## Development of the drought stress experimental system in the field with ridges

The frequency and damage of droughts have been increasing in recent years, threatening the world's food supply. To develop drought-tolerant crops, many drought studies have been conducted, mainly in the laboratory, and the drought stress response mechanisms of plants have been elucidated at the molecular level. On the other hand, it has been pointed out that the drought stress response of plants in the field differs in some respects from the response mechanisms that have been elucidated in the laboratory, and there are still many unknowns. In the development of drought-tolerant crops, it is essential to conduct drought tolerance tests and elucidate the drought response mechanisms of plants in the field, but it is not easy to reproduce a constant drought environment in the field where the environment fluctuates irregularly.

To overcome the various problems associated with drought trials in the field, we focused on "ridges." During 6 years of trials, we showed that the volumetric water content (VWC) in ridges (ridge height, 30 cm) was consistently lower than that in the flats (Fig. 1A). We also demonstrated that there was no significant difference in the contents of nutrients, nitrogen (N), phosphorus (P), and potassium (K) between the flats and ridges, both at the beginning and at the end of the soybean growing season (Fig.1B). We compared soybean growth in this system. The aboveground biomass of plants grown on ridges was clearly reduced compared with that of plants grown on flats; consequently, the yield of soybean grown on ridges was also reduced compared with that of soybean grown on flats (Fig. 2). The negative effect of ridges on plant growth on the ridges was mainly due to lack of water (Fig. 3). Together, these observations demonstrate that ridges are a valuable tool for inducing conditions that mimic mild drought stress in the field.

Although the height and width of the ridges need to be considered depending on the target plants, soil type, and desired level of drought stress, the developed drought stress experimental system is applicable to fields in various regions of the world and is expected to facilitate the selection and production of drought-tolerant lines.

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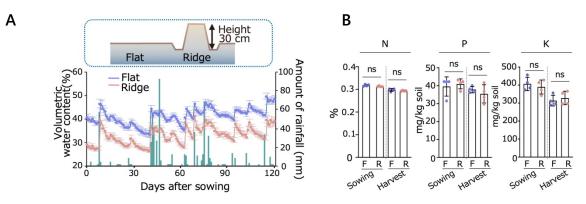
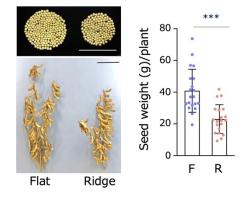


Fig. 1. Soil moisture variability and soil nutrient composition in the field drought stress experimental system with ridges

(A) Time course of soil VWC in the flats (no ridges) and 30-cm high ridges during soybean-growing season. A 30-cm-long soil moisture sensor (TDR) and a temperature sensor were inserted in each test plot, and data were recorded over time by a data logger. n = 4, error bars indicate SD. Green bars indicate precipitation. (B) Nutrient contents of soil in flats and ridges at soybean sowing and harvesting times. n = 4, error bars indicate SD, and ns indicates no significant difference (Student's t-test).



## Fig. 2. Soybean growth in the field drought stress experimental system with ridges

Morphology and yield at harvest of soybean grown in this drought stress experimental system. The left side shows the flats (F), and the right side shows the ridges (R). Bars indicate 10 cm in the pictures. n = 20, error bars are SD, and asterisks indicate significant differences (\*\*\*P < 0.001, Student's t-test).



Irrigation

Rainfed

## Fig. 3. Irrigation treatment for the field drought stress experimental system with ridges

Growth of soybean about 7 weeks after sowing, taken aerially by drone. Ridges are indicated by yellow arrows. The two rows surrounded by dotted lines indicate one replication including the flat and ridge areas, for a total of four replications of the trial. On the right is the rainfed area (no watering) and on the left is the test area that was irrigated to the same test design as the rainfed area on the right (irrigated area). The white box in the center contains the data loggers connected to the soil moisture sensors inserted in each row.

Reference: Nagatoshi et al. (2023) *Nature Communications* 14: 5047. © The Author(s) 2023. The figures were reprinted/modified from Nagatoshi et al. (2023).

