

Effect of foliar application of α -tocopherol on vegetative growth and some biochemical constituents of two soybean genotypes under salt stress

N Rahmawati and R I M Damanik

Faculty of Agriculture, Universitas Sumatera Utara, Padang Bulan, Medan 20155, Indonesia
E-mail : nini@usu.ac.id

Abstract. Foliar spray of plant growth regulating compounds including antioxidants is an effective strategy to overcome the adverse effects of environmental constraints on different plants. A field experiment was conducted on May – July 2017 at the experimental farm in Paluh Merbau Village Deli Serdang (EC 6 – 7 dS/m). The aim was to study the effects of foliar spray of α -tocopherol (0, 250, 500, 500 ppm) on vegetative growth and some chemical constituents of 2 soybean genotypes (Grobogan x Grobogan and Grobogan x Anjasmoro) under salt stress (EC 6 – 7 dS/m). Most of morphological and biochemical parameters were significantly affected by application of α -tocopherol. The α -tocopherol at 500 ppm recorded the best value of root fresh weight, shoot and root dry weight, number of leaves, chlorophyll b, and soluble protein content. There was significant difference found between plants treated with α -tocopherol in terms of number of branch, shoot fresh weight, and chlorophyll a. Soybean genotypes showed diverse morphology and physiological responses to salt stress. Grobogan x Anjasmoro genotype was salt-sensitive based on all variable, while Grobogan x Grobogan genotype was more tolerant based on morphological and biochemical characters.

1. Introduction

Soybean is a strategic crop plant grown to obtain edible oil and forage. Nevertheless, soybean production may be limited by environmental stresses such as soil salinity [1]. 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-1 [2]. High salt imposes damages in the whole life cycle of soybean. The difference in a plant response to a given level of salinity is dependent on the concentration and composition of ions in solution as well as the genotype that is exposed to the salinity [3]

The degree of salt tolerance of soybean germplasms varies among different developmental stages [4]. The essential processes leading to plant adaption to salt stress include control of water loss through stomata, metabolic adjustment, toxic ion homeostasis, and osmotic adjustment [5;6]. Soybean germplasms display a spectrum of salt tolerance capability from high to low [4].

Application of antioxidants including vitamins has gained considerable attention for alleviating the negative impact of water and salinity stress on plants in terms of plant growth and yield quantity and quality [7].

Alpha-tocopherol is a small molecule that is synthesized in the plants, mainly concentrated in plastids, and is one of the most effective single-oxygen quenchers [8]. Tolerance to salt stress, chilling stress, UV-B stress and pollutant stress is partly correlated with tocopherol content [9]. Alpha-



tocopherol is a strong antioxidant that assists in maintaining membrane stability [9], intracellular signaling, and transport of electrons in the photosystem-II system [10]) and is photoprotective in nature.

Foliar spray with α -tocopherol on faba bean plants induced increase in growth parameters, yield components, chlorophyll a, b and carotenoids content [11]. Tocopherols play a role in a range of different physiological phenomena including plant growth and development, senescence, preventing lipid peroxidation and to interact with the signal cascade that convey abiotic and biotic signals [12; [13].

The aim was to study the effects of foliar spray of α -tocopherol (0, 250, 500, 500 ppm) on vegetative growth and some chemical constituents of 2 soybean genotypes (Grobogan x Grobogan and Grobogan x Anjasmoro) under salt stress (EC 6 – 7 dS/m)

2. Materials and methods

Field experiment was conducted on May – July 2017 at the experimental farm in Paluh Merbau Village Deli Serdang (EC 6 – 7 dS/m). The experiment was arranged in a randomized block design with two factors, the first, factor is the genotype of soybean (Grobogan x Anjasmoro and Grobogan x Grobogan) and the second factor is the concentration of α - tocopherol (0, 250, 500 and 750 ppm) with three replicates per each treatment with sampling. The research started from land preparation, planting, application of exogenous antioxidant, maintenance, fertilizing and analysis morphological and biochemical parameters.

In application of exogenous antioxidant treatment plots, the whole shoots of plants were foliar application with different concentration of exogenous antioxidant. For the control treatment, plants were sprayed with water. Foliar application of exogenous antioxidant was applied once a week, started from second week after planting.

The study of morphology characters were began when the plants were 14 days. The observation on number of branch and number of leaves were measured every week began 2 week after planting (WAP) until 5 WAP. Shoot and root fresh weight, shoot and root dry weight were the plants were 6 WAP. The observation on biochemical constituents (chlorophyll a, chlorophyll b and soluble protein content) were the plants were 6 WAP.

The data were analyzed statistically using F-test and then following by Duncan Multiple Range Test (DMRT) at 5 % level.

3. Results and discussion

The foliar application of α -tocopherol significantly increased root fresh weight, shoot dry weight, and root dry weight compared with application of 250 ppm α -tocopherol and untreated plants (Table 1). The previous study reported that application of α -tocopherol increased fresh weight of shoots and roots in *Hibiscus rosasineses* L. Plants [14].

Table 1. Effect of application α -tocopherol on shoot and root weight in soybean genotype under salt stress

Treatment	Shoot fresh weight (g)	Root fresh weight (g)	Shoot dry weight (g)	Root dry weight (g)
Control	12.06	5,39 b	3.56 b	1.33 b
α -tocopherol 250 ppm	12.22	5.71 ab	3.70 ab	1.37 b
α -tocopherol 500 ppm	12.89	6.17 a	4.20 a	1.69 a
α -tocopherol 750 ppm	12.17	6.04 ab	3.62 ab	1.37 b

Note: Mean values by the same letter in same column do not significantly differ based on DMRT at 5% level

Shoot and root dry weight in grobogan x grobogan genotype significantly different from grobogan x anjasmoro genotype (Table 2). Maximum leaf shoot dry weight and root dry weight obtained in grobogan x grobogan genotype lead to increasing 15.43% and 11,76%, respectively. The growth of

root and shoot is the most important parameter for salt tolerance because roots are in direct contact with the soil and absorb water from soil and shoot supply it to the rest of the plant. For this reason, root and shoot length provides an important clue to the response of plants to salt stress [15]. Suppression of plant growth under saline conditions may either be due to the decreasing availability of water or to the increasing toxicity of NaCl associated with increasing salinity [16].

Table 2. Root fresh weight, root dry weight and root volume in two soybean genotype under salt stress

Genotype	Shoot fresh weight (g)	Root fresh weight (g)	Shoot dry weight (g)	Root dry weight (g)
Grobogan x Grobogan	12.77	5.87	4.04 a	1.52 a
Grobogan x Anjasmoro	11.90	5.79	3.50 b	1.36 b

Note: Mean values by the same letter in same column do not significantly differ based on DMRT at 5% level

Data in Table 3 indicated exogenous α -tocopherol application has significant effect on number of leaves. Although α -tocopherol treatment at a concentration of 500 ppm gives the highest increase in number of leaves compared with others treatment. Nevertheless, application of α -tocopherol has no significant effect on number of branch. None of different genotype led to significant changes in the number of branch and leaves. Meanwhile, data presented in Table 4 showed that number of branch and leaves increased in grobogan x grobogan genotype. Leaf injury and death is probably due to the high salt load in the leaf that exceeds the capacity of salt compartmentation in the vacuoles causing salt to build up in the cytoplasm to toxic levels [16;17]. Ludlow and Muchow [18] also reported that reduced leaf growth and accelerated leaf senescence are the common responses to water stress. The reduction in leaf number under salinity and water stress resulted from premature leaf senescence and defoliation. The other study reported, the reduction in number of internodes and number of branches could severely be affected by salinity [19] (Change et al., 1994).

Table 3. Effect of application α -tocopherol on number of branch and number of leaves in soybean genotype under salt stress

Treatment	Number of branch	Number of leaves
Control	2.00	6.33 b
α -tocopherol 250 ppm	2.17	6.67 b
α -tocopherol 500 ppm	2.50	11.33 a
α -tocopherol 750 ppm	2.33	6.67 b

Note: Mean values by the same letter in same column do not significantly differ based on DMRT at 5% level

Table 4. Number of branch and number of leaves in two soybean genotype under salt stress

Genotype	Number of branch	Number of leaves
Grobogan x Grobogan	2.33	8.17
Grobogan x Anjasmoro	2.27	7.33

Note: Mean values by the same letter in same column do not significantly differ based on DMRT at 5% level

Chlorophyll content can be considered as one of the indices to screen plant growth. Application of α -tocopherol at a concentration of 500 ppm gives the highest increase in all biochemical characters (Table 5). Foliar application of α -tocopherol has significant effect on chlorophyll b and soluble protein content, but no significant effect on chlorophyll a. In plants, tocopherols are believed to protect chloroplast membranes from photooxidation and help to provide an optimal environment for the photosynthetic machinery [9]. El-Quesni et al. [14] reported that foliar application of α -tocopherol increased chl a, chl b and carotenoids content compared to untreated plants. El-Bassiouny et al. [11]

reported that foliar spray of α -tocopherol on faba bean plants also increased chl a, b and carotenoids content. In the other study Orabi and Abdelhamid [20] showed that although salinity (sea water) induced considerable suppression in total proteins in the seeds of faba bean, foliar application of α -tocopherol at the rate of 50 or 100 mg L⁻¹ caused a significant increase in protein content but in both stressed and nonstressed plants.

Table 5. Effect of application α -tocopherol on chlorophyll a,b and soluble protein content in soybean genotype under salt stress

Treatment	Chlorophyll a	Chlorophyll b	Soluble protein
Control	1.04	0.40 b	0.26 b
α -tocopherol 250 ppm	1.40	0.62 b	0.29 ab
α -tocopherol 500 ppm	1.51	1.13 a	0.36 a
α -tocopherol 750 ppm	1.37	0.48 b	0.26 b

Note: Mean values by the same letter in same column do not significantly differ based on DMRT at 5% level

Table 6. Chlorophyll a,b and soluble protein content in two soybean genotype under salt stress

Genotype	Chlorophyll a	Chlorophyll b	Soluble protein
Grobogan x Grobogan	1.44	0.75	0.30
Grobogan x Anjasmoro	1.22	0.57	0.28

Note: Mean values by the same letter in same column do not significantly differ based on DMRT at 5% level

There were no significantly different in two genotype. Generally, biochemical constituents increased in grobogan x grobogan genotype compared than grobogan x grobogan genotype. Chlorophyll a, b and soluble protein increased 18.03%, 31.58%, and 7.14 respectively (Table 6). The decrease in chlorophyll content under salinity conditions is reported by Yasar et al. [21], Kusvuran [22], and Nazarbeygi et al. [23]. In the salt tolerant genotypes the chlorophyll content was protected probably because of the high antioxidant enzyme activities that prevented degradation of leaf chlorophyll. It is well known that soluble protein content is a significant indicator of physiological status of plants. Golezani and Noori [24] reported that protein content of three soybean cultivars was decreased by increased salinity levels and they attributed that to the disturbance in nitrogen metabolism. Also, the decrease in protein content by salt stress may be due to the effects of sodium chloride on protein synthesis [25].

4. Conclusions

Evaluation of morphology and biochemical parameters indicated that application of exogenous α -tocopherol with different concentration induced the salt tolerance and increased vegetative growth of salt resistance soybean genotype. The highest growth of soybean genotype under salt stress was with the application of α -tocopherol 500 ppm. Grobogan x Anjasmoro genotype was salt-sensitive based on all variable, while Grobogan x Grobogan genotype was more tolerant based on morphological and biochemical characters.

The research was supported by Universitas Sumatera Utara under the scheme of Penelitian TALENTA USU 2017 with contract number : 5538/UN5.1.R/PPM/2017, 22 May 2017

References

- [1] Ghassemi-Golezani K., Ghanehpour S, Dabbagh Mohammadi-Nasab A 2009 Effects of water limitation on growth and grain filling of faba bean cultivars *Journal of Food Agriculture and Environment* Vol **7** pp 442–447
- [2] Ashraf M 1994 Breeding for salinity tolerance in plants *Crit. Rev. Plant Sci.* Vol.**13** pp 17-42
- [3] Cramer G R 1992 Response of a Na-excluding cultivar and a Na-including cultivar to varying Na/Ca *J. Exp. Bot.* Vol. **43** pp 857–864
- [4] Phang T H, Shao G and Lam H M 2008 Salt tolerance in soybean *Journal of Integrative Plant Biology* Vol. **50** (10) pp 1196-1212

- [5] Hasegawa P M, Bressan R A, Zhu J K and Bohnert H J 2000 Plant cellular and molecular responses to high salinity *Ann Rev Plant Biol.* Vol. **51** pp 463-99
- [6] Munns R and Tester M 2008 Mechanisms of salinity tolerance *Ann Rev Plant Biol.* Vol. **59** pp 651-81
- [7] Sadak M S and Dawood M G 2014 Role of ascorbic acid and α tocopherol in alleviating salinity stress on flax plant (*Linum usitatissimum* L.) *J Stress Physiol Biochem* Vol **10** pp 93-111
- [8] Fryer M J 1992 The antioxidant effects of thylakoid vitamin E (α -tocopherol) *Plant Cell Environment* Vol **15** pp 381-392
- [9] Munné-Bosch S and Alegre L 2002 The function of tocopherols and tocotrienols in plants *Critical Reviews in Plant Sciences* Vol **21** pp 31-57
- [10] Munné-Bosch S and Falk J 2004 New insights into the function of tocopherols in plants *Planta* Vol **218** pp 323-326
- [11] El-Bassiouny H M S, Gobarah M E and Ramadan A A 2005 Effect of antioxidants on growth, yield, savism causative agents in seeds of *Vicia faba* L. plants grown under reclaimed sandy soils *J. Agr. Pak.* Vol **7**(4) pp 653-659
- [12] Sattler S E, Gilliland L U, Magallanes-Lundback M, Pollard M and Penna D D 2004 Vitamin E is essential for seed longevity and for preventing lipid peroxidation during germination *Plant Cell* Vol **16** pp 1419-1432
- [13] Baffel S O and Ibrahim M M 2008 Antioxidants and accumulation of atocopherol induce chilling tolerance in *Medicago sativa*. *Int. J. Agric. Biol.* Vol **10**(6) pp 593-598
- [14] EL-Quesni F E, Abd E L-Aziz N and Maga M K 2009 Some studies on the effect of Ascorbic Acid and α -tocopherol on the growth and some chemical composition of *Hibiscus rosasinensis* L. at Nurbaria *Ozean J. Appl. Sci.* Vol. **2**(2) pp 159-167
- [15] Jamil M and Rha E S 2004 The effect of salinity (NaCl) on the germination and seedling growth of sugar beet (*Beta vulgaris* L.) and cabbage (*Brassica oleracea capitata* L.) *Korean J. plant es.* Vol.b pps 226–232
- [16] Munns R 2003 Comparitive physiology of salt and water stress *Plant Cell Environ.* Vol. **25** pp. 239–250
- [17] Munns R, James R A and Läuchli A 2006 Approaches to increasing the salt tolerance of wheat and other cereals *J. Exp. Bot.* Vol **57** pp1025–1043
- [18] Ludlow M M and Muchow R C 1990 A critical evaluation of traits for improved crop yields in water-limited environments *Adv. Agron.* Vol **43** pp 107-153
- [19] Chang R, Chen Y W, Shao G H and Wan C W 1994 Effect of salt stress on agronomic characters and chemical quality of seeds in soybean *Soybean Science* Vol **13** pp 101-105
- [20] Orabi SA and Abdelhamid MT 2014 Protective role of α -tocopherol on two *Vicia faba* cultivars against seawater-induced lipid peroxidation by enhancing capacity of anti-oxidative system *J. Saudi Soc Agric Sci* Vol **15** pp 145-154
- [21] Yasar F, Ellialtioglu S and Yildiz K 2008 Effect of salt stress on antioxidant defense systems, lipid peroxidation, and chlorophyll content in green bean *Russian J. Plant Physiol.* Vol **55**(6) pp 782-786
- [22] Kusvuran S, Ellialtioglu S, Yasar F and Abak K 2007 Effects of salt stress on ion accumulations and some of the antioxidant enzymes activities in melon (*Cucumis melo* L.) *Inter. J. Food Agric. Environ.* Vol **2**(5) pp 351-354
- [23] Nazarbeygi E, Yazdi H L, Naseri R and Soleimani R 2011 The effects of different levels of salinity on proline and A-, B- chlorophylls in canola *Amer-Eurasian J. Agric. Environ. Sci.* Vol **10**(1) pp 70-74
- [24] Golezani K G and Noori M T 2011 Soybean Performance under Salinity Stress Soybean - *Biochem Chem Physiol.* Vol b pp 631-642
- [25] Omar M S, Yousifn D P, Al-Jibouri A J M, Al-Rawi M S and Hameed M K 1993 Effects of gamma rays and sodium chloride on growth and cellular constituents of sunflower

(*Helianthus annuus* L.) callus cultures *J. Islamic Acad. Sci* Vol. **6** (1) pp 69-72