Clarifying the genetic diversity of *Amaranthus tricolor* 'Hiyuna,' a traditional Asian vegetable

Amaranthus tricolor L., called 'hiyuna' in Japanese, is used as a traditional leafy vegetable in Asia. A. tricolor is resistant to major diseases, is more tolerant to environmental stresses, and is a rich source of nutrients such as iron and calcium, as well as vitamin C and beta-carotene. Yet despite this diversity and excellent nutritional quality, breeding of improved cultivars is widely neglected in modern breeding. A. tricolor accessions are conserved in the World Vegetable Center (WorldVeg) genebank (https://avrdc.org) and in the USDA National Plant Germplasm Information System (https://www.ars-grin.gov). These accessions hold a wide variety of genetic variations and useful agronomic traits with a high potential for breeding improved cultivars (Fig. 1). Some studies have reported on the evaluation of *A. tricolor* genetic resources, but the diversity conserved in both genebanks has not yet been systematically evaluated.

In this study, we evaluated the genetic diversity in the collection of A. tricolor accessions conserved by the WorldVeg and USDA genebanks based on genome-wide single-nucleotide polymorphisms (SNPs) developed using double-digest RAD-Seq (ddRAD-Seq), and created a core collection, which is valuable in improving crop breeding programs for performing extensive evaluations with minimal materials.

We analyzed the genetic diversity and population structure among 465 A. tricolor accessions with SNPs developed by ddRAD-Seq. We identified a set of 5,638 SNPs without missing data in 440 accessions in order to establish a breeding platform. We analyzed genetic diversity by principal coordinate analysis (PCoA) of the accessions using this marker set. The 377 A. tricolor accessions clustered into 4 main subpopulations (Q1–Q4) and an admixture group (Fig. 2). The proportion of accessions from India, Bangladesh, and China in Q1, Q2, and Q3 was significantly higher than that in other countries, and the proportion of the admixture group in all accessions was highest in accessions from Southeast Asia, especially Indonesia and Malaysia (Fig. 3). In addition, we created a core collection of 105 accessions representing the genetic diversity of 377 source accessions. This core collection is available for research and breeding through the WorldVeg genebank.

Marker selection breeding using the SNP markers and core collection obtained this time will pave the way for the development of breeding techniques and new varieties to improve nutritional value, eating quality, and yield. It is also expected to contribute to sustainable vegetable production in tropical and subtropical regions.

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Fig. 1. Phenotypes of *A. tricolor* accessions cultivated at World Veg

The photos exhibit phenotypic diversity, including leaf color, shape, and height. We cultivated 465 *A. tricolor* genetic resources.

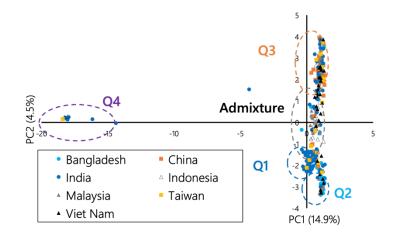


Fig. 2. Principal coordinate analysis (PCoA) of *A. tricolor* accessions based on genome-wide SNPs

Seven countries from which at least 10 accessions are derived are shown. The plots are colored to illustrate the origin of the accessions and to show the clustering according to population structure and geography.

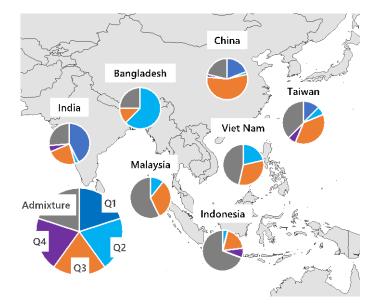


Fig. 3. Classification and geographic distribution of *A. tricolor* genetic resources using SNP markers The pie graphs show the

proportions of accessions of specific subpopulations in each country.

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