## Genetic distinctiveness of Japanese native *Saccharum spontaneum* and its importance as a genetic resource for sugarcane improvement

Sugarcane (*Saccharum* L.) is a globally important crop used not only for sugar production but also for bioenergy production to realize a low-carbon society. However, sugarcane production is increasingly threatened by climate change, which is expected to cause greater instability in yields and productivity. To address this issue, further improvement of sugarcane is required; however, breeding has been constrained by the limited genetic diversity of existing cultivars and breeding materials. To overcome these challenges, it is essential to expand genetic diversity and introduce valuable traits by effectively utilizing untapped wild genetic resources.

*Saccharum spontaneum* is widely distributed across tropical and subtropical regions of Asia, including both continental and island areas, and possesses a wide range of useful traits such as stress tolerance and excellent ratooning ability. Modern sugarcane cultivars have been developed through interspecific hybridization between *S. officinarum* and *S. spontaneum*. However, despite the recognized importance of *S. spontaneum*, there has been limited information on the specific contributions of its germplasm to the genetic background of modern cultivars.

In this study, we analyzed the genetic diversity of a total of 390 sugarcane genetic resources collected from all over the world. These included 135 *S. spontaneum* (including four Japanese native accessions), as well as *S. robustum* (45 accessions), *S. barberi* (15 accessions), *S. sinense* (6 accessions), and *S. maximum* (8 accessions), along with 96 modern cultivars (including seven Japanese cultivars).

The analysis revealed that *S. spontaneum* can be classified into three groups based on geographic distribution: Continental Asia Type (e.g., India and Thailand), Southeast Asia Type (e.g., Indonesia and Oceania), and Northeast Asia Type (e.g., Japan, China, and Taiwan) (Figs. 1 and 2). Furthermore, the *S. spontaneum* genomes of the Continental and Southeast Asia Types have made substantial contributions to the genetic background of modern sugarcane cultivars worldwide. In contrast, the contribution of the Northeast Asia Type was extremely limited and detected only in a small number of modern cultivars from Japan and China (Fig. 3). These findings indicate that *S. spontaneum* from Northeast Asia, particularly Japanese native accessions and Japanese cultivars with the genome, has significant importance and potential for expanding genetic diversity and introducing novel traits in future sugarcane breeding.

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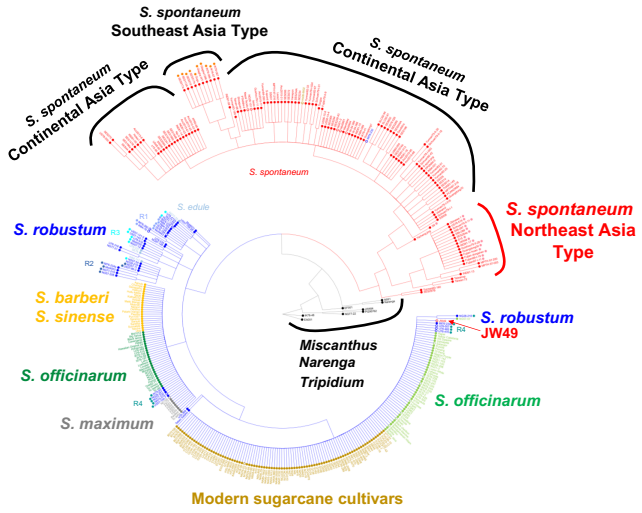


**Fig. 1. Molecular phylogenetic tree of sugarcane genetic resources worldwide based on mitochondrial genome sequences**

The mitochondrial genomes were assembled into single circular sequences using GetOrganelle, and phylogenetic inference was performed using MrBayes.

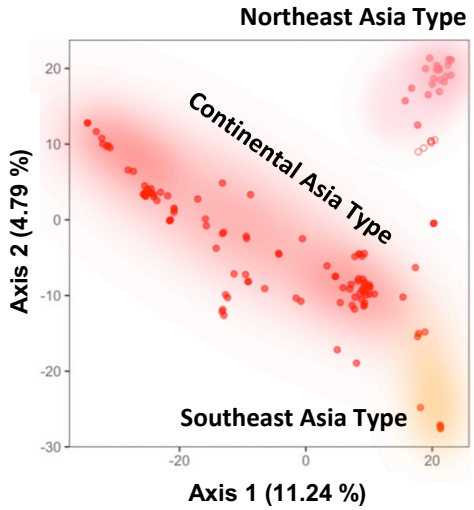


**Fig. 2. Principal coordinate analysis (PCoA) of *S. spontaneum* based on single-nucleotide polymorphism (SNP) data from nuclear genomes**

PCoA is an analytical method used to visualize distances among data points; greater distances between plots indicate greater genetic divergence. Axis 1 explains the largest proportion of variation among lineages, while Axis 2, which is orthogonal to Axis 1, explains the next largest proportion. The values in parentheses indicate the proportion of variance explained.

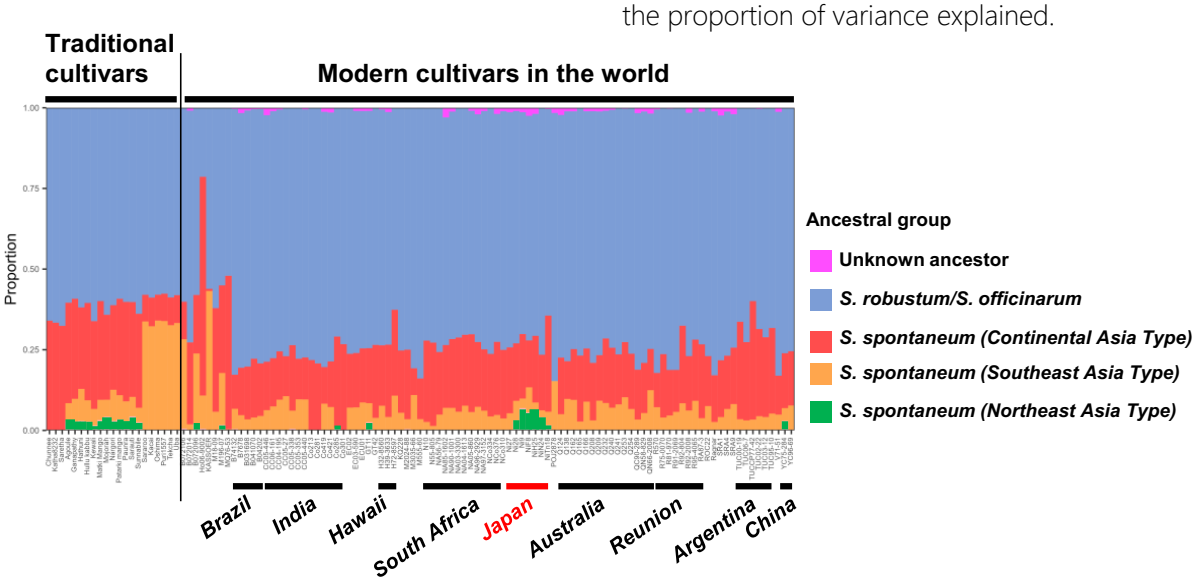


**Fig. 3. Proportions of ancestral species genomes in modern and traditional sugarcane cultivars**

The genomic proportions of each ancestral group were estimated by population structure analysis using species-specific DNA fragments. "Unknown ancestor" indicates previously unidentified ancestral species. Traditional cultivars classified as *S. barberi* and *S. sinense* were cultivated prior to the development of modern cultivars.