Effect of Drought on Storage Root Development and Gene Expression Profile of Sweetpotato under Greenhouse and Field Conditions

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ABSTRACT. Greenhouse and field culture systems were used to study the effect of drought conditions on the storage root (SR) formation in ‘Beauregard’ sweetpotato (Ipomoea batatas). In the greenhouse culture system, drought was simulated by withholding water for 5 and 10 days after transplanting (DAT) cuttings in dry sand. Control plants received water at planting and every 3 days thereafter. In the field studies, natural drought conditions and selective irrigation were used to impose water deprivation during the critical SR formation period. Greenhouse drought for 5 and 10 DAT reduced the number of SRs by 42% and 66%, respectively, compared with the controls. Field drought resulted in a 49% reduction in U.S. #1 SR yield compared with the irrigated condition. Quantitative real-time polymerase chain reaction (PCR) analysis showed differential expression of a set of sweetpotato transcription factors and protein kinases among greenhouse-grown plants subjected to well-watered conditions and water deficit during 5 DAT. A significant enhancement of expression was observed for known drought stress-associated genes such as an abscisic acid-responsive elements-binding factor, dehydration-responsive element-binding factor, and homeo-domain-zip proteins. Members of calcium-binding proteins showed differential expression under drought stress. For the first time it is reported that knotted1-like homeobox and BEL1-like genes showed altered expression in response to drought stress under a greenhouse condition. In summary, the results suggest that water deprivation during the SR formation period influences root development and expression patterns of stress-responsive genes and those previously found associated with SR formation in sweetpotato.

Drought stress represents a global constraint for sweetpotato production because most of the sweetpotato production occurs in semiarid regions (Saraswati, 2007). Considering the complexity of the physiological and genetic mechanisms associated with stress tolerance, a genomics-based understanding of the stress response of sweetpotato will help us develop strategies to sustain its productivity in stressful environments (Boyer, 1982). Candidate drought-responsive genes, identified through genomics research, will have great use in widening the natural allelic variation and its possible use for crop improvement (Rus et al., 2006). However, sweetpotato has lagged with respect to studies leading to the identification of its drought-responsive genes. One study reported 12 genes in response to drought stress in white fibrous roots (Kim et al., 2009), and most of these genes are similar to ones that are known to be associated with dehydration response in many other species. A dehydration responsive element-binding protein gene, a member of the AP2 (Apetala2)/EREBP (ethylene-responsive element binding protein) family, has been characterized in drought-stressed roots and stems of sweetpotato (Kim et al., 2008). Evidence of the genetic basis of sweetpotato tolerance to drought comes from studies carried out in vitro along with field and greenhouse experiments (Ekanayake and Dodds, 1993; Ricardo, 2011). Genes associated with antioxidant activity were up-regulated in leaves under drought and salt stress (Kim et al., 2013). Similarly, transcripts of late embryogenesis abundant proteins, known to be associated with abiotic stress responses, were increased with lignification in sweetpotato tissue cultures (Park et al., 2011). These studies collectively demonstrate that abiotic cues result in transcriptional changes in sweetpotato. However, gene expression under stress has not been studied in early stages of storage root development, before thickening of roots.

Sweetpotato SRs develop from adventitious roots emerging from root primordia located on stem nodes. Unknown intrinsic
and extrinsic factors trigger thickening of these adventitious roots, the process known as SR initiation. SR initiation has been defined as the formation of a cambium ring and appearance of anomalous cambia around discrete xylem and protoxylem elements within 20 d of the emergence of an adventitious root (Togari, 1950). Greenhouse and field culture systems in ‘Beauregard’ validated that SR initiation consistently occurred during the first 20 d after transplanting (Villordon et al., 2009a, 2009b). Preformed primordia that produce adventitious roots with pentarch, hexarch, or septarch steles have the potential to develop into SRs (Villordon et al., 2009b). However, damage to the root primordia, unfavorable edaphoclimatic conditions, and age of nodes influence the total SR in a cultivar.

Although previous molecular studies during the past decade have reported several candidate genes to be up-regulated or expressed preferentially in SRs, no single gene has been shown to be solely responsible for conversion of the adventitious roots into storage organs (Kim et al., 2002, 2005; Ku et al., 2008; Noh et al., 2010; Tanaka et al., 2008; You et al., 2003). Most of these genes have either regulatory roles as transcription factors or are involved in carbohydrate and protein metabolism during the development and thickening of the SRs. Firon et al. (2013) have recently demonstrated down-regulation in the expression of key genes of the phenylpropanoid biosynthesis pathway on the change in root fate from fibrous root to a storage organ. In addition, precise control at the level of gene expression of regulators of meristematic tissue identity and maintenance, up-regulation of cell-division regulators, and down-regulation of specific GRAS [GAI (gibberellin acid-insensitive), RGA (repressor of GAI), SCR (scarecrow)] family members in the SR-initiation process were indicated.

The present study was undertaken to examine the effect of drought stress on the SR formation under greenhouse and field conditions and to characterize the expression of a selected set of genes in root tissues of drought-stressed vs. non-stressed plants. The genes included in this study were selected based on their 1) role in gene regulation and calcium signaling during developmental and physiological processes; 2) enrichment in root libraries (GenBank; Firon et al., 2013; Schafleitner et al., 2010); 3) role in drought stress responses and hormone signaling; and 4) functional homology to genes known to be involved in storage organ development in other crops such as potato [Solanum tuberosum (Reddy et al., 2002)] and cassava [Manihot esculenta (de Souza et al., 2004)].

Materials and Methods

**Plant material.** Sweetpotato plant cuttings (~20 cm long) were obtained from generation 0 (derived from in vitro cultures) virus-tested, greenhouse-grown ‘Beauregard’ for greenhouse studies and virus-tested generation 1 seed roots in plant beds for field studies.

**Drought studies under greenhouse conditions.** Unrooted cuttings (n = 9 for each of the three treatments) were prepared and transplanted the same day in dry sand in cylindrical tubes (50 x 9.82 cm) under greenhouse conditions. Plants were grown inside the greenhouse under a day/night temperature regime of 27/21 °C for Trial 1 (30 Jan. 2011 to 10 Mar. 2011) and 28/25 °C for Trial 2 (9 May 2011 to 21 June 2011), respectively, at 50 to 70 μmol·m⁻²·s⁻¹; no artificial lighting was provided. Our earlier pilot experiments indicated that the cuttings could survive at least 10 DAT into dry sand under controlled conditions.

Drought stress was imposed by withholding water for 5 and 10 DAT and control plants received water the same day of transplanting (0 DAT). Subsequently, stressed plants received water every 3 d, whereas the control plants received water every 3 d throughout the experiment. Each treatment included nine plants. Cuttings were watered (400 mL) every 3 d after the initial 5- and 10-d treatment period. Water-soluble 20N–8.7P–16.6K solution (Peters Professional Soluble Plant Food; Scotts-Sierra, Marysville, OH) was applied (0.374 g/200 mL) at 12 and 22 DAT. Four weeks after transplanting, the plants were evaluated for the number of SRs (width greater than 1.5 mm) and the width and weight of the SRs. Thin pigmented roots (width less than 1.5 mm) were included in the total count of SRs (SRCount1) because we considered them as putative-forming-developing SRs. A second count of SRs (SRCount2) excluded these pigmented SRs. Maximum diameters of SRs were measured with a caliper. The experiments were repeated twice.

A second study was carried out in the greenhouse to evaluate expression of a set of selected genes (Table 1) in total roots from initially drought-stressed (no water for 5 DAT) and well-watered (control) plants. The initial water stress at planting may resemble unfavorable drought conditions met by farmers, where cuttings are left in soil without watering until rainfall arrives. Fertilizer was applied at 12 DAT. Roots from these plants were sampled at 14 DAT in triplicate by pooling roots from three plants and then the roots were frozen in liquid nitrogen. Root tissues were stored at –80 °C; the experiment was repeated twice.

**Drought studies under field conditions.** Field experiments were conducted in the Summer 2010 and 2011 in well-drained research fields at Chase, LA (lat. 32°6’ N, long. 91°54” W). The soil taxonomic class was fine-silty, mixed, active, thermic Typic Glossaquolls. There were three planting dates in 2010 (12 May, 27 May, and 3 June) and two planting dates in 2011 (19 May and 1 June). In each year, natural rainfall deficits during May and June created conditions where soil moisture in the root zone was near the wilting point for the soil type used in the studies. The rainfall pattern during the time of experiments in 2010 and 2011 is shown in Figure 1. Rickard and Fitzgerald (1969) defined agricultural drought as existing when the soil moisture in the root zone is at or below wilting point. During June to Aug. 2011, portions of northeastern Louisiana, including the location of the field experiments, had record low values for the Palmer hydrological drought index in the 117-year record (Blunden and Arndt, 2012). These growing conditions were used to compare SR yield from plots with drought-stressed (non-irrigated) vs. plots that were irrigated to maintain soil moisture at 50% of field capacity (FC) during the SR initiation phase, which can occur as early as 13 DAT in ‘Beauregard’ grown in field plots (Villordon et al., 2009b). Field preparation activities, including fertilizer rates, herbicide, and insecticide applications, were similar in each year as previously described (Villordon et al., 2009b, 2011). Supplemental overhead irrigation was supplied with a traveling irrigation sprinkler if a rainfall event did not occur in irrigated plots.

In each planting date, two plots were designated as irrigated vs. non-irrigated plots. Plot size was 12 rows x 30 m on 1-m centers. The plots were separated by a buffer zone equivalent to 12 rows. Supplemental irrigation was based on soil moisture sensor data and irrigation was applied when soil moisture at the 15-cm depth approached 25% of FC. A 16% volumetric water content (VWC) represented 50% of FC in this study. This soil moisture range has previously been calibrated (Constantin et al.,
| Gene     | Annotation                 | Forward primer          | Reverse primer          | Amplicon (bp) | Accession*          | Entry*    |
|----------|----------------------------|-------------------------|-------------------------|---------------|---------------------|-----------|
| *IbGRF* | General regulatory factor 2 | agctgacctcgatctgcaac    | cagtagtggcgatttcctgagc  | 210           | JP117273.1, CO500345.1 | S_PBL_c1096 |
| *IbDREB1* | DREB subfamily A-4        | cggcgatgatgaagcctga    | catatccaccgaacaatttgc   | 137           | JP128692.1*         | S_PBL_c2263* |
| *IbHB1*  | Homeobox-leucine zipper protein | ggtggaagaggttgtgtcagc | cagtcacactcgcctgttgc   | 210           | JP115003.1          | S_PBL_c3587 |
| *IbHB2*  | Homeobox 7                 | caggagctgagaaggtttatg   | ctcctcacgtctcttgtg      | 139           | DC880529.1          | S_PBL_c3430 |
| *Ibkn2*  | Knotted1-like homeobox protein | ttggagagcgagctgttagt   | attgatgatgtcgctggctt   | 209           | AB283028.1          |           |
| *Ibkn3a* | Knotted1-like homeobox protein | cgcctagcccataaacatcataa | ccacagtgagatacaataaga   | 212           | JP112770.1          | S_PBL_c31412 |
| *Ibkn3b* | Knotted1-like homeobox protein | cgcctagcccataaacatcataa | ccacagtgagatacaataaga   | 215           | AB283029.1          |           |
| *IbEF1a* | Elongation factor 1-alpha  | tctggaagaatgtgtagctgg  | cagttgggccctcttgcaac    | 165           | JP106582.1, EE883896.1 | S_PBL_c1695 |
| *IbTAP*  | 2A phosphatase associated protein | tggcgtgctgatcacaatcatac | cggcggcagacaacacaggtgg  | 217           | JP106582.1, EE883896.1 | S_PBL_c1695 |
| *IbSnRK* | snf1-related protein kinase | atgtgggtgctgatcacaatcatac | cggcggcagacaacacaggtgg  | 217           | JP106582.1, EE883896.1 | S_PBL_c1695 |
| *IbCRF1* | Cytokinin response factor  | cccataaggggaaggaaacct  | gggggttctctgcatacttcttgc | 183           | JP130502.1          | S_PBL_c3693 |
| *IbCRF2* | Cytokinin response factor  | cccacagatacatacattcattc | aataatgtcggactgtacctgc   | 211           | JP151420.1, JP158246.1* | S_PBL_c5300 |
| *IbAREB* | ABRE binding factor        | gcaactcgatcagcttgctg   | ttctgccctcttactctc      | 188           | JP112269.1          |           |
| *IbCDPK3* | Calcium dependent protein kinase | ctgtgatcgcagcaacgggag  | aagaggtttgtcaggtggcatttc | 93            | JP106466.1          | S_PBL_c41342 |
| *IbBEL1* | BEL1-like homeodomain      | tgagacgcattgataggttgcgt | gaaggcaagtcagagatgacta  | 213           | JP123314.1, EE881162.1 | S_PBL_c43041 |
| *IbBEL2* | BEL1-like homeodomain      | tctctcataatactctctccaact | agggtttgcaagatggtgagat  | 248           | JP106847.1, DV038045.1 | S_PBL_c4966 |
| *IbBEL3* | BEL1-like homeodomain      | ggtggtgtgtgctggtggata  | ccataagctgtctgtgactgtcttgc | 252           | JP106340.1          | S_PBL_c25157* |
| *IbCBP1* | Calcium-binding EF-hand protein | gacccgtcggcagctaacacttgc | tgtttctcctcctaagctgacta  | 259           | JG699346.1         | S_PBL_c97041 |
| *IbCBP2* | Calcium-binding EF-hand protein | gacccgtcggcagctaacacttgc | tgtttctcctcctaagctgacta  | 259           | JG699346.1         | S_PBL_c97041 |

*Accession number in GenBank, expressed sequenced tags, transcriptome shotgun assembly databases.
*Entry identifier in database of Finn et al., 2013.
*Entries in GenBank of *IbRF2* and *IbDREB1* genes are a fragment of whole unpublished assembled transcript.
*Entry identifier corresponding to *IbBEL3*, *IbCBP1*, *IbDREB1*, and *IbTAP* genes are partial fragment of whole unpublished assembled transcript.
Fig. 1. Rainfall data of Chase, LA, during the experiments of 2010 and 2011 to study the effect of drought stress on storage root yield of sweetpotato (National Oceanic and Atmospheric Administration, 2014).
Table 2. Effect of drought stress under greenhouse conditions on sweetpotato storage root growth.

| Treatment duration (d)* | SRCount1 [mean ± se (no. roots)]* | SRCount2* | SRWeight [mean ± se (g)] | Width [mean ± se (mm)]* |
|-------------------------|------------------------------------|------------|--------------------------|------------------------|
|                         | Trial 1                             | Trial 2    |
| 0                       | 5.9 ± 0.4 a<sup>a</sup>            | 4.0 ± 0.4 a<sup>a</sup> |
| 5                       | 3.3 ± 0.2 b<sup>b</sup>            | 2.8 ± 0.2 b<sup>b</sup> |
| 10                      | 2.4 ± 0.4 c<sup>c</sup>            | 1.3 ± 0.2 c<sup>c</sup> |
|                         |                                    | 1.9 ± 0.42 c   |
|                         | 1.9 ± 0.42 c<sup>c</sup>          | 1.9 ± 0.42 c   |
|                         | 1.9 ± 0.42 c<sup>c</sup>          | 1.9 ± 0.42 c   |
|                         |                                    | 3.5 ± 0.6 c    |
|                         |                                    | 3.2 ± 0.5 c    |
|                         |                                    | 2.1 ± 0.2 c    |

*Data on sweetpotato storage root number, weight, and width under control (well-watered) and drought (no watering for 5 and 10 d after transplanting), Trial 1 from 30 Jan. to 10 Mar. 2011; Trial 2 from 9 May to 21 June 2011.

*Number of days without receiving first watering after transplanting.

*Number of storage roots including the pigmented roots (i.e., putative initiating storage roots with an estimated maximum diameter less than 1.50 mm).

*Number of storage roots excluding the pigmented roots.

*Average of all measurements of maximum width of the storage roots.

Reductions were 42% and 29% in Trials 1 and 2, respectively, between 5 DAT drought stress vs. control plants. The reduction in SRCount1 at 10 DAT drought stress was greater compared with that at 5 DAT drought stress with a reduction of 59% and 67% in both Trial 2 and Trial 1, respectively. The SRCount2 at 10 DAT drought stress showed a reduction of 71% in Trial 1 and 62% in Trial 2.

All plants reached similar foliar growth at the time of harvest and no death occurred as a result of drought stress under greenhouse conditions. Plants under drought stress showed moderate to severe stress effects in terms of reduction of weight and the maximum diameter of the SRs compared with the control. For example, the weight of the SRs was 50% and 73% less in plants experiencing 5 and 10 DAT drought stress, respectively, in comparison with the control in Trial 1. Reductions were more pronounced in Trial 2.

**Drought stress studies under field conditions.** The results of field experiments of combined data from 2010 and 2011 seasons showed that the yield of sweetpotato plants experiencing drought stress under field conditions was reduced significantly (Table 3). The yield reduction was most pronounced for the important U.S. #1 grade; non-irrigated plots showed a 49% yield reduction compared with the irrigated plots. The total marketable root yield showed similar trends with a reduction of 43% in the non-irrigated plots compared with the control. The jumbo and medium grades were not significantly different and consistent with high replication variability typically encountered in sweetpotato yield studies.

**Gene expression under greenhouse drought stress.** Ten of 19 genes tested were found up-regulated in drought-stressed roots with fold change expression of at least 1.4 relative to control (IbAREB, IbBEL1, 2, 3, IbCBP2, IbCRF1, IbHB1, 2; IbKn2, 3a), and only two genes (IbCBP1 and IbCDPK3) were down-regulated (Fig. 2). Expression levels for IbBEL2, IbKn2, and IbCRF2 genes correspond to a single set of triplicate sample treatments, and for the rest of the genes, the expression levels are as described in “Materials and Methods” (two independent sets of triplicate samples). Of the up-regulated genes, nine were transcription factors and the other (IbCBP2) coded for a calcium-binding protein.

Three genes, IbHB2 (encoding a homeobox protein), IbCRF1 (encoding a protein similar to the Arabidopsis thaliana calcium-binding protein), and IbAREB (encoding an abscisic acid-responsive elements-binding factor), showed very high accumulation of their transcripts under drought stress compared with the control. The greatest increase in abundance of transcript under drought stress was observed for IbHB2.

**Bell** (IbBEL1, IbBEL2, and IbBEL3) and **KNOX** (IbKn3a) transcription factors were up-regulated 1.4- to 2.2-fold in roots of plants that were under drought stress. Two genes, IbDREB1 and IbGRF, did not show significant differences in their expression between roots of drought-stressed and control plants, whereas expression changes of IbSnRK and IbTAP were inconsistent (data not shown).

Of the down-regulated genes, only IbCBP1, encoding a calcium-binding protein, showed the greatest reduction of expression up to 80% (fold change 0.22) in roots under greenhouse drought stress compared with the control, and a slight decrease (fold change 0.76) of transcript abundance was observed for IbCDPK3, a gene putatively encoding a calcium-dependent protein kinase (Fig. 2).

Table 3. Combined storage root yield of sweetpotato in response to irrigation treatments under field conditions after 110 d (in 2010) and 130 d (in 2011) of growth, Chase, LA.

| Treatment | U.S. #1 | Medium | Jumbo | Total marketable |
|-----------|---------|--------|-------|------------------|
| Irrigated | 26.5 a<sup>a</sup> | 12.4 a  | 6.4 a | 45.2 a          |
| Non-irrigated | 13.0 b | 9.9 a | 2.8 a | 25.8 b          |
| P value | <0.0001 | 0.2 | 0.2 | <0.0001          |

<sup>a</sup>Sizes of roots: U.S. #1 51 to 89 mm in diameter, 76 to 229 mm long; medium (canner): 25 to 51 mm in diameter, 51 to 178 mm long; jumbo: larger than U.S. #1 in diameter, length or both, and without objectionable defects. Total marketable represents the summation of all grades.

<sup>b</sup>Means with the same letter within a column are not significantly different by Fisher’s exact test at P < 0.05.
Fig. 2. Expression profile of 14 genes in sweetpotato roots from 2-week-old plants under greenhouse drought stress (5 d after transplanting) vs. control by quantitative real-time polymerase chain reaction. Error bars represent SEM of three replicates in a single experiment. Fold change in expression of the genes was determined by normalizing the values against that of the reference gene IbEF1a and calculated relative to the control that was set to 1.0.

Discussion

Drought and storage root. Drought stress under greenhouse conditions significantly reduced the number, size, and weight of sweetpotato SRs compared with the control plants (Table 2). The 5 DAT treatments showed a 30% to 42% reduction in SR number across the two studies. SR numbers were reduced further (up to 66%) by extending the drought period to 10 DAT. Results at 5 and 10 DAT were consistent over two sets of experiments and demonstrated the effect soil moisture could exert on SR formation. Preliminary experiments carried out in growth chambers in 2008 and 2009 under controlled conditions of humidity, daylight, and temperature had a similar outcome (Solis, 2012).

The 2010 and 2011 growing seasons in Louisiana were characterized by prolonged periods without rainfall, especially during the critical period of SR initiation in field-grown ‘Beauregard’ (Villordon et al., 2009b). This growing environment allowed for the comparison of irrigated vs. non-irrigated treatments on SR initiation and subsequent SR bulking without the confounding effects of natural rainfall events.

In each year, there was marginal soil moisture (less than 25% to 50% of FC) during the transplanting phase in the non-irrigated plots. This allowed for some SR initiation; however, the lack of additional soil moisture up to 30 DAT impacted further development, resulting in low SR count and delayed SR development. At harvest (110 to 130 DAT), irrigated plots showed over a 100% increase in U.S. #1 yield relative to non-irrigated (up to 30 DAT) plots (Table 3). Adequate soil moisture after 30 d did not overcome the effects of the initial drought. These data corroborated earlier findings of Togari (1950) that provided evidence that environmental and management variables during the first 20 DAT exert considerable influence on SR formation, dictating the future yield of the crop.

The reduction of yield and quality of roots observed in the non-irrigated field and the reduced number, diameter, and weight of SRs under greenhouse drought conditions support our hypothesis that lack of soil moisture irrepairably alters root development toward non-storage-forming roots.

Gene expression. Several key genes were shown to be involved in sweetpotato SR development by comparing storage and non-storage-forming roots (Firon et al., 2013; Kim et al., 2002, 2005; Ku et al., 2008; Noh et al., 2010; Tanaka et al., 2008; You et al., 2003). Kim et al. (2009) focused primarily on the identification and study of genes from fibrous roots under drought stress at the late growth stage. The present study focused on gene expression profile of 2-week-old total root pools from non-stressed and drought-stressed plants (5 DAT) at an early stage of growth. The genes included in this study were selected based on comparative analysis of the available sweetpotato root transcriptome (Firon et al., 2013), expressed sequence tag sequences deposited at GenBank and PlantGDB, and sequences from leaf and stem libraries of drought-stressed sweetpotato plants (Schafleitner et al., 2010).

Homeobox leucine zippers, AP2/EREBP, and abscisic acid responsive-like genes. Among the genes that had the highest up-regulation in sweetpotato roots under drought stress were IbHB2, IbCRF1, and IbAREB (Fig. 2). The sweetpotato gene IbHB2 is an ortholog of ATHB7 (At2g46680) and a member of the homeobox leucine zipper transcription factors (HD-Zip). Transcripts of ATHB7 accumulated in response to water stress in A. thaliana (Olsson et al., 2004; Soderman et al., 1996). HD-Zip genes are implicated in both developmental changes and stress responses in A. thaliana (Hjellström, 2002; Lee and Chun, 1998; Soderman et al., 1999) and cassava (Lokko et al., 2007) and dehydration tolerance in the root and leaves of resurrection plant Craterostigma plantagineum (Deng et al., 2002). Furthermore, gradual reduction of expression of the cotton (Gossypium hirsutum) HD-Zip gene (GhHB1) with development of roots and its induction in response to abscisic acid and salt (Ni et al., 2008) indicated that HD-Zip genes play important roles in both morphogenic processes as well as stress responses of plants.

The sweetpotato gene IbCRF1 that showed the second highest increase in expression (fold change of 6) under drought stress is similar to AP2/EREBP genes (Riechmann and Meyerowitz, 1998) and cytokinin response genes (Rashotte and Goertzen, 2010). Expression of many members of the AP2/EREBP gene family is altered in response to abiotic (Chen et al., 2007; Kim et al., 2008; Kizis et al., 2001; Xiong et al., 2002) and biotic stresses (Lin et al., 2007). Although CRF-like genes have not been studied previously in sweetpotato, cytokinins were shown to induce SR organs in sweetpotato (Eguchi and Yoshida, 2008). Therefore, it is presumed that IbCRF1, being a cytokinin responsive gene, could play an important role in SR development under water stress.

Homeobox Bell and Knox I genes. KNOX (knotted1-like homeobox) genes have been previously found to be associated with the formation of SRs (Tanaka et al., 2008). In the present study, three BELL (BEL1-Like) genes IbBEL1, IbBEL2, and IbBEL3, and KNOX genes Ibkn1, Ibkn2, and Ibkn3 were up-regulated ≈1.4 to two times in 2-week-old sweetpotato roots in response to drought stress (Fig. 2). In sweetpotato, two variants of Ibkn3 (Ibkn3a and Ibkn3b) were identified (Solis, 2012). Ibkn3a showed ≈2-fold higher expression under drought over...
a control, whereas *Ibkn3b* was slightly down-regulated under drought stress (fold change of 0.88). BELL and KNOX proteins are known to interact during potato tuberization (Chen et al., 2003), *IbCRF1* and *IbCRF2*, under drought stress. Further functional characterization of the cytokinin signaling by *KNOX* and *BELL* genes is required in sweetpotato to understand the mechanism of their role in abiotic stress response and SR development.

**Calcium signaling genes.** In the present study, the *IbcBP2* gene, an ortholog of *A. thaliana* igt9 (At2g33990) encoding calmodulin binding protein, was up-regulated (fold change of 2.12), whereas two other genes encoding calcium-binding proteins, *IbcBP1* and *IbcDPK3*, were down-regulated under drought stress. CDPK-like genes were shown to be involved in potato tuberization (Poovaiath et al., 1996; Reddy et al., 2002). Similarly calcium-binding proteins such as CDPKs and calcineurin-B-like genes were shown to be induced by drought and salt stresses (Jimenez et al., 2008). Therefore, it remains to be seen if strict regulation of CDPK-like genes is required to trigger SR formation in sweetpotato by modulating other downstream-stress-related genes under drought stress (Albrecht et al., 2003; Cheong et al., 2003).

**Other regulatory genes.** Genes such as *IbGRF* and *IbDREB1* did not show significant differences between roots of drought-stressed and control plants, but these genes (*IbDREB1, IbGRF, IbSnRK*, and *IbTAP*) were shown to be abundant in SR libraries compared with that from lignified roots based on their digital expression profile in sweetpotato root (Solis, 2012). Inconsistent expression observed for *IbSnRK* and *IbTAP* between replicates in the present study was attributed to the difference in greenhouse temperatures during growth stages and at root sampling.

**Conclusions**

The present study showed drought stress significantly affects SR number and development in sweetpotato. The expression of genes (*IbHB2, IbCRF1*, and *IbAREB*), not previously documented in sweetpotato, were significantly altered in response to drought stress as observed in other species. This work further demonstrated, for the first time, that genes such as *IbBEL1, IbBEL2, IbBEL3, IbHB2*, and *Ibkn3a* are stress-responsive genes and are up-regulated in roots under greenhouse drought stress conditions. The overall results indicated that genes known to be associated with the onset of bulking are sensitive to drought stress and the consequence is a diminished number of sweetpotato SRs under stress. Altered expression of transcripts of signaling genes (*IbCBP1, IbCBP2*, and *IbcDPK3*) could be related to the reduced number of SRs. Detailed studies are required to precisely determine the role of up-regulated genes in SR under drought stress, which could potentially be used as biomarkers to identify the effect of water stress on SR development.

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