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Assessment of Physiological and Phytochemical Responses of *Althaea rosea* L. to Salinity Stress

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ABSTRACT

Background: This research was conducted to evaluate carbohydrate variation and physiology mechanisms against salinity stress. Seeds of *Althaea rosea* L. were sowed in pot with four levels salinity (0, 4, 8 and 12dSm^{-1}) using completely randomized block design with three replications. Salinity stress stimulated the accumulation starch in chloroplast which resulted in a greatly content of starch, total sugars in leaves and mucilage of roots and leaves. Reduced leaf area and number of stomata were occurred under salt stress. Furthermore, determination of ion concentrations roots and leaves in saline soil, showed statistically significant increase Na^+ and K^+. High storage of epicuticular wax load of leaves was found in 12dSm^{-1}. Also, amount of total tannin root was highest under 8dSm^{-1}. Phenol compound in root was not significant, but this content in leave greatly decreased in high level saline condition. Considering these results, we suggest that salinity stimulated proportion of soluble carbohydrate, starch and mucilage.

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INTRODUCTION

Mucilage is major part of the plant polysaccharides fraction that is typically found in many plant species and widely reported in the Malvaceae, including in Althea and Abelmoschus [1]. Mucilage probably assumes a plenty of physiological functions in plants [2]. Because of water binding capacity hydroxyl groups in the polysaccharide core, mucilage can hydrate and accumulate high water [3] therefore responses to abiotic stresses [4]. In various species of the Malvaceae family, have been reported many mucilages, According to the previous papers, roots and leaves of *Althaeu officinalis* L. and *Althaea rosea* are major source of mucilage storage [5]. Mucilage content of Marshmallow oscillated between 17% and 35% of its total weight. Mucilage storage in roots varies between 20 and 30 % but amounts of starch (35%), sugar (5-10%) and tannins (2%) were estimated. Basic structures of Marshmallow mucilage are composed by rhamnose, arabinose, galactose, glucose, mannose and xylose [6]. Phytochemical screening in *Cinnamomum tamala* leaves mucilage showed the presence of tannins and sugars [6]. Mucilage are used in industry and pharmaceutical cases [7]. Tannins are water-soluble phenolic compound that known as phenolic secondary metabolites. Phenolic compounds are known active antioxidant. It act as a direct antioxidant, which operate as hydrogen donor [8]. Epicuticular wax (EWL) deposition in plants cause to adapt against salinity [9]. Epicuticular waxes help leaves to maintain water by reducing cuticular transpiration [10].

MATERIAL AND METHODS

This experiment was carried out in, randomized complete block with three replications. *Althaea rosea* L. was planted in pot with ten kilogram of silt- clay soil. They were daily irrigated. After appearance of seedlings, four levels of salt treatment were performed (0, 4, 8 and 12 dS m^{-1}). Roots and leaves samples were collected after reproduction period.

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Soluble sugars and starch:

Soluble sugars were assessed using the phenol-sulfuric method of Dubois et al. (1956) with 80 % (v v^{-1}) ethanol and centrifuged for 10 min at 10,000 g to pellet insoluble fraction [11]. After ethanol extractions were evaporated, 10 ml distilled water was added and then, combined with $Ba(OH)_2$ (0.3 N) and 5 percent ZnS04. Thus samples were centrifuged 3000 rpm for 10 min and added 1 ml phenol 5 percent and 5 ml of sulfuric acid (98%) in 2 ml Supernatant. Finally extraction absorbance was determined at 485 nm (Biowave II UV/Visible). Measurement of non-structural carbohydrate and starch content has been undertaken on pellets, after ethanol extractions were reduced to glucose enzymatically according Palacio et al. (2007a) method [12]. Measurement of non-structural carbohydrate and starch content has been undertaken on pellets.

Mucilage:

Mucilage was extracted from10 g powder dry samples with boiling in 200 ml of distilled water acidified (pH 3.5 with 0.2 N HCl). The solutions were put in water bath (90 - 95^oC) for 12h and then they were kept at room temperature for 12h. The precipitated mucilage was separated through Whatman filter paper. After adding 96% ethanol, mucilage was stored over night at 4^oC and determined on a gravimetric basis [13].

Epicuticular wax loads:

EWL was determined using Ebercon et al. (1977) method, based on acidic potassium dichromate ($K_2Cr_2O_7$) reaction with epicuticular wax that cause to colour change. Ten pieces of leaf, each of 1cm^2, were used for the extraction of waxes [14]. The EWL were recorded on basis a calibration curve using different concentrations of polyethylene glycol-6000 at 590 nm (Biowave II UV/Visible). The results were calculated as g m^{-2}.

Total phenolics and Tannin content:

Total phenol content was done following the method of Makkar et al. [15]. Samples (50 µL) were made up to 500 µL using distilled water. Then, 250 µL of Folin-Ciocalteu reagent was added into the test tube followed by 1.25 mL of sodium carbonate solution. The tubes were vortexed before incubated in the dark for 40 min. Absorbance was read at 725 nm using spectrophotometer (Biowave II UV/Visible).

Using the method of Makkar et al. (1993) for determination of non-tannin phenolics, 100 mg PVPP was weighed in test tubes before being added with 1.0 mL distilled water and 1.0 mL of the extracted sample [15]. The tubes were vortexed before kept at 4°C for 15 min. Then, the tubes were vortexed again before centrifuged at 3000 rpm for 10 min. The supernatant was collected and measured for absorbance at 725 nm using spectrophotometer (Biowave II UV/Visible). The tannin content was calculated as follows:

(Total phenolics) – (non-tannin phenolics) = tannin:

Total phenolic and tannin content were expressed as gallic acid equivalents through the calibration curve of gallic acid (Sigma, USA) with the concentration range of 0-100 mg mL^{-1}.

Sodium and potassium concentration:

Na^+ and K^+ concentrations were measured according (Williams and Twine, 1960) leaves and roots were dried in 60^oC for 48 h. Then 1 gr of samples were grinded and burned in 560^oC to get ash then digested in 10 ml of 1N HCL [16]. The concentration of Na^+ and K^+ were obtained using a flame photometer (Jevway Pfp7)

Results:

There were significant differences ($p<0.01$) in the leaf area and number of stomata (Table 1). Salinity substantially decreased leaf area and stomata conductance in leaf (Figure 1A).

Difference in leaves cuticular wax accumulation was significant in response to salinity (Table 1). A greater density of wax was observed on leaves of plants that had been exposed to non saline water (Figure 2).

Tannin contents of root were significantly differed according the salt concentrations applied (Table 2). The tannin production in root was increased with increasing salt concentration in 8 ds m^{-1} (Figure 3A) but tannin root in 12 dS m^{-1} was significantly lowest than other treatments. We observed lower phenol in leaves from plants subjected to 8 dS m^{-1} events in comparison to non saline plants. Mucilage increased significantly ($P<0.01$) in leaves and roots in response to salt stress. The significant increase of Mucilage in the leaf and root were in response to higher doses of salts associated, 12 dS m^{-1}, (Figure 4). Our results establish that starch shoot was the only notable difference between 12 dS m^{-1} than control (Figure 5B). Whereas, no significant differences were found between salinity levels of root against salt stress (Figure 5B). Similarly, allocation of starch in roots seems to be significantly greater than stem-sprouters. Based on this observations (Figure 5A), in high salinity levels, cumulative sodium in leaves was significantly more than roots ($p<0.01$) (Table 1). Althea root in expose to 12 dS m^{-1}, K^+ concentration was notably enhanced in comparison leaf, but in non-saline and low salinity treatments. K^+ deposition in the root were significantly higher than leaf ($p<0.01$) (Table1).

Table1: physiological traits, Na^+ and K^+ leaves and roots of *Althaea rosea* under salinity stress.

Source of variation	df	Leaf area (Cm^2)	Number of stomata	Wax (g m^{-2})	Na(µmol.g^{-1} root DM)	Na^+ (µmol.g^{-1} leaf DM)	K^+ (µmol.g^{-1} root DM)	K^+ (µmol.g^{-1} leaf DM)
Salinity levels	3	84011.8**	1.68**	48198766.1**	2.63**	20.69 **	265848.1**	225754.9**

Table 2: Phenolics content[a] of roots and leaves of *Althaea rosea* under salinity stress.

Source of variation	df	Tannin Root	Tannin Leaf	Phenol Root	Phenol Leaf
Salinity levels	3	2905.86**	1405.49^{ns}	182.85^{ns}	1599.57*
Error	6	90.312	1014.9	65.79	180.35

[a]Expressed as mg gallic acid equivalent/mg dry weight
Means marked by (*) are significantly different ($p<0.05$), ns (non significant)

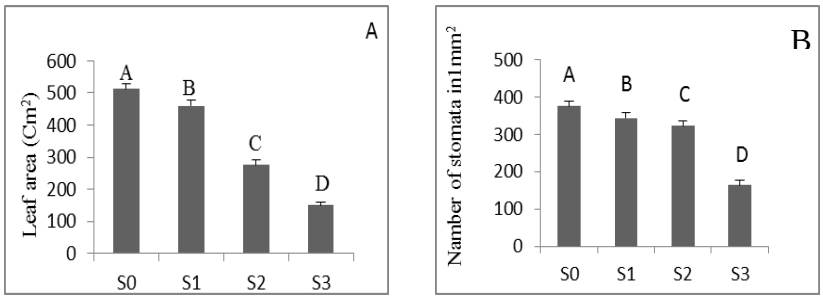

Fig.1: A) Leaf area (Cm^2) B) Number of stomata in $1mm^2$ *Althaea rosea* under different salinity stress.

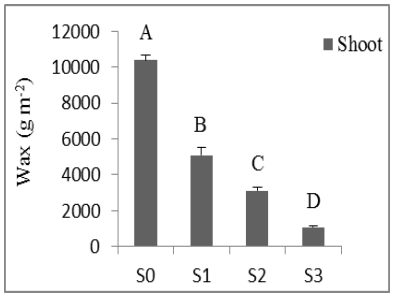

Fig. 2: Epicuticular wax loads in leaf of *Althaea rosea* under different salinity stress.

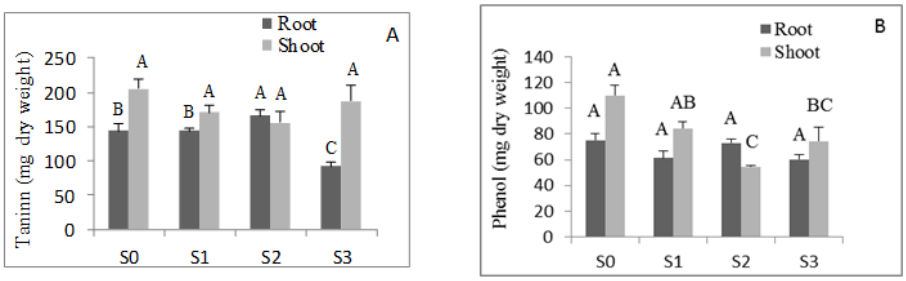

Fig. 3: A) Tannin (mgDW) B) Phenol (mgDW) in shoot and root of *Althaea rosea* under different salinity stress.

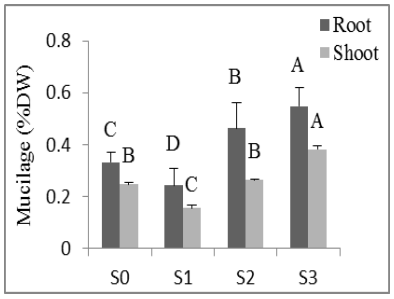

Fig. 4: Mucilage(%DW) in shoot and root of *Althaea rosea* under different salinity stress.

Discussions:

Salinity induced lower cuticular wax on leaves. Total wax load of leaves in 12 dS m^{-1} level was lowest. The ratio accumulation cuticular wax of sever salinity stress to control was 10.23%. Kimberly et al. (2006) found correlation between periodic dehydration stress, cuticular wax load and reduced stomata conductance [17]. Also they concluded that total wax load of young leaf in control was 2.5 fold after exposure to stress. Salinity in case of membrane integrity loss, decrease [18]. Tannins are polyphenolic compounds that widely distributed in higher plants [19]. It was observed by Odjegba and Alokolaro (2013) that tannin in salinized plants was significantly lower than drought-stressed plants [20]. Phenolic compounds are aromatic compounds that play various biological roles [21]. Generally, when plants are salinity stressed, Variation in secondary metabolite content can cause significant changes in resource availability such as carbon and other nutrients. Therefore, increasing secondary metabolites like total phenol, total alkaloids and tannins cause to reduce fertility and excess of carbon store in borage at salinity [22]. A few studies have been reported on the evaluation salinity effects on the total phenol in some medicinal plants. For example, results proved that total phenol in the olive oil increased with high NaCl levels in irrigation water [23]. Several studies investigating described that salinity significantly affected on mucilage content [22]. Furthermore, as was recently observed Mucilage increased in leaves and roots in response to salt stress [2]. It may be taken in consideration that condensed tannins combine to polysaccharides and leading to plant cell-wall hardening and delay in digestion and decomposition of conducting tissues [24]. Carbohydrates such as phosphorylated sugars perform a crucial role in regulating root initiation. [12]. Kaya et al. results showed that high NaCl had a noxious effect on membrane integrity in root. Also, K^+ was restricted from roots to leaves against salinity stress [25]. Utilization of sugar could be limitating factor growth, carbohydrates will accumulate in sensitive genotype but in salinity tolerant genotype, soluble carbohydrate increased, accumulation of sucrose could exert feedback on CO_2 metabolism [26].

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