

Anatomical, Physiological, and Molecular Responses of Sweetpotato Native Periderm to Preharvest Water Deprivation During Early-storage Root Bulking

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Abstract. Preharvest irrigation cutoff to promote skin set is a common management practice in irrigated commercial sweetpotato production, but its effects on native periderm, the developmentally formed periderm of intact storage roots before harvest, are poorly understood. We evaluated anatomical, physiological, and molecular responses of native periderm to controlled short-term irrigation/fertigation withdrawal during the early storage root bulking stage (30–50 days after transplanting) in ‘Bayou Belle’, ‘Beauregard’, and ‘Orleans’ sweetpotato. Greenhouse experiments compared well-watered plants with plants subjected to 5 days of withdrawal (WD5) or 10 days of withdrawal (WD10) before harvest. Native periderm structure was characterized from transverse sections stained with phloroglucinol-HCl, and postharvest weight loss was measured for 5 weeks. The relative expressions of *IbPAL*, *IbCCoAOMT*, *IbCAD*, and *IbACO* were quantified in well-watered and WD10 roots. Withdrawal increased circularity, an index of reduced outer-boundary irregularity, and produced a more continuous native periderm with fewer expansion-associated discontinuities. Percent lignified tissue increased 1.9-fold to 3.6-fold relative to well-watered controls, depending on cultivar and treatment duration. By week 5, cumulative weight loss was 38% to 45% in well-watered roots compared with 21% to 24% after WD5 and 20% to 30% after WD10. *IbPAL* expression increased 1.6-fold to 3.8-fold across cultivars, whereas *IbCCoAOMT*, *IbCAD*, and *IbACO* responses were genotype-dependent. These coordinated responses are consistent with accelerated native periderm maturation during early bulking and support the use of greenhouse-grown roots as a controlled developmental system. Because nutrient delivery was also interrupted during irrigation withdrawal, the response cannot be attributed to the water status alone; therefore, field validation and experiments that separate water from the nutrient supply are needed.

Introduction

Preharvest irrigation cutoff, often described commercially as field curing, is used in irrigated sweetpotato (*Ipomoea batatas*) production to promote skin set and reduce postharvest losses (Smith et al. 2009). In potato (*Solanum tuberosum*), skin set is defined as the acquisition of resistance to skinning injury or excoriation as the native periderm matures (Lulai and Freeman 2001). This transition occurs as the

phellogen becomes less susceptible to fracture, with strengthening and thickening of phellogen cell walls increasing adhesion between the phellem and underlying tissues (Lulai 2007; Lulai and Freeman 2001). Although an equivalent anatomical definition has not been established for sweetpotato, studies of sweetpotato commonly evaluate skin set using related measures such as skin adhesion, skinning incidence or severity, and force required to peel the skin (Villavicencio et al. 2007; Wang et al. 2013).

Therefore, the potato periderm maturation framework provides a useful model for evaluating whether preharvest water deprivation accelerates native periderm maturation in sweetpotato.

In sweetpotato, commercial and field observations suggest that preharvest soil moisture influences skin set. In California growing regions, irrigation is commonly reduced or ended 2 to 4 weeks before harvest to facilitate field curing and skin set (Smith et al. 2009). Experimental preharvest curing and earlier field studies likewise showed that drier preharvest conditions were associated with increased skin adhesion, altered periderm anatomy, greater lignification, reduced postharvest weight loss, and improved shelf life (Parmar et al. 2017; Villavicencio et al. 2007; Webster et al. 1973). These studies support a practical relationship between preharvest water status and storage root skinning resistance. However, direct evidence linking controlled water deprivation to native periderm structure, lignification, gene expression, and postharvest performance is limited. Among the anatomical changes associated with periderm maturation, lignification is of particular interest because it contributes to structural reinforcement and has been linked with sweetpotato skin adhesion and periderm development (van Oirschot et al. 2006; Villavicencio et al. 2007; Walter and Schadel 1983). This relationship provides a mechanistic rationale for evaluating the extent of periderm lignification together with the expression of lignin biosynthesis genes in response to preharvest water deprivation.

Comparable evidence from other storage organs supports a developmental link between the preharvest water status and barrier or postharvest performance. Potato tuber periderm resistance varies with the plant water status (Braue et al. 1983), irrigation affects skin blemishes and phellem formation (Jiang et al. 2022), and withholding irrigation alters tuber water fluxes and growth (Huntenburg et al. 2025). Controlled water deficit alters cassava storage root growth and physiology (Duque and Setter 2013), while drought-associated lignification in cassava has been linked to cinnamyl alcohol dehydrogenase (CAD)-related regulation (Yan et al. 2021). In sugarbeet, severe preharvest drought affects subsequent root respiration and susceptibility to storage rot (Lafta et al. 2024). Together, these studies indicate that storage organs retain measurable developmental and postharvest responses to preharvest water status.

We hypothesized that controlled preharvest water deprivation accelerates native periderm maturation by promoting the transition from an actively expanding periderm to a more continuous and structurally reinforced protective barrier. This study evaluated the anatomical, physiological, and molecular responses of early-bulking storage roots of ‘Bayou Belle’, ‘Beauregard’, and ‘Orleans’ sweetpotato to controlled water deprivation and assessed the utility of greenhouse-grown early-bulking storage roots as a model system for studying native periderm development.

Materials and Methods

Plant materials and growing conditions.

Greenhouse experiments were conducted at the Louisiana State University AgCenter Sweet Potato Research Station in Chase, LA, USA (lat. 32°36'N, long. 91°42'W). Virus-tested generation 1 vegetative cuttings of the sweetpotato cultivars Bayou Belle, Beauregard, and Orleans served as the planting material for all experiments. Vegetative cuttings were established in 10-cm-diameter polyvinyl chloride pots filled with washed river sand and maintained under greenhouse conditions similar to those described for studies of sweetpotato storage root development (Villordon et al. 2012). Plants received 0.5× Hoagland's no. 1 solution (nitrate only) (Hoagland and Arnon 1950). Growth substrate moisture was measured with ECH2O soil moisture sensors (Model EC-5; Decagon Devices Inc., Pullman, WA, USA) inserted vertically at depths of 2 to 7 cm. Sensor-based data from all experimental treatments were collected at hourly intervals throughout the experiments. Greenhouse temperatures were maintained at approximately 29 °C during the 14-h photoperiod and 18 °C during the 10-h dark period. Supplemental lighting was provided when necessary to maintain a 14-h photoperiod. Relative humidity ranged from 60% to 80%. These experimental approaches have been used to validate the timing of storage root initiation in the cultivar Beauregard (Villordon et al. 2009, 2012) and measure root architecture responses to biotic and abiotic variables (Villordon et al. 2012, 2013; Villordon and Clark 2018). The growing conditions and treatment schedules used in each experiment are summarized in Table 1.

Experimental design and developmental stage. Four independent but complementary greenhouse experiments were conducted between 2024 and 2026 to evaluate anatomical, physiological, and molecular responses of native periderm to short-term preharvest water deprivation (Table 1). Native periderm anatomy was evaluated during Expt. 1 and Expt. 4.

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Table 1. List of experiments. Plantings were staggered by treatment in all studies. Plants subjected to 10 d of water deprivation were established 10 d before well-watered controls (WWCs), while plants subjected to 5 d of water deprivation were established 5 d before the WWC.¹

Expt.	Study	Planting date of WWC	Date harvested	Measurements/sampling
1	A	2 Jan 2025	1 Feb 2025	Anatomical measurements
	B	3 Feb 2025	5 Mar 2025	Anatomical measurements
2	A	3 Feb 2025	22 Apr 2025	Weight loss measurements
	B	3 Mar 2025	20 May 2025	Weight loss measurements
3		15 Apr 2025	15 May 2025	Tissue sampling for gene expression experiments
4	A	20 Jan 2026	19 Feb 2026	Anatomical measurements
	B	23 Mar 2026	22 Apr 2026	Anatomical measurements

¹Staggered planting was used because preliminary experiments showed that imposing 5 d or 10 d of water deprivation on plants established on the same date slowed or arrested storage root bulking. The schedule approximately matched the duration of active fertigated growth among treatments and allowed comparisons at the designated early bulking stage on a common sampling date.

Postharvest weight loss was evaluated during Expt. 2. Gene expression was evaluated during Expt. 3. Planting dates were staggered by treatment in all experiments to align storage roots at the designated early bulking stage on a common sampling date. Plants assigned to the 10-d withdrawal treatment were established 10 d before the well-watered controls, and plants assigned to the 5-d withdrawal treatment were established 5 d before the controls. Preliminary experiments showed that imposing 5-d or 10-d withdrawal on plants established on the same date slowed or arrested storage root bulking, which would have produced treatment-associated differences in root size and developmental stage at a common harvest. Therefore, staggered planting was used to approximately match the duration of active fertigated growth among treatments while permitting comparisons at a common early-bulking developmental stage. The early bulking stage, which corresponds to the period of rapid cambial development described by Torgari (1950), was selected because storage roots undergo active secondary growth and native periderm development at this stage. These roots were not assumed to be developmentally equivalent to commercially mature field-harvested roots. At sampling, storage roots measured 6 to 10 mm in diameter in anatomical studies (Expt. 1 and Expt. 4) and 10 to 25 mm in the weight loss study (Expt. 2). Each experiment was arranged in a randomized complete block design. One plant grown in one pot constituted the experimental unit. The anatomical and postharvest weight loss experiments each included four independent biological replicate plants per cultivar × treatment combination. One to two storage roots were sampled from each replicate plant, depending on storage root availability; roots from the same plant were treated as subsamples and did not increase the biological nitrogen. Root subsamples were included within plant in the statistical analysis, with plant identity retained as the nesting/random factor. The gene expression experiment included three independent biological replicate plants per cultivar × treatment combination, with three technical quantitative polymerase chain reaction (qPCR) replicates per biological sample.

Water deprivation treatments. Plants were assigned to a well-watered control (WWC), 5-d water deprivation (WD5) treatment, or 10-d water deprivation (WD10) treatment. Under WWC, substrate was maintained at or above 50% of field capacity [approximately 8%–12% volumetric water content (VWC)]. The WD5 treatment typically reduced substrate moisture to approximately 4% to 6% VWC, and the WD10 treatment typically reduced it to approximately 2% to 4% VWC. These VWC values are similar to previously reported ranges for sand substrate (Hillel 1998). Withdrawal treatments were imposed by discontinuing irrigation/fertigation, whereas WWC plants continued to receive complete 0.5× Hoagland solution (PhytoTech Labs, Lenexa, KS, USA). Because the staggered planting schedule matched the duration of active fertigated growth before sampling, WD5 and WD10 plants were exposed to similar days of Hoagland nutrition (PhytoTech Labs) relative to the WWC plants over the experimental period. Nevertheless, further nutrient delivery was interrupted during the terminal 5-d or 10-d withdrawal interval. Nutrient acquisition in a drying substrate is strongly dependent on water availability because declining water content restricts transpiration-driven mass flow and diffusion of ions to the root surface (Hu and Schmidhalter 2005). Therefore, we interpreted WD5 and WD10 as short-term progressive dry-down treatments resulting from the withdrawal of irrigation/fertigation and recognized that the effects of water and nutrients cannot be completely separated during the terminal withdrawal interval. Comparable controlled environment studies have imposed drought following Hoagland-based nutrition (PhytoTech Labs) by withholding irrigation (Chapman et al. 2022; Fort et al. 2017; Langlé-Argüello et al. 2019; Krishnan and Merewitz 2022).

Periderm anatomical analysis (Expt. 1 and Expt. 4). Native periderm anatomy was evaluated in Expt. 1 and independently repeated in Expt. 4 (Table 1). Plants were sampled on a common harvest date at the designated early bulking stage (Table 1). One to two storage roots were sampled from each replicate plant. A transverse free-hand section with a thickness of approximately 50 to 100 µm was cut from the longitudinal midpoint of each freshly harvested

storage root. The midpoint was used as a standardized anatomical position among cultivars and treatments. Longitudinal variation along the storage root was not evaluated. Sections were stained immediately with freshly prepared phloroglucinol-HCl and examined by light microscopy under identical microscope, illumination, and camera settings. Images were analyzed using ImageJ (Schneider et al. 2012). To measure discontinuity of the outer phellem layer, the circularity measurement in ImageJ was used to quantify the irregularity of the surface boundary as follows:

$$\text{Circularity} = \frac{4\pi A}{P^2} \quad [1]$$

where A is the area and P is the perimeter. Circularity was used as a quantitative index of outer boundary irregularity. Circularity approaches 1.0 as an object becomes more circular and decreases with increasing perimeter irregularity (Schwendy et al. 2019; Willemse et al. 2018). Lignified tissue was segmented using the Labkit machine learning plugin (Arzt et al. 2022). Segmented images were converted to binary masks, and the total lignified area was determined using the Analyze Particles function. Total storage root cross-sectional area was measured from manually defined regions of interest that encompassed the entire cross-section. Percent lignified tissue was calculated as follows:

$$\% \text{ Lignified tissue} = \frac{\text{Total segmented lignified area}}{\text{Total storage root cross sectional area}} \times 100 \quad [2]$$

Postharvest weight loss measurements (Expt. 2). Postharvest weight loss was evaluated in Expt. 2 (Table 1). Plants were sampled on a common harvest date at the designated early bulking stage (Table 1). Four independent replicate plants were used per cultivar $\times$ treatment combination, with one to two storage roots sampled per replicate when available. Roots from the same plant were treated as subsamples. Roots were weighed immediately after harvest and stored under ambient laboratory conditions (approximately 27°C and 50%–70% relative humidity) without artificial curing. Fresh weight was recorded weekly for 5 consecutive weeks. Weight loss was expressed as a percentage reduction from initial fresh weight. The assay was used

as a functional measure of barrier performance in the early-bulking developmental system rather than as a simulation of commercial storage of mature roots.

Gene expression analysis (Expt. 3). Gene expression was evaluated in Expt. 3 (Table 1). Plants were sampled on a common harvest date at the designated early bulking stage (Table 1). Preliminary anatomical and physiological analyses showed that both water deprivation treatments elicited similar responses relative to WWC, with the WD10 treatment producing the greatest differences in native periderm structure and postharvest weight loss. Consequently, gene expression analyses were performed using only the WWC and WD10 treatments. Immediately after harvest, a 2- to 3-mm-thick transverse disc was removed from the midpoint of each storage root. The disc was cored through its thickness using a cork borer with a diameter that approximated the inner boundary of the native periderm. Removal of the central core yielded an intact outer ring of tissue predominantly consisting of native periderm with minimal underlying cortical tissue. Three independent plants constituted the biological replicates for each cultivar $\times$ treatment combination. The excised tissue was immediately frozen in liquid nitrogen and stored at -80°C until RNA extraction. Total RNA was extracted using a modified Trizol method (Park et al. 2012). First-strand cDNA synthesis and quantitative real-time PCR (qRT-PCR) were performed as described by Arce et al. (2024). Each cDNA sample was analyzed in three technical qPCR replicates. These replicates were used to assess assay precision and were not treated as independent biological observations. Relative transcript abundances of *IbPAL*, *IbCCoAOMT*, and *IbCAD* were quantified by qRT-PCR using previously published primers with *IbCOX* as a reference gene (Park et al. 2012). Primer sequences are provided in Table 2. Relative gene expression was calculated using the $2^{-\Delta\Delta\text{Ct}}$ method after normalization (Livak and Schmittgen 2001). Preliminary qRT-PCR assays that targeted ethylene-related genes, including *IbERF* and putative 1-aminocyclopropane-1-carboxylate (ACC) synthase (*IbACS*) and ACC oxidase (*IbACO*) genes, were evaluated as follow-up markers of ethylene-associated responses. The ACC synthase and ACC oxidase catalyze key steps in ethylene biosynthesis (Wang et al. 2002). Candidate *IbACS* and

IbACO target sequences were identified from the Sweetpotato Genomics Resource (Wu et al. 2018), and primers were designed based on predicted coding sequences using NCBI Primer-BLAST (Ye et al. 2012). The *IbACO* assay produced consistent amplification across biological samples and was retained for analysis. Then, qRT-PCR amplification, normalization to *IbCOX*, and relative expression analysis were performed as previously described.

Statistical analysis. Statistical analyses were performed using R (R Core Team 2019). The anatomical (Expt. 1 and Expt. 4), postharvest weight loss (Expt. 2), and gene expression (Expt. 3) datasets were analyzed independently. When one to two roots were sampled from a replicate plant, root measurements were treated as subsamples within a plant, and plant identity was retained as the nesting/random factor in mixed-effects analyses; individual roots were not treated as independent biological replicates. Linear mixed-effects models were fitted using the lme4 and lmerTest packages. Cultivar, water deprivation treatment, and their interaction were treated as fixed effects, whereas experimental run (study/planting event) was treated as a random effect. This run effect refers to independent experimental repetitions and is distinct from the intentional 5-d and 10-d treatment-specific staggering within each experiment. We failed to detect an experimental run effect; hence, the 2-year anatomical data were combined. For the postharvest weight loss study, storage week was included as a repeated measure, and cultivar, water deprivation treatment, storage week, and their interactions were evaluated. An analysis of variance was used to test fixed effects. When treatment effects were significant ($P \leq 0.05$), estimated marginal means were calculated using the emmeans package and compared using Tukey's honestly significant difference test. Gene expression data were analyzed separately for each cultivar $\times$ gene combination using Welch's two-sample t test on ΔCt values. Relative expression values calculated by the $2^{-\Delta\Delta\text{Ct}}$ method are presented as the means $\pm$ standard error, whereas statistical inference was based on ΔCt values. Model assumptions of normality and homogeneity of variance were evaluated by visual inspection of residual and quantile-quantile plots. Percentage data were transformed when necessary to satisfy model assumptions; however, untransformed means are presented throughout for clarity.

Table 2. Names and primer sequences of genes analyzed using quantitative real-time polymerase chain reactions.

Gene	Name	Forward (F; top) and reverse (R; bottom) primer sequence (5'-3')	Reference
<i>IbPAL</i>	Phenylalanine ammonia-lyase	F: GGCGAGCACGAGAAGAATGT R: ATGGCAGGGTTTCCGTTCTC	Effendy et al. (2013)
<i>IbCCoAOMT</i>	Caffeoyl-CoA O-methyltransferase	F: CCGGTTCTTGACCAGATGAT R: TTCCACAGGGTGTTGTCGTA	He et al. (2021)
<i>IbCAD</i>	Cinnamyl alcohol dehydrogenase	F: AGCTGGTAATGGTTGGCAGTC R: TCCAAAGCCGTGTTGACATA	He et al. (2021)
<i>IbACO</i>	1-aminocyclopropane-1-carboxylate oxidase	F: ACTCTGCATAGGGTGTGGC R: TTCATCGTCTCGAACCTCGG	This study ⁱ

ⁱPrimers in this study were designed based on candidate target sequences identified through the Sweetpotato Genomics Resource using NCBI Primer-BLAST. The Sweetpotato Genomics Resource is based on the annotated Ipomoea reference genome described by Wu et al. (2018). Primer-BLAST was used for primer design and specificity checking, as described by Ye et al. (2012).

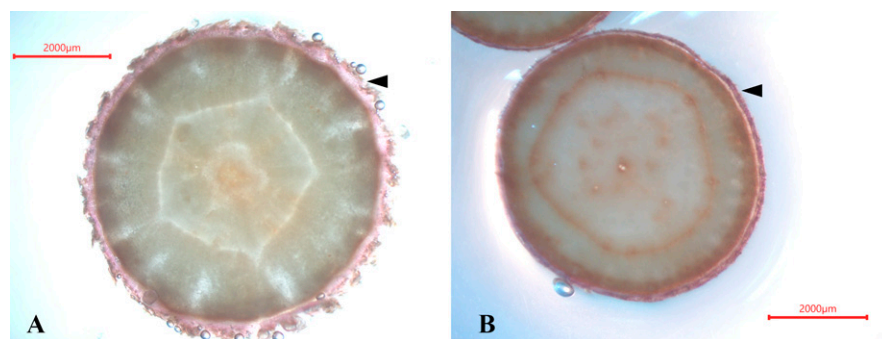


Fig. 1. Representative transverse sections from the midpoint of early-bulking 'Beauregard' sweetpotato storage roots maintained under well-watered conditions (A) or subjected to 10 d of preharvest water deprivation (B). Arrowheads indicate discontinuities in the outer native periderm of the well-watered root (A) and the comparatively continuous outer periderm of the water-deprived root (B). Sections were stained with phloroglucinol-HCl. Scale bars = 2000 µm.

Results and Discussion

Preharvest irrigation/fertigation withdrawal alters native periderm structure in early-bulking storage roots. Anatomical characterization revealed contrasting native periderm states among treatments. Well-watered roots retained visible expansion-associated discontinuities in the developing native periderm, whereas WD treatments produced a more continuous outer boundary and increased phloroglucinol-HCl

staining (Fig. 1). A quantitative image analysis showed significant increases in circularity and percent lignified tissue following withdrawal (Fig. 2). Relative to well-watered controls, percent lignified tissue increased 1.9-fold to 3.6-fold, depending on cultivar and treatment duration. Therefore, the magnitude of the response varied among cultivars, but the anatomical direction of change was consistent, with a transition from an actively expanding and discontinuous periderm to a more continuous

and lignified protective barrier. These changes are consistent with a developmental transition toward skin set rather than direct evidence that commercial field skin set had been achieved.

These observations are consistent with previous reports of preharvest environmental conditions influencing sweetpotato skin set. Webster et al. (1973) found that peeling resistance increased under warm, dry soil conditions. Villavicencio et al. (2007) subsequently showed that environmental conditions associated with greater skin adhesion also altered periderm anatomy, lignin content, and phellogen characteristics. Similar discontinuous and continuous lignification states have been observed in field-grown sweetpotato roots (Van Oirschot et al. 2006). Therefore, the present greenhouse response corresponds biologically to field-observed periderm phenotypes; however, it should not be interpreted as field validation of the magnitude or timing of the response.

The anatomical changes also provide a useful comparison with the potato periderm skin set model described by Lulai and Freeman (2001). Immature potato periderm contains an active phellogen with thin, mechanically fragile radial walls that permit tissue separation, whereas maturation is accompanied by phellogen inactivation and wall thickening that stabilize the periderm. In sweetpotato, the present response was characterized by increased lignification and greater continuity of the native periderm. Thus, although the structural mechanisms may differ between potato and sweetpotato, both systems involve developmental transition from a mechanically vulnerable and actively developing periderm toward a more stable protective barrier. Potato tuber periderm resistance also varies with the plant water status (Braue et al. 1983), irrigation affects phellogen formation and skin blemishes (Jiang et al. 2022), and soil drying alters tuber water fluxes and growth (Huntburg et al. 2025).

Preharvest irrigation/fertigation withdrawal reduces postharvest weight loss. Postharvest weight loss increased during the 5-week storage period in all cultivars, but roots from WD treatments consistently lost less weight than well-watered controls (Fig. 3). By week 5, cumulative weight loss of well-watered roots ranged from 38% to 45% compared with 21% to 24% after WD5 treatment and 20% to 30% after WD10 treatment. Across cultivars, withdrawal reduced cumulative week 5 weight loss by approximately 33% to 50%. The lower water loss coincided with the anatomical transition toward a more continuous and lignified native periderm, thus providing a functional correlate of the structural response.

The association between barrier development and water loss has precedent in other storage organs. In sugarbeet, wound healing studies showed that the development of suberized and lignified barrier tissues coincided with reduced root water loss, whereas incomplete barrier formation was associated with greater weight loss (Fugate et al. 2016). Although wound periderm and native sweetpotato periderm are distinct tissues, the common

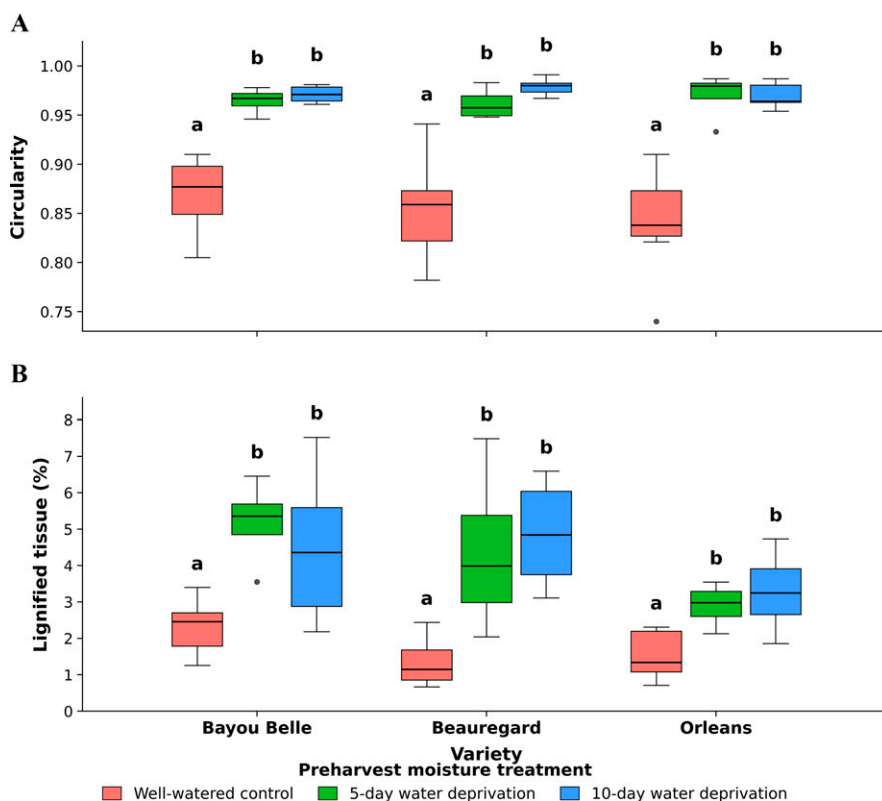


Fig. 2. Native periderm responses to preharvest water deprivation in early-bulking 'Bayou Belle', 'Beauregard', and 'Orleans' sweetpotato storage roots. (A) Circularity of transverse storage root sections. Values approaching 1.0 indicate a more circular cross-section with a smoother and more continuous outer boundary. (B) Percentage of lignified tissue from transverse sections stained with phloroglucinol-HCl and quantified using digital image segmentation. Plants were maintained under well-watered conditions (control) or subjected to water deprivation for 5 or 10 d before harvest. Boxes represent the interquartile range. Horizontal lines indicate the median. Whiskers extend to 1.5-times the interquartile range. Points indicate outliers. Within each cultivar, treatments sharing the same letter are not significantly different according to Tukey-adjusted comparisons at $P \leq 0.05$.

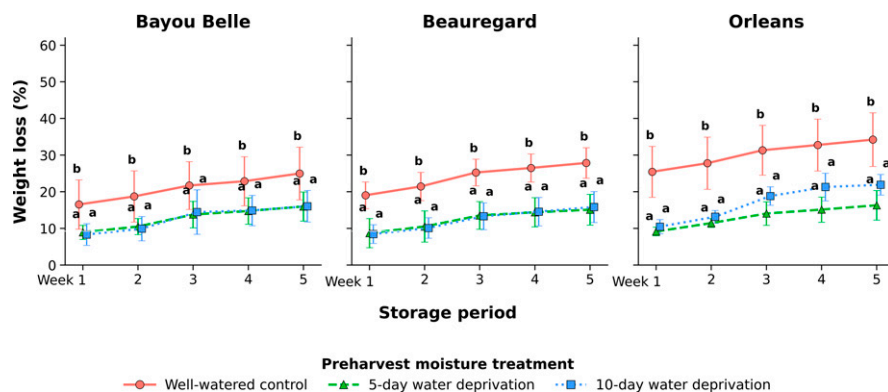


Fig. 3. Cumulative postharvest weight loss of storage roots from 'Bayou Belle', 'Beauregard', and 'Orleans' following preharvest irrigation treatments. Plants were maintained under well-watered conditions (control) or subjected to irrigation withdrawal for 5 or 10 d before harvest. Storage roots were held under ambient laboratory conditions, and weight loss was determined weekly for 5 weeks. Values are means $\pm$ standard deviation. Means sharing the same lowercase letter within each week are not significantly different according to Tukey-adjusted comparisons ($P \leq 0.05$).

functional relationship supports the broader interpretation that structural barrier maturation can improve water retention. Severe preharvest drought in sugarbeet also altered postharvest respiration and susceptibility to storage rot (Lafta et al. 2024), thus demonstrating that the preharvest water status can carry over into postharvest storage physiology. The relatively high absolute weight loss observed here reflects the small early-bulking greenhouse-grown roots and ambient storage conditions.

However, the assay was intended as a comparative measure of barrier function rather than a simulation of commercial storage of mature roots.

Preharvest irrigation/fertigation withdrawal induces cultivar-specific regulation of lignin biosynthetic genes. Water deprivation altered expression of lignin biosynthesis genes in a cultivar-dependent manner (Fig. 4). Specifically, *IbPAL* increased 1.6-fold to 3.8-fold relative to well-watered controls across the

three cultivars, indicating a common response at the entry point of the phenylpropanoid pathway. In contrast, *IbCCoAOMT* and *IbCAD* responses differed among cultivars. These molecular responses coincided with increased lignified tissue and greater continuity of native periderm, thus supporting the activation of phenylpropanoid metabolism during the observed developmental transition.

The increase in *IbPAL* expression is consistent with that reported by previous studies that showed activation of the phenylpropanoid pathway during sweetpotato wound periderm formation. Lignin biosynthesis requires coordinated activity of upstream phenylpropanoid genes, intermediate monolignol-modification genes, and downstream genes involved in monolignol formation and polymerization (Boerjan et al. 2003; Bonawitz and Chapple 2010). Effendy et al. (2013) demonstrated transcriptional activation of lignin-associated genes during sweetpotato wound healing. Subsequent transcriptomic studies documented coordinated induction of phenylpropanoid metabolism and cell wall remodeling during curing (Ji et al. 2022; Xuan et al. 2024). The present results extend this molecular context to the native periderm before harvest. In cassava, drought-responsive lignification has been mechanistically linked to CAD-related regulation (Yan et al. 2021), providing an additional storage root precedent for coordinated drought and lignin responses.

Although *IbPAL* induction was common across cultivars, downstream regulation varied. The contrasting *IbCCoAOMT* and *IbCAD* responses suggest that cultivars may achieve increased native periderm lignification through different regulatory routes. Similar genotype-dependent regulation of lignin biosynthetic genes has been reported during wound healing in sweetpotato (Effendy et al. 2013; Ji et al. 2022) and during periderm maturation in potato (Vulavala et al. 2019). Thus, the shared anatomical phenotype does not necessarily imply identical molecular regulation among cultivars.

Water deprivation is associated with increased expression of an ethylene-forming ACC oxidase gene. As a follow-up assay to evaluate whether ethylene biosynthesis was associated with native periderm maturation, relative expression of *IbACO* was quantified in well-watered and WD10 treatment roots (Fig. 5). The *IbACO* expression increased significantly in 'Bayou Belle' and 'Orleans', whereas 'Beauregard' showed a large but variable increase that was not statistically significant. Therefore, the response provides evidence of genotype-dependent regulation of an ethylene biosynthesis gene during the same interval in which native periderm continuity and lignification increased.

A possible role for ethylene metabolism is supported by earlier studies of sweetpotato. St. Amand and Randle (1989) reported that ethylene production preceded wound lignification and wound periderm formation. Preharvest ethephon application increased skin lignin/suberin content and resistance to skinning in another study (Wang et al. 2013). Curing-mediated

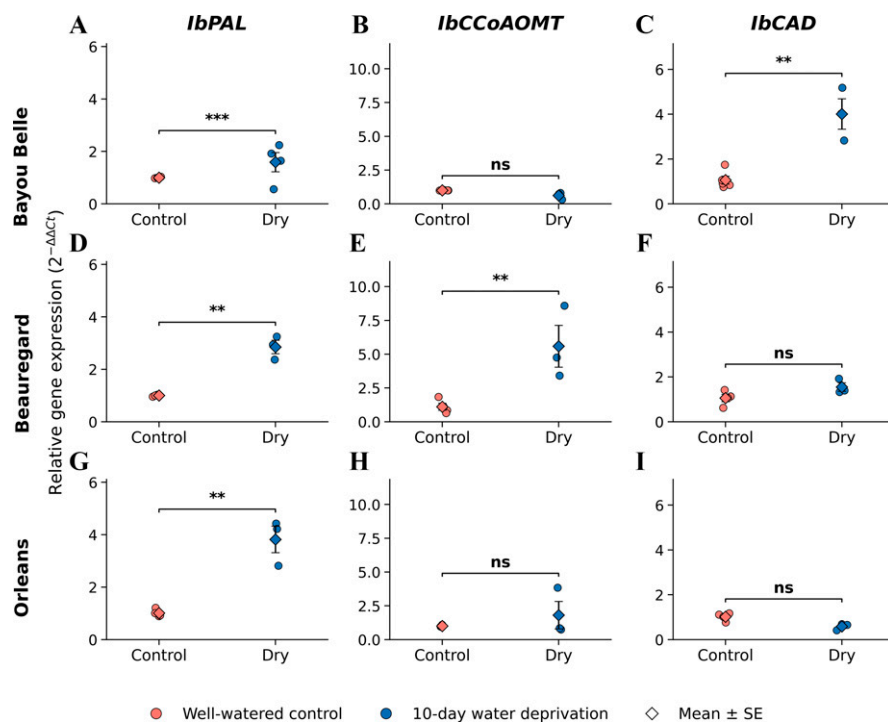


Fig. 4. Relative expression of *IbPAL*, *IbCCoAOMT*, and *IbCAD* in developing sweetpotato storage roots of 'Bayou Belle', 'Beauregard', and 'Orleans' subjected to well-watered control conditions or 10 d of preharvest water deprivation. Gene expression was quantified by a quantitative real-time polymerase chain reaction and normalized using the comparative $2^{-\Delta\Delta CT}$ method with *IbCOX* as the reference gene. Each point represents an individual biological replicate ($n = 3$). Diamonds indicate treatment means and error bars represent $\pm$ standard error. Statistical significance was determined by Welch's t test using ΔCt values. Differences between treatments within each cultivar are indicated as follows: NS, *, **, *** nonsignificant or significant at $P \leq 0.05$, 0.01, or 0.001, respectively.

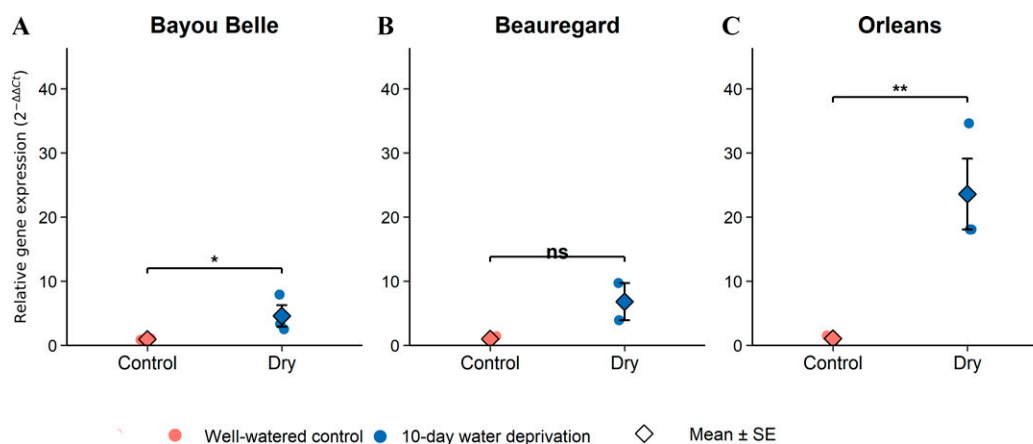


Fig. 5. Relative expression of *IbACO* in developing sweetpotato storage roots of 'Bayou Belle,' 'Beauregard,' and 'Orleans' subjected to well-watered control conditions or 10 d of preharvest water deprivation. Gene expression was quantified by quantitative RT-PCR and normalized using the comparative $2^{-\Delta\Delta CT}$ method. Each point represents an individual biological replicate ($n = 3$); diamonds indicate treatment means, and error bars represent $\pm$ standard error. Statistical significance was determined by Welch's t test using ΔCT values. Differences between treatments within each cultivar are indicated as follows: NS, *, ** nonsignificant or significant at $P \leq 0.05$ or 0.01 , respectively.

wound healing studies also showed activation of cell wall remodeling and phenylpropanoid-related pathways (Ji et al. 2022; Xuan et al. 2024). However, ethylene is not necessarily required for all periderm-associated responses. In potato, wound-induced ethylene is part of the tuber wound response, but it is not required for wound-induced suberization (Lulai and Suttle 2004). Because ACO catalyzes the final oxygen-dependent step in ethylene biosynthesis, increased *IbACO* expression is consistent with increased ethylene biosynthetic capacity (Houben and Van de Poel 2019); however, transcript abundance alone does not demonstrate increased ethylene production or signaling. Direct measurements of ethylene evolution, ACC concentration, and responses to ethylene agonists or inhibitors are required to establish causality.

Integrating anatomical, physiological, and molecular responses. Across the independent experiments, the anatomical, physiological, and molecular responses converged on a common developmental interpretation. Short-term withdrawal was associated with fewer visible periderm discontinuities, a 1.9-fold to 3.6-fold increase in percent lignified tissue, 33% to 50% reduction in cumulative week 5 weight loss, and 1.6-fold to 3.8-fold increase in *IbPAL* expression, together with cultivar-specific responses of *IbCCoAOMT*, *IbCAD*, and *IbACO* (Figs. 1–5). These coordinated responses are consistent with accelerated native periderm maturation during early-storage root bulking. Importantly, the experiments included developing greenhouse-grown roots rather than commercially mature field-grown roots; therefore, the value of the system is its ability to resolve developmental responses under controlled conditions rather than the ability to directly reproduce commercial skin set.

Limitations and implications of the developmental system. Two limitations should be considered when interpreting these results. First, the greenhouse system was designed for mechanistic control and did not reproduce the environmental heterogeneity, developmental maturity, or storage root size in commercial

fields. Second, water delivery and nutrient delivery remained coupled during the terminal withdrawal interval because irrigation was supplied as Hoagland solution (PhytoTech Labs). The staggered planting design reduced one potential source of bias: WD10 treatment and WD5 treatment plants were established 10 d and 5 d earlier, respectively; therefore, the duration of active fertigated growth was approximately matched among treatments and roots could be compared at the designated early bulking stage. Thus, WD plants were not simply exposed to 5 d or 10 d of fewer of Hoagland nutrition (PhytoTech Labs) over the experimental period. However, further nutrient addition ceased during the final WD5 treatment or WD10 treatment interval; therefore, the design did not experimentally separate water from nutrient effects during the terminal dry-down. Interruption of nutrient addition during a dry-down is not physiologically equivalent to imposing a defined nutrient omission treatment. As substrate water content declines, nutrient movement and uptake are constrained by reduced transpiration-driven mass flow and diffusion (Hu and Schmidhalter 2005). This coupling is consistent with that observed in controlled studies that imposed drought after Hoagland-based nutrition (PhytoTech Labs) by withholding irrigation (Chapman et al. 2022; Chen et al. 2015; Fort et al. 2017; Krishnan and Merewitz 2022; Langlé-Argüello et al. 2019). Accordingly, the present treatments were interpreted as short-term progressive dry-down treatments resulting from the withdrawal of Hoagland fertigation (PhytoTech Labs) while recognizing that altered nutrient acquisition may contribute to the integrated response. Factorial experiments that independently manipulate the water supply and nutrient supply and include tissue nutrient measurements and validation in mature field-grown roots are required to partition these effects and establish practical relevance.

Conclusion

Short-term preharvest withholding of irrigation/fertigation was associated with a more

continuous native periderm, increased lignification, reduced postharvest weight loss, and cultivar-specific expression of lignin-associated and ethylene-associated genes in storage roots in the early bulking stage. These coordinated responses support the use of early-bulking greenhouse-grown roots as a controlled developmental system for native periderm research. The results are consistent with published evidence regarding potato, cassava, and sugarbeet, thus showing that the preharvest water status can influence storage organ development and subsequent function. The magnitude and practical relevance of the sweetpotato response require validation in mature field-grown roots and experiments that separate water availability from nutrient delivery.

References Cited

- Arce LI, Labonte D, Villordon A, Gregorie JC. 2024. Differential expression of phosphate starvation-responsive genes among sweetpotato cultivars during establishment and onset of storage root formation. *J Am Soc Hortic Sci.* 149(6):311–319. <https://doi.org/10.21273/JASHS05433-24>.
- Arzt M, Deschamps J, Tobias P, Schmied C, Pietz S, Schmidt D, Tomancak P, Haase R, Jug F. 2022. LABKIT: Labeling and segmentation toolkit for big image data. *Front Comp Sci.* 4:777728. <https://doi.org/10.3389/fcomp.2022.777728>.
- Boerjan W, Ralph J, Baucher M. 2003. Lignin biosynthesis. *Annu Rev Plant Biol.* 54:519–546. <https://doi.org/10.1146/annurev.arplant.54.031902.134938>.
- Bonawitz ND, Chapple C. 2010. The genetics of lignin biosynthesis: Connecting genotype to phenotype. *Annu Rev Genet.* 44:337–363. <https://doi.org/10.1146/annurev-genet-102209-163508>.
- Braue CA, Wample RL, Kolattukudy PE, Dean BB. 1983. Relationship of potato tuber periderm resistance to plant water status. *Am Potato J.* 60(11): 827–837. <https://doi.org/10.1007/BF02853972>.
- Chapman C, Rossi S, Yuan B, Huang B. 2022. Differential regulation of amino acids and nitrogen for drought tolerance and poststress recovery in creeping bentgrass. *J Am Soc Hortic Sci.* 147(4):208–215. <https://doi.org/10.21273/JASHS05215-22>.
- Chen Y, Yu J, Huang B. 2015. Effects of elevated CO₂ concentration on water relations and

- photosynthetic responses to drought stress and recovery during rewetting in tall fescue. *J Am Soc Hortic Sci.* 140(1):19–26. <https://doi.org/10.21273/JASHS.140.1.19>.
- Duque LO, Setter TL. 2013. Cassava response to water deficit in deep pots: Root and shoot growth, ABA, and carbohydrate reserves in stems, leaves and storage roots. *Tropical Plant Biol.* 6(4):199–209. <https://doi.org/10.1007/s12042-013-9131-3>.
- Effendy J, La Bonte DR, Baisakh N. 2013. Identification and expression of skinning injury-responsive genes in sweetpotato. *J Am Soc Hortic Sci.* 138(3):210–216. <https://doi.org/10.21273/JASHS.138.3.210>.
- Fort K, Fraga J, Grossi D, Walker MA. 2017. Early measures of drought tolerance in four grape rootstocks. *J Am Soc Hortic Sci.* 142(1):36–46. <https://doi.org/10.21273/JASHS03919-16>.
- Fugate KK, Ribeiro WS, Lulai EC, Deckard EL, Finger FL. 2016. Cold temperature delays wound healing in postharvest sugarbeet roots. *Front Plant Sci.* 7:499. <https://doi.org/10.3389/fpls.2016.00499>.
- He S, Wang H, Hao X, Wu Y, Bian X, Yin M, Zhang Y, Fan W, Dai H, Yuan L, Zhang P, Chen L. 2021. Dynamic network biomarker analysis discovers IbNAC083 in the initiation and regulation of sweet potato root tuberization. *Plant J.* 108(3):793–813. <https://doi.org/10.1111/tpj.15478>.
- Hillel D. 1998. *Environmental soil physics*. Academic Press, San Diego, CA, USA.
- Hoagland DR, Arnon DI. 1950. The water-culture method for growing plants without soil (2nd ed) [Database]. California Agricultural Exp Stn Circ 347.
- Houben M, Van de Poel B. 2019. 1-Aminocyclopropane-1-carboxylic acid oxidase (ACO): The enzyme that makes the plant hormone ethylene. *Front Plant Sci.* 10:695. <https://doi.org/10.3389/fpls.2019.00695>.
- Hu Y, Schmidhalter U. 2005. Drought and salinity: A comparison of their effects on mineral nutrition of plants. *Z Pflanzenernähr Bodenkd.* 168(4):541–549. <https://doi.org/10.1002/jpln.200420516>.
- Huntenburg K, Pflugfelder D, Koller R, Dodd IC, van Dusschoten D. 2025. Diurnal water fluxes and growth patterns in potato tubers under drought stress. *Plant Soil.* 507(1-2):269–282. <https://doi.org/10.1007/s11104-023-06108-1>.
- Ji CY, Kim YH, Lee CJ, Park SU, Lee HU, Kwak SS, Kim HS. 2022. Comparative transcriptome profiling of sweetpotato storage roots during curing-mediated wound healing. *Gene.* 833:146592. <https://doi.org/10.1016/j.gene.2022.146592>.
- Jiang M, Shinnars-Carnelley T, Gibson D, Jones D, Joshi J, Wang-Pruski G. 2022. Irrigation effect on yield, skin blemishes, phellogen formation, and total phenolics of red potatoes. *Plants (Basel).* 11(24):3523. <https://doi.org/10.3390/plants11243523>.
- Krishnan S, Merewitz E. 2022. Variation in creeping bentgrass cultivar responses to drought stress. *HortTechnology.* 32(2):87–89. <https://doi.org/10.21273/HORTTECH04957-21>.
- Lafta AM, Eide JD, Khan MFR, Finger FL, Fugate KK. 2024. Severe preharvest drought elevates respiration and storage rot in postharvest sugarbeet roots. *J Agronomy Crop Sci.* 210(4):e12718. <https://doi.org/10.1111/jac.12718>.
- Langle-Argüello L, Martínez-Gutiérrez GA, Santiago-García PA, Escamirosa-Tinoco C, Morales I, Enríquez-del-Valle JR. 2019. Nutrient solutions and drought in plant growth and fructans content of *Agave potatorum* Zucc. *HortScience.* 54(9):1581–1584. <https://doi.org/10.21273/HORTSCI14129-19>.
- Livak KJ, Schmittgen TD. 2001. Analysis of relative gene expression data using real-time quantitative PCR and the 2^{-ΔΔCT} Method. *Methods.* 25(4):402–408. <https://doi.org/10.1006/meth.2001.1262>.
- Lulai EC. 2007. The canon of potato science: 43. Skin-set and wound-healing/suberization. *Potato Res.* 50(3-4):387–390. <https://doi.org/10.1007/s11540-008-9067-4>.
- Lulai EC, Suttle JC. 2004. The involvement of ethylene in wound-induced suberization of potato tuber (*Solanum tuberosum* L.): A critical assessment. *Postharvest Biol Technol.* 34(1):105–112. <https://doi.org/10.1016/j.postharvbio.2004.05.012>.
- Lulai EC, Freeman TP. 2001. The importance of phellogen cells and their structural characteristics in susceptibility and resistance to excoriation in immature and mature potato tuber (*Solanum tuberosum* L.) periderm. *Ann Bot.* 88(4):555–561. <https://doi.org/10.1006/anbo.2001.1497>.
- Park SC, Kim YH, Ji CY, Park S, Jeong JC, Lee HS, Kwak SS. 2012. Stable internal reference genes for the normalization of real-time PCR in different sweetpotato cultivars subjected to abiotic stress conditions. *PLoS One.* 7(12):e51502. <https://doi.org/10.1371/journal.pone.0051502>.
- Parmar A, Kirchner SM, Sturm B, Hensel O. 2017. Pre-harvest curing: Effects on skin adhesion, chemical composition and shelf-life of sweetpotato roots under tropical conditions. *East Afr Agric For J.* 82(2-4):130–143. <https://doi.org/10.1080/00128325.2017.1340141>.
- R Core Team. 2019. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria.
- Schneider CA, Rasband WS, Eliceiri KW. 2012. NIH Image to ImageJ: 25 years of image analysis. *Nat Methods.* 9(7):671–675. <https://doi.org/10.1038/nmeth.2089>.
- Schwendy M, Unger RE, Bonn M, Parekh SH. 2019. Automated cell segmentation in Fiji[®] using the DRAQ5 nuclear dye. *BMC Bioinformatics.* 20(1):39. <https://doi.org/10.1186/s12859-019-2602-2>.
- Smith TP, Stoddard S, Shankle M, Schultheis J. 2009. Sweetpotato production in the United States, p 287–323. In: Loebenstein G, Thottappilly G (eds). *The sweetpotato*. Springer, Dordrecht, the Netherlands. https://doi.org/10.1007/978-1-4020-9475-0_14.
- St. Amand PC, Randle WM. 1989. Ethylene production and wound healing in sweet potato roots. *HortScience.* 24(5):805–807. <https://doi.org/10.21273/HORTSCI.24.5.805>.
- Togari Y. 1950. A study of tuberous root initiation in sweetpotato. Tokyo Nat Agric Exp Stn Bull 68. [in Japanese with English summary].
- van Oirschot QEA, Rees D, Aked J, Kihurani A. 2006. Sweetpotato cultivars differ in efficiency of wound healing. *Postharvest Biol Technol.* 42(1):65–74. <https://doi.org/10.1016/j.postharvbio.2006.05.013>.
- Villavicencio LE, Blankenship SM, Yencho GC, Thomas JF, Raper CD. 2007. Temperature effect on skin adhesion, cell wall enzyme activity, lignin content, anthocyanins, growth parameters, and periderm histochemistry of sweetpotato. *J Am Soc Hortic Sci.* 132(5):729–738. <https://doi.org/10.21273/JASHS.132.5.729>.
- Villordon A, Clark C. 2018. Variation in root architecture attributes at the onset of storage root formation among resistant and susceptible sweetpotato cultivars infected with *Meloidogyne incognita*. *HortScience.* 53(12):1924–1929. <https://doi.org/10.21273/HORTSCI10746-18>.
- Villordon A, LaBonte D, Firon N, Carey E. 2013. Variation in nitrogen rate and local availability alter root architecture attributes at the onset of storage root initiation in ‘Beauregard’ sweetpotato. *HortScience.* 48(6):808–815. <https://doi.org/10.21273/HORTSCI.48.6.808>.
- Villordon A, LaBonte D, Solis J, Firon N. 2012. Characterization of lateral root development at the onset of storage root initiation in ‘Beauregard’ sweetpotato adventitious roots. *HortScience.* 47(7):961–968. <https://doi.org/10.21273/HORTSCI.47.7.961>.
- Villordon AQ, La Bonte DR, Firon N, Kfir Y, Pressman E, Schwartz A. 2009. Characterization of adventitious root development in sweetpotato. *HortScience.* 44(3):651–655. <https://doi.org/10.21273/HORTSCI.44.3.651>.
- Vulavala VKR, Fogelman E, Faigenboim A, Shoseyov O, Ginzberg I. 2019. The transcriptome of potato tuber phellogen reveals cellular functions of cork cambium and genes involved in periderm formation and maturation. *Sci Rep.* 9(1):10216. <https://doi.org/10.1038/s41598-019-46681-z>.
- Walter WM, Schadel WE. 1983. Structure and composition of normal skin (periderm) and wound tissue from cured sweet potatoes. *J Am Soc Hortic Sci.* 108(6):909–914. <https://doi.org/10.21273/JASHS.108.6.909>.
- Wang KLC, Li H, Ecker JR. 2002. Ethylene biosynthesis and signaling networks. *Plant Cell.* 14(Suppl):S131–S151. <https://doi.org/10.1105/tpc.001768>.
- Wang X, Arancibia RA, Main JL, Shankle MW, LaBonte DR. 2013. Preharvest foliar applications of ethephon increase skin lignin/suberin content and resistance to skinning in sweetpotato storage roots. *HortScience.* 48(10):1270–1274. <https://doi.org/10.21273/HORTSCI.48.10.1270>.
- Webster EG, Wright ME, Austin ME. 1973. Sweetpotato periderm failure in response to soil moisture and temperature. *HortScience.* 8(3):226–227. <https://doi.org/10.21273/HORTSCI.8.3.226>.
- Willemse J, Büke F, van Dissel D, Grevink S, Claessen D, van Wezel GP. 2018. SPARTICLE, an algorithm for the analysis of filamentous microorganisms in submerged cultures. *Antonie Van Leeuwenhoek.* 111(2):171–182. <https://doi.org/10.1007/s10482-017-0939-y>.
- Wu S, Lau KH, Cao Q, Hamilton JP, Sun H, Zhou C, Eserman L, Gemenet DC, Olukolu BA, Wang H, Crisovan E, Godden GT, Jiao C, Wang X, Kitavi M, Manrique-Carpintero NC, Vaillancourt B, Wiegert-Rininger K, Yang X, Bao K, Schaff J, Kreuze J, Grunberg W, Khan A, Ghislain M, Ma D, Jiang J, Mwanga ROM, Leebens-Mack J, Coin LJM, Yencho GC, Buell CR, Fei Z. 2018. Genome sequences of two diploid wild relatives of cultivated sweetpotato reveal targets for genetic improvement. *Nat Commun.* 9(1):4580. <https://doi.org/10.1038/s41467-018-06983-8>.
- Xuan H, Cheng J, Pang L, Yin L, Guan Y, Cheng J, Lu X, Lu G. 2024. Physiological-biochemical characteristics and a transcriptomic profiling analysis reveal the postharvest wound healing mechanisms of sweet potatoes under ascorbic acid treatment. *Foods.* 13(16):2569. <https://doi.org/10.3390/foods13162569>.
- Yan Y, Wang P, Lu Y, Bai Y, Wei Y, Liu G, Shi H. 2021. MeRAV5 promotes drought stress resistance in cassava by modulating hydrogen peroxide and lignin accumulation. *Plant J.* 107(3):847–860. <https://doi.org/10.1111/tpj.15350>.
- Ye J, Coulouris G, Zaretskaya I, Cutcutache I, Rozen S, Madden TL. 2012. Primer-BLAST: A tool to design target-specific primers for polymerase chain reaction. *BMC Bioinformatics.* 13:134. <https://doi.org/10.1186/1471-2105-13-134>.