Field manual for the rapid estimation of cowpea leaf stomatal conductance

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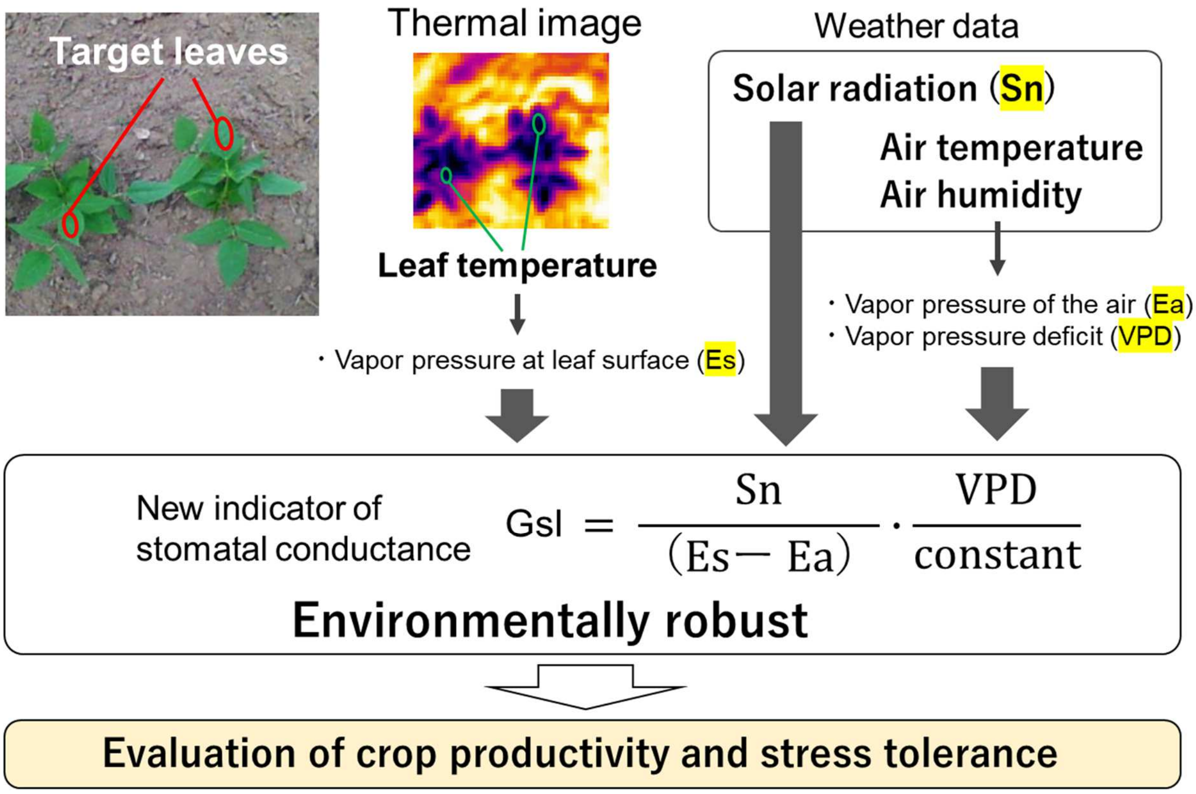
Transforming African Agriculture

Summary



A thermal camera can be easily applied to measure leaf temperature-based indicators, such as stomatal conductance (g_s), in large plant populations consisting of different varieties and under different treatments. However, a major difficulty in measuring these indicators is their fluctuation under different meteorological conditions. In this study, a new indicator of g_s, GsI, was developed. The calculation of GsI includes the following factors: leaf and air temperatures, relative humidity, and solar radiation. The leaf temperature of the cowpea plants can be measured using a low-cost thermal camera to ensure a cost-effective method. Relative to the actual (measured) g_s, GsI proved to be more stable than other indicators of stomatal conductance—such as the temperature difference between leaf and air—irrespective of solar radiation, air temperature, and relative humidity conditions. As no reference temperature is needed for the calculation of GsI, it can be easily applied to large populations of plants.

The three steps of measurement

- Take a thermal image using the name board
- Obtain the leaf temperature from the software
- Calculate the GsI

1. Introduction

Drought stress is one of the most common yield constraints in cowpea production. Leaf stomatal conductance is widely used as an indicator of plant water status and growth, especially under mild to moderate drought stress conditions, in which a higher stomatal conductance is closely related to improved yield. Recent improvements in genotyping technologies demand phenotypic information for large numbers of plants from cross-populations, breeding materials, and genetic resources. Remote-sensing technologies are available to meet this demand, and infrared thermal imaging is now widely used for the evaluation of stomatal conductance and transpiration rates at a scale varying from a single leaf to the canopy. Several indicators of stomatal conductance using thermal imaging have been developed, such as the air-leaf temperature difference (T_a-T_s), crop water stress index (CWSI), and standard deviation of canopy temperature (CTSD). A difficulty with these indicators is that the relationship between leaf temperature and stomatal conductance may vary strongly with variations in solar radiation, air temperature, humidity, and wind speed. Therefore, it is difficult to estimate stomatal conductance from leaf temperature over a wide range of meteorological conditions. Meteorologically robust, rapid, and simple indicators are desirable for the evaluation of stomatal conductance in field studies. In this study, a new indicator for stomatal conductance, GsI, was developed. This indicator is the result of a modification to the theoretical equation for stomatal conductance, as it aims to simplify the calculation by making several assumptions.

2. Equipment

Figure 1. FLIR C2 thermal camera

Several types of thermal cameras are available for the evaluation. Recently, the cost of infrared thermal cameras has decreased, and an inexpensive model is available at less than 500 USD. The FLIR C2 and C3 models (FLIR Systems, Wilsonville, USA) are examples (Figure 1) of inexpensive thermal cameras that scan wavebands at 7.5–14-μm intervals with an image size of 80 × 60 pixels. The size of this device is 12.5 cm (width) × 8 cm (length), and the weight is

130 g, making it suitable for field operations. The resolution of the temperature detection is 0.1 °C. The emissivity should be set to 0.99. The original thermal images are automatically converted to a high-contrast images through multi-spectral dynamic imaging (MSX), which enables the evaluators to easily detect the temperature data of the target point (Figure 2). The battery is rechargeable and lasts for several hours. Full charging is recommended before the measurement.

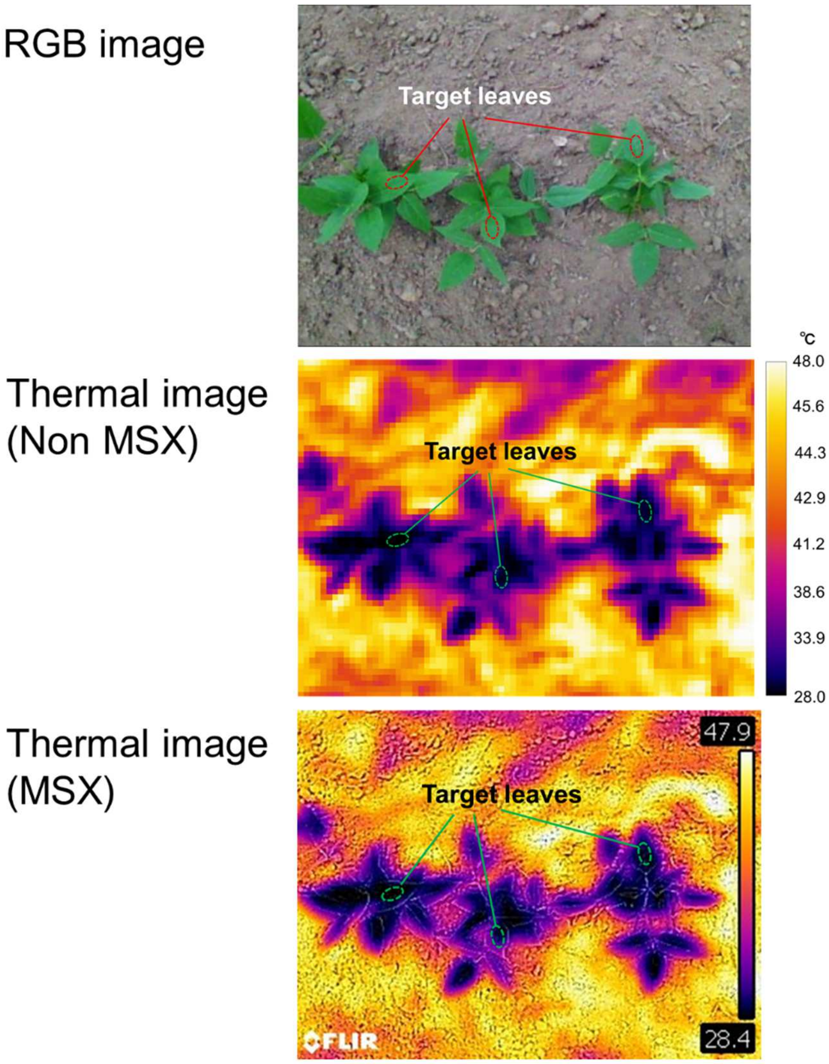


Figure 2. RGB image (above), thermal image with non-MSX (middle), and thermal image with MSX (below).

In our field trials, we found that because we targeted single leaves on each sample plant at a close range, an image of 4,800 pixels was sufficient for leaf temperature determination. Therefore, although several types of high-resolution thermal cameras are commercially available, we used a low-cost thermal camera with a relatively low resolution (60 × 80 pixels). Additionally, the use of such low-cost cameras allows the method to be used for trait evaluations—not only as part of basic scientific studies but also for local agronomic and breeding programs, especially in developing countries. Furthermore, multiple low-cost thermal cameras can be used simultaneously to reduce the evaluation time; however, in this case, temperature calibration is required to adjust the reading temperature among the different cameras.

3. Field application

3-1. Preparation

- Thermal camera

Many models of thermal cameras are available, but those that are small and light are most suitable. (FLIR C2 was used here). The battery should be fully charged before use.

- Name board or sample tags

The names of the target sample or plot need to be included in the photographed image. This is because FLIR C2 does not have the option of adding remarks for each image (Figure 3). The leaf temperature data should correspond to the sample or plot names in the data analysis.

- Steps or ladders (if necessary)

The thermal image should be taken from 1 to 1.5 m above the target leaf. If the target plant has an elect plant shape, steps or ladders are needed to maintain a 1-m distance during the later growth period.

- Weather station

Automatic weather station to record solar radiation, air temperature, and relative humidity. Recording interval of 5-10 minutes is preferable but day average data is also substitutable.

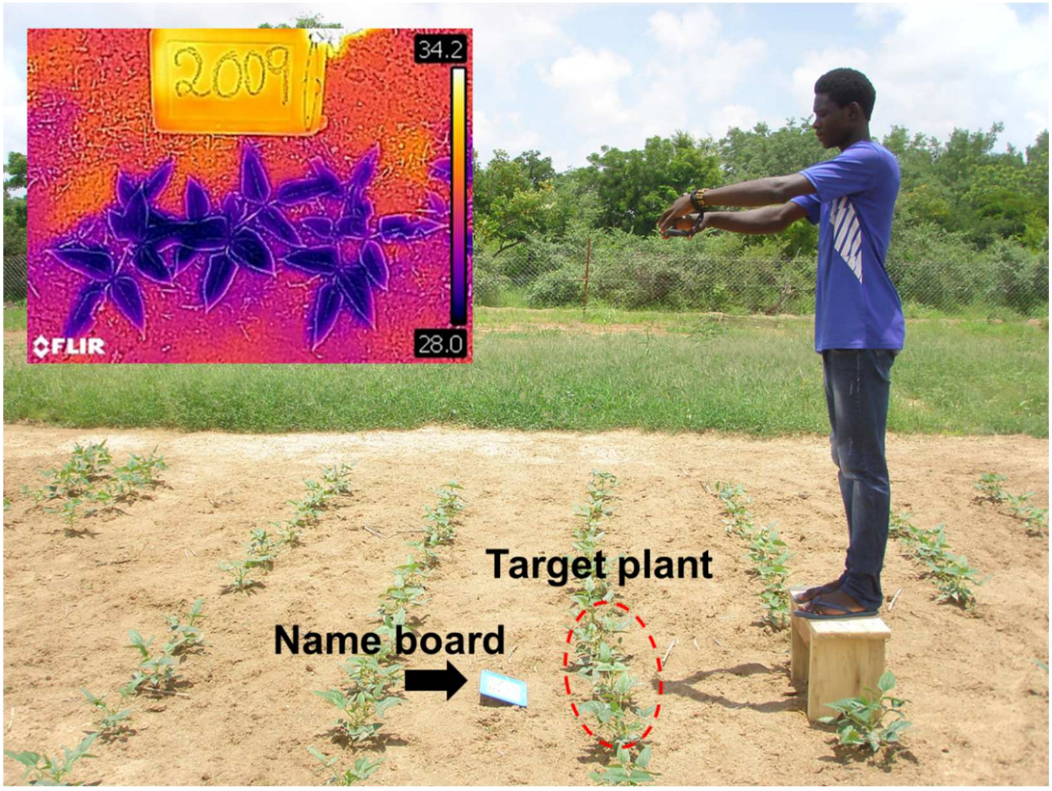


Figure 3. Acquiring thermal image in the field

3-2. Measurement

In the field trial, infrared thermal images of the cowpea plants were taken with a thermal camera between 10:00 and 12:00 h. Three contiguous plants were randomly selected within an experimental plot, and the corresponding thermal images were taken from a distance of 1–1.5 m above the plant for a resolution of 0.9 cm per pixel (Figure 3). The temperature at the central point of a fully expanded trifoliate leaf was obtained for each of the three plants from a thermal image using dedicated software (FLIR Tools, Figure 4).

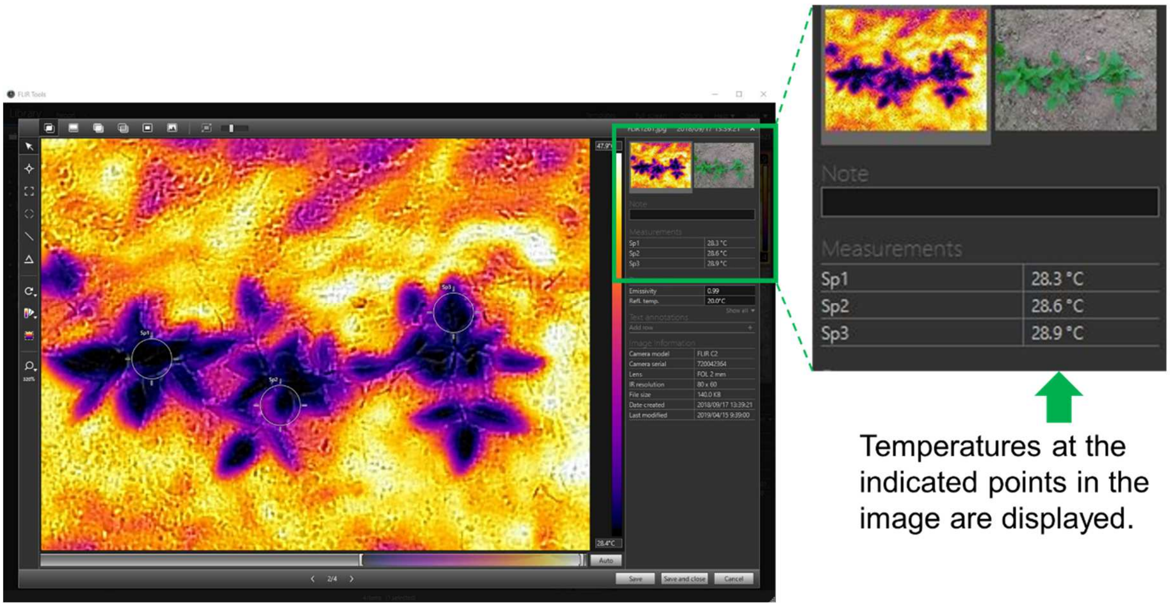


Figure 4. Image analysis using the FLIR Tools software

The meteorological conditions (air temperature, relative humidity, and solar radiation) at the time of photography are needed for the GsI calculation. These parameters can be measured at a weather station installed at the experimental site. The 5–10-min interval records are desirable, but the day average is also applicable if short-interval data are not available.

3-3. Calculation of GsI

The GsI is calculated using the following equation:

$$\text{Stomatal condustance } (g_s) \propto \text{GsI} = \frac{R_s}{(e_s - e_a)} \cdot \frac{\text{VPD}}{C_p\ \rho} \qquad (1)$$

where R_s and VPD are the solar radiation ($W \cdot m^{-2}$) and vapor pressure deficit (hPa), respectively, and e_s and e_a are the vapor pressure (hPa) at the leaf surface and the surrounding air, respectively. The $C_p\rho$ is volumetric heat capacity of the air, and is used as a constant value of

1216 J m^{-3} °C. The term e_s-e_a is obtained from the saturated vapor pressure at leaf temperature (D_{T_s}; hPa), air temperature (D_{T_a}; hPa), and relative humidity (RH; %) using Equation 2. The saturated vapor pressure at temperature T (D_T; hPa) is calculated using Equation 3. The VPD is calculated from Equation 4.

$$e_s - e_a = D_{T_s} - D_{T_a} \cdot \frac{RH}{100} \quad (2)$$

$$D_T = 6.108 \exp\left(\frac{17.3\,T}{237.3+T}\right) \quad (3)$$

$$VPD = D_{T_a}\left(1 - \frac{RH}{100}\right) \quad (4)$$

The relationship between GsI and stomatal conductance measured using a leaf porometer (SC-1, METER Environment, Pullman, USA) is shown in Figure 5. Stomatal conductance was evaluated for cowpea leaves on days with different meteorological conditions. In comparison with the air-leaf temperature difference (T_a-T_s), the advantage of GsI is its stable relationship with stomatal conductance. In contrast, T_a-T_s showed a strong relationship with stomatal conductance under similar meteorological conditions, but we found that the slope (or constant of the regression) was largely different when the meteorological conditions were largely different. The stable relationship of GsI with stomatal conductance makes it possible to compare GsI values among different environments.

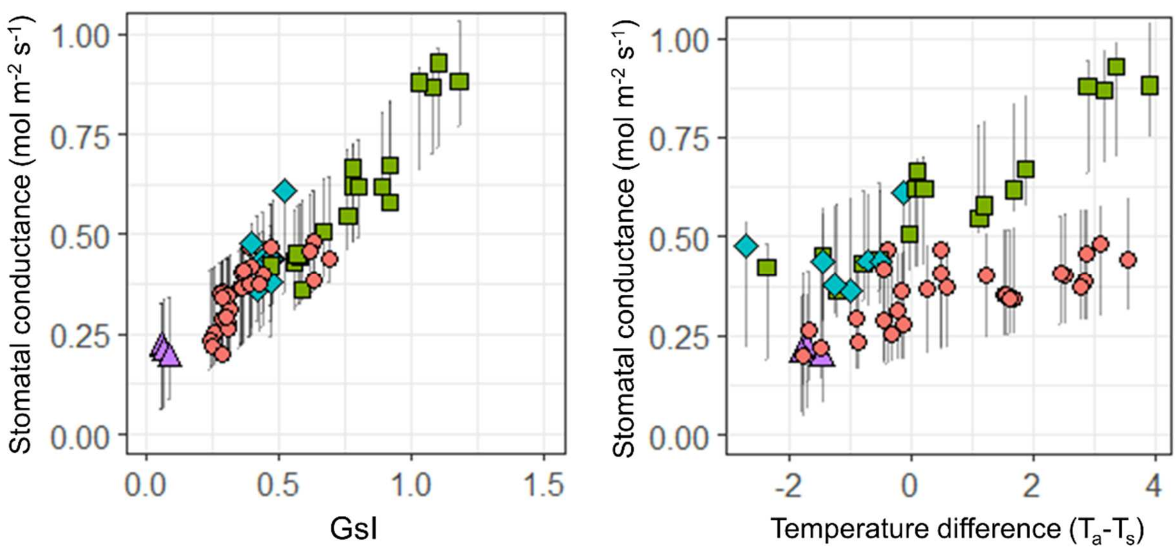


Figure 5. Relationships between stomatal conductance and GsI (left) and the temperature difference (right). Different symbols represent measurement dates with different meteorological conditions. Bars at each point in the scatter plot represent the 95% interval of the predicted distribution.

3-4. Environments not suitable for application

The accuracy of GsI is largely reduced in conditions that result in low stomatal conductance, such as heavy cloud cover and rain. In addition, plants growing under severe environmental conditions are not suitable for GsI evaluation. This is because the effect of wind speed is prominent only for the lower range of stomatal conductance, and this effect becomes much smaller for stomatal conductance larger than 0.2 $mol \cdot m^{-2} \cdot s^{-1}$. Hence, GsI is not suitable for estimating low stomatal conductance, although this issue was not thoroughly examined in this study. GsI is the most accurate estimate in the range from moderate to high stomatal conductance (approximately > 0.2 $mol \cdot m^{-2} \cdot s^{-1}$), in which GsI is a useful indicator for estimating plant growth and water status.

4. Examples of field application

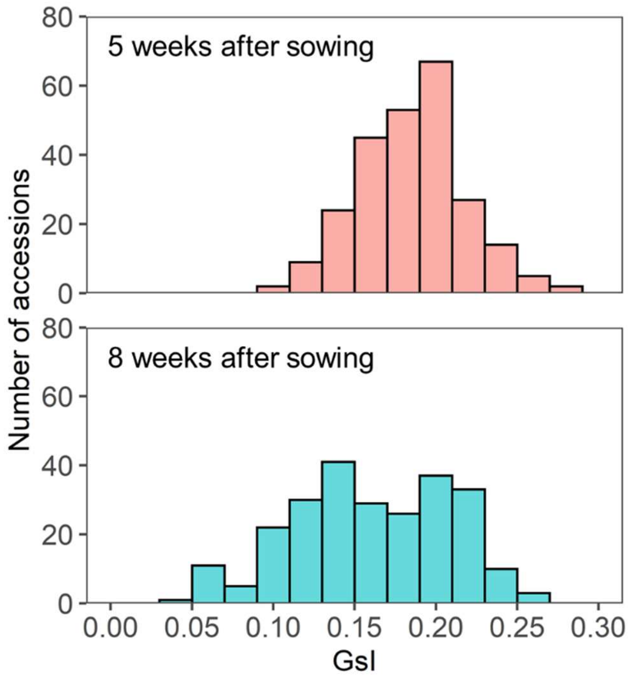


Figure 6. Genetic variability and GsI among 248 accessions of cowpea genetic resources. The distribution of GsI is shown at two vegetative growth stages: 5 weeks after sowing (top) and at the beginning of maturity, 8 weeks after sowing (bottom).

GsI was evaluated for 248 accessions of the cowpea mini-core subset from the world cowpea germplasm collection developed at IITA. Thermal images were taken for 744 plants (248 accessions × 3 replications) at 5 and 8 weeks after sowing, corresponding to the vegetative growth stage and beginning of maturity, respectively. Each image included three plants, and the GsI was separately calculated for each of them corresponding to a total of 4,464 plants. We found large genetic variability for GsI estimates among the 248 accessions of cowpea genetic resources under study (Figure 6). Cowpea is grown in diverse climactic environments, ranging from humid to arid regions; thus, stomatal responses to a given environment are thought to differ depending on the origin of the genotype. GsI values at 8 weeks after sowing tended to be lower than those obtained at 5 weeks after sowing, owing to the onset of plant senescence. Thus, GsI evaluation at the vegetative growth stage is suitable for determining the maximum values of stomatal conductance for each accession.

Another example of time-course changes in GsI in the 12 cowpea accessions is shown in Figure 7. Each point represents the mean of 15 data samples consisting of three plants and five replications. In total, 1,260 plants (15 sample × 12 accessions × 7 time points) were analyzed. Such information is useful for understanding the varietal response of stomatal conductance to changing environments.

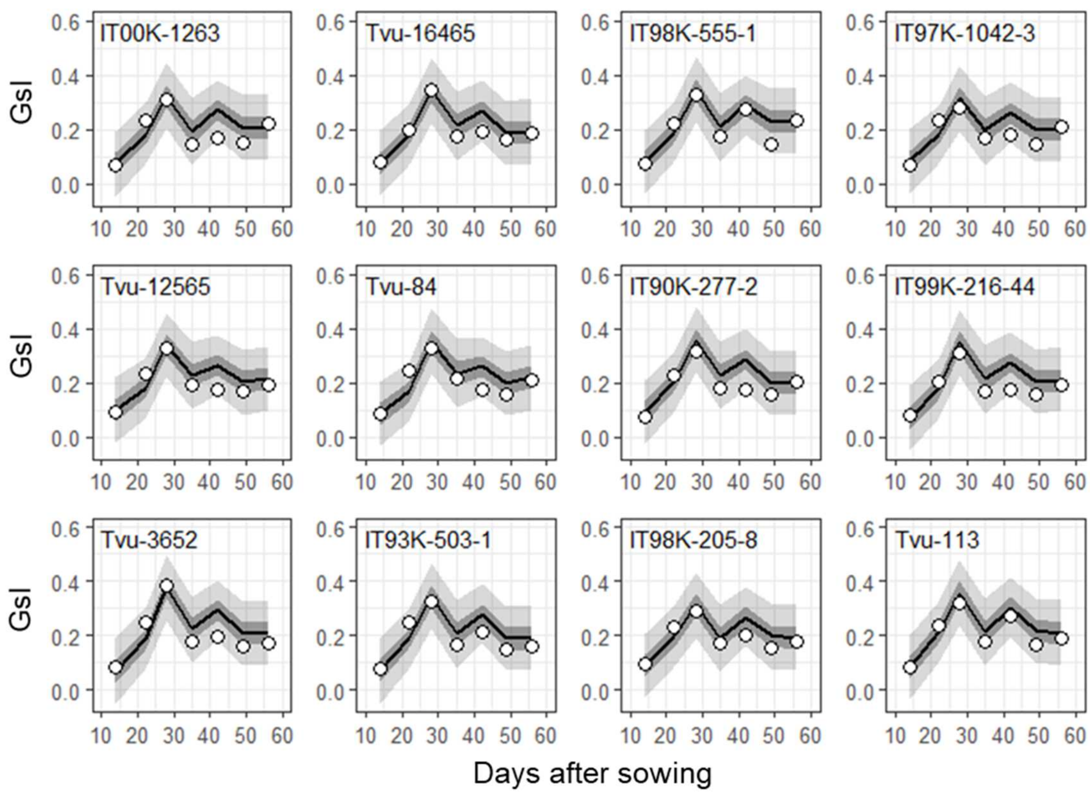


Figure 7. Time-course changes of the GsI in 12 cowpea accessions. Solid line represents the estimation by the state space model. Areas of dark gray and light gray represent the 50% and 95% confidence intervals of the model estimation, respectively.

Publications

Iseki, K., & Olaleye, O. (2019). A new indicator of leaf stomatal conductance based on thermal imaging for field grown cowpea. Plant Production Science, 23(1), 136–147. https://doi.org/10.1080/1343943X.2019.1625273

Iseki, K., & Olaleye, O. (2019). A new indicator of leaf stomatal conductance based on thermal imaging. JIRCAS Research Highlights, https://www.jircas.go.jp/en/publication/research_results/2019_b02

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