

The Effects of Different Levels of Salinity on Proline and A-, B- Chlorophylls in Canola

¹Ehsan Nazarbeygi, ²Hussein Lari Yazdi, ¹Rahim Naseri and ³Reza Soleimani

¹Young Researchers Club, Islamic Azad University, Ilam Branch, Ilam, Iran

²Islamic Azad University, Boroujerd Branch, Boroujerd, Iran

³Ilam Agriculture and Natural Resources Research Center, Soil and Water Research Institute, Agricultural Research, Education and Extension Organization, Iran

Abstract: In order to evaluation of response of canola to different levels of salinity an experiment was done in research laboratory of Islamic Azad University of Borujerd in Iran. Culturing seeds in the special dishes was done after sterilizing seeds by sodium hypochlorite solution 20%. Seedlings transferred to the Hogland- Arnon sub-power culture environment. After 24 hours from culturing, the seedlings were placed in the different salt medium (0, 75, 100 and 150 mM) as salinity treatments on Hayola and RGS. After 20 days the contents of a- and b- chlorophylls and proline were tested in the plant roots and leaves. Collected data analysis and mean comparison were done by SPSS software and Duncan's Multiple Range Test (DMRT). Results showed that salinity stress had significant effect on increase of a- and b- chlorophylls and content of proline ($p<0.01$). The amount of decreasing b-chlorophyll was more rather than a-chlorophyll. Decrease in roots and leaves was more in Hayola 401 cultivar than RGS cultivar. Salinity induced significant increase of proline content in leaf and root ($p<0/01$). Hayola cultivar had more ability to salinity stress tolerance compared with RGS.

Key word: Canola • Salinity stress • A, b chlorophylls and Proline

INTRODUCTION

Desertification and Salinization phenomena increase is quick in a global scale and currently affect more than 10% of arable lands which results in a decline of the yield of major crops greater than 50% [1]. Every year more and more lands become non-productive due to salt accumulation. Therefore, understanding the mechanisms of plant tolerance to salinity stress is important [2]. Salinity stress is more important stress which can limit plant productivity [3]. According to estimating american environment organization, about 20% agriculture lands are affected by salinity stress [4]. Studies showed that 2 million hectare added annually to saline soils [5]. High salinity afflicts about 95 million hectares of land worldwide [6] and adversely affects germination, growth, physiology and productivity by causing ionic and osmotic stresses as well as oxidative damage [7]. Moreover, salt stress has also been found responsible for an increased respiration rate, ion toxicity [8], decreased biosynthesis of chlorophyll [9] and inefficiency of

photosynthesis [10], all of which ultimately leading to lowered economic productivity. In general salinity is excess existing the soluble salts and mineral matters in water and soil solution that resulted in accumulation salt in rhizome area and plant can't enough uptakes water from soil [11]. Much salinity resulted from NaCl cause to at least three problems: 1- Osmotic pressure of external solution become more than osmotic pressure of plant cells which is require to regulating osmotic pressure to preventing dehydration by plant cells. 2- uptake and transform of nutrition ions such as potassium and calcium, by excess sodium would make problems. 3- High Na and Cl rates would cause to direct toxic effects on enzymic and membranous systems. One of the effects of salinity stress is reducing photosynthetic activity which caused to decreasing b and a chlorophylls and reducing CO_2 uptake and photosynthetic capacity [12]. According to studies, chlorophyll content and photosynthetic rate in salinity stress decreased in Mustard plant in compared to control plants [13]. Proline is used as an enzymic protector that contributes in macromolecules structure and is main

source of energy and nitrogen to confront salinity. Many researchers believed that proline accumulation in plants involved in resisting to salinity stress [14, 15]. The research aimed to study harmful effects of salinity stress on rapeseed plant and finding mechanism and introducing more resistance variety.

MATERIALS AND METHODS

This study conducted using seeds of RGS and Hayola 401 cultivars in research laboratory of Boroujerd Azad Eslamic University- Iran. Planting medium was included sub power Hogland- Arnon environment. Used dishes had 650 ml capacity. Experiments conducted in laboratory temperature and pH regulated 6.5 to all planting environment. In order to ventilation, the dishes were airing 2 hours every day. A light and dark period was 16 and 8 hours, respectively. Used NaCl concentration included 0 (Control), 75 (Low salinity), 100 (Medium salinity) and 150 (High salinity) mM which all conducted in three replication with randomly design. After 20 days, a- and b- chlorophylls rate measured using Arnon, [16] method. In this manner that 0.2 g leaf removed from plant and grinded it with 10 ml 80% Acetone by mortar and centrifuged to be until segregation operation and then find extract volume increased to 20 ml by adding 10 ml 80% acetone. For estimating of a and b chlorophylls accumulation, solution absorption measured with using 663 and 645 nm waves using spectrophotometer according to weight of samples based per wet weight mg. For measurement of Proline content, 0.5 gr vegetarian matter grinded in 10 ml 3% Solphosalicylic acid using Bates [17] method. The solution purified and 2 ml taken off from any solutions, then 2 ml ninhydrin acid agent and 2 ml acetic acid added to theme. Tubes soaked in Ban Mary for 1h in 100 C° and then kept for 30 m in ice bath, then 4 ml toluene added to tubes and two separate layers formed after shaking tubes and keeping them for 20 s. Finally colored layer absorption during 520 nm waves and proline content measured using standard curve. Collected data was extracted and statistical analysis was done by SPSS software. Analysis of variance and Duncan's Multiple Range Test was used for comparison of means.

RESULTS AND DISCUSSION

Obtained result from experiment showed that a- and b- chlorophylls reduced significantly due to increasing NaCl concentrations ($p < 0.01$), this reduction was more in

RGS cultivar in comparison with Hayola 401 cultivar. Comparing these cultivars indicated that Hayola 401 is more resistance to salinity stress than RGS (Fig. 1(a,b)).

According obtained results from the study, proline content of cultivars leaf and root increase significantly ($p < 0.01$) due to increasing NaCl rate. Increasing was more in 150 mM NaCl treatments than other treatments. Comparing both cultivars indicated that increasing proline content at Hayola 401 leaf and root is more than RGS (Fig. 2(a,b)). In general observed that Hayola 401 is more stable than RGS.

Many environmental factors control chlorophyll synthesis in plant. Existing these factors as limiting factors cause to disordering synthesizing chlorophyll and appearing chlorosis in plant. NaCl stress decreased total chlorophyll content of the plant by increasing the activity of the chlorophyll degrading enzyme: chlorophyllase [18], inducing the destruction of the chloroplast structure and the instability of pigment protein complexes [19]. The decrease in chlorophyll contents under saline conditions is reported by Iqbal *et al.* [20] and Ashraf *et al.* [21]. Results of various studies showed that a, b chlorophylls content, total chlorophyll and carotenoid reduced in leaf of wheat plants which grown in saline soil and this reduction is due to chlorophyllase activity enzymes in plant affected by salinity. a and b chlorophylls rate decreased due to salinity stress, chlorophyll reduction can attributed to changing Nitrogen metabolism direction to forming compounds such as proline which used to regulating osmotic [22]. Khan *et al* [23] reported that there is significant correlation between total chlorophyll and alfalfa yield and saline conditions. They suggested that a, b and total chlorophyll decrease due to increasing salinity in alfalfa cultivars. a- and b- chlorophylls content reduce in respond to salinity stress, this may be for forming protolytic enzymes such as chlorophyllase which responsible to decompose chlorophyll and damaging photosynthetic structures, is other cause at this reduction [24]. It is therefore proven that soil salinity had negative effects on the growth and photosynthetic metabolism of *B. napus*.

Accumulation of solutes especially proline, glycine-betaine and sugars is a common observation under stress condition [25-27]. Proline is an important osmolyte which synthesizing in many micro organisms and plants exposed to salinity and drought stress, thus it as a osmotic protector in plant. Proline accumulating in plants exposed salinity stress is due to low activity of oxidant enzymes [28].

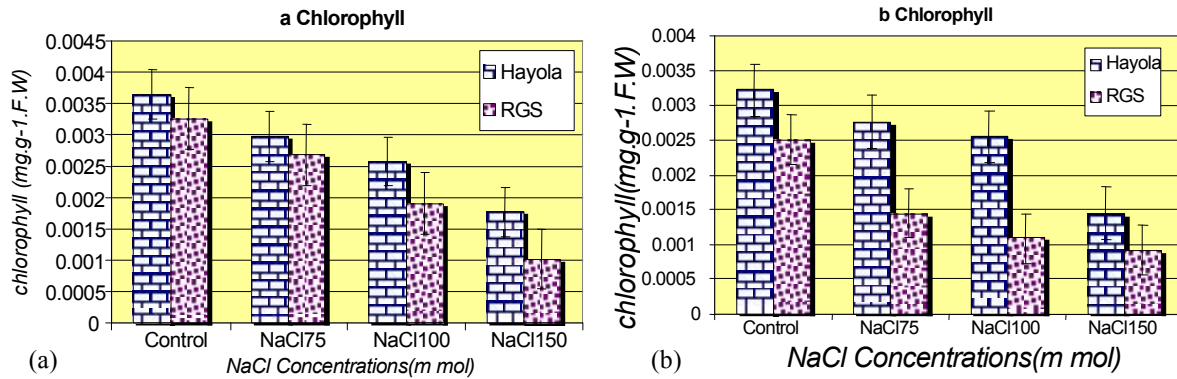


Fig. 1(a,b): a) chlorophyll changes of both cultivars under different NaCl concentrations.
b) chlorophyll changes of both cultivars under different NaCl concentrations.

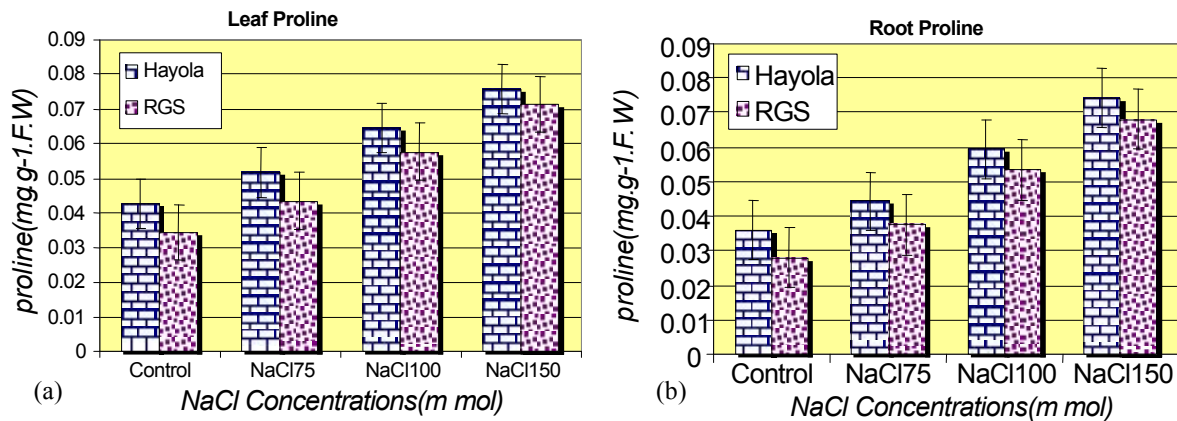


Fig. 2(a,b): a) Leaf Proline content changes in two cultivars under different NaCl concentrations.
b) Root Proline content changes in two cultivars under different NaCl concentrations.

Proline accumulating in leaf and particularly roots of tomato plant is use as a sensitivity index to salinity which possibly use to supporting different salinity rates [29]. Increasing proline is important for osmosis compatibility but also to preserving carbohydrates sink in chloroplasts. Valia *et al.* [30] suggested that increasing proline under saline conditions caused to less existing glutamate in biosynthesizing chlorophyll, thus it helped to producing proline for plant bearing more salinity conditions.

CONCLUSION

Because saline soils caused to reducing significantly plant productivity in agronomic lands and plants growth in vegetative covers, salinity problems must consider as a vital problem to reducing damages of salinity by studding and various investigations. During this experiment, Hayola 401 cultivar resistant defined in

compared to RGS cultivar, thus using this cultivar recommended.

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