

Can Foliar Molybdenum Compensate for Damage to Barley Because of Draught Stress?

Sareh Mahdavi

Department of Agriculture, Jahrom Branch, Islamic azad university, Jahrom, Iran.

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To evaluate the quantitative and qualitative responses of barley to foliar molybdenum in drought conditions, an experiment was conducted in 2013 in the Research Farm of Islamic Azad University of Shiraz. The experiment was iterated four times as split plots in the form of statistical design of randomized blocks. Main plots involved three levels of irrigation regimes including normal irrigation, mild stress (no irrigation at the beginning of grain filling) and high stress (no irrigation at early flowering). Subplots involved foliar molybdenum on three levels including foliar molybdenum with pure water, foliar molybdenum with 1% (10 per thousand) and 2% (20 per thousand) sodium molybdate. Results showed that stopping irrigation during reproductive growth significantly influences grain yield and most components of wheat grain yield. Grain yield decreased by 27% and 12% during no irrigation treatment at the beginning of flowering and no irrigation treatment at early grain filling, respectively, compared with normal irrigation. Foliar molybdenum increased grain yield and many components of yield. Compared to control treatment, 1% and 2% foliar molybdenum increased grain yield by 6% and 7%, respectively. Effect of foliar molybdenum was significant on grain protein. Grain protein increased by 38% and 15% during no irrigation treatment at the beginning of flowering and no irrigation treatment at early grain filling, respectively, compared with normal irrigation. In general, results of this study indicate the helpful role of molybdenum on improved quality of barley grain as well as alleviated damage of draught stress.

Key words: molybdenum, mild stress, foliar, grain protein, sodium molybdate

One of the main stresses reducing agricultural production is drought stress. Drought stress prevents plant photosynthesis, leading to serious changes in chlorophyll content and photosynthetic structures. One of the reasons that environmental stresses such as drought reduce growth and photosynthetic ability of the plant is the imbalanced production of oxygen free radicals and defensive mechanisms of these radicals leading to accumulation of ROS and oxidative stress

induction, damage to proteins, membrane lipids and other cellular components. Under environmental stresses such as drought, high activity of antioxidant enzymes and high content of non-enzymatic antioxidants are essential for plant tolerance to stress (Fu and Huang, 2001).

In many parts of the world, access to water is limited and drought process as an environmental stress is most likely to occur at any time (Rajaram *et al.*, 1996). With an average annual rainfall of 260 mm, Iran is located in arid and semi-arid area (Chassesemi *et al.*, 1995). With current trends in population growth, food production will not definitely meet *all* needs in future; taking into account the importance of grains as the main food source for people, any research on drought stress would be valuable.

* To whom all correspondence should be addressed.

In Iran, Barley grows in different climatic conditions and it is exposed to environmental stresses at different stages of development. A significant proportion of the barley crop is related to dryland which are constantly exposed to stress. Barley plant is resistant to drought and salinity; in arid areas where rainfall is insufficient, barley replaces wheat (Khodabandeh, 1990; Rasmusson, 1985). In semi-arid areas, spring barley varieties often face with water scarcity and drought in the final stages of their growth.

Stopping irrigation in milky and dough stages increased water use efficiency and decreased dry matter and grain yield in wheat genotypes (Ghodsi *et al.*, 2006). The reports show that humidity stress after anthesis rapidly decreases grain-filling duration followed by grain weight loss (Mohammadi *et al.*, 2006). Drought stress imposed after the formation of the terminal spikelet, which coincides with the early stem elongation in wheat, decreased the number of fertile spike and the number of grains per spike dry weight (no seeds) by about 50%, while stress imposed at anthesis caused a decreased spike dry weight by 58 to 94% (Robertson and Guinta, 1994).

Drought stress can accelerate the flowering and ripening of wheat. Stress at flowering and grain filling shortens filling duration (Jajarmi, 2009).

Molybdenum is an essential trace element for plants. The amount of molybdenum in the Earth's crust and the soil is very little. The amount of molybdenum in the planet Earth is estimated on average 2.3 mg/kg of which approximately 10% is plant usable. Molybdenum adsorption depends on soil pH; it is low in the pH 7 or higher, though it increases as pH decreases. The plants which respond to molybdenum fertilizer include legumes, cabbage family, pasture wheat and several types of herbs. Higher amount of molybdenum in the soil does not influence plant growth, while large concentrations of molybdenum in forage influences copper metabolism in animals. Mo shortage in plants and molybdenum-induced copper deficiency in animals have been reported in certain geographical areas of the world. Molybdenum is adsorbed as molybdate for plants. Little is known about molybdenum transport in plants. Its transfer is likely to happen in wood vena as molybdate or

complex with amino acids and sugars. On the other hand, the mobility of molybdenum in plants is relatively good; this is why molybdenum focuses in phloem vena and parenchymal cells. Research suggests a positive effect of molybdenum on increasing the efficiency of plant. Abdol El-Samad *et al* (2005) reported that wheat yield increased by the foliar molybdenum. Studying the effect of molybdenum application on activity of two enzymes, reductase and nitrogenase, in wheat under drought stress, they reported that the total weight of dry matter and nitrogen yield decreased significantly by drought stress. This reduction was due to the stress decreasing reductase and nitrogenase activity. Mo application significantly increased crop growth rate, total nitrogen yield and protein, potassium and magnesium content under stress. These authors stated that the positive significant effect of molybdenum on the activity of nitrate reductase and nitrogenase increased nitrogen metabolism and general growth of the plant. However, level of this positive effect was more for nitrate reductase than for nitrogenase.

Reduction of plant photosynthesis under drought stress has been reported in many studies; this reduction is due to stomatal and non-stomatal factors affecting photosynthesis (Kocheva and Georgiev, 2003). Closure of stomata and reduction of stomatal conductance decreases CO₂ entering the leaf and thus decreases CO₂ fixation (Molnar *et al.*, 2002). Non-stomatal factors such as the reduction in electron transfer reduced activity of the Calvin cycle enzymes including rubisco and decreased ATP production from non-stomatal factors that have been cited for the decline in photosynthesis (Parry *et al.*, 2002). Besides reducing the amount of photosynthesis, drought stress reduces the leaf surface (Paulsen, 2003). Drought stress reduces current photosynthesis in grain filling stage; due to greater demand for assimilates for grain filling, remobilization of assimilates from shoot increases for grain filling (Blum, 1998). The Mediterranean climate, particularly hot and dry climates such as Iran, the occurrence of drought stress during pollination and grain filling decreases grain yield. The purpose of this study is to evaluate the effect of molybdenum application on barley yield under drought stress.

MATERIALS AND METHODS

The present study was conducted in agricultural years 2012-2013 at the Agricultural Research Station of Islamic Azad University of Shiraz, eastern 52°48' longitude and northern 29°52' latitude, altitude 1595 meters above sea level. The project was iterated three times as split plot based on randomized complete blocks. The examined factors included the three levels of irrigation regimes: normal irrigation, mild stress (no irrigation at the beginning of grain filling) and high stress (no irrigation at early flowering); and three levels of foliar molybdenum: foliar with pure water, foliar with 0.10% (10 per thousand) and 0.20% (20 per thousand) ammonium molybdate. Irrigation regimens were performed on main plots and foliar molybdenum was applied in marginal plots.

The test was conducted in a 60×40m land including 36 6×2.4m plots. The distance was 0.6m between marginal plots and 3m between main plots. Distance of 3 meters was considered between iterations. Ridge or row spacing was 60cm and plant density was determined as 450 plants per square meter. Land preparation operations were conducted in fall 2011. Before the test, the soil was sampled from 0-30cm in depth and samples were taken to the laboratory for chemical and physical properties of soil (Table 1). The average temperature and rainfall in this season of the year, according to the annual report of the weather station, is given in Table 2.

The studied site was fallow in the past year. Land preparation operation involving ploughing, one disc and two perpendicular levellers was performed at late October. Phosphorus fertilizer (80kg per ha) from triple super phosphate, potassium fertilizer (50kg per ha) from potassium sulphate and Nitrogen (20kg per ha) from Urea were consumed. Total phosphorus and potash fertilizers and one-third of nitrogen were used at planting and the remaining nitrogen fertilizer was used while the rapid growth of wheat at the beginning of stalk (mid-March) and early heading (March 30) was used as exuberance. Planting operations were performed on November 4 and 5. Number of seeds needed for planting was 450 seeds per square meter.

Foliar micronutrient molybdenum was applied twice in the beginning of stem elongation (appearance of the first node in the lowest part of

the stem coinciding with the second half of March) and early heading (Exit of the awn tip of the flag leaf sheath). Foliar (hexammonium molybdate) was applied at the desired concentrations using a 12-lit motorized backpack sprayer. To determine grain yield, half a meter high and low plots was taken as marginal effects from the two central rows of the each plot; the remaining area (6m) were harvested to determine grain yield and biological performance. Yield components (grain weight, number of spikes per unit area, number of grains per panicle, plant height, panicle weight, harvest index and days to maturity) were measured at the end of growing season. Protein content was measured using Informatic 8600 (NIR). To do this, 20g whole-wheat flour prepared by hammer mill apparatus was placed in a special chamber. The amount of flour was enough to completely cover the screen sensitive to infrared light. By turning the machine on, the percentage of protein was studied and the results were presented. Protein yield was calculated by multiplying the percentage of protein in the grain yield.

To determine relative water content (RWC) of leaf, 3 top leaves of each plant were cut off in the middle of the day; of them, 3 same-size circle discs were removed and were immediately weighed by an accurate scale (-thousandth of g) (weight wet); then samples were soak in distilled water for full turgidity. All this time, the dishes were sealed at constant temperature. Then, the samples were dried and then weighted (turgidity weight). Samples were dried in aluminium pans for 8 hours in a 104°C dryer to obtain their dry weight. Relative water content was measured by the following formula.

TW: turgidity weight; DW: dry weight; FW: fresh weight; $RWC = (FW - DW / TW - DW) \times 100$

Data analysis was performed by SAS software and comparison of traits was performed by Duncan's multiple range test.

RESULTS AND DISCUSSION

Yield components were affected by irrigation stress; however, the effect of irrigation treatments on plant height, peduncle length and leaf relative water content was not significant. This difference was significant for biological function (5%) and for other traits (1%). In addition, the effect

of foliar molybdenum was significant for all traits except for biological function. The effect was significant for protein function (5%) and for other traits (1%), as shown in Table 3.

The number of fertile tillers

The number of fertile tillers decreased by drought stress. Stress at the beginning of flowering and early grain filling decreased the average number of fertile tillers by 17% and 8%, respectively, compared to normal irrigation. There was a significant difference between severe irrigation and mild stress (Table 3). Although tillering is considered a desirable trait, stress in early flowering makes most tillers never produce panicles and reach the yield. Therefore, their production is not only profitable for the plant, but it may be harmful due to the consumption of photosynthetic matter (Rebetzke *et al.*, 2002). Foliar molybdenum in the early stages of stem elongation and early grain filling led to increased production and greater number of fertile tillers. The number of fertile tillers increased from 381.2 in foliar pure water treatment to 398.7 and 409.4 for 1% and 2% foliar molybdenum, respectively (Table 4). However, there was no significant difference between the two treatments. Studies suggested that micronutrient deficiency decreased antioxidant enzyme activity and thus increased the susceptibility of plants to environmental stresses (Cakmak, 2000). Mo effectiveness in improving the damage caused by stress for no irrigation treatment was more tangible at flowering stage at a concentration of 0.5%.

The number of grains per panicle

Number of grains per panicle decreased at different levels of stress (by 10% under mild and 28% under severe stress, compared to normal irrigation) (Table 4). The number of grains per panicle reduced by 20% under severe stress compared to mild stress. Sharp drop in the average number of seeds under no irrigation conditions at the beginning of flowering is due to the effect of dehydration on seed inoculation process, the result

of which was not compensatory in the next steps. However, stopping irrigation at the beginning of grain filling did not affect the number of seeds; the significant decrease of this trait in the considered treatment was mainly due to grain falling caused by rapid drying of panicle. Reducing the number of formed seeds is caused by infertility of the first and second florets within each spikelet; water stress decreases the number of grains formed in the upper and lower regions (Duggan, 2000). Both concentrations of foliar molybdenum significantly increased the number of grains per panicle; however, there was no significant difference between the two concentrations. Auxiliary role of molybdenum in improving and increasing the number of grains per panicle under severe stress was more evident; slope of increase, especially for the first concentration of foliar molybdenum was considerable. Increase in the number of grains due to application of 1% molybdenum was 16% in the normal irrigation treatment and 19% in the severe stress treatment (Table 4). This could reflect the compensatory effect of low concentrations of molybdenum in order to avoid a severe drop in yield under stress conditions.

Grain weight

Grain weight loss was observed in both mild and severe stress; however, severity of reduction was completely different for both stress levels. Mean grain weight decreased by 24% and 11% under mild and severe stress, respectively, compared with normal irrigation treatment. The reduction in grain weight was 16% under mild stress compared to severe stress (Table 4). Sarbarzeh *et al* (2008) reported a significant reduction in grain weight as a result of drought stress at flowering (Sarbarzeh *et al*, 2008). Several reports show an increase in grain weight by irrigation, especially in the grain-filling period by increasing the transfer of assimilates to grain (Shafazadeh *et al.*, 2004; Trethowan & Reynolds, 2007). Effect of foliar molybdenum was significant

Table 1. Characteristics of the tested soil (0-30cm in depth)

Test	ECD s/m	PH	OC %	Lime %	Total N %	P ppm	K ppm	Clay %	Silt %	Sand %	Tex	Saturated humidity %SP
Depth 0-30	5.71	7.7	0.77	10.3	0.015	5.4	240	26.4	30	43.6	Sandy loam	30.24

Table 2. precipitation and average temperature in Karaj area (October 23- June 21)

May 22- June 21	April 21- May 21	March 21- April 20	February 20- March 20	January 21- February 19	December 22- January 20	November 22- December 21	October 23- November 21	Whether parameters
24.9	18.6	17.7	6.4	2.4	1.9	9.8	12.9	Average temp.
1.6	34.9	79.1	67.7	28.9	41.5	4.1	31.5	Precipitation

Table 3. ANOVA analysis of fertile tillers per square meter, number of grains per panicle, grain weight, grain yield, biological function, leaf relative water content, protein content and protein function

Sources of change	Degrees of freedom	M.S mean-square							
		Number of fertile tillers	number of grains per panicle	grain weight	grain yield	biological function	leaf relative water content	protein content	protein function
Iteration	3	0.91	0.725	0.775	456790	2641284.8	17.898	0.053	15440.3
Irrigation	2	29827.3**	393.37**	287.44**	9119188**	2641284*	8.231 ns	14.59**	12790**
A error	6	20.12	7.714	7.555	411917.3	2290830	25.87	0.10	6568.4
Foliar	2	1610.10*	72.481*	13.76*	598891.4*	227400 ns	50.731*	3.04**	29209**
Mutual effect	4	87.2**	9.537*	6.39	198124.4ns	282136 ns	1.67 ns	0.55*	967.5 ns
B error	18	11.62	1.852	1.240	1179652	1307733.9	1.324	0.11	1247.6
C.V (%)		11.89	15.3	13.9	16.9	19.6	18.6	21.2	19.7

ns: no significant difference; * and **: significant difference in 5% and 1%, respectively

Table 4. Comparison of the average number of fertile tillers, number of grains per panicle, seed weight, grain yield, biological function, leaf relative water content, protein content and protein function

Treatment	Mean MS							
	Number of fertile tillers	number of grains per panicle	grain weight	grain yield	biological function	leaf relative water content	protein content	protein function
Irrigation								
Normal irrigation	417.2 a	35.2 a	38.4 a	4453 a	12390 a	58c	8.6 c	697.3 a
Mild stress	385 b	31.7 b	34.4 b	3956 b	11900 a	65 b	10.3 b	624.4 b
Severe stress	349.4 c	25.5 c	29.2 c	3280 c	9990 b	69.72 a	11.92 a	614.3 b
Foliar								
Pure water	381.2 b	28 b	33.3 b	3750 b	11910 a	61.89 b	9.37 b	617.5 b
1%	398.7 a	33.3 a	34.2 ab	3989 a	11780 a	67.06 a	11.40 a	701.8 a
2%	409.4 a	34.4 a	35.8 a	4065 a	11370 a	69 a	11.54 a	695.8 a

In each column means with the same letters are not significant

on grain weight (5%). Application of 2% foliar molybdenum significantly increased grain weight; however, there was no significant difference between concentrations 1% and 5%. In addition, there was no difference between the concentration 1% and pure water.

Grain yield

Through no irrigation treatment, Grain yield decreased by 27% and 12% at the beginning of the flowering and at the beginning of the grain filling compared to normal irrigation (Table 4). There was a significant difference between mild stress and severe stress treatments. Although both treatments represent no irrigation during reproductive stage of the plant, the severity of damage to wheat yield was quite different and those yield components which are influenced by stress under one of the two conditions were different. A major factor in reducing grain yield under stress during the reproductive period is the reduced duration of grain filling (Mogensen *et al.*, 1992; Kobota, *et al.*, 1999). Determination of the main critical factor in reduction of grain yield under drought stress depends on the stage and the time when stress occurs as well as stress intensity. In the recent study, reduced grain yield was associated with shortened duration of grain filling, grain weight loss and reduced number of grains per main panicle. There was an increase in grain yield (6% and 7%, respectively) under 1% and 2% foliar molybdenum compared with control treatment. There were no significant differences between those two molybdenum concentrations (Table 4). The absorption and conversion of nitrate into organic nitrogen require several enzymes. Nitrate is converted to ammonium by nitrate reductase and nitrite reductase. Then, ammonium enters into amino acids, glutamine and other amino acids by glutamine synthetase. Nitrate nitrogen is absorbed by nitrate reductase (which can be related to photosynthesis or respiration); then it is converted to nitrite. At the following steps, nitrite is converted to ammonia in the cytoplasm or chloroplast and then it is combined by carbon skeleton (alpha-Ketoglutaric acid), to make the first amino acid, glutamic acid. Any deficiency in the activity of enzymes or factors contributing to this process causes nitrate accumulation and conversion will not happen; factors such as inactivity of nitrate reductase enzyme, dryness, coldness, toxins,

excessive use of nitrogen fertilizers, molybdenum and manganese deficiency can cause nitrate accumulation (Randall, 1969). By interfering with the activity of these enzymes, Molybdenum accelerates the process and reduces the damage to growth and ultimately performance.

Biological function

Biological function was significantly under influence on irrigation treatments (Table 3); biological function decreased by 20% under severe stress treatment compared to the control treatment (Table 4). There was no significant difference in biological function under mild and severe stresses. Through severe stress, the plant first sacrificed a number of its potential tillers for adaptation to stress conditions; secondly, the plant lost photosynthesis and growth opportunities by shortening reproductive period to escape from stress. Finally, this reduced the reproductive period of the plant along with less tillers and less grain per panicles. The effect of the concentration 1% was not significant on this trait. Foliar molybdenum increased the biological function; however, this increase was not significant for any of the foliar concentrations. Foliar molybdenum increased the general growth of shrubs and accelerated the onset of reproductive phase which was associated with the effect of stress on shortened reproductive period. These two simultaneous factors led molybdenum to prevent only the severe drop of grain yield and biologic function under severe stress treatments (Ghafarian *et al.*, 2012).

Leaf relative water content

RWC is the ratio of water content under stress to the water content of the completely swollen plants. Effect of irrigation regimes was significant on leaf relative water content; leaf relative water content increased by 17% and 12% under no irrigation treatment at early flowering and early grain filling compared with normal irrigation (Table 4). The difference was significant between mild and severe stresses. Studying wheat plant, Ahmadi and Ceiocemardeh (2004) reported that varieties under drought stress showed 20-30% higher relative water content. RWC of wheat leaf increased as concentrations of molybdenum increased; this measure increased from 61% in the control treatments, to 67% and 69% for 1% and 2% foliar concentrations, respectively. However, there was no significant difference between 1% and 2%

concentrations. RWC is a good indicator of plant water in selection for drought resistance. Molybdenum plays a positive role in increasing nitrogenase and nitrate reductase activity as well as potassium ion concentration to enhance plant tolerance to stress and improve RWC.

At the beginning of stem elongation and flowering by accelerating stem growth and leaf production and helping better availability of essential elements such as potassium, phosphorus and nitrogen, foliar Mo appears to be capable of increasing the plant ability to uptake and retain more water. The role of molybdenum in phosphorus and potassium availability to plants, especially during periods of rapid growth of leaves, increased the ability of plant to withstand the stress conditions (Ghafarian *et al.*, 2012).

Protein content increased as stress increased. Protein content increased by 38% and 15% under no-irrigation treatment at the beginning of flowering and grain filling, respectively, compared with normal irrigation (Table 4). In normal irrigation, seed protein content was at its lowest. Increase in protein content due to drought was predictable. Reduction of wheat grain weight under stress, caused by a reduction in starch reserves during grain filling (due to a significant reduction in starch synthesis enzymes) increased the protein content of the grain size. It should be noted that the increase in protein concentration due to environmental stresses does not necessarily mean higher quality of wheat; although the composition and protein concentration influences quality of wheat, protein concentration has a larger effect on flour quality compared to its composition (Souza *et al.*, 1994). Thus, environmental stresses such as drought and salinity increasing protein concentration reduce wheat quality due to changes in the ratio of accumulated amino acids. Protein content was also influenced by foliar molybdenum. Application of 1% and 2% molybdenum increased protein content of wheat grain by 22% and 23% in comparison with the case in which molybdenum was not applied. There was no significant difference between 0.5 and 1% foliar concentrations (Table 4).

Molybdenum is one of many compounds of molibdoflavoproteins such as nitrate reductase which participates in nitrogen metabolism.

Molybdenum causes rapid increase in nitrate reductase activity of molybdenum-deficient plants. Activity of this enzyme provides nitrogen required for the plant and increases seed protein (Ozturk and Aydin, 2004).

Protein function

Protein function was affected by irrigation regimes (Table 3); the highest protein function was related to normal irrigation (747.3 kg per hectare). Protein function decreased under Different levels of stress. Grain protein function decreased by 11% and 13% under mild and severe stress treatments, respectively, compared with normal irrigation. Despite the increased protein content under the drought, the reduction in the final function of grain protein under stress conditions was a significant drop in grain yield under stress treatments (Table 4). Application of foliar Mo significantly influenced grain protein function. As Table 4 shows, 1% and 2% foliar concentrations increased protein function by 14% and 12% compared to normal irrigation in which Mo was not used. Mo increased grain yield and protein content whereby protein function; however, drought stress treatments decreased grain yield while increasing protein content; these two overlapped their negative effects. Therefore, the highest protein function was obtained from normal irrigation treatments with 1% and 2% foliar, followed by 2% normal irrigation (Ghafarian *et al.*, 2012).

CONCLUSION

This experiment showed that Mo plays a considerable role in increasing production and development of stems and leaves through foliar at the beginning of stem elongation and its helpful effect on increasing leaf surface and relative growth rate and thus increasing barley yield. Based on the obtained results, the treatment could significantly improve drought tolerance in barley. In case of some traits, there was no significant difference between low and high molybdenum concentrations; this suggests molybdenum as an effective element at low concentrations. In conclusion, the results of this study indicate the beneficial role of molybdenum in improving barley grain quality and alleviating the damage caused by the drought stress.

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