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Full Length Research Paper

Genotypic variation in morphological and physiological response of soybean to waterlogging at flowering stage

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The experiment was conducted in greenhouse conditions at Vietnam National University of Agriculture to investigate genotypic variation in morphological and physiological responses of five soybean cultivars to waterlogging at the flowering stage. Seeds of each cultivar were sown in pots containing 6 kg dry soil. At early flowering stage, treatment pots were subjected to waterlogging by maintaining the water level of 3 cm above the soil surface for ten days. The results showed that the nodule number, nodule dry weight, chlorophyll content, carbon exchange rate, dry matter accumulation and nitrogen content were significantly reduced in waterlogging conditions. Among soybean cultivars, D140 and D912 showed better root length, nitrogen accumulation and total biomass under waterlogging conditions. Breeding for higher root growth, maintaining high nitrogen accumulation and total biomass could be an important strategy to improve soybean cultivar for waterlogging tolerance.

Key words: Flooding, flowering stage, nodule bacteria, soybean.

INTRODUCTION

Waterlogging stress has injured plant growth and caused yield loss in the world's crop production (Ronsenzweig et al., 2002). In soybean, photosynthesis (Cho et al., 2006), nitrogen fixation (VanToai et al., 1994; Shimamura et al., 2003; Henshaw et al., 2007a), biomass and grain yield (Bacanamwo et al., 1999; Henshaw et al., 2007b; Rhine et al., 2010) were reduced as a result of waterlogging effects. Grain yield loss of soybean was estimated about 30% by waterlogging stress at V2 stage, while it exceeded 93% when waterlogging occurred at R3 stage (Linkemer et al., 1998).

Lack of oxygen in flooded soil area inhibited root respiration completely (Blom and Voesenek, 1996). There was a strong relation between the developments of root system with top growth (Yang et al., 2012; Sallam and Scott, 1987; Araki et al., 2012). Waterlogging stress firstly affected root system and at the later stages shoot growth was influenced (Sakazono et al., 2014). When plant was subjected to waterlogging stress, their common response changed in alternation of stomatal behavior and photosynthesis rate. However, these changes could be at different levels, because of difference in waterlogging tolerance ability in various species and cultivars. The rapid reduction in photosynthesis activity was observed in waterlogging intolerant species such as maize (Zaidi et al., 2003) or wheat (Malik et al., 2001), whereas increase or less reduction in photosynthesis rate which were due to higher root development were found in barley (Pang et al., 2004), lotus (Stricker et al., 2005), lucerne glass (Irving et al., 2007), wetland plants (Li et al., 2007), which had higher root development under waterlogging condition.

In soybean, Sakazono et al. (2014) found that short-term waterlogging during early growth stage inhibited root elongation and root branching in most of soybean genotypes, but not in waterlogging tolerant soybean lines. This suggested that slow development of root system under waterlogging condition could restrict plant growth, but waterlogging tolerant soybean cultivar might maintain growth by maintaining development of root system. Root elongation under waterlogging stress could be an important factor contributing to waterlogging tolerance in soybean. Moreover, normal root was replaced by adventitious root which was formed under waterlogging

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conditions (Thomas et al., 2005; Colmer and Voenesk, 2009). The formation of adventitious root could contribute to plant growth during the waterlogging stress (Rich et al., 2012). Monitoring of root growth characters is an important strategy to improve waterlogging tolerance ability in soybean plants. Previous study showed that maintaining root growth at seedling stage is important for soybean growth under water stress (Sakazono et al., 2014). However, there is little information on genotypic variation in root characteristics in response of soybean genotypes to waterlogging at flowering stage.

In Vietnam, soybean cultivation after harvesting rice is an effective solution to expanse soybean production to meet the domestic demand. However, waterlogging by the excessive water in the soil of paddy field as well as frequent heavy rain during seedling and flowering stages could badly affect the growth and yield, as well as constrain soybean production (Hattori et al., 2014). Therefore, studying the mechanism to determine useful characteristics for breeding soybean cultivars to improve waterlogging tolerance is extremely necessary. This study was conducted to investigate the effects of waterlogging at flowering stage on root development and physiological traits of various soybean cultivars.

MATERIALS AND METHODS

Materials and experimental design

Five soybean cultivars named DT84, D140, D912, AK03 and K7833 were used in this study. DT84 is a widely planted cultivar in Vietnam. D140 and D912 were new recommended cultivars developed by the Department of Industrial and Medicinal Plant Science, Faculty of Agronomy, Vietnam National University of Agriculture. AK03 was developed by Legumes Research and Development Center, Vietnam. K7833 was introduced from Asian Vegetable Research and Development Center.

The experiment was conducted in greenhouse conditions at Vietnam National University of Agriculture (latitude 21°00'N and longitude 105°56', 7 m above mean sea level) in Spring season and was repeated in Autumn season in 2014. Seeds of each cultivar were sown separately in pots containing each 6 kg of dry soil per pot. Potting base contained 0.1 g N + 0.45 g P_2O_5 + 0.3 g K_2O. A total of 160 pots (25 × 25 × 20 cm) were used in each season. Waterlogging treatments and control under normal condition were applied. At the first day of flowering stage, waterlogging was imitated by applying water and kept at 3 cm of water level above the soil surface during 10 days. The 2×5 factorial experiment was arranged in a Randomized Complete Bock Design with four replications.

Data collection

Soybean plants were sampled at ten days after treatment. Leaf photosynthesis rate was recorded (µmol CO_2/m^2 leaf/s) by using LICOR-6400 (USA) at the youngest fully expanded leaf from 11:00 to 13:00 h, with the following specification/adjustment at 30°C, CO_2 concentration of 370 ppm; light density of 1500 µmol/m^2/s and humidity of 60%.

Morphological root traits were analysed by WinRhizo system (Regent Instruments, Inc., Quebec City, Canada) with an image scanner (Epson Perfection V700/V750 2.08A, Epson, Long Beach, CA, USA) set to a resolution of 400 dpi. Nodule number was counted and then nodule dry weight was determined after drying at 80°C for 48 h or until constant weight. Measurements of chlorophylls (a, b) were conducted by using a spectrophotometer (Spectro 2000 RS, USA) according to the method of Arnon (1949). The 0.2 g of fresh leaves samples were cut and extracted overnight with 80% acetone at -5°C. The extract was centrifuged at 10000 g for 5 min. Absorbance of the supernatant was read at 645, 663 and 480 nm using a spectrophotometer (Spectro 2000, USA). Nitrogen accumulation was analysed by using Automatic Digestion System (DKL 20 is interactive with JP Recirculating Water Pump) and UDK 149 VELP Automatic Kjeldahl Distillation and Titration. Total dry matter accumulation was determined after drying samples at 80°C for 48 h or until constant weight.

Data analysis

Mean data for each trait over two growing seasons was used for variance analysis using Systat 13. The mean values were compared using the Least Significant Difference (LSD) test.

RESULTS

Effect of waterlogging on total biomass

Total dry weight of all soybean plants were decreased by 11% to 32% in waterlogging stress conditions as compared to under normal conditions. There was a clear genotypic variation in the total dry weight in response of soybean cultivars to waterlogging stresses. The significant losses in total biomass were observed in DT84, AK03 and K7833 cultivars. In contrast, total biomasses were maintained in other cultivars D140 and D912 (Figure 1).

Effect of waterlogging on root growth

Nodule number and nodule dry weight of all soybean cultivars were significantly reduced under waterlogging stress condition in comparison to those under control conditions. In fact, the reduction of nodule dry weight ranged from 29 to 39% in waterlogging condition as compared with those in non-stress condition. The number of bacterial nodules of soybean cultivars reduced by

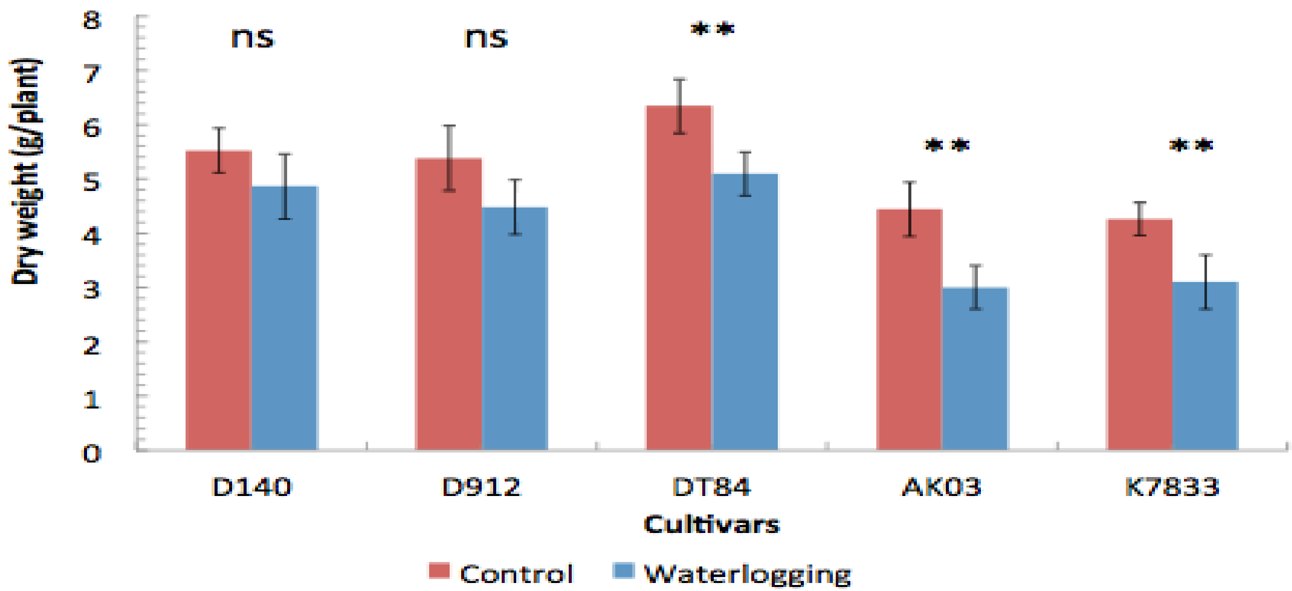

Figure 1. Effects of waterlogging on total dry weight of five soybean cultivars.
T test: **; p<0.05, ns, p>0.05.

Table 1. Effects of waterlogging on nodule number and nodule dry weight of five soybean cultivars.

Cultivar	Nodule number (nodules plant^{-1})			Nodule dry weight (g plant^{-1})		
	Control (a)	**Waterlogging (b)**	**b/a (%)**	**Control (a)**	**Waterlogging (b)**	**b/a (%)**
D140	23.25	19.23	83	0.49	0.33	67
D192	24.50	19.46	79	0.48	0.34	71
DT84	23.60	17.54	74	0.46	0.31	67
AK03	20.75	14.70	71	0.39	0.25	64
K7833	21.40	15.11	71	0.38	0.23	61
$LSD_{0.05}$(C)		*1.41*			*1.26*	
$LSD_{0.05}$(W)		*3.34*			*3.22*	
$LSD_{0.05}$(C×W)		*3.72*			*4.07*	

waterlogging from 17 to 29% and was more severe in AK03 and K7833 (29%) (Table 1).

There was significant genotypic variation in response of root traits to waterlogging conditions among the studied soybean cultivars. Under waterlogging conditions, root lengths of D140 and D912 increased significantly by 25% and 34%, respectively as compared to those in control conditions. By contrast, there was no clear change in root lengths of DT84, AK03 and K7833 (Figures 2 and 3). In this study, root diameter of all cultivars tended to increase under waterlogging conditions, but the difference was not at a significant level of 0.05 (Figure 3).

Effect of waterlogging on chlorophyll content

Chlorophyll a content significantly decreased in all studied soybean cultivars (16% to 26%), while chlorophyll b content did not change under waterlogging in comparison to the control (Figure 4).

Physiological response to waterlogging stress

Leaf gas exchange (LGE) characteristics of all soybean cultivars were significantly affected by waterlogging, but with different degrees. In fact, LGE of soybean cultivars significantly reduced under waterlogging condition, though less reduction of LGE was found in AK03 and K7833 cultivars.

There was genetic variation in nitrogen content of soybean cultivars in response to waterlogging conditions. Nitrogen content of DT84, AK03 and K7833 significantly reduced, whereas those of DT140 and D912 did not change under waterlogging conditions as compared to the control (Figure 6).

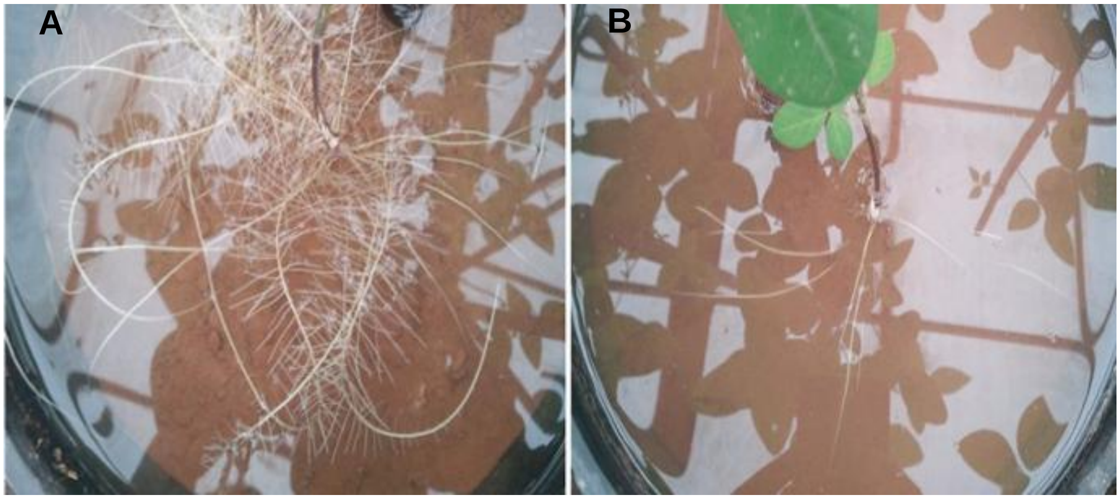

Figure 2. Effects of waterlogging on soybean root system of D912 (A) and DT84 (B).

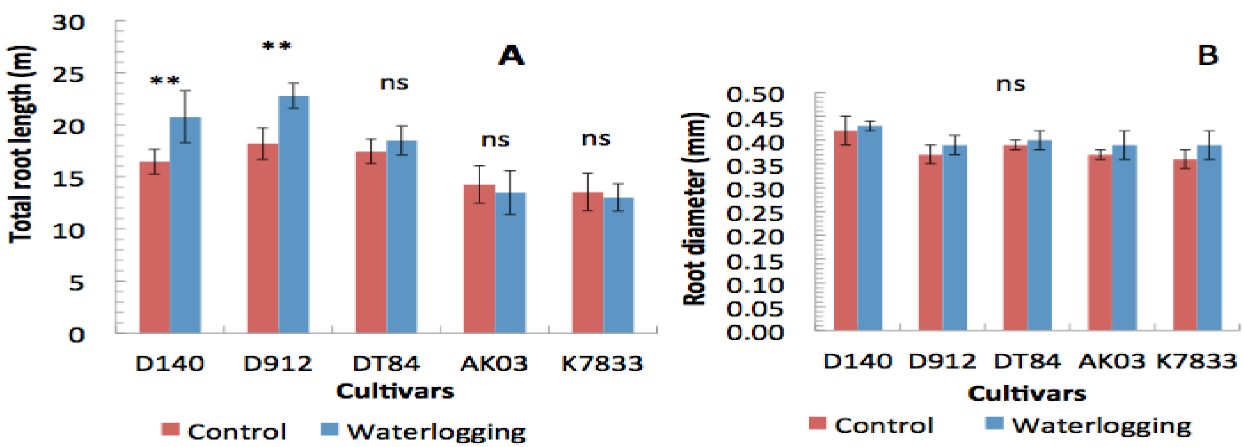

Figure 3. Effects of waterlogging on total root length (A) and root diameter (B) of five soybean cultivars.
T test: **; p<0.05, ns, p>0.05.

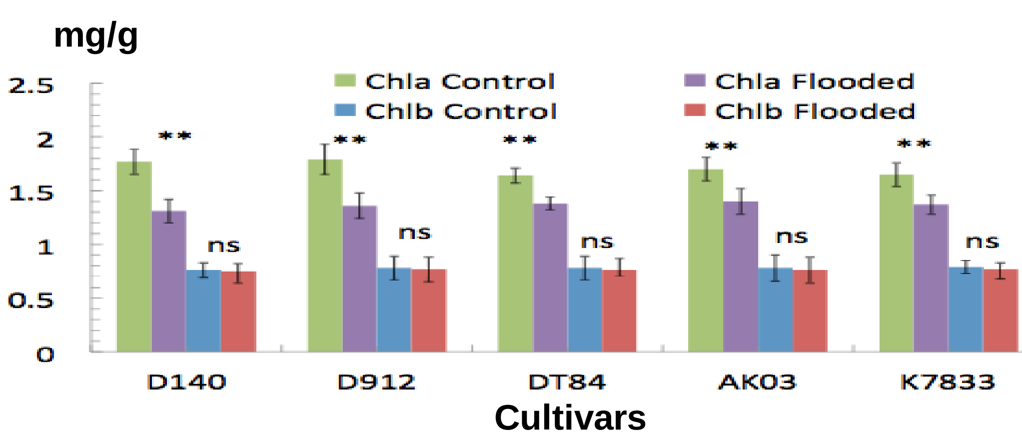

Figure 4. Effects of waterlogging on chlorophyll content of five soybean cultivars.
T test: **; p<0.05, ns, p>0.05.

DISCUSSION

In this investigation, five soybean genotypes had similar response to waterlogging in several studied traits such as chlorophyll a and b content, root diameter, nodule number, nodule dry weight and leaf gas exchange, but there was a clear variation in total biomass, root length and nitrogen content among the studied cultivars. The reduction of total biomass was observed in all cultivars under waterlogging treatment. This result is in agreement with the previous findings in wheat (Malik et al., 2001), mung bean (Ahmed et al., 2002), maize (Zaidi et al., 2004), barley (Pang et al., 2004) and soybean (Henshaw et al., 2007b). However, the significant reductions in total dry weight were found in D140 and D912 cultivars. The variation in dry weight in response to waterlogging was also found in soybean and groundnut (Miura, 2012).

Root growth traits are strongly affected by many environmental factors and very sensitive to non favorable conditions including oxygen shortage. Under waterlogging condition, concentration of available oxygen decreased rapidly because of slow diffusion of oxygen in water (Armstrong, 1979); as a result the respiration of root would be influenced (Drew, 1992). Moreover, diffusion of oxygen in flooded soil caused entrapment of gases produced in root, including gaseous hormone ethylene. This hormone was known to have a negative effect on root cell expansion (Strader et al., 2010). In several field crops such as wheat (Mano and Omori, 2007); maize (Zaidi et al., 2003) or tomato (McNamara and Mitchell, 1990), the adventitious roots were formed to replace normal root development under waterlogging condition. In soybean, the formation of adventitious root during waterlogging was reported in previous studies (Thomas et al., 2005; Bacanamwo and Purcell, 1999; Shimura et al., 2003; Henshaw et al., 2007a). In this study, DT140 and D912 quickly formed adventitious root system after one-week waterlogging treatment (Figure 2). Similar result was found in previous study that the higher root development under waterlogging indicates higher waterlogging tolerant in the soybean plant (Sakazono et al., 2014). Waterlogging reduced the number and weight of nodules as compared with those in control conditions. Miura (2012) also found similar results in the investigation of the ‘effects of flooding stress in root of wild groundnut (*Glycine soyja*) and soybean (*Glycine max*)’. Moreover, our study revealed that effects of waterlogging were more severe in AK03 and K7833 which had lower nodule number, root length and total biomass as well as slower adventitious formation than other cultivars. This could indicate that the higher bacterial nodules and the change in root morphology with adventitious root formation and higher root length facilitated root development to help soybean plant to survive under waterlogging conditions.

Waterlogging damaged membrane, electrolyte leakage, and decreased chlorophyll concentration led to less green leaves as signs of abscission (Kozlowski, 1997; Islam and MacDonald, 2004). This finding agrees with the finding of this study with significant reduction of chlorophyll a concentration in soybean leaves. Moreover, in this investigation, chlorophyll a content reduced more than chlorophyll b when soybean cultivars were subjected to waterlogging stress. Similar results were found in previous reports on okra (Ashraf and Arfan, 2005) and scrub (Zhao et al., 2014).

Previous studies reported that stomata conductance is the major factor influencing photosynthesis rate under waterlogging conditions (Huang et al., 1994; Malick et al., 2001; Ahamed et al., 2002; Ashraf, 2003; Stricker et al., 2005). Ahamed et al. (2002) investigated that the photosynthesis rate of mung bean decreased rapidly by progressive waterlogging at vegetative stage, and waterlogging might cause a fast decline in the photosynthesis rate by a mechanism independent to stomatal closure. In this study at early flowering stage, the reduction of photosynthesis rate was found with different levels in various cultivars with different root length. The lower leaf photosynthesis rate of AK03 and K7833 may possibly be regulated by root length development (Figure 5).

A previous study reported that waterlogging inhibited dry matter accumulation and nitrogen fixation of soybean (Bacanamwo and Purcell, 1999). On the other hand, waterlogging affected the nitrogen uptake of soybean plant (Puiatti and Sodek, 1999). Therefore, total nitrogen content decreased significantly, and yellowing of leaves was found under waterlogging condition. Our results were in line with those of previous studies with reductions of nitrogen and chlorophyll content in DT84, AK03 and K7833 cultivars. However, significant difference between nitrogen content of D140 and D912 under waterlogging conditions and control condition were not detected in this study. The contrast results could be attributed to the secondary aerenchyma formation in soybean plant (Shimamura et al., 2003).

In conclusion, responses of soybean cultivar to waterlogging at early flowering stage decreased in most growth parameters, except for total biomass, root length and nitrogen accumulation. Better growth of D140 and D912 cultivars under waterlogging conditions were associated with longer root development and maintaining higher nitrogen accumulation. The reduction in growth under waterlogging of AK03 and K7833 varieties could be attributed to the decreases in root growth and later chlorophyll content. The results indicated that the higher bacterial nodules and the change in root morphology with adventitious root formation and higher root length facilitated root development to help soybean plants to survive under waterlogging conditions.

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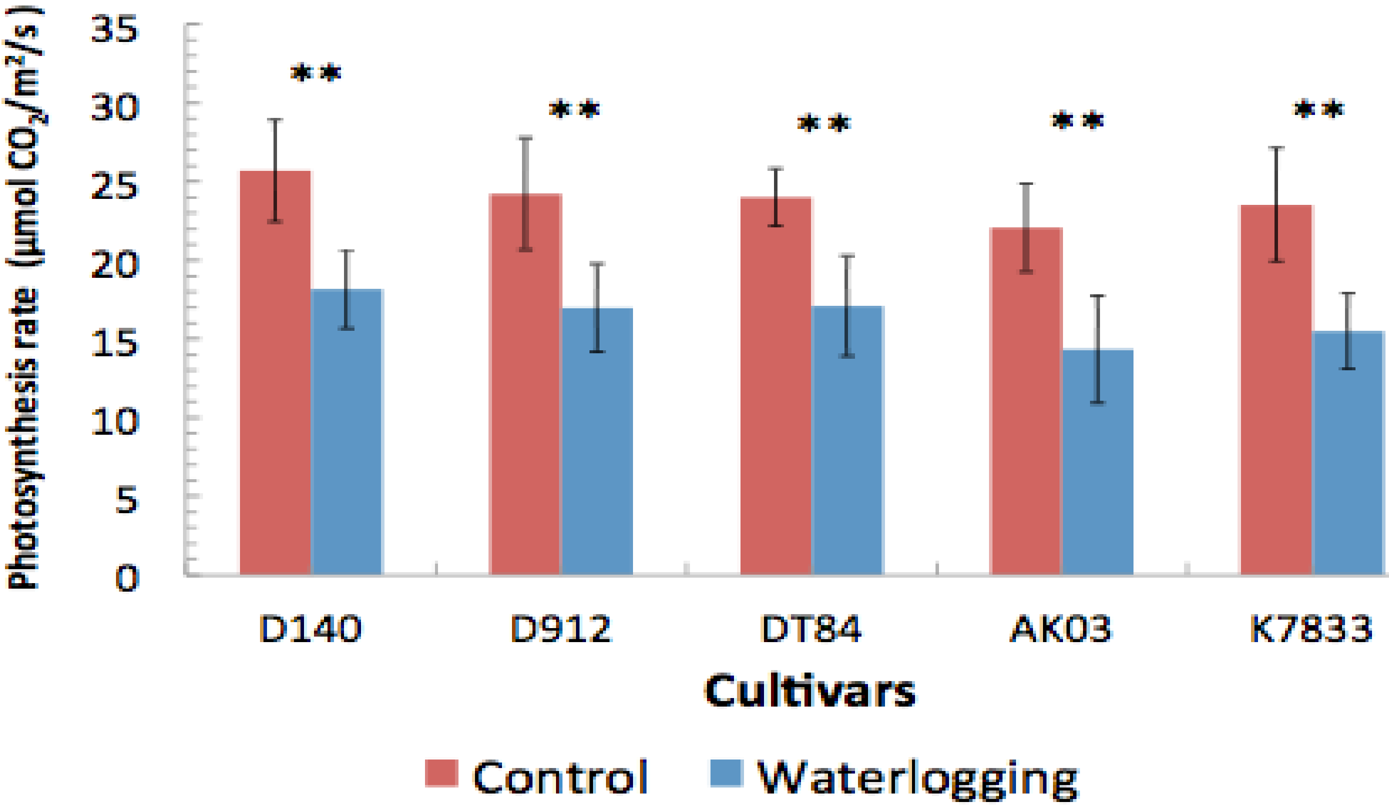

Figure 5. Effects of waterlogging on photosynthesis rate of five soybean cultivars.
T test: **; p<0.05.

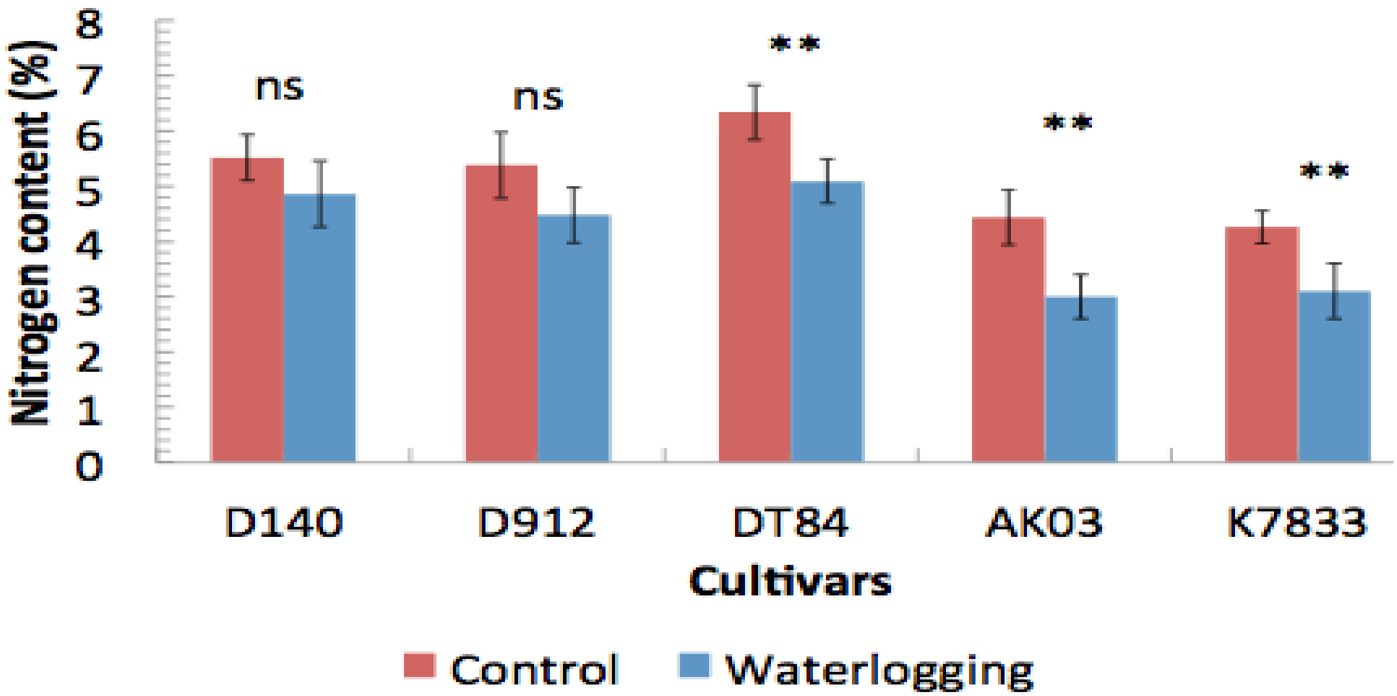

Figure 6. Effects of waterlogging on nitrogen content of five soybean cultivars.
T test: **; p<0.05.

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