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# Response of three new *Atriplex* species (*Atriplex* spp.) to drought and its recovery

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ABSTRACT

The present study was conducted to evaluate drought stress and its recovery on the growth and biochemical changes of three *Atriplex* species. The experiment included three *Atriplex* species and four irrigation regimes. The results showed that light (75% field capacity) and severe drought (50% field capacity) caused significant reduction in the growth of all three *Atriplex* species, and no irrigation destroyed *Atriplex leucoclada* and *Atriplex canescens*. Recovery could compensate some loss of weight in all three species, especially *Atriplex lentiformis*, so that there were significant differences between the control and 75% field capacity (FC) treatments. Tissue moisture content percentage in the control, 75% and 50% FC showed no significant difference. No irrigation caused a significant reduction in the moisture percentage of *A. lentiformis*; however, recovery alleviated considerable parts of this loss. Water deficit treatments (75% and 50% FC) increased the activity of catalase, peroxidase and superoxide dismutase, but no irrigation reduced the activity of these enzymes in *A. lentiformis*, which had the highest antioxidant enzymes activity. Dry weight had a positive and significant correlation with moisture content percentage and antioxidant enzymes activity. Generally, drought stress, depending on the stress levels, reduced growth and increased antioxidant enzymes levels; recovery, depending on the species and stress levels, could compensate some of these changes. *A. lentiformis* had the highest drought resistance and higher recovery ability, which might be due to higher activity of antioxidant enzymes.

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## 1. Introduction

Arid and semi-arid regions are characterized by low erratic rainfall between 250 and 500 mm $y^{-1}$, periodic droughts and different associations of vegetative cover, which generally includes short grass or shrub vegetation. Therefore, those ecosystems are susceptible to rapid land cover change resulting in a reduction in productivity and moisture conditions [34]. Arid and semi-arid regions comprise about 61% of Iran's land surfaces, and have a rainy period that typically runs from November to May, followed by a dry period between June and October. In this country, the rains are highly variable in time, space, amount and duration. Thus, water is one of the most important limiting factors for biological and agricultural activities in Iran [31]. Since their early transition from aquatic to terrestrial environments, plants have coped with periodic and unpredictable drought stress [46]. Drought stress may cause damage to cells, either directly or indirectly, through the formation of activated oxygen species (AOS) [30]. Under water stress conditions, levels of these antioxidants have shown increases, decreases, or no effect, depending on the species, duration of drought stress, the antioxidant investigated and the temporal sequence of antioxidant responses [41]. This is a growing concern for agriculture and forestry activities, considering the increasingly unpredictable nature of rainfall ([35,46]), which translates into important economic losses [10]. Members of the genus *Atriplex* have long been considered suitable for the restoration of degraded lands with precipitation of lower than 200 mm $y^{-1}$, useful for game habitat, re-vegetation, and some of them possess interesting properties for use as fodder [44]. Thus, several million hectares have been planted throughout the world.

*Atriplex leucoclada* L. is the native halophyte species that has been planted most often. Belonging to the Chenopodiaceae family and possessing the C4 metabolic pathway, it is widely distributed in non-saline as well as highly saline soils [14]. *Atriplex lentiformis* and *Atriplex canescens* are North American C4 shrubs, which were brought to Iran in 1963 from the north of Mexico and the north and west of the United States of America [14]. The adaptations of this species to drought conditions make them a particularly invaluable species for use as forage for livestock, wildlife habitats and also in re-vegetation and range-enhancement projects around the world [29,42].

The majority of studies on *Atriplex* spp. investigated morphological and physiological responses under salt stress [6,7,18,21] and the potential use of this genus as a fodder for livestock [29,42]. However, published information on drought stress and recovery in *Atriplex* spp. is lacking.

*Abbreviation:* AOS, activated oxygen species; FC, field capacity; CAT, catalase activity; SOD, superoxide dismutase activity; POD, peroxidase activity; APX, ascorbate peroxidase activity.

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The present study was planned to investigate 1) the effect of drought on growth, physiological and biochemical parameters of the three *Atriplex* species 2) possible differences in recovery ability among the three *Atriplex* species under different irrigation regimes 3) the correlation between growth parameters and other measured traits.

## 2. Material and methods

### 2.1. Plant materials and stress treatments

Three *Atriplex* species: *A. lentiformis*, *A. leucoclada and A. canescens*, have been used in this study. Uniform and healthy seeds of the test plants were purchased from Pakan Bazr Co. (Isfahan, Iran) and stored at 4 °C. After incubation, five seeds were planted in each 4 L capacity pot, which were filled with a mixture of medium texture sand and clay (1:2). The main characteristics of soil were: pH, 7.07; electrical conductivity (EC) 0.05 ds $m^{-1}$; organic carbon (OC) 0.83%, %; Total N, 0.080 g $kg^{-1}$; K 476 mg $kg^{-1}$ and P 16.5 mg $kg^{-1}$.

After emergence, the plants were thinned to three per pot. Pots were transferred to the greenhouse. The greenhouse at the Research and Application Station of the Faculty of Agriculture, Shiraz University (Shiraz, Iran) (29°43′ N and 52°35′ W) was covered with double-layer acrylic glazing, oriented north to south, and equipped with a pad-and-fan cooling system, an under-bench misting system for humidification, and a natural-gas-forced hot air heating system. The greenhouse had a floor area of 108 $m^2$, with 2.5 m gutter height and 4.3 m peak height. The day and night temperatures in the greenhouse were maintained at 28/22 $\pm$ 2 °C with a natural lighting condition. All pots were kept weed-free by hand-hoeing.

The pots were irrigated at field capacity (FC) until two weeks after planting. Drought stress was imposed on the plants two weeks after sowing by withholding irrigation (including four irrigation regimes: 100% FC as control, 75% FC, 50% FC and no irrigation) for four weeks followed by re-watering for two weeks. Thus, the trial was composed of: four weeks of drought period (two to six weeks after planting) and two weeks recovery period (six to eight week after planting). We have chosen drought and recovery periods according to Eric et al.[11] and Sapeta et al.[38].

### 2.2. Growth parameters

Plant growth parameters including: plant height (cm), moisture content (%) and dry weight (g), were measured at 14 (before drought stress), 42 (end of drought stress, before recovery period) and 56 (after recovery period, end of trial) days after planting. Dry weight was measured after drying in a hot air oven at 75 °C for 2 days.

### 2.3. Activities of antioxidant enzymes

At the end of each treatment, antioxidant enzymes activity was measured. Fresh leaf tissues (500 mg) were titrated in 10 mL phosphate buffer [pH 7.8; 50 mM] using an ice cooled sterilized pestle and mortar. The extract was centrifuged at 15,000 $\times g$ for 15 min at 4 °C. The supernatant was separated in another autoclaved Eppendorf tube and used for determining the activities of enzymes, superoxide dismutase (SOD), peroxidase (POD) and catalase (CAT). Inhibition in photoreduction of nitroblue tetrazolium (NBT) was used to assess the activity of SOD. The reaction mixture (1 mL) [500 μL phosphate buffer (pH 7.8), 0.5 mL distilled $H_2O$, 100 μL methionine, 50 μL NBT and 50 μL sample extract] in cuvettes was kept under light for 20 min. The optical density of the irradiated aliquot was read at 560 nm. Following Giannopolitis and Ries [16], SOD enzyme activity per unit was based on the amount of enzyme that inhibited 50% of NBT photoreduction. The method of Chance and Maehly [8] was used for determining CAT and POD activities. HEPES buffer (25 mM, pH 7.8) containing 0.2 mM EDTA, 2% PVP and 2 mM ascorbate was used for ascorbate peroxidase activity (APX) (EC 1.11.1.11) extraction. Enzyme activity was determined according to the protocol of Zhu et al. [47]. The activities of all four enzymes were expressed as U $mg^{-1}$ protein.

### 2.4. Statistical analysis

A factorial experiment arranged in a randomized complete block design with six replicates was used. The statistical differences among species were determined through analysis of variance (ANOVA). Fisher's Least Significant Difference (LSD) method was used for separation of the means. Analysis was performed with the statistical software SAS Program Version 9.1.3 [39] (SAS Institute Inc., Cary, NC, USA).

## 3. Result

### 3.1. Growth

The results of the effects of water deficit regimes on plant height of three *Atriplex* species are summarized in Fig. 1. Plant height was decreased significantly under light (75% FC) and severe (50% FC) drought in all three *Atriplex* species, whereas no irrigation destroyed *A. canescens* and *A. leucoclada*. Plant height in the three species increased till 14 days after planting. Plant height of *A. lentiformis* under no irrigation and severe drought (50% FC) continued to grow with little increase when

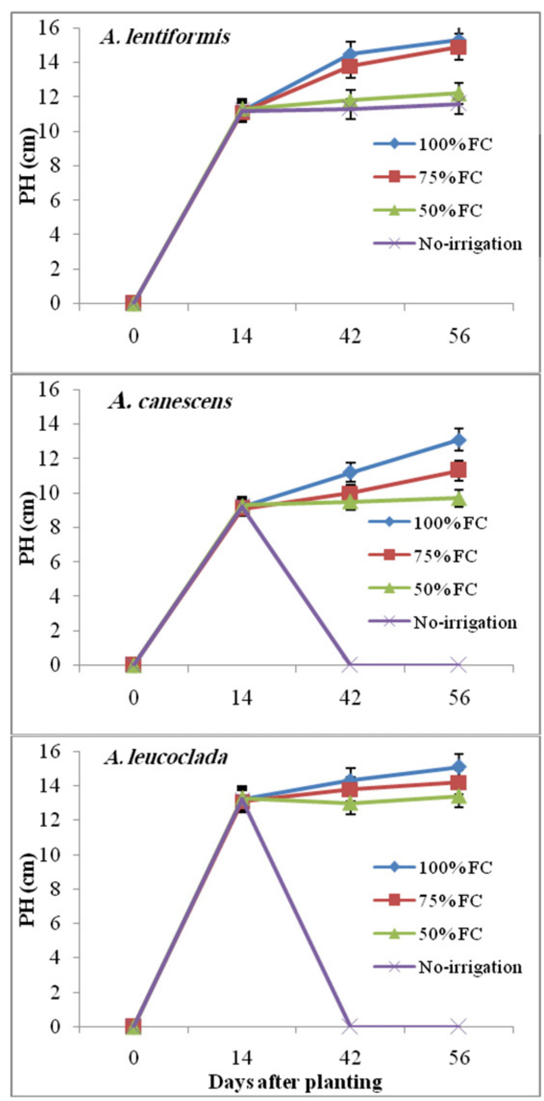


**Fig. 1.** PH, plant height in three *Atriplex* species under different irrigation regimes.

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