Production and Physiological Characters of Soybean Varieties Under Drought Stress with Application of Nitrogen Sources

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Abstract
Soybean is a legume sensitive to drought conditions resulting in decreased yield and seed quality. Drought stress on plants also inhibits nitrogen uptake. The objective of the research was to determine production and physiological characters of soybean varieties under drought stress through nutrient N management. This research used a randomized block design with 3 factors and 3 replications. The first factor was soybean varieties (Anjasmoro, Wilis and Sinabung). The second factor was drought stress conditions consisted of 50%, 60% , 70% and 80% of field capacity (FC). The third factor was the application of N consisted of (1). Without application of N fertilizer (control); (2). N fertilizer dose of recommendation (50 kg Urea/ha); (3). Inoculation of *Bradyrhizobium* sp. ; (4). Organic N sources (straw compost 10 tons/ha) ; (5). Organic N sources (farmyard manure 10 tons/ha). The results suggest that Anjasmoro variety improved dry weight of seed per plant compared with Willis and Sinabung. Increased drought stress (80-50 % of FC) resulted in a decrease in dry weight of seed per plant. Sources of N in the form of urea or *Bradyrhizobium* sp. increased the dry weight of seeds per plant compared with treatment N sources straw and manure compost. The interaction between *Bradyrhizobium* sp. or Urea and Anjasmoro variety improved dry weight of seeds per plant.
Keywords : drought stress, nitrogen, production, physiological character, soybean

Introduction

Soybean (*Glycine max* (L.) is the most important legum crop of the world and a major source of protein, energy, polyunsaturated fat, fibres both for humans and livestock. In addition to being a source of macronutrients and minerals, soybeans contain secondary metabolites such as isoflavones (Sakai and Kogiso, 2008), saponins, phytic acid, oligosaccharides, goitrogens (Liener 1994) and phytoestrogens (Ososki and Kennelly, 2003). Yield in soybean is highly affected by drought stress, particularly when the stress occurring during flowering and early pod expansion (Liu, 2004 ; Liu *et al.*, 2004 ; Purcell *et al*, 2004), shortens plant, suppressing the development of soybean root and shoot (Hamim *et al.*, 1996; Soepandi *et al.*, 1997), accelerate flowering and harvest age (Jusuf *et al.,* (1993), reducing the number of filled pods (Soepandi *et al.*, (1997), lowering the number of seeds/plant and seed weight per unit (de Souza *et al.*, 1997) and lower seed yield of soybean (Jusuf *et al.*, 1993; Soepandi *et al.*, 1997). Drought stress on plants also inhibits nitrogen, phosphorus and potassium uptake in plants. Generally, drought reduces both nutrient uptake by the roots and transport from the roots to the shoots, because of restricted transpiration rates and impaired active transport and membrane permeability (Tanguilig *et al.*, 1987 ; Hu and Schmidhalter, 2005).

Nitrogen is the mineral element that plants require in the largest amounts and a constituent of many plant cell components, including amino and nucleic acids (Hu and Schmidhalter, 2005). It required in the synthesis of chlorophyll, and also the formation and growth of the vegetative parts of the plant.

Fertilizer management can strongly affect crop productivity under conditions of drought. Thus, the addition of nutrients can either enhance or decrease plants resistance to drought or have no effect at all, depending on the level of water availability. Drought stress condition can affect nitrogen uptake in plants. Therefore, it is necessary to manage N fertilizer through organic N, inorganic N and N biological (bacteria N fixation). Inorganic N fertilizer has a high nutrient content of N (Urea contains 46 % N). Organic N fertilization has advantages in improving soil structure, does not damage the environment but has a very low N content, so if it only use organic N fertilization is needed very much organic fertilizer N per area. In addition, the use of organic N fertilizer for crops is necessary to ensure that the organic N fertilizer materials have decomposed well, because often times the use of organic N will have no effect on the plant as a source of organic N is not decompose properly (Hartatik and Widowati, 2006). In addition, the use of bio-fertilizers on dry land is indispensable for biological fertility in dryland soybean crop is generally low and is one type of legume that is active in tie up N from the air, so that the inoculation of *Bradyrhizobium* sp. is an effort to reduce dependence on chemical fertilizers (Prihastuti, 2012).

An early study of the relationships between water availability and the N-fertilizer responses from Bloem *et al.* (1992) demonstated that application of additional N enhanced wheat yield only when the drought was not severe. While drought conditions may reduce soil-N mineralization, thus lowering the N availability. Tanguilig *et al*. (1987) also demonstrated that a reduced crop N uptake may also be attributed to a decreased transpiration rate to transport N from roots to shoots. Based on the background, this research was conducted to determine the production and physiological characters of soybean varieties under drought stress through nutrient N management.

Materials and Methods

Set up of the experiment

The research was conducted at the screen house Faculty of Agriculture, University of Sumatera Utara, Medan, Indonesia on February to May 2012. Soil for the research was taken from dry land Sambirejo Village (District of Binjei, Langkat).

Experimental design and crop management

This research used a randomized block design with 3 factors and 3 replications. The first factor was soybean varieties (Anjasmoro, Wilis and Sinabung). The second factor was drought stress conditions consisting of 50%, 60% , 70% and 80% of field capacity (FC). Drought stress treatment of 80% of FC is the control treatment because it was considered optimal for the growth of soybean (Chen *et al.*, 2006; Sumarno and Mansuri, 2007). The third factor was the application of N consists of (1). Without N fertilizer (control); (2). N fertilizer dose of recommendation (50 kg Urea/ha); (3). Inoculation of *Bradyrhizobium* sp.); (4). Organic N sources (straw compost 10 tons/ha); (5). Organic N sources (farmyard manure 10 tons/ha). There were 3 x 4 x 5 x 3 = 180 experimental units. Each experimental unit consisted of 4 plants so there were 540 polybags.

Three soybean varieties (Anjasmoro, Wilis and Sinabung) was used as source of seeds. The soil dried for 15 days, then crushed and screened with a diameter of 6 mm sieve. Before planting, soil limed with dolomite 500 kg/ha and incubated for 2 weeks. A total of 10 kg weight of air-dry soil put in poly bag size 30 cm x 40 cm. Poly bag had previously been covered with plastic. Isolates of *Bradyrhizobium* sp. indigenous origin dry land village Sambirejo obtained by isolated first. Based on the pre- study results the isolates of *Bradyrhizobium* sp. potential. Propagation of *Bradyrhizobium* sp. isolate conducted in Soil Biology Laboratory, Faculty of Agriculture, University of Sumatera Utara by using yeast extract mannitol medium in a 500 ml flask were shaken 150 rpm at room temperature for 48 hours. *B. japonicum* inoculation were mixed with soybean seed just before planting in the shade in the morning. Bacterial isolates (density of 10^8 cells/ml) in 1 ml/poly bag was also given to the ground in a poly bag. Straw compost made with bio-activator *Trichoderma harzianum*.

The transparent plastic shade was made inside the screen house with a size of 12 mx 16 m made of bamboo and wire bonded. It was divided into 3 blocks. Determination of soil moisture content to determine the weight of air-dry soil to be put into polybag done by drying method. Determination of water content at field capacity (FC) was conducted using method describe by Altricks (Foth, 2004). P and K fertilizer application was done for all the plants at planting time (0 days after planting/DAP) according to the dose of 150 kg TSP/ha and 75 kg KCl/ha. Urea 50 kg/ha applied at twice i.e. a half of dose on planting time (0 DAP) and a half of dose on 30 DAP. Cow manure and straw composting was done at planting time according to treatment, administered by mixing fertilizer with planting medium.

Drought stress treatment carried out according to treatment was 50% , 60% , 70% and 80 % of FC. Plants treated 80% of FC up to 21 DAP, after that plants treated according to drought stress treatment until harvest time. Weeding is done manually by pulling weeds. Prevention of pest attacks carried out by using organic pesticides. Harvesting is done if the soybean crop has shown that the criteria have brown skin pods and stems and leaves have dried up variables observations.

Variables observed and statistical data analysis

Variables observed was chlorophyll, shoot and root N content and seed dry weight per plant. Chlorophyll content measured by using Opti-Sciences CCM 200 plus Chlorophyl meters (Opti-Sciences, 2011). Shoot and root N measured by Kjedhal method. If there were significant differences in the variance analysis followed by Duncan's Multiple Range Test (p = 0.05).

Results and Discussion

Soil, Farmyard manure and Straw Compost Characteristics

The characteristics of soil had low pH H_2O 5.0, low of N content (0.14%), low C organic content (1.02%), sufficient P (27.4 mg kg^{-1}), sufficient K (0.47 me/100), sufficient Ca (8.75 me/100), low cation exchange capacity (15.7 cmol(+)kg^{-1}, high Mg (3.69 me/100). The straw compost characteristic were C organic content 22.5 %, N total 1.33%, C/N ratio 16.9, P_2O_5 total 2.9%, K_2O 9.45. The characteristics of farmyard manure had the C organic content 17.98 %, N total 1.04%, C/N ratio 17.28, P_2O_5 total 0.79%, K_2O 0.30.

Physiological characters of soybean

Chlorophyll Content

Anjasmoro and Sinabung varieties gave the highest of chlorophyll content compared to Wilis. Application of Urea tent to increase the chlorophyll content compared to other treatments. Interaction between Anjasmoro variety and 60% of FC gave the highest of chlorophyll content, but interaction between Sinabung variety and 70% of FC the lowest of chlorophyll content (Table 1).

Shoot N content

Anjasmoro variety provide the highest shoot N content compared to Sinabung and Wilis. Farmyard manure treatment improved shoot N content significantly than other treatments, but the treatment of without N gave the lowest shoot N content. Drought stress treatment did not give effect significantly different on shoot N content (Table 2).

Root N content

Urea treatment improved the root N content significantly compared to other treatments. Manure treatment gave the lowest root N content. Interaction Anjasmoro variety with straw compost produced the highest levels of N roots than other treatments, but not significantly different from the interaction of Anjasmoro variety and aplication of Urea. The interaction between Urea and 70% of FC produced the highest levels of N roots (Table 3).

Table 1. Chlorophyll content of three soybeans varieties under drought stress with application of source of N

Treatment	Drought stress (% of FC)				Mean
	50	60	70	80	
Variety	……………………………unit/0.71 cm^2 …………………………				
Anjasmoro	33.14	35.48	31.70	34.03	33.53a
Sinabung	31.22	28.12	27.33	29.38	35.53a
Wilis	27.37	28.98	28.83	28.00	29.01b
Source of N					
Without N	29.89	31.57	29.20	28.72	29.85
Urea	30.58	30.75	31.49	31.66	31.12
Bradyrhizobium sp.	30.62	29.80	30.50	29.95	30.22
Farmyard manure	30.31	30.72	29.27	30.64	30.23
Straw compost	31.46	31.46	25.96	31.39	30.07
Mean	30.57	30.86	29.28	30.47	

Note : Different letters represent significant differences at Duncan's Multiple Range Test (p = 0.05)

Table 2. Shoot N content of three soybeans varieties under drought stress with application of source of N

	Drought stress (% of FC)				Mean
	50	60	70	80	
Variety	………………………% of shoot dry weight………………				
Anjasmoro	3.86	3.89	4.02	3.68	3.86x
Sinabung	3.43	3.48	3.61	3.40	3.48z
Wilis	3.94	3.71	3.52	3.26	3.61y
Source of N					
Without N	3.51	3.51	3.49	3.29	3.45d
Urea	3.72	3.79	3.63	3.47	3.65c
Bradyrhizobium sp.	3.57	3.14	3.36	3.57	3.41d
Farmyard manure	3.83	4.17	4.22	3.65	3.97a
Straw compost	4.10	3.85	3.89	3.26	3.77b
Mean	3.75	3.69	3.72	3.45	

Note : Different letters at the same group treatment represent significant differences at Duncan's Multiple Range Test (p = 0.05)

Table 3. Root N content of three soybeans varieties under drought stress with application of source of N

	Drought stress (% of FC)				Mean
	50	60	70	80	
Variety	…………………………% of root dry weight ………………				
Anjasmoro	2.20	2.24	2.35	2.16	2.24x
Sinabung	1.92	2.05	1.91	1.83	1.93y
Wilis	1.79	1.94	1.93	2.06	1.93y
Source of N					
Without N	1.96cde	1.99cde	2.08abcd	1.96cde	2.00l
Urea	1.99cde	2.34ab	2.36a	2.03cde	2.18j
Bradyrhizobium sp.	2.20abc	2.05bcde	1.81e	2.13abcd	2.05k
Farmyard manure	1.89de	1.95cde	1.78e	1.92cde	1.88m
Straw compost	1.82e	2.05bcde	2.29abc	2.03cde	2.05k
Mean	1.97	2.08	2.06	2.01	

Note : Different letters at the same group treatment represent significant differences at Duncan's Multiple Range Test (p = 0.05)

The application of Urea gave the highest root N content, while application of farmyard manure gave the lowest root N content. Interaction between Anjasmoro and application of straw compost gave the highest root N content, while the interaction between Sinabung variety and *Bradyrhizobium* sp. provide the lowest of root of N (Table 4).

Table 4. Root N content of three soybean varieties with the application of N sources

Variety	Source of N					Mean
	Without N	Urea	*Bradyrhizobium* sp.	Farmyard manure	Straw compost	
	……………………………………% ……………………………………					
Anjasmoro	2.13de	2.36ab	2.27bc	2.01f	2.41a	2.24x
Sinabung	2.05ef	2.01f	1.81h	1.91g	1.86h	1.93y
Wilis	1.82h	2.18cd	2.06ef	1.73i	1.87h	1.93y
Mean	2.001	2.18j	2.05k	1.88m	2.05k	

Note : Different letters represent at the same group treatment significant differences at Duncan's Multiple Range Test (p = 0.05)

Production of soybean

Seed dry weight per plant

Based on Table 9 shown that Anjasmoro variety produced the highest dry seed weight per plant compared with Sinabung and Wilis. Treatment of 70-80% of FC increased the dry weight of seeds per plant significantly compared with 50% and 60% of FC. In all varieties is seen that the dry seed weight per plant tent to increase with increasing% KL.

Table 5. Seed dry weight of three soybean varieties under drought stress with the application of N sources

	Drought stress (% of FC)				Mean
	50	60	70	80	
Variety	……………………………g ………………………….				
Anjasmoro	3.84	3.47	4.85	5.51	4.42a
Sinabung	3.49	3.83	4.17	4.78	4.07b
Wilis	2.81	3.44	4.12	4.34	3.68c
Source of N					
Without N	3.43	2.67	5.62	5.61	4.33
Urea	3.33	3.70	3.72	3.70	3.61
Bradyrhizobium sp.	2.48	5.25	3.87	4.48	4.02
Farmyard manure	3.34	3.51	3.85	4.95	3.91
Straw compost	4.87	4.00	3.79	5.16	4.46
Mean	3.49y	3.83y	4.17x	4.78x	

Note : Different letters at the same group treatment represent significant differences at Duncan's Multiple Range Test ($p < 0.05$)

Based on the research results chlorophyll content influenced by varieties (Table 1). Anjasmoro had the chlorophyll content higher than Willis and Sinabung. Treatment of 50 -80% of FC has not responded significantly different with chlorophyll cntent. Chlorophyll content related to the status of N in plant indicated by the shoot N content (Richardson *et al.*, 2002). The low chlorophyll content also indicated the low shoot N content. This is evidenced in this research that treatment without N application produced low chlorophyll content (Table 1) and also lowest shoot N content (Table 2), because the treatment without N application led to a shortage of plant N that leaves become paler due to the loss of chlorophyll which is pigments in photosynthesis. As already reported by Ashari (2006) that Nitrogen is one of the components of chlorophyll which plays an important role in photosynthesis. N deficiency symptoms are most obvious and commonly seen is a reduction in leaf green color due to the loss of chlorophyll, the green pigment that plays a role in photosynthesis. N is an essential element for plant growth which is a protein constituent of nucleic acids, stimulate overall growth, constituent protoplasm, chlorophyll molecules , nucleic acids and amino acids which are the building of protein.

Based on the limits of nutrient sufficiency and deficiency according to the results of the analysis of the shoot N content, in general it is in a state of deficiency because a value less than 4.2 % (Hardjowigeno, 2003). In this research, treatment of varieties significantly affect on shoot and root N content (Tables 2 and 3). Shoot N content in Anjasmoro higher than Wilis and Sinabung. This phenomenon is related to the ability of each variety in absorbing N nutrients from the soil. There are two forms of N fixation by plant roots namely ammonium and nitrate. Nitrogen is absorbed mostly in the form of nitrate ions are stored directly in the vacuole cells of root, storage organs (fruit), stems or leaves . The rest which are not stored in the vacuole would be reduced to NO_2 (nitrite) converted into ammonia (NH_3) by enzymes nitritreductase.

N sources significantly affect the levels of shoot and root N content. Farmyard manure provided the highest levels of shoot N content but application of Urea provided the highest levels of root N content. Farmyard manure is source of organic N that affect the physical, biological and chemical soil, improving soil aeration because the pores that exist in the soil increased so much aeration, related to percolation and the availability of water, fertilizer replacement and restore soil fertility due to nutrient taken.

Anjasmoro variety provided dry weight of seed per plant (Table 5) higher than Wilis and Sinabung. This related to genetic factors of each variety, in which the number of pods vary depending on variety, fertility and plant spacing. However, Anjasmoro variety produced the lowest amount of filled pods but it had dry weight of seeds per plant and dry weight of 100 seed were higher than Wilis and Sinabung, because Anjasmoro is large seed soybean varieties (dry weight of 100 seeds > 13 g). It can be understood that the dry weight of 100 seeds of Anjasmoro (13.51 g) is very different from Wilis (10.64 g) and Sinabung (10.33 g). The large size of the seed Anjasmoro variety presumably because of the concentration of photosynthesis in seed filling and the type of determinate growth, in which the R1 phase growth stalled. In line with the statement Akunda (2001) that the large seed size caused by the concentration of photosynthesis in seed filling . Seed yield is the total photosynthate is partitioned into seeds ,and the magnitude is the product of the rate of dry matter accumulation in the seed with the seed filling period and number of seeds.

Dry weight of seeds per plant (Table 5) increased with increasing % of FC. It is because the pod filling phase (R5 and R6) are very sensitive to water availability. The development of seed on R5 is characterized by a rapid increase in seed weight and nutrient accumulation, lasting up to R6. At pod filling phase occurs R6 but still immature. Soybean seed filling takes place during the final phase of maturation seed. This phase are largely influenced by the availability of water (Kim and Chung , 2007; Dhaubhadel *et al* , 2007). Therefore, the lower availability of water in the pod filling phase will result in increasing lower number of filled pods that also resulted in a decrease in dry weight of seeds per plant and 100 seed weight. Differences in the dry matter yield response have been reported by many researchers (Daneshian and Zare, 2005 ; Manalavan et al, 2009 ; Hamayun, *et al.*, 2010) that the decrease in yield with increasing drought stress during flowering and seed filling stage resulted in a decrease in the number of pods due to miscarriage during flowering so affect the number of seeds per pod. Beheshti and Fard (2010) have reported that the reduction in photosynthesis is a major factor limiting yield and all yield components . The process of photosynthesis is impaired resulting in decreased seed yield, harvest index and other components. Plants that are experiencing drought stress during seed filling phase, while photosynthesis is not enough for needs, so the plants will use the sink compound stored assimilates from other parts of the plant such as seeds and stems, resulting in a decrease in dry weight of seeds and stems.

Based on the research, it concluded that Anjasmoro variety improve the character of dry weight of seed per plant compared with Willis and Sinabung. Increased drought stress (80-50 % of FC) resulted in a decrease in dry weight of seed per plant and dry weight of 100 seeds but increases leaf proline content. Sources of N in the form of urea or *Bradyrhizobium* sp. increased the dry weight of seeds per plant compared with treatment N sources straw and manure compost. The interaction between Bradyrhizobium sp. or Urea and Anjasmoro variety improved dry weight of seeds per plant.

Acknowledgments

We gratefully thank to M. Iqbal Suyudi, M. Juandi and Heru Yosua Anugerah for their support in the research.

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