UC Davis
The Proceedings of the International Plant Nutrition Colloquium XVI

Title
Effects of molybdenum on Endogenous Hormone Contents in winter wheat under low temperature stress

Permalink
https://escholarship.org/uc/item/2x73g2t5

Authors
Xuecheng, Sun
Hu, Chengxiao
Tan, Qilin
et al.

Publication Date
2009-05-04

Peer reviewed

eScholarship.org Powered by the California Digital Library
University of California

1 INTRODUCTION

Molybdenum (Mo) is an essential element for higher plants. In China, more than 446 million ha arable land was Mo-deficient, which is becoming a limiting factor for wheat production in many provinces of China (Hu et al. 2002). Wang et al. (1989) found that Mo-deficiency was a major factor causing leaf-yellowing and tiller death on wheat in winter, and low temperature stress accelerated the development of Mo deficiency symptoms. Further studies showed that Mo application enhanced cold-resistance of winter wheat (Li et al. 2001; Vankova-Radeva et al. 1997). Several papers tried to analyze the physiological basis of cold resistance enhanced by Mo application from the changes of lipid composition (Yaneva et al. 1995), antioxidative enzymes (Sun et al. 2006b), nitrogen-containing compound (Hu et al. 2002) and photosynthetic characteristics (Sun et al. 2006a) in wheat. Phytohormones, especially ABA, are well known to be involved in regulating plant responses to cold stress. Mo has a close relationship with phytohormones balance. AO, one of Mo-containing enzymes, has been considered to catalyze the final step in the biosynthesis of ABA and IAA (Kaiser et al. 2005). However, little is known about the relationship of Mo and endogenous hormone under low temperature stress in plant. In order to improve our understanding of the mechanisms of cold resistance arising from Mo application in winter wheat, effects of Mo on endogenous hormone in winter wheat under low temperature stress are investigated in this paper.

2 MATERIALS AND METHODS

Wheat growth and sample collection

Two cultivars of winter wheat (Triticum aestivum L.), a Mo efficient cultivar 97003 (abbreviated as 97003) and a Mo inefficient cultivar 97014 (abbreviated as 97014), which differ in molybdenum uptake and distribution (Yu et al. 2002), were grown for 40 days in a modified Hoagland solution in a controlled-climate chamber at 15/12 °C (day/night) with a 14 h photoperiod at a light intensity of 400μmol·m^{-2}·s^{-1} and 70% air relative humidity. The nutrient solutions consisted 4mmol/L $Ca(NO_3)_2·4H_2O$, 6mmol/L KNO_3, 1mmol/L $NH_4H_2PO_4$, 2mmol/L $MgSO_4·7H_2O$, 100μmol/L EDTA-Fe, 46μmol/L H_3BO_3, 9μmol/L $MnCl_2·4H_2O$, 4μmol/L $ZnSO_4·7H_2O$, 0.5μmol/L $CuSO_4·5H_2O$. 0 and 1μmol/L $Na_2MoO_4·2H_2O$ was respectively added to the nutrient solutions as -Mo and +Mo treatments. After 40 days of germination, the temperature in controlled-climate chamber was adjusted to 5/2°C (day/night) for cold treatment. The first fully expanded leaves from –Mo and +Mo treatments were collected on 0, 2, 4 and 6 d of low temperature stress, frozen in liquid nitrogen and then stored at -80℃ for future analysis. Four biological replicates were prepared for each treatment.

Tissue extraction and AO Activities Analysis

AO activities were assayed according to Sagi *et al.* (1999). AO activity was expressed as $nmol^{-1}$ DCIP mg^{-1} protein min^{-1}. Soluble proteins in the assays were measured (Bradford 1976) using crystalline BSA as a reference.

Endogenous hormone contents determination

The methods for extraction and purification of the four hormones and

analysis of ABA, IAA, GA_3 and ZT were all carried out according to Wang et al (2008).

Analysis of Mo in Plant

Mo contents in plant was determined by using polarographic catalytic wave analysis with JP-2 oscilloscope polarograph in 0.25 mol·L^{-1} sulphuric acid, 0.1 mol·L^{-1} benzohydroxyacetic acid and saturated sodium hypochlorous acid solution (Wan et al. 1988).

Statistic Analysis

The data are presented as the averages of four replicates. Results were analyzed by GLM with Duncan multiple comparison using SAS v6.12 (SAS Institute, Cary, NC).

3 RESULTS

3.1 Mo concentrations in the leaves of winter wheat under low temperature stress

Table 1 Mo contents in leaves of -Mo and +Mo winter wheat under low temperature stress (μg·g-1)

Cultivars	Treatment	Days for low temperature stress(d)			
		0	2	4	6
97003	-Mo	0.048B	0.040B	0.047B	0.042B
	+Mo	2.242A	2.149A	2.261A	2.252A
97014	-Mo	0.034C	0.037B	0.039C	0.038C
	+Mo	2.217A	2.250A	2.165A	2.229A

Different letters in a column indicate significant differences at $P<0.01$ as determined by ANOVA followed by Duncan's test.

Molybdenum concentrations in Mo-fertilized plants leaves were all significantly higher than those in Mo-deficient plants after 0, 3, 6, 48 hrs of low temperature stress for both cultivars (Table 1).

3.2 Effects of Mo on AO activities in the leaves of winter wheat under low temperature stress

Application of Mo resulted in the increase of aldehyde oxidase (AO) activity in the leaves of both cultivars 97003 and 97014 during low temperature stress (Fig.1). With the prolongation of low temperature stress, the AO activities in leaves have a slight increase first, and then decrease slightly.

3.3 Effects of Mo on Endogenous Hormone Contents in leaves of winter wheat under low temperature stress

Comparing to the control, Mo application induced an obvious increase of the ABA and IAA contents in the leaves of both cultivar 97003 and 97014 (Fig. 2A and Fig. 2B) after 0, 2, 4, and 6 d of low temperature stress. No significant difference in GA_3 contents was detected between –Mo and +Mo treatments at 0, 2, 4 day of low temperature stress and Mo application significantly decreased gibberellin (GA_3) contents at 6 day of low temperature stress(Fig.2 C). Similarly, No significant difference in zeatin (ZT) contents was observed between –Mo and +Mo treatments at 0 and 2 day of low temperature stress and Mo application significantly increased zeatin (ZT) contents at 4 and 6 day of low temperature stress(Fig.2 D). With the

prolongation of low temperature stress, the ABA, IAA, and ZT contents in leaves have a slight increase first, and then decrease dramatically, the GA_3 contents leaves increased under continuous chilling stress.

3.4 Effects of molybdenum on the ABA/GA3 in leaves of winter wheat under low temperature stress

Molybdenum application increased the ABA/GA3 in leaves of winter wheat for both cultivars whether before or after low temperature stress. The ABA/GA3 in leaves peaked at 2 days of low temperature stress, and then had a continuous decrease for both cultivars (Fig.3).

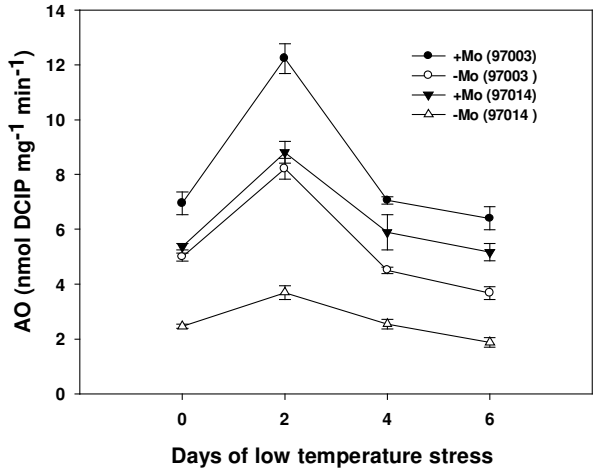

Fig.1 Effects of molybdenum on aldehyde oxidase (AO) activities in Mo-efficient winter wheat cultivar 97003 and Mo-inefficient winter wheat cultivar 97014 under low temperature stress.

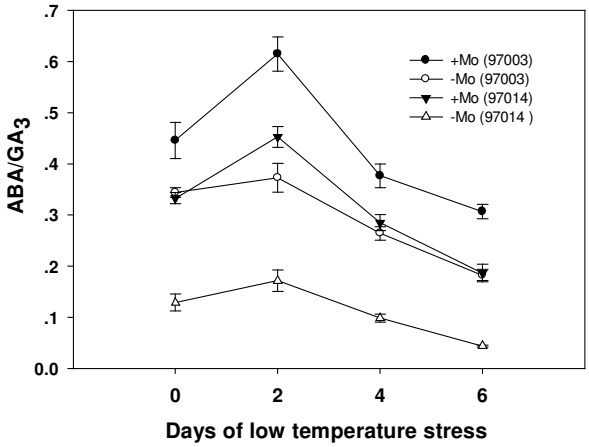

Fig. 3 Effects of molybdenum on ABA/GA_3 in Mo-efficient winter wheat cultivar 97003 and Mo-inefficient winter wheat cultivar 97014 under low temperature stress

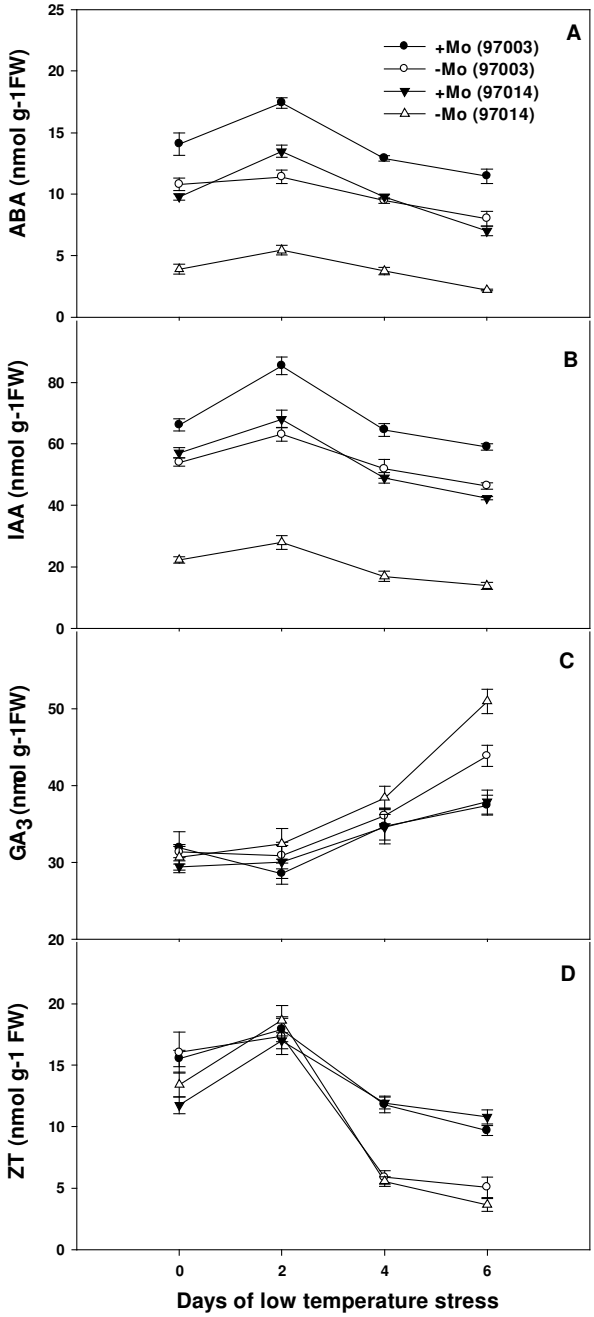

Fig .2 Effects of molybdenum on ABA (A), IAA (B) GA3 (C) and ZT (D) in Mo-efficient winter wheat cultivar 97003 and Mo-inefficient winter wheat cultivar 97014 under low temperature stress

4. DISCUSSION

4.1 Molybdenum affected the homeostasis of endogenous hormone via AO activities under low temperature stress

Molybdenum is involved in many physiological and biochemical processes since it is the prosthetic component of molybdoenzymes such as nitrate reductase (NR), aldehyde oxidase (AO), xanthine dehydrogenase (XDH), and sulfite oxidase (SO). AO plays an important role in the biosynthesis of phytohormones. AO catalyzes the last step of abscisic acid (ABA) and indole-3-acetic acid (IAA) synthesis has been verified in many plants such as Arabidopsis thaliana (Seo, 2000), maize (Katalin Barabas, 2000), tomato (Min, 2000) and pea (Zdunek-Zastocka, 2008). In our experiments, Mo application induced a significant increase in AO activities, ABA and IAA contents at 0, 2, 4, 6 day of low temperature stress. The results also showed that the changing tendency of AO activities, ABA and IAA contents was similar: they all have a slight increase first, and then decrease dramatically with the prolongation of low temperature stress in both cultivars. These results above verified that Mo regulated the ABA and IAA biosynthesis via AO in winter wheat. However, it is quite different in response of GA_3 and ZT to Mo. No significant difference in GA_3 and ZT contents was detected between -Mo and +Mo treatments in the earlier stage of low temperature stress, and Mo application decreased GA_3 contents, and increased ZT contents significantly until the later stage (4 d or 6 d) of low temperature stress. So we can infer that the response of ABA and IAA to Mo deficiency was prior to that of GA_3 and ZT. Interaction between plant hormones may influence the hormonal balances. The dramatic decrease in ABA and IAA levels in Mo deficient winter wheat may affect the homeostasis of endogenous hormones, so we observed that the ABA/GA_3 in –Mo treatment decreased dramatically with continuous exposure to low temperature.

4.2 Molybdenum regulated cold resistance via the changes of endogenous hormone in winter wheat

Phytohormones play critical roles in regulating plant responses to cold stress. Low ABA level results in a wilty appearance on plants through excessive transpiration and loss of stomatal control, altered seed dormancy, and impaired defenses responses to environmental stress such as low temperature stress (Kaiser et al. 2005). Many reports showed that ABA might activate basic leucine zipper (bZIP) transcription factors, and then regulate ABA-dependent cold-responsive (COR) gene in Arabidopsis and wheat (Kobayashi et al. 2008; Xiong et al. 2002).In this experiment, we found that ABA levels in +Mo treatment were higher than those in –Mo treatment for both cultivars. Further study indicated Mo regulated COR genes (*Wrab15, Wrab17, Wrab18,* and *Wrab19*) expression in winter wheat from the ABA-dependent signal pathway (unpublished data), which meant that Mo may regulated COR gene expression by ABA. IAA is also involved in regulating the cold resistance in plants(Rietveld et al. 2000). Zhao et al. (2000) found that the IAA content in the freezing tolerant cultivars was significant higher than that in the freezing sensitive cultivars during the overwintering period. In our experiment, the IAA contents in +Mo treatments were higher than those in –Mo treatments, which

was consistent with higher cold resistance in –Mo winter wheat. A significant decrease in the GA_3 content was also observed in +Mo winter wheat at 6 day of low temperature stress. In winter wheat, a large decrease in GA_3 content was associated with increased cold-hardiness (Waldman et al. 1975). Zeatin (a cytokinin) levels have been shown to decrease significantly in plants under low temperature stress. Our results showed that long period (4 d and 6 d) of exposure to low temperature stress resulted in a sharp decrease in zeatin contents both in –Mo and +Mo winter wheat and zeatin contents in +Mo treatment were significantly higher than those in -Mo treatments. Hormonal balances, especially the ABA/GA balance, have been considered to be closely related with cold resistance. In this study, the increase of ABA/GA_3 in +Mo winter wheat for both cultivars may enhance the cold resistance under low temperature stress.

In general, Mo regulated the biosynthesis of ABA and IAA, and then affected the homeostasis of endogenous hormone of winter wheat. Change in levels and balance of hormone regulated the expression of hormone-responsive genes. However, hormone-responsive signaling transduction in plants is a complex regulatory network. Further research is needed to evaluate the role of Mo in the regulatory network.

Acknowledgments

Financial support by National Natural Science Foundation of China (NSFC, No. 30671232) is greatly acknowledged.

References

Bradford M M. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Analytical Biochemistry, 1976, 72: 248-254.

Hu C, Wang Y and Wei W. Effect of molybdenum applictions on concentrations of free amino acids in winter wheat at different growth stages. Journal of Plant Nutrition, 2002, 25: 1487-1499.

Kaiser B N, Gridley K L and Ngaire B. The role of molybdenum in agricultural plant production. Annals of Botany, 2005, 96: 745-754.

Katalin Barabas N, Omarov R T, Erdei L and Herman Lips S. Distribution of the Mo-enzymes aldehyde oxidase, xanthine dehydrogenase and nitrate reductase in maize (Zea mays L.) nodal roots as affected by nitrogen and salinity. Plant Sci, 2000, 155: 49-58.

Kobayashi F, Maeta E, Terashima A, Kawaura K, Ogihara Y and Takumi S. Development of abiotic stress tolerance via bZIP-type transcription factor LIP19 in common wheat. J Exp Bot, 2008, 59: 891-905.

Li W, Wang Z, Mi G, Han X and Zhang F. Molybdenum deficiency in winter wheat seedlings as enhanced by freezing temperature. Journal of Plant Nutrition, 2001, 24:1195-1203.

Min X, Okada K, Brockmann B, Koshiba T and Kamiya Y. Molecular cloning and expression patterns of three putative functional aldehyde oxidase genes and isolation of two aldehyde oxidase pseudogenes in tomato. Biochim Biophys Acta, 2000, 1493: 337-341.

Rietveld P, Wilkinson C, Franssen H, Balk P, van der Plas L, Weisbeek P and Douwe de Boer A. Low temperature sensing in tulip (Tulipa gesneriana L.) is mediated through an increased response to auxin. Journal of Experimental Botany, 2000, 51: 587-594.

Sagi M, Fluhr R and Lips S H. Aldehyde oxidase and xanthine dehydrogenase in a flacca tomato

mutant with deficient abscisic acid and wilty phenotype. Plant Physiology, 1999, 120: 571-578.

Seo M, Peeters A J, Koiwai H, Oritani T, Marion-Poll A, Zeevaart J A, Koornneef M, Kamiya Y and Koshiba T. The Arabidopsis aldehyde oxidase 3 (AAO3) gene product catalyzes the final step in abscisic acid biosynthesis in leaves. PNAS, 2000, 97: 12908-12913.

Sun X, Hu C, Tan Q and Gan Q. Effects of molybdenum on photosynthetic characteristics in winter wheat under low temperature stress(in Chinese). Acta Agronomica Sinica, 2006a, 32: 1418-1422.

Sun X C, Hu C X and Tan Q L. Effects of molybdenum on antioxidative defense system and membrane lipid peroxidation in winter wheat under low temperature stress. Journal of Plant Physiology and Molecular Biology, 2006b, 32: 175-182.

Vankova-Radeva R V, Yaneva I A, Baidanova V D, Lvov N P, Karasev G S and Trunova T I. Molybdenum-induced increase in frost tolerance of winter wheat grown on acidic soil. Russian Journal of Plant Physiology.1997, 44: 204-209.

Waldman M, Rikin A, Dovrat A and Richmond A E 1975 Hormonal regulation of morphogenesis and cold-resistance II. effect of cold-acclimation and of exogenous abscisic acid on gibberllic acid and abscisic acid activities in Alfalfa (Medicago sativa L.) seedlings. Journal of Experimental Botany 1997, 26: 853-859.

Wan Y X, Liu X D and Li Z Y. Determination of soil available molybdenum and plant molybdenum by polarographic catalytic wave analysis (in Chinese). Soil Sci Rep China, 1988, 1: 43-46.

Wang C, Yang A, Yin H and Zhang J. Influence of water stress on endogenous hormone contents and cell damage of maize seedlings. Journal of Integrative Plant Biology, 2008, 50: 427-434.

Xiong L, Schumaker K S and Zhu J K. Cell signaling during cold, drought, and salt stress. Plant Cell, 2002, 14: 165-183.

Yaneva I, Vunkova-Radeva R, Stefanov K, Tsenov A, Petrova T and Petkov G. Changes in lipid composition of winter wheat leaves under low temperature stress: effect of molybdenum supply. Biologia Plantarum, 1995, 37: 561-566.

Yu M, Hu C X and Wang Y H. Molybdenum efficiency in winter wheat cultivars as related to molybdenum uptake and distribution. Plant and Soil, 2002, 245: 287-293.

Zdunek-Zastocka E. Molecular cloning, characterization and expression analysis of three aldehyde oxidase genes from Pisum sativum L. Plant Physiol Biochem, 2008, 46: 19-28.

Zhao C J, Kang S J, Wang J H, Guo X W, Li H X. Study on relations between plant endogenous hormones and cold resistance in wheat. Acta Agronomica Boreal Sinica, 2000, 15:51-54.