Chlorophyll Content Of Soybean As Affected By Foliar Application Of Ascorbic Acid And Inoculation Of Arbuscular Mycorrhizal Fungi In Saline Soil

Nini Rahmawati, Rosmayati, Delvian, Mohammad Basyuni

Abstract: Salt stress limits soybean production in vast areas worldwide. It has been found that increases salinity resulted in decrease of chlorophyll contents in soybean leaves. Effect of foliar application of ascorbic acid and inoculation of mycorrhizal arbuscular fungi on chlophyll content of soybean was studied in saline soil with EC 4– 5 mmhos/cm. The experiment was laid out in split-split plot design with three replications. The treatments consisted of two variety of saline tolerant soybean (non-selected soybean and F4 selected soybean) in main plots, ascorbic acid concentration (0 and 500 ppm) in sub plots. Mycorrhizal arbuscular fungi inoculum namely without inoculum (control), Glomus sp-1, Glomus sp-2, Glomus sp-3, Glomus sp-4, Glomus sp-5, and combined of all mycorrhizal inoculum were the sub-sub plot treatments. The parameters observed were chlorophyll a, b and total by using Henry and Grime (1993) method. The results showed that F4 selected soybean tolerant salinity, foliar application of ascorbic acid, inoculation of myorrhizal arbuscular fungi and interaction all of treatment significantly increased chlorophyll a, b and total chlorophyll of soybean leaves in saline soil.

Index Terms: ascorbic acid, chlorophyll, mycorrhizal arbuscular fungi, soybean, saline

1 INTRODUCTION

Salinity is one of the most important environmental factors that cause reduction in plant growth, development and productivity worldwide. During stress conditions the endogenous levels of growth regulators became low, which can be overcome by their exogenous application. Exogenous application of plant growth regulators, fertilizers, and on enzymatic antioxidants has been successfully used to minimize the adverse effects of salinity on plant growth and yield (Tuna, et al, 2008; Kaya et al, 2010) [1,2].

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- *Many studies have been reported on the use of growth regulators or mycorrhizal fungi in decreasing harmful effects of environmental stress. Mycorrhiza fungi increase growth, photosynthetic pigments and photosynthesis of host plants by better mineral nutrition. They cause chlorophyll organs of plant to grow by absorbing required carbon, giving nutriments to plant and increasing efficiency of photosynthesis (Enteshari,and Hajbagheri, 2011).*

Ascorbic acid (AA) is regarded as one of the most effective growth regulators against abiotic stresses (Conklin, 2001) [3]. AA is water-soluble antioxident, protecting biologically important macromolecule from oxidative damage caused by hydroxyl radicals, superoxide, and singlet oxygen. In addition to its importance in photoptotection and regulation of photosynthesis. AA plays an important role in regulation of cell cycle and several fundamental processes of plant growth and development (Dehghan et al, 2011) [4]. Golan-Goldhirsh et al. 1995 [5] on soybean indicated that, plants treated with ascorbic acid showed increases in photosynthesis processes. Many studies have been reported on the use of growth regulators or mycorrhizal fungi in decreasing harmful effects of environmental stress. Mycorrhiza fungi increase growth, photosynthetic pigments and photosynthesis of host plants by better mineral nutrition. They cause chlorophyll organs of plant to grow by absorbing required carbon, giving nutriments to plant and increasing efficiency of photosynthesis (Enteshari,and Hajbagheri, 2011) [6]. Netondo et al. (2004) [7] reported that photosynthetic activity decreases when plants are grown under saline conditions leading to reduced growth and productivity. The reduction in photosynthesis under salinity can be attributed to a decrease in chlorophyll content (Jamil et al. 2007) [8]. Salinity can affect chlorophyll content through inhibition of chlorophyll synthesis or an acceleration of its degradation (Netondo et al. 2004; Jamil et al. 2007) [7,8]. It has been found that increases salinity resulted in decrease of chlorophyll contents in soybean leaves. Salinity causes decrease both in growth and in net photosynthesis of higher plants. There is evidence in the literature suggesting that the composition and fuction of plants may under go changes in response to salinity (Sharma and Hall, 1990; Moales at al., 1992; Esse and Al-Ani, 2001) [9,10,11]. Soybean is important crop in our world because of it's high protein and oil content. Wide genetic variability exists among different cultivars of soybean (Ghassemi-Golezani et al. 2009) [12]. Soybean is classified as a moderately salt-tolerant crop and the final yield of soybean will be reduced when soil salinity exceeds 5 dS/m (Ashraf 1994) [13]. Soybean germplasms display a spectrum of salt tolerance capability. A vast collection of soybean genotypes was screened in order to identify genetic materials showing high tolerance to salt stress (Shao et al. 1986) [14]. This research was carried out to evaluate changes in chlorophyll content in leaves of soybean in response to salinity stress as affected by foliar application of ascorbic acid and inoculation of arbuscular mycorrhizal fungi.

2 MATERIALS AND METHODS

The field experiment was conducted in saline soil in in Percut Sei Tuan sub district, Regency of Deli Serdang North Sumatera Indonesia with EC 4-5 mmhos/cm on February – July 2013. The experiment was laid out in split-split plot design with three replications. The treatments consisted of two variety of saline tolerant soybean (non-selected soybean and F4 selected soybean) in main plots, ascorbic acid (AA) concentration (0 and 500 ppm) in sub plots. Mycorrhizal arbuscular fungi (AMF) inoculum namely without inoculum (control), Glomus sp-1, Glomus sp-2, Glomus sp-3, Glomus sp-4, Glomus sp-5, and combined of all mycorrhizal inoculum were the sub-sub plot treatments. Foliar application treatment gave to plant from 2 weeks after planting until a week before harvesting. For determination of chlorophyll concentrations, leaf samples (0.1 g) from fully expanded leaflets of each plant were collected and macerated in a mortar with 10 mL of 80% aqueous acetone (v/v). Then, extracts were used to measure the absorbance at 649nm and 665nm. The chlorophyll a [Chl a], b [Chl b] and total [Chl total] concentrations were estimated using equations based on the specific absorption coefficients as reported by Henry dan Grime (1993) [15].

$$\text{Chlorophyll a} = \{(12.7 \times A663) - (2.69 \times A645)\}/10$$
$$\text{Chlorophyll b} = \{(22.9 \times A645) - (4.68 \times A663)\}/10$$
$$\text{Chlorophyll total} = \{(8.02 \times A663) + (20.2 \times A645)\}/10$$

The chlorophyll concentrations were expressed as mg/g. All data were subjected to Analysis of Variance (ANOVA) using SAS statistical software. Also, means were separated by Duncan's Multiple Range Test (DMRT) at 5% level of significance.

3 RESULTS AND DISCUSSION

The result showed chlorophyll a, b and chlorohyll total in F4 selected soybean tolerant salinity higher than the unselected (Figure 1, 4, 7). The increased are respectively 33.14%, 54.35% and 39.69% for each parameter. Foliar application of ascorbic acid increased chlorophyll a, b and total chlorophyll each 11.23%, 25% and 16.15% than control treatment (Figure 2, 5 and 8). Salinity contributed to a reduction in chlorophyll a and b contents of soybean varieties, this decrease being more drastic in clorophyll a than in chlorophyll b. Also, inoculation of mycorrhizal arbuscular fungi increased chlorophyll content in soybean leaves. Figure 3, 6 and 9 show the highest chlorophyll content is the inoculation treatment that uses a mixture of all types of mycorrhiza. Interaction between foliar aplication of ascorbic acid and inoculation of mycorrhizal arbuscular fungi on chlorophyll a, chlorophyll b and total chlorophyll in F4 selected soybean tolerant salinity significantly increased. (Tabel 1, Tabel 2 and Tabel 3). Decreasing chlorophyll content of soybean leaves with increasing salinity could be related to increasing the activity of chlorophyll degrading enzyme, chlorophyllase (Jamil et al. 2007) [8], and the destruction of the chloroplast structure and the instability of pigment protein complexes (Singh and Dubey ,1995) [16].

Tabel 1. Effect of foliar application of ascorbic acid and inoculation of arbuscular mycorrhizal fungi on chlorophyll a (mg-1 FW)

Arbuscular Mycorrhizal Fungi	Non-selected soybean		F4 selected soybean tolerant salinity	
	Without AA	AA foliar application	Without AA	AA foliar application
Without AMF	1.32s	1.45rst	1.91klm	1.96
Glomus sp-1	1.39st	1.52qrs	1.96jk	2.24ef
Glomus sp-2	1.58pqr	1.59pqr	2.06hij	2.38cd
Glomus sp-3	1.65opq	1.71nop	2.10ghi	2.45c
Glomus sp-4	1.77mno	1.87klm	2.17efgh	2.48bc
Glomus sp-5	1.80lmn	2.01ijk	2.21efg	2.59ab
Mix of all inoculum	1.92jkl	2.13fghi	2.31de	2.68a

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

Tabel 2. Effect of foliar application of ascorbic acid and inoculation of arbuscular mycorrhizal fungi on chlorophyll b (mg-1 FW)

Arbuscular Mycorrhizal Fungi	Non-selected soybean		F4 selected soybean tolerant salinity	
	Without AA	AA foliar aplication	Without AA	AA foliar aplication
Without AMF	0.72o	0.85mno	1.06ijkl	1.29def
Glomus sp-1	0.75o	0.93lmn	1.22fgh	1.32def
Glomus sp-2	0.75o	0.99ijklm	1.21fgh	1.39de
Glomus sp-3	0.81no	1.07ijkl	1.24fg	1.56c
Glomus sp-4	0.81no	1.09hijk	1.28ef	1.71b
Glomus sp-5	0.95klmn	1.10hij	1.33def	1.82b
Mix of all inoculum	0.98ijklm	1.13ghi	1.42d	1.97a

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

In plants, oxidative stress and signs of senescence include loss of chlorophyll and protein and decline in membrane permeability, all of which lead to a progressive reduction in photosynthetic capacity. Salt stress leads to an increase in free radicals in chloroplasts and destruction of chlorophyll molecules by ROS, which results in reduction of photosynthesis and growth. Singlet oxygen atoms and O2−

radicals predominantly attack double-bond-containing compounds, thus damaging the chloroplast membrane system and photosynthetic reaction centers (Zhang et al. 2003) [17]. An increase in reactive oxygen species generation due to abiotic stress such as salt stress would result in further damage to PSII. Reactive oxygen species can also cause serious damage to organelles such as chloroplasts, mitochondria and plasma membranes. In consequence, efficiency of PSII decreases. This in turn may result in decrease damage to chloroplasts and PSII via scavenging of ROS by ascorbic acid (Dolatabadian and Jouneghani, 2009) [18]. Soybean plant inoculated by the treatment of combined of all mycorrhizal inoculum gave the highest leaf chlorophyll content and the control treatment (without inoculation of AMF) gave the lowest chlorophyll content. Similar results were reported for peanut (Al-Khaliel, 2010) [19] and garlic (Borde et al, 2010) [20]. Higher chlorophyll content may reflect the higher photosynthetic rate necessary to support carbon cost of arbuscular mycorrhizae associations. Increased photosynthetic of arbuscular mycorrhizae plant may be mediated by increased P nutrition, which obviously could given rise to an increase in plant growth (Al-Khaliel, 2010) [19]. The plant is stimulated by colonization to a limititation of Na transport towards leaves, and leads to better functioning of chloroplast and photosynthetic efficiency (Borde et al, 2010) [20].

Tabel 3. Effect of foliar application of ascorbic acid and inoculation of arbuscular mycorrhizal fungi on total chlorophyll (mg-1 FW)

Arbuscular Mycorrhizal Fungi	Non-selected soybean		F4 selected soybean tolerant salinity	
	Without AA	AA foliar application	Without AA	AA foliar application
Without AMF	2.05p	2.29o	2.97j	3.25hi
Glomus sp-1	2.14p	2.46mn	3.18i	3.56e
Glomus sp-2	2.32n	2.58m	3.27gh	3.76d
Glomus sp-3	2.46mn	2.77jk	3.35fg	4.02c
Glomus sp-4	2.59lm	2.96jk	3.45f	4.19c
Glomus sp-5	2.75kl	3.11i	3.54ef	4.41b
Mix of all inoculum	2.90jk	3.26h	3.72de	4.65a

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

Soybean genotype responded differently to salinity. The reduction of chlorophyll content of soybean studied. Less reduction in chlorophyll content was observed in F4 selected soybean tolerant salinity than non-selected soybean. Our result in agreement with Moussa (2006) [21] who reported that salt stress significantly decreased both chlorophyll a and b. An experiment done by Ashrafuzzaman et al (2000) [22] showed with increasing salinity chlorophyll a, b and total chlorophyll were reduced.

4 CONCLUSION

Less reduction of chlorophyll a, b and total chlorophyll was observed in F4 selected soybean tolerant salinity. Foliar application of ascorbic acid significantly increase chlorophyll a and total chlorophyll of soybean leaves. Inoculation of arbuscular mycorrhizal fungi significantly increased chlorophyll content of soybean leave in saline soil. The treatment of combined of all mycorrhizal inoculum gave the highest chlorophyll content compared the other treatment.. Interaction between foliar aplication of ascorbic acid and inoculation of mycorrhizal arbuscular fungi on chlorophyll a, chlorophyll b and total chlorophyll in F4 selected soybean tolerant salinity significantly increased

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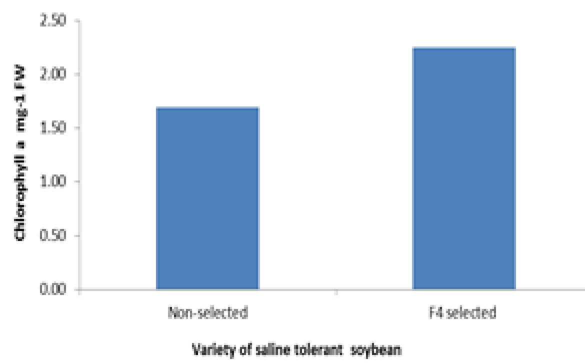


Figure 1. Effect of variety of saline tolerant soybean on chlorophyll a

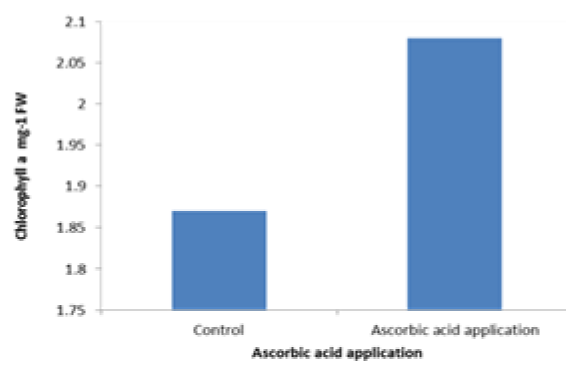


Figure 2. Effect of ascorbic acid foliar application on chlorophyll a

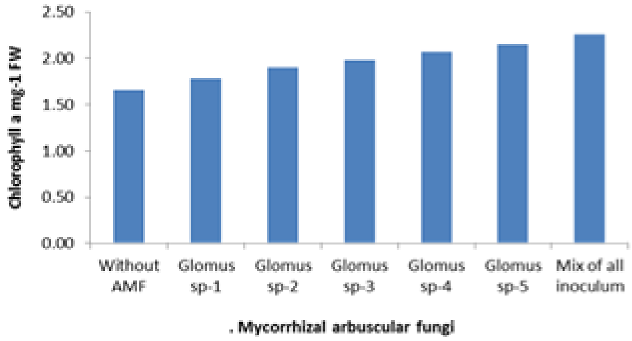


Figure 3. Effect of inoculation of mycorrhizal arbuscular fungi on chlorophyll a

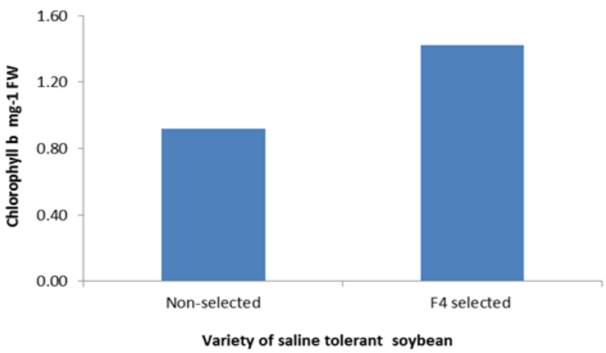
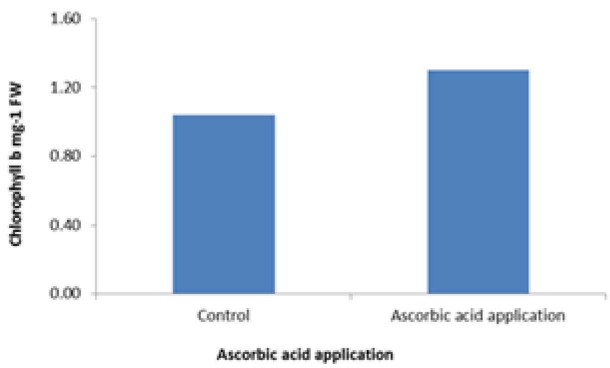


Figure 4. Effect of variety of saline tolerant soybean on chlorophyll b



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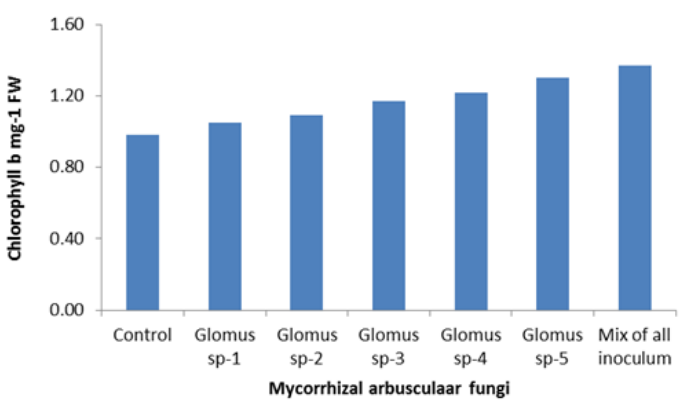

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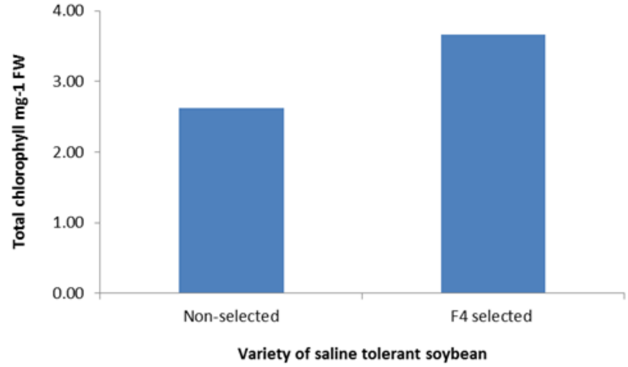

Figure 7. Effect of variety of saline tolerant soybean on total chlorophyll

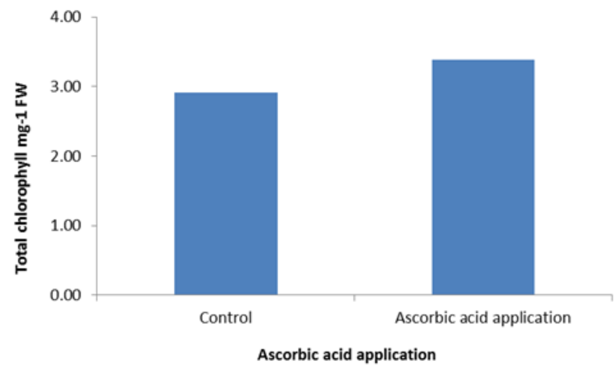

Figure 8. Effect of ascorbic acid foliar application On total chlorophyll

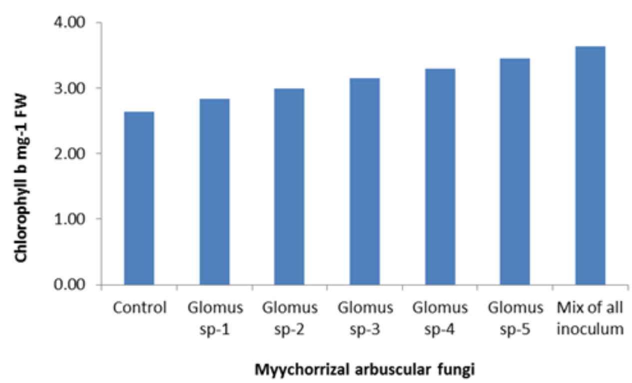

Figure 2. Effect of inoculation of mycorrhizal arbuscular fungi on total chlorophyll