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Enhance the allelopathic potential of two rice cultivar (*Oryza sativa* L.) by foliar application of salicylic acid under salinity stress

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Abstract

In order to investigate the effect of salicylic acid under salinity stress as an allelopathy stimulator in rice cultivars a hydroponic and pot experiment was carried out in factorial arrangement. Rice cultivars (Tarom and Neda) were grown under different levels of salinity stress (0, 4dS/m and 8dS/m) until 5 leaf stage, also at the same time the salicylic acid was used as foliar (0, 1% and 2%). The root exudates were collected and used as a growth bio-inhibitor in barnayardgrass pot culture. The highest inhibitory effects on chlorophyll, leaf area, root and nitrogen contents of barnyardgrass was obtain when the SA (%2) + EC (4dS/m) treatment was used (with 38%, 60%, 38% and 37.5% inhibition, respectively). Also, chlorophyll content was closely correlated with nitrogen content. Therefore, it may be possible to recommend that, the foliar application of SA 2% will be suitable for better stand of rice seedlings and more allelopathic potential on barnyardgrass in moderate saline conditions of paddies.

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Introduction

Rice is the staple food for more than three billion people all around the world (FAO, 2012). But unfortunately, its production likewise is characterized by heavy use of herbicides and fungicides, which may cause environmental problems in the paddy ecosystem, make it necessary to used numerous weed management options. Thus, the best way to control paddies weed in an environmentally acceptable is to develop cultivation of plants with high allelopathic potential or applying a mechanism to increase this ability. Plants can release allelopathic substances into their environment as volatiles, root exudates or degradation of residuals, directly (Olofsdotter, 2002). Allelopathy was defined as any direct or indirect influence of released chemicals from plan organelles to another living plant (Rice 1984). A large number of rice cultivars ability to inhibit the growth of several plant species was found (Chung *et al*, 2001; Chung and shin 2007; Kato-Noguchi 2004; Chung *et al* 2007; Berendji, 2008; Heidarzade, 2010). Chung *et al*, (2001) evaluated the allelopathic potential of 44 rice cultivars on barnyardgrass (Echinochloa crus-galli L.). They proposed differences among the cultivars for allelopathic inhibition on barnyardgrass. Jung *et al* (2004) studied the allelopathic potential of rice residues against barnyardgrass and observed inhibitory effect was varied between different parts of rice plant. Chung *et al* (2003) compared 114 rice cultivars in terms of allelopathic potential of leaves, straw, and hull extracts on seed germination and seedling growth of barnyardgrass. They reported higher inhibitory effects of straw extracts in comparison with hulls and leaves.

Secondary plant metabolites such as terpenoids, steroids, phenolic acids, 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 (Heidarzade, 2012). Phenolic compounds are the most widely studied with regard to their phytotoxicity (Zeinali *et al*, 2013). Meanwhile, the studies showed that taking some strategies can help and improve the releasing of these substances by plants (Zeinali, 2013). 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 *et al*, 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 *et al*, 2006). The evidence indicated that plant allelochemicals (phenolic compounds) increased under stress conditions such as low O2 and high temperature (Rashid *et al*, 2005).

Thus, the current study aimed to evaluate the alteration of allelopathic potential of root exudates of two rice cultivars in response to salicylic acid and salinity stress against growth characteristics of barnayardgrass

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/m²/s light intensity). This method is adapted from Heidarzade *et al.* (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 as irrigated water in barnyardgrass pot cultivation. The growth parameters such as Seedling emergence, Leaf area, Root content, Chlorophyll contents and Nitrogen content for barnyardgrass were determined 30 days after planting.

Total Nitrogen Content Determination

Total Kjeldahl Nitrogen (TKN) method (Isaac and Johnson, 1976) was used for nitrogen determination, (Kjeltec Auto1030 Analyzer, Foss Tecator AB, Hoganas, Sweden). Total reduced nitrogen was determined by using a micro Kjeldahl procedure with sulphuric acid, digestion catalyst and conversion of organic nitrogen into ammonium form.

Chlorophyll content

For green pigments determination porra (1989) method was used. So fresh leaf samples (0.025 gr for each treatment) were Freeze Dried by liquid nitrogen then homogenized and poured into a 15 mm polycarbonate tubes, finally 8 cc methanol (100%) was added to each them. For extraction all tubes were stored at a dark place for 24 hours. The chlorophyll content was determined by spectrophotometer by the following equation:

Chlorophyll concentration (a+b) = (0.0202 x A645) + (0.00802 XA663).

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

The results of analysis of variance (Table 1) indicated that, the treatments (B) had a significantly effect on all studied traits at 1% probability level and also cultivars showed a same effect with the exception of Root content.

Table 1. Analysis of variance for the inhibitory effect of rice root exudates on barnyardgrass traits.

		Mean Square				
S.O.V	df	Seedling Emergence	Leaf Area	Root Content	Chlorophyll content (a+b)	Nitrogen content
Cultivar (A)	1	36.8**	138.44**	1.36^{ns}	1.19*	30.5**
Treatment (B)	3	174.25**	346.83**	13.49**	33.16**	55.8**
A×B	3	1.39^{ns}	7.21^{ns}	0.24^{ns}	0.44^{ns}	6.91**
Error	24	1.06	3.69	0.46	0.26	0.4
CV (%)		5.17	11.04	10.05	5.15	4.3

**, Significantly at 1% probability level.

Seedling Emergence

Having knowledge from seedling emergence rate is one of the key factors for acceptable seedling establishment. The results of means comparison showed a significantly differences between studied treatment. So the lowest amount of barnyardgrass seedling emergence (with 8.5%) was related to SA (%2) + EC (4dS/m) treatment and the highest was observed in SA (%1) + EC (8dS/m) treatment (with 44%) compare to control (fig. 1). Meanwhile, Tarom cultivar showed a higher inhibitory effect compare to Neda cultivar.

Leaf area

According to the results (mean comparisons), all studied treatments showed significantly differences on leaf area of barnyardgrass compare to control. So the highest inhibitory effects was obtain from SA (%2) + EC (4dS/m) treatment (with more than 60% inhibition compare to control) and the lowest was related to SA (%1) + EC (8dS/m) compare to control (fig. 2).

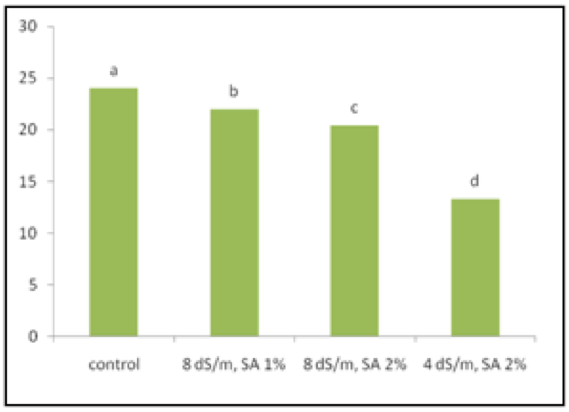

Fig. 1. Seedling emergence rate of barnyardgrass in response to treatments.

Root content

In term of root content there was no significantly difference between SA (%1) + EC (8dS/m) and control treatment (no inhibitory effect) but the results showed the high inhibitory effect (with 38%) for SA (%2) + EC (4dS/m) on root contents of barnyardgrass (fig. 3).

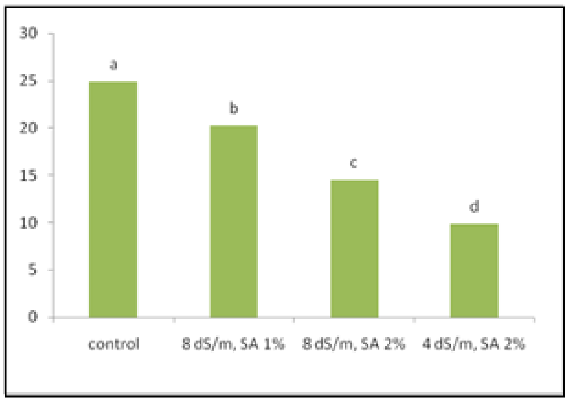

Fig. 2. Leaf area of barnyardgrass in response to treatments.

Chlorophyll content (a+b)

According to the importance of photosynthesis pigments (Chlorophyll) in plant photochemical reactions, therefore its alteration reflects the physiological state of plant and notice will be suitable for monitoring and selecting the best treatment or conditions. In term of our results, the highest chlorophyll content was obtained in control (with 12.6 mg/g). Also the highest inhibitory effect (with %38 inhibition) was related to SA (%1) + EC (8dS/m) treatments.

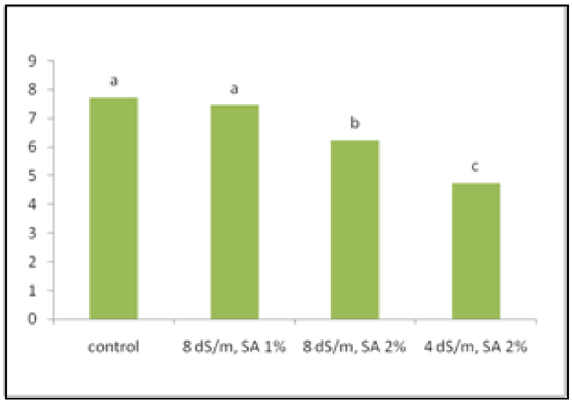

Fig. 3. Root content of barnyardgrass in response to treatments.

Nitrogen content

Nitrogen (N) is the main essential macronutrients for the growth of crops, and is a major component of chlorophyll and protein. According to our results (fig. 5), like the other traits the highest inhibitory effects on leaf nitrogen content of barnyardgrass was related to SA (%2) + EC (4dS/m) treatment (with 37.5% of inhibition).

Discussion

Environmental limiting factors can affect plants growth, development and production. Therefore, crops survival is strongly dependent on its ability to adaptation to environmental stresses (Robert-Seilaniantz *et al.* 2011). Sometimes, in ecosystems, combination of abiotic and biotic stresses (weeds) could be the main limiting factor. Plants can sense these conditions and taking strategy against them. One of these strategies is releasing some secondary metabolites from plants to their environments (Bertin *et al.* 2003). Plants may employ allelopathy in their interaction with biotic associates. Allelopathic potential helps them invade and compete within natural plant communities (Callaway and Aschehoug 2000, Qasem and Foy 2001, Kohli *et al.* 2006). Also, allelopathy had a close relationship with the other stress (Such as salinity). Having knowledge about these relationships is essential to adopting best practices for using of natural ability of plants. Salinity stress as one of the main plant growth limiting factors can changes the synthesis pathways of secondary metabolites such as allelochemicals (Gutbrodt *et al*, 2011). Between cereals rice is well known as allelopathic crop with suppressive impacts on barnyardgrass (as the main paddy weed) (Chung *et al*, 2001; heidarzade, 2010).

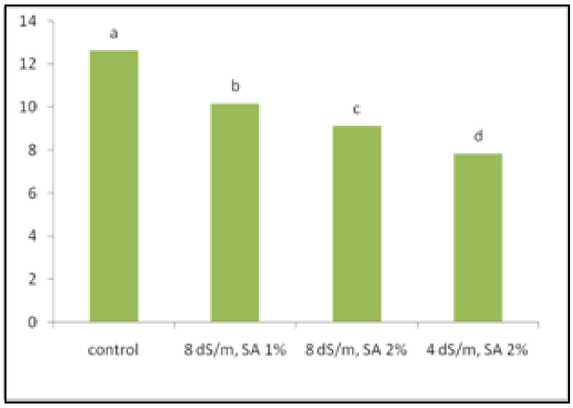

Fig. 4. Chlorophyll content of barnyardgrass in response to treatments.

Rashid *et al* (2005) reported that hound's-tongue seed allelochemicals (phenolic compounds) increased under stress conditions such as low O2 and high temperature and exposing in UV-B radiation. Zeinali *et al.* (2013) suggested that salinity stress in presence of salicylic acid could enhance the allelochemicals content of rice root exudates and improve the allelopathic ability against barnyardgrass (seed germination properties).

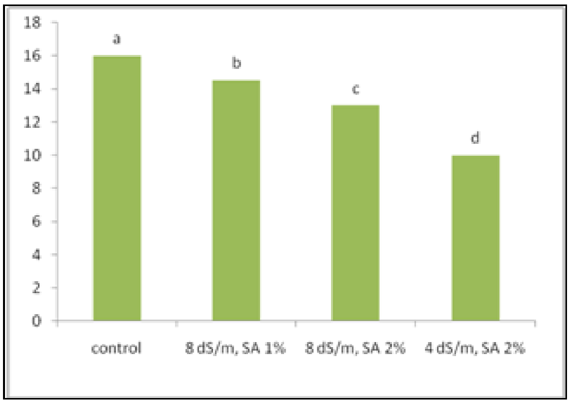

Fig. 5. Nitrogen content of barnyardgrass in response to treatments.

On the other hand, 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, other metabolites synthesis pathway, effect on opening and closing of stomata and respiration (Davies, 2004). 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 *et al.* (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 metal stress (Mishra and Choudhuri, 1999). Foliar application of salicylic acid induced tolerance to heat (Dat *et al*, 1998), frostbite (Janda *et al*, 1999) and salinity (Borsani *et al*, 2001) damages. As mentioned, the environmental stresses induce the production of reactive oxygen species in plant chloroplasts and other cell organelles (Reigosa *et al*, 2006).

The role of allelopathic compounds were suggested in many researches (Taiz and Zeiger, 2010; Heidarzade *et al*, 2010; Heidarzade *et al*, 2012; Esmaeili *et al*, 2012; Zeinali *et al*, 2013), but very few studies have been devoted to effect of abiotic stresses in physiological and biochemical (such as allelochemicals) alteration in plants. Although the previous studies indicated that the high yield capacity and high growth rate cultivars could produce more allelopathic compounds in compare to those with a poor yield. But our results didn't match with the mentioned results. So that the Tarom cultivar with low yield ability in compare to neda, showed the higher inhibitory effects on barnyardgrass growth stage properties. Whereas, our previous result on barnyardgrass germination properties also indicated that root exudates of Neda (high yield capacity cultivar) had the highest inhibitory potential.

In this study, we tried to investigate the interactions of salinity and salicylic acid treatments on allelopathic ability of rice root exudates against growth properties of barnyardgrass.

Conclusion

Consequently, all studied traits were closely associated with each other. So chlorophyll content showed more and positive correlation with nitrogen content, and also the lowest amounts of each trait were closely correlated by SA (%2) + EC (4dS/m) treatment. Hence, in moderate saline conditions of paddies, it may be possible to recommend that, the foliar application of SA 2% will be suitable for better growth of rice seedlings and allelopathic potential against barnyardgrass.

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