Cell Wall Proteome in the Maize Primary Root Elongation Zone. II. Region-Specific Changes in Water Soluble and Lightly Ionically Bound Proteins under Water Deficit1\[W\][OA]

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Cell Wall Proteome in the Maize Primary Root Elongation Zone. II. Region-Specific Changes in Water Soluble and Lightly Ionically Bound Proteins under Water Deficit\(^1\)\^[W]\^[OA]

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Previous work on the adaptation of maize (\textit{Zea mays}) primary roots to water deficit showed that cell elongation is maintained preferentially toward the apex, and that this response involves modification of cell wall extension properties. To gain a comprehensive understanding of how cell wall protein (CWP) composition changes in association with the differential growth responses to water deficit in different regions of the elongation zone, a proteomics approach was used to examine water soluble and loosely ionically bound CWPs. The results revealed major and predominantly region-specific changes in protein profiles between well-watered and water-stressed roots. In total, 152 water deficit-responsive proteins were identified and categorized into five groups based on their potential function in the cell wall: reactive oxygen species (ROS) metabolism, defense and detoxification, hydrolases, carbohydrate metabolism, and other/unknown. The results indicate that stress-induced changes in CWPs involve multiple processes that are likely to regulate the response of cell elongation. In particular, the changes in protein abundance related to ROS metabolism predicted an increase in apoplastic ROS production in the apical region of the elongation zone of water-stressed roots. This was verified by quantification of hydrogen peroxide content in extracted apoplastic fluid and by in situ imaging of apoplastic ROS levels. This response could contribute directly to the enhancement of wall loosening in this region. This large-scale proteomic analysis provides novel insights into the complexity of mechanisms that regulate root growth under water deficit conditions and highlights the spatial differences in CWP composition in the root elongation zone.

Roots often continue to grow under water deficits that completely inhibit shoot and leaf elongation (Sharp and Davies, 1979; Westgate and Boyer, 1985), and this is considered an important mechanism of plant adaptation to water-limited conditions (Sharp and Davies, 1989). Investigation of the mechanisms of root growth adaptation to water deficit is important for improving plant performance under drought, because water resources for agriculture are becoming increasingly limited.

The physiology of maize (\textit{Zea mays}) primary root elongation at low water potentials has been studied extensively (for review, see Sharp et al., 2004), which has provided the foundation for an understanding of the complex network of responses involved. Analysis of the relative elongation rate profile within the root elongation zone showed that under severe water deficit, elongation rates are fully maintained in the apical few millimeters but progressively inhibited as cells are displaced further from the root apex (Sharp et al., 1988; Liang et al., 1997). To help understand the maintenance of elongation in the apical region of roots growing under water deficit conditions, Spollen and Sharp (1991) measured the spatial distribution of turgor pressure and found that values were uniformly decreased by over 50% throughout the elongation zone of water-stressed compared to well-watered roots. These results suggested that water stress results in an increase in longitudinal cell wall extensibility in the apical region, which was confirmed by direct measurement of...
acid-induced extension (Wu et al., 1996). In contrast, cell wall extension properties are inhibited in the basal region of the elongation zone in water-stressed compared to well-watered roots (Wu et al., 1996; Fan and Neumann, 2004; Fan et al., 2006). Additional studies with the same experimental system demonstrated that activities of two cell wall proteins (CWPs) with known or proposed wall loosening properties, expansins, and xyloglucan endotransglucosylase/hydrolase (XTH), were increased specifically in the apical few millimeters of water-stressed compared to well-watered roots (Wu et al., 1994, 1996), providing a biochemical basis for the increase in cell wall extensibility (Wu and Cosgrove, 2000). At the transcript level, three expansin genes were up-regulated in the apical region and down-regulated in the basal region of the elongation zone of water-stressed roots, correlating with the increase and decrease of extensibility in these regions, respectively (Wu et al., 2001).

Since cell wall extensibility changes are likely to involve multiple components and processes, the previous work provided a limited understanding of the cell wall biology of root growth regulation under water deficits. In this study, a proteomics approach was used to expand our understanding of the CWPs that change in abundance in the elongation zone of water-stressed roots. By combining spatial analysis of the CWP changes with knowledge of the elongation rate patterns we aimed to gain further insight into the CWPs that are potentially involved in controlling the responses of cell elongation.

Proteomics approaches are increasingly being applied to identify large numbers of proteins from cell walls (Robertson et al., 1997; Blee et al., 2001; Chivasa et al., 2002; Watson et al., 2004; Bayer et al., 2006; Zhu et al., 2006). However, to our knowledge, no cell wall proteomics studies have focused on the involvement of CWPs in the response of root growth to water deficit conditions. Various methods have been developed to extract CWP fractions that may be loosely to very tightly bound to the cell wall matrix (Fry, 1988). The fraction 1 CWPs described in this study represent those proteins that are soluble in apoplastic fluid or lightly ionically bound to the cell walls. Proteomic studies of fraction 1 CWPs have been performed in leaves of several species (Haslam et al., 2003; Boudart et al., 2005), including a study of tobacco (Nicotiana tabacum) in which it was shown that 20 fraction 1 CWPs changed in abundance in response to salt stress (Dani et al., 2005). These studies not only revealed very different protein compositions in different species, but also reflected the variety of functions of fraction 1 CWPs.

In a previous study, an infiltration and centrifugation method was optimized for the extraction of fraction 1 CWPs from the elongation zone of the maize primary root with minimal cytosolic protein contamination (Zhu et al., 2006). In this study, this method was used to gain a more comprehensive understanding of how the composition of fraction 1 CWPs changes in association with the differential responses of cell elongation to water deficit in different regions of the elongation zone. The results reveal major and predominantly region-specific changes in protein profiles between well-watered and water-stressed roots that provide novel insights into the processes involved in regulating the root growth response to water stress.

RESULTS

Two-Dimensional Gel Analysis of Water Deficit-Responsive Fraction 1 CWPs in Different Regions of the Root Elongation Zone

Fraction 1 CWPs were extracted from four contiguous regions within the apical 20 mm of the primary root of maize seedlings grown under well-watered or water-stressed conditions. As shown in Figure 1, relative elongation rates were completely maintained under water deficit in the apical 3 mm region (R1); the 3 to 7 mm region (R2) exhibited maximum elongation rates in well-watered roots but progressive inhibition of elongation under water deficit; in the 7 to 12 mm region (R3), elongation decelerated in well-watered roots and was completely inhibited under water deficit; the 12 to 20 mm region (R4) was nonelongating in both well-watered and water-stressed roots. Two well-watered controls were collected, a temporal control in which well-watered roots were harvested at the same time as the water-stressed roots (48 h after transplanting), and a developmental control in which well-watered roots were harvested at 24 h after transplanting when they had reached the same length as the...
water-stressed roots. The two controls were important to help identify proteins that showed true change in abundance in response to water stress, because the protein composition might also vary with root development under well-watered conditions. Previous work in a different cultivar showed that the spatial pattern of relative elongation rate was almost identical in the two well-watered controls (Liang et al., 1997).

The extracted CWPs were separated by two-dimensional electrophoresis (2-DE) and stained with SyproRuby. Despite the large number of roots used for CWP extraction (750 roots per sample), very low amounts of protein were available for this analysis due to the extremely small proportion of total cellular proteins comprised by the extracted fraction 1 CWPs (approximately 0.01%), as well as the small regions of the root from which the proteins were extracted. However, the extraction procedure provided a relatively uncomplicated protein fraction, making these samples ideal for gel analysis. Furthermore, even though the total amount of protein loaded on each gel was low, individual proteins could be visualized because of the relatively small numbers of proteins in the extracts. On average, 157 spots were visualized from gels containing protein from R1, 340 from R2, 384 from R3, and 224 from R4. Representative 2-DE gel images for each region of each treatment are shown in Figure 2; gel images of replicate samples were closely comparable in spot patterns and intensity.

To be considered in our within-region analyses, protein spots needed to be present (or absent) in all three replicate gels in a specific region of each treatment. This approach allowed analysis in specific regions of proteins that did not necessarily yield reproducible results in one or more other regions. For R1 to R3, the numbers of spots that were reproducibly present in one or more treatments are shown in Figure 3. The results of the gel analysis are not shown for R4 because this was outside of the elongation zone in both well-watered and water-stressed roots (Fig. 1). However, R4 was used for abundance analysis of proteins that were stress responsive in one or more of the other regions (see below). In each region, the greatest proportion of spots was common to all treatments, while the two well-watered controls consistently had more spots in common with each other than with the water-stressed roots. In R1, 34 protein spots were found in both well-watered controls but not in the water-stressed roots and five were unique to the water-stressed roots. In R2, 22 protein spots were found in both well-watered controls and not in the water-stressed roots, 18 were unique to water-stressed roots, and of 150 spots common to the stressed roots and to one or both controls, the abundance of 14 increased and 17 decreased under water stress. In R3, 32 spots were found in both well-watered controls but not under water stress, nine were unique to the water-stressed roots, and of 108 spots common to the stressed roots and to one or both controls, the abundance of 26 increased and 23 decreased under water stress (Fig. 3). In proportion to the total number of proteins found in each region, the largest percentage change in protein abundance in water-stressed compared to well-watered roots was found in R1.

The changes in abundance of CWPs due to water deficit in R1, R2, and R3 were predominantly region specific. When the stress-responsive protein spots were compared by spot matching across the regions, 44 of the 68 spots in R1, 48 of the 71 spots in R2, and 57 of the 90 spots in R3 were responsive only in those specific regions (Fig. 4). Only 12 protein spots were stress responsive in all three regions. The large number of region-specific changes in protein abundance in response to water stress emphasizes the importance and effectiveness of the spatial analysis approach that we used to study the CWPs in the root elongation zone.

Identification of the Water Deficit-Responsive Fraction 1 CWPs

The proteins whose abundance changed significantly in response to water deficit in R1, R2, and/or R3 were excised and identified using mass spectrometry. The protein identifications are shown in Supplemental Table S1; confident identifications were based on multiple peptide matches and significant Mascot scores. Fifty three, 49, and 63 proteins were identified from the 68, 71, and 90 water deficit-responsive protein spots in R1, R2, and R3, respectively (Fig. 3). In most cases, each protein spot was identified as a single protein; those spots that yielded more than one confident identification are marked with an asterisk in Supplemental Table S1; confident identifications were based on multiple peptide matches and significant Mascot scores. Fifty three, 49, and 63 proteins were identified from the 68, 71, and 90 water deficit-responsive protein spots in R1, R2, and R3, respectively (Fig. 3). In most cases, each protein spot was identified as a single protein; those spots that yielded more than one confident identification are marked with an asterisk in Supplemental Table S1; confident identifications were based on multiple peptide matches and significant Mascot scores. Fifty three, 49, and 63 proteins were identified from the 68, 71, and 90 water deficit-responsive protein spots in R1, R2, and R3, respectively (Fig. 3).
there was a discrepancy between the observed and expected $M_r$, which may be the result of protein modification, incomplete genome/protein databases for maize, or alternate splicing products (Sun et al., 2005). Also, the identification of the same protein from different spots (e.g. spots 141, 142, 150, 402) indicates possible protein isoforms for which posttranslational modifications may have occurred, or these may be isoforms that arise from multigene families. The complete data is accessible at http://rootgenomics.missouri.edu/proticdb-1.2.1/Protic/home. To display these data we used the PROTICdb database (Ferry-Dumazet et al., 2005).

A large number of the proteins for which we found water stress-induced changes in abundance are known to be localized in cell walls, including two putative oxalate oxidases and two probable germin protein 4s, one α-1-arabinofuranosidase, one α-1,4-glucan-protein synthase, two β-galactosidases, two putative chitinases, three endo-1,3;1,4-β-D-glucanases, three putative α-galactosidase preproteins, four β-1,3-glucanases, five XTHs, four xylosidases, 12 β-D-glucosidases, and 18 peroxidases. In addition, most of the identified proteins (69%) have a putative N-terminal signal peptide that may lead to protein targeting into the secretory pathway (Nielsen et al., 1997), and 11% were predicted to be nonclassical secretory proteins that may be targeted to cell walls using an alternative pathway (Bendtsen et al., 2004; Supplemental Table S1). It should be noted that expansins, which were shown previously to exhibit altered activity and gene expression in the maize primary root elongation zone under water deficit (Wu et al., 1996, 2001), were not identified. Expansins are tightly bound to the cell wall and are not expected to be present in fraction 1 CWP extracts (McQueen-Mason et al., 1992).

Functional Classification and Region Specificity of Water Deficit-Responsive Fraction 1 CWPs

To better understand the biological processes encompassed by the identified proteins, the proteins were classified in five categories based on their annotations and potential functions in the cell wall (Supplemental Table S1). Of the 152 proteins identified from R1 to R3, 30 were categorized as being involved in reactive oxygen species (ROS) metabolism, 35 were in the category of defense and detoxification, 39 were related to hydrolase activity, 17 were related to carbohydrate metabolism, and 31 were considered as other/unknown. Within each functional category, changes in protein abundance in water-stressed compared to well-watered roots are shown for R1 to R3 in Figures 5 to 9. To allow for quantitative comparisons between the proteins, the data are expressed as fold-change values (log$_{10}$ scale) using mean values from the two well-watered controls and a background value of 0.001 when a protein spot could not be visualized in a particular treatment. For spots that yielded multiple identifications (Supplemental Table S1), only the most confident identification is presented in Figures 5 to 9.

Because the root elongation zone was shorter in the water-stressed roots (Fig. 1), some of the changes in protein abundance observed in R2 and R3 of water-stressed compared to well-watered roots could be attributed to the differences in stage of cell development between the treatments rather than to specific responses to water stress. Thus, stress-responsive proteins in R2 (decelerating region under stress) and R3 (initial nongrowing region under stress) were also compared to R3 and R4, respectively, in well-watered roots, which exhibited comparable developmental stages. Changes in protein abundance in R2 under
water stress that were consistent when compared to both well-watered R2 and R3 were considered to be independent of the shortening of the elongation zone; such proteins are marked with an asterisk in Figures 5 to 9. Similarly, consistent responses in water-stressed R3 when compared to well-watered R3 and R4 were considered to be independent of the change in developmental stage. Since elongation rates were the same in R1 under well-watered and water-stressed conditions, all changes in protein abundance between the stressed and well-watered treatments in R1 were considered to be specific responses to water stress.

In all functional categories, the changes in abundance of CWPs due to water deficit were predominantly region specific (Figs. 5–9). Where the same protein was stress responsive in more than one region, the direction of the response (increase or decrease in abundance) was the same in most cases, although there were several exceptions where proteins exhibited opposite responses in different, and sometimes adjacent, regions. Changes in CWP abundance under water deficit in the category of ROS metabolism are shown in Figure 5. Of the 20 proteins identified in this category from R1 to R3, a greater number exhibited increases rather than decreases in abundance in R1 and R2. The abundance of one superoxide dismutase [Cu-Zn], one putative oxalate oxidase, one probable germin protein, and three peroxidases increased in R1. The putative oxalate oxidase was consistently more abundant throughout the three regions, whereas the other proteins increased in abundance in R1 specifically. In R3, a greater number of proteins decreased rather than increased in abundance in the stressed roots, although the abundance of a different putative oxalate oxidase (same accession number but different spot) and a putative peroxidase increased greatly.

Of the 26 proteins in the category of defense and detoxification, a majority exhibited decreases in abundance in water-stressed compared to well-watered roots in R1, R2, and R3 (Fig. 6). In the category of hydrolases, in which a total of 33 stress-responsive proteins were identified, 13 of the 18 proteins in R1 exhibited a decrease in abundance under water stress (Fig. 7). These responses, including two XTHs and two endo-1, 3;1,4-β-D-glucanases, were specific to R1, with the exception of another XTH (spot 190) that decreased in abundance in all three regions. A fourth XTH (spot 1,650) exhibited a small increase in abundance specifically in...
R2, although this change was likely attributable to the change in developmental profile. All five of the proteins in the hydrolase category that increased in abundance in R1 of the water-stressed roots were identified as β-D-glucosidases. Four of these proteins also increased in abundance in R2 and R3.

There were 10 stress-responsive proteins identified in the category of carbohydrate metabolism (Fig. 8), of which five of the six proteins in R1 and R2 decreased in abundance, whereas four of nine increased in abundance in R3. Notably, all three soluble acid invertases increased in abundance in R2 or R3. There were 21 proteins in the category of other/unknown (Fig. 9). Most of these proteins (19 out of 21) showed region-specific responses to water stress. For example, a α-1, 4-glucan-protein synthase increased in abundance only in R1, and two legumin-like proteins increased in abundance only in R2.

Spatial Distribution of Fraction 1 CWPs in Well-Watered and Water-Stressed Roots

To reveal the spatial distribution of abundance along the elongation zone of the water deficit-responsive proteins, gel images of all regions of all treatments were normalized, which allowed for across-region comparisons of protein abundance. Only those protein spots that were reproducibly present (or absent) in all three replicates in all regions of all treatments were selected for this analysis, restricting the analysis to 73 of the 110 proteins for which fold-change responses are presented in Figures 5 to 9. The results are shown in Figure 10 and Supplemental Figures S1 to S5.

The abundance profiles along the elongation zone varied greatly for the different proteins both within and between protein families as well as between the well-watered and water-stressed treatments (in general the profiles were very similar in the two well-watered controls). As an example of the diversity within a protein family, the spatial distributions of

![Figure 4. Distribution among R1 to R3 of water deficit-responsive fraction 1 CWPs, showing that the changes in protein abundance in water-stressed compared to well-watered roots were predominantly region specific.](image)

| Spot No. | Accession No. | ID | Region 1 (0-3 mm) | Region 2 (3-7 mm) | Region 3 (7-12 mm) |
|----------|---------------|----|------------------|------------------|------------------|
| 381      | 134598        | Superoxide dismutase [Cu-Zn] | * | * | * |
| 386      | 50917909      | Putative oxalate oxidase | * | * | * |
| 395      | 34962528      | Probable germin protein 4 | * | * | * |
| 385      | 50920595      | Putative ascorbate peroxidase | * | * | * |
| 177      | 55761027      | Class III peroxidase 80 precursor | * | * | * |
| 157      | 25044849      | Peroxidase | * | * | * |
| 354      | 575605        | Cationic peroxidase isozyme 40K precursor | * | * | * |
| 218      | 575605        | Cationic peroxidase isozyme 40K precursor | * | * | * |
| 3336     | 55760921      | TPA: class III peroxidase 27 precursor | * | * | * |
| 1849     | 56412205      | Ascorbate peroxidase | * | * | * |
| 1254     | 31429827      | Putative peroxidase | * | * | * |
| 1601     | 55760921      | TPA: class III peroxidase 27 precursor | * | * | * |
| 1599     | 55760921      | TPA: class III peroxidase 27 precursor | * | * | * |
| 1944     | 50917909      | Putative oxalate oxidase | * | * | * |
| 759      | 15011986      | Putative peroxidase P7X | * | * | * |
| 1149     | 475336        | Superoxide dismutase [Cu-Zn] | * | * | * |
| 918      | 50920595      | Putative ascorbate peroxidase | * | * | * |
| 921      | 2997688      | Ascorbate peroxidase | * | * | * |
| 547      | 50939495      | Putative peroxidase | * | * | * |
| 1436     | 485953        | Glutaredoxin | * | * | * |

![Figure 5. Responses to water stress (WS) in R1 to R3 of fraction 1 CWPs in the category of ROS metabolism. To allow quantitative comparisons between the proteins, the data are expressed as fold-change values (log 10 scale) using mean values from the two well-watered (WW) controls and a background value of 0.001 when a protein spot was not visualized in a particular treatment. The changes in protein abundance that are marked with an asterisk are considered to be independent of developmental changes associated with the stress-induced shortening of the elongation zone (Fig. 1) and, therefore, likely to be specific responses to water stress (see text for details of this analysis).](image)
abundance of nine β-D-glucosidases are presented in Figure 10. Spot 355 and 400 showed expression only in R1, and moreover exhibited opposite stress responses with spot 355 decreasing in abundance under water deficit while spot 400 increased in abundance. Spots 1041 and 3514 were mainly present only in R2, and also showed opposite responses to water deficit with spot 1041 decreasing and spot 3514 increasing in abundance. Spot 409 was expressed in R2 and R3 of well-watered roots and increased in abundance in R1, R2, and R3 of water-stressed roots. Spot 3502 was expressed in R3 and R4 of well-watered roots, but the expression shifted to R2 in water-stressed roots. Spots 97, 99, and 305 were expressed in all four regions of well-watered roots with a gradual increase in abundance with increasing distance from the root apex. Water deficit caused a large increase in abundance of these proteins in R1 to R3, with the highest abundance occurring in R2 in each case. This example highlights the spatial complexity of the growth zone of maize roots and may also suggest differences in function between members of this large gene family.

Increase in Apoplastic ROS in R1 of Water-Stressed Roots

As detailed above, several of the CWPs whose abundance increased in R1 of the water-stressed roots were classified as being involved in ROS metabolism (Fig. 5), suggesting that the amount of apoplastic ROS may have been altered. In particular, the increased abundance of superoxide dismutase and of two putative oxalate oxidase/germin proteins suggested that the production of hydrogen peroxide (H$_2$O$_2$) may be greater in this region of water-stressed compared to well-watered roots.

Two methods were used to evaluate the prediction from the proteomic data of increased apoplastic ROS levels in R1 of water-stressed roots. First, H$_2$O$_2$ content was measured in apoplastic fluid extracted from R1 of well-watered and water-stressed roots using the same procedures used to extract the CWPs. The results showed that the apoplastic H$_2$O$_2$ content doubled in the water-stressed roots (Fig. 11). Since the extracts from the water-stressed roots had increased abundance of peroxidases (Fig. 5), additional experiments were conducted to assess whether differences in peroxidase activity may have differentially affected the H$_2$O$_2$ quantification between the treatments. The extracts from the water-stressed roots indeed had higher peroxidase activities compared to those from well-watered roots (data not shown). However, the estimated consumption of H$_2$O$_2$ by peroxidase activity represented only a small fraction of the H$_2$O$_2$ content of the extracts, and would not have appreciably affected the H$_2$O$_2$ measurements.

Measurements of ROS can be variable under different conditions and with different methods (Tarpey and Fridovich, 2001). Accordingly, to verify the finding of increased apoplastic H$_2$O$_2$ in R1 of water-stressed roots...
compared to well-watered roots, a novel technique was developed to image apoplastic ROS levels in situ using the fluorescent indicator dye 2′,7′-dichlorodihydrofluorescein (H$_2$DCF; custom designed by Molecular Probes). The dye was modified in such a way that it should be impermeable to the plasma membrane (see details in “Materials and Methods”). Confocal images of the root epidermis provided evidence of pronounced apoplastic ROS levels in R1 of water-stressed roots, whereas there was little ROS staining in the same region of well-watered roots (Fig. 12). The ROS levels in water-stressed roots were similar at several time points examined (36, 48, and 60 h after transplanting; data not shown), indicating that the increase in ROS was not a transient event. Evidence of apoplastic localization of H$_2$DCF staining was provided both by analysis of the pattern of staining in consecutive focal planes (Supplemental Video S1) as

| Spot No. | Accession No. | ID                          |
|----------|---------------|-----------------------------|
| 409      | 4096602       | β-D-glucosidase             |
| 301      | 4096602       | β-D-glucosidase             |
| 400      | 4096602       | β-D-glucosidase             |
| 99       | 4096602       | β-D-glucosidase             |
| 97       | 4096602       | β-D-glucosidase             |
| 190      | 1885310       | Endoxyloglucan transferase (XTH) |
| 194      | 33521218      | β-galactosidase             |
| 64       | 4731111       | Exohydrolase II             |
| 201      | 3822036       | Endo-1,3-1,4-β-D-glucanase  |
| 171      | 31432825      | Putative α-galactosidase preprotein |
| 67       | 18025340      | α-L-arabinofuranosidase/β-D-xylosidase isoenzyme ARA-1 |
| 466      | 1885310       | Endoxyloglucan transferase (XTH) |
| 196      | 1885310       | Endoxyloglucan transferase (XTH) |
| 57       | 4731111       | Exohydrolase II             |
| 58       | 4731111       | Exohydrolase II             |
| 212      | 3822036       | Endo-1,3-1,4-β-D-glucanase  |
| 170      | 31432825      | Putative α-galactosidase preprotein |
| 315      | 50918070      | β-glucosidase               |
| 302      | 435313        | β-glucosidase               |
| 3527     | 3822036       | Endo-1,3-1,4-β-D-glucanase  |
| 3524     | 4096602       | β-D-glucosidase             |
| 3514     | 435313        | β-glucosidase               |
| 1650     | 57753593      | Xyloglucan endo-transglycosylase/hydrolase (XTH) |
| 1230     | 7939623       | Putative β-galactosidase    |
| 776      | 4731111       | Exohydrolase II             |
| 3482     | 61614851      | β-galactosidase             |
| 3359     | 34894342      | Putative β-xylosidase       |
| 3163     | 18025342      | β-D-xylosidase              |
| 1041     | 4096602       | β-D-glucosidase             |
| 3161     | 34894342      | Putative β-xylosidase       |
| 422      | 435313        | β-glucosidase               |
| 343      | 7671447       | β-xylosidase-like protein   |
| 362      | 4731111       | Exohydrolase II             |

Figure 7. Responses to water stress in R1 to R3 of fraction 1 CWPs in the category of hydrolases. See the legend of Figure 5 for full description.

Figure 8. Responses to water stress in R1 to R3 of fraction 1 CWPs in the category of carbohydrate metabolism. See the legend of Figure 5 for full description.
well as by comparison to the different staining pattern obtained using 5-(and-6)-carboxy-2',7'-H2DCF diacetate (carboxy-H2DCFDA), an unmodified dye that is permeable to the membrane. The membrane-permeable dye showed distinct staining of the cytoplasm and cellular organelles (Fig. 12, inset), whereas there was no evidence of intracellular staining using H2DCF. It should be noted that in contrast to the root epidermal cells, detached and mature root cap cells showed obvious cytoplasmic and nuclear staining with H2DCF (Fig. 12), probably due to impairment of plasma membrane integrity associated with the onset of apoptosis. The distinct staining pattern of the root cap cells further reinforces the conclusion that H2DCF staining of the root epidermal cells in R1 was restricted within the apoplast.

Efforts were also made to analyze apoplastic ROS levels in R2 using H2DCF staining. However, the results suggested that the use of the dye in R2 was unreliable. There was often evidence of cytosolic staining in both the well-watered and water-stressed roots, indicating some degree of membrane permeability to the dye in the epidermal cells of this region. Thus, these results are not presented.

DISCUSSION

Identification and Spatial Distribution of Fraction 1 CWPs in the Root Elongation Zone

Using validated methods for fraction 1 CWP extraction from the maize primary root elongation zone (Zhu et al., 2006) in combination with mass spectrometry analysis, a total of 152 proteins were identified from water stress-responsive protein spots on 2-DE gels from three regions that exhibited distinct responses of elongation rate to water stress. In agreement with the findings of our previous study (Zhu et al., 2006), in which the analysis was limited to the whole elongation zone of well-watered roots with no spatial resolution, the results indicate that the method effectively enriched for CWPs with minimal cytosolic contamination. First, a large number of the proteins identified in this study have also been isolated from cell walls using other approaches, and many of these proteins are related to cell wall metabolism and structural modification (Fry, 1988). Second, 105 of the 152 proteins (69%) have an N-terminal signal peptide, whereas it was previously shown that only 3% of proteins identified in an extract of total soluble proteins from the root elongation zone have signal peptides (Zhu et al., 2006). In addition, increasing evidence suggests that proteins can be secreted into cell walls without having a signal peptide (Voigt and Frank, 2003; Slabas et al., 2004; Juarez-Diaz et al., 2006). Consistent with this model, 17 of the proteins were predicted to be so-called nonclassical secretory proteins (Bendtsen et al., 2004; Zhu et al., 2006), and among the 30 remaining proteins, 19 were previously identified in cell walls including two malate dehydrogenases, two glyoxalases I, three UDP-Glc pyrophosphorylases, and 12 b-D-glucosidases (Gross, 1977; Fry, 1988; Li et al., 1989; Chivasa et al., 2002; Pitarch et al., 2002; Kleczkowski et al., 2004; Watson et al., 2004).

Among the 152 identified proteins, 61 (40%) were not identified in our previous study of the cell wall proteome from the elongation zone of well-watered roots (Zhu et al., 2006). The presence of many newly identified proteins was due partly to the imposition of water stress that increased the abundance of particular
proteins, and partly to the spatial profiling that may have resulted in an enrichment of proteins in specific regions of the elongation zone.

The spatial distributions of protein abundance in relation to the elongation rate profiles provide insight into potential functions for some of the proteins (Fig. 10; Supplemental Figs. S1–S5). This spatial resolution also helps to distinguish the function of different proteins in the same family. As shown in Figure 10, nine spots that were identified as \( \beta \)-D-glucosidase showed widely varying spatial profiles in well-watered roots, implying that the proteins may have different roles associated with specific processes in the different regions. The differential responses of these proteins to water stress further support the notion that these proteins function differently. Another example of significant variation both in abundance profiles and in the pattern of response to water stress between proteins in the same family is provided by the peroxidases (Supplemental Fig. S1). Potential functions of \( \beta \)-D-glucosidases and peroxidases are discussed further in the following sections.

It should be noted that the R1 samples included the root cap in addition to the apical 3 mm of the root (additional washing steps were included to remove the majority of border cells from the root cap periphery). Thus, a fraction of the proteins identified in R1 may be extracellular proteins derived from the root cap. A comparison was made with a recent report on the pea (\textit{Pisum sativum}) root cap secretome (Wen et al., 2007), which revealed that only four of the root cap proteins matched with the 53 proteins identified in R1. These results suggest that the contribution of proteins secreted from the root cap was minor.

**Figure 10.** Spatial distribution of protein abundance for nine \( \beta \)-D-glucosidases in R1 to R4 of roots grown under well-watered (WW24 [developmental control] and WW48 [temporal control]) or water-stressed (WS48) conditions. The fitted lines were smoothed using the SPLINE method. Different letters indicate significant differences at the \( P < 0.05 \) level.
Increased Apoplastic ROS in Water-Stressed Roots—Potential Role in Enhanced Cell Wall Loosening

One large group of CWPs that showed changes in abundance under water stress are related to ROS metabolism (Fig. 5). ROS, including superoxide radicals, \( \text{H}_2\text{O}_2 \), and hydroxyl radicals, are normally produced in various cell compartments including the apoplast (Mittler et al., 2004). Increased ROS production often occurs under abiotic and biotic stress conditions, including water deficit, and can be associated with oxidative damage (Iturbe-Ormaetxe et al., 1998). Thus, it is possible that the changes in proteins associated with ROS metabolism may play a role in scavenging ROS and thereby preventing oxidative damage to the root cell walls and plasma membrane under water stress conditions (Apel and Hirt, 2004). However, it is notable that in R1, the six proteins in this category that increased in abundance included two putative oxalate oxidase/germin proteins and a superoxide dismutase, which contribute to \( \text{H}_2\text{O}_2 \) production (Lane, 1994; Pignocchi and Foyer, 2003), and two peroxidases/peroxidase precursors, which can also contribute to ROS production including the generation of hydroxyl radicals from \( \text{H}_2\text{O}_2 \) (Schweikert et al., 2000; Liszkay et al., 2003; Passardi et al., 2004). Thus, these changes in protein abundance suggested that apoplastic ROS levels may have increased in R1 of the water-stressed roots. This hypothesis was tested and confirmed both by quantification of \( \text{H}_2\text{O}_2 \) content in extracted apoplastic fluid and by in situ imaging of apoplastic ROS (Figs. 11 and 12).

The increase in apoplastic ROS in R1 under water deficit conditions could be involved in the enhanced longitudinal extensibility of the cell walls in this region of water-stressed compared to well-watered roots (Wu et al., 1996) and, thereby, in the maintenance of cell elongation despite reduced turgor pressure (Spollen and Sharp, 1991). Recent studies suggest that generation of hydroxyl radicals from \( \text{H}_2\text{O}_2 \) (by either the Fenton reaction or peroxidase activity) can play a direct role in cell wall loosening via polysaccharide cleavage (Fry, 1998; Fry et al., 2001; Liszkay et al., 2003), and there is evidence for this activity in the elongation zone of leaves (Rodriguez et al., 2002) and primary roots (Liszkay et al., 2004) of well-watered maize. Further, there is evidence that salinity-induced inhibition of leaf expansion in maize is associated with reduced apoplastic ROS production (Rodriguez et al., 2004, 2007). However, to our knowledge, up-regulation of this mechanism of wall loosening in the response of root growth to water stress has not been investigated. Indeed, enhanced cell wall loosening as an adaptive response to stress conditions has been little studied, being limited to investigations of the maintenance of elongation in water-stressed roots (for review, see...
Stiffening by oxidative cross-linking of wall phenolics may be involved in other processes including cell wall loosening. In R3 under water stress, these peroxidases decreased in abundance compared to well-watered roots. This could represent a mechanism to redirect resources and energy for abiotic stress adaptation. However, a few proteins in this category, including two polygalacturonase inhibitor proteins in R1 and an osmotin in R3, increased in abundance under water stress. These particular proteins may be important in the general defense responses of stressed plants. Osmotin, for example, was shown to be involved in responses to NaCl desiccation, ethylene, abscisic acid (ABA), and UV treatments as well as in resistance to fungal and viral attack (Liu et al., 1994).

The largest group of water stress-responsive CWPs identified in this study was the category of hydrolases (Fig. 7). In R1, the abundance of most hydrolases decreased markedly in the water-stressed roots. It was previously shown that the deposition rate and content of cell wall mass per unit length of root was decreased in the elongation zone of water-stressed compared to well-watered maize primary roots (Wu et al., 1994). Therefore, an overall reduction in cell wall hydrolases may reflect lower cell wall polysaccharide metabolism in water-stressed roots. However, three XTHs and two endo-1,3;1,4-β-D-glucanases, which are endocleavage type hydrolases and implicated in cell wall loosening processes (Nishitani and Tominaga, 1991; Fry et al., 1992; Kim et al., 2000), also decreased in abundance in R1 of the water-stressed roots. The decreased abundance of the XTHs, in particular, was unexpected since a previous study using the same experimental system (although with a different genotype) showed that the total extractable xyloglucan endotransglucosylase activity (XET; one of the two known activities of XTH proteins; Rose et al., 2002) was greater in R1 and R2 of water-stressed compared to well-watered roots (Wu et al., 1994), suggesting that XTH may be involved in the stress-induced enhancement of cell wall extensibility in the apical region. Moreover, the abundance profiles of these XTHs showed a close correlation with the spatial distribution of elongation rate in the well-watered roots (Supplemental Fig. S3). One of the possible explanations for the discrepancy between the XET activity and XTH protein abundance is that the XET activity was assayed from total soluble protein extracts and may not represent the activity of XTH specifically in the cell walls. It is also possible that XET activity was regulated at the posttranslational level. Thus, subtle protein modifications, which were not detected in this study, might lead to increased XET activity in water-stressed roots despite a decrease in protein abundance.

Interestingly, the only proteins in the hydrolase category that increased in abundance in R1 of the water-stressed roots were a group of five β-D-glucosidases (Figs. 7 and 10). Although exocleavage-type hydrolases are implicated in regulating cell elongation (Huber and Nevins, 1981; Kim et al., 2000), the β-D-glucosidases identified in this study may not play the same role since most of these proteins also increased in abundance in R2 and R3 under water stress where cell elongation had slowed or ceased, respectively (Fig. 1). There is evidence that β-D-glucosidase activity in the root apoplast functions in releasing free ABA from the ABA conjugate ABA-Glc ester, which may serve as a long-distance transport form (Hartung et al., 2002; Sauter et al., 2002; Lee et al., 2006; Schroeder and Nambara, 2006). ABA accumulates to high concentrations in the root elongation zone under water-stressed conditions, particularly toward the apex (Saab et al., 1992), and the accumulation is required for the maintenance of root elongation (Saab et al., 1990; Sharp et al., 1994; Sharp, 2002). Accordingly, the stress-induced increase in abundance of several β-D-glucosidases may be involved in ABA release from conjugated ABA transported to the elongation zone.

Apoplastic β-D-glucosidases have also been implicated in lignin synthesis. One of the proposed mechanisms for lignin synthesis is that monolignols are synthesized inside the cell and secreted into the cell wall as monolignol glucosides, where β-D-glucosidases hydrolyze the glucoside and release monolignols for lignin synthesis (Whetten et al., 1998). In this case, the β-D-glucosidases may play roles in reducing cell wall extensibility and inhibiting cell elongation in R2 and...
R3 of the water-stressed roots. Consistent with this possibility, Fan et al. (2006) published evidence for increased lignin metabolism in association with reduced cell wall extensibility in the basal region of the elongation zone in water-stressed maize primary roots.

Among the carbohydrate metabolism-related CWPs, three acid invertases increased in abundance in R2 and R3 of the water-stressed roots (Fig. 8). Interestingly, Tang et al. (1999) reported that antisense repression of a cell wall invertase resulted in a drastic decrease in taproot growth and development in carrot (Daucus carota). The smaller taproots were associated with a lower level of carbohydrate in roots but an elevated level of Suc and starch in leaves, suggesting that cell wall invertase plays an important role in Suc partitioning. In maize seedlings, primary root growth is dependent on the Suc supply from the kernel, and in particular, water-stressed roots accumulate a significant amount of sugars for osmotic adjustment in the elongation zone (Sharp et al., 1990). Thus, an increase in cell wall invertase (and activity) could create a strong sink to enhance Suc transport from the kernel to the roots to maintain a supply of sugars for root growth and stress adaptation.

The CWPs in the category of other/unknown further reflect the complexity of the response to water stress (Fig. 9). There are indications of regulation of protein modification involving α-1,4-glucan protein synthase, carbohydrate modification involving putative β-N-acetylhexosaminidase, and lipid modification, presumably associated with the plasma membrane, involving lipases. The involvement of many other proteins in the stress response remains unknown. For example, two legumin-like proteins were up-regulated specifically in R2. Legumin-like proteins are seed storage proteins and are usually found in seed endosperm (Meakin and Gatehouse, 1991). It is not clear whether these proteins perform a similar role for nutrient storage in the root elongation zone or whether they perform different roles. Further work is necessary to address the functionality of these proteins.

CONCLUSION

The results reveal major and predominantly region-specific changes in fraction 1 CWP composition in the elongation zone of water-stressed compared to well-watered roots. Stress-induced changes in CWPs are involved in multiple processes that regulate the pattern of response of cell elongation within the elongation zone. In particular, the protein identifications predicted that apoplastic ROS levels are increased in the apical region of the elongation zone in water-stressed roots, which was confirmed by quantification of H2O2 and in situ imaging. This response could contribute directly to the known enhancement of wall loosening in this region and, thereby, the maintenance of cell elongation despite reduced turgor pressure. Future studies of the tightly ionically bound and covalently bound CWP fractions will provide additional insight into the complexity of mechanisms that regulate root growth under water stress.

MATERIALS AND METHODS

Maize Seedling Culture, Harvest, and Extraction of Water Soluble Plus Lightly Ionically Bound CWPs

Maize (Zea mays 'FR697') seeds were surface sterilized in 0.3% NaOCl solution for 15 min, rinsed with distilled water, and imbibed for 24 h in aerated 1 mM CaSO4. The seeds were germinated in vermiculite (no. 2A, Therm-O-Rock East Inc.), which was well moistened with 1 mM CaSO4 for 28 h at 29°C and near-saturation humidity in the dark (Spollen et al., 2000). Seedlings with primary roots approximately 10 mm in length were transplanted to plastic containers containing vermiculite at water potentials of −0.03 MPa (well watered) or −1.6 MPa (water stressed), which were obtained by thorough mixing with different amounts of 1 mM CaSO4. Vermiculite water potentials were measured by isopiestic thermocouple psychrometry (Boyer and Knippling, 1965). The seedlings were then grown under the same conditions until the primary roots were harvested at 24 h (developmental control, roots of the same length as the water-stressed treatment) and 48 h (temporal control, roots of the same age as the water-stressed treatment) after transplanting in the well-watered treatment, and at 48 h after transplanting in the water-stressed treatment. The apical 20 mm of each root was sectioned into four regions (distances are from the root cap junction): R1, 0 to 3 mm plus the root cap; R2, 3 to 7 mm; R3, 7 to 12 mm; R4, 12 to 20 mm. Transplanting and harvesting were performed using a green safelight (Saab et al., 1990).

Immediately after harvest, the root segments were transferred into 20 mM ice-cold K2HPO4 solution (pH 6.0). The segments were then rinsed twice with distilled, deionized water and twice with 0.01 M MES buffer; it should be noted that these steps probably removed the majority of border cells from the root cap periphery (Wen et al., 2007). Water soluble and lightly ionically bound CWPs were then extracted according to the method optimized for the maize primary root elongation zone by Zhu et al. (2006). At each harvest of each treatment, three batches of 50 segments per region were used for CWP extraction; the extracts from the three batches were combined to produce a subsample. Five subsamples were pooled for each of three replicate samples per region for each treatment (i.e., CWPs were extracted from a total of 750 segments per sample).

Protein Separation by 2-DE

Prior to 2-DE, the CWP samples were precipitated overnight at ~70°C with 10% (w/v) TCA, and the pellets were washed three times with ice-cold methanol and dried briefly. The proteins were quantified using either Bio-Rad RCDC Protein Microassay (Bio-Rad) or RediPlate EZQ Protein Quantitation kit (Molecular Probes) according to the manufacturer’s recommendations. Samples of 7 μg protein were solubilized in 185 μL of an isoelectric focusing buffer (Sequential Extraction buffer 3; Bio-Rad). 2-DE was carried out as previously described (Zhu et al., 2006). After electrophoresis, gels were fixed in 7% acetic acid, 10% methanol for 1 h, and stained overnight with SyproRuby (Molecular Probes). The stained gels were washed in 7% acetic acid, 10% methanol for 1 h, and rinsed with water.

Image Analysis of 2-DE Gels

Protein spots were visualized using the TYPHOON 9410 system (Amersham Biosciences). Gels containing the three replicate samples from each treatment and each region were analyzed with Phoretix 2D Evolution software (Nonlinear Dynamics) enabling spot detection, quantification, and spot matching across different gels. The automatic spot detection and matching was followed by a manual correction. Experimental M/s were calibrated using commercial molecular mass standards run in a separate marker lane on the 2-DE gels, and the experimental pIs were calibrated according to Bio-Rad IPG strip specifications.

After subtracting background with the nonslip mode (margin 45), spot volumes were normalized by dividing each spot volume by the total volume of all spots present in all gels. The normalized spot volumes were used to determine the quantitative variation of protein expression across the treatments.
and regions. Region-specific water deficit-responsive spots were identified after normalizations within the batch of nine gel images (three replicates for each of three treatments) from each region. All 36 gel images were normalized for across-region comparisons of protein abundance. To determine the statistical significance of changes in protein abundance, a multiple comparison of means (Student-Newman-Keuls test) was performed with normalized protein spot volume as variables and control or treatment as factors, and a confidence level of 95% (SAS 5.1, SAS Institute Inc.). For proteins that were not detected in one condition when compared to another, a protein abundance of 0.001, which was the minimal value of spot intensity, was used for calculation of fold-change.

**Protein Identification by HPLC-Electrospray Quadrupole Time-of-Flight Mass Spectrometry**

Proteins differentially expressed were excised from gels and digested as described in Zhu et al. (2006). Peptide separation was performed by nanoflow HPLC (Ultimate). Five microliters of protein digests were loaded onto a C18 precolumn (LC Packing) for desalting and concentrating. Peptides were then eluted from the precolumn and separated on a nanoflow analytical C18 column and analyzed using an ABI QSTAR XL (Applied Biosystems/MDSci) as described in Zhu et al. (2006). Time-of-flight mass spectrometry spectra and product ion spectra were acquired using Analyst Q5 software. The peptide tandem mass spectra were searched against the maizeseq.org MAIZE EST database (www.maizeseq.org) using MASCOT search engine (http://www.matrixscience.com). Unambiguous identification was judged by the number of peptide sequence tags, sequence coverage, mowse score, the quality of tandem mass spectrometry spectra, and reproducibility of identifi-
cation across gels.

**Apoplastic H$_2$O$_2$ Assay**

Apoplastic fluid from R1 of roots grown under well-watered or water-stressed conditions for 48 h was extracted by the vacuum infiltration and centrifugation method described in Zhu et al. (2006) except without the desalting procedure. The H$_2$O$_2$ content was quantified using an Amplex Red Hydrogen Peroxide/Peroxidase Assay kit (Molecular Probes).

**In Situ Imaging of Apoplastic ROS**

A novel dye, H$_2$DCF, was custom synthesized for this study (Molecular Probes). The dye is a derivative of carboxy-H$_2$DCFDA, which is a fluorescent indicator of intracellular ROS (Maxwell et al., 1999), in which the acetone groups (which allow the molecule to cross the plasma membrane) have been cleaved. Therefore, H$_2$DCF should be restricted within the cell wall and apoplastic space.

Seedlings were grown in vermiculite under either well-watered or water-stressed conditions as described above. At 36, 48, or 60 h after transplantation, the seedlings were removed from the vermiculite and the tips of intact primary roots were immersed in a staining agarose solution. The solution was prepared as a combination of 1% high- and 1% low-gelling temperature agarose (1:1) in 1 mM CaSO$_4$, which solidified at approximately 30°C. The solution was allowed to cool and as it approached 30°C, H$_2$DCF and the fluorescent membrane probe FM 1-43 [4-(4-(dibutylamino) styryl) pyridinium dibromide; Molecular Probes] were added at final concentrations of 30 M and 50 M, respectively. Roots were then immersed immediately before the onset of solidification. For the staining of water-stressed roots, the water potential of the agarose solution was adjusted to ~1.6 MPa (the same water potential as the vermiculite in which the roots were growing) by addition of melibiose to avoid imposing an osmotic shock to the roots. Melibiose was chosen for this purpose because of evidence that it is neither hydrolyzed nor taken up by cells (Dracup et al., 1986). The agarose staining solution was allowed to cool and as it approached 30°C, agarose staining solution was developed to minimize potential diffusion of that it is neither hydrolyzed nor taken up by cells (Dracup et al., 1986). The shock to the roots. Melibiose was chosen for this purpose because of evidence that it is neither hydrolyzed nor taken up by cells (Dracup et al., 1986).

The epidermal cells were imaged for H$_2$DCF fluorescence using two-photon laser-scanning confocal microscopy (Zeiss LSM NLO 510 combined with a Coherent, Chameleon 720–950 nm tunable two photon laser) at 750 nm infrared excitation wavelength, and the emission was captured between 500 to 550 nm. The images were scanned without scan averaging (fast acquisition) using a 10X (EC Plan-Neofluar NA 0.3) objective together with 1.9 digital zoom under 512 pixel resolution in XY. The power, gain, offset, and detector gain levels were optimized and kept constant between different experimental samples in a given day. FM 1-43 and carboxy-H$_2$DCFDA imaging processes were the same as for H$_2$DCF with the exception of excitation and emission wavelengths, which were 543 nm and 565 to 615 nm, and 488 nm and 500 to 550 nm, respectively. All images were processed by LSM 5 Image Examiner under identical conditions.

**Supplemental Data**

The following materials are available in the online version of this article.

**Supplemental Figure S1.** Spatial distribution of abundance for CWPs in the category of ROS metabolism.

**Supplemental Figure S2.** Spatial distribution of abundance for CWPs in the category of defense and detoxification.

**Supplemental Figure S3.** Spatial distribution of abundance for CWPs in the category of hydrolases.

**Supplemental Figure S4.** Spatial distribution of abundance for CWPs in the category of carbohydrate metabolism.

**Supplemental Figure S5.** Spatial distribution of abundance for CWPs in the category of other/unknown.

**Supplemental Table S1.** Identities of water deficit-responsive protein spots from the 2-DE gels of water soluble and lightly ionically bound (fraction 1) CWPs.

**Supplemental Video S1.** Consecutive focal planes of the merged confocal image of a water-stressed root presented in Figure 12.

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Zhu et al.
Supplemental Figure S1 (page 1 of 3)
Supplemental Figure S1 (page 3 of 3)
Supplemental Figure S2 (page 1 of 3)
Supplemental Figure S2 (page 2 of 3)
Supplemental Figure S2 (page 3 of 3)
Supplemental Figure S3 (page 1 of 3)
Supplemental Figure S3 (page 2 of 3)
Supplemental Figure S4 (page 1 of 1)
Supplemental Figure S5 (page 1 of 3)
Supplemental Figure S5 (page 2 of 3)
Spot 1334 Profilin 5

Spot 1158 Putative ubiquitin-conjugating enzyme family protein

Supplemental Figure S5 (page 3 of 3)
**Supplemental Figure S1.** Spatial distribution of protein abundance for 14 fraction 1 CWPs in the category of ROS metabolism in the apical 20 mm of primary roots grown under well-watered (water potential of -0.03 MPa) or water-stressed (water potential of -1.6 MPa) conditions. Well-watered roots were harvested at 24 h (WW24; developmental control, roots of the same length as water-stressed roots) and 48 h (WW48; temporal control, roots of the same age as water-stressed roots) after transplanting. Water-stressed roots were harvested at 48 h after transplanting (WS48). Three replicates were analyzed for each treatment. The fitted lines were smoothed using the SPLINE method. Different letters indicate significant differences at the P<0.05 level. The panels are presented from left to right in the same order as the listing of proteins in Figure 5 of the main body of the paper.

**Supplemental Figure S2.** Spatial distribution of protein abundance for 17 fraction 1 CWPs in the category of defense and detoxification in the apical 20 mm of primary roots grown under well-watered or water-stressed conditions. See the legend for Supplemental Figure S1 for full description. The panels are presented from left to right in the same order as the listing of proteins in Figure 6 of the main body of the paper.

**Supplemental Figure S3.** Spatial distribution of protein abundance for 15 fraction 1 CWPs in the category of hydrolases in the apical 20 mm of primary roots grown under well-watered or water-stressed conditions. See the legend for Supplemental Figure S1 for full description. The panels are presented from left to right in the same order as the listing of proteins in Figure 7 of the main body of the paper.

**Supplemental Figure S4.** Spatial distribution of protein abundance for four fraction 1 CWPs in the category of carbohydrate metabolism in the apical 20 mm of primary roots grown under well-watered or water-stressed conditions. See the legend for Supplemental Figure S1 for full description. The panels are presented from left to right in the same order as the listing of proteins in Figure 8 of the main body of the paper.
**Supplemental Figure S5.** Spatial distribution of protein abundance for 14 fraction 1 CWPs in the category of other/unknown in the apical 20 mm of primary roots grown under well-watered or water-stressed conditions. See the legend for Supplemental Figure S1 for full description. The panels are presented from left to right in the same order as the listing of proteins in Figure 9 of the main body of the paper.

**Supplemental Video S1.** Consecutive focal planes (each 3 µm in thickness) of the merged confocal image from R1 (approximately 1.5 mm from the apex) of a water-stressed root presented in Figure 12 of the main body of the paper. The root was stained for apoplastic ROS using H$_2$DCF (green fluorescence) and with the membrane probe FM 1-43 (red fluorescence) to visualize the cellular structure. In contrast to the apoplastic localization of ROS staining with H$_2$DCF in the root epidermis, several detached and mature root cap cells showed cytoplasmic and nuclear staining with H$_2$DCF.
Supplemental Table S1. Identities of water deficit-responsive protein spots from the 2-DE gels of water soluble and lightly ionically-bound (fraction 1) CWPs

Spots were identified by HPLC-electrospray quadrupole time-of-flight mass spectrometry, and proteins were classified in five functional categories. SP refers to the presence of a signal peptide sequence predicted by SignalP (v3) with a P value threshold greater than 0.900. NSP indicates non-classical secretory proteins predicted by SecretomeP 1.0b (http://www.cbs.dtu.dk/services/SecretomeP-1.0) with an NN score greater than 0.600. Identifications and accession numbers (protein GI number) are from the National Center for Biotechnology Information (NCBI) database. The score, number of matched peptides and percent coverage are taken directly from the Mascot Daemon report. Theoretical molecular mass (in kD) and pI were estimated based on the top protein sequence obtained by BLASTX. Proteins were identified from R1 to R3 in the present study or by image cross comparison with the master gels (MG) in Zhu et al. (2006). Protein spots from R4 were not used for identifications. Protein identifications marked with an asterisk are one of the multiple protein identifications obtained for that spot number.
| Spot No. | Identification | Experimental Mass | Theoretical Mass | Accession No. | Mascot Score | No. of Peptides | Coverage % | SP | NSP | Identified from Region(s) | Organism Matched          |
|----------|----------------|-------------------|------------------|--------------|--------------|----------------|------------|----|-----|----------------------------|---------------------------|
| 157      | Peroxidase     | 39 8.2            | 36 8.0           | 25044849     | 151          | 3 10           | x          | R3 |     | Ananas comosus              |                           |
| 176      | Malate dehydrogenase* | 37 6.3         | 35 8.7           | 19880701     | 147          | 3 12           | x          | R1 |     | Oryza sativa                |                           |
| 176      | Putative peroxidase 1 precursor* | 37 6.3         | 35 7.3           | 52076453     | 168          | 3 13           | x          | R1 |     | Oryza sativa                |                           |
| 177      | Class III peroxidase 80 precursor | 37 6.6         | 34 6.9           | 55701027     | 177          | 3 26           | x          | R1 |     | Oryza sativa                |                           |
| 218      | Cationic peroxidase isozyme 40K precursor | 24 6.7         | 36 8.7           | 575605       | 60           | 3 12           | x          | R1 |     | Nicotiana tabacum            |                           |
| 354      | Cationic peroxidase isozyme 40K precursor | 36 9.2         | 36 8.7           | 575605       | 431          | 8 36           | x          | R1 |     | Nicotiana tabacum            |                           |
| 381      | Superoxide dismutase [Cu-Zn] | 17 5.9         | 15 5.6           | 134598       | 89           | 4 23           | x          | MG | 45  | Zea mays                    |                           |
| 385      | Putative ascorbate peroxidase | 25 5.5         | 27 5.4           | 50920595     | 274          | 6 20           | x          | R1 |     | Oryza sativa                |                           |
| 386      | Putative oxalate oxidase | 23 5.6         | 24 9.0           | 50917909     | 135          | 2 8            | x          | R1 | R2 | Oryza sativa                |                           |
| 395      | Probable germin protein 4 | 23 6.8         | 22 7.8           | 34902528     | 128          | 2 11           | x          | R1 |     | Oryza sativa                |                           |
| 547      | Putative peroxidase | 47 7.3         | 40 7.2           | 50939495     | 173          | 3 20           | x          | R3 |     | Oryza sativa                |                           |
| 735      | Putative peroxidase P7X* | 36 5.1         | 34 6.9           | 15011986     | 116          | 2 7            | x          | R3 |     | Oryza sativa                |                           |
| 759      | Putative peroxidase P7X | 34 7.5         | 34 6.9           | 15011986     | 58           | 2 8            | x          | R3 |     | Zea mays                    |                           |
| 918      | Putative ascorbate peroxidase* | 26 5.2         | 27 5.4           | 50920595     | 170          | 4 12           | x          | R3 |     | Oryza sativa                |                           |
| 921      | Ascorbate peroxidase* | 26 5.1         | 27 5.5           | 2997688      | 79           | 3 15           | x          | R3 |     | Zantedeschia aethiopica     |                           |
| 1149     | Superoxide dismutase [Cu-Zn] | 17 5.6         | 15 5.6           | 4753356      | 83           | 4 18           | x          | MG | 45  | Zea mays                    |                           |
| 1254     | Putative peroxidase | 40 3.9         | 36 4.7           | 31429827     | 224          | 4 11           | x          | R2 |     | Oryza sativa                |                           |
| 1436     | Glutaredoxin    | 11 8.3           | 12 7.1           | 485953       | 348          | 4 44           | x          | R3 |     | Oryza sativa                |                           |
| 1599     | TPA: class III peroxidase 27 precursor | 31 7.0         | 33 8.4           | 55700921     | 149          | 4 18           | x          | R2 |     | Oryza sativa                |                           |
| 1601     | TPA: class III peroxidase 27 precursor | 31 8.2         | 33 8.4           | 55700921     | 465          | 9 43           | x          | R2 |     | Oryza sativa                |                           |
| 1663     | Putative germin-like protein* | 28 8.0         | 24 8.5           | 50251393     | 132          | 2 24           | x          | R2 |     | Oryza sativa                |                           |
| 1849     | Ascorbate peroxidase | 25 5.4         | 20 7.5           | 56412205     | 66           | 3 14           | x          | R3 |     | Pennisetum glaucum          |                           |
| 1902     | TPA: class III peroxidase 27 precursor * | 34 8.1         | 33 8.1           | 55700921     | 150          | 3 9            | x          | R3 |     | Oryza sativa                |                           |
| 1944     | Putative oxalate oxidase | 22 5.6         | 24 9.0           | 50917909     | 121          | 2 11           | x          | R3 |     | Oryza sativa                |                           |
| 3185     | Thioredoxin h * | 11 4.1           | 13 4.9           | 12082335     | 63           | 2 15           | x          | R2 |     | Oryza sativa                |                           |
| 3336     | TPA: class III peroxidase 27 precursor | 15 4.6         | 33 8.4           | 55700921     | 68           | 2 5            | x          | R2 |     | Oryza sativa                |                           |
| Gene ID | Description                                           | Mw | pH | pI | Accession | EC | Status | Organism          |
|---------|-------------------------------------------------------|----|----|----|-----------|----|--------|-------------------|
| 3464    | Malate dehydrogenase*                                 | 35 | 5.9| 35 | 8.7       | 19880701 | 2   | 6     | R2 Oryza sativa    |
| 3465    | Putative purple acid phosphatase*                     | 34 | 6.0| 37 | 5.3       | 31429892 | 143 | 3     | 10 x R2 Oryza sativa |
| 3505    | Putative ascorbate peroxidase*                        | 23 | 6.5| 27 | 5.4       | 50920595 | 137 | 3     | 12 x R2 Oryza sativa |
| 3505    | Probable germin protein 4*                             | 23 | 6.5| 22 | 7.8       | 34902528 | 203 | 3     | 18 x R2 Oryza sativa |

**Defense and detoxification**

| Gene ID | Description                                           | Mw | pH | pI | Accession | EC | Status | Organism          |
|---------|-------------------------------------------------------|----|----|----|-----------|----|--------|-------------------|
| 50      | Putative Bplo*                                         | 79 | 6.1| 65 | 6.1       | 34897712 | 52  | 2     | 4 x R1 Oryza sativa |
| 74      | Putative syringolide-induced protein B13-1-1*          | 72 | 7.7| 63 | 7.7       | 50899710 | 183 | 5     | 10 x R1 Oryza sativa |
| 125     | β-1,3-glucanase                                        | 51 | 5.8| 49 | 6.3       | 924953   | 197 | 4     | 10 x R1 Triticum aestivum |
| 134     | Putative early nodulin 8 precursor                    | 45 | 7.5| 42 | 7.3       | 50938787 | 471 | 10    | 29 x R1 R2 R3 Oryza sativa |
| 138     | Rdc1:aspartyl protease family                         | 44 | 7.9| 45 | 6.9       | 32972250 | 109 | 4     | 4 x MG spot 19 Oryza sativa |
| 141     | Rdc1                                                  | 45 | 8.6| 45 | 7.1       | 49532749 | 118 | 3     | 9 x R1 Oryza sativa |
| 142     | Rdc1                                                  | 44 | 7.3| 45 | 7.1       | 49532749 | 409 | 8     | 20 x R1 R3 Oryza sativa |
| 150     | Rdc1                                                  | 44 | 7.7| 45 | 7.1       | 49532749 | 261 | 5     | 14 x R1 R3 Oryza sativa |
| 183     | Polygalacturonase inhibitor protein                   | 36 | 7.8| 36 | 7.5       | 18148925 | 486 | 8     | 25 x R1 Citrus sp. cv. Sainum |
| 185     | Polygalacturonase inhibitor protein                   | 36 | 7.3| 36 | 7.5       | 18148925 | 575 | 10    | 45 x R1 Citrus sp. cv. Sainum |
| 190     | Polygalacturonase inhibitor protein*                  | 34 | 6.8| 36 | 7.5       | 18148925 | 133 | 3     | 9 x R1 Citrus sp. cv. Sainum |
| 216     | γ-glutamyl transpeptidase                             | 25 | 6.8| 61 | 9.5       | 928934  | 97  | 3     | 14 x R1 Arabidopsis thaliana |
| 244     | Putative dirigent protein*                            | 12 | 4.5| 20 | 8.7       | 42454402 | 93  | 2     | 22 x R1 Saccharum officinarum |
| 358     | Putative chitinase                                    | 28 | 8.6| 32 | 6.5       | 55168113 | 380 | 7     | 24 x R1 Oryza sativa |
| 401     | Putative early nodulin 8 precursor                    | 45 | 6.9| 42 | 7.3       | 50938787 | 242 | 4     | 24 x R1 Oryza sativa |
| 402     | Putative early nodulin 8 precursor*                   | 43 | 6.9| 42 | 7.3       | 50938787 | 273 | 6     | 18 x R1 Oryza sativa |
| 407     | Polygalacturonase inhibitor protein                   | 34 | 7.1| 36 | 7.5       | 18148925 | 183 | 4     | 16 x R1 Citrus sp. cv. Sainum |
| 735     | Putative phytocyanin protein, PUP2*                    | 36 | 5.1| 27 | 5.2       | 52076874 | 102 | 2     | 7 x R3 Oryza sativa |

3
| Gene Name                                      | ORF Length (bp) | E-value (Bit Score) | ORF Start (AA) | ORF Length (AA) | MSA Score | Identity (AA) | Accession Number | Organism       |
|-----------------------------------------------|-----------------|--------------------|----------------|-----------------|-----------|---------------|-----------------|----------------|
| Glyoxalase I                                  | 37932483        | 138                | 32             | 5.7             | Z. mays   | R3            | 820             | **Zea mays**    |
| Putative class III chitinase RCB4             | 31432078        | 44                 | 31             | 4.6             | O. sativa | R3            | 840             | **Oryza sativa** |
| Probable β-1,3-glucanase                      | 7489680         | 147                | 50             | 5.6             | T. aestivum | R3            | 1071            | **Triticum aestivum** |
| Putative β-1,3-glucanase                      | 50905193        | 157                | 51             | 5.1             | O. sativa | R2            | 1100            | **Oryza sativa** |
| Osmotin                                       | 21207583        | 171                | 15             | 8.7             | P. ciliare | MG spot 46    | 1257            | **Pennisetum ciliare** |
| Putative disease resistance                   | 34899372        | 125                | 14             | 4.4             | O. sativa | R3            | 1334            | **Oryza sativa** |
| Putative class III chitinase RCB4             | 31432078        | 44                 | 31             | 4.6             | O. sativa | R3            | 1337            | **Oryza sativa** |
| Probable β-1,3-glucanase                      | 55168113        | 132                | 28             | 8.0             | O. sativa | R2            | 1663            | **Oryza sativa** |
| Lactoylglutathione lyase                      | 40336524        | 141                | 14             | 5.6             | A. thaliana | MG spot 50    | 1739            | **Arabidopsis thaliana** |
| Polygalacturonase inhibitor protein           | 1143381         | 103                | 23             | 6.4             | O. sativa | R3            | 1917            | **Actinidia deliciosa** |
| Cysteine proteinase inhibitor                 | 809608          | 81                 | 11             | 5.1             | Z. mays   | R2            | 2669            | **Zea mays**    |
| Polygalacturonase inhibitor                   | 34895198        | 61                 | 11             | 4.1             | O. sativa | R2            | 3185            | **Oryza sativa** |
| Glyoxalase I*                                 | 37932483        | 138                | 32             | 5.7             | Z. mays   | R2            | 3214            | **Zea mays**    |
| Putative Bplo*                                | 34897712        | 189                | 76             | 5.8             | O. sativa | R2            | 3420            | **Oryza sativa** |

**Hydrolases**

| Gene Name                                      | ORF Length (bp) | E-value (Bit Score) | ORF Start (AA) | ORF Length (AA) | MSA Score | Identity (AA) | Accession Number | Organism       |
|-----------------------------------------------|-----------------|--------------------|----------------|-----------------|-----------|---------------|-----------------|----------------|
| Exhydrolase II*                               | 4731111         | 201                | 78             | 6.9             | Z. mays   | R1            | 53              | **Zea mays**    |
| Exhydrolase II                                | 4731111         | 140                | 77             | 6.5             | Z. mays   | R1            | 57              | **Zea mays**    |
| Exhydrolase II*                               | 4731111         | 192                | 78             | 6.8             | Z. mays   | R1            | 58              | **Zea mays**    |
| Exhydrolase II*                               | 4731111         | 167                | 76             | 7.4             | Z. mays   | R1            | 62              | **Zea mays**    |
| Exhydrolase II                                | 4731111         | 100                | 76             | 6.3             | Z. mays   | R1            | 64              | **Zea mays**    |
| α-L-arabinofuranosidase/β-D-xylosidase isoenzyme ARA-1 | 18025340    | 70                 | 71             | 5.2             | H. vulgare | R1          | 67              | **Hordeum vulgare** |
| β-D-glucosidase                               | 4096602         | 362                | 59             | 5.6             | Z. mays   | R1            | 97              | **Zea mays**    |
| β-D-glucosidase*                              | 4096602         | 362                | 59             | 5.6             | Z. mays   | R1            | 98              | **Zea mays**    |
| β-D-glucosidase                              | 4096602         | 56                 | 60             | 6.2             | Z. mays   | R1            | 99              | **Zea mays**    |
| Putative α-galactosidase preproprotein        | 31432825        | 264                | 39             | 6.1             | O. sativa | R1            | 170             | **Oryza sativa** |
| Putative α-galactosidase preproprotein        | 31432825        | 158                | 38             | 5.8             | O. sativa | R1            | 171             | **Oryza sativa** |
|   | Enzyme Name                                      | Identity | PI  | MW  | pI  | EC  | Entry    | Composition | Species               |
|---|-------------------------------------------------|----------|-----|-----|-----|-----|----------|-------------|-----------------------|
| 190| Endoxyloglucan transferase (XTH)*               | 34       | 6.0 | 34  | 6.4 | 422 | 10       | 27          | R1                    |
| 194| β-galactosidase                                 | 32       | 5.4 | 89  | 6.6 | 33521218 | 47       | 2           | R1                    |
| 196| Endoxyloglucan transferase (XTH)                | 32       | 6.8 | 34  | 6.4 | 1885310  | 407      | 9           | R1                    |
| 201| Endo-1,3-1,4-β-D-glucanase                     | 29       | 7.0 | 33  | 7.2 | 3822036  | 189      | 4           | R1                    |
| 212| Endo-1,3-1,4-β-D-glucanase                     | 28       | 6.7 | 33  | 7.2 | 3822036  | 243      | 3           | R1                    |
| 301| β-D-glucosidase                                 | 58       | 5.5 | 64  | 6.2 | 4096602  | 122      | 4           | R1                    |
| 302| Exhydrolase II                                  | 77       | 6.7 | 68  | 6.6 | 4731111  | 178      | 3           | R3                    |
| 335| β-glucosidase                                   | 56       | 9.0 | 57  | 9.0 | 50918079 | 224      | 5           | R1                    |
| 343| β-xylosidase-like protein                       | 71       | 5.7 | 87  | 6.4 | 7671447  | 100      | 2           | R3                    |
| 400| β-D-glucosidase                                 | 58       | 5.3 | 64  | 6.2 | 4096602  | 208      | 6           | R1                    |
| 406| Endoxyloglucan transferase (XTH)                | 35       | 7.1 | 34  | 6.4 | 1885310  | 186      | 5           | R1                    |
| 409| β-D-glucosidase*                                | 58       | 5.2 | 64  | 6.2 | 4096602  | 228      | 6           | R1 R2 R3              |
| 422| β-glucosidase                                   | 59       | 5.4 | 65  | 6.2 | 433513   | 42       | 5           | R1                    |
| 776| Exhydrolase II                                  | 72       | 6.9 | 68  | 6.6 | 4731111  | 330      | 5           | R2                    |
| 920| Endoxyloglucan transferase (XTH)*               | 60       | 6.8 | 34  | 6.4 | 1885310  | 155      | 4           | R2                    |
| 1041| β-D-glucosidase                                 | 53       | 5.6 | 64  | 6.2 | 4096602  | 135      | 4           | R2                    |
| 1230| Putative β-galactosidase                        | 43       | 6.4 | 93  | 6.8 | 7939623  | 329      | 7           | R2                    |
| 1650| Xyloglucan endo-transglycosylase/hydrolase (XTH)| 30       | 6.0 | 31  | 4.7 | 57753593 | 115      | 2           | R2                    |
| 3161| Putative β-xylosidase                           | 98       | 5.6 | 87  | 5.2 | 34894432 | 290      | 6           | R2                    |
| 3163| β-D-xylosidase                                  | 69       | 5.5 | 83  | 6.4 | 18025342 | 121      | 2           | R2                    |
| 3214| β-galactosidase                                 | 32       | 5.2 | 84  | 6.0 | 61614851 | 77       | 2           | R2                    |
| 3228| Putative α-galactosidase*                       | 39       | 6.6 | 46  | 8.1 | 31432825 | 210      | 4           | R2                    |
| 3359| Putative β-xylosidase                           | 99       | 5.7 | 87  | 5.2 | 34894432 | 213      | 5           | R2                    |
| 3482| β-galactosidase                                 | 32       | 5.0 | 84  | 6.0 | 61614851 | 70       | 2           | R2                    |
| 3502| β-glucosidase                                   | 18       | 5.6 | 64  | 6.2 | 435313   | 88       | 8           | R2                    |
| 3514| β-glucosidase                                   | 55       | 5.4 | 64  | 6.2 | 435313   | 109      | 8           | R2                    |
| 3524| β-D-glucosidase                                 | 58       | 5.6 | 64  | 6.2 | 4096602  | 409      | 11          | R2                    |
| Entry  | Description                                      | Mass | Value 1 | Value 2 | Value 3 | Value 4 | Value 5 | Value 6 | Value 7 | Value 8 | Value 9 | Value 10 | Value 11 | Value 12 | Value 13 | Value 14 | Value 15 | Value 16 | Value 17 | Value 18 | Value 19 | R Value  | Species/Genus                                |
|--------|--------------------------------------------------|------|---------|---------|---------|---------|---------|---------|---------|---------|---------|---------|---------|---------|---------|---------|---------|---------|---------|---------|---------|---------|----------------|----------------|----------------|----------------|----------------|----------------|
| 3527   | Endo-1,3-1,4-β-D-glucanase                       | 29   | 7.0     | 33      | 7.2     | 3822036 | 166     | 3       | 13      | x       |         |         |         |         |         |         |         |         |         |         |         | R2 R3 | Zea mays                          |
| 116    | Putative cytosolic 6-phosphogluconate dehydrogenase |      |         |         |         |         |         |         |         |         |         |         |         |         |         |         |         |         |         |         |         | R3     | Zea mays                          |
| 164    | Aldose-1-epimerase-like protein                  | 40   | 7.1     | 39      | 9.4     | 2739168 | 335     | 7       | 29      | x       |         |         |         |         |         |         |         |         |         |         |         | R1     | Nicotiana tabacum                  |
| 409    | UDP-glucose pyrophosphorylase*                    | 58   | 5.2     | 52      | 5.5     | 37729658| 521     | 10      | 25      |         |         |         |         |         |         |         |         |         |         |         |         | R2     | Bambusa oldhamii                |
| 479    | Enolase                                          | 55   | 5.3     | 48      | 5.2     | 22273   | 104     | 7       | 23      | x       |         |         |         |         |         |         |         |         |         |         |         | R2     | Zea mays                          |
| 636    | Glyceraldehyde-3-phosphate dehydrogenase         | 39   | 7.4     | 36      | 6.9     | 34517179| 71      | 2       | 7       | x       |         |         |         |         |         |         |         |         |         |         |         | R2     | Zea mays                          |
| 904    | UDP-glucose pyrophosphorylase*                    | 28   | 4.6     | 52      | 5.5     | 37729658| 166     | 3       | 5       |         |         |         |         |         |         |         |         |         |         |         |         | R3     | Bambusa oldhamii                |
| 904    | Putative 6-phosphogluconolactonase*              | 28   | 4.6     | 52      | 5.5     | 37729658| 166     | 3       | 5       |         |         |         |         |         |         |         |         |         |         |         |         | R3     | Oryza sativa                     |
| 918    | Triosephosphate isomerase 1*                     | 26   | 5.2     | 27      | 5.7     | 168647  | 139     | 2       | 9       |         |         |         |         |         |         |         |         |         |         |         |         | R3     | Zea mays                          |
| 921    | Triosephosphate isomerase 1*                     | 26   | 5.1     | 27      | 5.7     | 168647  | 139     | 2       | 9       |         |         |         |         |         |         |         |         |         |         |         |         | R3     | Zea mays                          |
| 959    | Putative ribose-5-phosphate isomerase*           | 25   | 4.3     | 27      | 5.1     | 50934597| 108     | 3       | 14      | x       |         |         |         |         |         |         |         |         |         |         |         | R3     | Oryza sativa                     |
| 1101   | UDP-glucose pyrophosphorylase                     | 53   | 5.5     | 52      | 5.5     | 37729658| 140     | 4       | 7       |         |         |         |         |         |         |         |         |         |         |         |         | R3     | Bambusa oldhamii                |
| 1955   | Soluble acid invertase                           | 21   | 5.8     | 70      | 6.4     | 31872118| 270     | 4       | 9       | x       |         |         |         |         |         |         |         |         |         |         |         | R3     | Saccharum hybrid cultivar          |
| 1956   | Soluble acid invertase                           | 21   | 5.9     | 70      | 6.4     | 31872118| 233     | 3       | 9       | x       |         |         |         |         |         |         |         |         |         |         |         | R3     | Saccharum hybrid cultivar          |
| 3228   | Fructose bisphosphate aldolase*                  | 39   | 6.6     | 46      | 7.5     | 295850  | 210     | 4       | 19      | x       |         |         |         |         |         |         |         |         |         |         |         | R2     | Oryza sativa                     |
| 3390   | Aldose-1-epimerase-like protein*                  | 38   | 7.6     | 39      | 9.4     | 2739168 | 198     | 4       | 16      | x       |         |         |         |         |         |         |         |         |         |         | R2     | Nicotiana tabacum                  |
| 3390   | Fructose bisphosphate aldolase*                  | 38   | 7.6     | 38      | 7.5     | 295850  | 191     | 4       | 17      |         |         |         |         |         |         |         |         |         |         |         | R2     | Zea mays                          |
| 3426   | Soluble acid invertase                           | 46   | 5.2     | 70      | 6.4     | 31872118| 186     | 3       | 6       | x       |         |         |         |         |         |         |         |         |         |         | R3     | Saccharum hybrid cultivar          |

Carbohydrate metabolism

Other/unknown

| Entry  | Description                                      | Mass | Value 1 | Value 2 | Value 3 | Value 4 | Value 5 | Value 6 | Value 7 | Value 8 | Value 9 | Value 10 | Value 11 | Value 12 | Value 13 | Value 14 | Value 15 | Value 16 | Value 17 | Value 18 | Value 19 | R Value  | Species/Genus                                |
|--------|--------------------------------------------------|------|---------|---------|---------|---------|---------|---------|---------|---------|---------|---------|---------|---------|---------|---------|---------|---------|---------|---------|---------|----------------|----------------|----------------|----------------|----------------|----------------|
| 50     | Putative subtilisin-like proteinase*              | 79   | 6.1     | 78      | 6.3     | 40538972| 47      | 2       | 2       | x       |         |         |         |         |         |         |         |         |         |         |         | R1       | Oryza sativa                     |
| 53     | Putative subtilisin-like proteinase*              | 78   | 6.9     | 78      | 6.3     | 40538972| 182     | 4       | 6       | x       |         |         |         |         |         |         |         |         |         |         | R1       | Oryza sativa                     |
| No. | Protein Name                                           | Mw  | pI  | E  | E2 | R1 | R2 | R3 |   |   |
|-----|-------------------------------------------------------|-----|-----|----|----|----|----|----|---|---|
| 58  | Putative subtilisin-like proteinase*                  | 78  | 6.8 | 78 | 6.3| 4  | 6  | x  |   |   |
| 62  | Putative subtilisin-like proteinase*                  | 76  | 7.4 | 78 | 6.3| 129| 3  | 4  | x |   |
| 74  | Putative subtilisin serine protease ARA12*            | 72  | 7.7 | 79 | 6.3| 23296832 | 132| 3  | 16|x |
| 98  | Putative β-N-acetylhexosaminidase*                    | 60  | 6.2 | 58 | 6.1| 50511452 | 301| 7  | 14|x |
| 155 | Putative integral membrane Yip1 family protein        | 42  | 8.7 | 40 | 7.7| 34912572 | 142| 3  | 11|x |
| 168 | α-1,4-glucan-protein synthase                         | 39  | 5.4 | 41 | 6.1| 134588146 | 173| 4  | 10|   |
| 244 | Profilin 5*                                           | 12  | 4.5 | 14 | 4.7| 11493677 | 68 | 2  | 31|x |
| 316 | Putative r40c1 protein                                | 37  | 6.7 | 42 | 6.7| 34902150 | 252| 5  | 19|   |
| 365 | Unknown                                               | 15  | 8.2 | 18 | 7.8| 13194668 | 110| 2  | 20|x |
| 604 | Putative 41 kD chloroplast nucleoid DNA binding protein| 43  | 4.4 | 54 | 8.4| 50943229 | 153| 3  | 9|x |
| 688 | Adenosine kinase                                      | 39  | 4.6 | 36 | 5.3| 4582878 | 309| 6  | 15|   |
| 855 | Putative lipase                                       | 31  | 7.8 | 38 | 7.9| 55297457 | 206| 4  | 12|x |
| 904 | Putative carboxypeptidase*                            | 28  | 4.6 | 52 | 5.9| 20197951 | 114| 2  | 5|x |
| 920 | Putative PS60*                                        | 60  | 6.8 | 60 | 8.8| 52076641 | 162| 5  | 7|x |
| 959 |oxidoreductase NAD-binding domain-containing protein*  | 25  | 4.3 | 30 | 8.5| 42571485 | 239| 5  | 19|x |
| 1020| Translationally controlled tumor protein-like protein | 22  | 4.0 | 19 | 4.7| 23955914 | 191| 5  | 38|x |
| 1158| Putative ubiquitin-conjugating enzyme family protein  | 17  | 6.8 | 17 | 6.9| 50725506 | 230| 5  | 38|x |
| 1334| Profilin 5*                                          | 14  | 4.4 | 14 | 4.7| 11493677 | 120| 3  | 33|x |
| 1334| Ubiquitin-like protein*                               | 14  | 4.4 | 11 | 5.1| 1668773 | 109| 2  | 25|   |
| 1434| Cytochrome b5 domain-containing protein-like          | 11  | 4.8 | 11 | 5.5| 50915542 | 105| 3  | 28|   |
| 1518| Putative leucine aminopeptidase                       | 58  | 5.4 | 64 | 6.5| 21206625 | 366| 13 | 16|x |
| 1902| Putative lipase*                                      | 34  | 8.1 | 38 | 8.4| 55297457 | 217| 4  | 12|x |
| 1948| OSJNBb0091E11.17                                     | 20  | 4.6 | 40 | 6.1| 50925937 | 100| 2  | 6|x |
| 3337| Putative lipase                                       | 18  | 5.2 | 38 | 7.9| 55297457 | 97 | 2  | 5|x |
| 3385| Putative lipoamide                                   | 51  | 6.4 | 59 | 6.5| 34894958 | 150| 4  | 9|   |

R1: Oryza sativa
R2: Arabidopsis thaliana
R3: Zea mays
R4: Pennisetum ciliare
| ID   | Description               | Length | Mw  | pI   | Score | Exon | Intron | Status | Species       |
|------|---------------------------|--------|-----|------|-------|------|--------|--------|---------------|
| 3420 | Putative subtilisin-like proteinase* | 76     | 5.8 | 78   | 6.3   | 40538972 | 224 | 3 | 7 | x | R2 | Oryza sativa |
| 3464 | Legumin-like protein*     | 35     | 5.9 | 38   | 5.8   | 28950668 | 173 | 4 | 14 | x | R2 | Zea mays    |
| 3465 | Legumin-like protein*     | 34     | 6.0 | 38   | 5.8   | 28950668 | 179 | 3 | 11 | x | R2 | Zea mays    |
| 3507 | Legumin-like protein      | 38     | 6.3 | 38   | 5.8   | 28950668 | 256 | 6 | 23 | x | R2 | Zea mays    |