Soybean production under drought stress with application of *Bradyrhizobium japonicum* induced by genistein

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Abstract. A research about production of soybean under drought stress with the application of *Bradyrhizobium japonicum* induced by genistein was conducted in green house, Faculty of Agriculture, University of Sumatera Utara. The aim of the research was to study the role of *B. japonicum* induced by genistein on soybean production. The experiment used Randomized Complete Block Design Factorial with three factors. The first factor was genistein treatments consisted of without and with genistein. The second factor was *B. japonicum* consisted of without *B. japonicum*, *B. japonicum* isolate 1, *B. japonicum* isolate 2 and *B. japonicum* isolate 3. The third factor was soil water content condition namely 40%, 60% and 80% of field capacity. The parameters observed were plant growth rate, relative growth rate, nett assimilation rate, dry weight/plant and dry weight of 100 seeds. The result research showed that the treatment of interaction between *B. japonicum* induced genistein under 80% of field capacity gave the higher plant growth rate, plant growth rate, relative growth rate, nett assimilation rate and dry weight of 100 seeds.
Keywords: *Bradyrhizobium japonicum*, production, soybean

Introduction

Soybean has a strategic potential in food security as a source of protein and high-quality of functional food. Soybean contain several biologically active components that may contribute individually or synergistically to the health of this plant (Edman, *et al*, 1989 ; Messina, 1995). Among the components of soybean that have been hypothesized to provide health benefits are protein, isoflavone, saponin, oils and fatty acids, trypsin inhibitors. Until now, domestic soybean needs can not be fulfilled and it is still depend on import. Because of that, the national efforts to increase soybean production both intensification and extensification as empowerment dry land are necessary to be done.

Dry land is a potential area to be developed as an agricultural land in terms of its wide. Extensive dry land for agriculture in Indonesia is estimated at 11,853,848 ha. Distribution of dry land covers 32% in Sumatra, 11% in Kalimantan, 14.6% in Sulawesi and 24% approximately in Java and Bali, 6% in Nusa Tenggara and Papua, 12,4% in Maluku. Dry land is classified as sub optimal land because the land is infertile, acidic reaction, contain of Al, Fe, and Mn are high, poor organic matter and nutrient of N, P, K, Ca, and Mg, drought stress especially during the dry season. This situation will affect the development of the morphology and physiology of the soybean. Soybeans are very sensitive to drought conditions result in decreasing yield and grain quality (Frederick *et al*, 2001). Drought stress causes plants shortened, suppressing the development of soybean root and shoot (Yusuf *et al*, 1993; Hamim *et al*, 1996; Soepandi *et al*, 1997), accelerate flowering and harvest (Yusuf *et al* (1993), reducing the number of pods containing (Soepandi *et al* (1997), decrease the number of seeds/plant and seed weight/plant (De Souza *et al*, 1997) and descrease of soybean yield (Yusuf *et al*, 1993; Soepandi *et al*, 1997). Soybean as other legumes have the ability to symbiosis with rhizobia bacteria. The soybean roots form the root nodules where the bacteria form N_2 fixation from the atmosphere (Gresshoff, 1993; Morgan *et al*, 2005). Rhizobia and soybean plants develop the mutualism symbiosis that is controlled by both the symbionts. However, environmental conditions such as low temperature, high salinity, low pH and drought can negatively affect the symbiosis between legumes and rhizobia (Napoles, 2009).

Drought stress is the main factor influences of root nodules symbiotic and decrease the formation of root nodules, reducing the size of the nodules and N fixation (Serraj *et al*., 2003; Streeter, 2003; Tajima *et al*, 2004). Serraj (2003) and Kurdalai *et al*, (2002) reported that there are many mechanisms involved in the physiological response, carbon shortage and C metabolism in nodules, limited oxygen and reduce the number of N fixation. In the exchange of molecules between legumes and microorganisms, legume root exudates the form of flavonoids and isoflavonoids molecules that induce expression of nod (nodulation) genes in rhizobia (Sugiyama, 2008). Genistein and daidzein are the main isoflavones types of soybean root exudates that are responsive to the induction of nod genes in Bradyrhizobium

(Kosslak *et al*., 1990). As a result, the bacteria produce lipo-oligosaccharide Nod Factors that precision of structure determines the host range and specifications associated with induction of plant responses that nodule formation is complete (Vijn *et al*., 1993; Stokkermans and Peters, 1994; Geurts *et al*, 2005).

Based on this background it is necessary to do the research on the role of isoflavonoids as a signaling molecule in overcoming drought stress on soybean symbiosis with Bradyrhizobium japonicum.

Materials and Methods

The study was conducted on June-December 2011 in the greenhouse of Faculty of Agriculture University of Sumatera Utara. Propagation of three isolates of B. japonicum 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. Research used Randomized Block Design Factorial consisted of 3 factors and 3 replications. The first factor that genistein treatment consists of (1) Without treatment genistein and (2). With the treatment of genistein (50 μM). The second factor is the treatment *Bradyrhizobium japonicum* consists of (1). Without B. japonicum, (2). B. japonicum isolates 1 (3). B. japonicum isolates 2, and (4). B. japonicum isolates 3. The third factor is the level of drought stress consisted of 40%, 60% and 80% of field capacity.

Land for the study was taken from upland village Sambirejo (Langkat). Before planting done liming with dolomite 500 kg / ha and incubated for 3 weeks. Soil research put into the polybag. Polybag previously been covered with plastic. Soybeans variety used is Anjasmoro. Inoculation isolate B. japonicum carried out in accordance with the treatment, by isolate mixed with soybean seed just before planting at the shade in the morning.

Solution of genistein (isoflavones) are used for pre-incubation of bacteria. Genistein sterile bacterial culture is added to B. japonicum 24 hours before inoculation of soybean seed. Determination of water content of the soil by drying method (oven), while determination of the water content of field capacity was conducted by Bouyoucos method. The variables consisted of observations of plant growth rate, relative growth rate, net assimilation rate, dry weight of 100 seeds, and seed weight/plant.

Results and Discussion

Result

Plant Growth Rate

Based on Table 1. showed that treatment B. japonicum, the interaction between genistein and drought stress, and the interaction between B. japonicum, genistein and drought stress significantly affect the plant growth rate. Treatment B. japonicum B2 showed the highest plant growth rate (0090 g / day) while the lowest was in treatment B1 and B3. Interaction between genistein 50 μM and drought stress 80% of field capacity gives highest plant growth rate compared to other treatments. The interaction of three factors that give the highest rate of plant growth rate are drought stress treatment 80% of field capacity, 50 μM genistein and without B. japonicum (0166 g / day)

Relative Growth Rate

Table 2 showed that treatment of B. japonicum and interaction between genistein and drought stress did not give a significant effect on relative growth rate, while the interaction between genistein, drought stress and B. japonicum significantly effect on plant growth rate. The treatment of B. japonicum B2 gave the highest relative growth rate compared to other treatments. Increased field capacity followed genistein treatment tent to increase the relative growth rate. Interactions treatment of genistein 50 μm, 60% field capacity of drought stress and B. japonicum B2 gave the highest relative growth rate (0081 g/day). Based on these facts showed that the treatment of genistein increased the plant tolerance to drought stress on soybean symbiosis with B. japonicum B2, as shown by increasing in its relative growth rate.

Table 1. Mean Values of Plant Growth Rate on effect *B. japonicum* Inoculation and Drought Stress

Genistein (μm)	Drought stress (% of field capacity)	*B. japonicum* (B) B0	B1	B2	B3	Average
0	40	0.086 d	0.068 defg	0.014 i	0.046 defgh	0.054d
	60	0.063 defg	0.044 fghi	0.058 defg	0.005 j	0.043e
	80	0.021 hi	0.030 ghi	0.080 de	0.068 defg	0.050de
50	40	0.071 def	0.085 de	0.072 def	0.056 defgh	0.071c
	60	0.047 defgh	0.039 fghi	0.162 ab	0.131 abc	0.095b
	80	0.166 a	0.086 d	0.153 abc	0.070 def	0.119a
Average		0.076b	0.059c	0.090a	0.063 c	

Note : Mean values by the same letter do not significantly differ base on DMRT (F = 5%.)

Table 2. Mean values of Relative Growth Rate on effect *B. japonicum* inoculation and drought stress

Genistein (μm)	Drought stress (% of field capacity)	*B. japonicum* (B) B0	B1	B2	B3	Average
0	40	0,085 a	0,063 abcdefg	0,011 l	0,040 fghijk	0,050
	60	0,070 abcde	0,020 ijkl	0,078 abc	0,003 m	0,043
	80	0,025 ijkl	0,023 ijkl	0,047 defghi	0,039 ghijkl	0,033
50	40	0,042 efghij	0,054 bcdefgh	0,049 defghi	0,039 ghijkl	0,046
	60	0,025 ijkl	0,027 hijkl	0,081 ab	0,059 abcdefg	0,048
	80	0,073 abcd	0,036 ghijkl	0,068 abcdef	0,030 hijkl	0,052
	Average	0,053	0,037	0,056	0,035	

Note : Mean values by the same letter do not significantly differ base on DMRT (F = 5%.)

Net Assimilation Rate

Based on Table 3 shows that treatment of B. japonicum, and the interaction between B. japonicum and drought stress significantly affect the nett assimilation rate, whereas drought stress did not significantly affect the nett assimilation rate. The treatment of drought stress 60% of field capacity tent to increase the highest nett assimilation rate (0.0050). Inoculation treatment B. japonicum B2 gave the highest nett assimilation rate compared with other treatments. The interaction between drought stress 60% of field capacity and B. japonicum B2 gave the highest nett assimilation (0.0105) while the lowest on the interaction of drought stress of 60% field capacity treatment B. japonicum B1 (0.0015).

Table 3. Mean values of Net Assimilation Rate on effect of *B. japonicum* inoculation and drought stress

B. japonicum (B)	Drought stress (% of field capacity) 40	60	80	Average
B0	0,0056 cd	0,0039 def	0,0066 bc	0,0054 b
B1	0,0054 cd	0,0015 g	0,0024 efg	0,0031 c
B2	0,0020 fg	0,0105 a	0,0078 b	0,0068 a
B3	0,0025 efg	0,0041 de	0,0025 efg	0,0030 c
Average	0,0039	0,0050	0,0048	

Note : Mean values by the same letter do not significantly differ base on DMRT (F = 5%.)

Dry weight of 100 seeds

Based on Table 4 showed that the effect of genistein, drought stress, and the interaction of these three factors did not significantly affect on dry weight of 100 seeds. Interaction among without giving genistein, drought stress 80% of field capacity and B. japonicum B2 and interaction among 50 μm of genistein, drought stress 80% of field capacity and without B. japonicum gave the highest dry weight of 100 seeds (13,32 g) while the lowest was on treatment without genistein, without B. japonicum and 40% of field capacity.

Table 4. Mean values of dry weight of 100 seeds on effect of genistein, drought stress and *B. japonicum*

Genistein (µm)	Drought stress (% of field capacity)	*B. japonicum* (B)				Average
		B0	B1	B2	B3	
0	40	10.03	11.23	10.82	10.30	10.60
	60	12.28	12.48	12.03	11.63	12.11
	80	10.45	12.92	13.32	12.33	12.25
50	40	10.70	10.47	11.05	10.33	10.64
	60	12.88	12.30	11.12	12.50	12.20
	80	13.32	12.68	12.87	11.90	12.69
Average		11.61	12.01	11.87	11.50	

Based on this research, the effect of drought stress tent to decrese the plant growth rate, relative growth rate, net assimilation rate and dry weight of 100 seeds. This is consistent with research that has been done Prihandarini (1991) that a critical state at the time of formation water rates will cause to decrease the number of flowers, pods and seed weight. Kiyatno (1993) stated that water stress will reduce net assimilation rate, root length, root dry weight, root-canopy ratio, number of roots, plant height, plant dry weight, number of pods and weight of 100 seeds. Other researchers Sariyah (1992) stated that increasing water stress will decrease of leaf water potential, relative growth rate, dry seed weight, plant height, number of pods and seed yield per plant.

Decreasing in the growth variables observed of soybean production due to increased drought stress due to suspected impaired nutrient transport from the leaves to the seeds. Water play an important role in the translocation of nutrients within the plant. Leaves as a source asimilat (source) and act as seed storage (sink). During seed development, asimilat transported from leaves to seeds. Other influences of water stress resulting asimilat movement speed decrease. Water loss in the plant tissue will decrease cell turgor, increasing the concentration of macro-molecules and water chemical compounds in plants (Mubiyanto, 1997). The role of water for the plants have consequences directly or indirectly, plant water deficit will affect the plant metabolism by resulting in impaired growth (Pugnaire and Pardos, 1999). The process of cell metabolism and all the biochemical reactions in plants took place in water media. Nodule formation and N fixation in plants sensitive to drought stress and have a negative effect on soybean yield (Serraj and Sinclair, 1998). As an evaluation of the effects of genistein as induced to B. japonicum on drought stress conditions can be concluded that the induction of genistein can reduce the effects of drought stress on root nodule formation.

Conclusions

1. B. japonicum has a role to enhance plant growth rate, relative growth rate, net assimilation rate and dry weight of 100 seeds.
2. Increasing the drought stress can decrase the plant growth rate, relative growth rate, net assimilation rate and dry weight of 100 soybean seeds.
3. Induction of genistein can reduce the effects of drought stress on root nodule formation by B. japonicum.

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