BIOLOGIA PLANTARUM 49 (4): 613-616, 2005

BRIEF COMMUNICATION

Different responses of two contrasting wheat genotypes to abscisic acid application

X. ZHANG, T. WANG and C. LI[1]

Chengdu Institute of Biology, Chinese Academy of Sciences, P.O. Box 416, Chengdu 610041, P.R. China

Abstract

Purpose of this study was to investigate different responses of two wheat genotypes (*Triticum aestivum* L.) from the wet and dry climate regions to exogenous abscisic acid (ABA) application under well-watered and water-stressed conditions. Exogenous ABA was applied to the leaves by spraying and changes in dry matter accumulation and allocation, endogenous ABA content and carbon isotope ratio ($\delta^{13}C$) were monitored. The ABA application significantly decreased stem height, total biomass, total leaf area, total grain mass and leaf area/mass ratio, and significantly increased root/aboveground biomass ratio, endogenous ABA content and $\delta^{13}C$ under well-watered and water-stressed conditions. Compared with the wet climate genotype, the dry climate genotype was more responsive to exogenous ABA application, resulting in lower stem height, total biomass, total leaf area, total grain mass and leaf area/mass ratio, and higher root/aboveground biomass ratio, endogenous ABA content and $\delta^{13}C$ under all experimental treatments.

Additional key words: dry matter accumulation and allocation, stable carbon isotope ratio, *Triticum aestivum*, water use efficiency.

Genetic adaptations and environmentally determined acclimations of plants to varying evapotranspirational demand and soil water availability are key processes that allow plants to survive and compete successfully in different environments (Ludlow 1989). Plants growing in arid and semi-arid climates often face some degree of drought stress. As a consequence, plants have developed various mechanisms to enhance their drought tolerance including well-developed root systems, osmotic adjustment, and certain leaf morphologies (reviewed by Kozlowski and Pallardy 2002). Moreover, abscisic acid (ABA), a well-known stress-inducible plant hormone and growth inhibitor, has long been studied as a potential mediator for induction of drought tolerance in plants (Davies and Jones 1991, Davies 1995, Zeevaart 1999). ABA seems to play a predominant role in the conversion of environmental signals into changes in plant gene expression (Rock 2000, Zhu 2002). It has been shown to be involved in promoting drought tolerance, both from the use of applied exogenous ABA to intact plants (Nayyar and Kaushal 2002, Nayyar and Walia 2003, Wang *et al.* 2003, Li *et al.* 2004, Yin *et al.* 2004), and from measurement of the endogenous ABA contents (Li and Wang 2003, Yin *et al.* 2005a, Zhang *et al.* 2005). However, in crop plants effect of exogenous ABA application on physiological and morphological properties under water deficits is studied relatively little, in particular change in water use efficiency in response to exogenous ABA application is not clear.

The relation between stable carbon isotope ratio and water-use efficiency has led to wide-spread use of isotopic analysis in plant physiological ecology. The stable carbon isotope ratio, expressed as $\delta^{13}C$, reflects the balance between mesophyll demand for carbon dioxide against the diffusive supply through the stomata (reviewed by Farquhar and Sharkey 1982, Farquhar *et al.* 1989). The stable carbon isotope ratio of plant tissue provides an integrated measurement of influencing photosynthetic gas exchange over the time when the carbon is fixed (Nativ *et al.* 1999, Li 2000, Yin *et al.* 2005b).

We are investigating the physiological basis involved

Received 27 October 2004, *accepted* 6 June 2005.

Abbreviations: ABA - abscisic acid; $\delta^{13}C$ - stable carbon isotope ratio.

Acknowledgements: The research was supported by the Program of “100 Distinguished Young Scientists” and “Knowledge Innovation Engineering” of the Chinese Academy of Sciences (No. KSCX2-SW-115).

[1] Corresponding author; fax: (+86) 28 85222753, e-mail: licy@cib.ac.cn or chunyang.li@helsinki.fi

in drought tolerance together with the role of ABA in these adaptive responses. In our earlier experiments with the dry and wet climate genotypes of wheat, we showed large differences between the genotypes in drought-induced adaptive responses. The dry climate genotype was more responsive to water stress than the wet climate genotype, resulting in lower plant growth and production, and higher ABA accumulation and δ^{13}C (Li *et al.*, unpublished). In this study, we further study the different responses of the dry and wet climate genotypes of wheat to ABA application under both well-watered and water-stressed conditions with the aim to understand the role of ABA in drought tolerance in crop plants.

Two wheat genotypes from contrasting habitats (No. 01 from dry climate region Lasa, 30°10′ N, 91°30′ E, annual rainfall 320 mm and No. 02 from wet climate region Nanjing, 31°20′ N, 118°18′ E, annual rainfall 1106 mm) were selected for the study. Seeds were sown on wet tissue paper in Petri dishes. After seed germination, the seedlings were put into small plastic pots (180 cm^3) and grown for about one month. One hundred healthy seedlings of uniform height were chosen from each genotype and each seedling was transferred to a 2 dm^3 pot containing sand (grain size 0.1 - 0.6 mm). The seedlings were grown in a controlled environment for the rest of the experiment. A total of 5 g slow release fertilizer (7 % N, 5 % P and 26 % K) was added to each pot during the experiment.

Two experimental designs were completed: *1*) well-watered condition, 50 pots of each genotype were watered to 100 % of field capacity by supplying an amount of water equal to transpiration losses every other day; *2*) water-stressed condition, another 50 pots of each genotype were maintained at 50 % of field capacity by watering every other day. In each genotype and treatment, half of the individuals were given exogenous ABA [(±)-cis, trans-abscisic acid, *Sigma Chemical Company*, St Louis, MO, USA] by spraying it on the leaves [cm^3 of 50 μM ABA in 0.5 % (v/v) Tween 20 per day and individual]. Another half of the individuals were sprayed 5 cm^3 water as control. Evaporation from the soil surface was prevented by enclosing the pots in plastic bags that were tied to the stems of the plants. Transpiration water loss was measured gravimetrically by weighing all pots every other day.

Leaf samples from each genotype and treatment were used for carbon isotope analysis. The samples were oven-dried for 24 h at 80 ºC and homogenised by grinding in a ball mill. Stable carbon isotope abundance in combusted samples was measured as described by Li *et al.* (2000).

Three replicate samples of leaves from each genotype and treatment were measured for ABA contents as described by Li *et al.* (2005).

Plants were harvested after six months, and divided into leaves, stem, grain and roots. Leaf area for each individual was determined by *Portable Laser Area Meter* (*CI-203*, *CID Inc.*, Vancouver, DC, USA). Biomass samples were dried to constant and weighed. Root/aboveground biomass ratio (root mass divided by leaves, stem and grain mass) and leaf area/mass ratio were then calculated. Data were subjected to analyses of variance using the *SYSTAT* statistical software package.

Dry matter accumulation and substrate allocation under water stress exhibited the primary signals for drought adaptation. Exogenous ABA application significantly affected plant growth properties, *i.e.* stem height (SH), total mass (TM), total leaf area (LA), total grain mass (GM), root/shoot mass ratio (RS) and leaf area/mass ratio (LAM) under both well-watered and water-stressed conditions (Table 1). Significant differences between the genotypes were found in these properties under all treatments. The dry climate genotype showed lower SH, TM, LA, GM and LAM and higher RS than the wet climate genotype. The interaction effect between exogenous ABA application and genotype was also significant in these properties (Table 1).

Exogenous ABA application not only affected plant growth and structure, but also significantly increased endogenous ABA content and δ^{13}C under both well-watered and stress-watered conditions (Table 1). Significant differences between the genotypes were found in ABA and δ^{13}C under all treatments. Compared with the wet climate genotype, the dry climate genotype had higher ABA and δ^{13}C. The interaction effect between exogenous ABA application and genotype was also significant in ABA and δ^{13}C (Table 1).

Similarly, exogenous ABA application has been shown to enhance drought tolerance in other herbaceous plants (*e.g.* Socias *et al.* 1997, Heschel and Hausmann 2001, Xu *et al.* 2002, Pandey *et al.* 2003/4, Wang *et al.* 2003). Study with woody plants also confirmed the role of ABA in drought tolerance (Li and Wang 2003, Li *et al.* 2004, Yin *et al.* 2005a, Zhang *et al.* 2005). Accordingly, ABA may function as a trigger to initiate the process for drought adaptation.

Similarly, in our earlier experiments with the dry and wet climate genotypes of wheat, we also found that large differences between the genotypes in drought-induced morphological and physiological responses. Compared with the wet climate genotype, the dry climate genotype responded to drought with an higher endogenous ABA accumulation, resulting in lower dry matter accumulation and greater allocation to roots, and higher water use efficiency (Li *et al.* unpublished). Therefore, differences between the genotypes in these responses could be related with the local climatic condition of the genotypes, and reflect a particular adaptation. The dry climate genotype, which naturally occurs in regions with a low rainfall during growing season, could be assumed to possess a high drought adaptation that is achieved at the expense of the growth. In contrast, the wet climate genotype, which occurs in regions with a high rainfall during growing season, could be assumed to utilize abundant soil water supplies and thus grow rapidly.

Table 1. Stem height (SH), total biomass (TM), total leaf area (LA), total grain mass (GM), root/shoot mass ratio (RS), leaf area/mass ratio (LAM), abscisic acid content (ABA) and stable carbon isotope ratio ($\delta^{13}C$) in two contrasting wheat genotypes as affected by exogenous ABA application. Means ± SE, *n* = 5. No. 01. - the genotype from the dry climate region; No. 02 - the genotype from the wet climate region.

Moisture	Treat.	Genotype	SH [cm]	TM [g]	LA [dm^2]	GM [g]	RS	LAM [$dm^2\ g^{-1}$]	ABA [$\mu g\ g^{-1}$(FM)]	$\delta^{13}C$ [$^0/_{00}$]
100 %	ABA	No. 01	65.49±0.82	6.35±0.27	3.17±0.11	0.46±0.03	0.66±0.07	1.55±0.08	2.54±0.12	-26.88±0.34
		No. 02	70.58±0.96	7.52±0.31	4.34±0.19	0.55±0.04	0.52±0.04	1.86±0.11	2.05±0.10	-29.01±0.37
	control	No. 01	74.63±1.08	8.02±0.37	3.96±0.15	0.53±0.07	0.54±0.07	1.96±0.09	0.67±0.07	-30.22±0.40
		No. 02	82.70±1.35	9.69±0.54	5.25±0.22	0.72±0.07	0.46±0.02	2.24±0.15	0.62±0.05	-30.59±0.38
50 %	ABA	No. 01	51.78±0.47	4.80±0.26	1.62±0.09	0.33±0.02	0.87±0.13	1.28±0.06	3.06±0.29	-24.21±0.29
		No. 02	59.42±0.61	5.92±0.29	2.38±0.13	0.42±0.03	0.74±0.08	1.43±0.12	2.39±0.21	-28.20±0.31
	control	No. 01	63.60±0.77	6.68±0.34	2.96±0.17	0.44±0.03	0.72±0.07	1.69±0.08	2.27±0.20	-27.47±0.27
		No. 02	70.17±0.86	7.76±0.38	3.53±0.20	0.61±0.04	0.67±0.06	1.82±0.14	1.83±0.16	-29.68±0.33

Plant structure has also usually been related with their natural habitats, our results suggested that the genotype from low rainfall regions allocated greater dry matter to roots than the genotype from high rainfall regions when water was a limiting factor. Since survival strategies are adaptations to the environmental conditions in which the plants have evolved, the detected differences in physiological and morphological responses may explain specific adaptation mechanisms within the species. Consistent with this view, to test for genetic variation in sensitivity to applied ABA in jewelweed (*Impatiens capensis*), Heschel and Hausmann (2001) experimentally manipulated soil water availability and sprayed ABA on inbred lines derived from sites with different water availabilities, and found that plants from a relatively dry site in Rhode Island responded to drought treatment by significantly increasing survivorship in subsequent drought compared with plants from a wet site, and ABA application affected water use efficiency more in the ”dry” origin than in the ”wet” origin. Similar results also reported in woody plants (Li and Wang 2003, Li *et al*. 2004, Zhang *et al*. 2004, Yin *et al*. 2005a). Accordingly, these environment-specific responses to water stress provided strong evidence for adaptive differentiation between the origins in many plants.

$\delta^{13}C$ seems generally to have a high correlation with plant growth, but the relationship may be positive or negative. Increasing plant $\delta^{13}C$ has been shown to either increase or decrease biomass productivity (Cregg 1994, Leroux *et al*. 1996, Osorio *et al*. 1998, Li *et al*. 2000, Yin *et al*. 2004, Zhang *et al*. 2005). When water is limited, plants that use a finite water supply more efficiency would grow more rapidly, in this case, high $\delta^{13}C$ would positively affect plant productivity. Another way to increase $\delta^{13}C$ is to close stomata partially, thus restricting photosynthesis relative to plants whose stomata are fullly open, this strategy would result in a negative correlation between $\delta^{13}C$ and plant productivity. At the present study, wheat genotypes probably followed the latter strategy.

The dry climate genotype exhibited a distinctly higher ABA and $\delta^{13}C$ as affected by drought than the wet climate genotype. Differences between the genotypes in ABA and $\delta^{13}C$ were less in well-watered treatment without applied ABA than water stress and/or applied ABA treatment, suggesting that relationships between physiological responses and drought adaptations were complicated by internal drought tolerance mechanisms and external environmental factors such as water availability and exogenous ABA application, or their interaction.

References

Cregg, E.: Carbon allocation, gas exchange, and needle morphology of *Pinus ponderosa* genotypes known to differ in growth and survival under imposed drought. - Tree Physiol. **14**: 883-898, 1994.

Davies, P.J.: Plant Hormones. Physiology, Biochemistry and Molecular Biology. 2nd Ed. - Kluwer Academic Publishers, Dordrecht 1995.

Davies, W.J., Jones, H.G.: Abscisic Acid: Physiology and Biochemistry. - BIOS Scientific Publishers, Oxford 1991.

Farquhar, G.D., Ehleringer, J.R., Hubick, K.T.: Carbon isotope discrimination and photosynthesis. - Annu. Rev. Plant Physiol. Plant mol. Biol. **40**: 503-537, 1989.

Farquhar, G.D., Sharkey, T.A.: Stomatal conductance and photosynthesis. - Annu. Rev. Plant Physiol. **33**: 317-345, 1982.

Heschel, M.S., Hausmann, N.J.: Population differentiation for abscisic acid responsiveness in *Impatiens capensis* (Balsaminaceae). - Int. J. Plant Sci. **162**: 1253-1260, 2001.

Kozlowski, T.T., Pallardy, S.G.: Acclimation and adaptive responses of woody plants to environmental stresses. - Bot. Rev. **68**: 270-334, 2002.

Leroux, D., Stock, W.D., Bond, W.J., Maphanga, D.: Dry mass allocation, water use efficiency and $\delta^{13}C$ in clones of *Eucalyptus grandis*, *E. grandis* × *camaldulensis* and *E. grandis* × *nitens* grown under two irrigation regimes. - Tree Physiol. **16**: 497-502, 1996.

Li, C.: Population differences in water-use efficiency of *Eucalyptus microtheca* seedlings under different watering regimes. - Physiol. Plant. **108**: 134-139, 2000.

Li, C., Berninger, F., Koskela, J., Sonninen, E.: Drought responses of *Eucalyptus microtheca* provenances depend on seasonality of rainfall in their place of origin. - Aust. J. Plant Physiol. **27**: 231-238, 2000.

Li, C., Wang, K.: Differences in drought responses of three contrasting *Eucalyptus microtheca* F. Muell. populations. - Forest. Ecol. Manage. **179**: 377-385, 2003.

Li, C., Wu, N., Liu, S.: Development of freezing tolerance in different altitudinal ecotypes of *Salix paraplesia*. - Biol. Plant. **49**: 65-71, 2005.

Li, C., Yin, C., Liu, S.: Different responses of two contrasting *Populus davidiana* populations to exogenous abscisic acid application. - Environ. exp. Bot. **51**: 237-246, 2004.

Ludlow, M.M.: Strategies of responses to water stress. - In: Kreeb, K.H., Richter, H., Hinkley, T.M. (ed.): Structural and Functional Response to Environmental Stress. Pp. 269-281. Academic Publishing, The Hague 1989.

Nativ, R., Ephrath, J.E., Berliner, P.R., Saranga, Y.: Drought resistance and water use efficiency in *Acacia saligna*. - Aust. J. Bot. **47**: 577-586, 1999.

Nayyar, H., Kaushal, S.K.: Alleviation of negative effects of water stress in two contrasting wheat genotypes by calcium and abscisic acid. - Biol. Plant. **45**: 65-70, 2002.

Nayyar, H., Walia, D.P.: Water stress induced proline accumulation in contrasting wheat genotypes as affected by calcium and abscisic acid. - Biol. Plant. **46**: 275-279, 2003.

Osorio, J., Osorio, M.L., Chaves, M.M., Pereira, J.S.: Water deficits are more important in delaying growth than in changing patterns of carbon allocation in *Eucalyptus globules*. - Tree Physiol. **18**: 363-373, 1998.

Pandey, D.M., Goswami, C.L., Kumar, B.: Physiological effects of plant hormones in cotton under drought. - Biol. Plant. **47**: 535-540, 2003/4.

Rock, C.D.: Pathways to abscisic acid-regulated gene expression. - New Phytol. **148**: 357-396, 2000.

Socias, F.X., Correia, M.J., Chaves, M., Medrano, H.: The role of abscisic acid and water relation in drought responses of subterranean clover. - J. exp. Bot. **48**: 1281-1288, 1997.

Wang, Z.L., Huang, B.R., Xu, Q.Z.: Effects of abscisic acid on drought responses of Kentucky bluegrass. - J. amer. Soc. hort. Sci. **128**: 36-41, 2003.

Xu, X., Zheng, G.Q., Deng, X.P., Medrano, H.: Effects of exogenous abscisic acid and water stress on the growth response of subterranean clover of different genotypes. - Acta bot. sin. **44**: 1425-1431, 2002.

Yin, C., Duan, B., Wang, X., Li, C.: Morphological and physiological responses of two contrasting poplar species to drought stress and exogenous abscisic acid application. - Plant Sci. **167**: 1091-1097, 2004.

Yin, C., Peng, Y., Zang, R., Zhu, Y., Li, C.: Adaptive responses of *Populus kangdingensis* to drought stress. - Physiol. Plant. **123**: 445-451, 2005b.

Yin, C., Wang, X., Duan, B., Luo, J., Li, C.: Early growth, dry matter allocation and water use efficiency of two sympatric *Populus* species as affected by water stress. - Environ. exp. Bot. **53**: 315-322, 2005a.

Zeevaart, J.A.D.: Abscisic acid metabolism and its regulation. - In: Hooykaas, P.J.J., Hall, M.A., Libbenga, K.R. (ed.): Biochemistry and Molecular Biology of Plant Hormones. Pp. 189-207. Elsevier Science, Amsterdam 1999.

Zhang, X., Wu, N., Li, C.: Physiological and growth responses of *Populus davidiana* ecotypes to different soil water contents. - J. arid Environ. **60**: 567-579, 2005.

Zhang, X., Zang, R., Li, C.: Population differences in physiological and morphological adaptations of *Populus davidiana* seedlings in response to progressive drought stress. - Plant Sci. **166**: 791-797, 2004.

Zhu, J.K.: Salt and drought stress signal transduction in plants. - Annu. Rev. Plant Biol. **53**: 247-273, 2002.