

Effect of Methyl Jasmonate in Alleviating Adversities of Water Stress in Barley Genotypes

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ABSTRACT: Study to find out the response of barley (*Hordeum vulgare* L.) genotypes (Yousef, Morocco) to foliar application of methyl jasmonate was carried out under water limited environment, at the Agricultural Biotechnology Research Institute of Iran (ABRII), Karaj during 2010-11. All plants were sprayed with 100 ppm methyl jasmonate, and expanded leaves were harvested and extracted 24 h and 72 h later. The wire house experiment was laid out in completely randomized design. Data regarding of physiological traits (abscisic acid and auxin contents, Stomatal conductivity, proline contents and relative leaf water contents) were studied in leaves of barley genotypes (Yousef as tolerant and Morocco as susceptible). The data so collected were analyzed statistically by analysis of variance (anova) technique and LSD at 1% probability was used to compare the differences among treatments' means. In both genotypes drought stress increased in abscisic acid contents and with methyl jasmonate application further enhanced. Auxin contents decreased by drought stress, but methyl jasmonate had no significant effect on its content in both genotypes. Stomatal conductivity decreased in both genotypes under drought, with simultaneous decrease was observed in methyl jasmonate- treated plants. Water stress caused to decrease in proline contents and exogenously applied methyl jasmonate led to a further increment. In both genotypes relative leaf water content were decreased in drought but alleviated with methyl jasmonate treatment. These beneficial effects led to improvement in physiological index under drought. These results suggest the involvement of methyl jasmonate in improving the drought tolerance of barley as an important cereal crop.

Keywords: barley, drought tolerance, methyl jasmonate

INTRODUCTION

Environmental stresses provoke numerous plant responses, varying from altered gene expression to metabolic processes. Maintaining higher plant productivity under environmental stresses is plausibly the main challenge facing modern agriculture (Gill and Tuteja 2010). Among the environmental stresses, drought is a major abiotic stress limiting agricultural crop production and is the most important stresses worldwide (Karami . 2013). Complete understanding of physio-biochemical responses of plants to drought is vital for improving plant tolerance mechanisms to drought stress (Jaleel . 2006). Generally, plants experience drought stress either when the water supply to roots becomes hard or when the transpiration rate becomes very high (Manivannan . 2007). Plants can avoid drought stress by improving water absorption or decreasing transpiration (Ruiz-Sánchez . 2007). The responses of plants to water deficit are observed in forms of phenological responses, morphological changes,

physiological alterations, and biochemical adaptations, such as changes in plant structure, growth rate, tissue osmotic potential and antioxidant defenses (Duan . 2007). Osmolytes accumulation is mandatory in plants for osmotic adjustment under water limiting conditions. But osmolyte accumulation mainly depends upon water status, crop growth stage and cultivar (Hong-Bo . 2006). Accumulation of osmolytes such as proline, helps maintaining cell water status, sub-cellular structures and protecting membranes and proteins from the denaturing effects of the osmotic stress (Ashraf and Foolad 2007). The improved level of accumulated proline in crop plants is generally correlated with drought tolerance. Relative leaf water content (RLWC) is an integrative index of plant water status which is used to evaluate the tolerance to water stress. Reduction in RLWC under drought stress leads to Stomatal closure (Gindaba . 2004) which further resulting in decreased CO₂ assimilation (Anjum . 2012b). The plant hormone abscisic acid (ABA) plays a major role in seed maturation and germination, as well as in adaptation to abiotic environmental stresses. ABA promotes Stomatal closure by rapidly altering ion fluxes in guard cells (Leung and Giraudat 1998). The plant hormone abscisic acid, as a stress signal, also increases as a result of water stress. Increasing evidence indicated that one mode of ABA action may be related to its role in the oxidative stress in plants (Little . 1997). Jasmonic acid (JA) and methyl jasmonate (MJ), as a group termed jasmonates, are regarded as endogenous regulators that play important roles in regulating accumulation of various secondary metabolites, plant growth and development. They are involved in signal transduction pathway of plant responses to several environmental stress factors (Gill and Tuteja 2010). It is now becoming evident that jasmonates can act as true plant hormones, which mediate in various aspects of development and stress responses (Creelman and Mullet 1997; Hasanloo . 2008). Exogenous application of jasmonates modulates several physiological responses leading to improved resistance against abiotic stresses (Karami . 2013). Barley (*Hordeum vulgare* L.) is one of the crops of old world agriculture and was one of the first domesticated cereals. Barley is one of the most important crops in arid and semiarid regions, as it is relatively resistant to drought and salinity and requires less water as compared to wheat and corn (Karami . 2013). Nevertheless, drought is an important abiotic stress for barley, which is often grown in environments where drought is common (Stanca . 1992). Furthermore, barley is also a model experimental system because of its short life cycle and morphological, physiological, and genetic characteristics (Tommasini . 2008). Drought is one of the major constraints to barley production worldwide. Compared to other cereals, barley is well adapted to drought through water use efficiency. Nevertheless, drought is an important abiotic stress for barley, which is often grown in environments where drought is common (Anjum . 2012a). The objective of this work was to investigate the effect of drought stress and methyl jasmonate on abscisic acid and auxin contents, Stomatal conductivity, proline contents and relative water contents in leaves of two barley genotypes. Barley cv. Yousef is improved and cultivated in temperate zones of Iran and is well adapted to drought stress (Abedini 2012; Karami . 2013) and Morocco 9-75 is considered to be sensitive to drought stress (Ceccarelli 2004).

MATERIALS AND METHODS

The experiment was carried out during summer 2011 in rain-protected greenhouse at the Agricultural Biotechnology Research Institute of Iran (ABRII) Karaj, Iran. The experiment conducted in completely randomized design (CRD) with three treatments (well-watered mild water stress and severe water stress) and three replications under field conditions. Seeds of spring barley (*H. vulgare* L.) cv Yousef and Morocco 9-75 were obtained from Seed and Plant Improvement Institute of Iran (SPII) and from Dr. StefaniaGrando (International Center for Agricultural Research in the Dry Area, ICARDA), respectively. The barley genotypes were germinated and grown in 3:1 peat: perlite, in a controlled environment chamber at 25°C under 16:8, light to dark regime. Half of the individuals at each life stage were designated control plants. Water treatments were 70%, 30% and 10% of water holding capacity of soil. Normal irrigation was continued in two leaf stage and then discontinued for plants under stress to achieve certain level of stress. Treated plants were sprayed with MJ solution (100 ppm) and control plants were sprayed with dH₂O. Treated plants were isolated from control plants in different growth chambers, under identical conditions, to avoid inter-plant communication through airborne MJ. The samples were collected from the expanded leaves in vegetative stage for physiological and molecular analysis for each treatment. Twenty-four and seventy-two hours after MJ treatment, expanded young leaves of all plants of each stage (70% and 10% WHC) were simultaneously harvested. The plants were sampled (all expanded leaves) after 24 and 72 hours of MJ application to assess the abscisic acid and auxin contents, Stomatal conductivity, proline contents and relative leaf water contents. After thorough washing, the leaves were frozen in liquid nitrogen and stored at 70 °C until used for analysis. The remaining plants were harvested at maturity to assess physiological traits.

Abscisic acid and auxin determination

Five grams of leaf tissue per sample were used for ABA extraction, according to the method reported by Kelen . (2004). Briefly, samples were homogenised in methanol 70% and stirred overnight at 4 °C. After filtration through a Whatman 0.45 m filter, the extracts were completely evaporated under vacuum and dissolved in water at pH 3.0. The solutions were partitioned with diethyl ether three times and then passed through anhydrous sodium sulphate. After evaporation of the apolar phase, the dry residue was dissolved in 3 mL of methanol and the solution was used directly for injections. Analysis were performed in three replicates for each treatment. The high performance liquid chromatography (HPLC) analysis was equipped with a LC Pump Plus, a PDA Plus detector and an Autosampler Plus injector. The column temperature was maintained at 25 °C. The mobile phase was acetonitrile-water (26:74) containing 30 mM phosphoric acid at pH 4.0, flow rate 0.8µL/min. For each extraction, three different injections in HPLC were performed. To quantify the ABA and IAA content, known amounts of pure standards (Sigma) were injected into HPLC system and an equation, correlating peak area to ABA and IAA concentration, was formulated (Iriti . 2009; Kelen . 2004).

Proline determination

Proline was determined following Bates . (1973). Briefly, 0.5 g of leaf samples was ground in 5 ml 3 % Anjum . 2011 Blackwell Verlag GmbH sulphosalicylic acid and the mixture was centrifuged at 10,000 g for 10 min; 2 ml filtrate was mixed with 2 ml acid-ninhydrin and 2 ml of glacial acetic acid in a test tube. The mixture was incubated in water bath for 30 min at 98 °C and allowed to cool at room temperature. The mixture was extracted with 5 ml toluene and the absorbance was recorded at 520 nm (Bates . 1973; Anjum . 2011).

Relative Leaf water content (RLWC) and stomatal conductivity measurement

Plant water status was determined by measuring the relative leaf water contents (RLWC) from five fully expanded leaves. After recording the fresh weight (fw), the leaf samples were put in the test tubes containing distilled water; turgid weight (tw) was then recorded after 24 h. Leaves were then put into electric oven till constant weight to determinate the dry weight (dw). The RWC was calculated using the following relation: $RWC = (fw - dw)/(tw - dw) \cdot 100$ (Anjum . 2011). Where fw is fresh leaf weight, tw is turgid leaf weight and dw is dry leaf weight, respectively. Stomatal conductivity of fully expanded leaves was determined using a Porometer (Delta-T AP4, Delta-T Devices, Cambridge, UK) between 8 and 9 a.m (Karami . 2013).

Statistical analysis

Dataset was statistically analyzed by analysis of variance (Anova) technique using software SAS (Ver9.2) and the main effects were analyzed and at a significance level of 0.01.

RESULTS AND DISCUSSION

ABA contents

The ABA contents was drastically increased under drought stress in both barley cultivars, however, this increment was lower in Yousef as compared to Morocco, while minimum ABA levels were recorded in plants raised under well watered conditions. Exogenously applied MJ led to a further increment in ABA contents in both genotypes. In water stress, an increment in ABA contents were observed by 340% in Yousef and also by 1001% in Morocco, respectively. ABA contents were increased by exogenous application of MJ by 517% in Yousef and also by 1387% in Morocco respectively under water-deficit conditions after 24 hours after application of MJ respectively. But in a measurement in after 24 h with 72 h of treatment, no significant changes for this parameter were observed (Fig. 1).

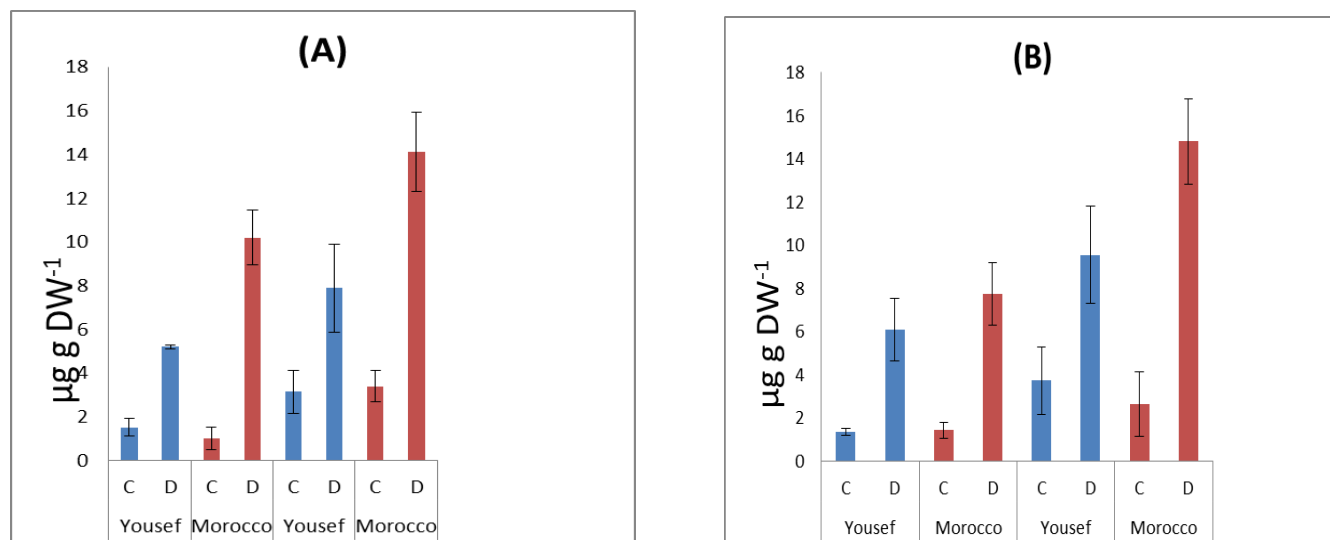


Figure 1. Influence of methyl jasmonate (MJ) application on ABA in two barley genotypes (Yousef and Morocco) under 24 hours (A) and 72 hours after methyl jasmonate application. C, control; D, drought; -MJ, no methyl jasmonate treatment; +MJ, with methyl jasmonate treatment

IAA contents

Water stress caused to decrease in IAA content (Table 1). In water stress a decrement in IAA content was observed by 46.4% and 46.2% respectively in Yousef and Morocco (Fig. 2). But the effect of MJ in IAA content in both genotypes was not significant (Table 1).

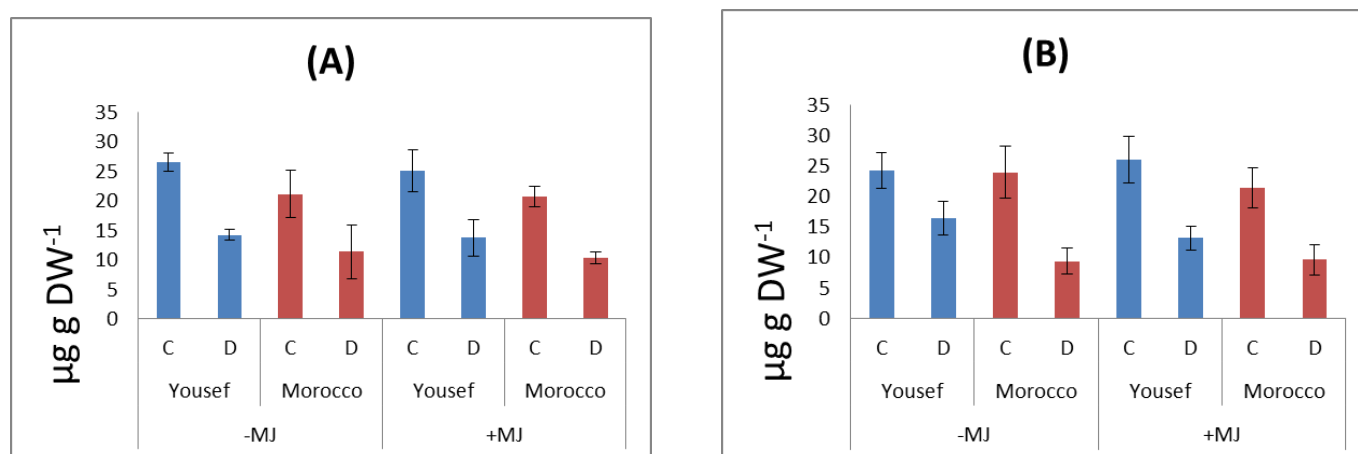


Figure 2. Influence of methyl jasmonate (MJ) application on IAA in two barley genotypes (Yousef and Morocco) under 24 hours (A) and 72 hours after methyl jasmonate application. C, control; D, drought; -MJ, no methyl jasmonate treatment; +MJ, with methyl jasmonate treatment

Stomatal conductivity

Drought reduced stomatal closure in barley. However, exogenously applied MJ led to a further decrement. In water stress a decrement in Stomatal conductivity by 48.7% and 71.2% were observed in Yousef and Morocco, respectively. Exogenous application of MJ led to an increment by 73% and 81% in Yousef and Morocco under water-deficit conditions after 24 hours after application of MJ. In a measurement in after 24 h with 72 h of treatment, no significant changes for Stomatal conductivity in both genotypes were observed (Fig. 3).

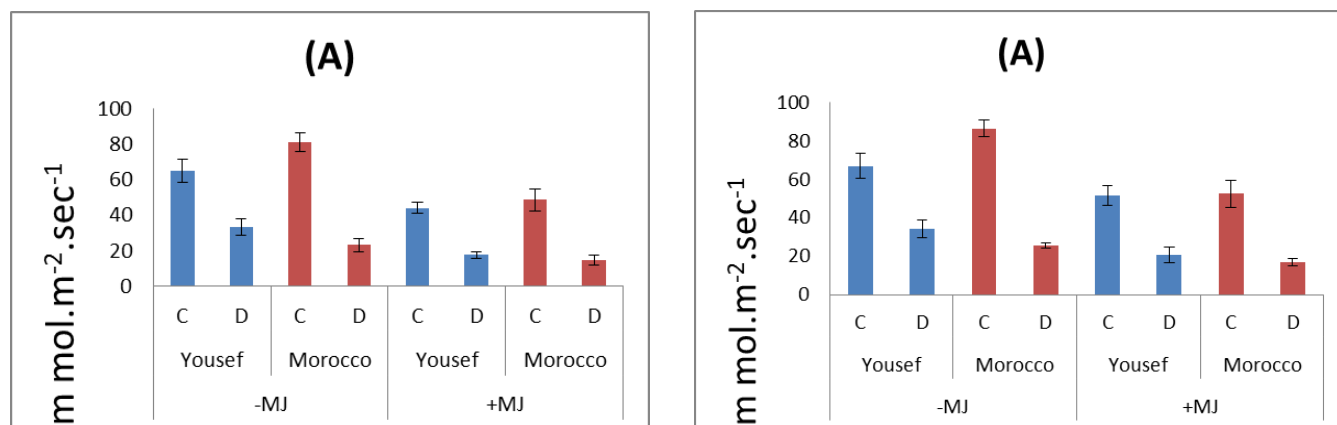


Figure 3. Influence of methyl jasmonate (MJ) application on Stomatal conductivity in two barley genotypes (Yousef and Morocco) under 24 hours (A) and 72 hours after methyl jasmonate application. C, control; D, drought; -MJ, no methyl jasmonate treatment; +MJ, with methyl jasmonate treatment

Proline

An increment in proline contents were observed by drought (Fig.). In water stress an increment in proline contents were observed by 488% and 479% respectively in Yousef and Morocco (Fig). The effect of MJ in proline contents after 24 h in both genotypes was not significant, but after 72 h, MJ application further enhanced the proline contents in both genotypes (Fig.). As a result, the proline contents were higher in stressed plants and the highest in stressed plants applied with MJ. Exogenous application of MJ led to an increase in proline contents by 693% and 536% in Yousef and Morocco respectively under water-deficit conditions after 72 h application (Fig. 4).

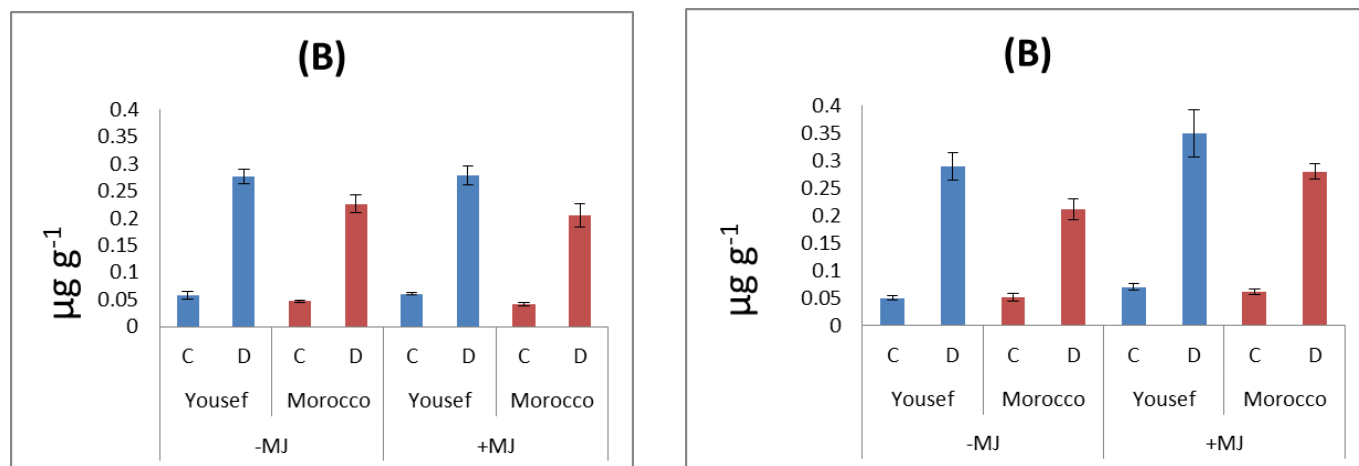


Figure 4. Influence of methyl jasmonate (MJ) application on proline content in two barley genotypes (Yousef and Morocco) under 24 hours (A) and 72 hours after methyl jasmonate application. C, control; D, drought; -MJ, no methyl jasmonate treatment; +MJ, with methyl jasmonate treatment

Relative leaf water contents (RLWC)

Over the experimental period, the progressive drought stress caused subsequent reduction in RLWC of barley plants as compared to well water control. The RLWC of the Yousef dropped from 87.9% at well watered condition to 67.9% at water stress. Nonetheless, the same values for Morocco were from 91.3% at well watered condition to 56.9% at water stress. Although the effect of MJ in RLWC after 24 h in both genotypes was not significant, after 72 h, drought-induced decrease in RLWC of barley plants was found to be increased by exogenous application of MJ under drought conditions. Exogenous application of MJ increased RLWC in Yousef and Morocco by 122% and 135% respectively in water stress condition after 72 h (Fig. 5).

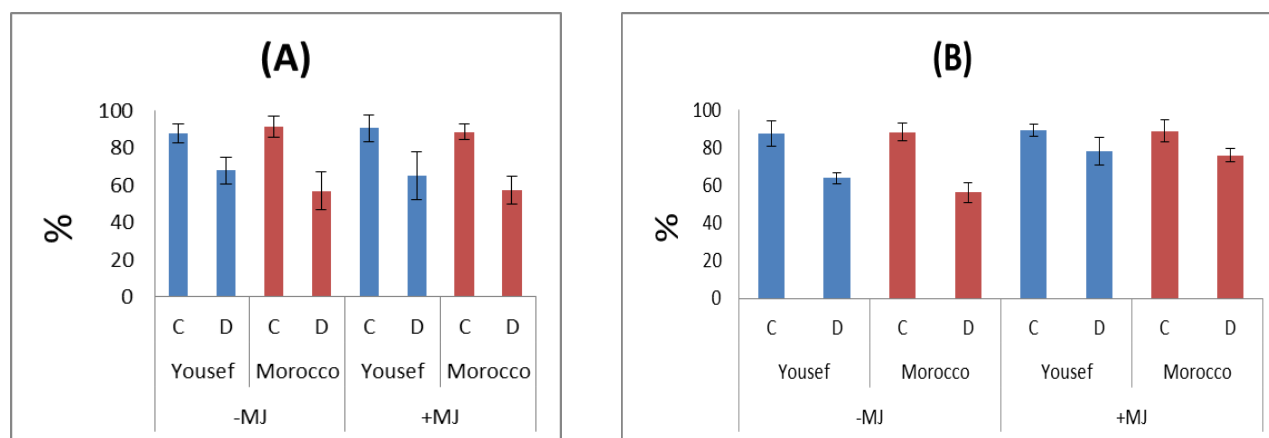


Figure 5. Influence of methyl jasmonate (MJ) application on RLWC in two barley genotypes (Yousef and Morocco) under 24 hours (A) and 72 hours after methyl jasmonate application. C, control; D, drought; -MJ, no methyl jasmonate treatment; +MJ, with methyl jasmonate treatment

Discussion

The plant floral scent methyl jasmonate (MJ) has been identified as a vital cellular regulator that mediates diverse developmental processes and defense responses against abiotic stresses (Cheong and Choi 2003). It has been reported that exogenously applied JA and MJ inhibited or promoted morphological and physiological changes in plants. The pleiotropic action of JA and MJ was in a concentration-dependent manner. Binns has reported that 100 μ M of MJ showed the best effective in physiological parameters and used this concentration as trial report (Binns 2001). In this study, we examined the effect of drought stress and methyl jasmonate on abscisic acid and auxin contents, Stomatal conductivity, proline contents and relative water contents in leaves of two barley genotypes. The results showed that twenty-four hours after MJ treatment is the best time for physiological studies such as ABA, IAA and Stomatal conductivity in both barley genotypes. It had no significant changes for evaluated parameters between 24 h and 72 h after MJ treatments for cited parameters that is in agreement with the results of Binnes . on effects of methyl jasmonate on *Echinacea pallida* (Binns 2001). But in our study for evaluation of MJ application on proline contents and relative leaf water contents, 72 h after treatment was a better time. This study indicates that water deficit led to substantial decrease in tissue water status (Fig. 5) in stressed plants. Cytosolic concentration of osmolytes is often increased in the species resistance to water deficit often, which not only helps in maintaining the tissue but also are involved in osmoregulation (Farooq . 2009a; Farooq . 2009b). In this study, one of the important osmolytes, proline, was substantially increased upon exposure to drought; interestingly, MJ triggered its biosynthesis further (Fig. 4). This increased proline might have helped to maintain high tissue water contents as indicated by higher values of RLWC. It is in agreement with the results of Anjum . on effects of drought and methyl jasmonate on soybean (Anjum . 2011). In our study MJ caused to increase in ABA levels in barley genotypes in both drought and well watered condition. It has been reported that higher levels of MJ in *Arabidopsis* caused to production of ABA. It is postulated that plants produce MJ during drought stress, which in turn stimulates the production of ABA (Kim . 2009). Although water stress caused to decrement in IAA levels, MJ application was not able to have significant effect on this hormone levels. We postulated that MJ acts in two different ways: in first way MJ cause to increment in ABA levels in barley genotypes that will led to stimulate the Stomatal closure. On the other way, MJ caused to increment in proline concentration. This two ways will cause to alleviating in RLWC decrementing by drought (Fig. 6). In conclusion, MJ application improved the barley performance under drought by modulating the tissue water contents, probably leading to improved crop performance.

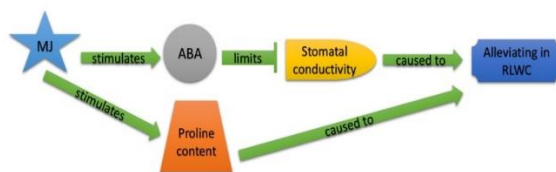


Figure 6. A schematic shape postulates that MJ acts in two different ways: in first way MJ cause to increment in ABA levels in barley genotypes that will led to stimulate the Stomatal closure. On the other way, MJ caused to increment in proline concentration. This two ways will cause to alleviating in RLWC decrementing by drought

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