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RESEARCH ARTICLE

Growth Response and Developing Simple Test Method for Waterlogging Stress Tolerance in Soybean

Kyung Hye Kim[1], Mi Jung Cho[1], Ji-Min Kim[1], Teaklim Lee[1], Jin Ho Heo[1], Ji Young Jeong[1], Juseok Lee[2], Jung-Kyung Moon[3], Sungteag Kang[1]*

[1] *Department of Crop Science & Biotechnology, Dankook University, Cheonan, 31116, Republic of Korea*
[2] *Bio-Evaluation Center, Korea Research Institute of Bioscience and Biotechnology, Cheongju, Chungbuk 28116, Republic of Korea*
[3] *National Institute of Crop Sciences, Rural Development Administration, Wanju-gun, Jeollabuk-do 55365, Republic of Korea*
Kyung Hye Kim and Mi Jung Cho contributed equally to this work.

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Abstract

The global climate changes and insufficient drainage system could cause the flooding with significant yield losses in crop production. Despite the severity of the flooding, the main reason for currently limited research progress is the lack of resistant resources. This study aimed to identify the main indicator to waterlogging stress and suggest a simple screening method for waterlogging resistant variety in soybean. We selected 3 resistance resources and 3 susceptible resources based on the results of mass field screen test, which was previously conducted using about 4,000 soybean genetic resources in RDA. Plant height, stem diameter, leaf chlorophyll content, root weight and adventitious root number and weight were used investigated as indicator. Significant changes by waterlogging treatment was not observed in the plant height and stem diameter. However, the chlorophyll content of first trifoliate leaf and emergence of adventitious roots showed a clear difference between the resistant and susceptible resources after 9 days of treatment, even though the growth of roots was reduced overall by waterlogging. The results indicated that the number of adventitious roots and the chlorophyll content of the first trifoliate leaf were an excellent indicator to distinguish resistance and susceptible resources. We also developed simple screening method for waterlogging in soybean. The resistance resource selection was possible within 6 days after waterlogging treatment in green house using the chlorophyll content of the first trifoliate leaf and the number of adventitious roots. The proposed simple screening method could save the time and labor for large scale field test.

Key words : Glycine max, waterlogging, flooding, adventitious root, chlorophyll

Introduction

Climate change causes extreme weather conditions in the agricultural sector, resulting in severe yield losses. In addition to the low availability of water causing drought stress, plants can also be affected by too much water. Flooding firstly restricts gas diffusion such as Oxygen and CO_2 between the plant and its surroundings due to physical properties. One key aspect of flooding is the 10,000-fold slower diffusion rate of gases in water compared with that in air (Bailey-Serres and Voesenek 2008). Lack of oxygen inside flooded plant parts leads to limit energy production in mitochondria. Low CO_2 availability in flooded leaves restricts photosynthesis. Therefore, flooding causes an energy crisis within plant cells. To be survived in flooding condition, plant have several strategies for avoidance oxygen deficiency by morphological and metabolic modifications (Mustroph 2018).

Flooding can be classified by two versions, waterlogging, where only the root system inside the soil is affected and submergence, where also parts or the whole shoot are under water. Waterlogging stress was frequently occurred in major cereal crops field because of heavy rainfall. Several species enduring waterlogging stress, have adventitious roots (Steffens and Rasmussen 2016). Adventitious roots are plant roots that form from any non-root tissue and are produced both during

Sungteag Kang (✉)
Email: kangst@dankook.ac.kr
Tel: +82415503621

normal development and in response to stress conditions, such as flooding, nutrient deprivation, and wounding. Adventitious roots have evolved to help plants tolerate a variety of stressful conditions by facilitating gas transport and water and nutrient uptake during flooding. Stress-induced adventitious roots formation was involved in ethylene biosynthesis (Steffens and Rasmussen 2016). Under aerated conditions, ethylene escapes from plant tissues because it is a gas. During waterlogging, water acts as physical entrapment causing it to accumulate ethylene inside the plant tissue. The development of adventitious roots by waterlogging is facilitated by ethylene, and high concentrations of ethylene increase the sensitivity of auxins derived from tissue organisms, in which adventitious roots form (Visser et al. 1996). Ethylene production was higher in resistant resources than in susceptible resource and disseminated to roots showed that root-centric aeration cells were more developed in resistant resources (Kim et al. 2015).

Soybean, which is one of the major agricultural crops, is particularly sensitive to flooding stress. The plant growth and grain yield are markedly reduced in flooded soil (Githiri et al. 2006). When soybean was treated with flooding at the vegetative growth stage or the reproductive stage, grain yield and quality were reduced compared to untreated soybean (Oosterhuis et al. 1990). Soybean showed various physiological and morphological responses, mainly due to the lack of oxygen needed for root respiration. Oxygen deprivation leads to the formation of toxic metabolites and nutrient deficiency, which causes wilting on the ground (ARMSTRONG et al. 1994). As the flooding continues, the short and soft adventitious roots are distributed on the surface due to lack of oxygen. As a result, nutrients and water absorption failures occur, and the weight of the root and the activity of the root nodules are reduced followed by, lowering the nitrogen fixation capacity (Fehr et al. 1971). Nutrient absorption by the waterlogging is depending on growth period, nutrient elements and damage site. Absorption of N, Ca, Mg and K is inhibited, resulting in decreased nutrient absorption and transfer (Bacanamwo and Purcell 1999). Furthermore, leaf chlorophyll content decreased and wilting phenomena from the bottom of the upper leaf yellowing leaves occurs (Lee et al. 2010).

This study was conducted to improve the efficiency of waterlogging resistance breeding program through selection of resistance genetic resources by waterlogging treatment, comparison of the growth characteristics of these resources, development of indicator and selection method for resistance resources.

Material and Method

Growth characteristics by waterlogging treatment

Growth characteristics of waterlogging resistance and susceptibility resources, three resistance resources (PI408105A, GCS2368, GCS0017) and three susceptibility resources (S99-2281, GCS3170, GCS2309) were selected and used by referring to the research report of RDA (Moon 2015). Before the waterlogging treatment, in the greenhouse of Dankook University, the horticultural soil and the nursery bed soil were mixed at the ratio of 3: 1 to the Wagner pot (1/1000a) of 174 cm in diameter and 197 cm in height, respectively, and cultivated until V_3 to V_4 stage after sowing in June 2015. In the greenhouse, 2m × 2m × 24cm (W × L × H) puddles were drilled and the plants were waterlogging in tap for 14 days (Fig. 1).

Several growth characteristics, plant height, stem diameter, leaf chlorophyll content, root weight and adventitious root number and weight were investigated. Plant height, stem diameter and leaf chlorophyll contents were investigated 3, 6, 9, and 12 days after treatment. The root weight and adventitious root number and weight were measured at 2 weeks after waterlogging treatment. The plant height was measured from the cotyledon to the top of the plant. The stem diameter was measured between the surface and the submerged area (3cm). The leaf chlorophyll content was measured using a chlorophyll meter (SPAD-502p, Minolta,


(A) (B)

Fig. 1. Small-scale waterlogging test. Control (A) and stressed (B) were compared to evaluate the growth characteristics.

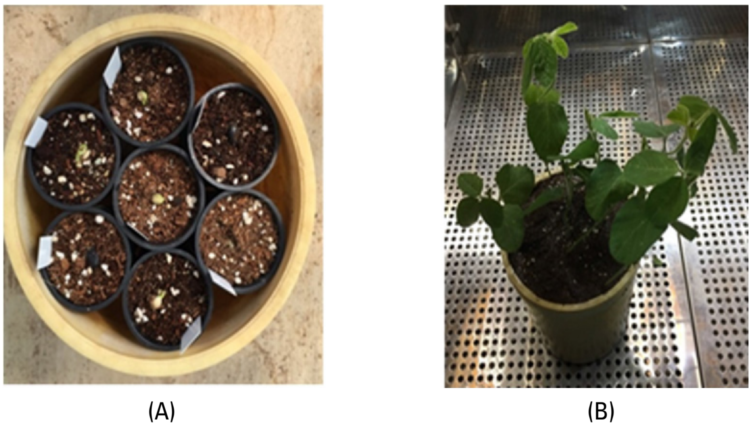
(A) (B)

Fig. 2. Simple screening method. Waterlogging treatment spot (A) and facility for the control (B) evaluate the growth and development characteristics for the resources selected.

Japan) with the mean value in 3 leaflets with 3 biological replications. The dry weight was measured after drying for 3 days in a dry oven at 40°C. The adventitious root was measured by the amount of irregularity generated roots in the area flooded over the surface of the soybean. Control treatments were applied with the conventional soybean cultivation method.

Development of simple screening test method

Resistance resources (PI408105A, GCS2368, GCS0017) and susceptible resources (S99-2281, GCS3170, GCS2309) were put into the Wagner pot for 5cm diameter and 18cm height in the growth area. The seeds were sowed, and the growth temperature was maintained at 25°C. In $V_3 \sim V_4$, 3cm of tap water was filled in the ground surface for 2 weeks and the growth temperature was maintained at 30 ± 3°C during waterlogging (Fig. 2). The growth characteristics measured by simple test method and the survey periods for the development were in the same ways as the method performed in the greenhouse.

Result

Growth characteristics under waterlogging treatment

Three resistance resources (PI4080105A, GCS2368, GCS0017) and three susceptible resources (S99-2281, GCS3170, GCS2309) were employed to investigate the growth characteristics after artificial waterlogging treatment at greenhouses in Dankook University.

The reduction of plant height by waterlogging treatment was not evident until the 3rd day of treatment, but after 6 days of treatment for both susceptible and resistant resources (Fig. 3). The Stem diameter was decreased by the waterlogging treatment in some resources but did not show a constant response for the entire resource (Fig. 4). Only GCS0017, resistant genetic resource, showed not significant difference between control and treatment in 3, 4, 6, 9 and 12 days after treatment.

The changes of chlorophyll content by waterlogging treatment at the first trifoliate leaf were significantly different between the resistance and susceptible resources. Especially, the chlorophyll content was not changed in resistance by waterlogging treatment, but the chlorophyll content significantly decreased in susceptible resources. The reduction in chlorophyll content at first trifoliate leaf caused by waterlogging in sensitive products occurred from the 3rd day after treatment (Fig. 5). The chlorophyll contents of the second trifoliate leaf showed a response to decrease significantly in the susceptible resources as in the case of the first trifoliate leaf. But PI408105A, the resistance resources showed chlorophyll decay by waterlogging treatment. All the susceptible resources showed significant decrease in chlorophyll content due to waterlogging treatment after 3, 6, 9, and 12 days of treatment. The longer the treatment days, the greater the reduction of chlorophyll content by waterlogging treatment (Fig. S1). Changes in the chlorophyll content at the third trifoliate leaf were decreased by waterlogging treatment and this phenomenon appeared in both resistance and susceptible resources. The longer the days of waterlogging treatment in susceptible resources, the greater the decrease in chlorophyll content (Fig. S2).

The adventitious roots generally occurred by waterlogging treatment, but the degree of adventitious roots was significantly different between resistance and susceptible resources. To evaluate the degree of adventitious roots, 3 criteria, number of adventitious roots, fresh weight, and dry weight, were used. The number of adventitious roots of resistance resources was about 30, but the number of adventitious roots of susceptible resources was about 5.

The fresh weight of the adventitious root was proportional to the number of adventitious roots, and the fresh weight was also high in resistance resources and low in susceptible resources (Fig. 6). It is considered that the resistance resource reduces the waterlogging damages by generating the adventitious roots more than the susceptible resources and facilitating the deficient oxygen supply due to the flooding. However, fresh weight and dry weight of roots showed a tendency to

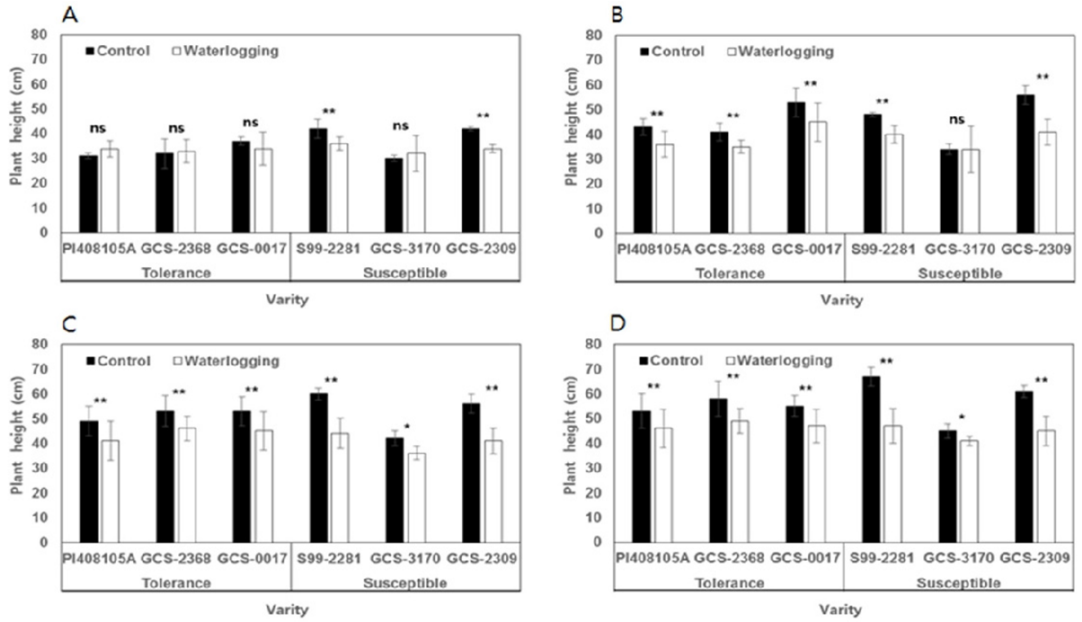

Fig. 3. Changes in plant height according to days induced by waterlogging treatment.
A: 3day after waterlogging treatment; B: 6day after waterlogging treatment; C: 9day after waterlogging treatment; D: 12day after waterlogging treatment

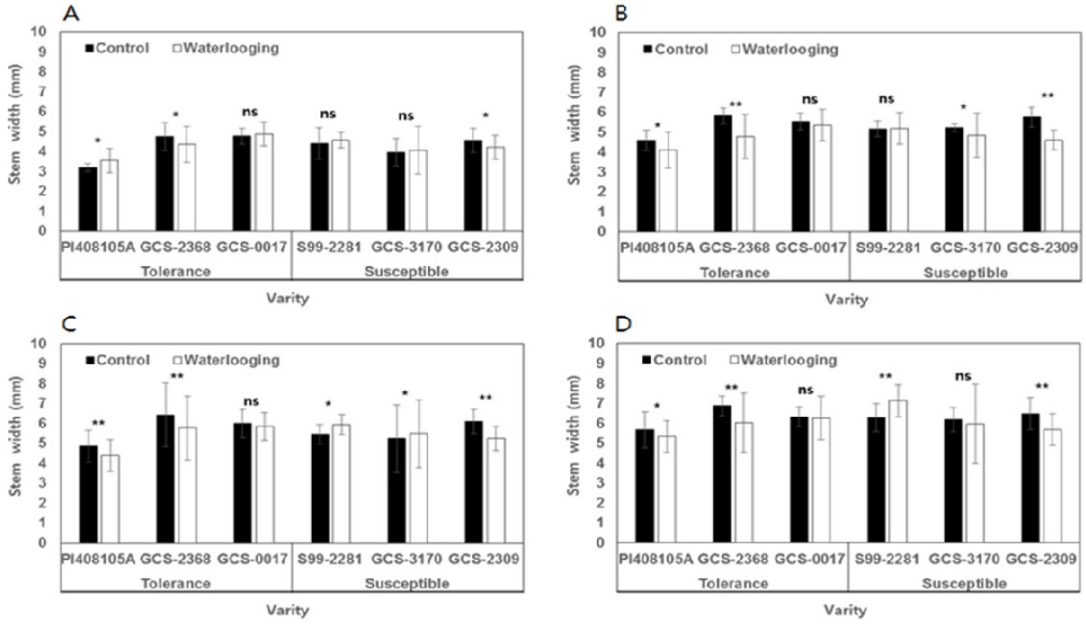

Fig. 4. Changes in stem width according to days induced by waterlogging treatment.
A: 3day after waterlogging treatment; B: 6day after waterlogging treatment; C: 9day after waterlogging treatment; D: 12day after waterlogging treatment

decrease by waterlogging treatment but did not show any difference between resistance and susceptible resources. This is because the waterlogging treatment inhibited the growth of roots and caused root rot, and these results were similar between the resistance and susceptible resources (Fig. 7).

Development of simple screening method

Three resistance resources and three susceptible resources were selected for the development of the simple screening method for waterlogging treatment. At a temperature of 30°C, tap water was filled 3cm from the surface of the ground and waterlogging for 2 weeks. The plant height, stem diameter, leaf chlorophyll content and the degree of adventitious roots were investigated.

There was no significant difference between the resistance and the susceptible resources in the total plant height and

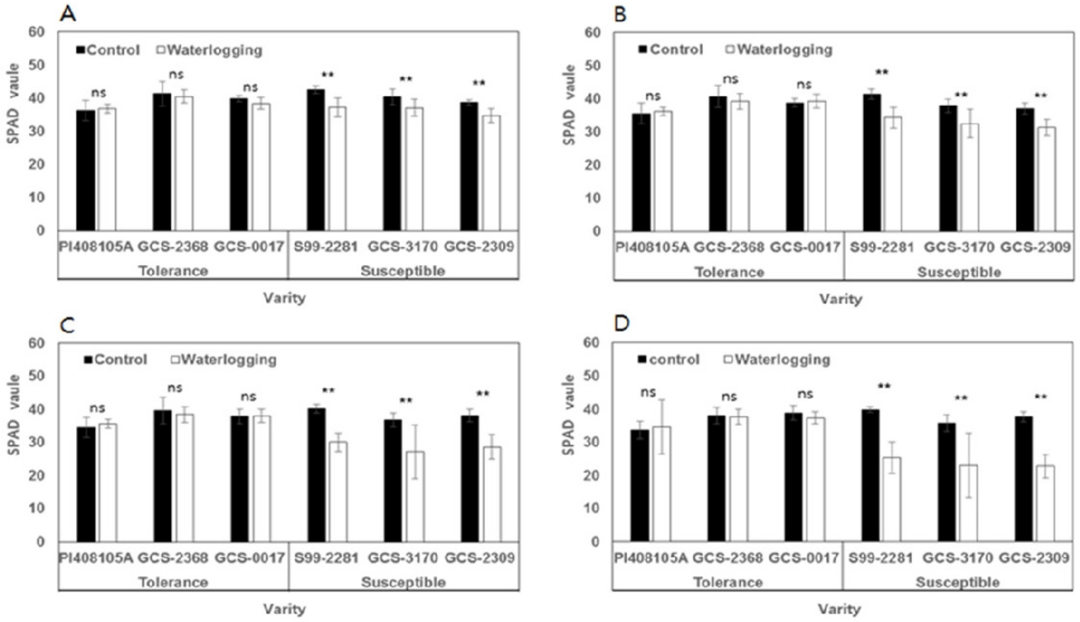

Fig. 5. Changes in 1st trifoliate leaf chlorophyll contents according to days induced by waterlogging treatment.
A: 3day after waterlogging treatment; B: 6day after waterlogging treatment; C: 9day after waterlogging treatment; D: 12day after waterlogging treatment

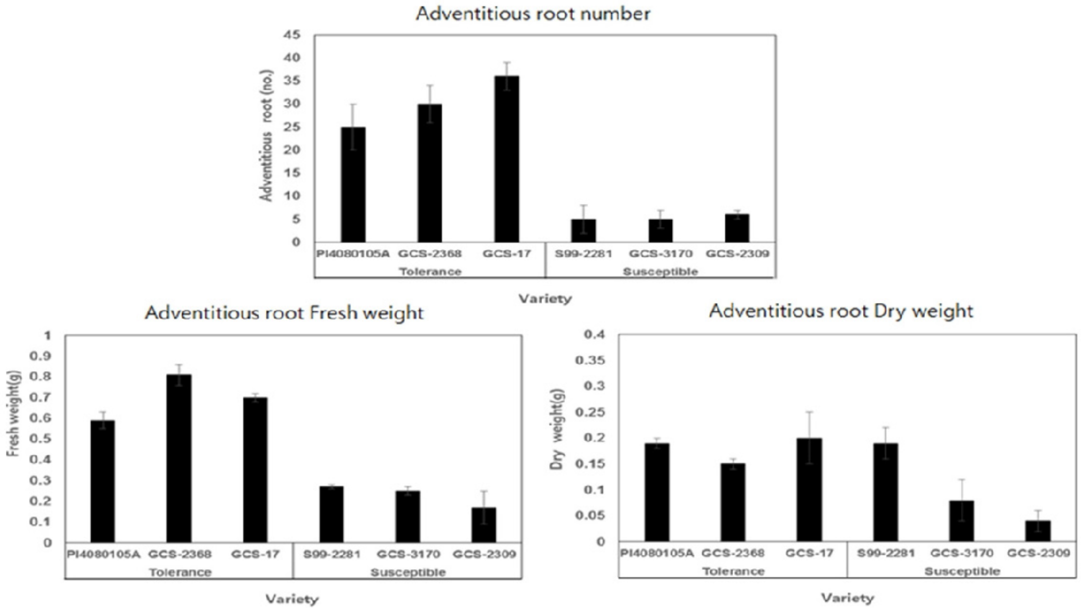

Fig. 6. Growth and development of adventitious root induced by waterlogging treatment.

stem diameter. Also, the longer the treatment days, the less the difference between the resistance and susceptible resources. As a result, it was found that plant height and stem diameter were not suitable as indicators of the difference between resistance and susceptible resources by waterlogging treatment.

The chlorophyll content of the leaves showed a tendency to decrease by waterlogging treatment. However, from the 6th day of treatment, there was a significant difference between the resistance and susceptible resources in the first trifoliate leaf, but the longer the treatment days, the consistent tendency was not shown between the resistance and susceptible resources. Therefore, the chlorophyll content of the leaves on the 6th day of waterlogging treatment is an indicator of the selection of resistant resources.

In the case of susceptible resources, the occurrence frequency of adventitious roots was very low in all treatment days. In the case of the resistance resources, the adventitious roots appeared on the 3rd day of treatment but the frequency of occurrence was not significantly different from the sus-

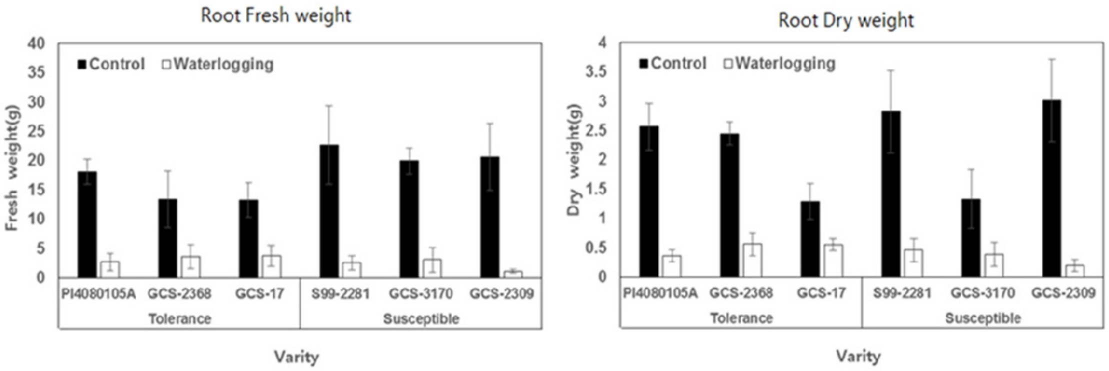

Fig. 7. Growth and development of root induced by waterlogging treatment.

Table 1. Major characteristics changes caused by the waterlogging stress based on simple screening method.

Trait		Day after treatment (days)							
		3		6		9		12	
		Tolerance1	Susceptible2	Tolerance	Susceptible	Tolerance	Susceptible	Tolerance	Susceptible
Plant height (cm)		32.4^{ns}	28.4	36.5^{ns}	31.9	38.6^{ns}	32.0	43.6^{ns}	33.5
Stem width (mm)		2.24^{ns}	2.15	2.64^{ns}	2.01	3.01^{ns}	2.65	3.12^{ns}	2.76
Chlorophyll Content (SPAD value)	1st trifoliate leaf	35.3^{ns}	33.2	34.6^{**}	31.1	33.0^{**}	30.7	28.1^{ns}	27.8
	2nd trifoliate leaf	37.3^{ns}	36.5	36.3^{ns}	35.7	34.3^{ns}	33.8	30.7^{ns}	30.4
	3rd trifoliate leaf	34.0^{ns}	33	36.5^{ns}	35.5	35.4^{ns}	35.1	31.4^{ns}	30.4
Adventitious root (no)		3^{ns}	0	10^{**}	0	13^{**}	0	25^{**}	0

1: Total average of tolerance resources (PI408105A, GCS-2368, GCS-0017)
2: Total average of susceptible resource (S99-2281, GCS-3170, GCS-2309)
ns: No significant differences in T-test
**: Significant differences at P<0.05 in T-test

ceptible resources. However, from the 6th day of treatment, the frequency of adventitious roots increased, indicating a significant difference from the susceptibility resources. Based on these results, it was confirmed that the resistance resources can be selected by artificially immersing the water at 30°C under the simple test method and investigating the chlorophyll content and the number of adventitious roots of the first trifoliate leaf after 6 days treatment (Table 1).

Discussion

Crop damage is occurring due to weather changes caused by global warming. In Korea, due to the weather conditions, there is damage due to heavy rainfall in the summer, which is the soybean growing season. In the case of flooding in the vegetative growth stage, the yield decreases to 43%. In the case of flooding in the reproductive growth stage, the problem with flooding is serious.

In addition, it suffers from flooding due to poor drainage of the field. In order to reduce flood damage, it is necessary to improve the cultivation environment. However, the development of waterlogging resistance resources has been evaluated as the most effective method. Therefore, this study was conducted to investigate the growth characteristics and develop simple test method of flooding. It was carried out to help early selection of resistant resources and to reduce the flood damage.

For the experiments, 3 resistance resources and 3 susceptibility resources were selected based on the results of the mass screen test, and a precision test was carried out by artificially flooding the greenhouses in Dankook University. Precision tests were carried out to investigate the effects of flooding on the growth of height, width, chlorophyll content, adventitious roots, fresh weight and dry weight of roots. Height and width were decreased by waterlogging treatment, but growth characteristics showed no significant difference between resistant and susceptible resources by waterlogging treatment. Also showed similar results to waterlogging treatments in which the height was reduced to 16 ~ 28% of that of untreated (VanToai et al. 2010). Therefore, it is concluded that the height and width are not suitable as flood resistance selection index. The chlorophyll content was the largest in the first trifoliate leaf, especially after 9 days of treatment, there was a clear difference between the resistant and susceptible resources. These results were similar to the difference in chlorophyll content of leaves from waterlogging treatment for 9 days in the vegetative growth period (Lee and Cho 2007). The degree of adventitious root, fresh weight and dry weight were changed by waterlogging treatment, and there was a significant difference between resistant and susceptible resources. The number of adventitious roots of resistant resources was about 30 and the number of adventitious roots of susceptible resources was about 5. The degree of adventitious roots by waterlogging treatment shows a significant difference in resistance and susceptibility resources. Fresh weight of the adventitious roots showed a significant difference between the resistance and susceptible resources, but the difference was not significant in the dry weight. Similar results are reported that flooding soybeans formed adventitious roots in the roots (Bacanamwo and Purcell 1999). Fresh weight and dry weight of adventitious roots showed a tendency to decrease by waterlogging treatment but did not show any tendency between resistance and susceptible resources. In the case of Valliyodan, adventitious roots fresh weight was significantly different on the 10th day of waterlogging treatment (Valliyodan et al. 2014). However, this experiment did not show much difference in adventitious roots fresh weight by flooding treatment. Decrease in the fresh weight of plants are reported due to waterlogging at the V4 stage (Oosterhuis et al. 1990). In contrast to waterlogging and non-treatment, it was similar with that of the previous study that the dry weight decreased 41-56% (Scott et al. 1989). It was concluded that the chlorophyll content of the first trifoliate leaf and the occurrence degree of adventitious roots were suitable as indicators of resistance to waterlogging.

As a result of the experiment using the simple test method developed using the Wagner pot, it can be seen the height and the width do not show any significant difference between the resistance resource and the susceptible resource, and thus are not suitable as the index indicating the difference between the resistance resource and the susceptibility resource. The chlorophyll content showed a significant difference between the resistant and susceptible resources in the first trifoliate leaf on the 6th day of treatment, but the longer the treatment days, the less the resistance and the susceptible resources showed. The adventitious roots showed a significant difference between the resistant and susceptible resources from the 6th day of treatment. Therefore, when the resistance resource is selected by using the simple test method, the degree of adventitious roots can be used as a selection index.

Acknowledgements

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Conflict of interest

The authors declare that they have no conflict of interest.

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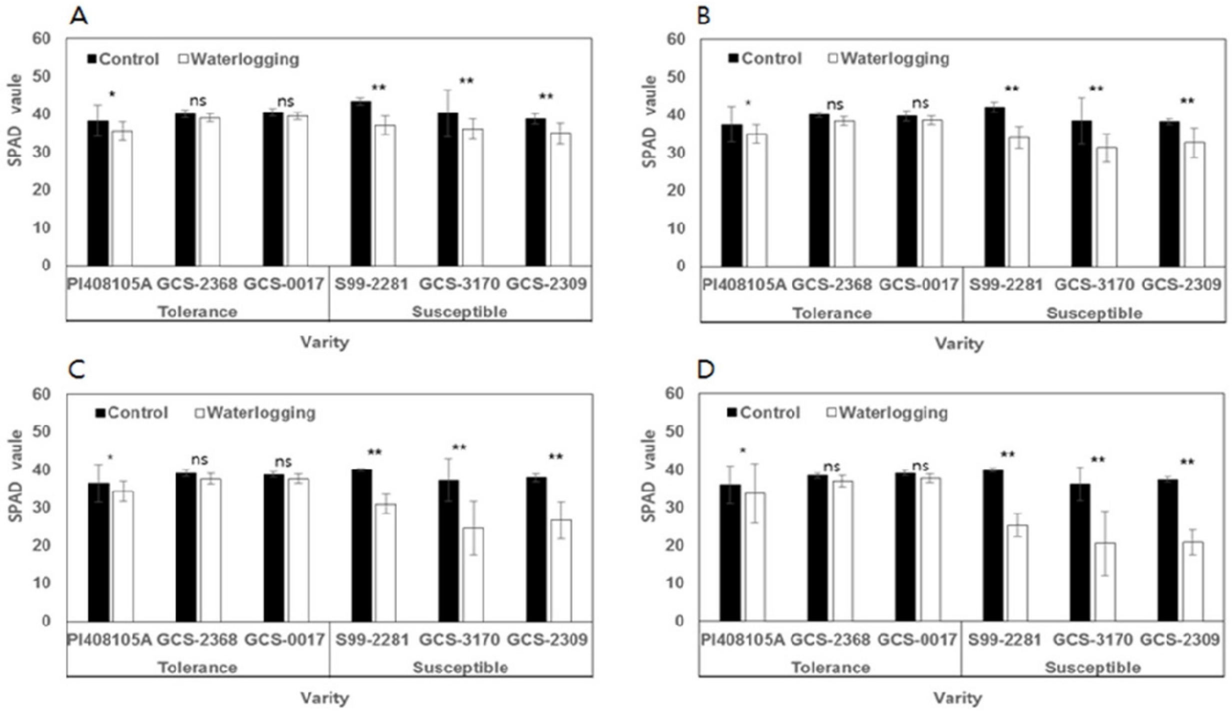

Fig. S1. Changes in 2nd trifoliate leaf chlorophyll contents according to days induced by waterlogging treatment.
A: 3day after waterlogging treatment; B: 6day after waterlogging treatment; C: 9day after waterlogging treatment; D: 12day after waterlogging treatment

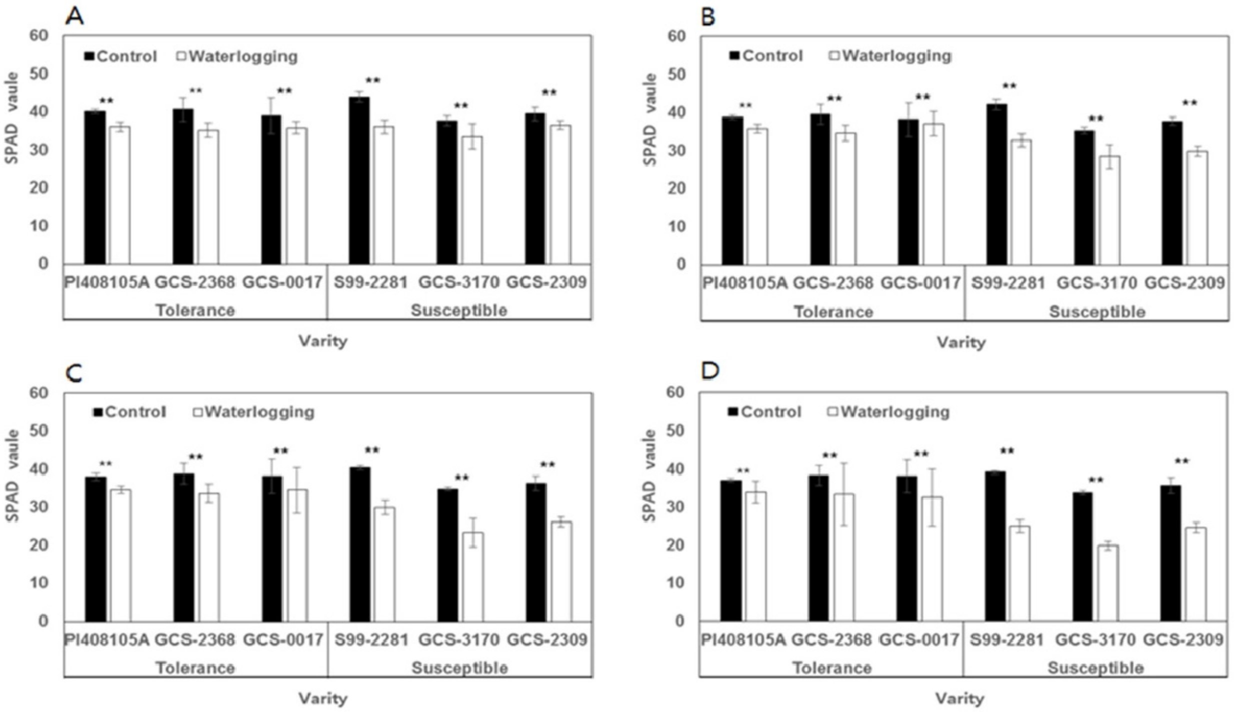

Fig. S2. Changes in 3rd trifoliate leaf chlorophyll contents according to days induced by waterlogging treatment.
A: 3day after waterlogging treatment; B: 6day after waterlogging treatment; C: 9day after waterlogging treatment; D: 12day after waterlogging treatment