Exogenous applied Allium sativum alleviate salinity induced stress in late sown wheat and alfalfa

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ABSTRACT

Allium sativum potential as medicine in homeopathy and allopathy have significant explored while its allelopathic potential remained unexplored. To investigate its stimulatory/inhibitory effect in late sown wheat and alfalfa crop under salinity stress, two pot experiments were performed under arid land climate in 2014-15. Salt stress was applied after four week of crop emergence, as saline water irrigation with concentrations of 4000 ppm and 6000 ppm along with control of 1000 ppm. To rectify the salt induced stress, exogenous application of garlic extract as foliar spray was used at reproductive stages booting flowering and grain filling. Garlic extract was applied at different concentrations viz., 10%, 20% and 30% of pure garlic extract. A distilled water (0% garlic extract) control was also set at each salinity level for both crops. Statistical analysis of the data revealed significant effect of salinity and garlic extract for each crops on growth, yield and root attributes except root length and root number in alfalfa that was non-significant for salinity stress. Interaction of salinity × garlic extract were non-significant for leaf area and root number for both experiments. Maximum concentration of exogenous foliar applied garlic extract reported an improvement of 70-77% in plant height, 47-85% in dry biomass accumulation, 08-26% in leaf attributes and 46-74% in root attributes at severe salinity stress level. Wheat spike length, spikelet per spike and per plant grain weight were also improved by this treatment. Garlic extract at 30% concentration can be adopted as saline water irrigation induced salinity stress regulating treatment in wheat and alfalfa crop.

Key words: Garlic extract, salinity stress, foliar application, allelopathy, arid climate.

Introduction

Importance of soil as critical resource to feed the world over the years cannot be neglected. Ecosystem services viz., carbon cycle, storage, cleaning, regeneration and biodiversity in natural flora are all supported by soil. The capacity for intensive farming resulted in over cultivation, over grazing and exposure to degradation and salinization. Currently more than 40% of agriculture land is seriously degraded and if this rate of soil loss continue, it will disappear most of the topsoil within next 60 years (Rengasamy 2010). More than 800 million hectares of cultivated land are salt affected (FAO 2008). Soil salinity problem may be due to saline, saline sodic or sodic soils. Salinity is not only associated with arid and semiarid soils but all types of soils may encounter this problem depending upon diverse morphological, physic-chemical and biological properties (Rengasamy 2006). The salinization may be attributed to chemical nature of weathered bed rock, soluble organic and inorganic compounds, rainfall accumulated salts and wind transported inorganic salts, overuse of chemical fertilizers and soil amendments, poor quality irrigation and capillary rise of shallow saline water (Rengasamy 2010). Intrusion of sea water to coast lands and plants ability to uptake and discharge salts in soil horizon also contribute to soil salinity development. Munns and Tester (2008) defined a two phase plant response to encounter salinity. A continuous osmotic phase that inhibit water uptake owing to osmotic pressure of saline solution which drop its potential energy and ionic imbalance or toxicity due to accumulation of specific ion over a longer period of time. Interactive effect of soil processes, soil structural stability, solute solubility, nutrient and water movement in root zone are key processes involved in plant growth and ionic uptake under saline conditions. Plant genetic potential for mitigation strategy also play vital role in adaptation and acclimatization in problematic soils.

Wheat is the 3rd largest cereal after rice and maize produced on 1/6th of total world cultivated area. Wheat supplies 20% of the daily uptake of calories in human food around the globe thus, directly influence the human survival and quality of life. About 2.5 billion people use wheat as an element source of energy and 1.2 billion as staple food (Gerber et al 2010). Wheat is currently under severe salinity stress and considerable loss in grain yield due to impaired growth under
improper nutrition as well as osmotic and drought stress are reported (Shahzad et al 2013). Wheat is moderately salt resistant and inhibited plant growth may be due to toxic effect of NaCl, inability of root system to stop ions entry and ion accumulation in shoot that slower down water uptake by xylem (Lambers 2003; Saqib et al 2012).

Alfalfa (Medicago sativa) is a perennial forage crop also known as queen of forages. It belongs to Fabaceae family and cultivated all around the world. It has the highest yield potential and nutritional value with its high protein content and digestibility. A versatile legume crop suitable for pasture, silage, hay, green chop, grazing, cover crop, organic fertilizer, soil improvement and soil conservation (Djilianov et al 2003; Kowsar et al 2008). Alfalfa is moderately salt tolerant as compared to other legumes (Munns and Tester 2008). Changing agricultural practices and increasing problem of soil aridity and salinity have threaten the alfalfa production (Castroluna et al 2014). Salinity stress is the most problematic and limiting factor for alfalfa production. Increased tolerance and stress management is the key concern for economical alfalfa production (Peel et al 2004).

Currently multiple approaches are employed to manage salt induced stress in field crops. Use of stress resistant cultivars, planting methods, seed priming, exogenous application of plant growth regulators and soil amendments are some common practices (Yusuf et al 2008; Zhang and Rue 2012). Exogenous foliar application of different growth hormones have significantly improved plant growth under salt stress when applied at critical growth stage (Rashad and Hussien 2014). Garlic (Allium sativum) is strongly aromatic bulb and among the oldest cultivated crop commonly used in cooking but effectively explored for its medicinal use in homeopathy and allopathy (Thomson and Ali 2003). Recently it’s been explored for anti-cancer activity (Lee et al 2013), antimicrobial activity (Ross et al 2001), anti-etiologic agent (Shams-Ghahfarokhi et al 2003), soil born fungus control (Sealy et al 2007), cholesterol reduction (Ackermann et al 2001), wound healing (Ejaz et al 2009), pathogen infested seed germination (Perelló et al 2013) and Immunomodulation and Anti-Inflammatory effect (Arreola et al 2015). So, current study is planned to investigate saline water stress on wheat and alfalfa crop. Stress mitigation through foliar application of garlic extract. Adjusting optimum level of garlic concentration for highest salinity management. Comparison of wheat and alfalfa growth yield and root attributes to changing levels of salinity stress and garlic extract concentrations.

Materials and Method

Two pot experiments were performed during the winter season 2014-15, under wire house of Department of Arid Land Agriculture, King Abdulaziz University at Hada Al-sham Jeddah, KSA. Same experimental conditions, pots for planting and planting time were used for wheat and alfalfa.

Pot Preparation

Top sandy loam soil from non-saline A horizon was collected from organic agriculture experimental field Hada al-sham KSA. Soil was air dried for 72 hr, cleaned for stones, straws and ground to pass a 5 mm sieve. Soil moisture content at 100% field capacity (23% w/w) were determine using pressure plate method (Klute and Dirksen 1986). Fertilizers as basal dose NH4NO3, (NH4)2HPO4, KH2PO4, ZnCl2 and H3BO3 were added at a rate of 300, 230, 150, 15, 15 mg pot⁻¹ respectively. Pots with a diameter of 15 cm and depth 36 cm were filled with 2500 g air dried soil which contain thoroughly mixed basal dose of fertilizer. Top 5 cm of each pot were layered with peat soil mixed in 1:1 ratio with sandy loam.

Planting

Prior to planting seeds of both crops were surface sterilized in 70% ethanol solution for two minutes. Sterilization was followed by soaking in 3% sodium hypo-chlorite for six minutes, then rinsed thrice with deionized water. Eight healthy seeds with similar size were sown in each pot and thinned to four per pot after one week of planting. Daily irrigation with non-saline water was applied until fourth week of planting.

Garlic Extract Preparation

Fresh healthy garlic bulbs were thoroughly washed and dried prior to extraction. Extraction was done by using diffusion extraction through decenter centrifuge (Beveridge and Rao 1997). Extract was sieved and packed into glass bottles covered with aluminum foil to avoid photo degradation. Concentration of 10%, 20% and 30% of garlic extract were prepared by dilution with distal water.

Experimental Design and Treatments

Both pot experiments were conducted in completely randomized design (CRD) with three replicates. Late planting of wheat and alfalfa was done at mid of January while optimum planting time at Hada Al-sham is first week of December. Treatments were applied after four weeks of planting until maturity. Irrigation of saline water was main plot treatment, while extract concentrations were sub plot treatment. Three levels of water salinity, S0; 1000 ppm, S1; 4000 ppm and S2; 6000 ppm and four levels of garlic extracts GE1; 0%, GE2; 10%, GE3; 20% GE4; 30% were used for comparison. Saline water irrigation was started from 4th week of planting to maturity. Garlic extract was foliar sprayed thrice at heading, booting and grain filling stages in wheat while from start of flowering for three consecutive weeks in alfalfa.
Data Recording

Wheat

Wheat agronomic and yield related traits were determined at crop maturity. Three plants from each pot were harvested and their averaged data were used as single repeat. Plant height, root length and spike length were recorded by measuring tape at maturity. Plant dry weight, root dry weight and grain weight per pot were obtained through laboratory analytical balance. Leaf number, spikelet per spike and root number were counted manually. Flag leaf area were measured through multiplying leaf length to averaged width of tip, middle and base. Unit of measurement for height and length were cm while for weight were g. flag leaf area were measured in cm$^2$. Root shoot ratio of dry plants were obtained by dividing root dry weight to shoot dry weight.

Alfalfa

Three randomly selected plants from each pot were harvested for data recording. Same agronomic parameters of alfalfa were measured by using same procedure as described for wheat. Number of leaflets per plant of alfalfa were recorded instead of number of leaf per plant. Each alfalfa leaf is multiple of three leaflets.

Statistical analysis

Completely randomized design was used with three replicates for both experiments. Data was statistically analyzed for analysis of variance (ANOVA) by using SAS 8.1. Revised least significant difference (RLSD) were computed @ p≤0.05 and p≤0.01 for statistically analyzing treatment means for comparison.

Results and Discussion

Wheat

Significant (p≤0.05) reduction in wheat growth and yield traits were documented for applied levels of saline water. Effect of salinity increased by increasing the concentration of salts in irrigated water. Garlic extract mitigated salinity induced stress by significantly (p≤0.01) improving all studied traits (Table 1). Interaction of salinity × garlic extract reported variable trend for different parameters. Interactive effect of salinity × garlic extract was highly significant (p≤0.01) for plant height, plant dry weight, spike length and grain weight per plant while significant (p≤0.05) for root length. Non-significant (p≥0.05) interaction was calculated for leaf number, flag leaf area, spikelet per spike, root dry weight and root count. Applied levels of 4000 ppm and 6000 ppm of saline water produced significant reduction in all studied parameters of wheat growth, yield and root attributes. Effect of severe salinity level (6000 ppm) was more pronounced as compared to 4000 ppm and 1000 ppm levels. Medium salinity level (4000 ppm) also resulted in significant reduction in most of the studied trait but a minute improvement for some studied traits were recorded as compared to control and severe salinity stress. Among these traits least improvement was in root dry weight. Highest variation/reduction was documented for flag leaf area, root number and spikelet per spike under highest level of salinity stress. Medium salinity stress produced stimulatory effect on plant dry weight, spike length, root dry weight and root number while severe salinity inhibited/reduced these parameters and intensity was more severe (up to 118%) for maximum salinity level as compared to medium salinity level Garlic extract significantly improved wheat characteristics for all levels of water salinity. Effect was more pronounced to mitigate medium salinity stress level as compared to severe salinity stress level. Different extract concentrations resulted in 31-52% and 26-153% improvement in plant growth and leaf traits, 23-64% and 18-86% in yield and yield contributors and 18-46% and 46-65% for root dynamics for medium and severe salinity stress respectively. All levels of GE brought improvement but maximum recovery was attained at highest concentration GE30% as compared to severe and non-saline treatment. Highest GE concentration produced almost non-significant results for medium salinity stress when compared to lowest salinity level for all studied traits except plant height, dry weight, spike length and root length. Maximum recovery was attained in flag leaf area and root dry weight where highest GE concentration was foliar sprayed. Garlic extract GE10% and GE20% produced almost non-significant recovery for severe salinity stress as compared to lowest salinity stress (1000 ppm). Root number and root dry weight were highest for medium saline water treatment when foliar sprayed with highest level of GE (GE30%).
Table 1. Effect of exogenous applied garlic extract on wheat growth, leaf and root attributes

<table>
<thead>
<tr>
<th>Salinity</th>
<th>Garlic Extract</th>
<th>Plant height cm</th>
<th>Plant dry weight g</th>
<th>Leaf number</th>
<th>Flag Leaf area cm²</th>
<th>Spike length cm</th>
<th>Spikelet per spike</th>
<th>Root dry wt g cm</th>
<th>Root length cm</th>
<th>Root number</th>
<th>Grain wt per plant g</th>
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<td>5.30 fg</td>
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<td>2.66 f</td>
<td>5.39 e</td>
<td>17.04 ef</td>
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<td>6.67 cd</td>
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<td>6.38 c</td>
<td>14.66 fg</td>
<td>2.64 e</td>
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Salinity   | GE           | **   | **   | **   | **   | **   | **   | **   | **   | **   | **   |

** and ** denote significance at the p<0.05 and p<0.01 probability level, respectively. ns: non-significant. CV: Coefficient of variation, LSD: Least significant difference, S: Salinity stress, GE; garlic extract foliar application, S0: Saline water with EC 1000ppm (control), S1: Saline water with EC 4000 ppm, S2: Saline water with EC 6000 ppm, EC: Electrical conductivity, Ppm: parts per million, Different GEs viz., GE0: Distil water (control), GE10: Garlic extract with 10% concentration, GE20: Garlic extract with 20% concentration, GE30: Garlic extract with 30% concentration, %: percentage.

Table 2. Effect of exogenous applied garlic extract on Alfalfa growth, leaf and root attributes

<table>
<thead>
<tr>
<th>Salinity</th>
<th>Garlic Extract</th>
<th>Plant height cm</th>
<th>Plant dry weight g</th>
<th>Leaf number</th>
<th>Leaf area cm²</th>
<th>Spikelet per spike</th>
<th>Root dry weight g</th>
<th>Root number</th>
<th>Grain wt per plant g</th>
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<td>0.89 def</td>
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<td>9.77 cd</td>
<td>2.52 fgh</td>
<td>8.66 ab</td>
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<td></td>
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<td>31.67 ab</td>
<td>1.33 cd</td>
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Table 3. Effect of exogenous applied garlic extract on Alfalfa growth, leaf and root attributes

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<tr>
<th>Salinity</th>
<th>Garlic Extract</th>
<th>Plant height cm</th>
<th>Plant dry weight g</th>
<th>Leaf number</th>
<th>Leaf area cm²</th>
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</table>

Salinity   | Garlic Extract | **   | **   | **   | **   | **   | **   | **   | **   | **   | **   |

* and ** denote significance at the p<0.05 and p<0.01 probability level, respectively. ns: non-significant. CV: Coefficient of variation, LSD: Least significant difference, S: Salinity stress, GE; garlic extract foliar application, S0: Saline water with EC 1000ppm (control), S1: Saline water with EC 4000 ppm, S2: Saline water with EC 6000 ppm, EC: Electrical conductivity, Ppm: parts per million, Different GEs viz., GE0: Distil water (control), GE10: Garlic extract with 10% concentration, GE20: Garlic extract with 20% concentration, GE30: Garlic extract with 30% concentration, %: percentage.
Alfalfa

Salinity stress documented remarkable reduction in alfalfa growth, leaf and root attributes while foliar application of garlic extract mitigates these salinity induced stresses. Main effects of salinity and garlic extract were highly significant (p≤0.01) for most of the studied traits except root number (p≤0.05) for garlic extract and plant height, plant dry weight, leaf area and root dry weight (p≤0.05) for salinity stress. Salinity stress reported non-significant effect (p≥0.05) on root length and number of roots per plant. Interaction of salinity stress × garlic extract were highly significant (p≤0.01) for root length and root dry weight, significant (p≤0.05) for plant dry weight and leaf number while non-significant for plant height, leaf area and root number. Salinity stress reduced plant height, plant dry weight, leaf number, root length and root number while increased root dry weight significantly. Increment in dry weight accumulation was higher under severe salinity stress compared to medium and low salinity stress treatment (Table 2). Medium salinity stress reduced plant dry weight and root number but inverse was true for severe salinity stress. On contrary severe salinity stress reduced while medium stress increased leaflet area as compared to low salinity stress treatment (control).

Garlic extract mitigated salinity induced effects and recovery was greater in pots under severe salinity stress than medium salinity stress except leaf and root number. Foliar application of GE produced non-significant improvement in alfalfa agronomic parameters in pots under low salinity stress (control) except plant height that was significant. Influence of garlic extract was concentration dependent and maximum recovery from salinity stressed pots were in those pots where 30% of GE were applied in both medium and severe saline backgrounds. On contrary, root length and number were higher for 20% GE under 4000 ppm and 6000 ppm salinity stress pots respectively. Garlic extract at maximum concentration reported 27-77% and 70-85% recovery for alfalfa growth traits, 66-85% and 8-84% in leaf attributes, 44-91% and 26-75% in root dynamics for medium and severe salinity treatments respectively. Highest root length (12.63 cm) and root dry biomass (4.83 g) were noted in pots under severe salinity stress when foliar sprayed with 30% concentration of GE.

Discussion

Each year 1.2% arable land is prone to salinization along with enhanced greenhouse effect which lead significant rise in annual mean temperature that is expected to be 1.1-6.4% higher at the end of 21st century globally (Parry 2007). Therefore, current studies are planned to mitigate the negative impact of salt and late season heat stress on wheat and alfalfa crop. Results of current experiment depicted that high salinity stress significantly reduced the plant height, dry matter accumulation leaf number, flag leaf area and root attributes in both wheat and alfalfa and grain contributing traits, spike length and spikelet per spike in wheat crop (Tab. 1-2). Reduction in these agronomic and yield contributing traits are denoted for severe effect of saline irrigation treatments and intensity of damage increases as the level of salinity increases in applied water (1000 ppm to 6000 ppm) (Mahmood et al 2012). High salinity stress produced 37% grain yield loss per plant that’s attributed to shorten spike length which produced less number of spikelet per spike and shrunken grain in wheat experiment. While a 14% reduction in alfalfa plant height and 11% reduction in leaf area that are major contributor to stem dry weight accumulation in forage are documented under highest salinity stress levels (Qiu et al 2014). An increased root dry weight were recorded in alfalfa crop which may be due to accumulation of salts in roots under higher salinity stress. Decreased number of leaf and leaf area may be attributed to shedding of old leaves that have hyper accumulated salts and decreased chlorophyll contents under salinity stress (Liu et al 2012; Singh and Gautam 2013). Qados (2011) has reported increase in fresh and dry weight accumulation in Viciafaba under NaCl stress.

Salinity bring several morphological, physiological and metabolic changes in plant that results in oxidative stress and over production of reactive oxygen species production. Reactive oxygen species production results in various biochemical changes at molecular level that include lipid peroxidation and membrane destruction. Plant initiate several enzymatic and non-enzymatic mechanisms to detoxify these reactive species (Li et al 2011). The antioxidant defense system include antioxidants such as, glutathione, ascorbic acid, proline and many other. Enzymes such as superoxide dismutase, catalase and peroxidase are major scavenger of O2− radicle to H2O2 and subsequently to H2O (Triantaphylides and Havaux 2009). Antioxidant system play key role in scavenging the oxidative damage induced by salinity stress.

Exogenous application of garlic extract significantly recovered the wheat and alfalfa from salt induced stresses. Effect of garlic extract was concentration dependent. Garlic extract bring greater stimulatory effect on salt affected treatments than non-saline. Recovery was greater in vegetative part than root attributes. Allelopathic effect of Allium sativum is tested for the first time on plant growth and yield of field crops. No previous study focusing on garlic potential to alleviate salt induced stress were found previously. Salt induced recovery under exogenous applied garlic extract may be due to different sulfur containing compounds like alliin (S-allyl cysteine sulfoxide) and alliinase are stable under dry environment and stable at 60 °C (Block 1985; Rybak et al 2004). This sulfur containing compound allicin can easily diffuse across phospholipid membrane and into the cytoplasm thus may serve as osmo-regulator under salt stress (Miron et al 2000).
Conclusion and Recommendations

Salt stress significantly affected both wheat (Poaceae) and alfalfa (Fabaceae) family crops. Exogenous foliar application of garlic extract at reproductive stages depicted remarkable recovery by alleviating salinity induced negative effects. Recovery was also concentration dependent and highest concentration of GE 30% resulted in maximum relief. Garlic extract at 30% concentration can be adopted as stress regulating treatment for wheat and alfalfa crop under saline conditions. Further research is needed to explore the stress regulating potential of Allium sativum by preparing different concentrations and combinations to optimize its dose. Screening and identification of allelochemicals responsible for salt stress alleviation is also potential subject for study.

References


