

# Review: Proteomic Techniques for the Development of Flood-Tolerant Soybean

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**Table 1.** Morphological and physiological effect of flooding stress on soybean.

Traits	Growth stage/ Flooding time	Observations	Reference
plant weight	2-day-old/3, 6, 12, 24 h	Fresh weight of germinating seed increased during the first 3 h of flooding and flood slowed down soybean growth.	Yin et al., 2014a
	3-day-old/5 days	Fresh weight of plant decreased almost 50% in flooded soybean compared with untreated soybean.	Khatoon et al., 2012a
	56-day-old/14 days	Plant height was 13 to 15% shorter under flooding than control.	VanToai et al., 2001
root, hypocotyl, cotyledon, leaf	2-day-old/1, 2, 3, 4 days; 5-day flood followed by 3-day recovery	Flooding inhibited root development, caused shorter length/less pigmentation of hypocotyl, and led detachment of cotyledon.	Shi et al., 2008; Hashiguchi et al., 2009; Nanjo et al., 2014
	3-day-old/3, 6 days	Flooding reduced length of shoot/root and number of secondary roots.	Khatoon et al., 2012b
	7-day-old/1, 2, 3 days	Flooding delayed root elongation, hypocotyl elongation, and development of first leaf.	Salavati et al., 2012
	8-day-old/7 days	Dry weight of root linearly decreased with length and surface area of root under flooding.	Sakazono et al., 2014
	28-day-old/7, 14, 21 days	Flooding decreased leaf area, while enhanced development of adventitious root.	Bacanamwo and Purcell, 1999
flowering time	V3-growth-stage/21 days	Flooding did not change days to flowering, whereas it increased days to maturity.	Githiri et al., 2006
yield	V3-growth-stage/21 days	Flooding reduced number of branch/pod/seed and seed weight; however, it increased 100-seed weight.	Githiri et al., 2006
	V5-growth-stage/3, 5, 7, 9 days	Number of pod/seed decreased when flooding was prolonged and seed yield reduced with stress duration of 3 days.	Jin-Woong and Yamakawa, 2006
	R1-growth-stage/14 days	Flooding was severe on grain yield with more than 60% reduced relative to control yield.	VanToai et al., 2001
aerenchyma formation	V1-growth-stage/28 days	Secondary aerenchyma formation was morphological acclimation to flooding and it supplied oxygen to root nodules.	Shimamura et al., 2002
	V12-growth-stage/10 days	Nitrogen metabolism in nodulated-soybean root partially recovered during prolonged flooding and it was associated with aerenchyma formation.	Thomas et al., 2005
cellular processes			
cell-wall reorganization	2-day-old/1, 2, 3, 4 days	Under flooding, plasma membrane contributed to cell-wall construction, and proteins related to cell-wall lignification and synthesis were impeded.	Nanjo et al., 2013; Komatsu et al., 2009; 2010; Yin et al., 2016
calcium signaling	2-day-old/3, 6, 12, 24, 48 h	Under flooding, calcium ion was elevated in root tip/cotyledon, and calcium signaling played roles in protein folding and energy metabolism.	Komatsu et al., 2013a; Yin et al., 2014b; Wang and Komatsu, 2016; 2017

<i>cell death</i>	2-day-old/1, 2, 3, 4, 6 days	Flooding caused injurious effect of cell death in root.	Nanjo et al., 2013; Komatsu et al., 2013b; Oh et al., 2014; Mustafa et al., 2015; Hashimoto et al., 2020
<i>protein metabolism</i>	2-day-old/3, 24, 48 h	Flooding inhibited protein synthesis, glycosylation, and folding <i>via</i> ribosomes, calnexin, and PDI-like proteins; however, it activated ubiquitin-mediated proteolysis.	Yanagawa and Komatsu, 2012; Yin et al., 2016; Wang and Komatsu, 2016; Komatsu et al., 2012; Mustafa and Komatsu, 2014
<i>RNA metabolism</i>	2-day-old/3, 6, 24 h	Flooding suppressed mRNA processing, and RNA-regulatory related proteins of glycine-rich RNA binding protein 3 and eukaryotic aspartyl protease triggered soybean tolerance to initial stress.	Yin et al., 2016; Yin and Komatsu, 2016
<i>energy metabolism</i>	2-day-old/2 days	Activated fermentation <i>via</i> alcohol dehydrogenase and biotinylation-mediated energy management responded to flooding.	Komatsu et al., 2013b; Wang et al., 2016a
<i>carbohydrate metabolism</i>	2-day-old/3, 24 h	Flooding caused imbalance of carbohydrate metabolism, while fructose conferred soybean tolerance towards initial flooding.	Nanjo et al., 2010; Wang et al., 2017
<i>metabolite accumulation</i>	2-day-old/2, 4 days	Flooding accelerated accumulation of citrate, 2-oxoglutarate, aconitate, succinate, fumarate, alanine, GABA, pyruvate, NAD, and NADH; however, it decreased ATP.	Komatsu et al., 2011; Nakamura et al., 2012; Wang et al., 2018
<i>hormone signaling</i>	2-day-old/3, 6, 12, 24, 48, 72 h	Ethylene biosynthesis and ABA signaling mediated protein phosphorylation in initial flooding.	Yin et al., 2014a; Komatsu et al., 2013c; Yin and Komatsu, 2015; Wang et al., 2016b

ABA, abscisic acid; GABA, gamma-aminobutyric acid; PDI, protein disulfide isomerase.

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**Table 2.** Plant-omic analysis to understand flood-response mechanisms on soybean.

Experimental materials	Growth stage/ Flooding time	Findings	Reference
<i>Proteomics</i>			
total cellular			
root tip, root, hypocotyl	2-day-old/3, 6, 12, 24 h	Initial flooding caused imbalanced accumulation of proteins involved in carbohydrate metabolism, while calcium-signal transduction protected soybean from stress.	Yin et al., 2014a; Nanjo et al., 2010
	2-day-old/2, 5 days	Under flooding, phosphatase 2A subunit-like proteins played roles on cell death in root tip, while isoflavone reductase and disease-related proteins were responsible for suppressed growth of soybean seedling.	Nanjo et al., 2013; Khatoon et al., 2012a
	14-day-old/3, 7 days	Soybean coped with waterlogging <i>via</i> regulation of carbohydrate consumption and programmed cell death.	Alam et al., 2010
leaf	6-day-old/2, 4, 6, 8 days	Waterlogging impaired metabolisms of photosynthesis/photorespiration and hydroxypyruvate reductase limited rate of photorespiration in soybean leaf.	Oskuei et al., 2017
root	2-day-old/3-day flood followed by 7-day recovery; 2-, 4-day flood followed by 4-, 6-, 8-day	Peroxidases and proteins related to lignification/cytoskeletal organization played roles in post-flooding recovery.	Salavati et al., 2012; Khan et al., 2014
	3-day-old/3, 6 days	Decreased proteins involved in destination and defense were associated with suppression of soybean growth under flooding.	Khatoon et al., 2012b
hypocotyl	2-day-old/2-day-flood followed by 2- and 4-day recovery	Activated glycosylation and secondary metabolism enabled soybean recovery from flooding-induced damage.	Khan et al., 2015
subcellular proteins			
cell wall/root, hypocotyl	2-day-old/2 days	Decreased lipoxygenases, germin-like protein precursors, glycoprotein precursors, and super oxide dismutase, leading to suppressed lignification <i>via</i> inhibited biosynthesis of ROS and JA under flooding.	Komatsu et al., 2010
nuclei/root tip, root	2-day-old/3 h	Initial flooding suppressed protein translation, and decreased zinc finger, glycine-rich protein, and rRNA-processing protein were affected by ABA-mediated phosphorylation.	Yin and Komatsu, 2015; 2016
	2-day-old/2 days	Under flooding, increased RACK1 and poly-ADP-ribosylation responded to stress; however, decreased zinc finger proteins, cell division cycle 5, and transduction played roles in tolerance.	Komatsu et al., 2013; 2014; Oh et al., 2014
mitochondria/root, hypocotyl	2-day-old/2 days	Flooding impaired electron-transport chains, and proteins involved in TCA cycle, glutathione, and aldehyde metabolism played roles in oxidation and peroxide scavenging.	Komatsu et al., 2011; Kamal and Komatsu, 2015

ER/root tip	2-day-old/2 days	Flooding suppressed protein synthesis in the ER and caused dysfunction of protein folding, leading to reduction of glycoproteins.	Wang and Komatsu, 2016; Komatsu et al., 2012
post-translational modifications			
phosphorylation/root tip, root, hypocotyl	2-day-old/3, 12, 24 h	Flooding altered phosphorylation status of proteins related to ethylene signaling, ABA response, protein folding, cell construction, and ATP generation.	Yin et al., 2014b; Yin and Komatsu, 2015; Nanjo et al., 2010; 2012
glycosylation/root tip, root	2-day-old/2 days	Flooding reduced glycoproteins, and glycosylation status of proteins related to stress, glycolysis, and protein degradation were predominantly affected.	Wang and Komatsu, 2016; Mustafa and Komatsu, 2014
nitrosylation/root	2-day-old/3, 9, 24 h	S-nitrosylation status of proteins involved in sugar-degrading contributed to anaerobic shift under flooding.	Hashiguchi and Komatsu, 2018
ubiquitination/root	2-day-old/2 days	Accumulation of CSN proteins enhanced degradation of ubiquitinated proteins under flooding.	Yanagawa and Komatsu, 2012
<i>transcriptomics/root, hypocotyl, cotyledon, leaf</i>	2-day-old/6, 12 h/root including hypocotyl	Functional disorder of acclimative response to flooding <i>via</i> transcriptional and posttranscriptional regulations was involved in flooding injury to soybean.	Nanjo et al., 2011
	4-day-old/10 days	Constitutive basal levels of transcripts related to sugar metabolism/fermentation were sufficient to breakdown carbohydrate reserves in hypocotyl and cotyledon of soybean under submergence.	Tamang et al., 2014
	V4-growth-stage/7 days	Alterations of genes involved in cell-wall precursor and starch/sugar metabolism served as adaptive response for soybean survival from flooding.	Chen et al., 2016
<i>metabolomics/root, hypocotyl</i>	2-day-old/2, 4 days	Flooding accelerated accumulation of citrate, succinate, aconitate, GABA, pyruvate, NAD, NADH, and alanine.	Komatsu et al., 2011; Nakamura et al., 2012
<i>bioinformatics/seedling</i>	2-day-old/2-7 days; flood for 2 or 4 days followed by 6-day recovery	Soybean Proteome Database was originally constructed based on gel-based proteomics, and it updated with integrated gel-free proteomics, genomics, transcriptomics as well as metabolomics.	Sakata et al., 2009; Ohyanagi et al., 2012; Komatsu et al., 2017

ABA, abscisic acid; CSN, COP9 signalosome; ER, endoplasmic reticulum; GABA, gamma-aminobutyric acid; JA, jasmonic acid; PTM, post translational modification; RACK1, receptor for activated protein kinase C1; ROS, reactive oxygen species; TCA, tricarboxylic acid. Data have been collected from 2010 to 2020.

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