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Full Length Research Paper

Abscisic acid content in roots and root characteristics of alfalfa under deficit irrigation

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The effect of drought stress on Abscisic acid (ABA) on roots and root characteristics of three alfalfa varieties (Aohan, Zhongmu No.1 and Suntory) was studied. The study was conducted in greenhouse of the Chinese Academy of Agricultural Sciences (CAAS), Beijing, China from September 2014 to May 2015. Alfalfa varieties were exposed to four irrigation levels of water-holding capacity viz 100% (w1) (control); 85% (w2); 70% (w3) and 55% (w4), which were considered as deficit irrigation treatments. The results showed that ABA was significantly affected by deficit irrigation treatments during different plant growth stages. With the exception of 90 and 105 days after transplanting, ABA increased after transplanting up to harvest in all treatments. Increasing deficit irrigation showed significant increase in ABA content in roots of alfalfa. The minimum content of ABA was obtained from w1 (44 ng/g.FW) and then increased to 56.6, 64.6 and 94.4 ng/g.FW for w2, w3 and w4 respectively on 105 days after transplanting. Moreover, the results showed that ABA content was differently affected among different varieties of alfalfa under different water stress levels. The maximum ABA content was 83.2, 61.7 and 49.9 ng/g.FW obtained with Aohan, Suntory and Zhongmu No.1 varieties respectively, at 105 days after transplanting. Highest water stress w4 (55%) reduced root length by 20.92%, lateral roots by 20.71%, root fresh biomass by 43.79% and root dry biomass by 37.96%. The root to shoot ratio was 1.9 times higher in water stressed plants compared with the control, indicating that water stress in alfalfa is affected more shoot growth than root one.

Key words: alfalfa, deficit irrigation, Abscisic acid (ABA), root characteristics.

INTRODUCTION

In recent years, drought has becomes a leading threat to agriculture in the world (Loss and Siddique 1994; Grando and Ceccarelli 1995). It is one of the main environmental stresses that limits plant growth and production (Lawlor and Cornic, 2002; Martínez et al., 2003; Tadina et al., 2007; Ren et al., 2007; Wu et al., 2009). Drought affects many *Medicago* species (Chebouti et al., 2001; Bouizgaren et al., 2011). Plants can avoid drought by having a root system adapted to drought (Morgan et al., 1986; Liu et al., 2005).

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Roots are the first part of a plant to sense water stress and its growth affects the answer to drought cues (Shimazaki et al., 2005). Root characteristics are defined as root length, root laterals number and root diameter are very important for good plant stand (Nanjo et al., 1999). Effect of water stress on root growth was studied in different crops such as wheat (Pritchard et al., 1991), maize (Wu and Cosgrove, 2000; Shimazaki et al., 2005) and barley (Sahnoune et al., 2004). One adaptation by plants to drought is the change in root to shoot ratio (Turner, 1997). Drought decreases growth of amaranth (Amaranthus spp.) in both root and shoot, but with less effect on root growth (Liu and Stützel, 2004). Lateral roots are one of the root system components which play an important role in water absorption. The development of lateral roots and growth are affected by complex interactions of different factors such as, environment, development and hormones (Casimiro et al., 2003; López-Bucio et al., 2003: Malamy, 2005), One important factor that affects the growth and development of roots to tolerate the environment such as water, drought, salt, cold, light, and temperature is ABA hormone (Signora et al., 2001; Sharp and LeNoble, 2002). ABA hormone is a key hormone that confers tolerance to environmental stresses. Plants increased ABA content when placed under water stress which effectively closes stomata to protect plants from adverse effects of drought (Schroeder et al., 2001; Rock et al., 2010; Mori and Murata., 2011) and to enhance drought resistance in crops (Schroeder et al., 2001; Wang et al., 2005; Shinozaki and Yamaguchi-Shinozaki, 2007). This study was conducted to evaluate the effects of deficit irrigation on abscisic acid content in roots and root characteristics of alfalfa.

MATERIALS AND METHODS

Plant material and destructive samples

The experiment was conducted in the green house of Animal Science Institute. Chinese Academy of Agricultural Sciences (CAAS), Beijing, China from September 2014 to May 2015. The treatments comprised of three varieties of alfalfa (Medicago sativa L.) viz Aohan, Zhongmu No.1 and Suntory. Seeds were germinated first on wet filter paper in petri-dishes. Four plants seeding were transplanted to each pots-culture (25 x 30 cm) with10 kg clay soil (pH 7.7%, organic matter 9.2 g kg⁻¹, total P 0.7 g kg⁻¹, total K 19 g kg⁻¹, bulk density 1.3 g cm⁻³ and field capacity 30%). The varieties were evaluated under four irrigation levels of water holding capacity viz: w1 (100%), w2 (85%), w3 (70%) and w4 (55%). The full water holding capacity w1 was considered as normal irrigation, while the other treatments w2, w3 and w4 were considered as deficit irrigation. Irrigation water was application (manually) commenced from the first day of transplanting and continued for the whole growing period of alfalfa (195 days).

Pot moisture contents measured daily by using HH2 moisture meter version 4.0 (Delta- T Devices Ltd. UK) to maintained the percentage of water-holding capacity (100, 85, 70 and 55%). Samples of roots were collected every 15 days and immediately frozen in liquid nitrogen and then stored at -80 °C for further ABA extraction and analysis. Samples of roots were carefully removed from the pot and washed several times in water to obtain the

complete roots. ABA was then extracted and purified according to Dobrev and Kaminek (2002) method and analyzed as described by Albacete et al. (2008). One homogenized gram of fresh root weight was placed in 5 ml of cold mixture of methanol, water and formic acid at pH 2.5 and was separated by centrifuging (20 000 g for 15 min after overnight extraction at -20°C. The root material was then subjected to generate more extract in 5 ml of the same solution for 30 min. Supernatants were filtered through Sep-Pak Plus†C18 (®Waters, Milford, MA, USA) to remove plant pigments and interfering lipids and evaporated to dryness. Residues were dissolved in 5 ml of methanol/water solution (1 to 4 ratio) using an ultrasonic bath and filtered by nylon membrane Millex filters (Ø 0.22 µm) (®Millipore, Bedford, MA, USA) then put in tubes adding extraction solution adjusting to 1.5 ml. Analyses were carried out on an HPLC/MS system consisting of an Agilent 1100 Series HPLC (Agilent Technologies, Santa Clara, CA, USA). Equipped with autosampler and connected to an Agilent Ion Trap XCT Plus mass spectrometer using ESI (an electrospray interface). It was filtered with Millex filters and injection, 100 µl of each fraction. For quantification of ABA calibration curves were constructed for component analyzed using internal standards: [²H₆]cis,transabscisic acid (©Olchemin Ltd, Olomouc). Root length (cm) and lateral roots number/plant were measured by using Scan Maker i800 plus (MICROTEK, shanghai, China). Fresh root biomass (g) was weighted with digital balance directly after sample of root collection and then dried at oven 60°C until a constant weight for determining dry biomass of roots. Root to shoot ratio were calculated by dividing fresh root weight by the fresh shoot weight.

Experimental design and statistical analysis

The experiment was laid out in complete randomized design (CRD) with four replications. Treatments were three varieties of alfalfa and four irrigation level of water holding capacity. Data were calculated and arranged as means and analysis using one way analysis of variance (ANOVA) followed by Duncan's multiple range test (P < 0.05).

RESULTS

Deficit irrigation and Abscisic acid (ABA) content

The effects of deficit irrigation treatments on the content of ABA in roots were shown in Figure 1. The results show that the content of ABA was affected by deficit irrigation at different plant growth stages. Increasing deficit irrigation showed a significant increase in ABA content. ABA content gradually increased with time under all irrigation treatments until 75 deficit davs after transplanting. From 75 days after transplanting, ABA content hastily decreased under w1, w2 and w3 for 30 days until it reached the lowest ABA content at 105 days after transplanting. From 105 days after transplanting ABA content tended to increase until harvest. Moreover, under w4, ABA content increased from 30 to 75 days after transplanting, and then declined until 90 days and remained to 105 days after transplanting, after that tended to increase until harvest. The minimum ABA content was obtained under w1 (44 ng/g.FW) and then increased to 56.6, 64.6 and 94.4 ng/g FW for w2, w3 and w4 at 105 days after transplanting, respectively.



Figure 1. Effect of deficit irrigation on Abscisic acid content in roots of alfalfa during different days after transplanting. w1, 100% of water holding capacity; w2, 85% of water holding capacity; w3, 70% of water holding capacity and w4, 55% of water holding capacity. In each days separately, the same letter are not significantly different (P < 0.05).

Abscisic acid (ABA) content and varietal response

The effect of alfalfa varieties on ABA content are presented in Figure 2. ABA content was affected by different varieties of alfalfa. ABA content progressively increased with time in all varieties up to 75 days after transplanting. ABA content under Zhongmu No.1 and Suntory decreased rapidly after 75 days after transplanting until reached its lowest activity level at 105 days after transplanting while Aohan decreased gradually. After that all the three varieties started to increase in ABA content and continued with that trend until harvest.

The minimum ABA content was 83.2, 61.7 and 49.9 ng/g .FW which was obtained by Aohan, Suntory and Zhongmu No.1 at 105 days after transplanting respectively. However, Zhongmu No.1 presented the lowest ABA content in all times, except for 180 and 195 days after transplanting in which was higher than Suntory while Aohan recorded the highest ABA content compared to the other two varieties.

Deficit irrigation and root characteristics

The effect of deficit irrigation treatments on root length, number of root laterals, fresh and dry weights of roots and root to shoots ratio are shown in Table 1. The results reveal that root length, number of root laterals, fresh and dry weights of roots were significantly reduced by increasing the deficit irrigation (P<0.05). The lowest reduction in root length was 9.3% followed by 12.7 and 20.9%. The least reduction in number of root laterals was 14.5% followed by 15.7 and 20.7%. Whereas the lowest reduction in root fresh weight was 9.9% followed by 11.1 and 43.8%. However, the least reduction in root dry weight was 7.3% followed by 21.7 and 38% was obtained by w2, w3 and w4 respectively, compared with control plants. Root to shoot ratio was significantly increased by increasing the deficit irrigation (P<0.05). The maximum increase in root to shoot ratio was 94.3%, followed by 78.6 and 30% in w4, w3 and w2 respectively compared to control treatment.

Root diameter was not significantly increased by increasing deficit irrigation. Irrigation of alfalfa plants by 55% of water holding capacity (W4) resulted in highest reduction in root diameter (5.3%) in comparison to the control plants (100% of water holding capacity).

Alfalfa varieties and root characteristics

The effect of alfalfa varieties on root length, number of root laterals, root diameter, fresh and dry weights of roots are shown in Table 2. Aohan resulted in significantly different root length, number of root laterals and dry root



Figure 2. Effect of three varieties of alfalfa on abscisic acid content, during different days after transplanting in roots of alfalfa. zh, Zhongmu No.1; ah, Aohan and su, Suntory. In each days separately, the same letter are not significantly different (P < 0.05).

Table 1. Effect of deficit irrigation on root characteristics and root to shoot ratio.

WHC%	Root length (cm)	No. of root lateral	Root diameter (cm)	Root fresh weight (g)	Root dry weight (g)	Root/shoot ratio
w1	457.23 ^a	1452.16 ^a	0.38 ^a	87.91 ^a	21.00 ^a	0.70 ^b
w2	418.45 ^b	1241.52 ^b	0.38 ^a	79.18 ^b	19.47 ^a	0.91 ^{ab}
w3	399.20 ^c	1224.93 ^b	0.36 ^a	78.15 ^b	16.45 ^b	1.25 ^a
w4	361.59 ^d	1151.38 ^c	0.36 ^a	49.42 ^c	13.03 ^c	1.36 ^a
SE ±	17.18	41.49	0.01	5.51	0.80	0.04

WHC%, water holding capacity; w1, 100% (control); w2, 85%; w3, 70% w4, 55%. The values in each column followed by the same letters are not significantly different (P < 0.05).

Table 2. Effect of alf	alfa varieties on	root characteristics.
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Varieties	Root length (cm)	No. of root lateral	Root diameter (cm)	Root fresh weight (g)	Root dry weight (g)	Root/shoot ratio
Zh	428.21 ^b	1202.92 ^b	0.38 ^a	81.18 ^a	19.01 ^a	1.06 ^a
Ah	357.92 [°]	1145.67 ^c	0.38 ^a	67.69 ^b	15.37 [°]	1.05 ^ª
Su	441.23 ^a	1453.90 ^a	0.35 ^a	72.12 ^b	18.08 ^b	1.06 ^a
SE ±	14.88	35.93	0.01	4.77	0.69	0.036

zh, Zhongmu No.1; ah, Aohan and su, Suntory. The values in each column followed by the same letters are not significantly different (P < 0.05).

biomass, while the minimum root diameter was observed in Suntory. Moreover, Zhongmu No.1 presented higher root fresh and dry weights compared with other two varieties. The highest root laterals number was observed in Suntory while no significant difference was observed in root to shoot ratio among all varieties.

DISCUSSION

Abscisic acid (ABA) is a hormone that indicates to endure environmental conditions such as drought and high salinity. Our study indicated that increasing deficit irrigation significantly increased ABA content. These results are in agreement with those reported by Brodribb and McAdam (2011) and McAdam and Brodribb (2012) on ferns (*Pteridium esculentum* and D. antarctica) and a lycophyte (*Selaginella kraussiana*). Outlaw (2003) reported that during drought stress ABA concentration was increased up to 30- fold.

Deficit irrigation reduced root length. This result is similar to that reported by Benlaribi et al. (1990); Ali Dib and Monneveux (1992) who attributed the reduction in root length and root fresh and dry weight to the reduction of turgor pressure in wheat. Similar results were observed in Albizzia seedlings (Nanjo et al., 1999), Erythrina seedlings (Nativ et al, 1999), Eucalyptus microtheca seedlings (Marron et al., 2002), and Populus species (Nautiyal et al., 2002). Moreover, Sacks et al. (1997) and Rao et al. (1993) found that under deficit irrigation, root growth of wheat and maize was not significantly reduced. The results showed that fresh and dry weights of roots were significantly reduced by increasing the deficit irrigation. Similar results were reported by Manivannan et al. (2007) and Lowlor and Cornic (2002) in pearl millet. Nicholas (1998) observed that in Avocado cultivars, water stress reduced the fibrous roots biomass. Under mild and severe water stress conditions, Populus species decreased root dry weight (Wullschleger et al., 2005). Similar results were observed in sugar beet (Pan et al., 2002). Furthermore, Amina et al. (2014) reported that drought reduced significantly the vegetative mass of the shoots and roots of wheat. Liu and Stützel (2004) observed that the effect of water stress was less in root growth than shoot one.

Our results showed that root to shoot ratio was significantly increased by increasing the deficit irrigation. Other studies demonstrated that under water-stress conditions, root-to-shoot ratio was increased to facilitate water absorption (Morgan 1984; Nicholas, 1998). Also Sharp and LeNoble (2002) and Manivannan et al. (2007) related this increase in water absorption to ABA content in roots and shoots.

Conclusion

Our results demonstrate that ABA was significantly

affected by deficit irrigation treatments and Alfalfa varieties. Increasing deficit irrigation showed significant increase in roots ABA content. Moreover, root length, laterals number, fresh and dry weights of roots were significantly reduced by increasing the deficit irrigation. The results showed that the root to shoot ratio was significantly increased by increasing the deficit irrigation, indicating that water stress in alfalfa crop affected more shoot growth than roots. Aohan presented the lowest root length, number of root laterals, dry root biomass and less root to shoot ratio. In contrast this variety had the highest ABA content.

Conflict of interests

The authors have not declared any conflict of interests.

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