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Waterlogging Effects on Nitrogen Accumulation and N_2 Fixation of Supernodulating Soybean Mutants

Jong-Tag Youn[1], Kyujung Van[2,3], Jae-Eun Lee[1], Wook-Han Kim[1], Hong-Tae Yun[1], Young-Up Kwon[1], Yong-Hwan Ryu[1], Suk-Ha Lee[2,3]*

[1]*National Institute of Crop Science, RDA, Suwon 441-857, Korea*
[2]*Department of Plant Science, Seoul National University, Seoul 151-921, Korea*
[3]*Research Institute for Agriculture and Life Science, Seoul National University, Seoul 151-921, Korea*

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Abstract

Soybean is sensitive to waterlogging stress, leading to reduce their growth and yield significantly. The objective of this study was to characterize the relative sensitivities of biomass accumulation and specific nodule activity under waterlogging stress between supernoduating mutants, 'SS2-2' and 'Sakukei 4' and their wild-type soybeans, 'Sinpaldalkong 2' and 'Enrei', respectively. Flooding treatment was performed to soybean plants grown in a pot by waterlogging for 15 days from the beginning bloom (R1) stage under natural light. The nodule number and weight were considerably decreased by waterlogging stress. The bleeding sap rate of waterlogging soybean plants was decreased by 78-80% in supernodulating mutants and 65-74% in their wild types compared to control plants. The relative ureide-N content was also decreased by waterlogging and the reduction was high in supernodulating mutants. This may cause the massive reduction of shoot and root dry weight and leaf area in waterlogged soybean plants. There was a varietal difference in response to the waterlogging stress. During the waterlogging, supernodulating mutants maintained higher spad value than their wild types. Particularly, the difference between soybean varieties was clear in low rank leaves from the top. Also, supernodulating mutants showed a weak waterlogging tolerance than their wild types. Under waterlogging conditions, massive nodules were considerably destroyed and specific nodule activity after waterlogging may not be recovered when compared to their wild-type soybeans. Supernodulating mutants showed lower seed yield than their wild types in waterlogging conditions.

Key words: nitrogen, soybean, supernodulating, ureide, waterlogging

Introduction

Recently, rice consumption in Korea has been decreasing and many rice fields have been converted for growing upland crops including soybean. In the converted fields that are not well drained, the excessive water stress during the growing season is the dominant reason for low yields in soybeans (Tian et al. 2005). Soybean is sensitive to waterlogging, which causes a decrease in photosynthesis (Chen et al. 1992), nutrient uptake (Sallam and Scott 1987), N_2 fixation (Minchin and Pate 1975; Sung 1993), and plant growth and yield (Russell et al. 1990; Smit et al. 1990). Short periods of flood irrigation can be detrimental to soybean if followed by untimely rainfall (Heatherly and Pringle 1991). On soils with poor drainage, canopy height, dry weight, and seed yield decreased linearly with duration of flood treatment for two to 14 days at both V4 and R2 growth stages (Fehr and Caviness 1977; Scott et al. 1989). When flood irrigation was applied at or after the R2 growth stage and water was allowed to stand for two days or more, yield was significantly decreased even in the absence of rainfall (Griffin and Sexton 1988).

Key physiological processes may be more sensitive to a particular stress than others, and therefore limit overall growth and productivity in a stressful environment. N_2 fixation was more sensitive to flooding than biomass accumulation in cowpea (Bacanamwo and Purcell 1999; Minchin and Summerfield 1976). The sensitivity of N_2 fixation to flooding was also revealed by N-fertilization studies. Buttery (1987) reported that soybean plants grown on nitrate were less sensitive to 30-day

* **To whom correspondence should be addressed**
Suk-Ha Lee
E-mail: sukhalee@snu.ac.kr
Tel: +82-2-880-4545 / Fax: +82-2-873-2056

flooding stress than plants relying on N_2 fixation, indicating the differential sensitivity to flooding between N_2 fixation and nitrate uptake and assimilation.

The detrimental effect of waterlogging was usually attributed to inadequate oxygen supply to sustain various root metabolisms (Kozlowski and Pallardy 1984). Waterlogging-induced soil anaerobosis was also harmful to nodule formation and function in several legume species (Bisseling et al. 1980; Hong et al. 1977; Huang and LaRue 1985; Turner et al. 1983). Decreased O_2 concentration in the rhizosphere during flooding offered at least two reasons that nitrate could ameliorate flooding stress relative to N_2 fixation (Bacanamwo and Purcell 1999). Firstly, nitrate could be used as an alternative to O_2 as an electron acceptor in hypoxic roots (Garcia-Novo and Crawford 1973; Reggiani et al. 1985). Secondly, respiratory energy demands for N_2 fixation and assimilation were higher than those for nitrate uptake and assimilation (Ryle et al. 1978). Consequently, hypoxic roots of plants supplying plants with nitrogen had less damage than hypoxic roots of plants dependent upon N_2 fixation. Andreeva et al. (1987) reported that waterlogging reduced nitrogenase activity and irreversibly altered ultrastructures of cells in soybean root nodules. Decreases in nitrogen accumulation by bean plants under waterlogged conditions might be due in part to reduced nodulation and decreased nitrogenase activity (Minchin and Pate 1975). The restriction of assimilatory nitrate reduction was mainly because the conversion of nitrate to ammonia in leaf tissue was coupled to the light reactions of photosynthesis (Hoff et al. 1992), which were inhibited by waterlogging.

Interest in N transport in the xylem has centered mainly on its relationship with metabolic processes occurring in other parts of the plant, particularly in the root system (Puiatti and Sodek 1999). While asparagine is the main organic form of transport of plants grown on nitrate (dependent on nitrate reduction and assimilation), ureides predominate in the transport of N in the xylem of symbiotic plants like soybean (dependent on atmospheric N_2 fixation) (Matsumoto et al. 1977). Moreover, nitrate applied to symbiotic plants leaded to the inhibition of N_2 fixation (Vessey et al. 1988) and a severe reduction in the transport of ureides (McClure and Israel 1979). Amino acids were much less affected, but the most clearly established change was the higher asparagine/glutamine ration for plants grown on nitrate (Pate et al. 1980).

The objective of this study was to characterize the relative sensitivities of nitrogen accumulation and N_2 fixation under waterlogging stress between supernoduating mutants and their wild-type soybeans.

Materials and Methods

Plant materials

Two supernodulating soybeans, 'SS2-2' and 'Sakukei 4', and their wild-types, 'Sinpaldalkong 2' and 'Enrei' were tested in this experiment. Five seeds were sown on June 16, 2005 in a plastic pot 15.9 cm in diameter and 19 cm in height. Pots were filled with volcanic ash soil without fertilization. The seedlings were thinned to one plant per pot after germination. The pots were divided into two groups at R1 growth stage. One group was continuously flooded at 3-4 cm above the soil surface for 15 days and the other was irrigated normally.

Measurements

Four seedlings for each treatment were sampled to measure the nodule number, leaf area, SPAD value, plant N content, xylem sap, and dry matter accumulation at four times; just before waterlogging initiation (R1 growth stage), 15 days after waterlogging treatment, 30 days after drainage and harvesting time. Total leaf area of each plant was measured by passing individual leaflets through a LI-COR LI-3100 leaf area meter (LI-COR, Lincoln, NE, USA). Leaf chlorophyll was determined from four leaves collected in the upper canopy of each plant using a SPAD-502 meter (Minolta Camera Co. Ltd., Japan). Collection and analysis of xylem bleeding sap was conducted using the procedure of Shimamura et al. (2003). The first drop of xylem sap at the cut surface was discarded to avoid contamination from damaged cells. Subsequent drops were collected into ice at 3-min intervals for 1 hour. The xylem sap in each plant was weighed and the bleeding sap rate was calculated. Four samples per treatment were stored at -30 ℃ in a freezer until analysis. Concentrations of amino-N, nitrate-N, and ureide-N (allantoin and allantonic acid) in the xylem sap were determined using the ninhydrin technique (Herridge 1984), 1:1 (mol) asparagine:glutamine standard the salicylic acid technique (Cataldo et al. 1975) and the method of Young and Conway (1942), respectively. The absorbance was analyzed on a spectrophotometer (UV-1200 UV-VIS, Shimadzu Co. Ltd., Kyoto, Japan). The number of nodules and dry weight were measured after drying in an oven at 80 ℃ for 48 hours. Shoot N content was measured using an N-C analyzer (Sumigraph NC-800, oxygen gas combustion - gas chromatography system, Sumika Chemical Analysis Service, Ltd., Japan). The outside structures of the nodules were observed with a stereoscopic microscope (SMZ-10, Nikon Co. Ltd., Tokyo, Japan). Then, transverse cross-sections 100-200 μm in thickness were cut on a plant microtome (MTH-1, Nippon Medical & Chemical Instruments Co. Ltd., Osaka, Japan) and viewed with a light microscope (OPTIPHOT-2, Nikon Co. Ltd., Tokyo, Japan).

Results

Biomass accumulation

Excessive water stress at R1 growth stage decreased dry matter production. The shoot and root dry matter accumulation was lower in the excessive-watered (EW) soybean plants compared to that of well-watered (WW) soybean plants (Table 1). The reduction of the shoot and root dry weight was greater at 30 days after drainage than that just after drainage as compared to that of WW soybeans. Shoot and root dry weight of supernodulating mutants, SS2-2 and Sakukei 4, were reduced more significantly

Table 1. Shoot and root dry weight (DW) of soybeans as affected by waterlogging treatment.

Water treatment	Variety	Shoot DW (g/plant)			Root DW (g/plant)		
		JBW†	JBD	30DAD	JBW	JBD	30DAD
Well watered	SS2-2	1.6	5.2	14.0	0.4	2.1	5.5
	Sakukei 4	1.6	5.7	13.8	0.6	2.6	5.9
	Sinpaldalkong 2	1.8	4.9	12.1	0.9	2.2	5.8
	Enrei	1.8	4.7	13.7	0.8	2.0	5.7
Excessive Watered	SS2-2	1.6	3.5	3.2	0.4	0.7	1.4
	Sakukei 4	1.6	3.5	3.5	0.6	1.0	2.1
	Sinpaldalkong 2	1.8	3.7	3.8	0.9	1.3	2.1
	Enrei	1.8	3.5	4.8	0.8	1.1	2.8
Analysis of variance‡							
P(W)		NS	<0.01	<0.01	NS	<0.01	<0.01
P(V)		NS	NS	<0.05	<0.01	<0.05	<0.05
P(W × V)		NS	NS	<0.05	NS	NS	NS
$LSD_{0.05}$		0.36	0.74	1.15	0.21	0.40	0.70

†JBW : Just before waterlogging, JBD : Just before drainage, 30DAD : 30 days after drainage.
‡W : water treatment; V: variety; NS: not significant.

than those of their wild types, Sinpaldalkong 2 and Enrei. Shoot dry weight was reduced by 33-39% in supernodulating mutants and 25-26% in their wild types just after waterlogging treatment, and 75-77% in supernodulating mutants and 65-69% in their wild types at 30 days after waterlogging treatment. The same trend was shown in root dry weight. The reduction of root dry weight by EW was 62-67% in supernodulating mutants and 41-45% in their wild types just after drainage, and 64-75% in supernodulating mutants and 51-64% in their wild types at 30 days after drainage.

Temporal change of nodulation

In general, the nodule formation of supernodulating mutant is much higher than that of its wild-type soybean (Fig. 1). However, nodule number and dry weight of soybeans was significantly decreased by waterlogging when compared to that of WW soybeans which increased continuously to 30 days after drainage (Fig. 2). The reduction of nodule number and weight by EW was greater in supernodulating mutants and 30 days after drainage than that of their wild-type soybeans and just after drainage. However, dry weight per nodule of waterlogged soybeans was significantly increased at 30 days after drainage compared to that of WW soybeans.

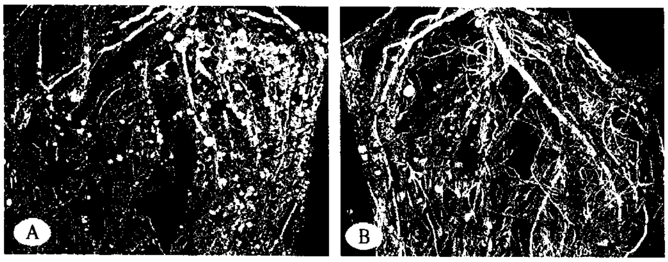

Fig. 1. The root of supernodulating mutant SS2-2 (A) and its wild-type soybean Sinpaldalkong 2 (B) at R3 stage.

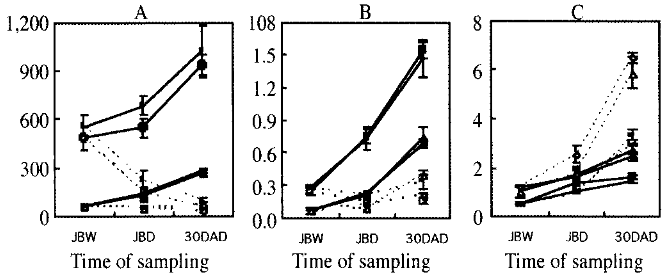

Fig. 2. Nodule number (A), nodule dry weight (B) and single DW (C) as affected by waterlogging treatment. SS: SS2-2 (○), SA: Sakukei 4 (□), SP: Sinpaldalkong 2 (◇), EN: Enrei (△). WW: well watered (filled mark), EW: excessive watered (open mark). JBW: Just before waterlogging, JBD: Just before drainage, 30DAD: 30 days after drainage. The vertical bars indicate standard deviation of three replication.

Table 2. Shoot nitrogen content of soybeans as affected by waterlogging treatment.

Variety	Shoot N content (mg plant⁻¹)					JBW ~ JBD		JBD ~ 30DAD	
	JBW†	JBD		30DAD					
		WW‡	EW	WW	EW	WW	EW	WW	EW
SS2-2	42	178	64	409	54	136	22	232	-11
Sakukei 4	54	176	56	413	65	122	2	237	9
Sinpaldalkong 2	38	105	52	272	66	67	14	167	14
Enrei	38	95	38	302	80	57	0	207	42
$LSD_{0.05}$	7	28	15	49	19	29	17	64.8	23

†JBW : Just before waterlogging, JBD : Just before drainage, 30DAD : 30 days after drainage.
‡WW : well watered, EW : excessive watered.

Table 3. Shoot nitrogen concentration of soybeans As affected by waterlogging treatment.

Variety	Shoot N concentration (mg N g⁻¹ DW)					JBW ~ JBD		JBD ~ 30DAD	
	JBW†	JBD		30DAD					
		WW‡	EW	WW	EW	WW	EW	WW	EW
SS2-2	27	31	16	30	19	4.2	-10.8	-1.1	2.7
Sakukei 4	33	34	19	29	16	1.1	-14.4	-4.9	-2.1
Sinpaldalkong 2	22	20	11	22	17	-1.3	-10.6	1.8	5.9
Enrei	21	21	14	23	18	0.5	-7.1	1.0	3.7
$LSD_{0.05}$	3.4	2.9	2.1	1.7	3.4	2.8	3.4	2.6	4.1

†JBW : Just before waterlogging, JBD : Just before drainage, 30DAD : 30 days after drainage.
‡WW : well watered, EW : excessive watered.

Nitrogen concentration

Shoot N content of WW plants increased rapidly (Table 2). In contrast, waterlogging treatment for 15 days rapidly reduced N-accumulation rate. There was no varietal difference in N accumulation except for Enrei during 30 days after drainage. Shoot N concentration of WW plants that recovered for 30 days were higher in supernodulating mutants than their wild-type soybeans (Table 3). However, shoot N concentration of EW soybeans was

Table 4. Leaf area of soybeans as affected by watgerlogging treatment.

Water treatment	Variety	Leaf area (cm² plant⁻¹) JBW†	JBD	30DAD	JBW ~ JBD	JBD ~ 30DAD
Well watered	SS2-2	264	753	884	489	204
	Sakukei 4	311	907	1286	596	379
	Sinpaldalkong 2	270	642	837	372	195
	Enrei	297	723	993	425	270
Excessive Watered	SS2-2	264	386	220	122	-166
	Sakukei 4	311	400	284	88	-116
	Sinpaldalkong 2	270	365	270	95	-95
	Enrei	297	390	338	93	-52
Analysis of variance‡						
P(W)		NS	<0.01	<0.01	<0.01	<0.01
P(V)		<0.01	<0.05	NS	NS	NS
P(W × V)		NS	NS	NS	NS	NS
$LSD_{0.05}$		31	145	248	147	325

†JBW : Just before waterlogging, JBD : Just before drainage, 30DAD : 30 days after drainage.
‡W: water treatment; V: variety; NS: not significant.

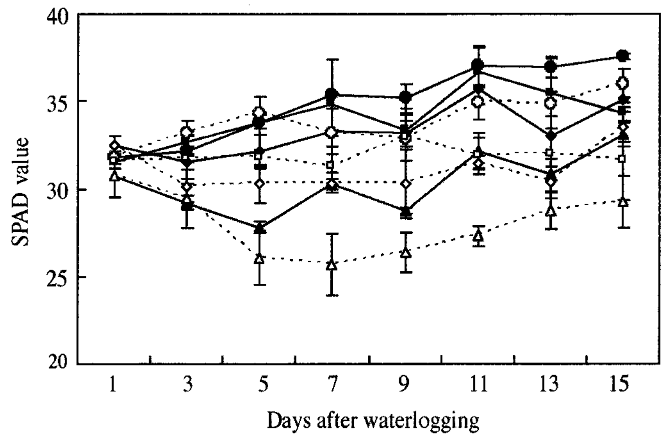

Fig. 3. SPAD value of the 2nd leaf of main stem in soybeans during waterlogging treatment. SS: SS2-2 (○), SA: Sakukei 4 (□), SP: Sinpaldalkong 2 (◇), EN: Enrei (△). WW: well watered (filled mark), EW: excessive watered (open mark). The vertical bars indicate standard deviation of three replication.

more decreased in supernodulating mutants. Wild-type soybeans showed a lower shoot N concentration in response to EW compared to supernodulating mutants, but did not show a varietal difference during 30 days after drainage.

Leaf area and spad value

Leaf area of WW soybeans increased constantly until 30 days after drainage, but that of EW soybeans decreased right after waterlogging (Table 4). Regardless of soybean genotypes, leaf area was reduced by waterlogging treatment. Supernodulating mutants showed greater reduction in leaf area than their wild-type soybeans. The 2nd leaf spad value of main stem increased steadily in WW condition (Fig. 3). Unlike WW soybeans, the spad value of EW soybeans increased slightly in the later period of waterlogging treatment, although the slight decrement was observed in the early period of waterlogging treatment. The leaf spad value of supernodulating mutants remained high during waterlogging compared to their wild-type soybeans, and the leaf spad value of EW soybeans was higher than that of WW. The leaf greenness by the leaf position on the main stem is presented in Fig. 4. The highest spad value of leaf was observed in the 3rd or 4th leaf from the top, and the reduction of spad value by waterlogging was increased at the low-positioned leaf from the top in wild-type soybeans.

Xylem sap component

Sap bleeding rate was much lower in EW plants than in WW plants (Table 5). A significant varietal difference was not found in EW but was in WW conditions. Although the Sakukei 4

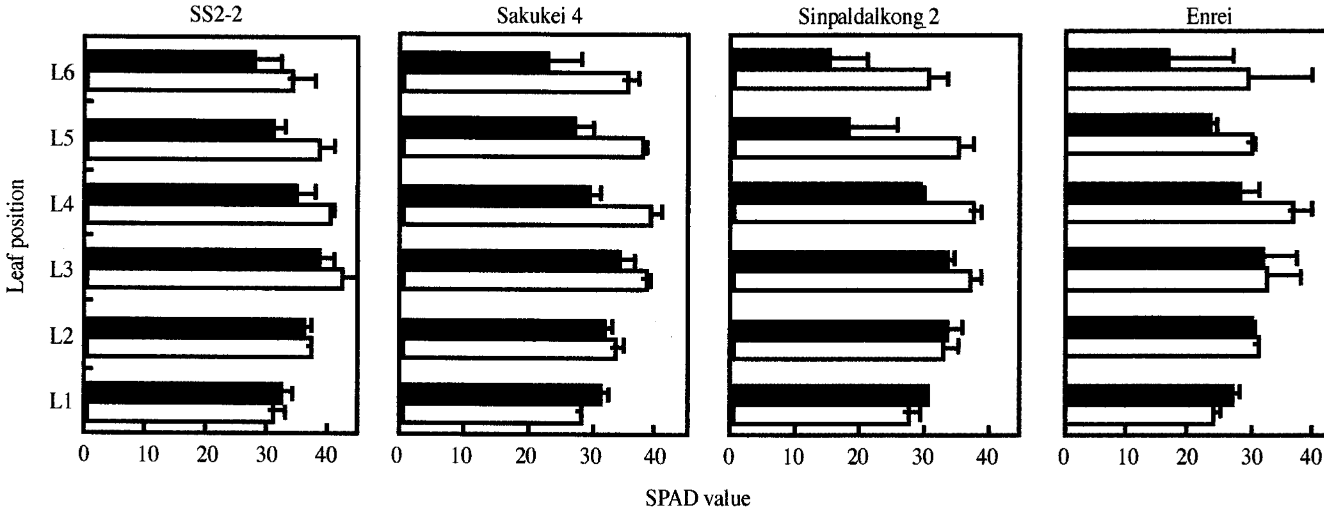

Fig. 4. SPAD values of leaves at different nodes on the main stem of soybeans. Well watered (open mark), Excessive watered (filled mark). L_{1+i} = leaf i node below L_1. The vertical bars indicate standard deviation of three replication.

Table 5. Bleeding sap rate of soybean hypocotyls as Affected by waterlogging treatment.

Variety	Bleeing sap rate (g h⁻¹ plant⁻¹)					JBW ~ JBD		JBD ~ 30DAD	
	JBW†	JBD		30DAD					
		WW‡	EW	WW	EW	WW	EW	WW	EW
SS2-2	0.36	0.47	0.10	0.44	0.27	0.10	-0.26	-0.03	0.17
Sakukei 4	0.44	0.68	0.14	0.76	0.28	0.24	-0.30	0.08	0.15
Sinpaldalkong 2	0.24	0.36	0.13	0.36	0.21	0.12	-0.11	-0.00	0.09
Enrei	0.33	0.58	0.15	0.43	0.26	0.25	-0.18	-0.15	0.11
$LSD_{0.05}$	0.11	0.13	0.04	0.10	0.08	0.05	0.09	0.10	0.05

†JBW : Just before waterlogging, JBD : Just before drainage, 30DAD : 30 days after drainage.
‡WW : well watered, EW : excessive watered.

showed a high sap bleeding rate at three sampling times in WW, sap bleeding rate was greatly reduced by waterlogging in SS2-2 and Sakukei 4. There was a significant difference in the relative ureide-N, α-amino-N, and nitrate-N to total N concentration between WW and EW plants (Table 6). The relative ureide-N content was decreased by waterlogging, especially in supernodulating mutants. The α-amino-N and nitrate-N content were increased by waterlogging, and again especially in supernodulating mutants.

Seed yield and harvest index

Shoot and seed dry weight decreased by waterlogging (Table 7). Shoot dry weight of EW supernodulating mutants was 30-41% that of WW plants. Shoot dry weight of wild-type soybeans was 45-51% of that in WW plants. The declining pattern of seed dry weight by EW was similar to the shoot dry weight. Harvest index of WW plants was greater in the supernodulating mutant, Sakukei 4, than in the wild type, Enrei. Harvest index increased only in EW wild-type soybeans.

Discussion

Inhibition of nodulation by waterlogging has been reported in several legume species (Andreeva et al. 1987; Bishnoi and Krishnamoorthy 1992; Khan 1974; Minchin and Pate 1975;). Sallam and Scott (1987) suggested that nodulation inhibition was attributed to the suppression of root growth and root hair development. In the anaerobic conditions, energy supply for the formation of nodules may be reduced (Sprent and Gallacher 1976). As shown in Fig. 2, both nodule number and weight increased rapidly in WW. This was in contrast to EW after waterlogging at R1 stage. However, this result was not in good agreement with previous studies (Nelson-Scheriber and Schreitzer 1986; Sung 1993), in which nodule number and weight built up rapidly after R1 in control plants, and increased ten days after waterlogging. In this study, waterlogged soybeans decreased nodule number by about 68-73% in supernodulating mutants, and about 60-65% in wild-type soybeans. Thirty days after drainage, the nodule number had not recovered, but the nodule weight slightly increased. This might be caused mainly by the increase in single nodule dry weight by 2.5 to 3.5 times (Fig. 2C).

Water/nutrient absorption and nitrogenase activity of root nodules were lowered by oxygen deficiency in the soil. Furthermore, CO_2 concentration in the flooded soil atmosphere could be increased, and biomass and elongation of soybean roots were inhibited by high CO_2 levels (Boru et al. 1997; Grable and Danielson 1965; Jackson 1979). In addition, the secondary aerenchyma might play a role in venting gases to the atmosphere as well as allowing oxygen to transport to growing points in soybean plants (Shimamura et al. 2003). As shown in Fig. 5, the formation of secondary aerenchyma and adventitious roots was shown in all soybeans after waterlogging. Sap bleeding rate of waterlogged soybean plants was restricted by 78-80% in supern-

Table 6. Relative-N content (%) to total N concentration in the xylem sap of soybeans as affected by waterlogging treatment.

Water treatment	Variety	Ureide-N (%)			α-amino-N (%)			Nitrate-N (%)		
		JBW†	JBD	30DAD	JBW	JBD	30DAD	JBW	JBD	30DAD
Well watered	SS2-2	93	95	85	6	5	15	0.8	0.5	0.4
	Sakukei 4	83	95	89	14	4	10	3.1	0.5	0.4
	Sinpaldalkong 2	75	94	89	22	5	11	3.1	0.6	0.6
	Enrei	46	94	88	43	6	11	10.8	0.4	0.5
Excessive Watered	SS2-2	93	52	68	43	45	24	10.8	2.7	7.5
	Sakukei 4	83	60	87	43	38	12	10.8	2.0	1.5
	Sinpaldalkong 2	75	89	80	43	8	17	10.8	2.4	2.8
	Enrei	46	73	84	43	26	16	10.8	1.3	0.6
Analysis of variance‡										
P(W)		NS	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
P(V)		<0.01	<0.01	<0.01	<0.01	<0.01	<0.05	<0.05	NS	<0.01
P(W × V)		NS	<0.01	<0.05	<0.01	<0.01	NS	<0.05	NS	<0.01
$LSD_{0.05}$		7.5	8.1	6.6	4.6	8.0	7.2	5.0	0.9	1.6

† JBW : Just before waterlogging, JBD : Just before drainage, 30DAD : 30 days after drainage.
‡ W: watertreatment; V: variety; NS: not significant.

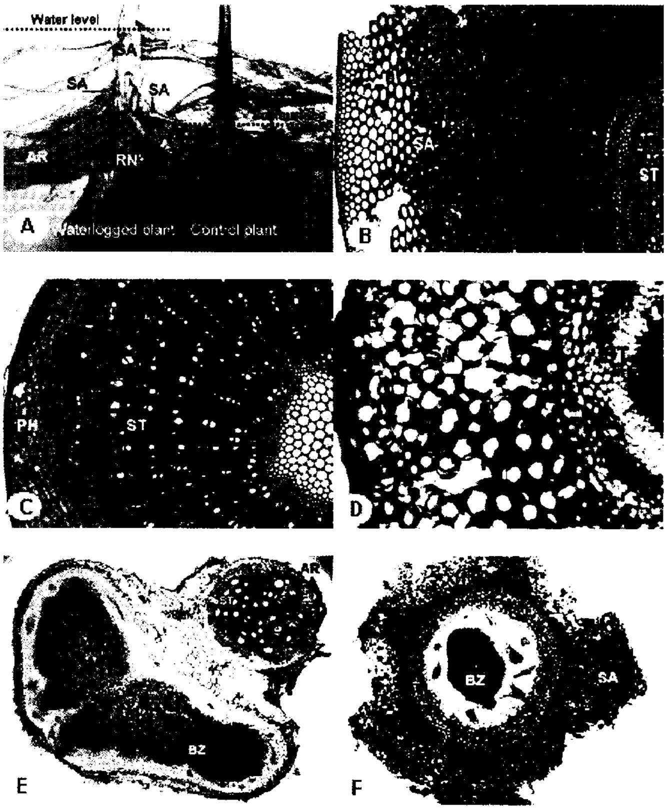

Fig. 5. Development of secondary aerenchyma (SA) in hyopcotyl, adventitious root (AR), and root nodules (RN) of soybean, cv. Sinpaldalkong 2, after 15 days of waterlogging. A: root system, B: flooded hypocotyl, C: irrigated hypocotyl, D: flooded adventitious root, E: irrigated adventitious root and root nodule, F: flooded root nodule, BZ: bacteroid zone, PH: phellem, ST: stele.

odulating mutants, and by 65-74% in wild types compared to control plants (Table 5). This might cause the remarkable reduction of shoot and root dry weight (Table 1), and leaf area (Table 4) in the waterlogged soybean plants.

Meanwhile, waterlogging treatment at R1 stage also inhibited the nitrogen fixing activities of nodules (Sung 1993). This might have resulted from the decrease in nitrogenase synthesis in the bacteroids (Bisseling et al. 1980), or by the curtailed carbohydrate metabolism of host tissue in the nodule (Huang and LaRue 1985). N_2 fixation was more inhibited by waterlogging stress than the biomass accumulation (Bacanamwo and Purcell 1999). This was consistent with our study. Shoot dry weight of waterlogged plants was 61-75% of control plants just before drainage and 25-35% after recovery for 30 days (Table 1). Similarly, shoot N content of waterlogged plants was 31-49% of control plants at just before drainage and 13-27 % at 30 days after drainage (Table 2). Also, the reduction of shoot N concentration (Table 3) by waterlogging was considered due to inhibited nitrogen fixing activities of nodules. This significant decrease in N content right after waterlogging for 15 days was evident by the decreased greenness of leaves (Figs. 3 and 4). Leaf greenness determined by the SPAD chlorophyll meter has been associated with leaf N in the low and medium leaf N concentration ranges (Dwyer et al. 1995). During waterlogging for 15 days, supernodulating mutants maintained more greenness than their wild

Table 7. Shoot and seed dry weight (DW) and harvest index (HI) of soybeans as affected by waterlogging treatment.

Varieties	Shoot DW (g plant^{-1})			Seed DW (g plant^{-1})			Harvest index		
	WW[†]	EW	EW / WW	WW	EW	EW / WW	WW	EW	EW / WW
SS2-2	8.0	3.2	0.40	6.0	2.5	0.43	0.43	0.44	1.04
Sakukei 4	9.5	2.8	0.29	8.5	2.6	0.31	0.47	0.48	1.02
Sinpaldalkong 2	7.5	3.8	0.51	5.6	3.1	0.56	0.43	0.45	1.05
Enrei	8.1	3.3	0.40	4.9	3.8	0.77	0.38	0.54	1.42
$LSD_{0.05}$	1.7	1.0	0.08	1.4	1.5	0.24	0.03	0.11	0.24

[†] WW : well watered, EW : excessive watered.

types (Fig. 3). The supernodulating mutants showed higher N content right after waterlogging for 15 days, whereas the N content and nodule weight were higher in wild-type soybeans 30 days after recovery by drainage.

N_2 fixation of soybean was the limiting factor under flooding condition that was further supported by the observation that soybean plants supplemented with nitrate were less flood sensitive than those relying on N_2 fixation as the N source. Inhibition of biomass and N accumulation by flooding was not greater under nitrate-supplemented soybean plants (Bacanamwo and Purcell 1999). In this study, nitrogen fertilizer was not applied. As this might cause nitrogen shortage at the reproductive growth stage, soybeans in this study might mainly depend on N_2 fixation as an N source. There were significant differences in nitrogen fixation ability among soybean genotypes and waterlogging treatment (Table 6). The relative-ureide N (%) to total N concentration was 94 to 95% among WW soybean plants whereas that of waterlogged plants was lower in supernodulating mutants (52 to 62%) than in wild types (73 to 89%), indicating that inhibition of N_2 fixation activity by waterlogging was more severe in supernodulating mutants than in wild-type soybeans. Although the supernodulating mutants showed higher shoot N content right after waterlogging, the specific nodule activity (SNA) of supernodulating mutants was lower than the wild types due to weak flood tolerance and their low SNA. Purcell and Sinclair (1995) also found that nodulated root respiration rates were approximately twice as great as respiration rates of roots without nodules.

Differences in the dry matter yield response have been reported by many researchers (Heatherly and Pringle 1991; Scott et al. 1989; Won et al. 2006; Youn et al. 2008). The dry matter yield was decreased by waterlogging and significant varietal differences were found in this experiment (Tables 1 and 7). Seed yield of supernodulating mutants was more adversely affected by waterlogging than that of their wild types. Lower seed yields were considered mainly due to the lesser specific nodule activity which may increase N deficiency and subsequent decreases in biomass accumulation.

In summary, the supernodulating mutants showed a weaker waterlogging tolerance than their wild types. Under waterlogging conditions, nodulation was considerably destroyed, and specific nitrogenase activity had not recovered well after waterlogging in supernodulating soybean plants. Supernodulating mutants showed lower seed yield than the wild types in waterlogging conditions. This might be caused mainly due to the lesser specific nodule activity and subsequent decrement of biomass accumulation. Further research is needed to evaluate the tolerance of supernodulating mutants in several environmental conditions.

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References

Andreeva IN, Swaraj K, Kozlova GI, Raikhman LA. 1987. Changes in ultrastructure and nitrogen-fixing activity of soybean nodules under the influence of flooding. Soviet Plant Physiol. 34: 427-435

Bacanamwo M, Purcell LC. 1999. Soybean dry matter and N accumulation responses to flooding stress, N sources and hypoxia. J. Exp. Bot. 50: 689-696

Bishnoi NR, Krishnamoorthy HH. 1992. Effect of waterlogging and gibberellic acid on nodulation and nitrogen fixation on peanut. Plant Physiol. Biochem. 28: 663-666

Bisseling T, van Staveru W, van Kammen A. 1980. The effect of waterlogging on the synthesis of nitrogen components in bacteroids of *Rhizobium leguminosarum* in root nodules of *Pisum sativum* L. Biochem. Biophys. Res. Commun. 93: 687-693

Buttery BR. 1987. Some effects of waterlogging and supply of combined nitrogen on soybean growth. Can. J. Plant Sci. 67: 69-77

Boru G, VanToai TT, Alves JD. 1997. Flooding injuries in soybean are caused by elevated carbon dioxide levels in the root zone. In MA Bennett, JD Metzger, eds., Proc. Fifth Natl. Symp. on Stand Establishment. 205-209

Cataldo DA, Haroon M, Schrader LE, Youngs VL. 1975. Rapid colorimetric determination of nitrate in plant tissue by nitration of salicylic acid. Commum. Soil Sci. Plant Anal. 6: 71-80

Chen JJ, Chen CL, Sung FJM. 1992. Flooding effect on photosynthesis in soybean leaves. J. Agri. For. 41: 17-26

Dwyer LM, Anderson AM, Ma BL, Stewart DW, Tollenaar M, Gregorich E. 1995. Quantifying the non-linearity in chlorophyll meter response to corn leaf nitrogen concentration. Can. J. Plant Sci. 75: 179-182

Fehr WR, Caviness CE. 1977. Stages of soybean development. Ext. Bull. 80. Iowa State University, Ames, IA

Garcia-Novo F, Crawford RMM. 1973. Soil aeration, nitrate reduction and flooding tolerance in higher plants. New Phytol. 72: 1031-1039

Grable A, Danielson RE. 1965. Effect of carbon dioxide, oxygen, and soil moisture suction on germination of corn and soybeans. Soil Sci. Soc. Am. Proc. 29: 12-18

Griffin JL, Sexton AM. 1988. Response of solid-seeded soybean to flood irrigation flood duration. Agron. J. 80: 885-888

Heatherly LG, Pringle III HC. 1991. Soybean cultivar's response to flood irrigation of clay soil. Agr. J. 83: 231-236

Herridge DF. 1984. Effects of nitrate and plant development on the abundance of nitrogenous solutes in root-bleeding and vacuum-extracted exudates of soybean. Crop Sci. 25: 173-179

Hoff T, Stummann BM, Henningsen KW. 1992. Structure, function and regulation of nitrate reductase in higher plants. Physiol. Plant 84: 616-624

Hong TD, Minchin FR, Summerfield RJ. 1977. Recovery of nodulated cowpea plants (*Vigna unguiculata* (L.) Walp.) from waterlogging during vegetative growth. Plant Soil 48: 661-672

Huang CY, LaRue TA. 1985. Effects of waterlogging on the nitrogen fixation and carbohydrate metabolism in nodules of soybean plant. Proc. Natl. Sci. Counc. B. ROC 9: 82-88

Jackson MB. 1979. Rapid injury to peas by soil waterlogging. J. Sci. Food Agric. 30: 143-152

Khan AG. 1974. The occurrence of mycorrhizae in halophytes, hydrophytes and xerophyte and endogone spores in adjacent soils. J Gen. Microbiol. 81: 7-14

Kozlowski TT, Pallardy SG. 1984. Effects of flooding on metabolism, In TT Kozlowski, ed, Flooding and plant growth. Academic Press, Orlando, pp 165-193

Matsumoto T, Yatazawa M, Yamamoto Y. 1977. Distribution and change in the contents of allantoin and allantoic acid in developing nodulating and non-nodulating soybean plants. Plant Cell Physiol. 18: 353-359

McClure PR, Israel DW. 1979. Transport of nitrogen in the xylem of soybean plants. Plant Physiol. 64: 411-416

Minchin FR, Pate JS. 1975. Effects of water, aeration and salt regime on nitrogen fixation in a nodulated legume: definition of an optimum root environment. J. Exp. Bot. 26: 60-80

Minchin FR, Summerfield RJ. 1976. Symbiotic nitrogen fixation and vegetative growth of cowpea (*Vigna unguiculata* (L.) Walp.) in waterlogged conditions. Plant Soil 45: 113-117

Nelson-Schreiber BM, Schreitzer LE. 1986 Limitations on leaf nitrate reductase activity during flowering and pod fill in soybean. Plant Physiol. 80: 454-458

Pate JS, Atkins CA, White ST, Rainbird RM, Woo KC. 1980. Nitrogen nutrition and xylem transport of nitrogen in ureide-producing grain legumes. Plant Physiol. 65: 961-965

Puiatti M, Sodek L. 1999. Waterlogging affects nitrogen transport in the xylem of soybean. Plant Physiol. Biochem. 37: 769-773

Purcell LC, Sinclair TR. 1995. Nodule gas exchange and water potential response to rapid imposition of water deficit. Plant Cell Environ. 18: 179-187

Reggiani R, Brambilla I, Bertani A. 1985. Effect of exogenous nitrate on anaerobic metabolism in excised rice roots. 1. Nitrate reduction and pyridine nucleotide pools. J. Exp. Bot. 36: 1193-1199

Russell DA, Wong DML, Sachs MM. 1990. The anaerobic response of soybean. Plant Physiol. 92: 401-407

Ryle GJA, Powell CE, Gordon AJ. 1978. Effect of source of nitrogen on the growth of Fiskeby soya bean: the carbon economy of whole plants. Annals Bot. 42: 637-648

Sallam A, Scott HD. 1987. Effects of prolonged flooding on soybean at the R2 growth stage. I. Dry matter and N and P accumulation. J. Plant Nutr. 10: 567-592

Scott HD, DeAngulo J, Daniels MB, Wood LS. 1989. Flood duration effects on soybean growth and yield. Agron. J. 81: 631-636

Shimamura S, Mochizuki T, Nada Y, Fukuyama M. 2003. Formation and function of secondary aerenchyma in hypocotyl, roots and nodules of soybean (*Glycine max*) under flooded conditions. Plant Soil 251: 351-359

Smit BA, Neuman DS, Stachowiak ML. 1990. Root hypoxia reduces leaf growth. Role of factors in the transpiration stream. Plant Physiol. 92: 1021-1028

Sprent JI, Gallacher A. 1976. Anaerobiosis in soybean root nodules under water stress. Soil Biol. Biochem. 8: 317-320

Sung FJM. 1993. Waterlogging effects on nodule nitrogenase and leaf nitrate reductase activities in soybean. Field Crops Res. 35: 183-189

Tian XH, Nakamura T, Kokubun M. 2005. The role of seed structure and oxygen responsiveness in pre-germination flooding tolerance of soybean cultivars. Plant Prod. Sci. 8: 157-165

Turner FT, Sij JW, McCauley GN, Chen CC. 1983. Soybean seedling response to anaerobiosis. Crop Sci. 23: 40-44

Vessey KJ, Walsh KB, Layzell DB. 1988. Oxygen limitation of N_2 fixation in stem-girdled and nitrate-treated soybean. Physiol. Plant 73: 113-121

Won JY, Ji HC, Cho JW. 2006. Nitrogen uptake and growth of soybean seedling under flooding stress. Kor. J. Crop Sci. 51: 118-122

Youn JT, Van K, Ryu YH, Shinji S, Lee CK, Kim SL, Seo SJ, Lee SH. 2008. Evaluation of nitrogen fixation between supernodulating soybean mutants and their wild-types using ^{15}N in field conditions. J. Crop Sci. Biotech. 11: 31-38

Young EG, Conway CF. 1942. On the estimation of allantoin by the Rimini-Schryver reaction. J. Biol. Chem. 142: 839-853