Analyses of flooding tolerance of soybean varieties at emergence and varietal differences in their proteomes

Yohei Nanjo [a,*], Hee-Young Jang [b], Hong-Sig Kim [b], Susumu Hiraga [a], Sun-Hee Woo [b], Setsuko Komatsu [a,*]

[a] NARO Institute of Crop Science, Tsukuba 305-8518, Japan
[b] Chungbuk National University, Cheong-ju 361-763, Republic of Korea

ABSTRACT

Flooding of fields due to heavy and/or continuous rainfall influences soybean production. To identify soybean varieties with flooding tolerance at the seedling emergence stage, 128 soybean varieties were evaluated using a flooding tolerance index, which is based on plant survival rates, the lack of apparent damage and lateral root development, and post-flooding radicle elongation rate. The soybean varieties were ranked according to their flooding tolerance index, and it was found that the tolerance levels of soybean varieties exhibit a continuum of differences between varieties. Subsequently, tolerant, moderately tolerant and sensitive varieties were selected and subjected to comparative proteomic analysis to clarify the tolerance mechanism. Proteomic analysis of the radicles, combined with correlation analysis, showed that the ratios of RNA binding/processing related proteins and flooding stress indicator proteins were significantly correlated with flooding tolerance index. The RNA binding/processing related proteins were positively correlated in untreated soybeans, whereas flooding stress indicator proteins were negatively correlated in flooded soybeans. These results suggest that flooding tolerance is regulated by mechanisms through multiple factors and is associated with abundance levels of the identified proteins.

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1. Introduction

Heavy and/or continuous rainfall in poor drainage soil areas often causes flooding conditions. The risk of flooding has risen due to increases in rainfall intensity associated with recent climate changes (Schiermeier 2011; Min et al., 2011; Pall et al., 2011; Bailey-Serres et al., 2012). Additionally, recent disruptions in climate patterns have also occasionally resulted in extreme rainfall in unexpected areas. Flooding conditions have a negative impact on crop production. Therefore, improving the flooding tolerance of crops is an important issue in agriculture. Excess water induces oxygen deprivation, which restricts energy production through mitochondrial oxidative phosphorylation in plants (Gibbs and Greenway, 2003). Flooding also affects the carbon dioxide level, temperature, soil constituents, soil-microorganisms and the light level of the environment surrounding the plant (Nabben et al., 1999; Boru et al., 2003; Jackson and Colmer 2005; Mommer et al., 2006; Kokuryu et al., 2010). Alteration of these environmental factors influences plant growth.

Soybean is a crop that is sensitive to flooding stress, which reduces both growth and yield (Russell et al., 1990; Oosterhuis et al., 1990). Flooding prior to germination causes rapid water uptake into the seed, resulting in its physical collapse (Nakayama et al., 2004). This injurious effect is associated with water content in the seed, and this effect can be ameliorated by adjusting its water content (Chida et al., 2009). Flooding after seed germination, during the seedling emergence stage, results in physiological damage due to oxygen deficiency (Nakayama et al., 2005). The response of soybean seedlings to flooding during emergence has been analyzed using omics technologies, such as transcriptomics, proteomics and metabolomics in an effort to clarify the molecular mechanisms associated with injury. Transcriptomic analyses showed that drastic changes in the expression of more than 6000 genes for photosynthesis, glycolysis, amino acid synthesis/degradation, protein degradation, cell wall synthesis, secondary metabolism, metabolite transport, cell organization, chromatin structure synthesis and transcription occur under flooding (Komatsu et al., 2009; Nanjo et al., 2011). Proteomic studies demonstrated that some proteins involved in fermentation, scavenging of reactive oxygen species, glycolysis, protein storage, and disease/defense were affected by flooding (Hashiguchi et al., 2009; Nanjo et al., 2012). Metabolomics showed that metabolites related to the tricarboxylic acid cycle, alanine synthetic pathway and gamma-amino butyric acid shunt are

* Corresponding authors. Tel.: +81 29 838 8693; fax: +81 29 838 8694.
   E-mail addresses: ynanjo@affrc.go.jp (Y. Nanjo), skomatsu@affrc.go.jp (S. Komatsu).

significantly accumulated in the root of soybean seedlings under flooding (Nakamura et al., 2012). Furthermore, a recent analysis of the response of a flooding-tolerant soybean mutant to flooding stress demonstrated that rapid activation of alcohol dehydrogenase and diminishing alterations in levels of proteins related to development, protein synthesis/degradation, secondary metabolism, and cell wall metabolism are associated with tolerance at this growth stage (Komatsu et al., 2013b). As with the above analysis, investigation of materials with different tolerance levels may provide crucial information to clarify the tolerance mechanism. Several analyses screening for flooding- or waterlogging-tolerant varieties at germination (Nakayama et al., 2004; Sayama et al., 2009), mid-vegetative (V4–V5 stage, Cho et al., 2013) and early reproductive (R2 stage, Van Toai et al., 2001) stages have been reported. Furthermore, subsequent analyses have also been performed to identify quantitative trait loci responsible for tolerance (Sayama et al., 2009; Cornelious et al., 2005). Although many efforts have been made to find and generate tolerant varieties, data regarding tolerance at the emergence stage is scarce.

In this study, the flooding tolerance of soybean varieties at the emergence stage was evaluated and which identified potential tolerant soybean varieties using a previously established method. The evaluation showed differences in the tolerance of varieties and, in combination with proteomics technology, identified the potential proteins associated with flooding tolerance of soybean seedlings.

2. Results

2.1. Evaluation of flooding tolerance of soybean varieties

To identify soybean varieties that show flooding tolerance at seedling emergence, 128 soybean varieties including Japanese, Korean, Chinese and USA cultivars were subjected to screening. Two-day-old soybeans were flooded for 5 days at a density of one seedling in 60 mL floodwater, and then transplanted to wetted sand. At 3 days after transplantation, the rate of plant survival, those lacking apparent damage and those with lateral root development, combined with the radicle elongation rate during the 3 days were calculated and used for subsequent PCA to condense the data. In the analysis, the first principal component explained 70.7% of total variance. The contribution ratio of the second, third and fourth principal components were 18.2%, 6.6% and 4.4%, respectively. The loadings for these values were 3.32 (elongation rate), 4.45 (survival rate), 4.28 (undamaged rate) and 3.85 (lateral root development rate), respectively; indicating that the survival rate is the greatest contributing factor. From this result, the first principal component score was defined as the FTI in this study. The FTI values of soybean varieties were distributed over a continuum and ranged from −3.54 to 2.61 (Fig. 1, Table 1). The varieties with the highest FTI values were Iyodaizu and Geumkangkong. These soybean varieties were able to survive and elongate without severe injurious effects after 5-day flooding treatment (Fig. 2). The varieties with an FTI value near 0 were Suzuyutaka and Chakaori, indicating that among the varieties tested, these showed moderate tolerance. Additionally, while these varieties were able to survive, they had visible injurious effects, such as brown coloration and suppression of both elongation and lateral root development at 3 days after transplantation. The varieties showing the lowest FTI values were Williams82 and Miyagioojiro. These flooding-sensitive varieties were unable to elongate after 5-day flooding treatment. Cotyledon detachment from the root and root structure collapse were observed, as well as cotyledon collapse and chlorosis. In addition to the analysis, averages of the rate values were also calculated. The average values showed a similar tendency to the FTI

values in terms of rank, indicating that the average values could be used for rough evaluation (Table 1, Suppl. Fig. S1).

2.2. Proteomic comparison of 15 selected soybean varieties

The results of evaluation showed differences between varieties. In this study, the varieties were defined as tolerant (FTI >1.30, value is a half of maximum value), moderately tolerant (1.30 > FTI > −1.77) and sensitive (FTI <−1.77). From these 3 categories, 15 varieties were selected for further analysis (Fig. 1). The tolerant varieties selected were: Iyodaizu, Geumkangkong, Sohokong, Dagikong, Pangsakong, Shirosaya and Ichihime. The moderately tolerant varieties selected were Enrei, Jack, Sachiyutaka, Fukuyutaka and Yumeyutaka. The sensitive varieties selected were Peking, Oosuzu and Williams82. These varieties were subjected to comparative proteomic analysis to clarify the molecular mechanism of flooding tolerance. Each 2-day-old soybean variety was flooded for 2 days, and proteins were extracted from radicles of the untreated 2-day-old soybeans and the 2-day-flooded soybeans. The proteins were digested with trypsin and lysylendopeptidase C, and the resulting peptides were subjected to LC–MS/MS analysis (Supplemental Fig. S2).

In the comparative proteomic analysis, protein levels were compared between Enrei and the other varieties under each condition. In the untreated and flooded soybeans, 1597 and 1517 proteins were identified, respectively. The relative protein quantity data were analyzed by PCA to identify trends between varieties. The PCA score plot did not show a trend correlating with the FTI (Supplemental Fig. S3). To identify flooding tolerance related proteins, the correlation between the quantitative ratios of these proteins and FTI values of varieties were analyzed using Pearson's correlation coefficient. Of the identified proteins in untreated soybean, 57 proteins were found to be correlated ($p < 0.05$) with the FTI values of those varieties (Suppl. Table S1). Ten proteins showed high significance ($p < 0.01$) in the correlated proteins (Fig. 3, Table 2, Suppl. Fig. S4). Of the 10 proteins, 7 were positively correlated and 3 were negatively correlated with FTI values. In flooded soybeans, 154 proteins were found to be correlated ($p < 0.05$) with FTI (Suppl. Table S2), and 37 proteins were significant ($p < 0.01$, Fig. 4, Table 3, Suppl. Fig. S5). Of the 37 proteins, 13 were positively correlated and 24 were negatively correlated with FTI. The proteins showing high correlation with FTI were functionally annotated (Fig. 5, Tables 2 and 3). The majority of negatively correlated proteins consisted of proteins belonging to cell wall degradation/modification, biotic stress and protein synthesis categories. Furthermore, proteins in these categories consisted of expansin-like B1, xyloglucan endotransglycosylase, rhamnogalacturonate lyase, polygalacturonase inhibiting proteins, kunitz family trypsin inhibitor proteins and ribosomal proteins.

3. Discussion

In this study, flooding tolerance at the seedling emergence stage of soybean varieties including Japanese, Korean, Chinese and USA cultivars was evaluated. In the evaluation, the FTI values, which were based on the survival rate, lack of damage, lateral root development and elongation after termination of flooding treatment, were considered representative of the flooding tolerance level. The flooding treatment condition employed was based on a method established using the soybean cultivar Enrei (Nanjo et al., 2013). The report describing the method showed that the density of the seedling in floodwater is a factor that determines the degree of flooding injury at the seedling emergence stage. During flooding, soybean seedlings absorb dissolved oxygen from floodwater, resulting in an extremely low oxygen environment. Enrei is a major