Nitrogen Uptake and Growth of Soybean Seedlings under Flooding Stress

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ABSTRACT : This experiment was carried out on plastic pots (40cm×25cm×30cm) filled with sand soil at greenhouse using two soybean cultivars with small seed; one was Pungsannamulkong (PSNK) recognized as a tolerant cultivar against excessive water stress and the other one was Sobaeknamulkong (SBNK) recognized as a susceptible cultivar. Seed was sown with 30 plants of 2 hills, and the amount of applied fertilizer was N; 3.0 g, P; 3.0 g, and K; 3.4 g per m^2 with all basal fertilizations. Plants were grown under photoperiod of natural light with day temperature of 31±5 °C and night temperature of 22±1 °C. The flooding treatment was done for 3, 5, 7 and 10 days by filling pots with tap water up to 1 cm above the level of the soil surface when plants were 2 days after emerging. Nitrogen uptake by leaves of soybeans decreased significantly by the flooding after 6 days. This significant reduction of N uptake by flooding was evidently recognized from the chlorosis of leaves. The dry matter of flooded soybean seedlings significantly decreased compared to non-flooded soybean seedlings at 10 days. The dry matter of roots also showed similar result of the shoot. Shoots had more N reduction than roots under the flooding. This N reduction was more pronounce in SBNK than in PSNK. Chlorophyll content of flooded soybeans showed decreasing or non-increasing tendency, and the reduction of chlorophyll content was more in SBNK than in PSNK from the flooding stress. Nitrate content of soybean seedlings with flooding stress showed decreasing tendency in shoot and root parts. Ammonium content, however, was higher in flooding stress compared to the non-flooding. Flooding caused a remarkable change in the AA (amino acid) composition and TAA (total amino acid) concentration in the leaves of soybean seedlings.

Keywords: flooding, soybean, N uptake, amino acid

Flooding from excessive rainfall is an important stress that reduces soybean growth and seed yield throughout the world. Natural flooding can be classified into two categories: (1) stream flooding, characterized by the overflow of rivers or creeks into a flood plain and (2) lowland flooding, characterized by inadequate surface drainage and slow soil permeability of depressional areas. Flooding causes premature senescence in soybean which resulted in leaf chlorosis, necrosis, defoliation, cessation of growth and reduced seed yield. Seed yields were generally similar when flood periods were 1~2 days but when flood periods were held longer than 2 days at R2 stage, average yield reduction was about 50% compared to non-treated plant (Griffin & Saxton, 1988). Also, on-farm researches indicated that a flooding for as little as three days at the early vegetative growth stages could kill soybeans (Sullivan *et al.*, 2001) and waterlogging for as little as two days at the V4 growth stage (Fehr & Caviness, 1977) reduced the seed yield by 18% (Scott *et al.*, 1989). The determinate soybean cultivars were more susceptible to prolonged excessive water stress during early reproductive growth than early vegetative growth (Griffin & Saxton, 1988).

On the other hand, due to the lower energy yield of fermentation compared to respiration a disturbance of the cellular energy supply may be assumed thereby affecting the maintenance of endergonic processes such as ion uptake. Oxygen deficiency under a flooding condition caused serious root injury to many land plants. Under these conditions, plants produced a number of glycolytic end products including ethanol, lactate, and various organic acids and amino acids derived from pyruvate (Drew, 1997).

Nitrogen has to be taken up in a large amount in order to maintain growth and development, because of its high abundance in plants (Marschner, 1995). In cowpea, the flooding decreased N_2 fixation relatively more than vegetative growth, and reduction of N_2 fixation was responsible for decreased vegetative growth (Minchin & Summerfield, 1976).

Therefore, this study was carried out to characterize the N uptake, NO_3 and NH_4 content and concentrations of N-containing compounds (amino acid, chlorophyll) of two soybean cultivars with different flood periods at early seedling stage.

MATERIALS AND METHODS

Plant culture and flooding treatment

This experiment carried out on plastic pots (40cm×25cm

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×30cm) filled with sand soil at greenhouse affiliated to Collage of Agriculture & Life Science, Chungnam National University in Korea using two soybean cultivars with small seed; one was Pungsannamulkong (PSNK) recognized as a tolerant cultivar of excessive water stress and the other one was Sobaeknamulkong (SBNK) recognized as a susceptible cultivar of excessive water stress.

Seed was sown with 30 plants of two hills and the amount of fertilizer applied was N; 3.0 g, P; 3.0 g, and K; 3.4 g per m^2 with all basal fertilizations. Plants were grown under photoperiod of natural light with day temperature of 31±5 °C and night temperature of 22±1 °C. The flooding treatment was done for 3, 5, 7 and 10 days by filling pots with tap water up to 1 cm above the level of the soil surface when plants were 2 days after emerging. Control plants remained in well watered (about 60% soil moisture) condition during the experiment.

Chlorophyll content

Chlorophyll content of soybean seedlings was measured by the method of Yoshida *et al.* (1972). The total chlorophyll of soybean seedlings flooded for 3, 5, 7 and 10 days was measured with a spectrophotometer (UV-120-02 Shimadzu) at 652 nm.

Nitrogen, nitrate and ammonium content

All samples were dried at 80 °C during 72 hrs in a dry oven and ground with 20 mesh to measure the total nitrogen content of flooded soybean seedlings for 3, 5, 7, and 10 days. Nitrogen uptake was determined by the modified Kjeldahl method. Nitrate content was determined by modified Cataldo *et al.* (1975) method. Also, ammonium content was determined by Chaney & Marbach (1962) method.

Amino acid concentration

The amino acid was analyzed using an amino acid analyzer (Biochrom 20, Phamarchia Biotech., UK) after filtered a dried powder sample of 0.2 g with a 0.45 μm filter. The dried powder sample was inserted into 6N-HCl and hydrolyzed for 24 minutes, and then it was decompression concentrated with a rotary evaporator and melted with 100 ml of distilled water before filtering.

RESULTS AND DISSCUSION

Flooding inhibited the biomass accumulation and N_2 fixation, but the inhibition of N_2 fixation occurred earlier and was more pronounce than the inhibition of biomass accumu-

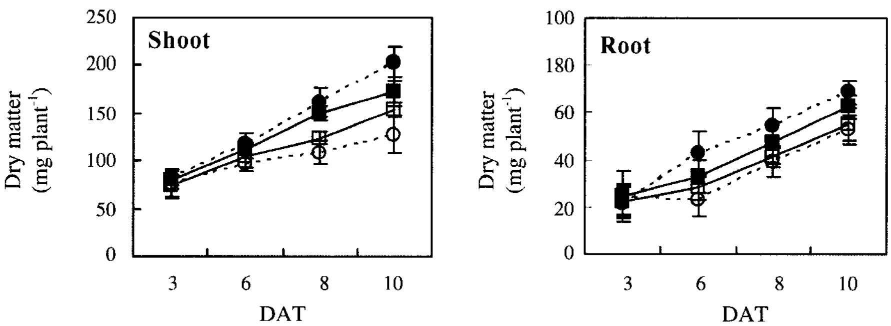

Fig. 1. Dry matter accumulation of soybean seedlings under different flooding periods. ■ ; PSNK control. □ ; PSNK treatment. ● ; SBNK control. ○ ; SBNK treatment. Bars indicates means ± SE. DAT; days after treatment.

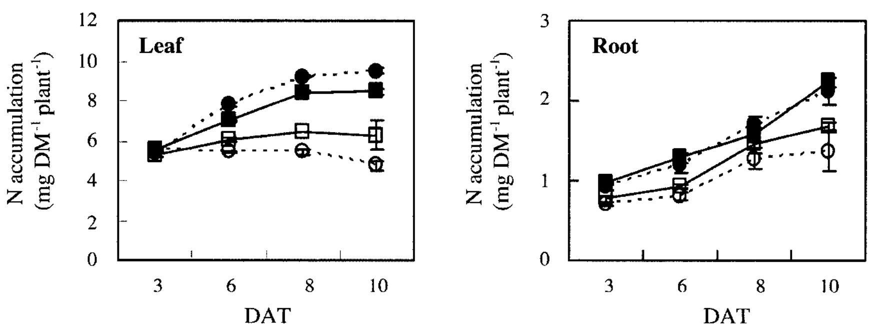

Fig. 2. Nitrogen uptake of soybean seedlings under different flooding periods. ■ ; PSNK control. □ ; PSNK treatment. ● ; SBNK control. ○ ; SBNK treatment. Bars indicates means ± SE. DAT; days after treatment.

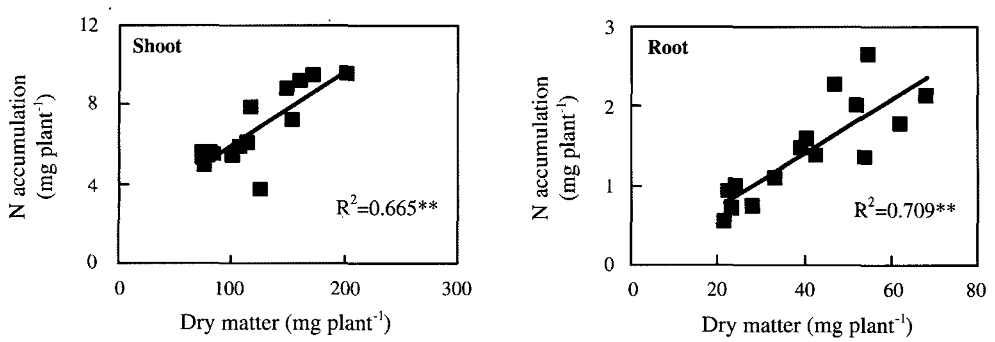

Fig. 3. Relationship between N uptake and dry matter of two soybean seedlings on flooding stress.

lation (Bacanamwo & Purcell, 1999).

There was no effect of flooding on leaf dry matter at 6 days (Fig. 1). However, nitrogen uptake of soybeans decreased significantly by the flooding after 6 days in leaves. This significant reduction of nitrogen uptake by flooding was evidently recognized from the yellowing of leaves (Fig. 2). Dry matter of flooded soybean seedlings significantly decreased when compared to non-flooded soybeans at 10 days (Fig. 1). At 10 days, the dry matter of shoot in flooded soybeans significantly decreased compared to non-flooded plants (Fig. 1). The reduction of dry matter by flooding was 11% and 37% in PSNK and SBNK, respectively, at 10 days. Although the dry matter of shoot of PSNK was lower than that of SBNK in non-flood plants, reduction of dry matter in PSNK was slightly smaller than that of SBNK. The dry matter of root showed a similar trend that of the shoot.

The nitrogen uptake reduction by the flooding may be caused by the decreased accumulation of the dry matter. Wilson (1988) suggested that under nutrient deficits, shoots were more starved than roots, and this could decrease photosynthesis and overall biomass accumulation.

In this experiment, shoots showed more nitrogen reduction than roots under the flooding (Fig. 2). After 8 days, the reduction of the nitrogen uptake of flooded plants was 33% and 17% in shoots and roots, respectively, relative to the control plants, and the reduction of the nitrogen uptake was 38% and 30% in shoot and root, respectively, after 10 days.

On other hand, with the flooding stress in soybean seedlings, the nitrogen uptake in shoots and roots of two cultivars reduced obviously at 6 days. This reduction was more pronounce in SBNK than in PSNK.

There also were highly significant differences between the nitrogen uptake and dry matter in root and shoot (Fig. 3). The coefficient of determination of root was higher (R^2 = 0.709**) than shoot (R^2 = 0.665**). Therefore, under flooding stress, the reduction of the nitrogen uptake tended to decrease the dry matter accumulation more in root than in shoot. The chlorophyll content of two soybean cultivars by

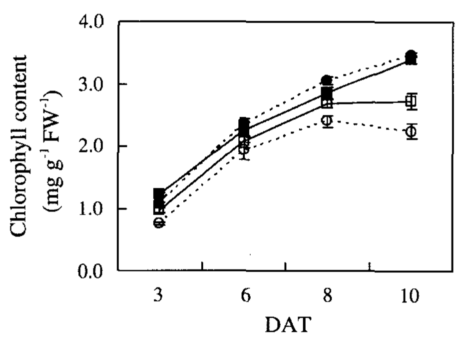

Fig. 4. Chlorophyll content of soybean seedlings under different flooding periods. ■ ; PSNK control. □ ; PSNK treatment. ● ; SBNK control. ○ ; SBNK treatment. Bars indicates means ± SE. DAT; days after treatment.

flooding stress showed increasing tendency up to 8 days compared to the controls, but after that, the chlorophyll content of flooded soybeans showed decreasing or non-increasing tendency.

The chlorophyll content in controls of SBNK and PSNK was 3.47 mg F.W.$^{-1}$ and 3.40 mg F.W^{-1} at 10 days, respectively, but the chlorophyll content of flooded soybean seedlings was 2.24 mg F.W.$^{-1}$ and 2.74 mg F.W.$^{-1}$ in SBNK and PNSK, respectively, at the same time. The reduction of chlorophyll content was more markedly in SBNK than in PSNK at the flooding stress (Fig. 4).

Yardanova & Popova (2001) reported that the chlorophyll content showed noticeable decrease after 3 days in barley seedlings with the flooding stress. And the flooding stress reduced the chlorophyll content of soybean compared to non-flooded plants at paddy field (Hideki *et al*, 1988). Ashraf & Rehman (1999) reported that there was a high correlation between the chlorophyll content of leaves and CO_2 uptake. According to Else *et al.* (1995), the flooding of 24 hours decreased the transpiration rate of tomato seedlings. Therefore, the reduction of the photosynthesis by reduced chlorophyll content of leaves could be declined the biomass

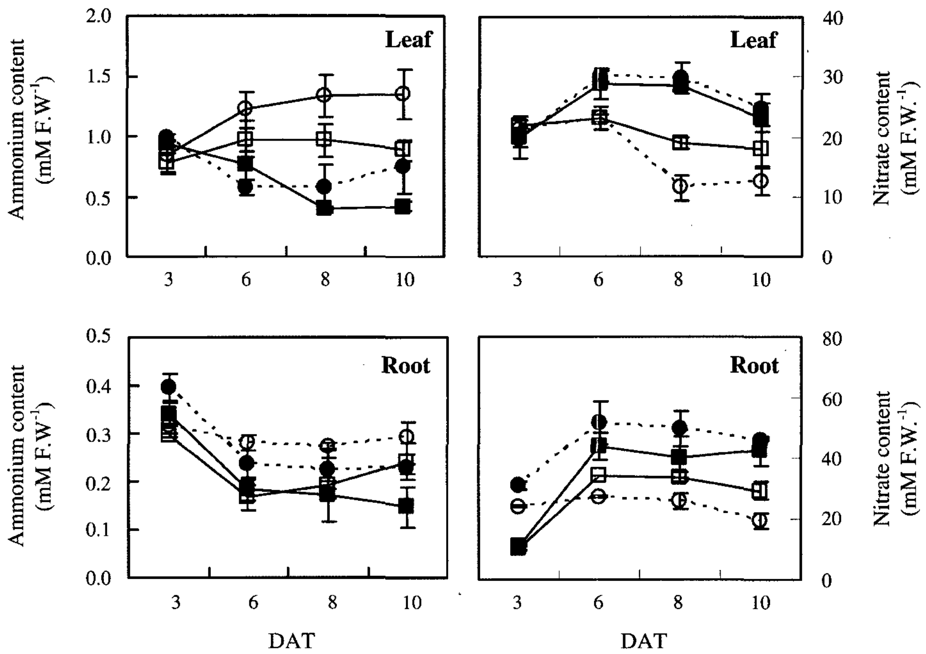

Fig. 5. Ammonium and nitrate content of soybean seedlings under different flooding periods. ■ ; PSNK control. □ ; PSNK treatment. ● ; SBNK control. ○ ; SBNK treatment. Bars indicates means ± SE. DAT; days after treatment.

Table 1. Amino acid concentration (μg g^{-1} DW^{-1}) of soybean seedlings at 10 days after flooding stress.

Amino acid	Leaf				Root			
	SBNK		PSNK		SBNK		PSNK	
	Con	Treat	Con	Treat	Con	Treat	Con	Treat
Aspartic acid	5.00	2.17	8.16	5.16	3.44	2.07	5.47	5.97
Serine	2.58	0.77	3.12	2.59	1.56	1.03	1.89	1.65
Glutamic acid	3.70	1.08	4.64	3.91	2.10	1.50	2.41	2.32
Histidine	2.06	0.61	2.45	2.12	1.06	1.10	1.46	1.37
Arginine	2.62	ND†	3.54	2.35	0.83	0.52	1.10	0.87
Threonine	2.52	0.02	3.09	2.28	0.49	0.50	0.74	0.71
Alanine	2.95	1.10	3.47	2.29	1.71	1.55	2.06	2.08
Proline	3.57	0.07	4.70	3.31	1.70	1.08	2.43	2.19
Cysteine	1.27	ND	1.60	0.80	0.68	0.79	0.99	0.77
Tyrosine	2.28	ND	2.56	1.47	ND	0.30	0.09	0.47
Valine	1.73	ND	2.28	1.35	0.49	0.56	0.81	0.74
Methionine	0.53	0.13	0.76	0.33	0.17	0.70	0.14	0.47
Lysine	1.10	0.40	1.88	1.54	0.97	0.85	1.02	0.70
Isoleucine	1.74	0.38	2.19	1.92	0.79	0.89	1.05	0.93
Leucine	3.66	0.30	4.63	3.30	1.14	1.34	1.61	1.41
Phenylalanine	3.86	0.32	4.17	3.29	0.90	1.15	1.30	1.37
TOTAL	41.19	7.35	53.24	38.01	18.03	19.93	24.58	24.02

†ND; not detected

accumulation with flooding stress.

In flooded soils, growth reduction of shoot and root was more closely related to the declining O_2 concentration in the soil solution than to the concentration of dissolved inorganic nitrogen (Alam, 1999). And there was a rapid depletion of NO_3^-, as free O_2 was quickly consumed by soil biota, development of anaerobic conditions, and loss of active soil, but N was freely promoted through denitrification in waterlogging soils.

In this experiment, the nitrate content of soybean seedlings with the flooding stress showed decreasing tendency in shoot and root parts. Ammonium content, however, was higher in flooding stress compared to non-flooding (Fig. 5). Also, under the flooding stress, ammonium content was less in PSNK than in SBNK, but nitrate content was higher in PSNK than in SBNK.

Flooding caused a remarkable change of amino acid (AA) composition and total amino acid (TAA) concentration of the leaves of soybean seedlings (Table 1). TAA of non-flooded soybean at leaves and root was 53.24 and 24.58, respectively, in PSNK, and was 41.19 and 18.03, respectively, in SBNK. TAA concentration of flooded soybean seedlings for 10 days was 7.35 on leaves and was 15.93 on root in SBNK, and it was 38.01 on leaves and was 24.02 on root in PSNK.

AA content of leaves was more in PSNK than in SBNK. Arginine, cysteine, tyrosine and valine were not founded in leaves of SBNK seedlings, and cysteine, tyrosine and valine were not detected in leaves of PNSK seedlings when the flood stress was applied for 10 days. Therefore, as concomitant with decreased N uptake, TAA concentration in leaves of the water-stress tolerant SBNK was significantly reduced by 10 days of flooding, but the effect of flooding stress in PSNK treatment was significantly less then in SBNK treatment.

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