Effect of indigenous mycorrhizal fungi on organic osmotic adjusment in soybean under salt stress

Nini Rahmawati, Rosmayati, Delvian, and Mohammad Basyuni

Faculty of Agriculture, University of Sumatera Utara

Coorresponding Author: nini@usu.ac.id

Abstract. Research about influence of indigenous mycorrhizal fungi on organic osmotic adjustment to salinity stress in soybean has been implemented in saline land in Percut Sei Tuan sub district, Regency of Deli Serdang with EC 4-5 mmhos/cm. This study aimed to determine the effect of indigenous mycorrhizal inoculation on organic osmotic adjusment in different soybean varities. This study used Split Plot Design with two plots. The main plot was soybean varieties consist of non-selected Grobogan variety (salt-sensitive variety) and F5 selected Grobogan variety (salt-tolerant variety). Split plot was mycorrhizal inoculum consists of 7 types of mycorrhizal inoculum namely without inoculum, *Glomus sp-1*, *Glomus sp-2*, *Glomus sp-3*, *Glomus sp-4*, *Glomus sp-5*, and combined of all mycorrhizal inoculum. The parameters observed were leaf proline content, reducing sugar and soluble protein. The results showed that leaf proline content, reducing sugar content and soluble protein were significantly different in soybean varieties, mycorrhizal significantly affected leaf proline content, reducing sugar and soluble protein, while the soybean varieties and mycorrhizal interaction significantly affected soluble protein content in leaves.

***Keywords*:** organic osmotic adjusment, mycorriza, soybean, salt stress

Introduction

Soybean (*Glycine max*) is one of the most important grain legumes. It represents not only an essential source of protein, oil and micronutrients in human and animal diets, but is also an attractive crop for the production of biodiesel (Pimentel and Patzek, 2008). Growth, development and yield of soybean are the result of genetic potential interacting with environment. Soybean seed production may be limited by environmental stresses such as soil salinity (Ghassemi-Golezani et al. 2009; Silvente et al. 2012).

Soybean is classified as a moderately salt-tolerant crop. Soybean germplasms display a spectrum of salt tolerance capability from high to low (Phang, et al 2008). Plant salinity tolerance can be assessed either as a reduction in relative plant growth rate after a prolonged exposure to a given concentration of salt or as a plant survival rate after a treatment with a defined concentration of salt (Munns, 2002). The inhibition of plant growth and even plant death by salinity is attributed mainly to osmotic stress, ionic toxicity, and nutritional imbalance (Kaymakanova et al., 2010; Wu et al., 2010).

In response to osmotic stress caused by salt or drought, plants could accumulate metabolites that act as compatible solutes to lower the cellular osmotic potential without affecting normal metabolic reactions (Hasegawa et al. 2000). The types of osmoprotectant metabolites and their relative contribution in lowering the osmotic potential differ greatly among plant species. Osmotic adjustment has been reported in legume with a high tolerance to water stress (Ford, 1984; Ashraf and Iram, 2005). Metabolic adjustments in response to the adverse environmental conditions may highlight pools of metabolites that play important roles in metabolism and physiology and may indicate which pathways have been perturbed by the stress. Osmotic adjustment, an important adapted mechanism of higher plants, depends on the accumulation of compatible solutes such as proline, soluble sugar, and glycinebetaine.

Several eco-physiological studies investigating the role of arbuscular mycorrhizal fungi symbiosis in protection against osmotic stresses have demonstrated that the symbiosis often results in altered rates of water movement into, through and out of host plants, with consequent effects on tissue hydration and plant physiology (Auge, 2001). Arbuscular mycorrhizal (AM) plants under osmotic stress conditions, should provide an insight into the role of the AM symbiosis in the process of osmotic adjustment during osmotic stress (Ruiz-Lozano, 2003). AMF can improve host physiological processes like water absorption capacity of plants by increasing root hydraulic conductivity and favourably adjusting the osmotic balance and composition of carbohydrates (Evelin et al, 2009; Ruiz-Lozano, 2003). Mycorrhizae were shown to have beneficial effects in delaying or coping with toxic effects caused by soil salinity by maintaining an overall physiological balance (Sharifi et al., 2007).

Materials and Methods

This research had conducted saline land in Percut Sei Tuan sub district, Regency of Deli Serdang with EC 4-5 mmhos/cm on February – Jay 2013. The experiment consisted Split Plot Design with two plots. The main plot was soybean varieties consist of non-selected Grobogan variety (salt-sensitive variety) and F5 selected Grobogan variety (salt-tolerant variety). Split plot was mycorrhizal inoculum consists of 7 types of mycorrhizal inoculum namely without inoculum, *Glomus sp-1*, *Glomus sp-2*, *Glomus sp-3*, *Glomus sp-4*, *Glomus sp-5*, and combined of all mycorrhizal inoculum. Inoculum of arbuscular mycorrhizal fungi isolated from saline soil.

The data were analyzed statistically using F-test and then following by Duncan Multiple Range Test (DMRT) at 5% level. The parameter observed were leaf proline content, leaf soluble sugar content, and leaf soluble protein content. Free proline content was measured spectrophotometrically according to the method of Bates et al. (1973). The protein content in the leaves was determined as described by Bradford (1976) method using bovine serum albumin as the standard. Soluble sugar content was determined by the anthrone method using succrose as standart.

Results and Discussion

Leaf Proline Content

Table 1 showed that inoculation of arbuscular mycorrhizal fungi significanly affect the leaf proline content in different varieties of soybean.

Table 1. Mean values of leaf proline content in different varieties of soybean and inoculum of arbuscular mycorrhizal fungi

Soybean Variety	Arbuscular Mycorrhizal Fungi							Average
	Control	*Glomus sp-1*	*Glomus sp-2*	*Glomus sp-3*	*Glomus sp-4*	*Glomus sp-5*	Mix of all inoculum	
Non-selected	111.73	108.31	106.75	104.34	103.59	100.76	99.32	104.97a
Selected	99.99	97.59	95.29	93.89	93.13	92.25	90.76	94.70b
Average	105.86a	102.95b	101.02bc	99.12cd	98.36cd	96.50de	95.04e	

Note : Mean values by the same letter do not significantly differ based on DMRT (F = 0.05)

Proline content of leaves was lower in mycorrhizal plant. Significant difference between mycorrhizal plant and non-mycorrhizal plant was observed. The treatment of combined of all mycorrhizal inoculum gave the lowest leaf proline content and the control treatment (without inoculation of AMF) gave the highest proline content, whereas the proline content increase 11.39%. Several authors reported that non-AM plants accumulated more proline that AM plants at various salinity levels, suggesting that proline accumulation in plants may be a sympton of stress in less salt-tolerant species or that this accumulation may be also due to salinity and not necessarily to mycorrhizal colonization. Stressed mycorrhizal plants osmotically adjust better than non-AM plants with a greater concentration of solutes of Na or synthesise more solutes such as proline. The higher accumulation of Na in AM peanuts in this study was for the osmoregulation purpose, while the reduced foliar proline in arbuscular mycorrhizal peanuts suggests that the fungus was able to alleviate the damage due to salt stress (Evelin et al, 2009; Al-Khaliel, 2010).

Variety of soybean showed significantly different leaf proline content, whereas the proline content in selected variety lower than non-selected variety. Celik and Atak (2012) reported more proline content in soybean salt-sensitive cultivar than salt-tolerant cultivar. Increased proline under salt stress has an adaptive significance as it lowers the generation of free radicals and thus reduces the lipid peroxidation linked membrane damage resulting in their stabilization The proline accumulation is an adaptive response related to salt-tolerance and it contributes to water status of plants under water or salt stress and act as free radical scavenger (Alia et al, 1995). The accumulation of proline in plants exposed to identical salt stresses appears to be cultivar specific in soybean. Proline levels in stressed plants were inversely correlated with capacity to withstand salinity stress (Moftah and Michel, 1987).

Leaf Soluble Sugar Content

Table 2 showed that selected variety of soybean significantly different from non-selected soybean variety. Leaf soluble sugar content in selected variety higher than non-selected. The salt tolerant lines had generally greater soluble sugars than the salt sensitive ones. Ashraf (1994) found salt tolerant wild populations with cultivated populations of *Melilotus indica* and *Eruca sativa*, that the former had significantly higher soluble sugars in their leaves than the latter salt sensitive populations at varying salt levels of the growth medium. However, this does not rule out a significant role of soluble sugars in salt tolerance nor a potential role for soluble sugar accumulation as an indicator for salt tolerance in breeding programs for some species. It suggests that salt-sensitive trifoliate soybean plants were not able to accumulate soluble sugars to enhance tolerance in response to salinity stress. Additionally, salt stress usually decreases the efficiency of photosynthesis in source tissues and thus may reduce the supply of soluble sugars to pool tissues under salt stress (Rosa et al., 2009).

Table 2. Mean values of leaf soluble sugar content in different varieties of soybean and inoculum of arbuscular mycorrhizal fungi

Soybean Variety	Arbuscular Mycorrhizal Fungi							Average
	Control	*Glomus sp-1*	*Glomus sp-2*	*Glomus sp-3*	*Glomus sp-4*	*Glomus sp-5*	Mix of all inoculum	
Non-selected	0.17	0.22	0.35	0.41	0.49	0.58	0.82	0.43b
Selected	0.48	0.57	0.62	0.70	0.74	0.82	1.09	0.72a
Average	0.32f	0.40e	0.48d	0.56c	0.62c	0.70b	0.95a	

Note : Mean values by the same letter do not significantly differ based on DMRT (F = 0.05)

Based on Table 2. showed that leaf soluble sugar content in non-mycorrhizal plant lower than mycorrhiza-plant. The plant inoculated with combined of all inoculum had higher leaf soluble sugar content. Zou et al (2013) reported at all the salt levels, mycorrhizal plants showed higher soluble sugar contents than non-mycorrhizal plants, which may enable mycorrhizal plants to maintain higher osmotic adjustment during salt stress. This is consistent with previous findings of Kumar et al. (2010), who observed higher soluble sugars in AM *Jatropha curcas* plants grown with 0.1, 0.2, 0.3, 0.4, and 0.5% NaCl, compared with non-AM control. Soluble sugars are important osmolytes for osmotic adjustment that are synthesized and accumulated in cytosol under salt stress (Nemati et al., 2011).

Leaf Soluble Protein Content

Table 3. showed that variety selected to salinity stress had higher leaf protein content than non-selected variety. Leaf soluble protein content in selected variety increased 12.89%. Soybean plant inoculated with mix of all inoculum had highest leaf soluble protein content and non-inoculated plant had lowest leaf soluble protein content. Interaction between soybean variety and arbuscular mycorrhizal fungi significantly affect the leaf soluble content. Interaction between selected soybean variety and mix of all AMF inoculum gave the highest protein content while the lowest on interaction non-selected variety and treatment without inoculation of AMF.

A higher content of soluble proteins has been observed in salt tolerant than in salt sensitive cultivars of barle, sunflower, finger millet, and rice (Ashraf and Harris, 2004). Soybean (*Glycine max*) cultivars differing in salt tolerance show different levels of proteins and amino acids when grown in the presence of NaCl. Salt-tolerant soybean cultivars Clark and Forest accumulate higher levels of soluble proteins, whereas sensitive cultivar Kint shows a decrease in the soluble protein level when grown in saline soils (Elsamad and Shaddad, 1997). Abdel-Fattah et al (2013) reported protein content increased in wheat with inoculation of AMF in comparison with control plants. Higher protein concentration may be due to higher efficiency of osmotic regulation mechanism in wheat plants which in turn prevent proteins reduction under salt stress, and synthesis of osmotin like protein or structural protein.

Table 3. Mean values of leaf soluble protein content in different varieties of soybean and inoculum of arbuscular mycorrhizal fungi

Soybean Variety	Arbuscular Mycorrhizal Fungi							Average
	Control	*Glomus sp-1*	*Glomus sp-2*	*Glomus sp-3*	*Glomus sp-4*	*Glomus sp-5*	Mix of all inoculum	
Non-selected	7.38f	8.03ef	8.2e	8.38cde	8.51cde	8.9cd	9.37bc	8.40b
Selected	8.09e	8.57cde	9.07bc	9.073bc	9.78b	10.56a	11.19a	9.48a
Average	7.74e	8.30de	8.64cd	8.73cd	9.15bc	9.73ab	10.28a	

Note : Mean values by the same letter do not significantly differ based on DMRT (F = 0.05)

Estrada et al (2013) investigated whether native AMF isolated from an area with problems of salinity and desertification can help maize plants to overcome the negative effects of salinity stress better than non-AM plants or plants inoculated with non-native AMF

Conclusions

From the results of the present investigation, it can be concluded that selected variety of soybean to salinity stress and inoculated with mix all of indigenous mycorrhizal had lowest proline content and highest of leaf soluble sugar and soluble protein content. Mychorrhiza protects the plants from salinity injuri by organic osmotic adjusment, thus alleviating salt damage and enhancing salt tolerance of the selected variety of soybean.

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