model M1005NA), using under 8 KeV, 5 mA for one minute. The films were developed and made into photographs. Process II: The microphotographs were subjected to image processing software (Image Analyzer, Photem IBAS, Karl Zeiss) which was previously programmed to allow one to calculate and to treat statistically the distribution and areas of silica bodies. Distribution maps of silica bodies in selected portions of the leaf blade, histograms and statistical analysis of the areas occupied by silica bodies, absolute frequency of silica bodies in a divided section of the leaf blade, and scanning photometry as indicated by reciprocal grey values in both sides of the midrib were also included in the analysis of the microphotographs.

Results and Discussion

Figs. 1, 2, and 3 are sample microphotographs obtained by soft X-ray irradiation for se-

lected portions of the leaf blade of the rice plant. Fig. 1 showed a dense distribution of silica bodies on both sides of the midrib in a flag leaf of cv. 'Akabo', Fig. 2 indicated a remarkable contrast in the distribution of silica bodies on the left and right, respectively, of the midrib in a flag leaf of cv. 'Iwaga'; namely, the right side had very few silica bodies, whereas the left side had many silica bodies. Fig. 3 indicated no silica bodies to be present on both sides of midrib in a flag leaf of cv. 'Eiko'. Figs. 4 to 7 represent samples of figures obtained by IBAS from the microphotographs of selected portions of the fifth leaf blades of cv. 'Tan-ginbozu'. Fig. 4 was a distribution map for silica bodies in selected 4,000 micronmeter length portion of the fifth rice leaf blade. It indicated that there were distributional differences in the density of silica bodies over the area of the leaf blade analyzed. Fig. 5 was a frequency histogram and corresponding statistical analysis showing the variation in number of silica bodies in the different areas examined. It showed that the largest area was $5,000 \mu\text{m}^2$ among the 428 silica bodies observed. Their average are was $841.66 \mu\text{m}^2$ with a standard deviation of 585.964. Fig. 6 was a three-dimensional display of the number of silica bodies in each section of which $6,000 \mu\text{m}$ length and $4,000 \mu\text{m}$ width of a selected portion of leaf blade was divided into 400 ($=20 \times 20$). This figure showed clearly the distributional difference for silica bodies according to the position of the section. Fig. 7 was a scanning photometry graph, indicating the reciprocal grey values obtained when making a transverse scan across a microphotograph of the leaf blade. Since the blackness of large vascular bundles in the midrib region was removed by the IBAS before it scanned, the reciprocal grey values here were relatively higher than for other portions of the blade. These, the middle large peaks in this figure, were those of large vascular bundles in the midrib area.

According to the method we reported here, it is easier to prepare specimens and to make microphotographs than with other

Fig. 4. A figure obtained by operation of a image analyzer, IBAS, showing distribution of silica bodies in selected 4,000 μm length portion of fifth rice leaf blade, cultivar 'Tan-ginbozu'. The blackness of large vascular bundles in the midrib portion was removed previously by IBAS.

methods cited earlier. It also is possible to put several leaf blades on one soft X-ray film at the same time to take a microphotograph because the area which the soft X-ray irradiates is about 12 cm diameter of circle. Moreover, from these X-ray micrographs, one is able to easily use

IBAS and statistics analysis based on the areas of distribution of silica bodies. Further, with IBAS, it may be possible to obtain similar information on other cells or tissues; for example, the thickness and blackness of vascular bundles, the range of costal zones and their blackness as compared with silica bodies. The leaves used in the experiments reported here were dried, and the microphotographs were paradermal ones. But, it is also easy to obtain microphotographs and to make image analyses based on cut sections, thus allowing one to detect the degree of silicification throughout the several tissues of the leaf (epidermis, mesophyll, sclerenchyma bundles, vascular bundles). Using this method, we are now undertaking several experiments on the silicification of different epidermal tissues, making a comparison among cultivars or ecological varieties of rice, as well as among different genera of Poaceae, and the influence of environmental conditions on them. We also are undertaking experiments on the surface structures of epidermal tissues. This series of investigations, in addition to

Fig. 5. A figure obtained by operation of image analyzer, IBAS, showing frequency histogram and statistics for the different classes of areas represented by silica bodies in a selected portion of fifth rice leaf blade, cultivar 'Tan-ginbozu'.

- Fig. 1. Microphotograph of a selected portion of leaf blade (lamina) of rice plant obtained by soft X-ray irradiation, showing a dense silica distribution of silica bodies (arrow) on both sides of the lamina midrib (M). The name of the rice cultivar is 'Akabo' (x 17).
- Fig. 2. Microphotograph of a selected portion of the rice plant leaf blade obtained by soft X-ray irradiation, showing very few silica bodies (arrow) at the right side of the midrib as compared with many present on the left side of the midrib (M). Cultivar is 'Iwaga' (x 17).
- Fig. 3. Microphotograph of a selected portion of the rice plant leaf blade obtained by soft X-ray irradiation. It shows no silica bodies to be present on both sides of the midrib (M). Cultivar is 'Eiko' (x 17).

Fig. 6. A figure obtained by-operation of a image analyzer, IBAS, showing distribution of silica body frequencies indicated by three dimensional plot in each divided section of a selected portion of fifth rice leaf blade, cultivar 'Tan-ginbozu'.

Fig. 7. A figure obtained by operation of a image analyzer, IBAS, Showing scanning photometry as indicated by reciprocal grey value in both sides of midrib in a selected portion of fifth rice leaf blade, cultivar 'Tan-ginbozu'. Note; BC: Bulliform cell containing silica body (-ies) inside, LV: Large vascular bundle.

the present studies, are bring performed in order to elucidate the problem of epidermal tissue formation and the adaptation of the epidermis of rice leaves and that of leaves of other grasses to the surrounding environment.

Acknowledgements

The present work was supported partially by a research Grant-in-aid from the Ministry of Education, Japan (No. 56480029). The authors wish to express their thanks to Dr. T. WADA, Nagoya University, for his discussion and advice on this subject. We also would like to thank Mr. T. KOGA and Mr. F. ISHIDATE of Karl Zeiss, Inc. for their support in the operation of the image analyzer, Photem IBAS.

References

1. AKAI, S. 1938. On the figures of leaves of the rice plant grown under a combination-

practice of several effective measures for the control of blast disease. Ann. Phytopath. Soc. Japan **7**: 173—191.

2. ILER, R. 1979. The Chemistry of Silica. John Wiley and Sons, Inc. New York.
3. KAWASHIMA, R. 1927. The preventing effect of SiO_2 for the "Imochi" sickness of paddy rice. Jour. Sci. Soil Mann. **1**: 86—91.**
4. KIDO, M. and YANATORI 1964. Studies of silicific tissues of leaf in rice plant by spondogram-hydrogen chloride treatment method. Proc. Crop Sci. Soc. Japan **33**: 115—118.**
5. MIYAKE, K. and M. IKEDA 1932. The relation between addition of silica and *Dactylaria* disease of rice plant. Jour. Sci. Soil. Mann. **6**: 53—76.**
6. SONI, S.L., P.B. KAUFMAN and R.A. JONES 1972. Electron microprobe analysis of the distribution of silicon and other elements in rice leaf epidermis. Bot. Gaz. **133**: 66—72.
7. SUZUKI, H. 1937. Studies on the relation between the anatomical characters of the rice plant and its susceptibility to blast disease. Jour. Coll. Agric. Tokyo Imp. Univ. **14**:

- 181—264.
8. TAKEOKA Y. and M. Shimizu 1980. Vegetative proliferations of floral spikelets in *Oryza sativa* L. IX. Transformation of lemma to foliage leaf according to the proliferation. Japan. Jour. Crop Sci. **49**: 81—87.*
9. YOSHIDA, S., Y. OHNISHI and K. KITAGISHI 1962. Histochemistry of silicon in rice plant. I. A new method determining the localization of silicon within plant tissue. Soil. Sci. Plant Nutr. **8**: 30—35.
10. ——— 1962. ———
- II. Localization of silicon within rice tissues. Soil Sci. Plant Nutr. **8**: 36—41.
11. ——— 1962. ———
- III. The Presence of cuticle-silica double layer in the epidermal tissue. Soil. Sci. Plant Nutr. **8**: 1—5.
12. ——— [1965. Chemical aspects of the role of silicon in physiology of the rice plant. Bull. Nat. Inst. Agr. Sci. **B 15**: 18—58.*
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- * In Japanese with English summary.
- ** In Japanese.

〔和 文 摘 要〕

軟X線・画像解析法による草本表皮系組織の珪質化に関する研究

第1報 機動細胞珪酸体の検出と測定方法について

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作物の恒常的な発育を保つ上で外気に接する表皮系組織の果たす役割は大きい。表面構造の変化と珪質化の面からこの組織の構造と機能を明らかにする目的で研究を行い、機動細胞に形成される珪酸体を迅速、簡便かつ広範囲に測定する方法を検討した。その結果、非晶質ゲル (amorphous gel, $\text{SiO}_2 \cdot n\text{H}_2\text{O}$) の形をとる珪酸体が軟X線をよく吸収する性質を利用して、葉内に分布する珪酸体の鮮明画像を得ることが可能になった。本法は次の二つの過程から成る。すなわち、1) 試料の調整から葉内の珪酸体画像を得る過程、2) 得られた画像写真から珪酸体の葉内分布パターン、面積などを計測する過程。

実験には土耕栽培による水稻品種短銀坊主の第5葉と、同じく赤坊、岩賀、栄光の各止葉を供試した。

過程1：採取した葉身をホルマリン・アルコール・酢酸混合液で5日間固定したのち水洗し、アイロンで乾燥する。この標本を黒色ビニール袋に密封したFG型フィルム上にのせ、8 KeV, 5 mA に調整した軟X線照射装置 (ソフテックス, M-1005 NA 型) のマイクロ照射部で1分間照射する。レンドールにより現像したネガフィルムを焼付けして珪酸体画像を得る。

過程2：珪酸体の画像写真を対話式画像解析システム (Photem IBAS, Zeiss) にかけて、調査項目を指定して必要とする図表を描かせる。

この方法による利点は、1) 植物組織の珪酸 (体) 同定のために開発された従来の灰像法、組織化学などの方法に比べてはるかに簡便迅速に試料調整ができる。そのため多数の試料を比較的短時間に処理することができる。2) 広範囲にわたる面積を撮影できるため葉身1枚の珪酸体分布を測定することができる。3) あらかじめプログラミングをしておけば画像解析システムにより分布密度、面積などの測定と統計処理を迅速に行うことができる。4) 組織切片の軟X線画像から珪酸体及び類似性物質の組織内分布を調べることができる。