

# Application News

## No. S52

Confocal Laser Scanning Microscope LSM  
Scanning Probe Microscope SPM / Atomic Force Microscope AFM

### Shape Observation and Hardness Evaluation of Soybean Leaf Trichomes by LSM and SPM

Soybeans are known to have different resistances to the larva of the common cutworm (*Spodoptera litura*), which is a type of herbivorous vermin, depending on the variety of the soybean plant. As one factor that governs resistance to this insect pest, the microscopic hairs called "trichomes" which grow from the surface of soybean leaves are considered to play a key role. Grasping the distinctive features of trichomes that display strong resistance can provide an indicator for the direction of future selective breeding. Therefore, in this study, the length, angle, and radius of the trichomes of four varieties of soybeans were measured using a confocal laser scanning microscope (LSM), and the hardness (amount of deformation with respect to load) of the trichomes was measured using a scanning probe microscope (SPM).

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#### ■ Shape Observation of Trichomes by LSM

The length, angle, and radius of the trichomes of four varieties of soybeans (IAC 100, Himeshirazu, Tamahomare, Hougyoku) were measured. Fig.1 shows an example of measurement of the length and angle, and Fig. 2 shows an example of measurement of the radius. In the 2-dimensional image (intensity image), the image of the entire trichome and the profile of the fine structure of the surface can be observed, and in the 3-dimensional image (height image), the size can be measured.

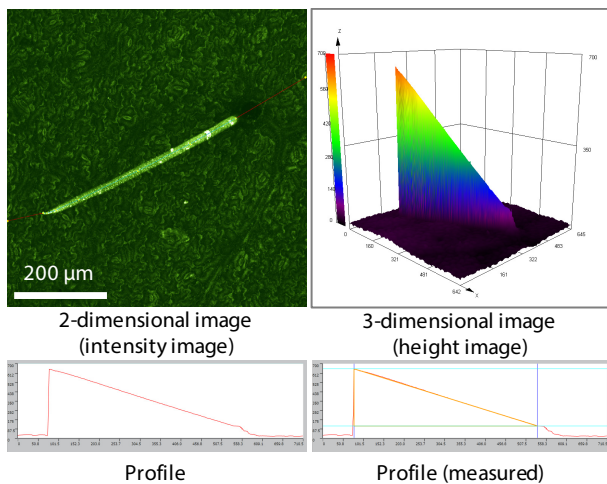


Fig. 1 Example of Measurement of Length and Angle

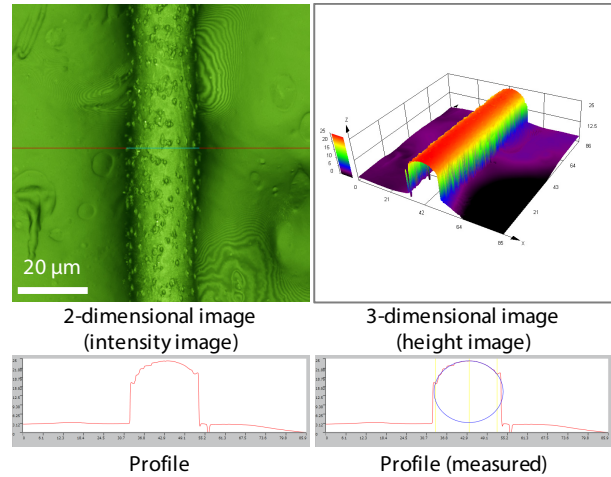


Fig. 2 Example of Measurement of Radius

Table 1 shows the results of the measurements of the length, angle, and radius. The length and angle are the average values of 15 trichomes of each variety of soybean, and the radius is the average of nine trichomes. From the measurement results, it can be understood that resistance increases as the length of the trichomes becomes longer and the angle becomes closer to the perpendicular. No correlation between resistance and the trichome radius was found, as the radius did not vary greatly among the four varieties.

Table 1 Measurement Results of Length, Angle, and Radius of Trichomes

	Resistance	Length [μm]	Angle [°]	Radius [μm]
IAC 100	<b>Strong</b>	758.2	50.8	10.3
Himeshirazu		680.3	35.1	10.3
Tamahomare		577.1	6.0	12.4
Hougyoku		<b>Weak</b>	547.3	3.2

### Evaluation of Trichome Hardness (Deformation with Respect to Load) by SPM

The hardness of the trichomes was evaluated by force curve measurements by the SPM. The sample trichomes were fixed lying flat on a smooth plate. The probe of the SPM was then pressed vertically onto the fixed trichome, and the amount of deformation of the trichome when pressed with the probe was measured. Fig. 3 shows the measurement procedure.

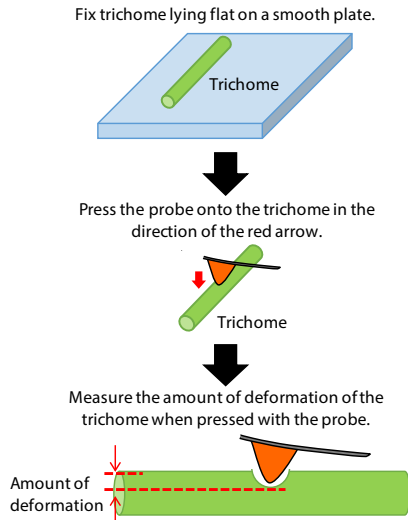


Fig. 3 Measurement Procedure

Fig. 4 shows the load-deformation curves obtained from the force curve measurements. The ordinate shows the load (force) applied to the trichome, and the abscissa shows the amount of deformation of the trichome.

When a load of the same magnitude is applied, the largest deformation occurs in the IAC 100 trichome, followed by the Himeshirazu trichome, whereas the Tamahomare trichome shows little deformation in comparison with the other two samples. In other words, the results show a trend in which resistance to the larva of the common cutworm, a type of herbivorous vermin, is higher in softer, more flexible trichomes (trichomes which deform flexibly under load).

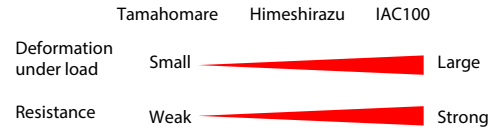
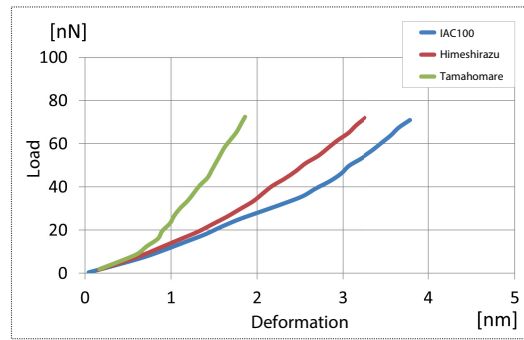


Fig. 4 Load-Deformation Curves and Relationship of Deformation and Resistance

### Conclusion

Trichomes are considered to be one factor that governs the resistance of soybeans to the larva of the common cutworm, which is a type of herbivorous vermin. Therefore, the length, angle, radius, and hardness (amount of deformation with respect to load) of the trichomes of four varieties of soybean plants were measured. As a result, it was possible to identify the length, angle, and hardness characteristics of trichomes that display stronger resistance.

Samples provided by:

Prof. Naoki Mori, Kyoto University Graduate School of Agriculture, Division of Applied Life Sciences  
Ministry of Education, Culture, Sports, Science and Technology (MEXT) Coordination Section, Grant-in-Aid for Research on Innovative Areas, "Innovative materials technology based on biodiversity"

<Reference>

R. Nakata, Y. Kimura, T. Yamamoto, S. Moriguchi, N. Yoshinaga and N. Mori: Induced Chemical and Physical Properties of Trichomes in the Resistant Soybean Leaves Against Spodoptera Litura Larvae, Shimadzu Hyoron, Vol. 73, No. 1-2 (Sep. 2016), 29-39

### Reference (Force Curve Measurement)

In evaluating hardness (amount of deformation with respect to load) in this experiment, force curves were measured by SPM. Fig. 5 shows the principle of operation of force curve measurement. The hardness evaluation was conducted in the portion indicated by ③.

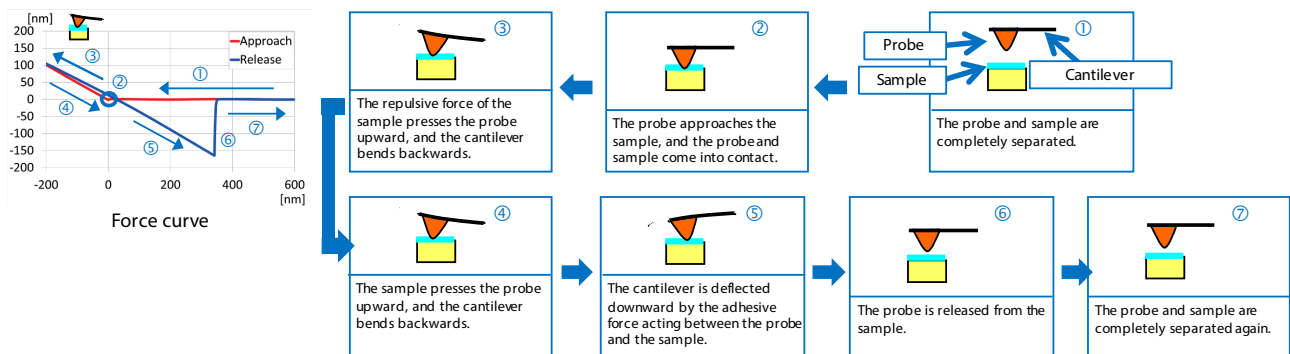


Fig. 5 Principle of Operation of Force Curve Measurement

First Edition: Sep. 2020



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