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Employing Aqueous Allelopathic Extracts of Sunflower in Improving Salinity Tolerance of Rice

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ABSTRACT

Possibility of improving salinity tolerance by allelopathy was studied in a lab study. Seeds of rice cultivars Super Basmati and Shaheen Basmati were primed in various concentrations (0, 5, 10, 20 & 25%) of allelopathic extract of sunflower for 48 h. Primed and non-primed seeds were sown at 0, 50 and 100 mM NaCl salinity levels. Seed priming with allelopathic plant water extract substantially improved the germination and early seedling growth of both rice cultivars under saline conditions. Sunflower plant water extracts improved germination percentage, decreased mean germination time and produces more number of leaves in both rice cultivars, nonetheless Super basmati performed better at 5% and Shaheen basmati performed better at higher concentrations at both salinity levels. Shaheen basmati performed well under saline conditions than Super basmati. © 2011 Friends Science Publishers

Key Words: Rice; Salinity; Allelopathy; Germination

INTRODUCTION

Soil salinity is a major threat to crop production in many areas of the world. It not only causes a reduced crop yield but also decreases the ability of soil to nourish the younger plants. Under saline conditions, seed germination is inhibited due to limited water uptake, because of reduced radical emergence (Hampson & Simpson, 1990). The problem is wide spread in Pakistan and needs a solution. Different strategies to overcome this problem are used including selection and breeding of resistant cultivars in different crop species (Ashraf, 2009).

Allelopathy is a phenomenon that includes the direct or indirect harmful or beneficial effects of a chemical from one plant on another (Ashrafi *et al*., 2008). A number of phytotoxic or growth promoting substances or allelochemicals have been found in different plant species and in different tissues of plants and soils (Ashrafi *et al*., 2008). Allelochemicals are usually the plant secondary metabolites that have a great potential as natural pesticides and as growth promoters (Chon & Kim, 2002). Many plant species, including some important crops, can produce and release allelochemicals into the environment to suppress the growth of other plants (Golisz *et al*., 2008). Allelochemicals may be secondary metabolites or waste products of the main metabolic pathways in plants and these may not be important in the primary metabolism essential for plant survival (Swain, 1977). Allelochemicals may inhibit the growth of plants at certain concentration or stimulate the growth of the same or different plants at different concentrations (Narwal, 1994).

Release and activity of allelochemicals may be affected by environmental factors such as soil moisture, soil pH, soil organic matter content or soil salinity (Al-Turki & Dick, 2003). Many researchers agree that higher concentrations of secondary metabolites or allelochemicals might result in a more resistance in plants against environmental stresses (Bergelson & Purrington, 1996; Stotz *et al*., 1999; Baldwin *et al*., 2002). Secondary metabolites may be induced in plants as a response to environmental stress like drought, nutrient deficiency or salinity (Siemens *et al*., 2002; Hoballah *et al*., 2004). Induction of secondary metabolites or allelochemicals may be important for the defense of plants in situations where several threats or stress factors act simultaneously (Gouinguené & Turlings, 2002; Hansson, 2004). It is now well established that release of allelochemicals has many advantages for many plants especially under environmental stressful conditions such as drought, nutrient deficiency, etc. (Inderjit & Mallik, 2002; Kong *et al*., 2002; Florentine, 2003). Phenolics or other allelopathic chemicals may influence soil nutrients and soil microbial ecology because of which plant growth pattern is affected (Inderjit & Asakawa, 1998). After the release of the phytochemicals, the plant is no longer in control of who receives the chemicals (Dicke *et al*., 2003).

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Among allelopathic crops, sunflower (*Helianthus annuus*) is well known for its chemical compounds (Ashrafi *et al*., 2008). Some water-soluble allelochemicals have been reported in sunflower, which inhibits the germination and growth of other species (Ashrafi *et al*., 2008). Several phenols and terpenes have been reported in various cultivars of sunflower (Spring *et al*., 1992; Macias *et al*., 2002). Allelochemicals from sunflower are mostly used as herbicides (Ashrafi *et al*., 2008). However, potential of these chemicals for the control of certain environmental stresses is yet to be explored. This laboratory study was conducted to investigate the potential of sunflower allelochemicals in improving the salinity tolerance in rice.

MATERIALS AND METHODS

Experimental details and seed materials: This laboratory trial was carried out in Seed Science Laboratory, Department of Agronomy, University of Agriculture, Faisalabad, Pakistan. Seeds of fine rice cultivar Super-Basmati and Shaheen Basmati were used. These seeds were obtained from Rice Research Institute, Kala Shah Kakoo, District Sheikhupura, Pakistan. The initial seed moisture content was 8.06% (on dry weight basis).

Treatments: For imposing treatment of sunflower water extract, field grown sunflower plant sticks were harvested at maturity and dried under shade for a few days. The well-dried plants were chopped into about 5 cm pieces with fodder cutter. Chopped plant material was dried in an oven at 70°C for 48 h. The oven-dried material was ground in a grinder and passed through a 40-mesh screen. The ground herbage was soaked in distilled water for 24 h at room temperature (30°C±4) in the ratio of 1 g herbage: 20 mL water. The water extract was obtained by filtering the mixture (herbage & water) through Whatman No. 42 filter paper and used a fresh either as such or diluted with distilled water to prepare different concentrations according to the treatment. Petri dishes were given a thorough washing with detergent using hot water as precautionary measure against pathogens and pollutants. Rice seeds were cleaned manually and physical purity was ensured.

Sunflower water extract was diluted with distilled water to prepare solutions of different concentrations (v/v): 5, 10, 15, 20 and 25% and in control treatment only distilled water was used. Seeds of rice cultivars Super Basmati and Shaheen Basmati were primed using these concentrations of sunflower water extract for 48 h. Fifteen seeds of both cultivars were grown in each Petri dish (9 cm diameter) replicated five times in completely randomized design. Filter paper (Whatman No. 42) was used as medium of germination. Salinity was induced by preparing of 50 mM and 100 mM solutions by dissolving 2.925 g and 5.85 g of NaCl in distilled water by using flask of 1000 mL and then made the volume up to the mark. Two ml of saline solution was applied in each petridish. The dishes were kept at room temperature (30°C±4) for seed germination.

Germination test: Germination counts were recorded daily for a period of 10 days and number of leaves and no. of roots were counted. Shoot length and root length was determined with scale. Fresh and dry weight was also recorded. Germination was observed daily according to the AOSA method (AOSA, 1990). The time to 50% germination (T_{50}) was calculated according to the following formula of Coolbear *et al*. (1984) modified by Farooq *et al*. (2005):

$$T_{50} = t_i + \left[\frac{N/2 - n_i}{n_j - n_i}\right](t_j - t_i)$$

Where N is the final number of germination and ni, nj are cumulative number of seeds germinated by adjacent counts at times ti and tj when ni < N/2 < nj.

Mean germination time (MGT) was calculated according to the equation of Ellis and Roberts (1981):

$$MGT = \frac{\sum Dn}{\sum n}$$

Where n is the number of seeds, which were germinated on day D and D is the number of days counted from the beginning of germination.

Germination index (GI) was calculated according to the AOSA (1983) using the following formula:

$$GI = \frac{\text{No. of germinated seeds}}{\text{Days of first count}} + \text{-------} + \frac{\text{No. of germinated seeds}}{\text{Days of final count}}$$

Germination energy was recorded on the 4th day after planting. It is the percentage of germinating seeds 4 days after planting relative to the total number of seeds tested (Ruan *et al*., 2002). Final germination percentage was calculated by dividing total germinated seeds to the total number of seeds sown multiplied by 100.

RESULTS

Application of allelochemicals with concentrations 5, 10, 15, 20 and 25% significantly affected the germination and other growth parameters of both rice cultivars. Regarding time taken for 50% germination, priming with allelochemicals reduced the time taken for 50% germination both under saline and non-saline conditions. Among all the allelochamicals, concentration of 15% was found to significantly reduce the time taken for 50% germination. The concentrations of allelochemicals above and below this level have same effect except the lowest concentration that was at par with that of control. The trend was same in both the cultivars. Among cultivars, Shaheen basmati take less time for completion of 50% germination (Fig. 1a). Same results were found in case of mean germination time (Fig. 1b).

Regarding germination energy, a concentration of allelochemicals of 20% gave significantly high germination energy under both saline and non-saline conditions. It was followed by 15% concentration of allelochemicals; however, it was at par with all other levels. The results were same in case of both rice cultivars (Fig. 2a). Similar results were observed in case of germination index (Fig. 2b), where a concentration of 20% allelochemicals was at the top and gave significantly high germination index under both conditions and with both rice cultivars. It was the case also regarding final germination percentage (Fig. 2c).

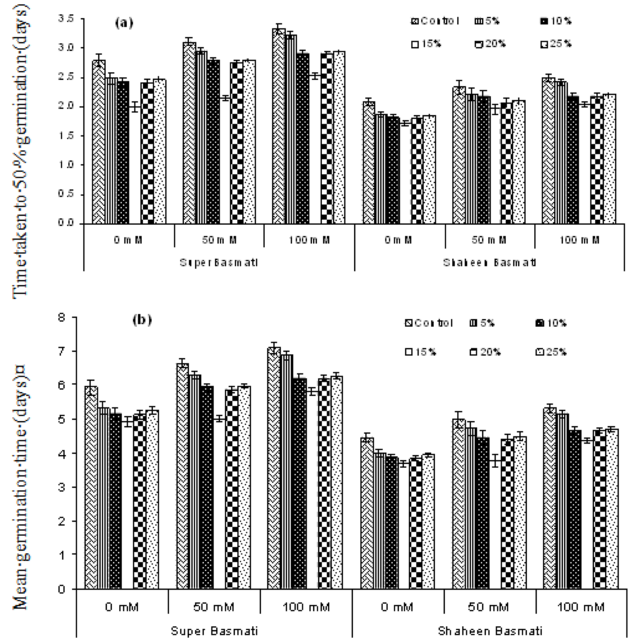
Allelochemicals perform the same for germination index in both rice cultivars and the salinity levels as in case of germination energy. However, a 20% concentration of allelochemicals perform better at a salinity level of 50 mM in Super basmati but it was at par with control or where no allelochemicals were used (Fig. 2b). Similar results were noted in case of final germination percentage. However, Shaheen basmati perform better in case of germination percentage than Super basmati (Fig. 2c).

Among different growth parameters, number of leaves and number of roots were recorded. In case of leaf score, under non-saline conditions, the results were not good by allelochemicals except a lowest dose of 5%, which was at par with control. Under conditions of 50 mM salt level, the number of leaves was increased with the increase in concentration of allelochemicals; however, these were at par with each other. Under high salinity level, a concentration of 10% remained better than all others, which were at par with each other (Fig. 3a). In case of Shaheen basmati, under non-saline conditions, a concentration of 15% performed better than all others. It was also better under saline conditions, but was at par with others (Fig. 3a). Regarding root number, 20% allelochemicals concentration was the best under either condition and with both the rice cultivars. It was followed by a concentration of 15% (Fig. 3b).

Results were similar in case of both shoot and root length (Fig. 4a & b). A concentration of 20% allelochemicals gave best results among all other treatments both under normal and saline conditions for both the rice cultivars. It was followed by the concentration of 15% and other allelochemicals treatments, under both salinity levels. All treatments including control were at par with each other for both the parameters (Fig. 4b).

Fresh weight of seedlings of super basmati under non-saline conditions was greater for 25% concentrations of allelochemicals were used. However, it was at par with the treatment, where no chemical was used. Nevertheless, in case of Shaheen basmati, a 10% concentration of allelochemicals gave significantly greater fresh weight than all other treatments. Under saline conditions, allelochemicals gave more fresh weight than control in both the rice cultivars but were at par with control (Fig. 5a). Regarding dry weight, allelochemicals gave significantly more dry weight under both saline and non-saline conditions in both rice cultivars (Fig. 5b). A concentration of 25% performed better amongst treatments especially in Super basmati than other treatments but at highest salinity level, a 20% allelochemical treatment was at the top. For Shaheen basmati, allelochemical concentration of 10% produced significantly more dry weight under non-saline conditions. Under saline conditions, it was at par with other treatments.

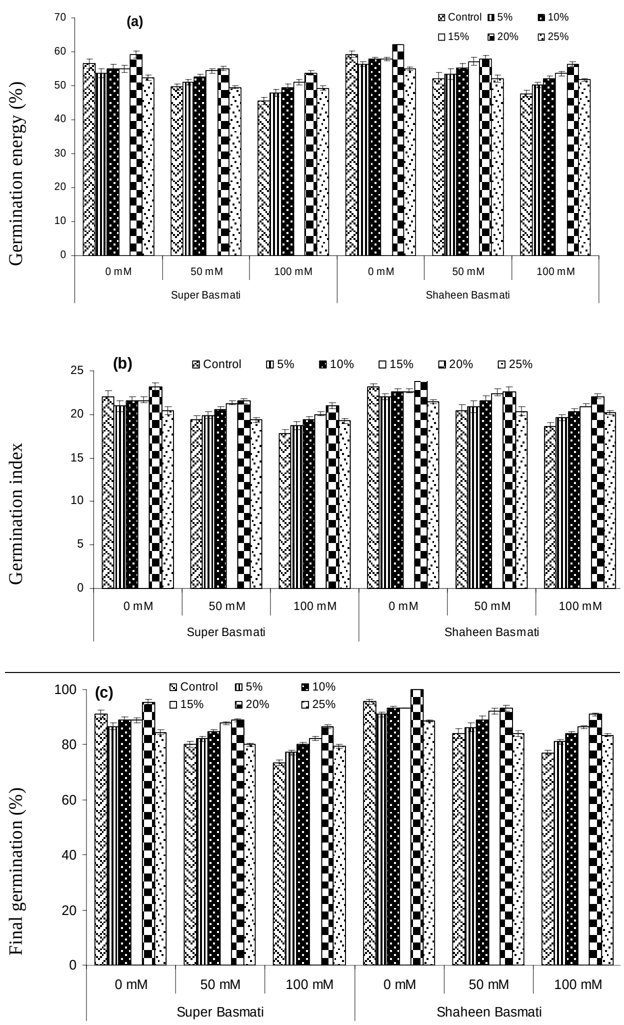
Fig. 1: Influence of seed soaking with sunflower water extracts on (a) time taken to 50 % germination and (b) mean germination time of two rice cultivars under salinity stress ± S.E



DISCUSSION

It is apparent from the present study that rice seed treatment with allelochemicals may perform some better results under saline conditions. Allelochemicals showed a little effect in case of germination related parameters and interestingly these have no inhibitory effect on the germination of seeds. However, a lower dose appeared to be beneficial for seeds and final germination percentage was increased a little by allelochemicals than control or where no allelochemicals were used for seed treatment. This was because, among the allelochemicals, certain secondary metabolites have a significant role in the important metabolism within the plant (Chon & Kim,

Fig. 2: Influence of seed soaking with sunflower water extracts on (a) germination energy (%), (b) germination index and (c) final germination of two rice cultivars under salinity stress ± S.E



2002). Other germination traits like germination energy and germination index were significantly improved by the application of allelochemicals and there may be a possibility that using some other type of allelochemicals may also improve these characters. Significant effect of allelochemicals in improvement of germination related characteristics is not studied or may not been reported. However, it is reported that some water-soluble allelochemicals in sunflower inhibit the germination and growth of other species (Ashrafi *et al*., 2008).

As given in results section, allelochemicals performed better in case of growth related traits, under saline conditions. Both nunber of leaves and roots were higher, where allelochemicals were applied than where no allelochamicals were used. Being secondary metabolites, allelochemicals have role in certain metabolisms of the plant and due to this character; these are used as plant

Fig. 3: Influence of seed soaking with sunflower water extracts on (a) leaf score and (b) root score of two rice cultivars under salinity stress ± S.E

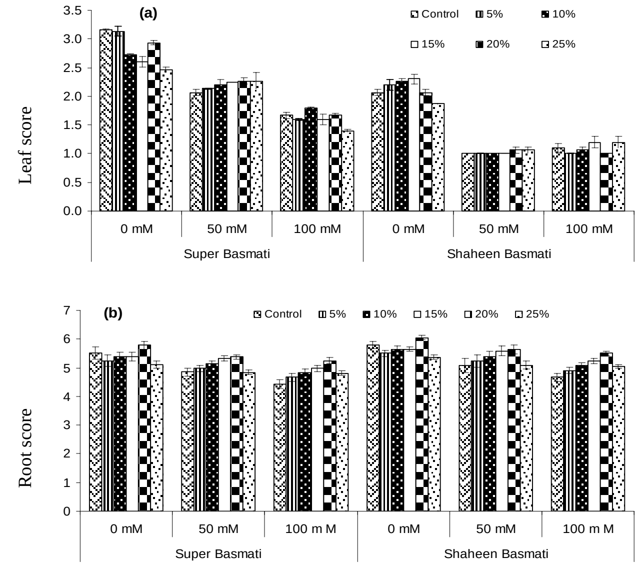
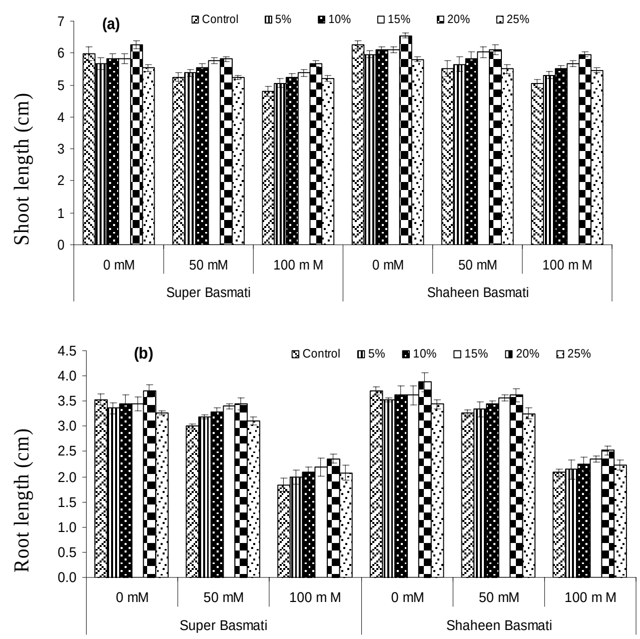


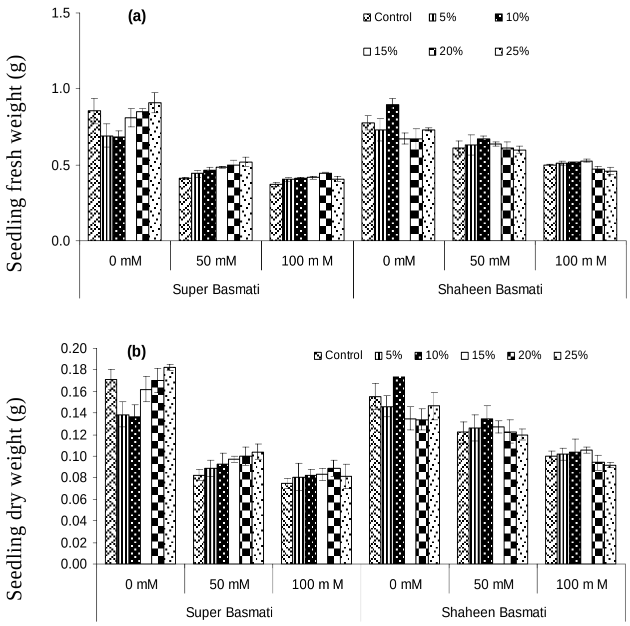
Fig. 4: Influence of seed soaking with sunflower water extracts on (a) shoot length and (b) root length of two rice cultivars under salinity stress ± S.E



protectants under stressful conditions (Bergelson & Purrington, 1996; Stotz *et al*., 1999; Baldwin *et al*., 2002).

A significant impact of allelochemcials was observed in both root and shoot length. Pedrol *et al*. (2006) stated that allelochemicals, being secondary metabolites, have significant impact on the growth of the plants, also under stressful conditions, although the exact mechanism of action is not known yet. It is known that

Fig. 5: Influence of seed soaking with sunflower water extracts on (a) seedling fresh and (b) dry weight of two rice cultivars under salinity stress ± S.E



allelochemicals induce the detoxifying enzymes and transporters, which are involved in the inactivation and sometimes complete elimination of certain toxins and metabolic processes like oxidative modification of functional groups, the formation of glucosyl, glutathione or malonyl conjugates and detoxification via specific membrane transport, leading to vacuolar sequestration or exocytose, are also supported by certain secondary metabolites (Pedrol *et al*., 2006).

Fresh weight of the seedlings was improved with the application of allelochemicals under non-saline conditions in both rice cultivars. However, under saline conditions these were at par with the control treatment. One reason may be that allelochemicals inhibit the growth of the plants and these chemicals may be one of phytotoxic type, which have inhibitory effect instead of any positive effect (Inderjit & Asakawa, 1998) and after the release of these phytochemicals, the plant can no longer control these chemicals (Dicke *et al*., 2003) as a result of which the growth of plant is affected.

In case of dry weight, the results of allelochemicals were encouraging as the dry weight of seedlings was more in treatments where allelochemicals were applied than that of control or where no chemical was used. Previously reported results are in favor of our results (Siemens *et al*., 2002; Hoballah *et al*., 2004).

The present study shows that allelochemicals have potential to cope with environmental stresses especially salinity problem. It also provides an opportunity for further investigations in this important field i.e., promotion of plant survival under stressful conditions. Allelochemicals from sunflower performed well under saline conditions and it is expected that these may play role in plant assistance under other stresses. Allelochemicals from other crops should be tested in this regard. By doing further in this way, may clear the role of allelochemicals for plant survival under stressful conditions.

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