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# Aquaporins are major determinants of water use efficiency of rice plants in the field

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ABSTRACT

This study aimed at specifying the reasons of unbalanced water relations of rice in the field at midday which results in slowing down photosynthesis and reducing water use efficiency (WUE) in japonica and indica rice under well-watered and droughted conditions. Leaf relative water content (RWC) decreased in the well-watered plants at midday in the field, but more dramatically in the droughted indica (75.6 and 71.4%) than japonica cultivars (85.5 and 80.8%). Gas exchange was measured at three points during the day (9:00, 13:00 and 17:00). Leaf internal $CO_2$ ($C_i$) was not depleted when midday stomatal depression was highest indicating that $C_i$ was not limiting to photosynthesis. Most aquaporins were predominantly expressed in leaves suggesting higher water permeability in leaves than in roots. The expression of leaf aquaporins was further induced by drought at 9:00 without comparable responses in roots. The data suggest that aquaporin expression in the root endodermis was limiting to water uptake. Upon removal of the radial barriers to water flow in roots, transpiration increased instantly and photosynthesis increased after 4 h resulting in increasing WUE after 4 h, demonstrating that WUE in rice is largely limited by the inadequate aquaporin expression profiles in roots.

## 1. Introduction

Rice is more sensitive to water deficit than other crop plants. In spite of the numerous studies, the physiological basis of rice sensitivity to water deficit is still poorly understood if dissected into processes rather than considered collectively [1]. Even if growing in water-saturated soil, a rice plant shows reduction in stomatal conductance ($g_s$), rates of transpiration ($E$) and photosynthesis ($A$) (collectively known as midday stomatal depression), which are typical symptoms of water deficit in the afternoon where transpiration demand is high on sunny days [2]. Midday stomatal depression has been reported in many species including rice and attributed mainly to reduction in stomatal conductance as a result of plant negative water status [3–5]. Recently, Zhang et al. [6] showed that midday stomatal depression is mainly linked to stem rather than to leaf water potential in sub-tropical trees. The negative water status at midday and subsequent stomatal depression slow down photosynthesis and accelerate photoinhibition which ultimately result in reducing biomass accumulation [7,8].

Midday stomatal depression in well-watered rice plants and their sensitivity to water deficit are attributed to excessive water loss by stomatal and non-stomatal transpiration from leaves [9–11] and inadequate water uptake from soil by roots [12–14]. However, recent evidence suggests that rice leaves are not specifically sensitive to water deficit as compared to maize leaves, based on the finding that the sensitivity of leaf elongation rates of different genotypes of rice were similar to that of maize when differences in root system were neutralized [15]. This indicates that the sensitivity of rice to water deficit and hence, the severe midday stomatal depression may be mainly due to its poor root system.

According to the composite model of water flow through roots [16], the path of water from soil through roots to leaves includes two sections. The first (radial flow) is the flow of water through different layers of root cells including epidermis, exodermis, cortex and endodermis. The second (axial flow) is the flow of water inside xylem vessels upward to the leaves. Within the radial component of water flow, water could pass through the cell walls (apoplastic), through plasmodesmata (symplastic) or through cell membranes (transmembrane flow). The two latter paths cannot be separated experimentally and are collectively termed as cell-to-cell pathway. Once early metaxylem vessels mature, the axial path of water flow will not be limiting to water movement [17–19].

Rice roots have been found to have high hydraulic resistance compared to maize roots apparently because rice roots have apoplastic barriers to radial water flow as shown by excessive suberin deposition in different cell layers of the root and the

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well-developed endodermis [13,20]. More specifically, Ranathunge et al. [21] showed that the hydraulic conductance of the outer parts of rice roots (epidermis, exodermis and the outermost cell layer of cortex) was 30 times higher than that of the overall root hydraulic conductance and that the endodermis was limiting to water flow through roots. It has been shown that the contribution of the cell-to-cell pathway to radial water flow through roots is negligible under high transpiration. However, under water deficit conditions, more than 40% of the water uptake by roots may be contributed by the cell-to-cell pathway if calculated on leaf elongation rate basis [22]. Even under conditions of high transpiration where bulk water flow through plant root tissues is apoplastic, the root exodermis and endodermis are exceptions where water has to go through the cell-to-cell path [23]. Based on the findings of Ranathunge et al. [21], the cell-to-cell path of water in the endodermis can be viewed as the bottle neck to water uptake by roots in rice and improving the root water uptake, therefore, requires increasing the permeability of the cell-to-cell path to water, precisely in the endodermis.

Aquaporins are a family of membrane major intrinsic proteins (MIP) that have been proved to facilitate water movement through membranes in plants [24–26]. They exist in the cell membrane (plasma membrane intrinsic proteins, PIPs), tonoplast (tonoplast intrinsic proteins, TIPs), peribacteroid membranes of $N_2$-fixing symbiotic root nodules (NIPs) and in the endoplasmic reticulum (small basic intrinsic membranes, SIPs). In rice, 33 aquaporin genes have been characterized, among which there are 11 PIPs and 10 TIPs [27]. PIPs are divided into two subgroups (PIP1s and PIP2s) based on sequence homology. PIP2 and TIP aquaporins have high water transport activity compared to PIP1s [28–30]. Nonetheless, PIP1 aquaporins have been suggested to form heterotetramers with PIP2s with increased water transport activity [31,32]. Some aquaporins have been reported to transport other physiologically important small molecules such as $CO_2$ [33], boron or silicon [25] or $H_2O_2$ [34].

Ohshima et al. [35] have reported that the aquaporin contents of the cell membranes and tonoplasts of the crassulacean acid metabolism (CAM) plant *Graptopetalum paraguayense* were extremely low compared to those of radish, which could account for the lower water permeability of *Graptopetalum* membranes. Evidence from numerous studies involving quantifying aquaporin expression and overexpression and/or down-regulation of aquaporin genes strongly supports a prominent role in increasing water permeability of different tissues in rice [36–39] and other plants [34,40,41]. However, the drought tolerance of transgenic plants overexpressing aquaporin genes varied where it increased in some cases [34,38,42] but was not affected or even decreased in some others [43,44]. Moreover, the correlation between the expression of aquaporins and midday stomatal depression, if any in rice or other plants, is yet to be understood. This highlights the need for genome-wide studies on the expression of aquaporins in relation to water status in field-grown plants in order to drive a more informative and precise conclusion on how to manipulate aquaporin genes to improve plant productivity.

Sakurai-Ishikawa et al. [45] have shown that most of root aquaporins in rice were upregulated during periods of high transpiration demand (beginning of the light period) probably to maintain a positive plant water status. However, the relevance of this information to plant responses in the field is limited because the authors grew the plants under non-saturating conditions of 370 $\mu$mole $m^{-2}$ $s^{-1}$ light intensity and 75% relative humidity (RH), where stomatal depression did not occur but rather the rates of transpiration increased progressively during the whole light period. Many reports have described the tissue localization and regulation of aquaporin expression in rice in relation to water status in order to dissect their function(s) in the regulation of plant water status during normal and water stressful conditions [10,37,39,46]. Nonetheless, the significance of aquaporin expression profiles in relation to whole plant water status, rates of photosynthesis and water use efficiency in the field remains elusive because in most previous studies, data on photosynthesis and water use efficiency in the field were not included. Furthermore, the responsiveness of rice root aquaporins that are expressed in the endodermis to transpiration demand in the field has not been characterized yet.

The objectives of the present study were to: (1) investigate the diurnal changes in gas exchange parameters in relation to expression profiles of leaf and root aquaporins in four rice cultivars (two japonica cultivars and two indica cultivars) with contrasting genetic backgrounds grown in the field, (2) implement previous information on tissue localization of aquaporins combined with their water transport activities in order to predict the water permeability of rice roots and leaves and hence, pinpoint the main possible reason(s) of midday stomatal depression, (3) analyse the diurnal changes in leaf soluble and insoluble sugar contents, a possible contributor to midday down-regulation of photosynthesis, as affected by changes in rates of photosynthesis, and (4) test the consequences of removing the barriers to radial water flow in the roots on the rates of transpiration and photosynthesis when stomatal depression is maximum. The data provide evidence that the rates of photosynthesis may be partly affected by inhibited sugar translocation but are independent of leaf internal $CO_2$ ($C_i$) and that both rates of photosynthesis and transpiration are tightly linked to the leaf water status which is in turn the product of aquaporin expression profiles in leaves and roots.

## 2. Materials and methods

### 2.1. Plant material

Four rice cultivars were used in this study, two japonica cultivars (Giza 178 and Sakha 101) and two indica cultivars (IR64 and PSL2). Giza 178 and Sakha 101 were obtained from the Agricultural Research Institute (Giza, Egypt) and IR64 was kindly supplied by the International Rice Research Institute (IRRI, Philippines). PSL2 was obtained from the Ministry of Agriculture of Thailand.

### 2.2. Plant growth and drought treatment

This experiment was carried out in the research field of Botany Department, Damietta University. The seeds of the four rice cultivars were sown outdoors in May (about 12 h photoperiod, 28/24 °C day night temperature, 2850 $\mu$mole $m^{-2}$ $s^{-1}$ maximum light intensity and 45% day RH) in soil prepared by mixing equal volumes of sandy and clay soil, to form a soil layer 30 cm deep. The plants were grown in randomized block design with six blocks for each cultivar. Each block was 2 × 2 m. The plants were watered every day at 8:00–9:00. At the age of 15 days, the plants were thinned to be 25 cm apart. Daily watering continued up to the age of 40 d. Then, water was withheld from three blocks of each cultivar which were used for drought treatment. The remaining three blocks of each cultivar kept receiving water every day and were used as a control. The water content of the soil was monitored several times a day (measured instantly by dividing the weight of a soil sample by its water-saturated weight) so that subsequent measurements and sampling were made when the soil water content was about 4% higher than the soil water content at which the plants started wilting (determined from a preliminary experiment as the weight of soil sample at wilting divided by the saturated soil weight). The soil water content at wilt was considered as the fraction of transpirable soil water, FTSW [47]. All blocks reached the stressful water content after 3 d of withholding water. To maintain the soil water content