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Investigation of Some Selected Rice Landraces for C4 Photosynthetic Traits

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Abstract

Achieving food security amidst rapid climate change necessitates an increase in rice production, a crucial food crop for over half of the global population. One promising approach in this regard is the introduction of the more efficient C4 photosynthesis into the rice plant, which is driven by C3 photosynthesis. However, despite numerous attempts, the absence of a leaf anatomy compatible with C4 photosynthesis still acts as a major roadblock to successfully implement the complex C4 biochemistry into the rice plant, and the identification of phenotypes with a naturally occurring C4-like leaf anatomy could potentially act as a stepping stone in the introduction of C4 photosynthesis into rice. Therefore, this study aims to investigate some selected Sri Lankan rice landraces for the presence of C4-like leaf anatomical characteristics, such as high leaf vein density, reduced mesophyll cell number, and low stomatal density, by comparing them with those of two representative C4 grass species, *Setaria italica* and *Echinochloa colona*. Leaf samples were collected and fixed six weeks post transplantation. For the measurement of vein density, leaf sections were cleared using a KOH gradient, and stomatal density measurement was carried out on both nail polish stomatal imprints and leaf sections cleared using an 85% Lactic acid: 1% chloral hydrate solution, depending on the species. Mesophyll cell counts were taken by clearing leaf sections using the same clearing solution. All the measurements were carried out on the photomicrographs captured using a digital light microscope. Each of these measurements were represented by 15 measurements of five leaf blades taken from five different plants. Our findings highlighted that, despite none of the landraces showing comparable leaf anatomical characteristics to *S. italica* and *E. colona*, interestingly, Pachchaperumal stood out for its highest vein density, lowest mesophyll cell number, and relatively lower stomatal density among the rice landraces tested, showing a possible adaptation to reduce transpiration and maintain the hydraulic integrity in the hot tropical microclimate of Sri Lanka, similar to the conditions under which C4 photosynthesis evolved. Apart from that, in our study, two other landraces, Hondarawalu and Niyanwee, were also selected as high vein density candidates. Overall, we present three candidates as a germplasm source for future studies in understanding the establishment of C4-compatible leaf anatomy in a C3 rice plant, namely, Hondarawalu, Niyanwee, and especially, Pachchaperumal, which could meaningfully contribute to the ongoing work of the development of C4 rice.

Keywords: *Food security, C4 photosynthesis, C4 rice, rice landraces, C4 leaf anatomy*