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Alterations in content of phenolic acids and growth parameters of two rice (*oryza sativa*) cultivars in response to salicylic acid under abiotic stress

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ABSTRACT: Present study was conducted to analyzing the allelochemicals by HPLC method in root exudates of two rice cultivars (Tarom and Neda) under salinity stress condition. The selected cultivars were grown in hydroponic system with different levels of salinity and foliar application of salicylic acid. The root exudates were quantified by HPLC method. The effects of treatments on some growth parameters of studied cultivars were also determined. Among the studied phenolic acids, P-coumaric acid just detected in tarom root exudates and *p*- hydroxybenzoic acid and vanillic acid were detected only in some combined treatments [such as EC (4 dS/m)+ SA (1%); EC (8 dS/m)+ SA (1%); EC (4 dS/m)+ SA (2%); EC 8 (dS/m)+ SA (2%)]. The most significant positive effect on leaf area was belonged to SA 2% (with 14% increase). The highest increase in chlorophyll contents was obtain when the SA (%2) + EC (4dS/m) was used (with %12.5 increase compare to control). Accordingly, if the salinity problem was observed in paddies it may be possible to recommend the foliar application of SA 2% for better stand of seedlings and more phenolic acids secretion.

***Keywords*:** Chlorophyll content, HPLC, phenolic acid, Tarom, Neda

INTRODUCTION

Rice (*Oryza sativa* L.) is one of the most important crops between cereals. It is the most widely consumed as a main food for a large part of the world's human population, especially in Asia. It is third-highest worldwide agricultural products, after sugarcane and maize (FAOSTAT, 2012).

Often, rice production is characterized by heavy use of herbicides and fungicides, which may cause environmental problems in the paddy ecosystem (Chung., 2001), and human health problems, make it necessary to diversify weed management options. Thus, the best way to control paddies weed in an environmentally acceptable and sustainable approach is to develop natural compounds like allelochemicals. Plants can release these chemicals into their environment as volatiles, root exudates or degradation of residuals, directly.

Grain wheat and rye are main sources of many bioactive compounds and contribute significantly to the total intake of cereals in many countries. Alkylresorcinols, benzoxazinoids, lignans, phenolic acids, phytosterols and tocols are common bioactive compounds present in these cereals (Andersson, 2013). Wu. (2001) have implicated 2,4-dihydroxy-7-methoxy-1,4-benzoxazin-3-one as an important allelochemical in wheat. Roth. (2000) found that prompt tillage of the mature sorghum stalks delayed development of the following wheat crop but did not affect grain yields, probably because allelopathic compounds degraded in the soil. Minorsky (2002) stated that barley (*Hordeum vulgare*) should be considered as a depressive prior crop for both durum wheat and bread wheat in a field cropping sequence. Moreover, sunflower (*Helianthus annuus*) showed effective weeds suppression and strong inhibitory effects on physiological processes of test plants (Bernat, 2004). Secondary plant metabolites such as terpenoids, steroids, phenols, coumarins, flavonoids, tannins, alkaloids, and cyanogenic glycosides, and their degradation products have been known to be involved in allelopathic phenomena, and are important in all agroecosystems (Reigosa, 2006). These compounds could play a valuable role in an integrated weed management system, potentially reducing the amount of synthetic herbicides required for weed control (Seal, 2004). Among these substances, phenolic compounds are the most widely studied with regard to their phytotoxicity (Zeinali, 2013). Meanwhile, the abiotic stress can change plant physiology, morphology, and chemistry characteristics, which in turn affects root and shoot growth and production of secondary metabolites (Taiz and Zeiger, 2010; Zeinali, 2013). Invasive plants may employ allelopathy in their interaction with biotic associates. Allelopathic influence helps them invade and compete within natural plant communities (Callaway and Aschehoug, 2000; Qasem and Foy, 2001; Kohli, 2006). The evidence indicated that hound's-tongue seed allelochemicals (phenolic compounds) increased under stress conditions such as low O2 and high temperature (Rashid, 2005). They also showed that dead hound's-tongue seeds release higher concentrations of phenolic compounds compared to live seeds. Furness. (2008) reported that the inhibitory effects of hound's-tongue leaf leachate on seedling growth of grasses increased when plants were exposed to UV-B radiation.

Accordingly, the main objectives of this study were to evaluate the alteration phenolic acids concentration and physiological growth parameters of Tarom and Neda rice cultivars under saline condition in presence of salicylic acid.

MATERIALS AND METHODS

Rice hydroponic culture system and treatments

After preparation of two rice cultivars (Neda and Tarom) from Rice Research Institutes of Iran (RRII, Amol, Mazandaran), the pure seeds were allowed to imbibe on moistened paper towels for 2 h. Filter paper (Whatman No. 42) containing 100 seeds were placed in sterilized 9 cm Petri in natural room condition for germination. For each cultivar, 50 uniformly grown rice seedlings (With 2 mm radical length) were selected and transferred into a plastofoam sheet (25.5 × 35.5 cm) which was allowed to float on distilled water (15l) inside a PVC container (26 × 36 × 18 height cm). Also for oxygen requirements, air pumps (Resun Ac-9906, China) prepared for each container. The container was placed in growth chamber (With 27/20°C day/night temperature, 70% RH and 440 µmoles/m^2/s light intensity). This method is adapted from Heidarzade . (2010). The seedlings in each container were nourished by Yoshida (1981) rice nutrient solution every five days until harvesting time. After 21 days of seedling transfer time, the salt was added to hydroponics solution at different levels of electrical conductivity (0, 4 and 8 dS/m). During the salinity stress the salicylic acid was applied as foliar (0, 1% and 2%). The root exudates of each container were collected separately to be used in quantification allelochemicals by HPLC method.

HPLC instrumentation for Quantification of phenolic acids in root exudates

A chromatographic system equipped with a 20 µl sample loop, degasser, quaternary pump, column oven and diode-array detector were used for phenolic acids analysis. Chemstation software was used for data processing. Analytical grade cinnamic acid, vanilic acid, ferulic acid, *p*-coumaric acid, *m*-coumaric acid, *p*-hydroxybenzoic and acid were purchased from E. Merck (Germany) for preparation of standards. All samples were filtered using 0.42 µm Millipore filters before injection and 20 µl of each was injected directly to HPLC system. Stock solutions of phenolic acid standards (1000 µg ml^{-1}) were prepared by dissolving appropriate amounts of analytes in methanol, placed in dark bottles and stored in a refrigerator at 4ºC until injection time.

Traits evaluation

The growth parameters such as leaf area, shoot length, shoot dry weight, root content, root dry weight and chlorophyll content were determined at mid-vegetative growth stage.

Statistical analysis

Experiment was evaluated in a factorial arrangement based on completely randomized design with two factors and four replications. Analysis of variance was performed for seedling growth parameters of studied rice cultivars by using the general linear model (PROC GLM) procedure in Statistical Analysis System (SAS) program (SAS Institute, 1997). Means were separated using the LSD test and statistical significance was evaluated at $P = 0.05$.

RESULTS AND DISCUSSION

Effect of treatments on phenolic acids content in root exudates

After optimization and determine the appropriate mode for chromatography, the specific amount of standards of each phenolic acids was injected into the system for calibration curves. All phenolic acids were identified according to their absorption time in HPLC column, the concentration of phenolic acids were determined (T-1). Cinamic acid and m-coumaric acid were detected among of all treatments and cultivars. P-coumaric acid just detected in Neda root exudates and *p*- hydroxybenzoic acid and vanillic acid were detected only in some combined treatments [such as EC (4 dS/m)+ SA (1%); EC (8 dS/m)+ SA (1%); EC (4 dS/m)+ SA (2%); EC (8dS/m)+ SA (2%)]. According to table-1, the total maximum concentration of phenolic acids detected in root exudates of Neda cultivar in SA (2%) +EC (8dS/m) treatment (with 482µg/l) and also the lowest concentrations of these substances belong to the Tarom cultivar in SA (2%) treatment (with 62 µg/l).

Table 1. quantities of detected plenolic acids (µg/l) in root exudates of two rice cultivars

Phenolic acid		p-hydroxy benzoic acid	Vanilic acid	p-coumaric acid	Ferulic acid	m-coumaric acid	Cinnamic acid	SUM
Cultivar	Treatment							
Tarom	Control	ND	ND	ND	79	114	87	280
	EC (4 dS/m)	ND	ND	ND	92	123	90	305
	EC (8 dS/m)	ND	ND	ND	ND	84	41	125
	SA (1%)	ND	ND	ND	ND	55	27	82
	SA (2%)	ND	ND	ND	ND	40	22	62
	4 dS/m+ SA 1%	10	ND	ND	96	129	93	318
	8 dS/m+ SA 1%	29	ND	ND	89	116	88	322
	4 dS/m+ SA 2%	46	15	ND	101	146	98	406
	8 dS/m+ SA 2%	20	ND	ND	93	105	75	293
Neda	Control	ND	ND	ND	79	125	96	293
	EC (4 dS/m)	10	ND	ND	92	135	99	300
	EC (8 dS/m)	12	ND	21	15	92	45	336
	SA (1%)	9	8	34	ND	61	30	185
	SA (2%)	13	12	ND	19	44	24	142
	4 dS/m+ SA 1%	11	10	65	96	142	102	112
	8 dS/m+ SA 1%	32	29	44	89	128	97	426
	4 dS/m+ SA 2%	51	46	15	101	161	108	482
	8 dS/m+ SA 2%	22	20	19	93	116	83	353

ND, not detection

Total length of seedlings

The results showed a significant effect of treatments on studied traits. So that, the highest positive effect on shoot lengths was belong to both SA 2% singly and its combination with salinity stress EC (4dS/m) (with 42 and 41cm, respectively). Between treatments, EC 8dS/m had the most negative effect on seedlings total length compare to control (33% reduction).

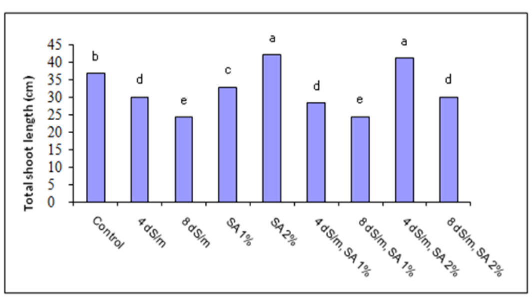

Figure 1. The effect of treatments on total shoot length, SA= salicylic acid

Leaf Area

According to the results (figure 2), the most significant positive effect on leaf area was belonged to SA 2% (with 14% increase). While the most negative effect on leaf area was related to 1 % salicylic acid + salt stress 8dS/m and salinity treatments 8dS/m (with %68 and %65, respectively).

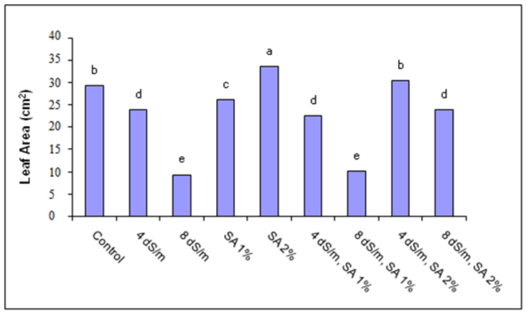

Figure 2. The effect of treatments on Leaf area, SA= salicylic acid

Root content

Based on mean comparison (Figure 3), the most significant positive effect on root content was related to SA (%2) + EC (4dS/m) (with %7 increase), Also the highest negative and significant impact on root contents was observed in EC (8dS/m) treatment (with %59 reduction compared to control). Also, the SA (1 and 2%) didn't have any significantly difference compare to control (figure 3).

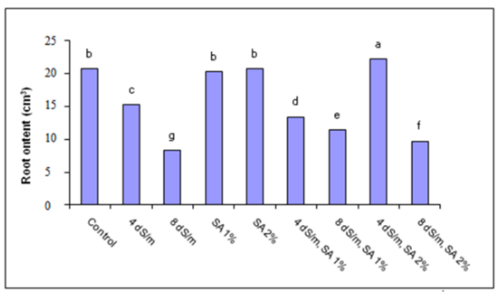

Figure 3. The effect of treatments on Root contents, SA= salicylic acid

Shoot dry weight

A significantly difference was observed between treatments on shoot dry weight (figure 4), the highest increase in shoot dry weight was related to SA (%2) + EC (4dS/m) treatment (with 15.5% increase), at same time the SA (2%) didn't have any positive effect on shoot dry weight compare to control. On the other hand the lowest amounts of shoot dry weight was belong to salt stress treatment at 8dS/m electrical conductivity.

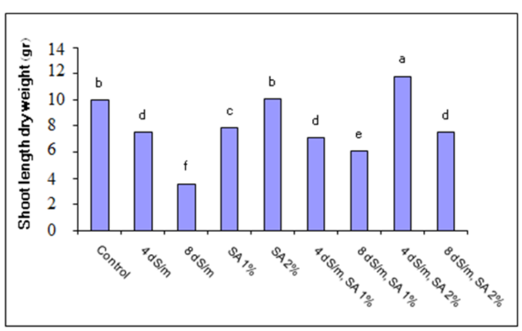

Figure 4. The effect of treatments on Shoot length, SA= salicylic acid

Chlorophyll content (a+b)

Mean comparisons (figure 5) of chlorophyll content indicated that, the highest increase in contents was obtain when the SA (%2) + EC (4dS/m) was used (with %12.5 increase compare to control). Also the minimum effects (with %36 reduction) were related to EC (4dS/m) and SA (%1) + EC (8dS/m) treatments.

Response of two studied rice cultivars to different treatments

According to mean comparisons (T-2), Neda cultivar showed a better response to treatments compare to Tarom cultivar (in term of all studied parameters).

Table 2. Response of cultivars in term of studied parameters

Trait cultivar	Shoot length (cm)	Leaf area (cm^2)	Root content (cm^3)	Shoot dry weight (gr)	Chlorophyll content (µg/g)
Neda	**35.6**	**28.31**	**18.09**	**8.91**	**11.59**
Tarom	30.16	24.06	15.35	7.58	9.85
LSD	0.88	0.70	0.53	0.22	0.28

Plants for their survival have different mechanisms for adapting to the environmental changes, such as morphological, physiological and molecular mechanisms (Bohnert , 1995). Salinity can affect physiological processes in plants, from germination to the maturity stage. Photosynthesis is a key pathway in plant physiology, that seriously influenced by salinity. Meanwhile, abscisic acid produced in response to salt stress is causes the stomatal closure and limiting the entry of carbon dioxide into the plant (Leung , 1994). Salt stress is a main cause of reactive oxygen species accumulation in cells and damage to membrane lipids, proteins and nucleic acids (Noctor and Foyer, 1998). Antioxidants are neutralizing these free radicals (such as ascorbic acid, tocopherol and glutathione) in plants (Zhang and Kirkham, 1994). Salicylic acid, as the endogenous growth regulator and natural phenolic compound plays a key role in regulation of plant physiological processes such as: flowering induction, growth and development, ethylene synthesis, effect on opening and closing of stomata and respiration. Also, salicylic acid regulates the senescing, signaling and gene expression of premature aging process in plant. Salicylic acid has a protective role in plants under environmental stresses. Bezrukova . (2001) suggested that Salicylic acid enhances the wheat seedlings establishment and its tolerance under tough condition (saline environment) and also showed positive effect on rice seedlings against heavy metals stress (Mishra and Choudhuri, 1999). Foliar application of salicylic acid induced tolerance to heat (Dat , 1998), frostbite (Janda , 1999) and salinity (Borsani , 2001) damages. As mentioned, the environmental stresses induce the production of reactive oxygen species in plant chloroplasts and other cell organelles. The oxygen free radicals may be converted to hydrogen peroxide by superoxide dismutase and then, ascorbate peroxidase and glutathione reductase is converted to water in chloroplasts (Reigosa , 2006).

Catalase has a cleaner role in term of ROS production (Taiz and Zeiger, 2010) during salt stress in rice. The main objective of present study was to identify which treatment can enhance the phenolic acid in rice root exudates. The role of phenolic acid compounds were suggested many times (Taiz and Zeiger, 2010; Heidarzade , 2010; Heidarzade , 2012; Esmaeili , 2012; Zeinali , 2013), but very few studies have been devoted to effect of abiotic stresses in physiological and biochemical (such as allelochemicals) alteration in plants. Also, studies have shown that the levels of metabolites production in plants is directly related to plant growth rate and plants size. Between our studied cultivars Neda as an improved cultivar showed high yield capacity and high growth rate compare to Tarom, therefore it could be concluded that the highest phenolic acid content in Neda cultivar will be positive correlated by its high growth rate ability compare to Tarom.

According to the results, if the salinity problem was observed in paddies it may be possible to recommend the foliar application of SA 2% for better stand of seedlings and more phenolic acids secretion. Finally, salicylic acid could be suitable and play a key role in moderate tough environment for better seedlings growth and development.

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