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Genetic variability of oxidase oxalate activity and elongation in water-stressed primary roots of diverse maize and rice lines

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A previous study of maize primary roots under water stress showed pronounced increases in oxalate oxidase activity and apoplastic hydrogen peroxide in the apical region of the growth zone where cell elongation is maintained. We examined whether increased oxalate oxidase activity in water-stressed roots is conserved across diverse lines of maize and rice. The maize lines exhibited varied patterns of activity, with some lines lacking activity in the apical region. Moreover, none of the rice lines showed activity in the apical region. Also, although the genotypic response of root elongation to water stress was variable in both maize and rice, this was not correlated with the pattern of oxalate oxidase activity. Implications of these findings for root growth regulation under water stress are discussed.

Under water-limited conditions, root growth is often maintained relative to shoot growth.[1-3] This response is an adaptive feature that allows plants to continue to access water as the soil dries.[4,5] The mechanisms underlying growth maintenance under water stress have been studied extensively in the primary root of maize seedlings (for review see refs. 6,7,8). The length of the root growth zone is approximately 12 mm under well-watered conditions, whereas under water stress, cell elongation is maintained in the apical few mm but is inhibited progressively further from the apex, resulting in a shortened growth zone.[9] These responses to water stress involve spatially differential regulation of cellular growth processes, including enhancement and inhibition of cell wall extensibility in the apical and basal regions, respectively.[10] Transcriptomic and cell wall proteomic analyses conducted with the different regions of the growth zone revealed primarily region-specific changes in water-stressed compared with well-watered roots.[11,12] In particular, several transcripts and proteins related to reactive oxygen species (ROS) production, including putative oxalate oxidases, increased in abundance in the apical region and oxalate oxidase activity was shown to increase markedly in the apical few mm.[13] Oxalate oxidase activity also increased, although to a lesser extent, beyond approximately 8 mm from the apex (beyond the growth zone). Oxalate oxidases catalyze the conversion of oxalate to CO_2 and hydrogen peroxide (H_2O_2), and in cereals are known to be cell wall localized.[14]

Recent results of Voothuluru and Sharp[13] demonstrated that apoplastic H_2O_2 levels increased specifically in the apical region of the growth zone in water-stressed maize primary roots, correlating with the maintenance of cell elongation and the pronounced increase in oxalate oxidase activity in this region. Apoplastic ROS can have growth regulatory functions including cell wall loosening as well as tightening,[15,16] and have also been shown to act as signaling molecules in various processes.[17] Accordingly, the increase in apoplastic ROS in the apical region of the growth zone is likely to play an important role in root growth regulation under water stress.

The spatial profiles of transcripts, cell wall proteins, oxalate oxidase activity and apoplastic H_2O_2 in the growth zone of water-stressed maize primary roots, as described above, were conducted in inbred line FR697, which is a temperate line and was selected because it exhibits a relatively high capacity to maintain primary root elongation at low water potentials.[18] Since maize is genetically diverse,[19] we wanted to determine if the increase in oxalate oxidase activity in the apical region of the growth zone of water-stressed primary roots was conserved across diverse lines. Twenty six inbred lines, constituting the parents of the maize nested association mapping (NAM) population and representing the diversity of maize,[20] were selected for analysis. The NAM parents include 10 temperate lines, three lines of mixed temperate and tropical ancestry, and 13 tropical lines (**Fig. 1**).

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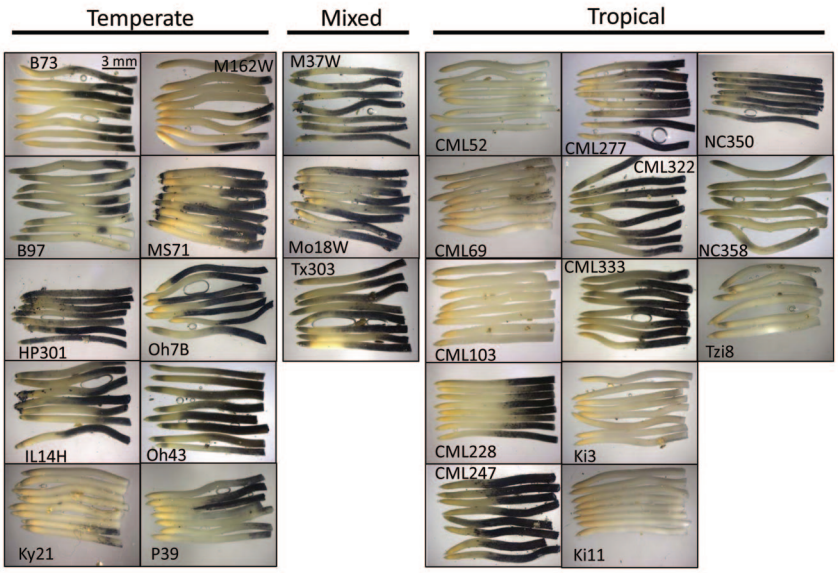

Figure 1. Oxalate oxidase activity determined by oxalate-dependent oxidation of 4-chloro-1-naphthol, seen as dark blue staining, in 12-mm apical segments of water-stressed primary roots of the maize NAM parental lines. Roots were harvested 48 h after transplanting to vermiculite at a water potential of -1.6 MPa. Growth conditions, water potential measurements and oxalate oxidase staining procedures were as described by Voothuluru and Sharp.[13] The illustrated roots are representative of 8–15 assayed roots per line. Experiments were repeated with a subset of 10 lines to confirm consistency of staining patterns.

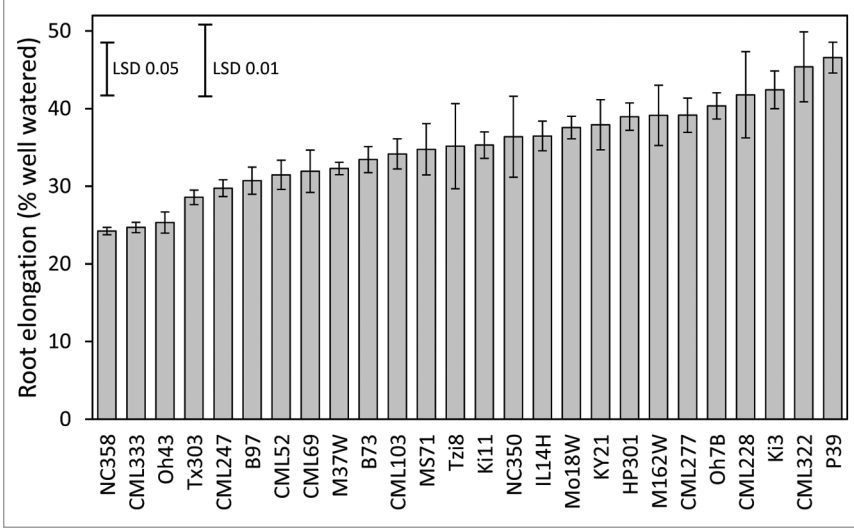

Figure 2. Relative maintenance of primary root elongation in the maize NAM parental lines during 48 h after transplanting to vermiculite at a water potential of -1.6 MPa. The results are calculated as a percentage of well-watered controls; the well-watered and water-stressed root elongation data are presented in **Figure S1**. Values are means ± SE of n = 13–25. Analysis of variance (PROC GLM, SAS version 9.3; SAS Institute) was used to compare the means among lines in each treatment. Least significant differences (LSD) for *P* values of 0.05 and 0.01 are shown. Experiments were repeated with a subset of 10 lines to confirm consistency of the root growth response.

After germination, the seedlings were transplanted into vermiculite at water potentials of -0.03 MPa (well-watered treatment) or -1.6 MPa (severe water stress treatment) and grown under conditions of minimal evaporation or transpiration (darkness and near-saturation humidity). This system allows for precise, steady and reproducible control of water deficit conditions.[9] In the well-watered treatment, none of the lines showed discernible staining for oxalate oxidase activity at any location within the apical 12 mm of the root (data not shown). In contrast, when grown under water-stressed conditions, the lines showed diverse spatial patterns of increased oxalate oxidase activity (**Fig. 1**). Except for lines M162W, B73 and P39, all the temperate lines exhibited oxalate oxidase activity within the apical 3 mm region (as previously observed for FR697[13]), with some lines showing more pronounced staining than other lines. In addition, all the temperate lines showed oxalate oxidase activity in the basal region (beyond 6 mm from the apex). The three mixed ancestry lines also showed increased oxalate oxidase activity in both the apical and basal regions. In contrast, the tropical lines showed several diverse patterns of oxalate oxidase activity: CML247, CML277, CML322, CML333, NC350 and NC358 showed staining in both the apical few mm as well as the basal region, Tzi8 showed staining only in the apical region, CML 228 showed staining only in the basal region, whereas CML52, CML69, CML103, Ki3 and Ki11 showed no oxalate oxidase activity throughout the apical 12 mm. It is not unexpected that the tropical lines show a broader range of responses as they have wider variability within their pedigrees and contain higher levels of diversity than temperate maize.[21]

To confirm that the varied oxalate oxidase staining observed in the apical region of the root growth zone among the lines did not result from differential penetration of the staining solution, the apical 1.5–3 mm region of a subset of 10 lines was stained after sectioning to allow direct access of the solution to the tissues. The results were consistent with those observed in the intact roots (data not shown).

The NAM parents also showed large variation in the response of primary root elongation to water stress, ranging from 24% (in NC358) to 47% (in P39) of the elongation observed in the respective well-watered controls (**Fig. 2**). To evaluate whether the response of root elongation was associated with the occurrence of oxalate oxidase activity in either the apical 3 mm or the basal region of the growth zone, the oxalate oxidase activity in the different regions was scored as described in **Table S1**. The results showed that the relative maintenance of root

elongation under water stress did not correlate with the occurrence of oxalate oxidase staining in either the apical or basal regions (R^2 values were ≤ 0.1).

The involvement of oxalate oxidases in biotic stress tolerance is common to different cereal species.[22] Therefore, to investigate whether increased oxalate oxidase activity in the root growth zone under water stress is a conserved response in cereals, we examined a range of diverse rice lines. Rice was chosen because of known variation in response to water stress and because of the availability, for future studies, of RNAi lines for all of the oxalate oxidases present in rice (J Leach, personal communication). Ten lines representing the diversity of rice (five japonica, four indica and one aus line) and known to have contrasting water use efficiency parameters[23,24] were selected for analysis. However, none of the lines showed any staining for oxalate oxidase activity in the apical few mm of the root under water-stressed conditions (**Fig. 3**). Interestingly, while the japonica lines also exhibited no or marginal staining beyond 6 mm from the apex, the indica and aus lines consistently exhibited limited staining in this region. It should be noted, however, that we have not characterized the dimensions of the growth zone in the rice primary root under either well-watered or water-stressed conditions, and it is possible that the staining in the basal region was beyond the zone of cell elongation. The rice lines exhibited greater variation in the response of primary root elongation to water stress than that observed in maize (**Fig. 4**). The least inhibited rice line was Cypress, which maintained 56% of the elongation of the well-watered control, whereas the poorest line (Moroberekan) showed only 22% maintenance of root elongation. There was no correlation between the occurrence of staining for oxalate activity in the basal region and the response of root elongation to water stress (R^2 = 0.005; staining scores are shown in **Table S1**).

Taken together, the maize and rice results suggest that oxalate oxidase-mediated mechanisms are involved in regulating the growth response in the apical region of water-stressed primary roots only in some maize lines and are not involved in any of the rice lines tested. Although a correlation between the occurrence of increased oxalate oxidase activity and root growth maintenance under water deficit conditions was not apparent, it should be emphasized that the results do not necessarily imply that the maize and rice lines that lack an oxalate oxidase response also lack the response of increased apoplastic ROS levels in the apical region of the growth zone.[13] Oxalate oxidase might not be a major contributor to apoplastic H_2O_2 production in these lines, and increased ROS levels could be achieved by increased activity of other ROS-producing enzymes. These alternative hypotheses could be tested by measuring apoplastic ROS levels in the rice and contrasting maize lines under water stress conditions. The relevance of increased oxalate oxidase activity to the response of root elongation under water stress can be examined by manipulating oxalate oxidase activity and documenting the effects on processes regulating root elongation. In a following paper, transgenic maize lines overexpressing oxalate oxidase[25] will be utilized for this purpose. The finding that maize has considerable genetic diversity in the response of oxalate oxidase activity, as well as elongation, in water-stressed roots could be useful not only in physiological studies but also in breeding programs to improve root growth traits under water deficit conditions.

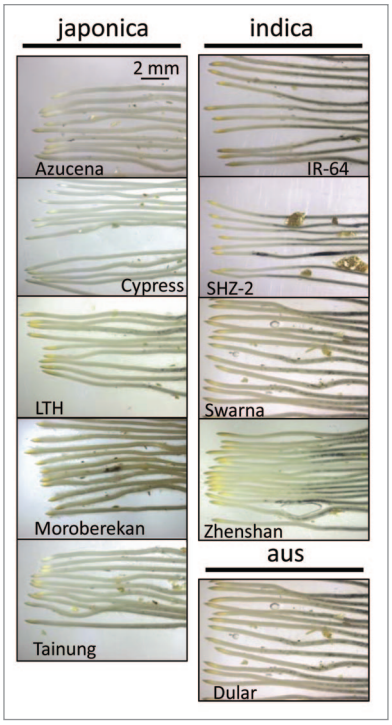

Figure 3. Oxalate oxidase activity determined by oxalate-dependent oxidation of 4-chloro-1-naphthol in 12-mm apical segments of water-stressed primary roots (n = 8–15) of diverse rice lines. Roots were harvested 48 h after transplanting to vermiculite at a water potential of -1.2 MPa. Note that rice has a relatively thin primary root, resulting in less hydraulic contact with the vermiculite growth media compared with maize. Therefore, the rice seedlings were grown at a higher water potential than was used for maize. Other growth conditions and measurement procedures were as described in **Figure 1**.

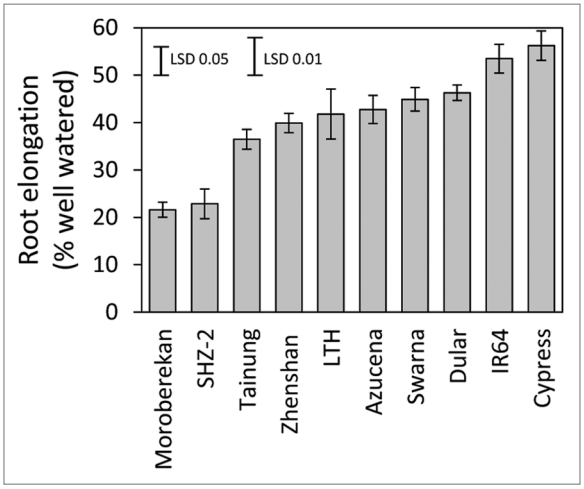

Figure 4. Relative maintenance of primary root elongation in diverse rice lines during 48 h after transplanting to vermiculite at a water potential of -1.2 MPa. The results are calculated as a percentage of well-watered controls; the well-watered and water-stressed root elongation data are presented in **Figure S2**. Values are means ± SE of n = 13–18. Statistical analysis was conducted as described in **Figure 2**; least significant differences (LSD) for *P* values of 0.05 and 0.01 are shown.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Acknowledgments

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Supplemental Material

Supplemental material may be found at: www.landesbioscience.com/journals/psb/article/23454

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