



Physiological and sucrose metabolic responses to waterlogging stress in balloon flower (*Platycodon grandiflorus* (Jacq.) A. DC)

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Abstract Waterlogging stress is a major limiting factor resulting in stunted growth and loss of crop productivity, especially for root crops. However, physiological responses to waterlogging have been studied in only a few plant models. To gain insight into how balloon flower (*Platycodon grandiflorus* (Jacq.) A. DC) responds to waterlogging stress, we investigate changes to sucrose metabolism combined with a physiological analysis. Although waterlogging stress decreased the photosynthetic rate in balloon flower, leaves exhibited an increase in glucose (ninefold), fructose (4.7-fold), and sucrose (2.1-fold), indicating inhibition of sugar transport via the phloem. In addition, roots showed a typical response to hypoxia, such as the accumulation of proline (4.5-fold higher than in control roots) and soluble sugars (2.1-fold higher than in control roots). The activities and expression patterns of sucrose catabolizing enzymes suggest that waterlogging stress leads to a shift in the pathway of sucrose degradation from invertase to sucrose synthase (Susy), which consumes less ATP. Furthermore, we suggest that the waterlogging-stress-induced gene *PlgSusyl* encodes the functional Susy enzyme, which may contribute to improving tolerance in balloon flower to waterlogging. As a first step toward understanding the waterlogging-induced regulatory mechanisms in balloon flower, we provide a solid

foundation for further understanding waterlogging-induced alterations in source-sink relationships.

Keywords Balloon flower · Invertase · Storage roots · Sucrose synthase · Waterlogging stress

Introduction

The intensification of rainfall due to global warming will result in more flash flooding and seasonal waterlogging. It has been estimated that 12% of all irrigated farmland could be waterlogged frequently in the near future, leading to an approximate 20% reduction in crop productivity (Tian et al. 2021). The important biological implication of waterlogging conditions is hypoxia (a deficiency of oxygen) or anoxia (the complete absence of oxygen) in the soil environment, resulting in limited mitochondrial aerobic respiration, suppressed root activity, and lack of energy in plants (Phukan et al. 2016). In response to oxygen limitation, plants undergo a metabolic switch from aerobic respiration to anaerobic fermentation, which uses pyruvate metabolized from starch through glycolysis as a starting substrate to generate adenosine triphosphate (ATP, Parent et al. 2008; Colmer and Voesenek 2009; Banti et al. 2013). However, the accumulation of lactate and ethanol, the main fermentation end products of lactate dehydrogenase and alcohol dehydrogenase, respectively, can also contribute to root injury and death (De Castro et al. 2022). In addition, waterlogging stress causes the overproduction of reactive oxygen species and reactive nitrogen species that potentially lead to DNA damage, protein degradation, lipid peroxidation, and cell membrane damage (Jethva et al. 2022).

Roots are the primary sufferer of waterlogging stress, and it has been well-reported that storage roots, such as

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sweet potato and cassava, are very sensitive to waterlogging, which affects their growth and yield (Kerdee et al. 2021; Pan et al. 2021b). In the case of ginseng, photosynthetic rate, root diameter, and root weight were significantly reduced when soils were waterlogged for more than three days (Suh et al. 2021). In addition, the incidence of cassava root rot disease and diseases in ginseng were higher in the rainy season (Kim et al. 2007; Akrofi et al. 2018), indicating that waterlogging stress, directly and indirectly, affects the production and yield of storage roots. To cope with waterlogging stress, plants have evolved various strategies including changes to morphology (e.g., the formation of adventitious roots or other aeration tissues, rapid elongation of apical meristematic tissue, and the formation of air films in the upper cuticle), physiology (e.g., endogenous hormone biosynthesis, respiration, and photosynthesis), and metabolism (e.g., primary and secondary metabolites). However, elucidation of the waterlogging response signaling pathways and tolerance mechanisms in storage roots remains an outstanding challenge in plant biology.

Balloon flower (*Platycodon grandiflorus* (Jacq.) A. DC), a single genus in the family Campanulaceae, is native to East Asia (Zhang et al. 2015). In Korea, balloon flower root is mostly used for medicinal and culinary purposes, and its production value amounted to over 100 million U.S. dollars in 2011 (Lee et al. 2014). Recently, platycodin D, an active ingredient derived from the roots of balloon flower, has been suggested as a potent natural product for preventing or treating COVID-19 infection by hindering membrane fusion (Kim et al. 2021), which has led to increasing interest in the plant. Balloon flower has a taproot system, and soil drainage has a significant impact on the yield of storage roots and the incidence rate of root disease (Lee et al. 1999). Although this indicates that balloon flower is sensitive to waterlogging stress, physiological and molecular responses to waterlogging stress in their roots remain unknown.

In this study, to fill this gap, physiological and molecular changes in balloon flower subjected to waterlogging stress were analyzed, and gene expression and enzyme activities in non-treated and waterlogged plants were compared to determine the effect of waterlogging stress on sucrose metabolism. Our systematic analysis provides the first step toward a functional dissection of changes in sucrose metabolism during the response of balloon flower to waterlogging stress.

Materials and methods

Plant materials and treatment

One-year-old roots of balloon flower (*P. grandiflorus* (Jacq.) A. DC) were transplanted into pots filled with soil and grown in a growth chamber at 24 °C and 50% relative humidity

under a long photoperiod (16 h light/8 h dark) at a photon flux density of 100 $\mu\text{mol m}^{-2} \text{s}^{-1}$ (fluorescent white lamps). Four weeks after transplanting, healthy, growing potted plants were placed in a plastic container filled with water for the waterlogging treatment. The water level was maintained at 3 cm above the soil surface, and the concentration of dissolved oxygen was less than 0.04 mmol/m³. Control plants remained in a well-watered condition throughout the experiment. Leaves (fully expanded leaves) and roots were collected at 0, 5, 9, 13, and 18 days after the treatment, quickly frozen in liquid nitrogen, and stored at –80 °C until analysis.

The physiological response of balloon flower to waterlogging stress

The effects of waterlogging stress on photosynthesis were determined by analyzing chlorophyll fluorescence using a pulsed modular fluorometer (FluorPen FP110). Fully expanded leaves were grounded, and chlorophyll (Chl) was extracted in 95% ethanol at 80 °C for 30 min, and the concentrations of Chl *a*, Chl *b*, and Chl *a + b* were calculated according to Czyczyło-Mysza et al. (2013).

The proline contents in leaves and roots were quantified using the acid ninhydrin method described by Eom et al. (2022), calculated based on a calibration curve derived from standard proline solutions, and expressed as ng/mg of fresh weight (FW).

The total content of ATP was analyzed using an EZ-ATP Assay Kit (DoGenBio Co., Seoul, Korea) according to the manufacturer's instructions, and measured using a fluorescence microplate reader equipped with a 535 nm excitation filter and a 595 nm emission filter.

Analysis of soluble sugar contents and sucrose-metabolizing enzymes

To analyze soluble sugar contents, 100 mg of ground samples were mixed with an equal volume of cold water. After centrifugation at 13,000 rpm for 15 min, the supernatants were transferred to a clean tube. Then sucrose, glucose, and fructose were analyzed using a Sucrose/D-Fructose/D-Glucose Assay Kit (Megazyme, Wicklow, Ireland), according to the manufacturer's instructions.

To determine the activities of sucrose-metabolizing enzymes, 0.1 g of ground leaf material was mixed in 1 mL of extraction buffer (200 mM HEPES, pH 7.5, 3 mM MgCl₂, 1 mM EDTA, 2% glycerol, and 0.1 mM PMSF) as described in Bonfig et al. (2006). After centrifugation, the supernatant was dialyzed and used to analyze the activities of the vacuolar invertase (V-inv), neutral/alkaline invertase (N-inv), and sucrose synthase (Susy). The pellet was washed three times, suspended in the above extraction buffer containing 1 M NaCl, and subjected to centrifugation. The supernatant

was dialyzed and used to determine the activity of cell wall invertase (C-inv). The activities of V-inv, N-inv, and C-inv were determined using GOD-POD reagent (Bonfig et al. 2006), and Susy activity was analyzed as described by Janner et al. (2015). Protein content was quantified using a Pierce™ BCA Protein Assay Kit (Thermo Fisher Scientific, USA).

In-silico analysis for identification of sucrose-metabolizing enzymes

To identify and enumerate the complete family of *P. grandiflorus* invertases (PlgInvs) and Susys (PlgSusys), BLAST searches ($p < 0.01$) were performed on *P. grandiflorus* genome sequence data (Kim et al. 2020) based on known plant invertase and Susy sequences. The obtained protein sequences were analyzed using domain searches (HMMER 3.0, <http://hmmer.org/>), predictions of subcellular localization (SignalP, <http://www.cbs.dtu.dk/services/SignalP/>; Protcomp, <http://www.softberry.com/berry.phtml>; CELLO, <http://cello.life.nctu.edu.tw/>), and molecular weight (MW, Compute pI/MW tool, http://web.expasy.org/compute_pi/). ClustalW and MEGA 11 software were used for multiple sequence alignments and phylogenetic analyses.

Quantitative real-time PCR (qRT-PCR) analysis

To investigate the expression of the sucrose-metabolizing enzyme genes, qRT-PCR analysis was used. The total RNA from the balloon flower leaves and roots were extracted according to the manufacturer's instructions (Favorgen, Ping Tung, TAIWAN) and reverse-transcribed into cDNA using oligo (dT) primer, which was then used for cloning or qRT-PCR. A final volume of 20 μ l reaction contains 10 μ l SYBR-Green Master Mix, 2 μ l cDNA template, 0.5 μ l each amplification primer, and 7 μ l H₂O. The qRT-PCR was performed with Toyobo SYBR-Green Master Mix (Toyobo, Co., Ltd., Osaka, Japan) under the following conditions: 95 °C for 3 min followed by 40 cycles of 95 °C for 10 s, 55 °C for 30 s, and 72 °C for 15 s. The balloon flower actin gene was used as the internal reference. The primer sequences are listed in Table S1. All analyses were performed in five biological replicates and two technical replicates.

Transient expression of PlgSusy1 in tobacco

The full-length *PlgSusy1* gene, amplified using gene-specific primers, was subcloned into a gateway binary vector pGWB505, and then transformed into *Agrobacterium tumefaciens* strain GV3101. For transient expression in tobacco (*Nicotiana benthamiana*), agro-infiltration was performed as described by Ji et al. (2021). Briefly, a single colony of *A. tumefaciens* GV3101 containing either PlgSusy1 or GUS

construct was inoculated into LB medium with antibiotics and grown overnight at 28 °C. The bacteria were collected by centrifugation and resuspended in 10 mM MES and 10 mM MgCl₂ containing 150 μ M acetosyringone to an OD600 of 1.0. Tobacco leaves were infiltrated with the bacterial suspension using a sterile 1 mL needleless syringe, and infiltrated leaves were used for the analysis of Susy activity. Transient expression was assessed in three biological replicates.

Statistical analysis

All the experiments were conducted for three independent replicates, and data were expressed as mean \pm standard error. Statistical significance for differences between treatments was analyzed using the Student's t-test. ***, ** or * indicate statistical significance at $p < 0.001$, $p < 0.01$ or $p < 0.05$, respectively.

Results and discussion

Physiological responses in Balloon flowers under waterlogging conditions

Waterlogging-induced hypoxia or anoxia dramatically affects plant growth, development, and survival (Pan et al. 2021a; De Castro et al. 2022). To investigate the effect of waterlogging stress on the growth of balloon flowers, a time course analysis was undertaken to measure stem growth under waterlogging and normal conditions. As shown in Fig. 1, waterlogging had a negative effect on the growth and development of balloon flower. The plant growth (stem length) stopped almost completely after 10 days of waterlogging onwards whereas the stem length of control plants increased linearly (Fig. 1a, b). Under waterlogging stress, root dry weight was 71% lower (control plants, 1,068 \pm 142 mg; waterlogged plants, 309 \pm 91 mg) than that of non-waterlogged plants (Fig. 1c). This indicates that waterlogging stress significantly impacts the yield of balloon flower roots.

Proline is a proteinogenic amino acid synthesized by the glutamate and ornithine pathways, and it plays a highly beneficial role in plants exposed to environmental stresses as an osmolyte, a free radical scavenger, a carbon and nitrogen reserve, and a signaling molecule (Szabados and Savaouré 2010; Hayat et al. 2012). Under waterlogging stress conditions, proline contents were significantly increased in the roots of plants including banana, peach, and rice (Cao et al. 2020; Xiao et al. 2020; Teoh et al. 2022). Similarly, we found that proline accumulated in the waterlogged roots of balloon flower. As shown in Fig. 2a, there was an approximately 4.5-fold increase in proline content in roots

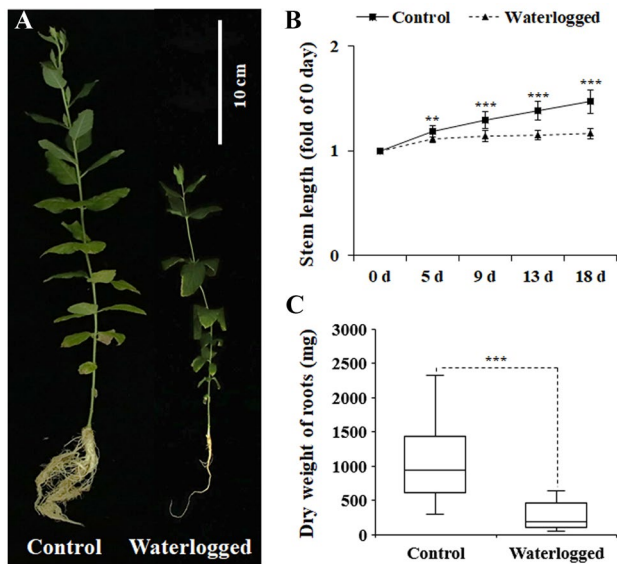


Fig. 1 Effect of waterlogging stress on the growth of balloon flower. The phenotypes (a), stem length (b) and dry weights of roots (c) were measured after 18 days of waterlogging stress treatment. Asterisks indicate significant differences (Student's t-test; $**p < 0.01$, and $***p < 0.001$) between non-treated and treated plants. The bars represent the mean \pm standard error ($n = 10$)

after waterlogging for 13 days. The accumulation of proline in response to waterlogging is mediated by stimulation of synthesis, loss of feedback inhibition, and the inhibition of xylem transfer (Olgun et al. 2008; Lothier et al. 2020).

The accumulated evidence suggests that there are physiological effects of waterlogging in leaves, even though waterlogging does not lead to hypoxic conditions in leaves. Under waterlogged conditions, the chlorophyll synthesis ability of leaves decreased as demonstrated by decreasing transcription levels and decreasing activity of chlorophyll metabolism-related genes (Yu et al. 2019; Zhang et al. 2021). As shown in Fig. 2b, the contents of Chl *a*, Chl *b*, and Chl *a + b* were significantly lower in the leaves of waterlogged plants than in the non-waterlogged plants. In addition, the chlorophyll fluorescence data showed that the reduced content of Chl led to a decrease in the maximal photochemical efficiency of photosystem II (Fv/Fm, Fig. 2c). Similar physiological responses have been seen in peanut, wheat, avocado, and cassava (Wu et al. 2015; Cao et al. 2022; Lin et al. 2022; Zeng et al. 2022).

Effect of waterlogging stress on sucrose metabolism

Photosynthetically generated sucrose is translocated to the roots where it is used for root development and growth (Kircher and Schopfer 2012; Kutschera and Briggs 2019). Therefore, we predicted that the waterlogging-induced photosynthetic inhibition would have decreased the level of

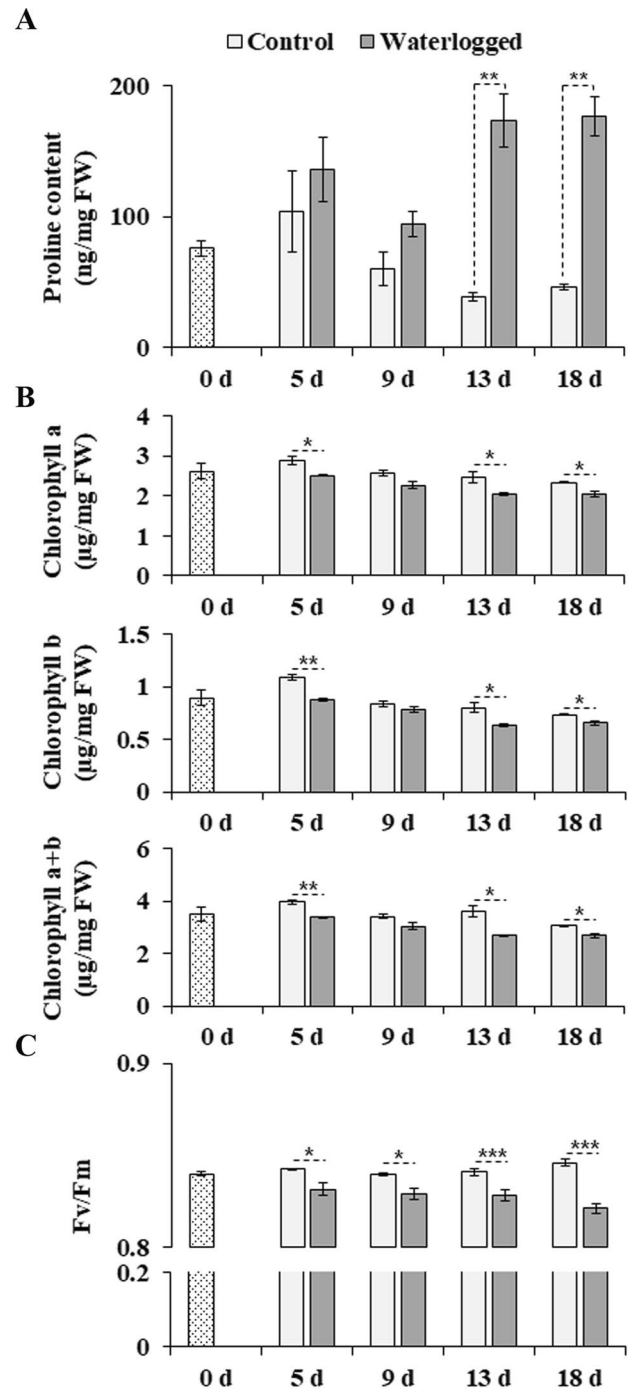


Fig. 2 Physiological response to waterlogging stress in balloon flower. Changes in root proline content (a), leaf chlorophyll content (b), and Fv/Fm values (c). Asterisks indicate significant differences (Student's t-test; $*p < 0.05$, $**p < 0.01$, and $***p < 0.001$) between non-treated and treated plants. The data are presented as means \pm SE ($n = 10$)

sucrose in roots, resulting in the observed reduction of root growth and development. To test this hypothesis, we analyzed the tissue contents of sucrose, glucose, and fructose.

Contrary to our prediction, the sucrose content was approximately 2.1-fold higher in the roots of waterlogged plants (18 days after waterlogging) than in control plants (Fig. 3). The roots of waterlogged plants also had higher levels of glucose (ninefold) and fructose (4.7-fold). In addition, although waterlogging stress was shown to decrease the photosynthetic rate in balloon flower, the sucrose, fructose, and glucose contents were greater in the leaves of waterlogged plants than in those of the control plants (Fig. 2c). In *Medicago truncatula*, the phloem loading rate was reduced by waterlogging stress (Lothier et al. 2020), indicating that the accumulation of sugar in leaves of waterlogged balloon flower should be mediated by the inhibition of sugar transport via the phloem. Under waterlogged conditions, sugars are required for the formation of new adventitious roots, which contain more aerenchyma than primary roots (Visser and Voesenek 2005; Qi et al. 2020). Furthermore, osmotic adjustment is a useful adaptation for hypoxia, maintaining water balance and turgor pressure (Olorunwa et al. 2022). For example, waterlogging-resistant genotypes accumulate higher contents of soluble sugars than susceptible genotypes under waterlogging stress (Sairam et al. 2009; Zhang et al. 2017, 2019; Olorunwa et al. 2022). This indicates that the accumulation of soluble sugars in the roots is an important component of the response to waterlogging stress.

Changes in activities of the sucrose catabolizing enzymes

Under waterlogging conditions, energy reserves decline due to decreased photosynthesis and O₂ availability (Barickman et al. 2019; Pan et al. 2021a). Thus, an increase in glucose should be required for the production of ATP to be used by energy-consuming pathways (Arora et al. 2017). Sucrose's

utilization requires either irreversible hydrolysis by invertase (EC 3.2.1.26, β -fructofuranosidase) or reversible cleavage by Susy (EC 2.4.1.13, Hyun et al. 2009). As shown in Fig. 4, the activities of V-inv and N-inv in the leaves of waterlogged plants were higher than in control plants, but C-inv and Susy activities were not changed by waterlogging stress. On the contrary, V-inv and N-inv activities were lower in the roots of waterlogged plants than in control plants, whereas Susy activity was significantly increased by the waterlogging treatment. Sucrose degradation by Susy is more energy efficient than sucrose degradation by invertase, saving up to two ATP molecules in the conversion of sucrose to hexose phosphates (Fedosejevs et al. 2014; Stein and Granot 2019). In the roots of balloon flower, ATP content had significantly decreased after 13 days of waterlogging stress (Fig. S1). Taken together, these results indicate that increasing Susy activity and decreasing invertase activity in the roots of balloon flower should be required for a higher cellular energy state to be established under waterlogging conditions.

Identification of the sucrose catabolizing enzymes in balloon flower

To identify the invertase and Susy genes, we analyzed the balloon flower genome data (Kim et al. 2020) using invertase and Susy sequences previously identified in other plants. After redundant sequences were removed, a total of 12 putative PlgInv—three in the PlgV-inv family, two in the PlgC-inv family, and seven in the PlgN-inv family—and four PlgSusy genes were found (Table 1). All sucrose catabolizing enzymes in balloon flower had a calculated MW ranging from 53.35 to 95.45 kDa (Table 1). Two PlgC-invs were predicted to contain the hydrophobic N-terminal signal peptide involved in the

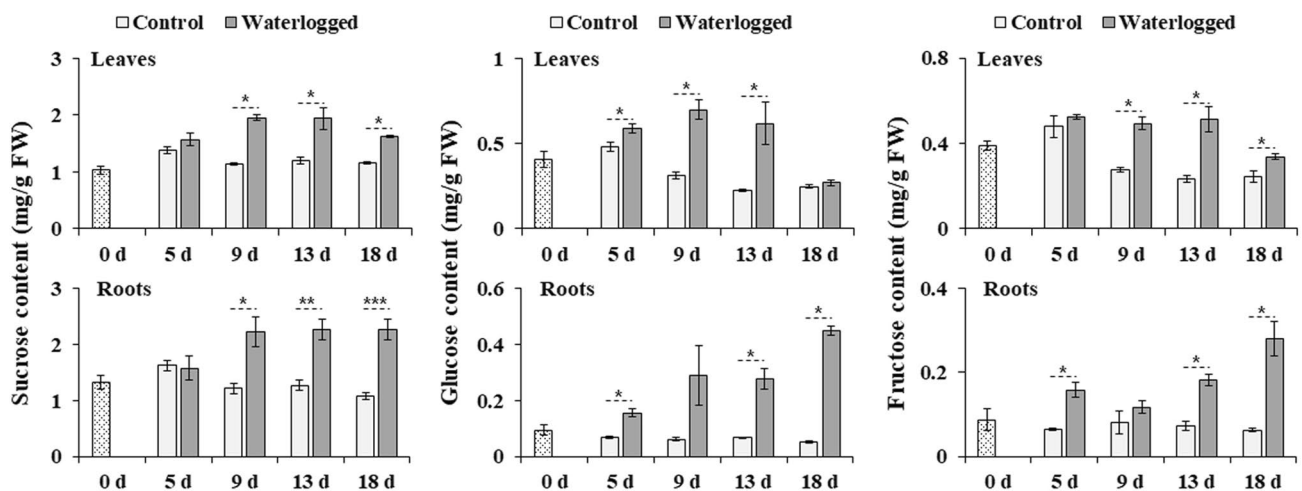


Fig. 3 Soluble sugar concentrations in leaves and roots of balloon flower. Asterisks indicate significant differences (Student's t-test; * $p < 0.05$, ** $p < 0.01$, and *** $p < 0.001$) between waterlogging stress -treated and non-treated plants. The data are presented as means \pm SE ($n = 10$)

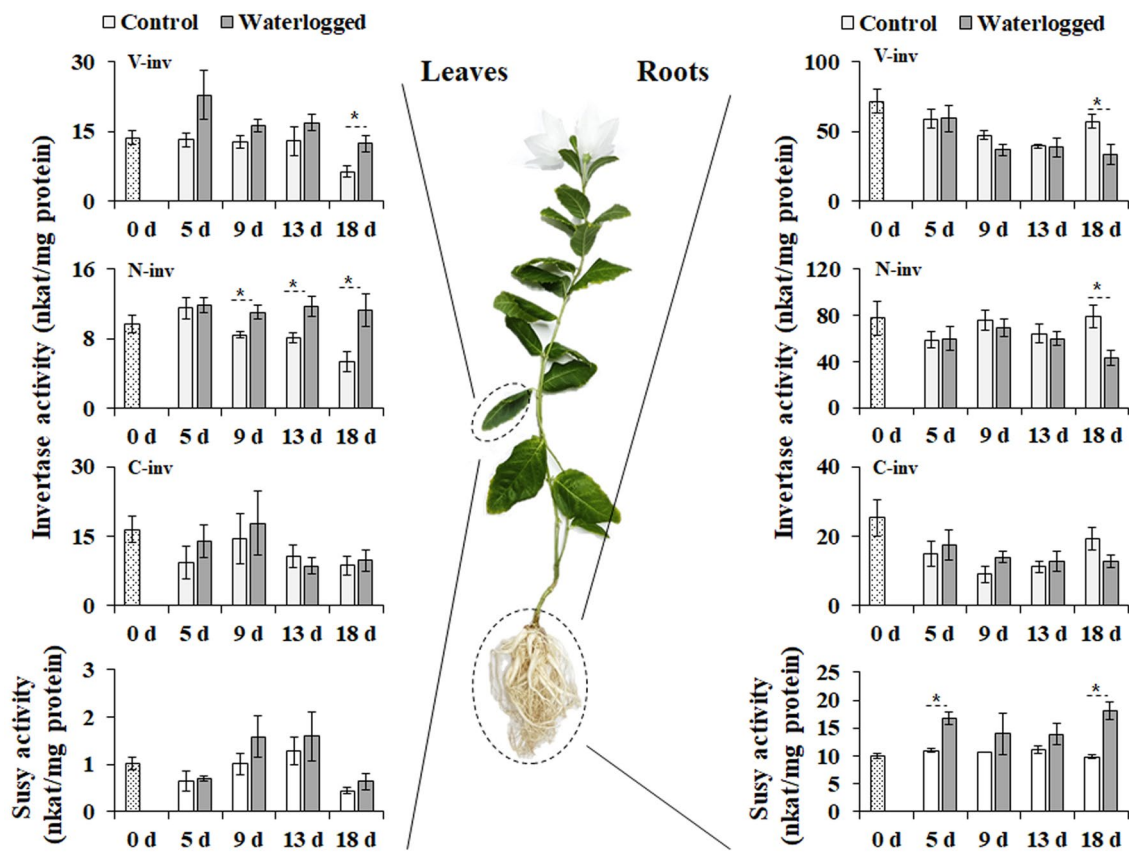


Fig. 4 Effect of waterlogging stress on the activities of sucrose catabolizing enzymes in balloon flower: vacuolar invertase (V-inv), neutral/alkaline invertase (N-inv), cell wall invertase (C-inv), and sucrose

synthase (Susy). Asterisks indicate significant differences (Student's *t*-test; * $p < 0.05$) between non-treated and treated plants

Table 1 Gene catalog of sucrose catabolizing enzymes in balloon flower

Name	Accession NO	CDS (bp)	Protein length	kDa	Subcellular Localization	Signal peptide
PlgC-inv1	PGJG292820	1761	586	66.58	Extracellular	0.7172
PlgC-inv2	PGJG300930	1725	540	61.42	Extracellular	0.9988
PlgV-inv1	PGJG013270	1950	649	72.42	Vacuole	0.0023
PlgV-inv2	PGJG348670	1932	643	71.75	Vacuole	0.0002
PlgV-inv3	PGJG348650	2556	851	95.45	Vacuole	0.159
PlgN-inv1	PGJG401200	1704	567	64.90	Cytoplasm	0.0004
PlgN-inv2	PGJG107430	1536	511	58.35	Cytoplasm	0.0008
PlgN-inv3	PGJG292460	2043	680	77.10	Cytoplasm	0.0005
PlgN-inv4	PGJG256660	1971	656	74.89	Cytoplasm	0.0031
PlgN-inv5	PGJG202660	1971	656	74.94	Cytoplasm	0.0035
PlgN-inv6	PGJG302980	1869	622	69.82	Cytoplasm	0.0015
PlgN-inv7	PGJG056720	2013	670	76.11	Cytoplasm	0.0436
PlgSusy1	PGJG315350	2427	808	92.84	Cytoplasm	0.0006
PlgSusy2	PGJG116750	2507	834	95.27	Cytoplasm	0.0255
PlgSusy3	PGJG020240	2412	803	91.02	Cytoplasm	0.0052
PlgSusy4	PGJG113500	2583	860	96.61	Cytoplasm	0.0004

secretory pathway to the cell wall (Table 1): a feature common to most C-invs in other plants (Ji et al. 2005;

Hyun et al. 2011). In addition, all PlgV-invs and PlgN-invs were predicted to have vacuole and cytoplasm

localization, respectively. Although Susy of azuki bean (*Vigna angularis*) are membrane-bound in a complex with cellulose synthase (Fuji et al. 2010), most plant Susys, including PlgSusys, are considered to be cytosolic proteins (Table 1; Stein and Granot 2019; Liao et al. 2022). As shown in Fig. S2, most PlgC- and PlgV-invs contained Glyco_hydro 32N (PF00251) and Glyco_hydro 32C (PF08244) domains, which corresponds to the N- and C-terminal domains of glycosyl hydrolase family 32 (Alberto et al. 2004). In addition, the multiple sequence alignment of PlgN-invs showed that they have a highly conserved glycoside hydrolase 100 (Glyco_hydro 100) domain, which is a trait common in N-invs of other plants (Dahro et al. 2016, 2021; Juárez-Colunga et al. 2018). Furthermore, PlgSusys are thought to have conserved domains for sucrose-synth (PF00862) and glycos-transf-1 (PF00534). Taken together, the presence of conserved domains described within each enzyme family (PlgV-invs, PlgC-invs, PlgN-invs, and PlgSusys) suggests that sucrose catabolizing enzymes in balloon flower likely have the same function as their homologous proteins.

To gain insight into the potential functions of sucrose catabolizing enzymes in balloon flower during responses to waterlogging stress, the expression pattern of each gene was analyzed by qRT-PCR. As shown in Fig. 5, Fig. S3 and Fig. S4, *PlgV-inv2* and *PlgN-inv3* were up-regulated in leaves by waterlogging stress, whereas *PlgC-inv1* and *PlgC-inv2* were down-regulated. In the roots of the waterlogged balloon flower, *PlgV-inv* transcripts decreased, indicating that decreasing activity of V-inv (Fig. 4) is mediated by the downregulation of *PlgV-invs*. In addition, the transcript level of *PlgSusy1* increased, while those of *PlgSusy3* and *PlgSusy4* decreased in the waterlogged roots. These observations indicate that increased activity of Susy by waterlogging stress could be related primarily to *PlgSusy1* expression in roots. Various genes encoding PlgN-invs were up-regulated in the roots of balloon flower by waterlogging stress (Fig. 5). However, N-inv activity was lower in the roots of waterlogged plants than that in control plants (Fig. 4). Post-transcriptional regulation by RNA-binding proteins, micro-RNAs, and the spliceosome and post-translational regulation by invertase inhibitors play an essential role in controlling sucrose metabolism (McKenzie et al. 2013; Wang et al. 2020; Coculo and Lionetti 2022). Non-correlation between transcriptional levels of *PlgN-invs* and N-inv activity indicates the importance of these regulations in the sucrose metabolism of balloon flower.

Transient gene expression using agro-infiltration offers a simple and efficient technique for the rapid production of recombinant proteins and the characterization of gene functions (Bashandy et al. 2015; Norkunas et al. 2018). To characterize the function of *PlgSusy1*, *Agrobacterium* cells carrying the *PlgSusy1* construct were infiltrated into the leaves

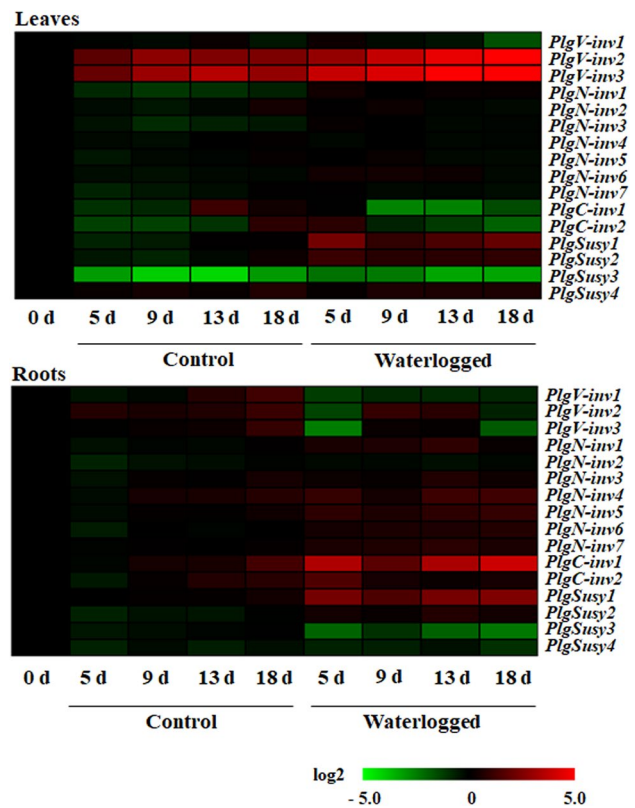


Fig. 5 The expression pattern of sucrose catabolizing enzymes in response to waterlogging stress in balloon flower. Transcript levels of each gene were normalized to actin. Relative expression level of each gene (Fig. S3 and S4) has been represented as heatmap of log₂ fold change

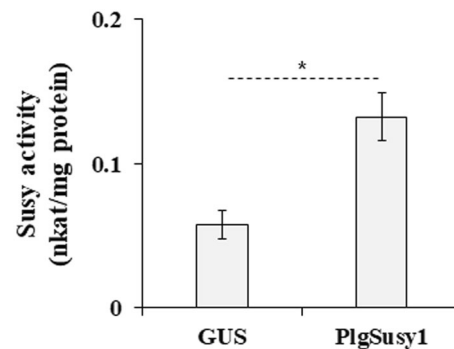


Fig. 6 Sucrose synthase activity of *PlgSusy1*. *PlgSusy1* or GUS (mock control) recombinant proteins transiently expressed in *N. benthamiana* leaves through agro-infiltration. Asterisks indicate significant differences (Student's t-test; **p* < 0.05)

of tobacco plants. As shown in Fig. 6, transient expression of *PlgSusy1* resulted in an increase in Susy activity, indicating that *PlgSusy1* encodes the functional Susy enzyme.

Conclusion

The present study provides an overview of the changes in sucrose metabolism in the leaves and roots of balloon flower under waterlogging conditions. Based on the analysis of sucrose catabolizing enzyme activities, we found that waterlogging stress leads to a shift in the pathway of sucrose degradation from invertase to SuSy in roots. Further, waterlogging-induced PlgSusy1 expression should play an important role in sucrose degradation to improve metabolic performance in hypoxia conditions. Taken together, our results provide a solid starting point for further understanding the physiological and molecular mechanisms underlying responses to waterlogging stress in root crops.

Author contributions HSJ and TKH conceived and designed the experiments. HSJ performed the experiments. HSJ and TKH wrote the manuscript.

Declarations

Conflict of interest The authors declare no conflict of interest.

References

- Akrofi S, Akuoko KO, Bour KB, Nyarko JA (2018) Farmers' knowledge and perception of the dry cassava root rot disease in Brong Ahafo region of Ghana. *Ghana J Agric Sci* 52:33–42
- Alberto F, Bignon C, Sulzenbacher G, Henrissat B, Czjzek M (2004) The three-dimensional structure of invertase (β -Fructosidase) from *Thermotoga maritima* reveals a bimodular arrangement and an evolutionary relationship between retaining and inverting glycosidases. *J Biol Chem* 279(18):18903–18910. <https://doi.org/10.1074/jbc.M313911200>
- Arora K, Panda KK, Mittal S, Mallikarjuna MG, Rao AR, Dash PK, Thirunavukkarasu N (2017) RNAseq revealed the important gene pathways controlling adaptive mechanisms under waterlogged stress in maize. *Sci Rep* 7(1):1–12. <https://doi.org/10.1038/s41598-017-10561-1>
- Banti V, Giuntoli B, Gonzali S, Loreti E, Magneschi L, Novi G, Paparelli E, Parlanti S, Pucciariello C, Santaniello A, Perata P (2013) Low oxygen response mechanisms in green organisms. *Int J Mol Sci* 14(3):4734–4761
- Barickman TC, Simpson CR, Sams CE (2019) Waterlogging causes early modification in the physiological performance, carotenoids, chlorophylls, proline, and soluble sugars of cucumber plants. *Plants* 8(6):160. <https://doi.org/10.3390/plants8060160>
- Bashandy H, Jalkanen S, Teeri TH (2015) Within leaf variation is the largest source of variation in agroinfiltration of *Nicotiana benthamiana*. *Plant Methods* 11(1):1–7. <https://doi.org/10.1186/s13007-015-0091-5>
- Bonfig KB, Schreiber U, Gabler A, Roitsch T, Berger S (2006) Infection with virulent and avirulent *P. syringae* strains differentially affects photosynthesis and sink metabolism in Arabidopsis leaves. *Planta* 225(1):1–12. <https://doi.org/10.1007/s00425-006-0303-3>
- Cao X, Wu L, Wu M, Zhu C, Jin Q, Zhang J (2020) Abscisic acid mediated proline biosynthesis and antioxidant ability in roots of two different rice genotypes under hypoxic stress. *BMC Plant Biol* 20(1):1–14. <https://doi.org/10.1186/s12870-020-02414-3>
- Cao M, Zheng L, Li J, Mao Y, Zhang R, Niu X, Geng M, Zhang X, Huang W, Luo K, Chen Y (2022) Transcriptomic profiling suggests candidate molecular responses to waterlogging in cassava. *PLoS ONE* 17(1):0261086. <https://doi.org/10.1371/journal.pone.0261086>
- Cocolo D, Lionetti V (2022) The plant invertase/Pectin Methylesterase inhibitor superfamily. *Front Plant Sci* 13:863892–863892. <https://doi.org/10.3389/fpls.2022.863892>
- Colmer TD, Voisenek LACJ (2009) Flooding tolerance: suites of plant traits in variable environments. *Funct Plant Biol* 36(8):665–581. <https://doi.org/10.1071/FP09144>
- Czyczylo-Mysza I, Tyrka M, Marcinińska I, Skrzypek E, Karbarz M, Dziurka M, Hura T, Dziurka K, Quarrie S (2013) Quantitative trait loci for leaf chlorophyll fluorescence parameters, chlorophyll and carotenoid contents in relation to biomass and yield in bread wheat and their chromosome deletion bin assignments. *Mol Breed* 32(1):189–210. <https://doi.org/10.1007/s11032-013-9862-8>
- Dahro B, Wang F, Peng T, Liu JH (2016) PtrA/NINV, an Alkaline/neutral invertase gene of *Poncirus trifoliata*, confers enhanced tolerance to multiple abiotic stresses by modulating ROS levels and maintaining photosynthetic efficiency. *BMC Plant Biol* 16(1):1–18. <https://doi.org/10.1186/s12870-016-0761-0>
- Dahro B, Wang Y, Alhag A, Li C, Guo D, Liu JH (2021) Genome-wide identification and expression profiling of invertase gene family for abiotic stresses tolerance in *Poncirus trifoliata*. *BMC Plant Biol* 21(1):1–18. <https://doi.org/10.1186/s12870-021-03337-3>
- De Castro J, Hill RD, Stasolla C, Badea A (2022) Waterlogging Stress Physiology in Barley *Agronomy* 12(4):780. <https://doi.org/10.3390/agronomy12040780>
- Eom SH, Ahn MA, Kim E, Lee HJ, Lee JH, Wi SH, Kim SK, Lim HB, Hyun TK (2022) Plant response to cold stress: cold stress changes antioxidant metabolism in heading type kimchi cabbage (*Brassica rapa* L. ssp *Pekinensis*). *Antioxidants* 11(4):700. <https://doi.org/10.3390/antiox11040700>
- Fedosejevs ET, Ying S, Park J, Anderson EM, Mullen RT, She YM, Plaxton WC (2014) Biochemical and molecular characterization of RcSUS1, a cytosolic sucrose synthase phosphorylated in vivo at serine 11 in developing castor oil seeds. *J Biol Chem* 289(48):33412–33424. <https://doi.org/10.1074/jbc.M114.585554>
- Fujii S, Hayashi T, Mizuno K (2010) Sucrose synthase is an integral component of the cellulose synthesis machinery. *Plant Cell Physiol* 51(2):294–301. <https://doi.org/10.1093/pcp/pcp190>
- Hayat S, Hayat G, Alyemeni MN, Wani AS, Pichtel J, Ahmad A (2012) Role of proline under changing environments: a review. *Plant Signal Behav* 7(11):1456–1466. <https://doi.org/10.4161/psb.21949>
- Hyun TK, Hoffmann A, Sinha AK, Roitsch T (2009) Tomato mitogen activated protein kinases regulate the expression of extracellular invertase Lin6 in response to stress related stimuli. *Funct Plant Biol* 36(12):1088–1097. <https://doi.org/10.1071/FP09136>
- Hyun TK, Eom SH, Kim JS (2011) Genomic analysis and gene structure of the two invertase families in the domesticated apple (*Malus × domestica* Borkh). *Plant Omics* 4(7):391–399
- Jammer A, Gasperl A, Luschn-Ebengreuth N, Heyneke E, Chu H, Cantero-Navarro E, Großkinsky D, Albacete A, Stabentheiner E, Franzaring J, Fangmeier A, van der Graaff E, Roitsch T (2015) Simple and robust determination of the activity signature of key carbohydrate metabolism enzymes for physiological phenotyping in model and crop plants. *J Exp Bot* 66(18):5531–5542. <https://doi.org/10.1093/jxb/erv228>
- Jethva J, Schmidt RR, Sauter M, Selinski J (2022) Try or die: dynamics of plant respiration and how to survive low oxygen conditions. *Plants* 11(2):205. <https://doi.org/10.3390/plants11020205>
- Ji X, Van Den Ende W, Van Laere A, Cheng S, Bennett J (2005) Structure, evolution, and expression of the two invertase gene

- families of rice. *J Mol Evol* 60(5):615–634. <https://doi.org/10.1007/s00239-004-0242-1>
- Ji HS, Bang SG, Ahn MA, Kim G, Kim E, Eom SH, Hyun TK (2021) Molecular cloning and functional characterization of heat stress-responsive superoxide dismutases in garlic (*Allium sativum* L.). *Antioxidants* 10(5):815. <https://doi.org/10.3390/antiox10050815>
- Juárez-Colunga S, López-González C, Morales-Eliás NC, Massange-Sánchez JA, Trachsel S, Tiessen A (2018) Genome-wide analysis of the invertase gene family from maize. *Plant Mol Biol* 97(4):385–406. <https://doi.org/10.1007/s11103-018-0746-5>
- Kerdee S, Kongsil P, Nakasathien S (2021) Waterlogging tolerance and recovery in canopy development stage of cassava (*Manihot esculenta crantz*). *Agrivita* 43(20):233–244. <https://doi.org/10.17503/agrivita.v43i2.2615>
- Kim JH, Lee SW, Min JY, Bae YS, Shin MU, Kim SB, Kim MK, Yeon CR, Lim JY, Kim HT (2007) Development of control system with fungicides against diseases of ginseng plant. *Res Plant Dis* 13(3):164–169. <https://doi.org/10.5423/rpd.2007.13.3.164>
- Kim J, Kang SH, Park SG, Lee Y, Kim OT, Chung O, Lee J, Choi JP, Kwon SJ, Lee K, Ahn BO, Lee DJ, Si Y, Shin IG, Um Y, Lee DY, Kim GS, Hong CP, Bhak J, Kim CK (2020) Whole-genome, transcriptome, and methylome analyses provide insights into the evolution of platycoside biosynthesis in *Platycodon grandiflorus*, a medicinal plant. *Hortic Res* 7:1–12. <https://doi.org/10.1038/s41438-020-0329-x>
- Kim TY, Jeon S, Jang Y, Gotina L, Won J, Ju YH, Kim S, Jang MW, Won W, Park MG, Pae AN, Han S, Kim S, Lee CJ (2021) Platycodin D, a natural component of *Platycodon grandiflorum*, prevents both lysosome- and TMPRSS2-driven SARS-CoV-2 infection by hindering membrane fusion. *Exp Mol Med* 53(5):956–972. <https://doi.org/10.1038/s12276-021-00624-9>
- Kircher S, Schopfer P (2012) Photosynthetic sucrose acts as cotyledon-derived long-distance signal to control root growth during early seedling development in *Arabidopsis*. *Proc Natl Acad Sci USA* 109(28):11217–11221. <https://doi.org/10.1073/pnas.1203746109>
- Kutschera U, Briggs WR (2019) Photomorphogenesis of the root system in developing sunflower seedlings: a role for sucrose. *Plant Biol* 21(4):627–633. <https://doi.org/10.1111/plb.12981>
- Lee YH, Park SR, Ryu JS, Lim ST, Ko BG, Yun HD (1999) Survey on cultural environment and soil morphological characteristics of *Platycodon grandiflorus*. *Korean J Soil Sci Fert* 32(3):215–222
- Lee S-W, Son Y-W, Shin Y-W, Cho Y-S (2014) The current status in Korea and future perspectives of balloon flower (*Platycodon grandiflorum*) in Gyeongsangnam-Do. *J Korean Soc Int Agric* 26:68–72. <https://doi.org/10.12719/KSIA.2014.26.1.68>
- Liao G, Li Y, Wang H, Liu Q, Zhong M, Jia D, Chunhui H, Xu X (2022) Genome-wide identification and expression profiling analysis of sucrose synthase (SUS) and sucrose phosphate synthase (SPS) genes family in *Actinidia chinensis* and *A. eriantha*. *BMC Plant Biol* 22(1):1–15. <https://doi.org/10.1186/s12870-022-03603-y>
- Lin SY, Chen PA, Zhuang BW (2022) The stomatal conductance and Fv/Fm as the indicators of stress tolerance of avocado seedlings under short-term waterlogging. *Agronomy* 12(5):1084. <https://doi.org/10.3390/agronomy12051084>
- Lothier J, Diab H, Cukier C, Limami AM, Tcherkez G (2020) Metabolic responses to waterlogging differ between roots and shoots and reflect phloem transport alteration in *medicago truncatula*. *Plants* 9:1–20. <https://doi.org/10.3390/plants9101373>
- McKenzie MJ, Chen RKY, Harris JC, Ashworth MJ, Brummell DA (2013) Post-translational regulation of acid invertase activity by vacuolar invertase inhibitor affects resistance to cold-induced sweetening of potato tubers. *Plant Cell Environ* 36(1):176–185. <https://doi.org/10.1111/j.1365-3040.2012.02565.x>
- Norkunas K, Harding R, Dale J, Dugdale B (2018) Improving agroinfiltration-based transient gene expression in *Nicotiana benthamiana*. *Plant Methods* 14(1):1–14. <https://doi.org/10.1186/s13007-018-0343-2>
- Olgun M, Metin Kumlay A, Cemal Adiguzel M, Caglar A (2008) The effect of waterlogging in wheat (*T. aestivum* L.). *Acta Agric Scand Sect B Soil Plant Sci* 58(3):193–198. <https://doi.org/10.1080/09064710701794024>
- Olorunwa OJ, Adhikari B, Brazel S, Popescu SC, Popescu GV, Shi A, Barickman TC (2022) Waterlogging during the reproductive growth stage causes physiological and biochemical modifications in the leaves of cowpea (*Vigna unguiculata* L.) genotypes with contrasting tolerance. *Plant Physiol Biochem* 190:133–144. <https://doi.org/10.1016/j.plaphy.2022.08.018>
- Pan J, Sharif R, Xu X, Chen X (2021a) Mechanisms of waterlogging tolerance in plants: research progress and prospects. *Front Plant Sci* 11:627331. <https://doi.org/10.3389/fpls.2020.627331>
- Pan R, Han H, Medison MB, Abou-Elwafa SF, Liu Y, Yang X, Zhang W (2021b) Aerenchyma formation in the root of leaf-vegetable sweet potato: programmed cell death initiated by ethylene-mediated H₂O₂ accumulation. *Physiol Plant* 173(4):2361–2375. <https://doi.org/10.1111/ppl.13587>
- Parent C, Capelli N, Berger A, Crèvecoeur M, Dat JF (2008) An overview of plant responses to soil waterlogging. *Plant Stress* 2(1):20–27
- Phukan UJ, Mishra S, Shukla RK (2016) Waterlogging and submergence stress: affects and acclimation. *Crit Rev Biotechnol* 36(5):956–966. <https://doi.org/10.3109/07388551.2015.1064856>
- Qi X, Li Q, Shen J, Qian C, Xu X, Xu Q, Chen X (2020) Sugar enhances waterlogging-induced adventitious root formation in cucumber by promoting auxin transport and signalling. *Plant Cell Environ* 43(6):1545–1557. <https://doi.org/10.1111/pce.13738>
- Sairam RK, Dharmar K, Chinnusamy V, Meena RC (2009) Waterlogging-induced increase in sugar mobilization, fermentation, and related gene expression in the roots of mung bean (*Vigna radiata*). *J Plant Physiol* 166(6):602–616. <https://doi.org/10.1016/j.jplph.2008.09.005>
- Stein O, Granot D (2019) An overview of sucrose synthases in plants. *Front Plant Sci* 10:95. <https://doi.org/10.3389/fpls.2019.00095>
- Suh SJ, Moon JW, Jang IB, Kim YC, Kim DH, Yu J (2021) Waterlogging duration and depth affect growth and photosynthetic properties of ginseng. *Korean J Med Crop Sci* 29(6):409–417. <https://doi.org/10.7783/KJMCS.2021.29.6.409>
- Szabados L, Savaouré A (2010) Proline: a multifunctional amino acid. *Trends Plant Sci* 15(2):89–97. <https://doi.org/10.1016/j.tplants.2009.11.009>
- Teoh EY, Teo CH, Baharum NA, Pua TL, Tan BC (2022) Waterlogging stress induces antioxidant defense responses, aerenchyma formation and alters metabolisms of banana plants. *Plants* 11(15):2052. <https://doi.org/10.3390/plants11152052>
- Tian LX, Zhang YC, Chen PL, Zhang FF, Li J, Yan F, Dong Y, Feng BL (2021) How does the waterlogging regime affect crop yield? A global meta-analysis. *Front Plant Sci* 12:634898. <https://doi.org/10.3389/fpls.2021.634898>
- Visser EJW, Voisenek LACJ (2005) Acclimation to soil flooding-sensing and signal-transduction. *Plant Soil* 274(1):197–214. <https://doi.org/10.1007/s11104-004-1650-0>
- Wang M, Zang L, Jiao F, Perez-Garcia MD, Oge L, Hamama L, Gouriérec JL, Sakr S, Chen J (2020) Sugar signaling and post-transcriptional regulation in plants: an overlooked or an emerging topic? *Front Plant Sci* 11:578096. <https://doi.org/10.3389/fpls.2020.578096>
- Wu X, Tang Y, Li C, Wu C, Huang G (2015) Chlorophyll fluorescence and yield responses of winter wheat to waterlogging at different growth stages. *Plant Prod Sci* 18(3):284–294. <https://doi.org/10.1626/pp.s.18.284>
- Xiao Y, Wu X, Sun M, Peng F (2020) Hydrogen sulfide alleviates waterlogging-induced damage in peach seedlings via enhancing

- antioxidative system and inhibiting ethylene synthesis. *Front Plant Sci* 11:696. <https://doi.org/10.3389/fpls.2020.00696>
- Yu Q, Shen Y, Wang Q, Wang X, Fan L, Wang Y, Zhang S, Liu Z, Zhang M (2019) Light deficiency and waterlogging affect chlorophyll metabolism and photosynthesis in *Magnolia sinostellata*. *Trees Struct Funct* 33(1):11–22. <https://doi.org/10.1007/s00468-018-1753-5>
- Zeng R, Cao J, Li X, Wang X, Wang Y, Yao S, Gao Y, Hu J, Luo M, Zhang L, Chen T (2022) Waterlogging tolerance and recovery capability screening in peanut: A comparative analysis of waterlogging effects on physiological traits and yield. *PeerJ* 10:e12741. <https://doi.org/10.7717/peerj.12741>
- Zhang L, Wang Y, Yang D, Zhang C, Zhang N, Li M, Liu Y (2015) *Platycodon grandifloras*—an ethnopharmacological, phytochemical and pharmacological review. *J Ethnopharmacol* 164:147–161. <https://doi.org/10.1016/j.jep.2015.01.052>
- Zhang P, Lyu D, Jia L, He J, Qin S (2017) Physiological and de novo transcriptome analysis of the fermentation mechanism of *Cerasus sachalinensis* roots in response to short-term waterlogging. *BMC Genomics* 18(1):1–14. <https://doi.org/10.1186/s12864-017-4055-1>
- Zhang Y, Ou L, Zhao J, Liu Z, Li X (2019) Transcriptome analysis of hot pepper plants identifies waterlogging resistance related genes. *Chil J Agric Res* 79(2):296–306. <https://doi.org/10.4067/S0718-58392019000200296>
- Zhang Y, Liu G, Dong H, Li C (2021) Waterlogging stress in cotton: damage, adaptability, alleviation strategies, and mechanisms. *Crop J* 9(2):257–270. <https://doi.org/10.1016/j.cj.2020.08.005>

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