# High water-use efficiency and associated accumulation of leaf metabolites in *Erianthus arundinaceus*

*Erianthus arundinaceus* (hereafter, *Erianthus*), a genetic resource of a closely related genus of sugarcane (*Saccharum* spp.), is believed to contribute to the improvement of drought tolerance in sugarcane through intergeneric crosses. However, there has been insufficient verification of the physiological determinants of drought tolerance in this species through comparison with sugarcane, and no progress has been made in searching for promising traits as selection indicators for drought tolerance. Research is needed on drought tolerance-related traits of this species, focusing not only on the root system but also on above-ground characteristics. Leaf water-use efficiency (WUE), which is the photosynthetic rate divided by the stomatal conductance, is considered to be one of the useful indicators. Here, in addition to WUE, we focus on related stomatal morphology and metabolites.

In a glasshouse at the TARF-JIRCAS, a pot experiment was performed under dry and wet conditions to investigate the response of WUE, primary metabolites in various plant parts by metabolomics, and stomatal morphology in sugarcane cultivar NiF8 and *Erianthus* accession JW630, and to search for useful traits in above-ground parts related to drought tolerance (Fig. 1).

Metabolomics of various plant organs and multivariate analysis (principal component analysis, hierarchical cluster analysis) showed that there is a linkage between parts of the plant with regard to metabolite composition, with interspecific differences and soil moisture effects clearly evident, particularly in the leaves. Regardless of soil moisture conditions, *Erianthus* exhibited higher WUE (Fig. 2a) and had fewer stomata on the lower side of its leaves (Fig. 2b). It also accumulated abundant betaine and γ-aminobutyric acid (GABA) in its leaves, which are involved in stomatal closure and stress responses (Fig. 3).

In addition to the known rooting ability, such as deep rooting, the above-ground leaf characteristics (stomatal density, metabolites) may be useful evaluation indicators with regard to the drought tolerance of *Erianthus*. On the other hand, the causal relationship between the high WUE of *Erianthus* (Fig. 2a) and the leaf traits should be investigated further. As a group of genetically distinct genotypes of *Erianthus* is known to exist, it is possible that there are genotypes that show different physiological responses to the present study, and studies focusing on the diversity of traits in the genetic resource population are underway. In the future, the possibility of simple selection for drought tolerance using these traits (stomatal density, leaf metabolites) as biomarkers will be tested using hybrid populations, etc., to improve the efficiency of drought tolerance breeding through the development of drought tolerance-related genetic markers.

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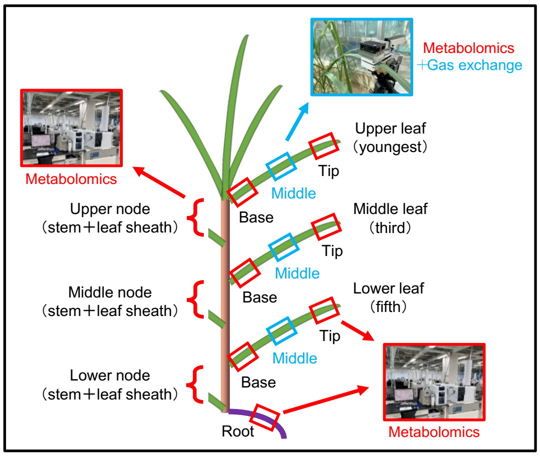


**Fig. 1. Targeted plant parts for analysis in the present study**

Gas exchange characteristics and stomatal morphology were targeted in the middle parts of the upper, middle, and lower leaves. For metabolomics, a total of 16 samples per plant were tested: nine leaves + three stems + three leaf sheaths + one root. All were sampled from the main stem.

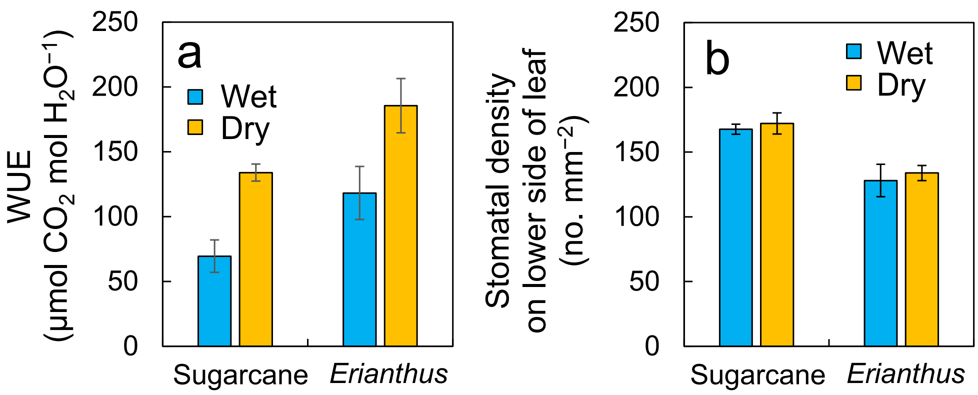


**Fig. 2. Interspecific differences in water use efficiency (WUE) calculated from leaf gas exchange parameters (a) and stomatal density on the lower side of leaf (b)**

Blue and orange bars represent soil wet and dry treatments, respectively. Error bars represent standard deviations (n=4). These are the measured values for the middle-aged leaves. WUE was measured at a light intensity of 500 μmol $m^{-2}$ $s^{-1}$. Interspecific differences are significant for each variable in each treatment (*t*-test, $p<0.01$).

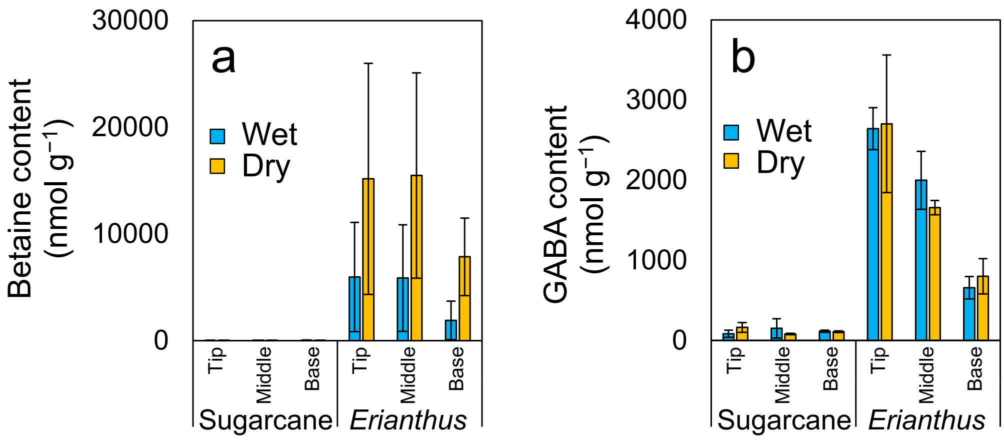


**Fig. 3. Interspecific differences in betaine content (a) and GABA content (b) at different leaf parts (tip, middle, and base).**

Blue and orange bars represent soil wet and dry treatments, respectively. Values are the means of three leaves; error bars represent standard deviations (n=3). Significant interspecific differences are found for each variable in each treatment (*t*-test, $p<0.05$), except for betaine in the wet plot.

Reference: Takaragawa & Wakayama (2024) *Planta* 260: 90.