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Isoflavone characters of three soybean varieties under drought stress with application of nitrogen sources

[1]Yaya Hasanah, [1]Tengku Chairun Nisa, [2]Hapsoh, [1]Hamidah Hanum

[1]Faculty of Agriculture, University of Sumatera Utara, Medan 20132, Indonesia;
[2]Faculty of Agriculture, University of Riau, Pekanbaru 29293, Indonesia.
Coorresponding Author: azkia_khairunnisa@yahoo.co.id

Abstract. Human interest in soybean greatly increased in recent years due to the positive effects of secondary metabolites in soybean such as isoflavones for human health. Isoflavones are phytoestrogens worked as the antioxidant and chemopreventive agents for estrogenic compounds that can inhibit cell proliferation. Isoflavones also serves to prevent heart damage, osteoporosis and menopause syndrome, prevent prostate cancer, colon cancer and breast cancer. Daidzein, genistein and glycitein are the major isoflavones in soybean. A research about isoflavone charácters of three soybean varieties under drought stress with application of Nitrogen sources was conducted in green house, Faculty of Agriculture, University of Sumatera Utara. The aim of the research was to study the effect of Nitrogen sources under drought stress on isoflavone characters of three soybean varieties. The experiment used a Factorial Randomized Block Design with three factors and three replications. The first factor was soybean varieties consisted of Anjasmoro, Wilis and Sinabung. The second factor was Nitrogen sources consisted of without N fertilizer application; Urea 50 kg/ha, *Bradyrhizobium sp.*, cow manure 10 t/ha, rice straw compost 10 t/ha. The third factor was soil water content condition namely 50%, 60%, 70 and 80% of field capacity. The parameters observed were isoflavone charcters consisted of genistein, daidzein, glycitein and isoflavone total. The result showed that Anjasmoro variety had the genistein, daidzein, glycitein and isoflavone total contents of soybean higher than Wilis and Sinabung. The application of Nitrogen sources increased the genistein, daidzein, glycitein and isoflavone total contents of soybean compared without application of Nitrogen sources. There is a distinct pattern in the increase in the isoflavone total content in Anjasmoro, Wilis and Sinabung with increasing the drought stress. At Anjasmoro variety, increasing drought stress caused the lower of isoflavone total content, while the varieties Sinabung and Wilis are the opposite pattern.

***Key Words*:** drought stress, isoflavones, Nitrogen, soybean, variety

Introduction

Soybean had 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 (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 (Purcell et al, 2004). Drought stress causes plants shortened, suppressing the development of soybean root and shoot (Hamim et al, 1996; Soepandi et al, 1997), accelerate flowering and harvest, 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 (Soepandi et al, 1997).

Drought also affects the quality of soybean seeds in terms of the content of secondary metabolites such as isoflavones. Isoflavones are important secondary metabolites are accumulated mainly in the seeds and leaves of soybean. Although it can also be found in the roots, stem, seed coat, embryo and pods (Dhaubhadel et al, 2007). There are three characters in major soybean isoflavone namely genistein, daidzein and glycitein. The isoflavones may be in the form of free molecules, unmodified molecules, but also sometimes found conjugated with malonyl-glucose or glucose and stored in the vacuole (Yu and McGonigle, 2005).

Soybean isoflavones is very beneficial for human health (Rochfort and Panozzo, 2007) and to the soybean plants themselves useful to plant defense and nodulation (Subramanian et al, 2007; Zhang et al, 2007). Levels of isoflavones in soybean seeds is regulated by a number of environmental and genetic factors (Mebrahtu et al., 2004). Induction of phenylpropanoid pathway, one branch in the synthesis of isoflavonoids with environmental factors have been reported extensively. For example, one genotype were planted in different years at the same location have up to 3-fold variation in isoflavone content (Wang and Murphy 1994) . Temperature is an abiotic factor of the much-studied effects on isoflavone content (Lozovaya et al .. 2005) . Nevertheless, the water regime (Bennett et al., 2004; Caldwell et al., 2005; Lozovaya et al., 2005), soil fertility (Vyn et al., 2002), ultraviolet (Beggs et al.,1985) and gamma radiation is also a factor affecting isoflavone content. One of the efforts to increase soybean isoflavone content in terms of the environment is the provision of nutrient N, because it plays the role in the formation of N protein, constituent of protoplasm, chlorophyll molecule, nucleic acids, and amino acids which are the building blocks of protein. The greater of protein content in the seed need more nitrogen (Ashari. 2006). Based on this background, the objective of the study was to determine the response of three soybean varieties that received drought stress on the management of N in terms of content of soy isoflavones.

Materials and Methods

The study was conducted on February to June 2012 in the greenhouse of Faculty of Agriculture University of Sumatera Utara. 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. Research used a Randomized Block Design Factorial consisted of 3 factors and 3 replications. The first factor that soybean variety consists of Anjasmoro, Wilis and Sinabung. The second factor is soil moisture condition namely 50%, 60%, 70% and 80% of field capacity. The third factor is N sources consists of without N fertilizer (control), N an organic fertilizer recommendation dose (50 kg urea/ha), biological N sources (inoculation of *Bradyrhizobium* sp.), organic N sources (straw compost 10 t/ha), organic N sources (farmyard manure 10 t/ha).

The soil was taken from dry land Sambirejo Village (Langkat). Before planting done liming with dolomite 500 kg/ha and incubated for 3 weeks. Soil for the research put into the polybag. Polybag previously been covered with plastic. Inoculation isolate *Bradyrhizobium* sp. carried out in accordance with the treatment, by isolate mixed with soybean seed just before planting at the shade in the morning. Determination of soil water content by drying method (oven), while determination of the water content of field capacity was conducted by Altrick method. The variables observed were genistein, daidzein, glycitein and isoflavone total. Concentration of genistein, daidzein and glycitein were determined using a high-performance liquid chromatography (HPLC) modified from Franke et al. (1995) Total isoflavone concentrations were summed from genistein, daidzein and glycitein contents.

Results and Discussion

Genistein

Based on Table 1 showed that N treatment tent to increase the genistein content compared to without giving N. Anjasmoro variety gave higher genistein content than Wilis and Sinabung. Both of the varieties Anjasmoro, Wilis and Sinabung showed that N treatment increased compared with the genistein content without giving N.

Table 1. Genistein content of three soybean varieties on effect of N sources

Source of Nitrogen	Variety			Average
	Anjasmoro	Wilis	Sinabung	
Without N	216.76	197.62	208.72	207.70
Urea	298.68	232.70	251.70	261.03
Bradyrhizobium.sp.	271.93	233.98	256.18	254.03
Farmyard manure	256.85	263.66	283.93	268.15
Rice Straw compost	263.84	256.85	225.04	248.58
Average	261.61a	236.96b	245.12ab	

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

Increase in the percentage of field capacity caused to decrease genistein content. In general, genistein content in Anjasmoro variety decreased with increasing percentage of field capacity up to 70% of field capacity, but in the varieties Sinabung, genistein content increased with percentage of field capacity up to 60% of field capacity (Table 2).

Table 2. Genistein content of three soybean varieties on effect of drought stress

Variety	Drought stress (% of field cacapcity)				Average
	50	60	70	80	
Anjasmoro	325.56a	229.52h	234.59gh	256.78c	261.61a
Wilis	236.13fg	262.81b	238.67f	210.22i	236.96b
Sinabung	224.17h	243.31e	260.96b	252.02d	245.12ab
Average	261.95	245.21	244.74	239.68	

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

Daidzein

Based on Table 3 showed that the source of N Bradyrhizobium sp. gives the highest content of daidzein, whereas treatment without N application and straw compost gives the lowest content of daidzein. Although not significant, the daidzein content of Wilis provide the highest, followed by Anjasmoro and Sinabung. Innoculation of Bradyrhizobium sp. and Anjasmoro tent to give the highest of daidzein content while rice straw compost and Sinabung tent to give the lowest of daidzein content.

Glycitein

Based on Table 4 showed that Urea and Bradyrhizobium sp. increased Glycitein content significantly. Increasing drought stress from 50% to 80% of field capacity caused to increase glycitein content. Interaction between Bradyrhizobium sp. and 80% of field capacity increased Glycitein content significantly, while the interaction between without giving N and 50% of field capacity gave the lowest Glyctein content.

Table 3. Daidzein content of three soybean varieties on effect of drought stress

Source of Nitrogen	Variety			Average
	Anjasmoro	Wilis	Sinabung	
	....................µg/g....................			
Without N	536.88	531.13	522.76	497.31b
Urea	543.55	595.68	568.84	569.17ab
Bradyrhizobium.sp.	636.32	606.29	604.76	518.69a
Farmyard manure	558.21	580.91	565.38	517.18ab
Rice straw compost	546.86	550.75	465.73	582.41b
Average	564.36	572.95	545.49	536.95

Note : Values by the same letter do not significantly differ base on Duncan Mutiple Range Test (F = 5%.)

Tabel 4. Glycitein content of soybean on effect of N sources and drought stress

Source of Nitrogen	Drought stress (% of field capacity)	Average

	50%	60%	70%	80%	
	……………µg/g……………				
Without N	34.87h(74)	43.53efg(93)	57.81ab(123)	46.93def	45.78b
Urea	39.82fgh(68)	57.87ab(98)	58.13ab(99)	58.84ab	53.67a
Bradyrhizobium.sp.	48.37de(75)	59.78ab(92)	53.20bcd(82)	64.67a	56.51a
Farmyard manure	43.52efg(92)	51.96bcd(110)	36.28h(77)	47.19de	44.74b
Rice straw compost	47.11de(124)	36.36h(96)	47.19de(125)	37.90gh	37.90b
Average	42.74b	49.90a	50.52a	51.11a	

Note : Values by the same letter do not significantly differ base on Duncan Mutiple Range Test (F=5%)

Isoflavone total

Based on Table 25 showed that the source of N Urea and Bradyrhizobium.sp. gave the highest total isoflavone content significantly compared without giving the source N. Drought stress had no significant effect, but there is a downward trend in total isoflavone levels by increasing percent of field capacity.. Drought stress 50% of field capacity gave the highest of isoflavone total content, while 80% of field capacity gave the lowest of isoflavone total content

Table 5. Isoflavone total content of soybean on effect of N sources and drought stress

Source of Nitrogen	Drought stress (% of field capacity)				Average
	50%	60%	70%	80%	
	……………µg/g……………				
Without N	784.30	801.41	774.44	741.86	775.50c
Urea	889.89	888.51	884.25	873.38	884.01ab
Bradyrhizobium.sp.	954.83	898.93	901.62	852.87	902.06a
Farmyard manure	871.39	907.07	875.90	818.85	868.30ab
Rice straw compost	844.86	844.42	767.57	851.77	827.16bc
Average	869.05	868.07	840.76	827.74	

Note : Values by the same letter do not significantly differ base on Duncan Mutiple Range Test (F=5%)

There is a distinct pattern in the increase in the isoflavone total content in Anjasmoro, Wilis and Sinabung with increasing the drought stress. At Anjasmoro variety, increasing drought stress caused the lower of isoflavone total content, while the varieties Sinabung and Wilis are the opposite pattern (Figure 1).

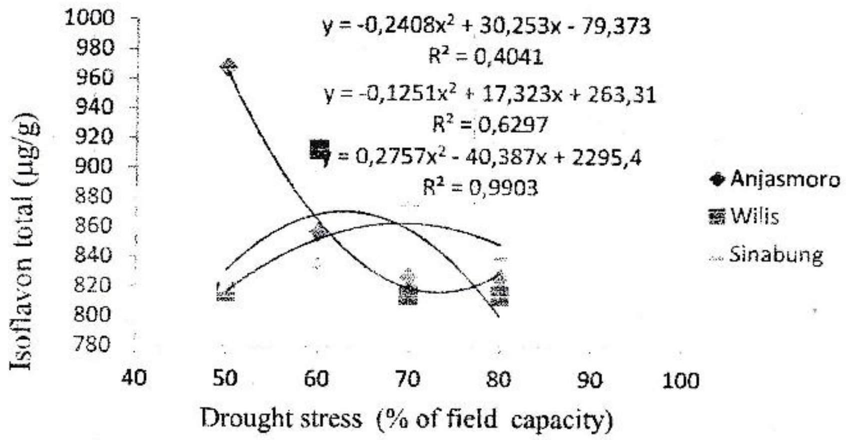

Figure 1. The relationship between total isoflavone soybean varieties with the availability of water

The higher genistein content on Anjasmoro than Willis but not significantly different compared to Sinabung isoflavones indicated that the formation was influenced by genetic factors. Hoeck et al. (2000); Mebrahtu et al, (2004); Gutierrez-Gonzalez et al., (2009), Murphy et al, (2009) reported that seed isoflavone levels are very varied and regulated by genetic and environmental factors, with the components of the interaction between the factors genetic and environmental. Tsukamoto et al. (1995) stated that in the seeds, about 90% of the total isoflavones contained in the cotyledon and hypocotyl.

Sources of N significantly affect genistein content compared without giving N. This indicates that the role of N in the formation of isoflavones in soybean. Function of N for plants was instrumental in the formation of amino acids and proteins. Biosynthetic pathways of isoflavones (genistein, daidzein and glycitein) begins with the formation of phenylalanine as the main precursor. Phenylalanine is one of the amino acid group, whereas N plays a role in the formation of amino acids. Therefore N application increased the isoflavone content compared without giving N.

Conclusions

Anjasmoro variety gave higher genistein content than Wilis and Sinabung. Innoculation of Bradyrhizobium sp. and Anjasmoro tent to give the highest of daidzein content while rice straw compost and Sinabung tent to give the lowest of daidzein content. Increasing drought stress from 50% to 80% of field capacity caused to increase glycitein content. Interaction between Bradyrhizobium sp. and 80% of field capacity increased Glycitein content significantly. Drought stress 50% of field capacity gave the highest of isoflavone total content, while 80% of field capacity gave the lowest of isoflavone total content.

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