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Review

Proteomic approaches to uncover the flooding and drought stress response mechanisms in soybean

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ABSTRACT

Soybean is the important crop with abundant protein, vegetable oil, and several phytochemicals. With such predominant values, soybean is cultivated with a long history. However, flooding and drought stresses exert deleterious effects on soybean growth. The present review summarizes the morphological changes and affected events in soybean exposed to such extreme-water conditions. Sensitive organ in stressed soybean at different-developmental stages is presented based on protein profiles. Protein quality control and calcium homeostasis in the endoplasmic reticulum are discussed in soybean under both stresses. In addition, the way of calcium homeostasis in mediating protein folding and energy metabolism is addressed. Finally, stress response to flooding and drought is systematically demonstrated. This review concludes the recent findings of plant response to flooding and drought stresses in soybean employed proteomic approaches.

Biological significance: Soybean is considered as traditional-health food because of nutritional elements and pharmacological values. Flooding and drought exert deleterious effects to soybean growth. Proteomic approaches have been employed to elucidate stress response in soybean exposed to flooding and drought stresses. In this review, stress response is presented on organ-specific manner in the early-stage plant and soybean seedling exposed to combined stresses. The endoplasmic reticulum (ER) stress is induced by both stresses; and stress-response in the ER is addressed in the root tip of early-stage soybean. Moreover, calcium-response processes in stressed plant are described in the ER and in the cytosol. Additionally, stress-dependent response was discussed in flooded and drought-stressed plant. This review depicts stress response in the sensitive organ of stressed soybean and forms the basis to develop molecular markers related to plant defense under flooding and drought stresses.

1. Introduction

Soybean is an important food crop containing abundant protein and vegetable oil [1]. Soybean is unique among crops, because it supplies protein equal in quality to that of animal sources [2]. Soybean is advantageous for biodiesel producing, which is converted from vegetable oil, because it is produced without or nearly zero nitrogen [3]. In addition, soybean is rich in phytochemicals such as isoflavones and phenolic compounds [4], which contributed to reducing the risk of heart/cardiovascular diseases, osteoporosis, and cancer [5]. Furthermore, it is possible for soybean to step into symbiosis with rhizobia to provide nitrogen for plant growth and development [6]. These findings document several aspects of soybean, including nutritional elements, biodiesel production, pharmacological values, and symbiosis potential.

Soybean production is affected by abiotic constraints, including weather-related phenomena, soil-nutrient availability, salinity, and photoperiod [2]. Annual global losses in crop production due to flooding are comparable to those caused by drought [7]. Flooding is composed of several underlying changes such as oxygen, carbon dioxide, reactive oxygen species (ROS), and phytotoxins inside plants and from environment [8]. Due to restricted gas exchange, deficit of energy/carbohydrate and accumulation of volatile ethylene occurred by flooding [9]. Drought poses as another constraint for plant growth and terrestrial ecosystem productivity [10]. Drought induced meristematic cells, reduced cell division [11], and limited cell elongation/expansion growth [12]. These findings indicate that flooding and drought are complex abiotic stressors affecting plant growth.

A series of findings were obtained in soybean with different exposure time to flooding and drought stresses using proteomic techniques (Fig. 1). With flooding duration, a plethora of biological processes underwent, including signal transduction, hormone regulation, transcriptional control, glucose degradation, sucrose accumulation,

Abbreviations: ER, endoplasmic reticulum; HSP, heat shock protein; PDI, protein disulfide isomerase; ROS, reactive oxygen species; SAM, *S*-adenosylmethionine

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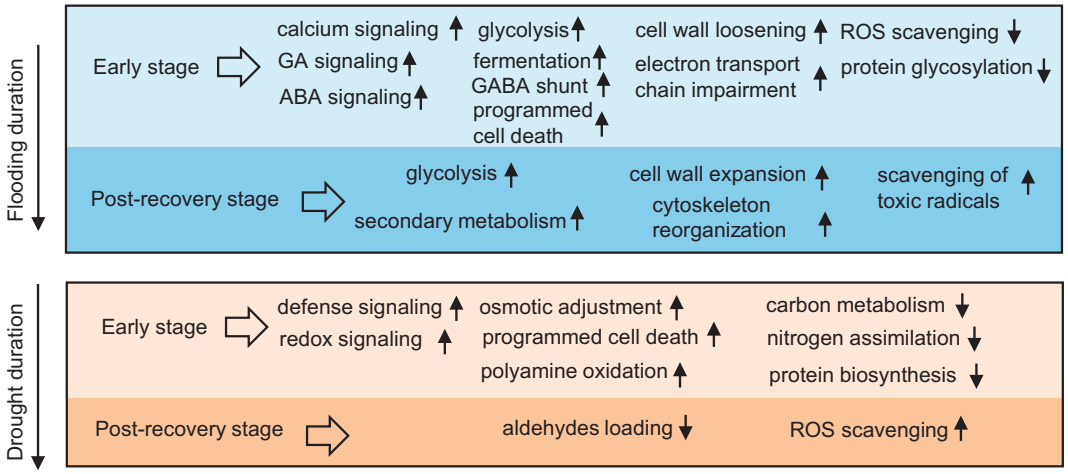

Fig. 1. Overview of cellular processes induced by flooding and drought in soybean. Cellular events in soybean exposed to flooding and drought were explored using proteomic approaches. The up- and down-arrows indicate the activated and suppressed metabolisms, respectively, induced by stress condition. Abbreviations are as follows: ABA, abscisic acid; GA, gibberellic acid; and GABA, gamma-aminobutyric acid.

alcoholic fermentation, mitochondrial impairment, proteasome-mediated proteolysis, and cell wall loosening [13]. Signal transduction of calcium [14] and hormone regulation of abscisic acid [15] as well as gibberellic acid [16] were activated by flooding. Moreover, fermentation [17] as well as gamma-aminobutyric acid shunt [18] were induced in flooded soybean. As reported, lignification [19] and electron transport chains [18] were altered; however, ROS scavenging [19] and protein glycosylation [20] were suppressed. Regarding post-flooding recovery, scavenging of toxic radicals [21], ATP generation/secondary metabolism [22], and cell wall metabolism/cytoskeleton reorganization [23] were responsible for recovery. These findings indicate that different strategies might be utilized in soybean under flooding conditions and during post-flooding recovery stage.

Enormous progress has been made in drought-stressed soybean employed proteomic approaches (Fig. 1). Osmotic adjustment, defense signaling, and programmed cell death were involved in drought adaptation [24]. In drought-stressed soybean, increased *S*-adenosylmethionine (SAM) synthetases played roles in redox signaling and polyamine oxidation [25]; however, decreased methionine synthase impaired seedling growth [26]. Moreover, carbon metabolism, nitrogen assimilation, and protein biosynthesis responded to drought in soybean nodules [27]. Additionally, peroxidase and aldehyde dehydrogenase took part in post-drought recovery by scavenging toxic ROS and reducing load of harmful aldehydes [28]. These studies indicate a variety of biological processes are in response to drought and such findings form the molecular basis of stress response in soybean. In respect to both stresses, signaling transduction plays roles in stressed plant and ROS scavenging participates in post-stress recovery.

Stress response displays on organ-specific manner, which provides detail information of cellular processes for plant growth and development [29]. Organelle protein profiles not only provide fundamental information of stress response, but also refine the knowledge of signaling pathways [30,31]. In the current review, findings from organ-specific and stage-dependent proteomic studies of flooded and drought-stressed soybean were presented. Subcellular proteomics of endoplasmic reticulum (ER) was summarized. In addition, the ER function and affected-cellular processes were bridged with calcium homeostasis in soybean. Stress response in soybean was addressed in respect to flooding or drought.

2. Morphological, biochemical, and physiological changes of soybean under flooding and drought stresses

Flooding and drought are adverse environmental conditions; and they inhibit soybean growth at both the early stage [25,32] and the seedling stage [26,33]. In early-stage soybean, dry weight of plant was reduced after 1-day exposure to combined stresses [32]; however, length of root including hypocotyl was decreased or increased in flooded or drought-stressed plant [25]. Regarding soybean seedling, flooding and drought significantly suppressed weight of plant and length of root including hypocotyl [26,33]. These results describe the stress effects on soybean growth and differ the changes of plant morphology such as length of root including hypocotyl in response to flooding and drought.

Furthermore, biochemical and physiological processes are illuminated in flooded and drought-stressed soybean beyond the morphological changes. A tight control over carbon metabolism to meet the required energy demand for alleviating stress impacts was exhibited in soybean, irrespective of the kind and severity of extreme-water conditions [34]. Reduction in net photosynthesis was induced by both stresses; however, starch granules and abscisic acid/stomatal conductance were responsible for flooding and drought, respectively [35]. Additionally, stress-response events were investigated in soybean employing proteomic analyses, in which flooding and drought stresses were concurrently conducted. Protein synthesis was suppressed in soybean exposed to combined stresses [20,25]; however, redox signaling and polyamine oxidation were differentially controlled *via* SAM synthetases [25]. Calcium homeostasis was the mediator for ER stress and it modified carbon metabolism through the regulation of pyruvate decarboxylase in soybean under combined stresses [32]. These results represent the affected events in stressed plant and shed light on the stress specificity underlying the difference in soybean morphology caused by flooding and drought stresses.

A plethora of processes were deciphered in flooded and drought-stressed soybean employing proteomic techniques (Fig. 1). Moreover, other approaches such as reversal genetic, biochemical, and metabolic analyses are employed to determine the highlighted events in response to flooding and drought. For example, activated fermentation was critical for flooding tolerance and alcohol dehydrogenase was key fermentative enzyme [36]. The effects of alcohol dehydrogenase were further validated by overexpression [37] and by biochemical analyses including *in situ* hybridization [38], Western blot [38], as well as enzyme assay [39]. Scavenging of ROS differed in soybean exposed to combined stresses; and it was determined by the changes of ascorbate peroxidase *via* Western blot, enzyme activity, and biophoton emission [40]. In addition, gamma-aminobutyric acid shunt occurred in stressed soybean [18] and increased accumulation of gamma-aminobutyric acid were examined through metabolomic approaches [41,42]. These results

indicate that proteomic techniques coupled with other approaches are important tools to contribute for the elucidation of affected processes in soybean under flooding and drought stresses.

3. Organ-specific response in soybean under flooding and drought stresses

Gene expression [43,44], metabolite accumulation [45,46], hormone signaling [47,48], and protein profile [26,33] shown organ specificity in plants. Gene expression of *salT* was rapidly upregulated in sheath and root; however, no induction was observed in leaf lamina of rice under osmotic stress [43]. In tomato, *pLE4* and *pLE25* represented abscisic acid-induced genes; however, gene expression was differentially regulated during seed development [44]. Raffinose, glucose, and proline accumulated in root, whereas fructose and sucrose were specifically increased in leaf of pepper during water deficit [45]. Ethylene acted at green/red stages in achene or at green/white stages in receptacle during the development of strawberry [48]. Organ-specific analysis of salt-stressed soybean indicated that protein abundance of fructokinase 2 decreased in root and hypocotyl; however, glyceraldehyde-3-phosphate dehydrogenase declined in hypocotyl and leaf [49]. These findings demonstrate organ specificity of plant during development or under stress stimuli.

3.1. Organ-specific response in the early-stage soybean under both stresses

Plants adapt to stress by regulating protein abundance on the organ-specific manner [29]. Growth rate of soybean was inhibited under flooding and drought; however, there was much difference in root morphology under these abiotic stresses [40]. In the early-stage soybean, root length was markedly suppressed by flooding, whereas root diameter was reduced by drought [25]. Besides morphological differences, the findings employing proteomic techniques in the early-stage soybean exposed to combined stresses were presented as organ-specific events (Fig. 2).

In root tip, fermentation or protein metabolism was activated under flooding or drought, and biotin-related metabolism responded to combined stresses [50]. Increased activity of alcohol dehydrogenase was reported in flooding-tolerant sorghum [51] and overexpression of *alcohol dehydrogenase 2* eliminated growth retardation of flooded soybean [37]. Under drought, abundance of proteins related to protein metabolism was increased in tolerant rootstock for citrus [52]. Class II aminoacyl tRNA/biotin synthetases superfamily protein and biotin/lipoyl attachment domain containing protein were reported as biotin-related proteins in response to combined stresses [50]. Aminoacyl-tRNA synthetases ensured translation of genetic code [53] and mediated protein synthesis [54]. Biotin/lipoyl attachment domain containing protein holds multidomain, in which biotin or lipoic acid was attached for protein biotinylation or lipoylation [55]. Histone biotinylation was mediator of glucose [56] and biotin shortage induced cell death/defense signaling under abiotic stress [57]. Collectively, these findings suggest that fermentation or protein metabolism might be critical for stress adaptation under flooding or drought. In addition, biotin-related proteins might play roles in gene regulation and stress signaling under both stresses.

In root, TOPLESS RELATED, NADH dehydrogenase subunit 7, and villin 4 showed converted protein abundance under combined stresses [50]. TOPLESS RELATED was general corepressor for plant development [58] and it associated with hormone response [59]. Formation of ROS was limited by NADH dehydrogenase [60]; however, impairment of electron transport chain occurred in flooded soybean [18]. Villins served as major actin filament-binding proteins to regulate actin dynamics [61] and villin 4 maintained cytoskeleton formation/cytoplasmic streaming to promote soybean growth under drought [50]. Overall, hormone response, ROS formation, and actin dynamics might be differently induced in the early-stage soybean under combined stresses.

3.2. Organ-specific response in soybean seedling under both stresses

Stress response is highly dependent on stress intensity, stress duration, and organ specificity. Signal transduction, membrane system, transport regulation, ROS scavenging, stress defense, and metabolic rearrangement were activated in root and leaf of soybean seedling exposed to short-term salt stress [62]. ATP production was reduced due to decreased glyceraldehyde-3-phosphate dehydrogenase in root, hypocotyl, and leaf of soybean under 1-week salt exposure [49]. In addition, systematic comparison among plant organs exposed to flooding or drought was explored using both gel-based [26,33] and gel-free [39] proteomic techniques. Under flooding, isoflavone reductase was commonly declined among root, hypocotyl, and leaf; and it responsible for decreased antioxidant efficiency [33]. Under drought, decreased methionine synthase was presented in root, hypocotyl, and leaf; and it impaired soybean growth through regulation of cell wall lignification [26]. Furthermore, under combined stresses, the tricarboxylic acid cycle was suppressed in root and leaf in soybean seedling; and beta-amylase 5 played roles in starch degradation to provide carbohydrate intermediates in leaf [39]. These findings suggest that antioxidant scavenging and energy provision might be induced in flooded and drought-stressed soybean seedling. The findings employing proteomic techniques in soybean seedling exposed to combined stresses were presented on the organ-specific manner (Fig. 2).

In root, proteins related to metabolism were mainly increased in flooded and drought-stressed soybean [26,33]. Pyruvate dehydrogenase was decreased in the tricarboxylic acid cycle in soybean under both stresses [39]. Citrate synthase and malate dehydrogenase were markedly declined by flooding, whereas succinyl-CoA synthetase was increased by drought [39]. *Pyruvate dehydrogenase* was downregulated in flooded soybean [63] and drought-stressed rice [64]. Citrate synthase converts acetyl-CoA to citrate [65]; and enhanced enzyme activity [66]/overproduction of citrate [67] conferred aluminum tolerance. *Malate dehydrogenase* was downregulated in root of flooded cucumber [68] and soybean [63]; however, its protein abundance was increased in *Arabidopsis* under osmotic stresses [69]. Certain threshold activity of succinyl-CoA synthetase activated gamma-aminobutyric acid shunt [70], which was also revealed in drought-stressed soybean [71]. These findings indicate that the tricarboxylic acid cycle is markedly affected by both stresses. In addition, compared to drought, flooding might pose serious effects on root exposed to short-term stress.

In hypocotyl, protein abundance of 14-3-3 was dramatically increased in soybean seedling exposed to combined stresses [39]. 14-3-3 proteins are conserved regulator and bind to a multitude of functionally diverse signaling proteins [72]. Under salt stress, inhibition of SOS2 interacting with 14-3-3 proteins conferred basal repression of the SOS pathway, indicating a quick re-association with SOS2 and other interacting partners in plant facing to changeable environment [73]. 14-3-3 protein was increased in flooded soybean [18] and it interacted with RACK1 [74]. Overexpression of *14-3-3* enhanced drought tolerance in transgenic cotton [75]. Additionally, in the tricarboxylic acid cycle, succinate dehydrogenase was decreased under combined stresses [39]. Succinate dehydrogenase played central role in respiratory metabolism [76]; and the upregulated-gene expression and increased-protein abundance were associated with plant tolerance to osmotic conditions [77,78]. These findings suggest that 14-3-3 proteins serve as signal integrators to induce biological processes to struggle against flooding and drought. In addition, succinate dehydrogenase might be limited enzyme controlling the tricarboxylic acid cycle in hypocotyl under both stresses.

In leaf, metabolism-related proteins were increased in soybean under flooding and drought [26,33]; however, energy-related proteins decreased in drought-stressed plant [26]. Additionally, the tricarboxylic acid cycle in soybean was suppressed by combined stresses [39].

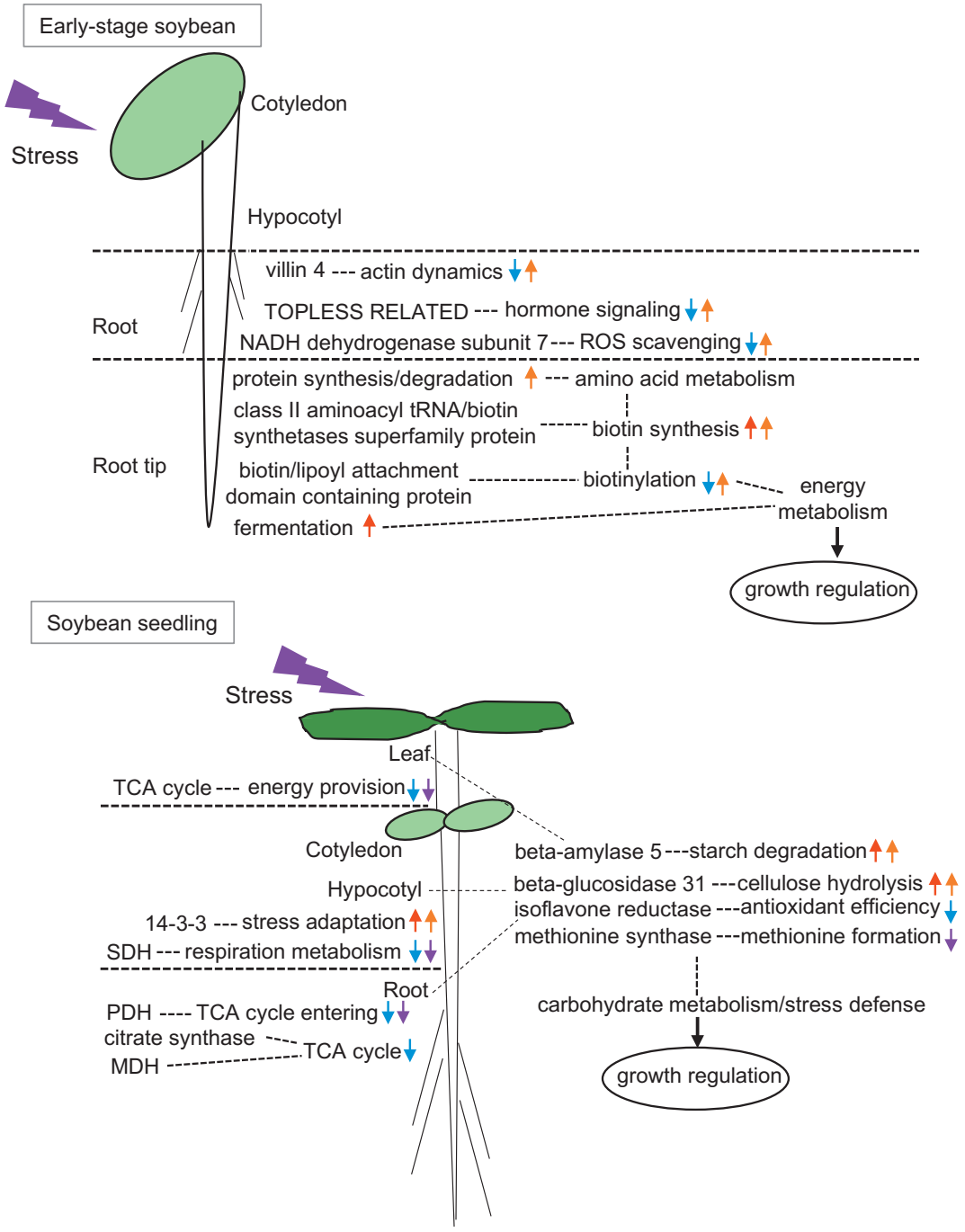

Fig. 2. Organ-specific representation of flooding and drought response mechanisms in soybean at early stage and seedling stage. The red and blue arrows indicate changes of protein abundance under flooding; orange and purple arrows indicate changes of protein abundance under drought; and up- and down-arrows indicate the increased and decreased changes of protein abundance, respectively, compared to unstressed soybean. Abbreviations are as follows: TCA, tricarboxylic acid; SDH, succinate dehydrogenase; PDH, pyruvate dehydrogenase; and MDH, malate dehydrogenase.

Mitochondria were assumed to be inoperative under flooding, because requirement for oxygen as the terminal electron acceptor was absolute [79]. In flooded soybean, electron transport chain was impaired [18] and oxidation/peroxide scavenging led to biophoton emission/oxidative damage [80]. A truncated tricarboxylic acid cycle was revealed under anoxic conditions; and the tricarboxylic acid cycle and oxidative phosphorylation were essential for energy producing rather than carbon skeletons under drought stress [81]. These findings suggest that energy producing might be hampered *via* the suppressed tricarboxylic acid cycle in stressed plant.

Fewer proteins were commonly affected in root, hypocotyl, and leaf in flooded and drought-stressed soybean such as isoflavone reductase, methionine synthase, beta-glucosidase 31, and beta-amylase 5 [26,33,39]. Decreased protein abundance of isoflavone reductase or methionine synthase impaired growth of flooded-soybean seedling [33] or drought-stressed plant [26]. beta-Glycosidase 31 was accumulated with stress duration and it associated with stress adaptation in soybean under combined stresses [39]. Moreover, beta-amylase 5 participated in starch degradation to mediate carbohydrate mobilization for energy provision [39]. Overall, these findings suggest that carbohydrate metabolism is activated in soybean-seedling stage under flooding and drought stresses.

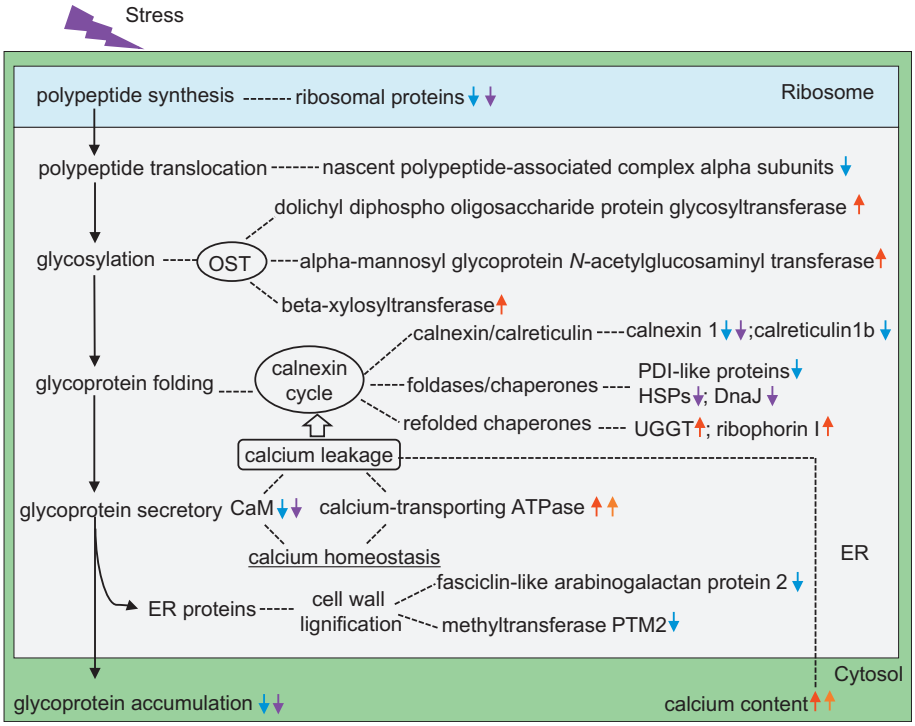

Fig. 3. Schematic representation of flooding and drought response mechanisms in the endoplasmic reticulum of soybean. The red and blue arrows indicate changes of protein abundance under flooding; orange and purple arrows indicate changes of protein abundance under drought; and up- and down-arrows indicate increased and decreased changes of protein abundance, respectively, compared to unstressed soybean. Abbreviations are as follows: OST, oligosaccharyltransferase; UGGT, UDP glucose:glycoprotein glucosyltransferase; and CaM, calmodulin-binding protein.

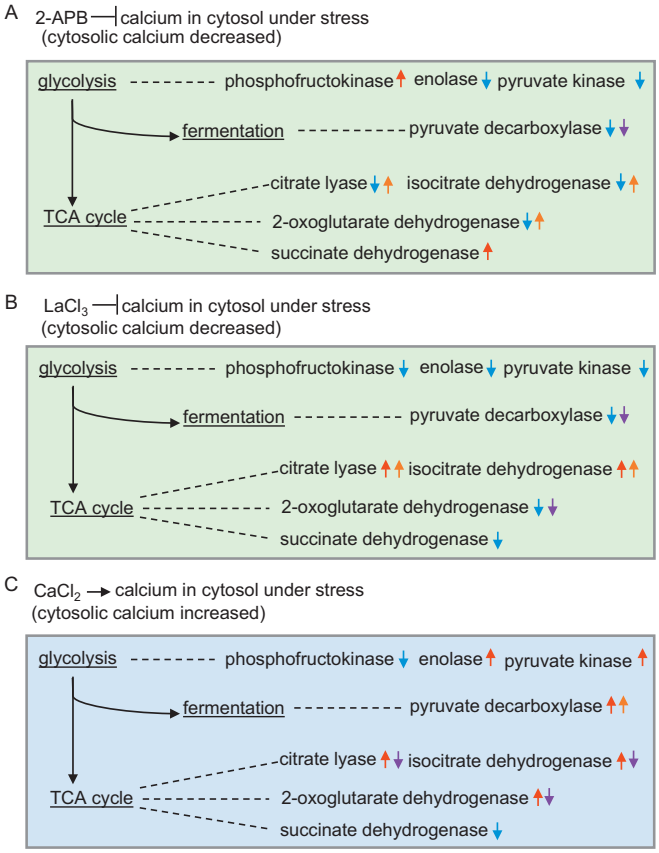

Fig. 4. Schematic representation of calcium response mechanisms in soybean under flooding and drought. 2-APB, $LaCl_3$, and $CaCl_2$ were used to change cytosolic calcium in soybean under flooding and drought. The red and blue arrows indicate changes of protein abundance under flooding; orange and purple arrows indicate changes of protein abundance under drought; and up- and down-arrows indicate increased and decreased changes of protein abundance, respectively, compared to stressed plant without chemical application. Abbreviations are as follows: TCA, tricarboxylic acid; and 2-APB, 2-aminoethoxydiphenyl borate.

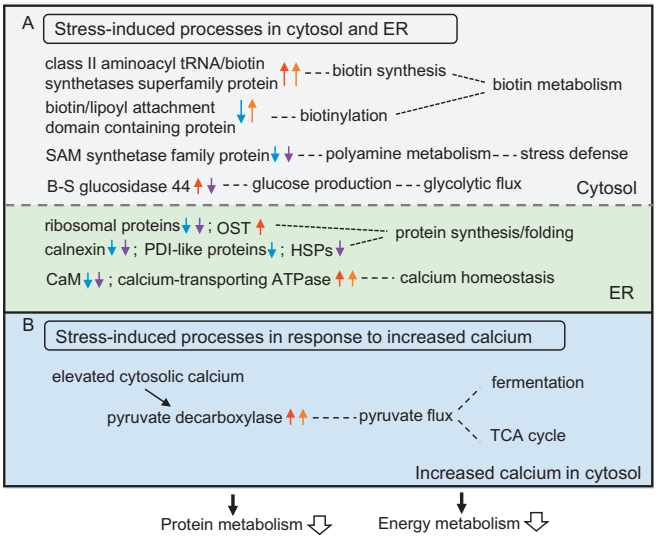

Fig. 5. Schematic representation of stress-induced processes and events in response to calcium in soybean under flooding and drought. The red and blue arrows indicate changes of protein abundance under flooding; orange and purple arrows indicate changes of protein abundance under drought; and up- and down-arrows indicate increased and decreased changes of protein abundance, respectively. The abundance of protein induced by stresses in cytosol and the ER was compared to unstressed plant (A). The abundance of protein in response to additional calcium was compared to stressed plant without reagent application (B). Abbreviations are as follows: OST, oligosaccharyltransferase; CaM, calmodulin-binding protein; and TCA, tricarboxylic acid.

4. Endoplasmic reticulum response in soybean under flooding and drought stresses

Cellular processes for environment sensing and stress response are highly organized [82]. Subcellular proteomics elucidates the functions of spatially organized proteins in specific organelle [83]. The ER consists of nuclear envelope and flattened peripheral sheets, which contain ribosomes and interconnected tubules extending throughout the cytoplasm [84]. The ER functions as a protein factory and calcium reservoir

Table 1
List of flooding-response proteins in soybean.

No.	Ratio	Protein ID	Location	Description
1	↓[a]	Glyma01g05580.1	Gm01: 5,372,500–5,376,619 (strand: −)	*S* adenosyl *L* methionine dependent methyltransferases superfamily protein
2	↓	Glyma01g35220.2	Gm01: 47,744,169–47,746,902 (strand: −)	Plant VAP homolog 12
3	↑	Glyma02g00550.1	Gm02: 328,488–333,545 (strand: −)	*S* adenosyl *L* methionine dependent methyltransferases superfamily protein
4	↑	Glyma02g01750.2	Gm02: 1,271,084–1,276,344 (strand: +)	Thioredoxin family protein
5	↓	Glyma02g04980.1	Gm02: 4,030,951–4,034,105 (strand: +)	RNA binding family protein
6	↓	Glyma02g10790.1	Gm02: 8,679,102–8,686,798 (strand: +)	Protein phosphatase 2A subunit A2
7	↑	Glyma02g11890.1	Gm02: 10,096,333–10,101,410 (strand: −)	Methyltransferase
8	↓	Glyma02g13330.1	Gm02: 11,601,786–11,605,281 (strand: +)	Reversibly glycosylated polypeptide 3
9	↑	Glyma02g43110.1	Gm02: 47,984,584–47,988,358 (strand: −)	*S* adenosyl *L* methionine dependent methyltransferases superfamily protein
10	↓	Glyma02g43460.1	Gm02: 48,222,057–48,226,061 (strand: −)	PDI like 2,2
11	↓	Glyma02g45030.1	Gm02: 49,443,006–49,447,931 (strand: +)	Putative mitochondrial RNA helicase 2
12	↓	Glyma03g00920.1	Gm03: 626,722–631,477 (strand: −)	NADH: cytochrome *b5* reductase 1
13	↓	Glyma03g03800.1	Gm03: 3,633,770–3,638,147 (strand: −)	Plant VAP homolog 12
14	↑	Glyma03g26090.1	Gm03: 33,434,531–33,438,525 (strand: +)	RAS 5
15	↑	Glyma03g33240.1	Gm03: 40,885,587–40,891,963 (strand: +)	Ca^{2+}-transporting ATPase
16	↓	Glyma03g33710.1	Gm03: 41,215,500–41,220,643 (strand: +)	DnaJ homolog subfamily
17	↓	Glyma03g37650.1	Gm03: 44,190,430–44,194,864 (strand: +)	DnaJ heat shock family protein
18	↑	Glyma03g39130.1	Gm03: 45,329,036–45,332,552 (strand: +)	Thioredoxin family protein
19	↓	Glyma03g41210.1	Gm03: 46,735,997–46,737,827 (strand: −)	Rotamase cyclophilin 2
20	↑	Glyma03g42070.3	Gm03: 47,374,627–47,378,345 (strand: +)	Cytochrome *b5* isoform E
21	↓	Glyma03g42150.1	Gm03: 47,428,796–47,435,494 (strand: −)	RNA binding family protein
22	↑	Glyma04g00200.1	Gm04: 7268–22,892 (strand: −)	α-1,3 mannosyl glycoprotein β 1,2 *N*-acetylglucosaminyl transferase putative
23	↑	Glyma04g01690.1	Gm04: 1,128,740–1,133,407 (strand: −)	Ribophorin I
24	↑	Glyma04g04810.1	Gm04: 3,567,380–3,577,561 (strand: +)	Ca^{2+}-transporting ATPase
25	↑	Glyma04g33740.1	Gm04: 39,489,463–39,495,115 (strand: +)	*S* adenosyl *L* methionine dependent methyltransferases superfamily protein
26	↓	Glyma04g38000.1	Gm04: 44,418,156–44,421,941 (strand: +)	Calnexin 1
27	↑	Glyma04g38190.1	Gm04: 44,589,289–44,604,291 (strand: -)	Phosphate deficiency response 2
28	↓	Glyma04g38590.1	Gm04: 44,957,860–44,971,142 (strand: +)	β-Galactosidase 10
29	↓	Glyma04g40090.1	Gm04: 46,218,142–46,227,070 (strand: +)	Nucleic acid binding OB fold like protein
30	↓	Glyma04g40750.2	Gm04: 46,699,533–46,705,586 (strand: +)	CTC interacting domain 11
31	↓	Glyma04g40760.1	Gm04: 46,724,021–46,729,598 (strand: +)	CTC interacting domain 11
32	↑	Glyma04g41010.1	Gm04: 46,901,418–46,904,354 (strand: −)	Cytochrome *b5* isoform E
33	↓	Glyma04g42690.1	Gm04: 48,376,626–48,381,064 (strand: −)	PDI like 1,2
34	↑	Glyma05g01010.1	Gm05: 609,594–611,534 (strand: +)	Malate dehydrogenase
35	↓	Glyma05g03320.1	Gm05: 2,547,518–2,552,812 (strand: −)	Purple acid phosphatase 27
36	↓	Glyma05g05460.1	Gm05: 4,767,337–4,771,477 (strand: −)	Glutamate dehydrogenase 2
37	↓	Glyma05g27980.1	Gm05: 33,852,909–33,854,869 (strand: +)	Rubber elongation factor protein (REF)
38	↓	Glyma05g28500.1	Gm05: 34,296,431–34,303,454 (strand: −)	Subtilisin like serine endopeptidase family protein
39	↑	Glyma05g29050.1	Gm05: 34,730,528–34,735,631 (strand: +)	Mitochondrial substrate carrier
40	↓	Glyma05g30330.1	Gm05: 35,722,786–35,726,536 (strand: +)	emp24/gp25L/p24 family/GOLD family protein
41	↓	Glyma05g33330.1	Gm05: 38,028,921–38,032,577 (strand: −)	Calnexin 1
42	↓	Glyma05g33700.1	Gm05: 38,278,126–38,280,829 (strand: +)	Receptor like kinase 1
43	↓	Glyma05g34900.1	Gm05: 39,094,615–39,098,883 (strand: +)	Arginosuccinate synthase family
44	↓	Glyma05g36600.1	Gm05: 40,426,908–40,430,703 (strand: −)	HSP 70 protein 5
45	↑	Glyma05g36620.1	Gm05: 40,443,124–40,447,225 (strand: −)	HSP 70 family protein
46	↓	Glyma05g38120.1	Gm05: 41,530,564–41,533,554 (strand: +)	UDP D glucose/UDP D galactose 4 epimerase 1
47	↑	Glyma06g00230.1	Gm06: 24,815–43,462 (strand: +)	α-1,3 mannosyl glycoprotein β 1,2 *N*-acetylglucosaminyl transferase putative
48	↑	Glyma06g01790.1	Gm06: 1,129,858–1,134,208 (strand: −)	Ribophorin I
49	↑	Glyma06g04900.1	Gm06: 3,460,618–3,467,695 (strand: +)	Ca^{2+}-transporting ATPase
50	↓	Glyma06g05770.1	Gm06: 4,127,859–4,132,398 (strand: +)	Nitrilase/cyanide hydratase
51	↑	Glyma06g12090.1	Gm06: 9,308,451–9,312,386 (strand: +)	PDI like 1,2
52	↓	Glyma06g14030.1	Gm06: 11,079,494–11,084,221 (strand: −)	CTC interacting domain 11
53	↓	Glyma06g14760.1	Gm06: 11,565,183–11,572,316 (strand: −)	Nucleic acid binding OB fold like protein
54	↑	Glyma06g16860.1	Gm06: 13,253,251–13,268,511 (strand: +)	Phosphate deficiency response 2
55	↓	Glyma06g17060.1	Gm06: 13,418,027–13,421,764 (strand: −)	Calnexin 1
56	↓	Glyma07g02470.1	Gm07: 1,682,404–1,690,170 (strand: +)	Protein phosphatase 2C family protein
57	↓	Glyma07g03220.1	Gm07: 2,263,708–2,268,409 (strand: −)	Inorganic H^+ pyrophosphatase family protein
58	↓	Glyma07g03930.1	Gm07: 2,782,652–2,789,177 (strand: +)	Dihydrolipoamide acetyltransferase long form protein
59	↑	Glyma07g05830.1	Gm07: 4,519,394–4,525,700 (strand: −)	Cytochrome *b5* isoform E
60	↓	Glyma07g11310.1	Gm07: 9,498,808–9,503,366 (strand: +)	B-S glucosidase 44
61	↓	Glyma07g35090.1	Gm07: 40,210,582–40,221,957 (strand: +)	Calmodulin binding protein
62	↑	Glyma07g35490.1	Gm07: 40,668,330–40,671,317 (strand: −)	emp24/gp25L/p24 family/GOLD family protein
63	↓	Glyma08g01480.1	Gm08: 939,512–942,346 (strand: −)	UDP D glucose/UDP D galactose 4 epimerase 1
64	↓	Glyma08g02100.1	Gm08: 1,432,092–1,438,807 (strand: +)	Monodehydroascorbate reductase 6
65	↓	Glyma08g02940.1	Gm08: 2,029,928–2,033,740 (strand: +)	HSP 70 family protein
66	↓	Glyma08g04460.1	Gm08: 3,149,759–3,155,385 (strand: +)	ATP dependent caseinolytic protease
67	↓	Glyma08g06020.1	Gm08: 4,278,474–4,281,591 (strand: −)	Receptor like kinase 1
68	↑	Glyma08g12200.1	Gm08: 8,865,640–8,870,375 (strand: +)	Mitochondrial substrate
69	↓	Glyma08g23550.1	Gm08: 17,944,940–17,951,613 (strand: −)	Protein phosphatase 2C family protein
70	↓	Glyma08g41220.3	Gm08: 41,236,106–41,240,034 (strand: −)	*S* adenosyl *L* methionine dependent methyltransferases superfamily protein
71	↓	Glyma08g42730.1	Gm08: 42,711,059–42,724,363 (strand: +)	α/β-Hydrolases superfamily protein
72	↓	Glyma08g43670.1	Gm08: 43,456,275–43,459,631 (strand: +)	β-1,2 xylosyltransferase
73	↑	Glyma08g43680.1	Gm08: 43,461,710–43,464,771 (strand: +)	β-1,2 xylosyltransferase
74	↑	Glyma08g47790.1	Gm08: 46,591,084–46,595,539 (strand: −)	Aldolase type TIM barrel family protein

(*continued on next page*)

Table 1 (*continued*)

No.	Ratio	Protein ID	Location	Description
75	↓	Glyma09g04980.1	Gm09: 3,758,897–3,769,175 (strand: −)	ABC transporter C family member 14-like
76	↑	Glyma09g07040.1	Gm09: 5,857,812–5,860,477 (strand: +)	Glutaredoxin family protein
77	↓	Glyma09g08120.1	Gm09: 7,185,807–7,188,643 (strand: +)	Subtilase family protein
78	↑	Glyma09g08830.1	Gm09: 8,241,631–8,249,457 (strand: +)	DnaJ/Sec63 Brl domains containing protein
79	↓	Glyma09g16690.1	Gm09: 20,098,535–20,099,611 (strand: +)	Chaperone protein htpG family protein
80	↑	Glyma09g25940.1	Gm09: 32,168,675–32,172,540 (strand: +)	Membrane associated progesterone binding protein 3
81	↑	Glyma09g29470.1	Gm09: 36,349,061–36,355,214 (strand: +)	Staurosporin and temperature sensitive 3 like b
82	↓	Glyma09g30910.1	Gm09: 37,693,814–37,698,798 (strand: −)	B-S glucosidase 44
83	↑	Glyma09g36560.1	Gm09: 42,263,808–42,266,564 (strand: +)	Chaperone regulator like protein
84	↑	Glyma09g38410.2	Gm09: 43,780,130–43,785,822 (strand: +)	Calreticulin 3
85	↑	Glyma10g00880.2	Gm10: 610,809–617,509 (strand: +)	*S* adenosyl *L* methionine dependent methyltransferases superfamily protein
86	↑	Glyma10g01820.1	Gm10: 1,317,528–1,323,204 (strand: +)	Thioredoxin family protein
87	↓	Glyma10g02370.1	Gm10: 1,629,329–1,637,165 (strand: −)	ABC transporter C family member 4-like
88	↑	Glyma10g04370.1	Gm10: 3,364,599–3,368,253 (strand: +)	*S* adenosyl *L* methionine dependent methyltransferases superfamily protein
89	↓	Glyma10g15910.1	Gm10: 18,699,198–18,719,524 (strand: +)	S formylglutathione hydrolase
90	↓	Glyma10g24620.1	Gm10: 32,175,274–32,180,564 (strand: −)	Potassium channel β subunit 1
91	↑	Glyma10g28880.1	Gm10: 37,780,349–37,784,273 (strand: +)	Inorganic H^+ pyrophosphatase family protein
92	↑	Glyma10g35450.1	Gm10: 43,648,036–43,656,146 (strand: +)	Ribophorin I
93	↓	Glyma10g35490.1	Gm10: 43,714,944–43,722,164 (strand: +)	Phosphoglucosamine mutase family protein
94	↑	Glyma10g36170.1	Gm10: 44,352,238–44,357,180 (strand: −)	PDI like 5,2
95	↑	Glyma10g39750.1	Gm10: 47,377,444–47,378,739 (strand: +)	Oligosaccharyltransferase complex
96	↓	Glyma10g42630.1	Gm10: 49,524,298–49,528,280 (strand: −)	GHMP kinase family protein
97	↓	Glyma10g43590.1	Gm10: 50,228,552–50,231,641 (strand: +)	Ras related small GTP binding family protein
98	↓	Glyma11g04650.1	Gm11: 3,182,802–3,186,273 (strand: +)	Peptidase M20/M25/M40 family protein
99	↓	Glyma11g11410.1	Gm11: 8,132,034–8,134,710 (strand: +)	Subtilisin like serine protease 2
100	↑	Glyma11g12800.1	Gm11: 9,149,894–9,154,262 (strand: +)	Dolichyl diphospho oligosaccharide protein glycosyltransferase 48 kDa subunit
101	↑	Glyma11g13460.1	Gm11: 9,552,340–9,559,012 (strand: +)	Calreticulin 3
102	↑	Glyma11g14970.1	Gm11: 10,720,268–10,721,423 (strand: +)	Pathogenesis related thaumatin superfamily protein
103	↓	Glyma11g15010.1	Gm11: 10,752,370–10,757,000 (strand: +)	UDP XYL synthase 6
104	↓	Glyma11g15120.1	Gm11: 10,824,406–10,828,605 (strand: +)	Ras related small GTP binding family protein
105	↓	Glyma11g19550.1	Gm11: 16,310,166–16,313,231 (strand: −)	UDP D apiose/UDP D xylose synthase 2
106	↓	Glyma11g20630.1	Gm11: 17,418,671–17,423,245 (strand: +)	PDI like 1,4
107	↓	Glyma11g31450.1	Gm11: 32,629,475–32,634,601 (strand: −)	Regulatory particle triple A ATPase 3
108	↓	Glyma11g31470.1	Gm11: 32,684,322–32,688,565 (strand: −)	Regulatory particle triple A ATPase 3
109	↑	Glyma11g34490.1	Gm11: 36,309,028–36,312,310 (strand: +)	Leucine-rich repeat receptor like protein kinase family protein
110	↓	Glyma11g35740.1	Gm11: 37,351,501–37,358,128 (strand: +)	Biotin/lipoyl attachment domain containing protein
111	↑	Glyma12g04950.1	Gm12: 3,287,891–3,292,128 (strand: +)	Dolichyl diphospho oligosaccharide protein glycosyltransferase 48 kDa subunit
112	↓	Glyma12g05460.4	Gm12: 3,635,192–3,641,756 (strand: +)	Calreticulin 3
113	↓	Glyma12g06970.1	Gm12: 4,751,921–4,752,781 (strand: −)	Dessication induced 1VOC superfamily protein
114	↓	Glyma12g07070.1	Gm12: 4,818,042–4,822,153 (strand: +)	Ras related small GTP binding family protein
115	↓	Glyma12g07260.1	Gm12: 4,957,660–4,961,664 (strand: +)	PDI like 1,4
116	↓	Glyma12g08930.1	Gm12: 6,693,116–6,695,951 (strand: +)	UDP D apiose/UDP D xylose synthase 2
117	↓	Glyma12g29550.1	Gm12: 32,985,108–32,989,483 (strand: +)	PDI like 1,4
118	↓	Glyma13g00780.1	Gm13: 493,912–501,516 (strand: +)	Galactose mutarotase like superfamily protein
119	↓	Glyma13g02870.1	Gm13: 2,823,368–2,830,952 (strand: −)	Peptidase M20/M25/M40 family protein
120	↓	Glyma13g03650.1	Gm13: 3,663,883–3,672,157 (strand: +)	Plant L ascorbate oxidase
121	↑	Glyma13g09130.1	Gm13: 10,116,237–10,116,628 (strand: +)	Thioredoxin family protein
122	↑	Glyma13g18630.1	Gm13: 22,300,713–22,304,473 (strand: +)	*S* adenosyl *L* methionine dependent methyltransferases superfamily protein
123	↓	Glyma13g23170.1	Gm13: 26,624,092–26,628,887 (strand: +)	Inorganic H^+ pyrophosphatase family protein
124	↑	Glyma13g30490.1	Gm13: 33,109,988–33,114,029 (strand: −)	Pyruvate decarboxylase 2
125	↓	Glyma13g32660.1	Gm13: 34,763,980–34,767,201 (strand: +)	Pyrophosphorylase 6
126	↓	Glyma13g40130.1	Gm13: 40,681,809–40,686,147 (strand: +)	PDI like 1,4
127	↓	Glyma13g40350.1	Gm13: 40,852,483–40,855,002 (strand: −)	PDI like 5,1
128	↓	Glyma13g40870.3	Gm13: 41,317,042–41,320,023 (strand: −)	RAB GTPase homolog 8A
129	↓	Glyma13g42270.1	Gm13: 42,303,058–42,305,143 (strand: +)	Pyridoxal 5 phosphate dependent enzyme family protein
130	↓	Glyma13g43430.2	Gm13: 43,121,828–43,126,858 (strand: +)	PDI like 16
131	↓	Glyma14g05520.1	Gm14: 3,948,810–3,952,784 (strand: +)	PDI like 2,2
132	↑	Glyma14g20360.1	Gm14: 23,336,396–23,337,161 (strand: +)	Thioredoxin family protein
133	↑	Glyma14g24090.1	Gm14: 28,767,127–28,771,904 (strand: −)	PDI like 1,1
134	↑	Glyma15g01880.1	Gm15: 1,245,193–1,250,273 (strand: −)	PDI like 1,6
135	↓	Glyma15g03120.1	Gm15: 2,171,866–2,174,277 (strand: −)	Pyridoxal 5 phosphate dependent enzyme family protein
136	↓	Glyma15g04560.2	Gm15: 3,179,074–3,182,655 (strand: +)	Ras related small GTP binding family protein
137	↓	Glyma15g12100.1	Gm15: 8,968,913–8,975,996 (strand: +)	Fumarylacetoacetase putative
138	↑	Glyma15g12880.1	Gm15: 9,554,380–9,557,776 (strand: +)	RAB GTPase homolog B1C
139	↓	Glyma15g13620.1	Gm15: 10,209,940–10,216,791 (strand: −)	Glycosyl hydrolase family protein
140	↓	Glyma15g15870.1	Gm15: 12,196,351–12,206,082 (strand: +)	ABC transporter C family member 14-like
141	↑	Glyma15g18310.1	Gm15: 15,003,048–15,005,641 (strand: +)	Glutaredoxin family protein
142	↑	Glyma15g20400.1	Gm15: 18,260,654–18,267,258 (strand: +)	DnaJ/Sec 63 Brl domains containing protein
143	↓	Glyma15g21890.1	Gm15: 20,279,967–20,282,605 (strand: −)	*S*-adenosylmethionine synthetase family protein
144	↓	Glyma16g00590.1	Gm16: 247,496–254,683 (strand: +)	Dihydrolipoamide acetyltransferase long form protein
145	↑	Glyma16g08410.1	Gm16: 7,765,711–7,774,194 (strand: −)	Staurosporin and temperature sensitive 3 like A
146	↓	Glyma16g17500.1	Gm16: 19,007,669–19,013,835 (strand: +)	*S* adenosyl *L* methionine dependent methyltransferases superfamily protein
147	↓	Glyma16g23010.1	Gm16: 26,638,616–26,641,814 (strand: +)	RNA binding family protein
148	↓	Glyma16g27030.1	Gm16: 31,081,828–31,086,763 (strand: +)	Tubulin α 3
149	↓	Glyma17g15740.1	Gm17: 12,459,363–12,463,520 (strand: −)	Glutamate dehydrogenase 2

(*continued on next page*)

Table 1 (*continued*)

No.	Ratio	Protein ID	Location	Description
150	↓	Glyma17g16850.1	Gm17: 13,635,703–13,638,881 (strand: −)	N. D.*
151	↑	Glyma17g34070.1	Gm17: 37,961,077–37,965,016 (strand: −)	Class II aminoacyl tRNA/biotin synthetases superfamily protein
152	↓	Glyma18g07030.1	Gm18: 5,730,116–5,732,839 (strand: +)	Cyclophilin 5
153	↑	Glyma18g09480.1	Gm18: 8,374,020–8,378,571 (strand: −)	β-1,2 xylosyltransferase
154	↓	Glyma18g12920.1	Gm18: 12,333,170–12,343,800 (strand: +)	HIS HF
155	↓	Glyma18g45500.1	Gm18: 55,246,255–55,246,808 (strand: −)	PDI like 1,2
156	↓	Glyma18g52450.1	Gm18: 61,052,857–61,057,770 (strand: −)	Ras related small GTP binding family protein
157	↓	Glyma18g52860.1	Gm18: 61,306,195–61,309,857 (strand: −)	O Glycosyl hydrolases family 17 protein
158	↑	Glyma18g53700.1	Gm18: 61,964,545–61,968,719 (strand: +)	Aldolase type TIM barrel family protein
159	↑	Glyma19g29720.1	Gm19: 37,451,136–37,455,763 (strand: +)	NADH: cytochrome *b5* reductase 1
160	↓	Glyma19g33140.1	Gm19: 40,772,865–40,774,370 (strand: −)	Ahal domain containing protein
161	↑	Glyma19g34890.1	Gm19: 42,485,179–42,490,482 (strand: +)	*S* adenosyl *L* methionine dependent methyltransferases superfamily protein
162	↑	Glyma19g35960.1	Gm19: 43,384,216–43,390,334 (strand: +)	Ca^{2+} transporting ATPase
163	↓	Glyma19g39710.1	Gm19: 46,301,684–46,304,736 (strand: +)	Amino acid dehydrogenase family protein
164	↓	Glyma19g40810.1	Gm19: 47,126,385–47,129,171 (strand: +)	*S*-adenosylmethionine synthetase 2
165	↑	Glyma19g41690.1	Gm19: 47,898,502–47,902,440 (strand: +)	Thioredoxin family protein
166	↑	Glyma19g44780.1	Gm19: 50,096,715–50,101,693 (strand: +)	Cytochrome *b5* isoform E
167	↓	Glyma19g44860.1	Gm19: 50,167,251–50,174,260 (strand: −)	RNA binding family protein
168	↓	Glyma20g01220.1	Gm20: 833,962–839,151 (strand: +)	Oxidoreductases acting on the aldehyde
169	↑	Glyma20g03930.1	Gm20: 3,862,190–3,865,257 (strand: −)	emp24/gp25L/p24 family/GOLD family protein
170	↓	Glyma20g12150.1	Gm20: 17,069,690–17,077,611 (strand: +)	Plant *L* ascorbate oxidase
171	↓	Glyma20g19000.1	Gm20: 26,702,308–26,707,310 (strand: −)	Potassium channel β-subunit 1
172	↓	Glyma20g23080.1	Gm20: 33,012,502–33,015,990 (strand: +)	Calreticulin 1b
173	↑	Glyma20g27980.1	Gm20: 36,953,778–36,956,758 (strand: −)	Oligosaccharyltransferase complex
174	↑	Glyma20g29660.1	Gm20: 38,514,463–38,517,385 (strand: −)	Membrane steroid binding protein1
175	↓	Glyma20g32030.1	Gm20: 40,646,909–40,654,749 (strand: −)	Phosphoglucosamine mutase family protein
176	↓	Glyma20g32320.1	Gm20: 40,930,276–40,936,570 (strand: +)	Ras related small GTP binding family protein

[a] Increased (up arrow) and decreased (down arrow) protein abundance in flooded plant was compared to 2-day-old unstressed soybean; Protein ID, according to Phytozome soybean genome database; Location, location was determined using DAIZUbase (http://daizu.dna.affrc.go.jp/) based on the identified proteins [20,32,39].

[85]. Protein folding is aided by factors in the ER and adverse environmental conditions induce misfolded proteins [86]. In addition, ER-based *N*-glycosylation was an important participant in stress response [87]. In the early-stage soybean, protein synthesis and glycosylation in the ER were affected by flooding [88,89]. Exposed to flooding and drought stresses, accumulation of glycoproteins was reduced; however, cytosolic calcium increased [20]. These findings elucidate that ER play pivotal roles in stressed plant such as protein synthesis, protein glycosylation, and calcium homeostasis. The findings employing ER proteomic techniques in soybean under flooding and drought conditions were summarized (Fig. 3).

4.1. Protein quality control in the endoplasmic reticulum of soybean under both stresses

ER is characterized as being devoid of membrane-bound ribosomes or studded with ribosomes [84]. Protein abundance of ribosomal proteins was decreased [88] and similar result was reported in drought-stressed soybean [20]. Ribosomal proteins play regulatory roles in tissue fate [90], plant development [91], and environment sensing [92]. Post-translational modifications of ribosomes are responsible for environmental conditions [93]. These findings indicate that protein abundance and post-translational modifications of ribosomal proteins mediate stress response of soybean under flooding and drought conditions.

Nascent polypeptides bearing glycosylated sites are folded in *N*-glycan dependent way [86]. *N*-glycosylation encompasses synthesis and modification of sugar moieties in the ER and Golgi; and ER-localized steps of *N*-glycan production play roles in stress response [87]. Flooding activated *N*-glycan synthesis, which mediated stress adaptation through increased protein abundance of oligosaccharyltransferases [20]. In addition, translocation of polypeptides into the ER was slowed down by increased nascent polypeptide-associated complex in flooded soybean [88]. Salt exposure caused osmotic sensitivity and reduced cell division in *Arabidopsis* of the mutant of *stt3a-1* and *stt3a-2*, which encode the subunit of oligosaccharyltransferase complex [94]. *N*-glycosylation functioned beyond protein folding and it was necessary for sufficient cell-wall formation in salt-stressed *Arabidopsis* [95]. These results suggest that *N*-glycosylation is involved in *N*-glycan synthesis, protein folding, and cell wall synthesis in plant under flooding and osmotic conditions.

N-glycosylated polypeptides bearing the monoglucosylated oligosaccharide are recognized by ER lectins such as calnexin and calreticulin [84]. Calnexin creates the environment for protein folding and regulates ER-mediated cell death [96]. Protein folding was suppressed by flooding and drought due to decreased calnexin [20]. Stress-induced programmed cell death was modulated by ER-response pathway in *Arabidopsis* under water deficit condition [97]. Cell death occurred in flooded soybean through the regulation of protein phosphatase 2A subunit-like proteins [98]. Besides calnexin and calreticulin, protein disulfide isomerase (PDI)-like proteins or heat shock proteins (HSPs) were major folding assistants in soybean under flooding or drought [20]. These findings address ER lectins and folding assistants in protein folding; and bridge ER stress and cell death regulation in soybean under flooding and drought.

The ER has quality control system to eliminate misfolded proteins from secretory pathway [99]. Regarding glycoprotein secretory, accumulation of glycoproteins was declined in stressed soybean under flooding and drought [20]. As presented, protein glycosylation was suppressed in flooded soybean [88]. Glycoproteins related to protein degradation, cell wall, and glycolysis were activated; however, stress-related proteins were decreased in flooded soybean [89]. Glycoprotein OS9 was component of ER-associated degradation machinery and it acted in protein degradation [100]. Suppressed activity of UDP-glucose:glycoprotein glucosyltransferase altered plant-vegetative development and impaired stress response under biotic and abiotic conditions [101]. These findings imply that flooding and drought decline glycoprotein accumulation in soybean. In addition, glycoproteins involved in glycolysis and protein quality control might promote stress response in stressed plant.

Table 2
List of drought-response proteins in soybean.

No.	Ratio	Protein ID	Location	Description
1	↓[a]	Glyma01g35220.2	Gm01: 47,744,169–47,746,902 (strand: −)	Early responsive dehydration stress protein
2	↓	Glyma02g04980.1	Gm02: 4,030,951–4,034,105 (strand: +)	RNA binding family protein
3	↓	Glyma02g10790.1	Gm02: 8,679,102–8,686,798 (strand: +)	Protein phosphatase 2A subunit A2
4	↓	Glyma02g13330.1	Gm02: 11,601,786–11,605,281 (strand: +)	Reversibly glycosylated polypeptide 3
5	↓	Glyma02g45030.1	Gm02: 49,443,006–49,447,931 (strand: +)	Putative mitochondrial RNA helicase 2
6	↓	Glyma03g03800.1	Gm03: 3,633,770–3,638,147 (strand: −)	Plant VAP homolog 12
7	↑	Glyma03g33240.1	Gm03: 40,885,587–40,891,963 (strand: +)	Ca^{2+}-transporting ATPase
8	↓	Glyma03g37650.1	Gm03: 44,190,430–44,194,864 (strand: +)	DnaJ heat shock family protein
9	↓	Glyma03g41210.1	Gm03: 46,735,997–46,737,827 (strand: −)	Rotamase cyclophilin 2
10	↑	Glyma03g42070.3	Gm03: 47,374,627–47,378,345 (strand: +)	Cytochrome *b5* isoform E
11	↓	Glyma03g42150.1	Gm03: 47,428,796–47,435,494 (strand: −)	RNA binding family protein
12	↓	Glyma04g38000.1	Gm04: 44,418,156–44,421,941 (strand: +)	Calnexin 1
13	↓	Glyma04g38590.1	Gm04: 44,957,860–44,971,142 (strand: +)	β-Galactosidase 10
14	↓	Glyma04g40090.1	Gm04: 46,218,142–46,227,070 (strand: +)	Nucleic acid binding OB fold like protein
15	↓	Glyma04g40750.2	Gm04: 46,699,533–46,705,586 (strand: +)	CTC interacting domain 11
16	↓	Glyma04g40760.1	Gm04: 46,724,021–46,729,598 (strand: +)	CTC interacting domain 11
17	↓	Glyma04g42690.1	Gm04: 48,376,626–48,381,064 (strand: −)	PDI like 1,2
18	↑	Glyma05g01010.1	Gm05: 609,594–611,534 (strand: +)	Malate dehydrogenase
19	↓	Glyma05g03320.1	Gm05: 2,547,518–2,552,812 (strand: −)	Purple acid phosphatase 27
20	↓	Glyma05g05460.1	Gm05: 4,767,337–4,771,477 (strand: −)	Glutamate dehydrogenase 2
21	↓	Glyma05g27980.1	Gm05: 33,852,909–33,854,869 (strand: +)	Rubber elongation factor protein (REF)
22	↓	Glyma05g28500.1	Gm05: 34,296,431–34,303,454 (strand: −)	Subtilisin like serine endopeptidase family protein
23	↑	Glyma05g29050.1	Gm05: 34,730,528–34,735,631 (strand: +)	Mitochondrial substrate carrier
24	↓	Glyma05g34900.1	Gm05: 39,094,615–39,098,883 (strand: +)	Arginosuccinate synthase family
25	↓	Glyma05g36600.1	Gm05: 40,426,908–40,430,703 (strand: −)	HSP 70 protein 5
26	↓	Glyma05g36620.1	Gm05: 40,443,124–40,447,225 (strand: −)	HSP 70 family protein
27	↓	Glyma05g38120.1	Gm05: 41,530,564–41,533,554 (strand: +)	UDP D glucose/UDP D galactose 4 epimerase 1
28	↓	Glyma06g05770.1	Gm06: 4,127,859–4,132,398 (strand: +)	Nitrilase/cyanide hydratase
29	↓	Glyma06g14030.1	Gm06: 11,079,494–11,084,221 (strand: −)	CTC interacting domain 11
30	↓	Glyma06g14760.1	Gm06: 11,565,183–11,572,316 (strand: −)	Nucleic acid binding OB fold like protein
31	↓	Glyma06g17060.1	Gm06: 13,418,027–13,421,764 (strand: −)	Calnexin 1
32	↓	Glyma07g02470.1	Gm07: 1,682,404–1,690,170 (strand: +)	Protein phosphatase 2C family protein
33	↓	Glyma07g03930.1	Gm07: 2,782,652–2,789,177 (strand: +)	Dihydrolipoamide acetyltransferase long form protein
34	↓	Glyma07g11310.1	Gm07: 9,498,808–9,503,366 (strand: +)	B-S glucosidase 44
35	↓	Glyma08g01480.1	Gm08: 939,512–942,346 (strand: −)	UDP D glucose/UDP D galactose 4 epimerase 1
36	↓	Glyma08g02100.1	Gm08: 1,432,092–1,438,807 (strand: +)	Monodehydroascorbate reductase 6
37	↓	Glyma08g04460.1	Gm08: 3,149,759–3,155,385 (strand: +)	ATP dependent caseinolytic protease
38	↑	Glyma08g12200.1	Gm08: 8,865,640–8,870,375 (strand: +)	Mitochondrial substrate
39	↓	Glyma08g23550.1	Gm08: 17,944,940–17,951,613 (strand: −)	Protein phosphatase 2C family protein
40	↓	Glyma08g42730.1	Gm08: 42,711,059–42,724,363 (strand: +)	α/β-Hydrolases superfamily protein
41	↑	Glyma08g47790.1	Gm08: 46,591,084–46,595,539 (strand: −)	Aldolase type TIM barrel family protein
42	↓	Glyma09g08120.1	Gm09: 7,185,807–7,188,643 (strand: +)	Subtilase family protein
43	↓	Glyma09g30910.1	Gm09: 37,693,814–37,698,798 (strand: −)	B-S glucosidase 44
44	↓	Glyma09g37860.1	Gm09: 43,388,629–43,392,321 (strand: −)	RAS 5
45	↓	Glyma10g15910.1	Gm10: 18,699,198–18,719,524 (strand: +)	S formylglutathione hydrolase
46	↓	Glyma10g24620.1	Gm10: 32,175,274–32,180,564 (strand: −)	Potassium channel β subunit 1
47	↓	Glyma10g35230.1	Gm10: 43,422,479–43,430,194 (strand: −)	Ras related small GTP binding family protein
48	↓	Glyma10g35490.1	Gm10: 43,714,944–43,722,164 (strand: +)	Phosphoglucosamine mutase family protein
49	↓	Glyma10g42630.1	Gm10: 49,524,298–49,528,280 (strand: −)	GHMP kinase family protein
50	↓	Glyma10g43590.1	Gm10: 50,228,552–50,231,641 (strand: +)	Ras related small GTP binding family protein
51	↓	Glyma11g04650.1	Gm11: 3,182,802–3,186,273 (strand: +)	Peptidase M20/M25/M40 family protein
52	↓	Glyma11g11410.1	Gm11: 8,132,034–8,134,710 (strand: +)	Subtilisin like serine protease 2
53	↓	Glyma11g11460.1	Gm11: 8,165,996–8,170,401 (strand: −)	Ascorbate peroxidase 3
54	↓	Glyma11g15010.1	Gm11: 10,752,370–10,757,000 (strand: +)	UDP XYL synthase 6
55	↓	Glyma11g15120.1	Gm11: 10,824,406–10,828,605 (strand: +)	Ras related small GTP binding family protein
56	↓	Glyma11g19550.1	Gm11: 16,310,166–16,313,231 (strand: −)	UDP D apiose/UDP D xylose synthase 2
57	↓	Glyma11g31450.1	Gm11: 32,629,475–32,634,601 (strand: −)	Regulatory particle triple A ATPase 3
58	↓	Glyma11g31470.1	Gm11: 32,684,322–32,688,565 (strand: −)	Regulatory particle triple A ATPase 3
59	↑	Glyma11g35740.1	Gm11: 37,351,501–37,358,128 (strand: +)	Biotin/lipoyl attachment domain containing protein
60	↓	Glyma12g06970.1	Gm12: 4,751,921–4,752,781 (strand: −)	Dessication induced 1VOC superfamily protein
61	↓	Glyma12g07070.1	Gm12: 4,818,042–4,822,153 (strand: +)	Ras related small GTP binding family protein
62	↓	Glyma12g08930.1	Gm12: 6,693,116–6,695,951 (strand: +)	UDP D apiose/UDP D xylose synthase 2
63	↓	Glyma13g00780.1	Gm13: 493,912–501,516 (strand: +)	Galactose mutarotase like superfamily protein
64	↓	Glyma13g02870.1	Gm13: 2,823,368–2,830,952 (strand: −)	Peptidase M20/M25/M40 family protein
65	↑	Glyma13g03650.1	Gm13: 3,663,883–3,672,157 (strand: +)	Plant L ascorbate oxidase
66	↓	Glyma13g10700.1	Gm13: 12,773,297–12,782,079 (strand: +)	Heat shock protein 70 (Hsp70) family protein
67	↑	Glyma13g23170.1	Gm13: 26,624,092–26,628,887 (strand: +)	Inorganic H^+ pyrophosphatase family protein
68	↓	Glyma13g30490.1	Gm13: 33,109,988–33,114,029 (strand: −)	Pyruvate decarboxylase 2
69	↑	Glyma13g32660.1	Gm13: 34,763,980–34,767,201 (strand: +)	Pyrophosphorylase 6
70	↓	Glyma13g40870.3	Gm13: 41,317,042–41,320,023 (strand: −)	RAB GTPase homolog 8A
71	↓	Glyma13g42270.1	Gm13: 42,303,058–42,305,143 (strand: +)	Pyridoxal 5 phosphate dependent enzyme family protein
72	↓	Glyma13g43430.2	Gm13: 43,121,828–43,126,858 (strand: +)	PDI like 16
73	↓	Glyma15g03120.1	Gm15: 2,171,866–2,174,277 (strand: −)	Pyridoxal 5 phosphate dependent enzyme family protein
74	↓	Glyma15g04560.2	Gm15: 3,179,074–3,182,655 (strand: +)	Ras related small GTP binding family protein

(*continued on next page*)

Table 2 (*continued*)

No.	Ratio	Protein ID	Location	Description
75	↓	Glyma15g12100.1	Gm15: 8,968,913–8,975,996 (strand: +)	Fumarylacetoacetase putative
76	↓	Glyma15g13620.1	Gm15: 10,209,940–10,216,791 (strand: −)	Glycosyl hydrolase family protein
77	↑	Glyma15g21890.1	Gm15: 20,279,967–20,282,605 (strand: −)	*S*-adenosylmethionine synthetase family protein
78	↓	Glyma16g00590.1	Gm16: 247,496–254,683 (strand: +)	Dihydrolipoamide acetyltransferase long form protein
79	↓	Glyma16g23010.1	Gm16: 26,638,616–26,641,814 (strand: +)	RNA binding family protein
80	↓	Glyma16g27030.1	Gm16: 31,081,828–31,086,763 (strand: +)	Tubulin α 3
81	↓	Glyma17g15740.1	Gm17: 12,459,363–12,463,520 (strand: −)	Glutamate dehydrogenase 2
82	↓	Glyma17g16850.1	Gm17: 13,635,703–13,638,881 (strand: −)	N. D.*
83	↑	Glyma17g34070.1	Gm17: 37,961,077–37,965,016 (strand: −)	Class II aminoacyl tRNA/biotin synthetases superfamily protein
84	↓	Glyma18g07030.1	Gm18: 5,730,116–5,732,839 (strand: +)	Cyclophilin 5
85	↓	Glyma18g12920.1	Gm18: 12,333,170–12,343,800 (strand: +)	HIS HF
86	↓	Glyma18g52450.1	Gm18: 61,052,857–61,057,770 (strand: −)	Ras related small GTP binding family protein
87	↓	Glyma18g52860.1	Gm18: 61,306,195–61,309,857 (strand: −)	O Glycosyl hydrolases family 17 protein
88	↑	Glyma18g53700.1	Gm18: 61,964,545–61,968,719 (strand: +)	Aldolase type TIM barrel family protein
89	↓	Glyma19g33140.1	Gm19: 40,772,865–40,774,370 (strand: −)	Ahal domain containing protein
90	↑	Glyma19g35960.1	Gm19: 43,384,216–43,390,334 (strand: +)	Ca^{2+} transporting ATPase
91	↓	Glyma19g39710.1	Gm19: 46,301,684–46,304,736 (strand: +)	Amino acid dehydrogenase family protein
92	↓	Glyma19g40810.1	Gm19: 47,126,385–47,129,171 (strand: +)	*S*-adenosylmethionine synthetase 2
93	↑	Glyma19g44780.1	Gm19: 50,096,715–50,101,693 (strand: +)	Cytochrome *b5* isoform E
94	↓	Glyma19g44860.1	Gm19: 50,167,251–50,174,260 (strand: −)	RNA binding family protein
95	↓	Glyma20g01220.1	Gm20: 833,962–839,151 (strand: +)	Oxidoreductases acting on the aldehyde
96	↑	Glyma20g12150.1	Gm20: 17,069,690–17,077,611 (strand: +)	Plant *L* ascorbate oxidase
97	↓	Glyma20g16070.1	Gm20: 22,168,983–22,178,286 (strand: −)	Heat shock protein 70 (Hsp70) family protein
98	↓	Glyma20g19000.1	Gm20: 26,702,308–26,707,310 (strand: −)	Potassium channel β-subunit 1
99	↓	Glyma20g32030.1	Gm20: 40,646,909–40,654,749 (strand: −)	Phosphoglucosamine mutase family protein
100	↓	Glyma20g32320.1	Gm20: 40,930,276–40,936,570 (strand: +)	Ras related small GTP binding family protein

[a] Increased (up arrow) and decreased (down arrow) protein abundance in drought-stressed plant was compared to 2-day-old unstressed soybean; Protein ID, according to Phytozome soybean genome database; Location, location was determined using DAIZUbase (http://daizu.dna.affrc.go.jp/) based on the identified proteins [20,32,39].

4.2. Calcium level in the endoplasmic reticulum of soybean under both stresses

The ER is engaged in protein synthesis, protein folding, lipid synthesis, drug detoxification, carbohydrate metabolism, as well as major roles in calcium homeostasis and signaling [84]. Calcium in the ER lumen mimics extracellular calcium to help protein assume stable conformation for secretion and provides a reservoir for release of calcium into cytoplasm [102,103]. Calcium homeostasis is maintained through distribution of calcium-binding proteins, calcium pumps, and calcium-release channels in the ER [85]. Calcium release from the ER was involved in the elevation of unfolded protein response in salt-stressed plant [104] and calcium application eliminated cell death in flooded soybean [14]. These findings emphasize the importance of calcium homeostasis in the ER and propose that calcium release from the ER might trigger stress response in plant.

Calcium-related signal transduction was induced in flooded soybean and it played important roles in early response to flooding [105]. Calcium was involved in drought tolerance through enhanced anti-oxidative activity in *Camellia sinensis* [106]. Notably, calcium release from the ER was involved in elevation of unfolded protein response and ROS participated in ER-associated degradation under osmotic stress in *Arabidopsis* [104]. Additionally, cytosolic calcium was increased during plant development and it further induced by flooding and drought stresses in soybean through the regulation of calnexin, calreticulin, calmodulin-binding proteins, and calcium-transporting ATPases [20]. Taken together, these findings suggest that calcium release from the ER is required to activate the processes such as unfolded protein response and ER-associated degradation to eliminate ER stress in plant under flooding and drought.

5. Calcium effects on soybean under flooding and drought stresses

Calcium is an important secondary messenger playing vital roles in stress signaling and increased cytosolic calcium induced by adverse conditions triggers downstream response to cope with stresses [107,108]. Calcium application promoted metabolism and ion transport to enhance hypoxia tolerance [109]. Plant pretreated by calcium displayed increased biomass and improved-drought tolerance [110]. Calcium regulated aquaporin in hydraulic mediation in response to flooding and drought [111]. Furthermore, calcium-dependent mitochondrial carries balanced mitochondrial-oxidative phosphorylation under flooding [112]. Moreover, cytosolic calcium was elevated in flooded and drought-stressed soybean [20]. These findings depict calcium homeostasis in the subcellular organelles such as ER and mitochondria; and indicate that calcium acts in metabolic regulation in stressed plant. The findings of calcium effects on stress response in soybean under flooding and drought were summarized based on proteomic results (Fig. 4).

5.1. Calcium participates in the metabolisms in the endoplasmic reticulum of soybean under both stresses

Steep electrochemical gradients for calcium gradients, which are important for signal transduction and metabolic processes in cytoplasm and organelles, exist across plasma membrane, tonoplast, and ER [113]. Under osmotic stress, unfolded protein response was induced and calcium released from the ER in *Arabidopsis* [104]. Increased cytosolic calcium disturbed ER cabinet for protein folding in soybean exposed to flooding and drought [20]. Inhibition of calcium channel in the ER and block calcium-ATPase in the plasma membrane reduced the elevated cytosolic calcium level in flooded and drought-stressed soybean [32]. These findings elucidate that increased calcium level in cytosol partially releases from the ER in stressed soybean.

Calcium gradients are critical for metabolic regulation in organelles; and calnexin, PDI-like proteins, HSPs, and thioredoxin family proteins presented as abundant ER proteins in response to calcium levels in stressed soybean [32]. It was revealed that calnexin was responsible for protein folding in soybean under flooding and drought [20]. Calcium ion elevated in flooded cotyledon of soybean and it played roles to induce HSP70-meidiated signal transduction [114]. HSPs were involved in protein quality control such as folding and refolding of stress-denatured proteins [115]. Taken together, these findings suggest that calcium gradients in the ER are disturbed by flooding and drought; and

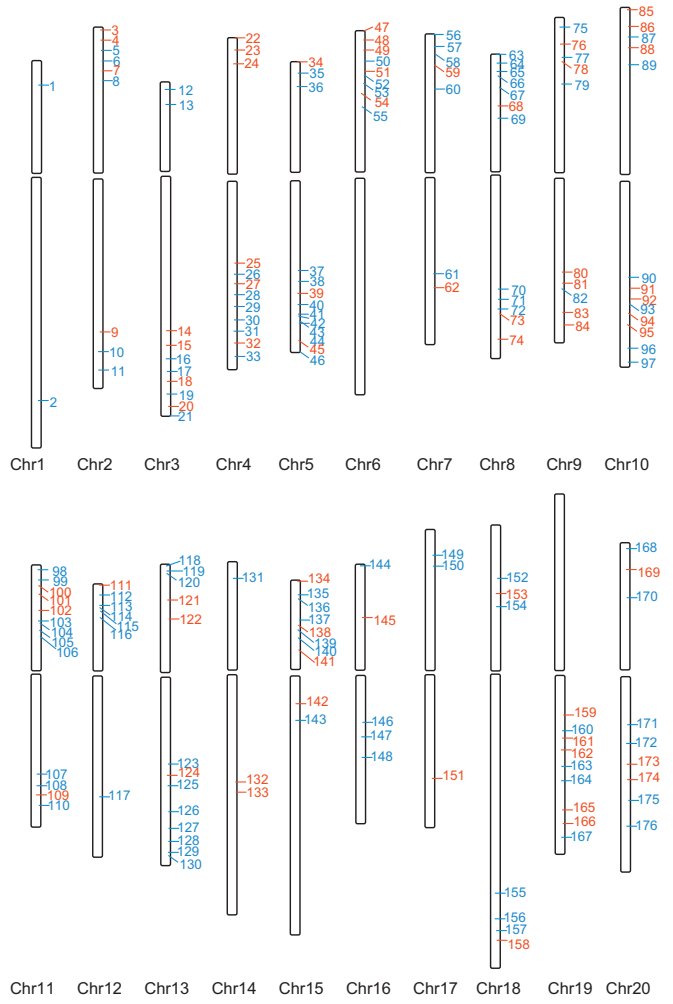

Fig. 6. Determination of flooding-response proteins in soybean. The location of flooding-response proteins was determined using DAIZUbase (http://daizu.dna.affrc.go.jp/) based on the identified proteins [20,32,39]. Red and blue colors indicate increased and decreased protein abundance, respectively, in flooded plant, compared to unstressed soybean. The information of flooding-response proteins was listed in Table 1.

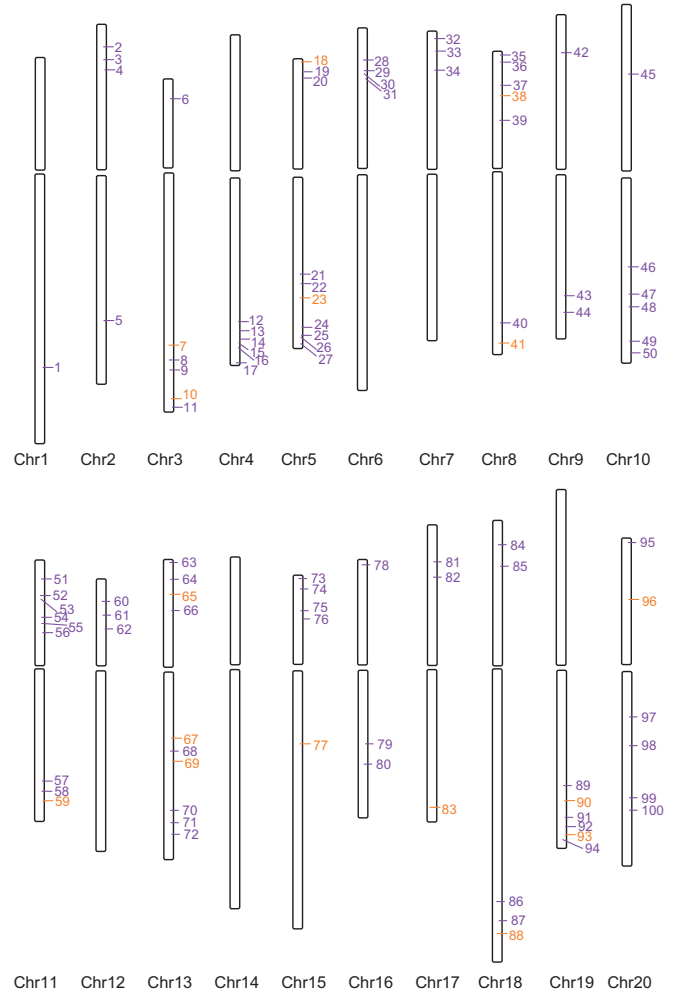

Fig. 7. Determination of drought-response proteins in soybean. The location of drought-response proteins was determined using DAIZUbase (http://daizu.dna.affrc.go.jp/) based on identified proteins [20,32,39]. Orange and purple colors indicate increased and decreased protein abundance, respectively, in drought-stressed plant, compared to unstressed soybean. The information of drought-response proteins was listed in Table 2.

protein folding is affected due to calcium release from the ER.

5.2. Calcium mediates energy metabolism in soybean under both stresses

Metabolism-related proteins were activated under stress conditions such as energy sensor Snf1-related protein kinase, which played modulatory role in hypoxia adaptation and salt tolerance [116]. Imbalance accumulation of proteins in carbohydrate metabolism caused flooding injury to soybean [17]. Mitochondrial proteins were associated with flooding response and considerable impairment to electron transport chain was exerted by flooding [18]. Energy management was responsive for stress adaptation through the regulation of biotin and biotinylation in soybean [20]. Besides, glycolysis, fermentation, the tricarboxylic acid cycle, and amino acid metabolism were indicated as calcium-response processes in soybean under flooding and drought [32]. These findings suggest that calcium has a variety roles in metabolic regulation and shed light on calcium homeostasis with energy-related metabolisms in stressed plant.

Low-oxygen stress caused energy and carbohydrate crisis, which was controlled through regulated consumption of carbohydrate and energy reserves [117]. Calcium transients were required for upregulated gene expression in sugar metabolism, glycolysis, and fermentation in response to flooding [65,118]. Under drought, calcium-mobilizing compound was involved in reduction in guard-cell turgor [119]. Phosphorylation of nodulin 26 was presented as part of osmotic adaptation in soybean nodules and it was catalyzed by calcium-dependent protein kinase [120]. Furthermore, the tricarboxylic acid cycle was activated to provide sufficient ATP for physiological activities in drought-stressed root [121]. Notably, glycolysis, fermentation, and the tricarboxylic acid cycle were examined as calcium-response metabolisms in soybean under flooding and drought; and especially pyruvate decarboxylase was indicated as switch enzyme for energy metabolism [32]. Taken together, these findings suggest that calcium mediates carbohydrate and energy metabolism in stressed soybean; and fermentation or the tricarboxylic acid cycle might serve as the major source of energy in respect to flooding or drought.

6. Stress-response proteins in soybean under flooding and drought stresses

Proteomics has enriched the knowledge of stress response in soybean [122]. Energy regulation and plant defense were essential to conquer flooding; however, osmotic adjustment, defense signaling, and programmed cell death played roles in drought adaptation [34]. Flooding and drought adversely affected vegetative and reproductive stages [123,124], whereas, a plethora of processes were induced by combined stresses in the early-stage soybean [50,32]. Furthermore, root tip was indicated as the sensitive organ in the early-stage soybean

exposed to combined stresses [50]. Although stress response was summarized [34,125], systematic comparison between flooding and drought in the sensitive organ of early-stage soybean is limited. Herein, the findings in root tip of soybean exposed to flooding and drought were presented (Fig. 5).

6.1. Comparison of stress-response processes in soybean under both stresses

Class II aminoacyl tRNA/biotin synthetases superfamily protein, biotin/lipoyl attachment domain containing protein, SAM synthetase family protein, and B-S glucosidase 44 responded to combined stresses in the root tip [50]. In cytosol, class II aminoacyl tRNA/biotin synthetases superfamily protein was increased under combined stresses; however, biotin/lipoyl attachment domain containing protein decreased or increased under flooding or drought, respectively [50]. With interactions with amino acids, tRNAs, and the universal cellular energy source of ATP, aminoacyl-tRNA synthetases represented the bridge between RNA and contemporary cellular milieu [126]. Class II aminoacyl tRNA/biotin synthetases superfamily protein was described as aspartate-tRNA ligase and mediated plant perception of beta-aminobutyric acid, which provided broad-spectrum disease protection in *Arabidopsis* [127]. Biotin/lipoyl attachment domain containing protein harbors the multidomain, in which biotin or lipoic acid was attached for protein biotinylation or lipoylation [55]. Biotin deficiency resulted in cell death and activation of defense signaling for abiotic stresses [57]. These findings suggest that biotin synthesis might be enhanced under combined stresses; however, biotinylation appears to be suppressed or activated in flooded or drought-stressed soybean.

SAM synthetase family protein was decreased in flooded and drought-stressed soybean [50]. SAM synthetase is the key enzyme catalyzing the formation of SAM, which is the precursor of ethylene and polyamine [128]. In flooded rice, ethylene accumulated and gene expression of *SNORKEL1* and *SNORKEL2* was induced to trigger internode elongation *via* gibberellin [129]. In flooded soybean, ethylene signaling played roles in stress tolerance *via* protein phosphorylation at the initial-flooding stress [130]. Besides, polyamines played pivotal roles in stress defense to various adverse environment [131] and polyamine oxidation responded to flooding and drought in the early-stage soybean [25]. These results indicate that ethylene steps into hormone interaction for flooding tolerance in rice or acts in protein phosphorylation in soybean at the initial flooding. On the other hand, polyamine metabolism might be associated with plant sensitivity to early-stage stresses through the regulation of decreased SAM synthetase family protein.

Imbalanced carbohydrate metabolism occurred in flooded soybean [17] and drought-stressed wheat [132]. B-S glucosidase 44 was increased in flooded soybean; however, converted trend was displayed in drought-stressed plant [50]. B-S glucosidase 44 was beta-glucosidase related protein and it played roles in cellulose hydrolysis by converting cellobiose to glucose [133]. beta-Glucosidases have various functions such as cell-wall modification, stress defense, phytohormone signaling, and secondary metabolism [134]. Sugar metabolism, glycolysis, and fermentation were activated by low-oxygen stress [65]; however, the tricarboxylic acid cycle served as source of energy provision under drought [121]. These findings suggest that B-S glucosidase 44 might play roles in glucose production to sustain energy provision in flooded soybean. However, the tricarboxylic acid cycle might be major process for energy metabolism in drought-stressed soybean.

Protein synthesis participated in stress tolerance in flooded soybean [135] and it was suppressed through decreased abundance of mRNA export/pre-ribosome biogenesis-related proteins [136]. Protein synthesis was inhibited by osmotic stress [137], either induced by polyethylene glycol or drought condition [26]. In the ER, calnexin provides cabinet for protein folding [138] and calcium homeostasis [85]. Calcium level is mediated by calmodulin-binding proteins [139], calcineurin B-like proteins [140], and calcium-dependent protein kinases [141]. In addition, calcium-transporting ATPase, either locating in the ER or in the plasma membrane [142], is associated with calcium content in the cell [113]. These findings represent the interaction between protein folding and calcium homeostasis, suggesting that maintain calcium level in the ER might facilitate protein folding in soybean under combined stresses.

Pyruvate decarboxylase exhibited inverted protein abundance in soybean under combined stresses; however, it was increased by elevated cytosolic-calcium level [32]. Proteins related to glycolysis and fermentation were induced by anaerobic conditions; and *pyruvate decarboxylase* was dramatically upregulated [143]. Overexpression of *pyruvate decarboxylase* enhanced its enzyme activity and increased ethanol production; and these results positively correlated with survival after flooding [144]. Although pyruvate decarboxylase was critical enzyme for fermentation, it also involved in stress signal and plant adaptation under drought [145]. Fast consuming of pyruvate was determined in dark-stressed plant, which was validated with enzyme activity of pyruvate decarboxylase [146]. In addition, pyruvate decarboxylase was indicated to switch energy metabolism in soybean in response to combined stresses [32]. These findings depict that increased cytosolic calcium induces the accumulation of pyruvate decarboxylase for stress adaptation under flooding and drought. Additionally, direction of pyruvate flux either to fermentation or to the tricarboxylic acid cycle might dependent on stress specificity of flooding or drought.

6.2. Determination of stress-response proteins in soybean under both stresses

Exploring the datasets derived from “-omics” to find functional unit or machinery could be used as the tool for plant breeding [147]. Quantitative trait loci mapping is an effective application of genomic-based approaches to improve sustainability and stability of yield under adverse conditions [148]. Plant breeding could benefit from the integration of “-omics” datasets and quantitative trait loci. Quantitative trait locus, which linked to marker Sat_064, was associated with growth improvement and grain yield for flooded soybean [149]. Molecular markers, including Satt226, Sat_044, Satt205-satt489, A489H, and B031-1, were linked to quantitative trait loci for drought tolerance in soybean [150]. More, integration of datasets derived from “-omics” addressed genes and pathways related to stress tolerance of flooding [125] and drought [151]. To overcome shortages of time consuming and labor intensive of conventional breeding [148], the location of stress-response proteins in soybean exposed to combined stresses was determined (Tables 1 and 2, Figs. 6 and 7). These results indicated that chromosomes 5, 10, 11, and 13 contained abundant flooding-response proteins (Fig. 6); and chromosomes 5 and 13 held more drought-affected proteins (Fig. 7). These findings suggest that chromosomes 5 and 13 might harbor many quantitative trait loci for stress response in soybean under flooding and drought.

Chromosomes 5 and 13 presented with abundant stress-response proteins under flooding and drought (Figs. 6 and 7) such as calnexin, PDI-like proteins, HSPs, and pyruvate decarboxylase. Exposed to combined stresses, calnexin, PDI-like proteins, and HSPs were associated with protein folding [20]; and pyruvate decarboxylase was reported as switch enzyme in energy metabolism [32]. Besides, calcium homeostasis was involved in protein folding and energy provision [32]. Pyruvate decarboxylase was calcium-response protein in soybean exposed to combined stresses and its protein abundance was further accumulated in presence of additional calcium in stressed plant [32]. Overexpression of *pyruvate decarboxylase* enhanced the survival of flooded plant [152]. Longer-root length was observed in drought-stressed soybean [25] and similar phenomenon displayed in the loss-of-function mutation of *pyruvate decarboxylase 1* [153]. Furthermore, oxygen deprivation [154] and osmotic conditions [155] elevated cytosolic calcium. Taken together, these findings suggest that elevating cytosolic calcium across certain threshold is potential for pyruvate carboxylase to switch pyruvate flux into energy metabolism to confer flooding and drought stresses in soybean.

7. Conclusion and future prospects

Flooding and drought stresses exert deleterious effects on soybean growth [25]. Considering the importance of soybean, clarification of the underlying mechanisms in response to combined stresses is absolutely needed. Organ-specific analysis indicated that root tip in the early-stage soybean was more sensitive to both stresses than other organs. Protein quality control and calcium homeostasis were disrupted in the ER of soybean exposed to combined stresses. Furthermore, increased-cytosolic calcium in stressed soybean was verified from the ER and it further induced the accumulation of pyruvate decarboxylase. These findings employing proteomic studies suggest that calcium homeostasis might represent the bridge between cytosol and subcellular compartment in plant cell of soybean-root tip in response to combined stresses. In addition, calcium release from the ER was required for unfolded protein response [104] and elevated cytosolic calcium directed pyruvate in stressed soybean [32], indicating the importance of calcium roles on protein metabolism and energy regulation to cope with flooding and drought stresses.

On the other hand, decreased-ribosomal proteins were responsible for suppressed-protein synthesis; however, heterogeneity in ribosomal proteins displayed different selectivity for translating sub-pools of transcripts in mammalian cell [156]. This sheds light on ribosome specificity in plant in response to stresses. Stress-dependent response was compared between flooding and drought; however, upstream events such as stress sensing and transduction are limited, suggesting that concerns might be placed on emphasis. Overall, elucidation of stress-response processes based on integrated datasets of “-omics”, clarification of responsive pathways from stress sensing to plant adaptation, and validation of protein function *in vivo* will aid in developing stress-tolerant soybean in the future.

Conflict of interest

The authors confirm that this article content has no conflict of interest.

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