Does root-sourced ABA play a role for regulation of stomata under drought in quinoa (Chenopodium quinoa Willd.)

Jacobsen, Sven-Erik; Liu, Fulai; Jensen, Christian Richardt

Published in:
Scientia Horticulturae

DOI:
10.1016/j.scienta.2009.05.019

Publication date:
2009

Document version
Early version, also known as pre-print

Sven-Erik Jacobsen, Fulai Liu, Christian Richardt Jensen:
Does root-sourced ABA play a role for regulation of stomata under drought in quinoa (*Chenopodium quinoa* Willd.)

The original paper can be found here:
Jacobsen, S.-E., F. Liu & C.R. Jensen. 2009. Does root-sourced ABA play a role for regulation of stomata under drought in quinoa (Chenopodium quinoa Willd.). *Scientia Horticulturae* 122, 281-287

http://dx.doi.org/10.1016/j.scienta.2009.05.019

Does root-sourced ABA play a role for regulation of stomata under drought in quinoa (*Chenopodium quinoa* Willd.)

Sven-Erik Jacobsen*, Fulai Liu, Christian Richardt Jensen

Copenhagen University, Faculty of Life Sciences, Department of Agricultural Sciences, Højbakkegaard Allé 13, DK-2630 Taastrup, Denmark.

*Corresponding author at: Copenhagen University, Faculty of Life Sciences, Department of Agricultural Sciences, Højbakkegaard Allé 13, DK-2630 Taastrup, Denmark.
Tel.: +45 35333388; fax: +45 35333478.
Email address: seja@life.ku.dk

Abbreviations: ABA, abscisic acid; FTSW, the fraction of transpirable soil water; LER, leaf expansion rate; PAR, photosynthetically active radiation, ψ_r, root water potential; ψ_l, leaf water potential. A_{max}, photosynthesis; g_s, stomatal conductance; $WUE_{Amax/gs,}$ photosynthetic water use efficiency.

Does root-sourced ABA play a role for regulation of stomata under drought in quinoa (*Chenopodium quinoa* Willd.)

Abstract

The Andean seed crop quinoa (*Chenopodium quinoa* Willd.) is traditionally grown under drought and other adverse conditions that constrain crop production in the Andes, and it is regarded as having considerable tolerance to soil drying. The objective of this research was to study how chemical and hydraulic signalling from the root system controlled gas exchange in a drying soil in quinoa. It was observed that during soil drying, relative g_s and photosynthesis A_{max} (drought stressed/fully watered plants) equalled 1, until the fraction of transpirable soil water (FTSW) decreased to 0.82 ± 0.152 and 0.33 ± 0.061, respectively, at bud formation, indicating that photosynthesis was maintained after stomata closure. The relationship between relative g_s and relative A_{max} at bud formation was represented by a logarithmic function (r^2 = 0.79), which resulted in a photosynthetic water use efficiency $WUE_{Amax/gs}$ of 1 when FTSW > 0.8, and increased by 50% with soil drying to FTSW 0.7–0.4. Mild soil drying increased slightly ABA in the xylem. It is concluded that during soil drying, quinoa plants have a sensitive stomatal closure, by which the plants are able to maintain leaf water potential (ψ_l) and A_{max}, resulting in an increase of WUE. Root originated ABA plays a role in stomata performance during soil drying. ABA regulation seems to be one of the mechanisms utilised by quinoa when facing drought inducing decrease of turgor of stomata guard cells.

Keywords:
Hydraulic signals
Chemical signals
Leaf growth
Stomatal conductance
Soil-water thresholds

1. Introduction

Agriculture in the Andean highlands is characterized by a high degree of risk due to drought, frost, wind, hail, and soil salinity. Water shortage arising from a combined effect of low rainfall, a relatively high evapotranspiration rate and poor soils with low water retaining capacity, is a major constraint to plant production (Jacobsen et al., 2003; Geerts et al., 2008).

There are two seasons, the rainy season for crop production from September to March, and the dry season, where also the risk of frost increases (Jacobsen et al., 2007). Drought occurs both as intermittent drought, which is highly unpredictable from year to year, and as terminal drought. Early drought after emergence may lead to a re-sowing and cause an increased risk for suffering from drought under seed filling, a delayed harvest and crop loss (Garcia et al., 2007).

The native seed crop quinoa (*Chenopodium quinoa* Willd.) which has been cultivated in the Andean region for several thousand years for the supply of highly nutritious food, tolerates several of the abiotic factors that constrain crop production in the Andes (Jacobsen and Mujica, 2001; Mujica et al., 2001; Bois et al., 2006; Jacobsen et al., 2006). However, research on the physiological mechanisms for resistance, and the response to actual stress levels conferred by the environment, has only recently been initiated. Initial results have demonstrated that quinoa tolerates drought through growth plasticity and tissue elasticity (Vacher, 1998), and inherent low osmotic potential (Jensen et al., 2000). Quinoa also avoids the negative effects of drought through its deep, dense root system, reduction of leaf area through leaf dropping, special vesicular glands, small and thick-walled cells adapted to large losses of water without loss of turgor, and stomatal closure (Jensen et al., 2000; Jacobsen et al., 2003). It is believed that quinoa yields can be stabilized with the help of deficit irrigation by applying only half of the irrigation water as required for full irrigation, replacing evapotranspired water (Geerts et al., 2008).

Increasing soil moisture deficit is normally accompanied by changes in root (ψ_r) and leaf water potential (ψ_l), xylem nitrate concentration, and xylem pH (Bahrun et al., 2002). Soil moisture represents the available resource of water, controlling plant growth and water use, including reduction of leaf area expansion and stomatal conductance during drought (Davies and Zhang, 1991). A study of the effect of progressive soil drying can be conducted by comparing plant responses as a function of

the fraction of transpirable soil water (FTSW). Earlier studies have shown a consistent relationship between plant physiological processes (e.g. leaf expansion, stomatal conductance, gas exchange) and FTSW under drought conditions, caused by a decrease in plant water status (Lecoeur and Sinclair, 1996; Soltani et al., 2000; Liu et al., 2007; Shahnazari et al., 2008).

Both chemical and hydraulic signals are operative and integrated in regulation of leaf growth and stomatal conductance when plants experience drought stress (Davies et al., 1994; Comstock, 2002). At mild soil water deficit chemical signals may be produced in roots and transported via the xylem to the shoot where they reduce leaf growth and stomatal conductance, resulting in a delay in plant water deficit (Dodd and Davies, 1996; Dodd et al., 2006; Bahrun et al., 2002). Changes in ABA and pH of the xylem have been considered to act as chemical signals during early stages of soil drying (Davies and Zhang, 1991; Bacon et al., 1998). When soil water deficit becomes more severe, hydraulic signals as a result of changes in hydrostatic pressure become significant, reducing stomatal conductance (Davies et al., 1994). The pattern of interaction and the time-course between the two signal types are still poorly understood (Comstock, 2002).

The objective of the present study was to investigate the physiological mechanisms, specifically the role of ABA, that may be involved in the control of stomatal aperture of quinoa during progressive soil drying, and to test the hypothesis that water use efficiency of quinoa was improved during mild soil water deficits.

2. Materials and methods

2.1 Plant material and growing conditions

A pot experiment was conducted at the experimental station of the Faculty of Life Sciences (LIFE), University of Copenhagen, Taastrup, Denmark in 2002. Quinoa (*Chenopodium quinoa* Willd.), cv. INIA-Illpa from Puno, Peru (3825 masl, 16°S, 70°W) was grown in pots (15-cm diameter by 50-cm tall). The pots contained 4 kg cultural substrate (GB-Pindstrup Substrates No.1, pH = 6.0) in a controlled environment greenhouse [day/night air temperature 20/14 ± 2°C; 60% relative humidity; 12 h photoperiod at 600 μmol m^{-2} s^{-1} PAR supplied by metal-halide lamps].

Four seeds per pot were sown on 28 June, 2002. When the first two leaves had emerged, thinning was carried out to one plant per pot. Pots were randomly arranged in the greenhouse.

2.2 Water treatments

Until start of the drought treatment the plants were irrigated daily with nutrient solution (Pioneer NPK Macro 14-3-23 + Mg combined with Pioneer Micro; pH = 5.5; EC = 1.3) to maintain full water holding capacity (WHC). Drought stress was imposed by withholding water and nutrients from pots at two growth stages. In the first experiment drought stress was imposed during the bud formation period (developmental stage 3-4; Jacobsen and Stølen, 1993), 33 days after sowing, and lasted for 16 days until all plant available water in the pots had been used. Start plant dryweight was in average 2.0 g. In the second study the drought stress treatment was imposed during late bud/flower initiation (developmental stage 7-8), 45 days after sowing, and lasted for 9 days. Start plant dryweight was in average 11.4 g. Plants that remained well watered at 100% WHC served as control plants. 100% WHC was defined as pot weight when drainage had stopped after saturation of the soil.

Water content in the pot was expressed as the fraction of transpirable soil water (FTSW). Total transpirable soil water (TTSW) was the difference between the pot weights at 100% WHC (pot weight about 6.6 kg) and when the transpiration rate of the stressed plants decreased to 10% of the control plants. The daily value of FTSW was estimated as the ratio between the amount of transpirable soil water still remaining in the pot and TTSW:

$$FTSW = (WT_n - WT_f)/TTSW \qquad (1)$$

where WT_n is the actual pot weight on a given date, and WT_f is the pot weight at the time when transpiration rate of stressed plants was 10% of the control plants (pot weight about 3.1 kg). The actual pot weight was obtained by weighing all pots daily during the drying cycle.

2.3 Measurement of biophysical parameters

After imposition of drought stress, g_s and A_{max} were measured on fully expanded upper canopy leaves (four leaves per plant, four plants per treatment) at midday with a LI-6200 portable photosynthesis system (LiCor Inc., Lincoln, NE, USA).

Four plants were harvested from each treatment, and plant leaf area was measured with a leaf area meter (model 3050A, LiCor Inc., Lincoln, NE, USA). Dry weight of plant parts was obtained after drying at 80 °C for 24 h. We calculated the photosynthetic water use efficiency ($WUE_{Amax/gs}$), defined as the ratio between the rate of photosynthesis (A_{max}) and stomatal conductance for water vapour (g_s). Leaf expansion rate (LER) was calculated as:

$$LER = (LA_2-LA_1)/(t_2-t_1) \quad (2)$$

where LA_1 and LA_2 are the leaf areas, and t_1 and t_2 are time (days) between two consecutive harvests. Relative LER (RLER) was calculated as:

$$RLER = (LER/LA_1)_{drought} / (LER/LA_1)_{control}$$

Leaf water potential ψ_l was measured at midday in a pressure chamber (Soil Moisture Equipment Corp., Santa Barbara, CA, USA, where one young, fully expanded leaf was placed with the leaf stalk protruding outside, and the leaf lamina inside the chamber. The leaf was immediately after measuring wrapped in aluminium foil and transferred into liquid nitrogen for storing at –80°C until required. Root water potential ψ_r was measured by pressurizing the potted plant in a Scholander pressure chamber. The entire pot was sealed into the chamber and the shoot was de-topped at 15-20 cm from the stem base. With the stem stump protruding outside the chamber, pressure was applied. The pressure was increased gradually until it equalled ψ_r of the plant.

2.4 Xylem sap collection and ABA determination

In the drying cycle three plants per treatments were harvested each day. At each harvest, xylem sap was collected by pressurizing the roots of the potted plant in a Scholander-type pressure chamber. The entire pot was sealed into the pressure chamber and the shoot was detopped at 15-20 cm from the stem base. With the stem stump protruding outside the chamber, a 0.3 MPa over pressure was applied. The cut surface was cleaned with pure water and dried with blotting paper. 0.5-1.0 ml of sap was collected using a pipette from the cutting surface into an Eppendorf-vial wrapped with aluminium. The sap was immediately stored at –80°C for chemical analysis. The xylem pH was determined after the sap was allowed to thaw for half an hour, using a pH meter (PHM95, pH meter, Radiometer Danmark A/S, Denmark). Xylem nitrate was measured

with a nitrate electrode (Nitrate Ion Selective Electrode, Radiometer Analytical S.A., France). Electrical conductivity was measured on a CDM Conductivity Meter, Radiometer, France. C and N were measured in an Elemental Analyzer Flash 1112, CE Instruments, Thermo Quest Italia S.p.A., Italy. The concentration of ABA in the xylem was analysed without further purification by an enzyme linked immunosorbent assay (ELISA) using a monoclonal antibody for ABA (AFRC MAC 252) according to Asch (2000). No cross-reaction of the antibody with other compounds in xylem sap was detected when tested according to Quarrie et al. (1988).

2.5 Data Analysis and Statistics

To facilitate data analysis, the measured values of relative g_s and WUE of the drought-stressed plants were expressed relative to the control plants, evaluated using a linear-plateau model. The relative values were

$$1 \text{ if } Ci \leq FTSW \leq 1 \qquad (3a)$$

$$1 - A \times (FTSW - Ci) \text{ if } FTSW \leq Ci \qquad (3b)$$

where A is the slope of the linear equation (3b), and Ci is the threshold of FTSW at which the measured traits started to diverge, i.e. increase or decline, from 1.

The data were subjected to analysis of variance procedures. To estimate A and Ci in the linear-plateau model (Equation 3), PROC NLIN (SAS Institute 1988) was employed. Coefficient of determination (r^2) was calculated for each curve as 1-SSE/CSS where SSE is the residual sum of squares and CSS is the corrected total sum of squares. Statistical separations between different plant physiological processes were based on comparisons of the 95% confidence intervals of the coefficients in Equation 3b (Soltani et al., 2000).

3. Results

3.1 Soil water status

Changes of water in the pots, measured as FTSW, during the drying cycle, are shown in Fig. 1. In the well-watered treatment, FTSW was maintained above 0.8. In the drought-stressed treatment, FTSW decreased over time until all the plant available

soil water was used, 12 days after imposition of stress in plants at bud formation. The cumulative water use in drought-stressed and well-watered plants at bud formation was similar during the first seven days of the drying cycle. After that there was a significant difference between droughted and control plants.

3.2 Gas exchange

In the well-watered control plants, stomatal conductance g_s decreased from 2 to 0.5 mol m^{-2} s^{-1} (Fig. 2a), with a simultaneous increase in A_{max} from 10 to 20 μmol m^{-2} s^{-1} (Fig. 2b). Under conditions of progressive drought, g_s was significantly lower than the controls 5 days after the onset of stress, and declined close to 0 at the end of the drying cycle (Fig. 2a). For A_{max} there was a minor, but significant difference between drought-stressed and control plants after 6-9 days, thereafter the drought treatment approached rapidly 0 (Fig. 2b).

The relationship between relative g_s and relative A_{max} was represented by a curvilinear logarithmic function (r^2 = 0.79), indicating an efficient A_{max} (Fig. 3). It resulted in a $WUE_{Amax/gs}$ of 1 when FTSW > 0.8, seen in the last graph in Fig. 4. $WUE_{Amax/gs}$ increased by 50% at FTSW 0.7–0.4.

Both A_{max} and g_s were affected by a decreasing soil water content, A_{max} less than g_s.

3.3 Leaf (ψ_l) and root water potential (ψ_r)

ψ_r decreased slightly as soil dried. The ψ_l of drought-stressed plants decreased only slightly to -1 MPa, always below ψ_r (Figs. 5a,b).

3.4 Leaf expansion rate (LER)

LER for plants at bud formation in the fully watered control was 200–500 mm^2 d^{-1} pl^{-1} for 10 days, whereafter it decreased to 0 (Fig. 6). Drought reduced LER to about 50% on average during the first 10 days when compared with the well-watered plants. LER of the droughted plants showed a continuous decease during the drought period, indicating a rapid response of LER to soil drying (Fig. 6).

3.5 ABA, xylem sap pH, nitrate and electrical conductivity

ABA in the xylem was constant at c. 150 and 200 pmol ml^{-1} (Fig. 7a). Drought increased ABA from 2 days after onset of stress, compared to the control treatment, and a large increase in ABA from the xylem occurred after 11 days.

The pH of xylem sap collected from plants at bud formation decreased from 6 to 5.5 during the experimental period, with pH of drought-stressed plants different from the control during days 1-5 (Fig. 7b). Xylem sap conductivity, which remained at 2-3 mS cm^{-1}, and xylem nitrate, did not change with soil drying (data not shown).

3.6 Leaf nitrogen and carbon

The N content of leaves was 5-6%, with a small but significant difference between drought-stressed and control plants (data not shown). In contrast, the C content of leaves was higher in the well-watered treatment (38%) compared to the drought-stressed treatment (34%) for plants at bud formation. Relative values of N and C both decreased with soil drying, whereas relative C/N remained constant.

In particular, an adequate supply of nitrate for assimilation to amino acids, together with photosynthesized carbon compounds and their availability for protein synthesis, is essential for metabolism. We found a high nitrogen content of 5-6% of newly developed leaves in quinoa. Total N, which was only slightly influenced by drought, was even higher than found in N-fixating legumes. The carbon content was significantly higher in the control plants than in drought-stressed plants at bud formation, and lower than for example in maize (Loomis and LaFitte, 1987).

3.7 Relationships between the relative values of biophysical parameters and FTSW

Transpiration was maintained until a threshold value of FTSW 0.58 was reached (Fig. 4). When FTSW decreased beyond a threshold value of 0.82, the values of relative g_s declined linearly, whereas A_{max} was maintained until a FTSW value of 0.33. Photosynthetic water use efficiency ($WUE_{Amax/gs}$) increased by ca. 50%, when soil water content decreased below 0.7 (Fig. 4). The parameters tested as a function of ψ_r gave a similar result.

4. Discussion

4.1 FTSW, leaf water potential and stomatal gas exchange

g_s was very sensitive to soil water deficit, similar to what was demonstrated for leaf expansion. The soil-water threshold of FTSW=0.82 for g_s, which was observed here (Fig. 4), was higher, that is stomata closing earlier, than in crops like soybean 0.64 (Liu et al., 2003), sunflower 0.40 (Tardieu and Davies, 1993), maize cultivars 0.39-0.60 (Ray and Sinclair, 1997), and chickpea 0.34 (Soltani et al., 2000). Many contradictory findings for stomatal closure under decreasing water potential have provided evidence that leaf conductance does not simply depend on epidermal turgor hydraulics (Loesch and Schulze, 1994). Stomata respond differently to long and short-term drought stress (Jensen et al., 1996), and also different soil types may influence the closure of stomata. The experimental method and soil type used here was identical to the soybean study (Liu et al., 2003).

The soil-water threshold for g_s was significantly higher than that for A_{max}. A linear model was tested also to be significant, demonstrating an efficient A_{max} even under continuous soil drying. These findings indicate that drought results in an increase in photosynthetic efficiency and WUE in quinoa (Fig. 4).

Previous results have indicated that gas exchange parameters of quinoa are within the normal range of other C3-plants such as lupin (Jensen et al., 1998) and barley (Mogensen et al., 1994), and that stomatal closure in field and greenhouse grown quinoa did not occur before ψ_l was below –1.2 to –1.6 MPa, for which reason quinoa was characterised as a crop tolerating dehydration (Jensen et al., 2000). In this study, with a different environment of another cultivar, the values for photosynthetic WUE were lower than reported for rape (Jensen et al., 1996) and sunflower (Freeden et al., 1991). Stomatal closure had already started before ψ_l reached –1 MPa in plants at bud formation. Development of g_s showing a decrease for drought-stressed and control plants, and the level of net photosynthesis was similar to that reported by Jensen et al. (2000).

The levels of ψ_l obtained were in accordance with the results of García et al. (1991) for quinoa, who showed that under irrigation predawn ψ_l was from -0.5 to -1.0 MPa, and in stressed conditions it was reduced to -1.5 MPa. Jensen et al. (2000) demonstrated a stable ψ_l for ten days, whereafter ψ_l in drought-stressed plants decreased to –2 MPa. In the present experiment, ψ_l was maintained at least for ten days, where it was still not below – 1MPa (Fig. 5b). In drought-stressed plants, stomatal closure began when ψ_l was -0.8 MPa, whereas ψ_r was only slightly affected by drought.

4.2 Leaf expansion rate (LER)

In previous papers we have shown that during mild soil drying root-generated ABA is transported to shoots decreasing leaf elongation rate and leaf stomata conductance in a number of species such as wheat (Ali et al., 1998), maize (Bahrun et al., 2002), soybean (Liu et al., 2003) and potato (Liu et al., 2005). In quinoa LER of well-watered quinoa plants was higher (up to 500 mm^2 d^{-1} $plant^{-1}$) than for soybean (max 270 mm^2 d^{-1} plant-1), grown under the same conditions with respect to soil type and pot size (Liu et al., 2003). LER under drought stress was significantly lower than the control from onset of drought (Fig. 6), and apparently more sensitive to drought than g_s (Fig. 2a). This is similar to observations in other crops where leaf expansion is more sensitive to soil water deficits than g_s (Boyer, 1970; Saab and Sharp, 1989; Sadras and Milroy, 1996). The soil-water threshold for leaf area expansion was shown to be 0.29 for soybean (Liu et al., 2003), chickpea 0.48 (Soltani et al., 2000), and field pea 0.40 (Lecoeur and Sinclair, 1996). For quinoa, the threshold value could not be calculated, but it was estimated to be close to 1. Plant leaf area was determined by both the area of individual leaves and the number of leaves, and drought may affect both. For this reason the development of leaf area as affected by drought stress at a whole plant level might be of more agronomic importance. Nevertheless, we observed that reduction in single leaf expansion and whole plant leaf area occurred at a similar soil-water status.

4.3 Xylem ABA, pH and conductivity

Quinoa, unlike many other crops, seems not to produce ABA in root tips as a consequence of a decreasing ψ_r because ABA increases before the decrease in ψ_r when soil dries. In other crops was shown a linear relationship between ABA and ψ_r, suggesting that the extent to which ABA accumulated in the xylem sap is dependent on ψ_r (Dodd and Davies, 1996; Liu et al., 2004; 2005). In quinoa ψ_r decreased slightly as soil dried, coinciding with an increase in ABA in the xylem, compared to the control, indicating that there was an effect of a mild soil water deficit on the production of ABA. The decreasing ψ_r and soil water content was followed by a rapid closure of stomata (low g_s) and a decreased LER, whereas the level of A_{max} was maintained for a longer time.

Drought stress has been demonstrated to reduce the activity of H^+-pumping ATPases associated with the root xylem being one of the causes of increased alkalinity of xylem sap that is often observed for plants under stress (Hartung and Radin, 1989; Wilkinson and Davies, 2002). Buffers adjusted to a "stressful" pH of between 6.4 and 7.0 can close stomata and reduce leaf growth in the intact plant (Wilkinson et al., 2007). Such interactions between ABA and pH allow the shoot to modify the response to a root signal as a function of local conditions (Wilkinson, 1999; Wilkinson et al., 1998; Wilkinson and Davies, 2002). In soybean, however, no obvious difference in pH between drought-stressed and fully-watered plants was observed (Liu et al., 2003). In this study there seems to be some effect of xylem pH, as pH increases in plants under drought stress days 1-5, although not higher than pH 6.3 (Fig. 7b).

4.4 Leaf nitrogen and carbon

The interaction between carbon dioxide and nitrate assimilation is of key importance for crop production. The supply of nitrate is crucial for leaf growth because of the role of proteins in the growth of cell walls and the cytoskeleton, and hence in cell expansion (Lawlor et al., 1988). N-deprivation was shown to decrease shoot water potential in barley (Dodd et al., 2002). An increased C-assimilation per unit N would increase biomass and the C/N ratio (Lawlor, 2002).

The C/N ratio of 6-7 was lower than the 14-25 ratio normally reported for plant material on dry weight basis. Under field conditions with slow soil drying it was shown that the N content in quinoa decreased from 5 to 3% under drought, because of limited uptake of N from the drying soil (Jensen et al., 2000). In this experiment we saw only a slight decrease from 6 to 4%, and a slight decrease in the relative N content. This corresponds to a rapid decline in LER following withdrawal of nitrate from the roots (McDonald and Davies, 1996).

5. Final discussion

Quinoa apparently uses another system for adapting to soil water deficits than found in maize showing interactions between N, ABA and xylem pH to stomata behaviour during soil drying (Wilkinson et al., 2007). Mechanisms used by quinoa to maintain turgor under increasing drought, when ABA apparently plays a minor role, could be:

1. Osmotic adjustment

It was shown in the previous study by Jensen et al. (2000) that there was no osmotic adjustment in the cultivar examined, however, it does not exclude the possibility that it can be found in other cultivars.

2. Antitranspirant compounds

A possible explanation for drought-induced stomatal closure is that quinoa produces other antitranspirant compounds than ABA in the xylem sap. Cytokinins as the classical antagonists of ABA, also as stomatal reactions are concerned, may play a role. When cytokinin transport is reduced in the xylem, for instance as a result of limited N supply, stomatal sensitivity to xylem ABA may be increased. This may explain an increase in tissue ABA sensitivity induced by N deficiency (Fusseder et al., 1992; McDonald and Davies, 1996). ABA/cytokinin ratios may change already under mild stress conditions, indicating that also in quinoa hormonal stress signals may exist and may play an important role. Ethylene can be an early drought-induced signal influencing leaf and shoot growth (Sharp and LeNoble, 2002; Sobeih et al. 2004). Both cytokinin and ethylene reactions should be studied in quinoa.

We conclude that during soil drying, quinoa plants, at least the cultivar studied, has a sensitive stomatal closure maintaining leaf water potential ψ_l and photosynthesis A_{max}, resulting in an increase of water use efficiency in plants. ABA root signalling plays some role in stomata performance. The apparent lack of significant root-sourced ABA regulation means that quinoa must depend also on hydraulic regulation through a change in turgor or other chemical substances yet to be studied.

Acknowledgements

This study was financed by the Danish Agricultural and Veterinary Research Council (SJVF). We thank the anonymous reviewers and the editor for the useful comments to the manuscript.

References

Ali, M., Jensen, C.R., Mogensen, V.O., 1998. Early signals in field grown wheat in response to soil drying. Australian Journal of Plant Physiology 25, 871-882.

Asch, F., 2000. Determination of abscisic acid by indirect Enzyme Linked Immuno Sorbent Assay (ELISA). Technical Report, Taastrup, Denmark: Laboratory for

Agrohydrology and Bioclimatology, Department of Agricultural Sciences, The Royal Veterinary and Agricultural University.

Bacon, M.A., Wilkinson, S., Davies, W.J., 1998. pH-regulated leaf cell expansion in droughted plants is abscisic acid dependent. Plant Physiology 118, 1507-1515.

Bahrun, A., Jensen, C.R., Asch, F., Mogensen, V.O. 2002. Drought-induced changes in xylem pH, ionic composition and ABA concentration act as early signals in field grown maize (*Zea mays* L.). Journal of Experimental Botany 53, 1-13.

Boyer, J.S. 1970. Leaf enlargement and metabolic rates in corn, soybean, and sunflower at various leaf water potentials. Plant Physiology 46, 233-235.

Bois, J.F., Winkel, T., Lhomme, J.P., Raffaillac, J.P., Rocheteau, A., 2006. Response of some Andean cultivars of quinoa (*Chenopodium quinoa* Willd.) to temperature: Effects on germination, phenology, growth and freezing. Eur. J. Agron. 25, 299-308

Comstock, J.P. 2002. Hydraulic and chemical signalling in the control of stomatal conductance and transpiration. Journal of Experimental Botany 53, 195-200

Davies, W.J., Zhang, J., 1991. Root signals and the regulation of growth and development of plants in drying soil. Annual Review of Plant Physiology and Plant Molecular Biology 42, 55-76.

Davies, W.J., Tardieu, F., Trejo, C.L., 1994. How do chemical signals work in plants that grow in drying soil? Plant Physiology 104, 309-314.

Dodd, I.C., Davies, W.J., 1996. The relationship between leaf growth and ABA accumulation in the grass leaf elongation zone. Plant, Cell and Environment 19, 1047-1056

Dodd, I.C., Munns, R., Passioura, J.B., 2002. Does shoot water status limit leaf expansion of nitrogen-deprived barley? J. Exp. Bot. 53, 1765-1770

Dodd, I.C., Theobald, J.C., Bacon, M.A., Davies, W.J., 2006. Alternation of wet and dry sides during partial rootzone drying irrigation alters root-to-shoot signalling of abscisic acid. Functional Plant Biology 33, 1081-1089

Freeden, A.L., Gamon, J.A., Field, C.B., 1991. Responses of photosynthesis and carbohydrate partitioning to limitations in nitrogen and water availability in field grown sunflower. Plant, Cell and Environ. 14, 963-970.

Fromard, L., Babib, V., Fleurat-Lessard, P., Fromont, J.-C., Serrano, R., Bonnemain, J.-L., 1995. Control of vascular sap pH by the vessel-associated cells in woody species. Plant Physiology 108, 913-918

Fusseder, A., Wartinger, A., Hartung, W., Schulze, E.-D., Heilmeier, H., 1992. Cytokinins in the xylem sap of desert grown almond (Prunus dulcis) trees: daily courses and their possible interactions with abscisic acid and leaf conductance. New Phytologist 122, 45–53.

Garcia, M., Raes, D., Jacobsen, S.-E., Michel, T., 2007. Agroclimatic contraints for rainfed agriculture in the Bolivian Altiplano. J. Arid Environments 71, 109-121

Geerts, S., Raes, D., Garcia, M., Vacher, J., Mamani, R., Mendoza, J., Huanca, R., Morales, B., Miranda, R., Cusicanqui, J., Taboada, C., 2008. Introducing deficit irrigation to stabilize yields of quinoa (*Chenopodium quinoa* Willd.). Europ. J. Agronomy 28 427–436

Hartung, W., Radin, J.W., 1989. Abscisic acid in the mesophyll apoplast and in the root xylem sap of water-stressed plants: the significance of pH gradients. Current Topics in Plant Biochemistry and Physiology 8, 110-124

Jacobsen, S.-E., Mujica, A., 2001. Quinua: Cultivo con resistencia a la sequía y otros factores adversos. In: Jacobsen S-E, Mujica A, Portillo Z editor. Memorias, Primer Taller Internacional sobre Quinua – Recursos Geneticos y Sistemas de Producción, 10-14 May 1999, UNALM, Lima, Peru. Editor CIP, p. 175-180.

Jacobsen, S.-E., Stølen, O., 1993. Quinoa - Morphology and phenology and prospects for its production as a new crop in Europe. Eur. J. Agron. 2, 19-29.

Jacobsen, S.-E., Mujica, A., Jensen, C.R., 2003. The resistance of quinoa (*Chenopodium quinoa* Willd.) to adverse, abiotic factors. Food Reviews International 19, 99-109.

Jacobsen, S.-E., Rodriguez, D., Mujica, A., Canahua, A., Amachi, F., Andersen, S., 2006. The role of quinoa and other crops in the Andean desert regions. Mountain Forum Bulletin, July 2006: p. 8-10

Jacobsen, S.-E., Monteros, C., Corcuera, L.J., Bravo, L.A., Christiansen, L.J., Mujica, A., 2007. Frost resistance mechanisms in quinoa (*Chenopodium quinoa* Willd.). European Journal of Agronomy, 471-475

Jensen, C.R., Mogensen, V.O., Mortensen, G., Andersen, M.N., Schjoerring, J.K., Thage, J.H., Koribidis, J., 1996. Leaf photosynthesis and drought adaptation in field-grown oilseed rape (*Brassica napus* L.). Aust. J. Plant Physiol. 23, 631-644.

Jensen, C.R., Mogensen, V.O., Poulsen, H.-H., Henson, I.E., Aagot, S., Hansen, E., Ali, M., Wollenweber, B., 1998. Soil water matric potential rather than water content determines drought responses in field-grown lupin (*Lupinus angustifolius* L.). Aust. J. Plant Physiol. 25, 353-363.

Jensen, C.R., Jacobsen, S.-E., Andersen, M.N., Nuñez, N., Andersen, S.D., Rasmussen, L., Mogensen, V.O., 2000. Leaf gas exchange and water relations of field quinoa (*Chenopodium quinoa* Willd.) during soil drying. European Journal of Agronomy 13, 11-25.

Lawlor, D.W., 2002. Carbon and nitrogen assimilation in relation to yield: mechanisms are the key to understanding production systems. J. Exp. Bot. 53, 773-787

Lawlor, D.W., Boyle, F.A., Keys, A.J., Kendall, A.C., Young, A.T., 1988. Nitrate nutrition and temperature effects on wheat: a synthesis of plant growth and nitrogen uptake in relation to metabolic and physiological processes. J. Exp. Bot. 39, 329-343

Lecoeur, J., Sinclair, T.R., 1996. Field pea transpiration and leaf growth in response to soil water deficits. Crop Science 36, 331-335.

Liu, F., Jensen, C.R., Andersen, M.N., 2003. Hydraulic and chemical signals in the control of leaf expansion and stomatal conductance in soybean exposed to drought stress. Functional Plant Biology 30, 65-73

Liu, F., Andersen, M.N., Jacobsen, S.-E., Jensen, C.R., 2004. Stomatal control and water use efficiency of soybean (*Glycine max* L. Merr.) during progressive soil drying. Environ. Exp. Bot. 54, 33-40

Liu, F., Jensen, C.R., Shahanzari, A., Andersen, M.N., Jacobsen, S.-E., 2005. ABA regulated stomatal control and photosynthetic water use efficiency of potato (*Solanum tuberosum* L.) during progressive soil drying. Plant Science 168, 831–836

Liu, F., Savic, S., Jensen, C.R., Shahnazari, A., Jacobsen, S.-E., Stikic, R., Andersen, M.N., 2007. Water relations and yield of lysimeter-grown strawberries under limited irrigation. Scientia Horticulturae 111, 128-132.

Loesch, R., Schulze, E.-D., 1994. Internal coordination of plant responses to drought and evaporational demand. In: Schulze E-D, Caldwell MM editor. Ecophysiology of Photosynthesis. Ecol. Stud. 100. Springer-Verlag, Berlin, 185-204

Loomis, R.S., LaFitte, H.R., 1987. The carbon economy of a maize crop exposed to elevated CO2 concentrations and water stress, as determined from elemental analyses. Field Crops Res. 17, 63-74

McDonald, A.J.S., Davies, W.J., 1996. Keeping in touch: responses of the whole plant to deficits in water and nitrogen supply. Advances in Botanical Research 22, 229–300.

Mogensen, V.O., Mortensen, G., Jensen, C.R., 1994. Photosynthesis of flag leaves and ears of field grown barley during drought. Eur. J. Agron. 3, 111-116.

Mujica, A., Jacobsen, S.-E., Izquierdo, J., 2001. Resistencia a factores adversos de la quinua. In: Mujica A, Jacobsen S-E, Izquierdo J, Marathee J editor. Quinua *(Chenopodium quinoa* Willd.) – Ancestral cultivo andino, alimento del presente y futuro. FAO, UNA-Puno, CIP. Santiago, Chile, p. 162-183.

Quarrie, S.A., Whitford, P.N., Appleford, N.E., Wang, T.L., Cook, S.K., Henson, I.E., Loveys, B.R., 1988. A monoclonal antibody to (S)-abscisic acid: its characterisation and use in a radioimmunoassay for measuring abscisic acid in crude extracts of cereal and lupin leaves. Planta 173, 330–339.

Ray, J.D., Sinclair, T.R., 1997. Stomatal closure of maize hybrids in response to drying soil. Crop Science 37, 803-807.

Sadras, V.O., Milroy, S.P., 1996. Soil-water thresholds for the responses of leaf expansion and gas exchange: A review. Field Crops Research 47, 253-266.

SAS Institute, 1988. SAS/STAT user's guide: Release 6.03 ed. SAS Inst., Inc., Cary, N.C.

Shahnazari, A., Ahmadi, S.H., Liu, F., Laearke, P.E., Plauborg, F.L., Jacobsen, S.-E., Jensen, C.R., Andersen, M.N., 2008. Nitrogen dynamics in the soil-plant system under deficit and partial root zone drying irrigation strategies in potatoes. European Journal of Agronomy 28/2, pp 65-73

Sharp, R.E., LeNoble, M.E., 2002. ABA, ethylene and the control of shoot and root growth under water stress. J. Exp. Botany 53, 33-37

Sobeih, W.Y., Dodd, I.C., Bacon, M.A., Grierson, D., Davies, W.J., 2004. Long-distance signals regulating stomatal conductance and leaf growth in tomato (Lycopersicon esculentum) plants subkected to partial root-zone drying. J. Exp. Bot. 55, 2353-2363

Soltani, A., Khooie, F.R., Ghassemi-Golezani, K., Moghaddam, M., 2000. Thresholds for chickpea leaf expansion and transpiration response to soil water deficit. Field Crops Research 68, 205-210.

Tardieu, F., Davies, W.J., 1993. Integration of hydraulic and chemical signalling in the control of stomatal conductance and water status of drought-stressed plants. Plant, Cell and Environment 16, 341-349.

Vacher, J.J., 1998. Responses of two main Andean crops, quinoa (*Chenopodium quinoa* Willd.) and papa amarga (*Solanum juzepczukii* Buk.) to drought on the Bolivian Altiplano: Significance of local adaptation. Agric., Ecosystems and Environ. 68, 99–108.

Wilkinson, S., 1999. pH as a stress signal. Plant Growth Regulation 29, 87–99.

Wilkinson, S., Davies, W.J., 2002. ABA-based chemical signalling: the co-ordination of responses to stress in plants. Plant, Cell and Environment 25, 195-210

Wilkinson, S., Corlett, J.E., Oger, L., Davies, W.J., 1998. Effects of xylem pH on transpiration from wild-type and flacca tomato leaves: a vital role for abscisic acid in preventing excessive water loss even from well-watered plants. Plant Physiology 117, 703–709.

Wilkinson, S., Bacon, M.A., Davies, W.J., 2007. Nitrate signalling to stomata and growing leaves: interactions with soil drying, ABA, and xylem sap pH in maize. Journal of Experimental Botany 58, 1705–1716

.

Figures

Fig. 1. Water use, measured as FTSW, during drying at bud formation.
Error bars represent standard error of the means (S.E.M.) (n = 8).

Fig. 2. Stomatal conductance (g_s) (Fig. 2a) and photosynthesis (A_{max}) (Fig. 2b) during drying. Error bars represent standard error of the means (S.E.M.) (n = 4).

Fig. 3. Relative photosynthesis (A_{max}) as a function of relative stomatal conductance (g_s). Error bars represent standard error of the means (S.E.M.) (n = 4).

Fig. 4. Relative transpiration, photosynthesis (A_{max}), stomatal conductance (g_s) and photosynthetic water use efficiency ($WUE_{Amax/gs}$) as influenced by soil drying. Fitted lines are from the linear-plateau model, eq. 3 (SAS Institute 1988)

Fig. 5. Root water (ψ_r) and leaf water potential (ψ_l) under soil drying. Error bars represent standard error of the means (S.E.M.) (n = 4).

Fig. 6. Leaf expansion rate (LER) under drought. Error bars represent standard error of the means (S.E.M.) (n = 4).

Fig. 7. ABA (Fig. 7a) and pH (Fig. 7b) in the xylem under soil drying. Error bars represent standard error of the means (S.E.M.) (n = 4).

Figures

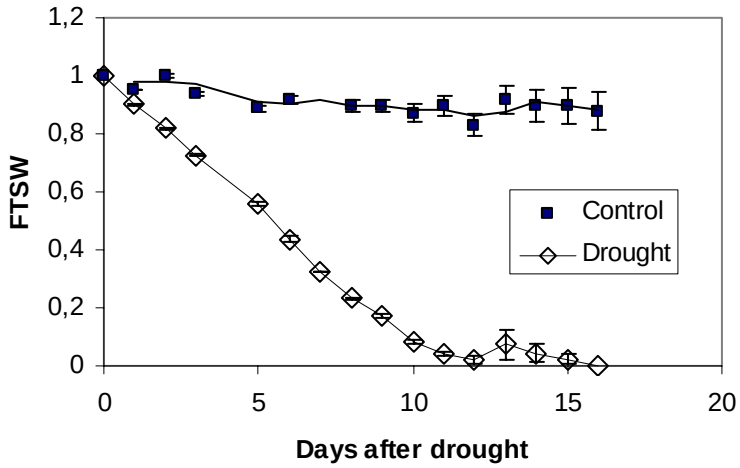

Fig. 1

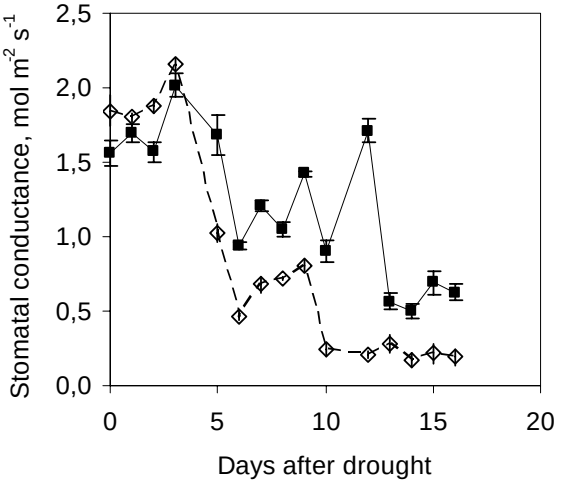

Fig. 2a

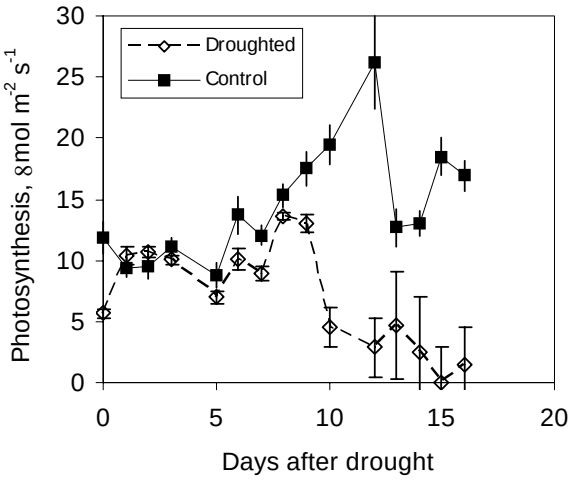

Fig. 2b

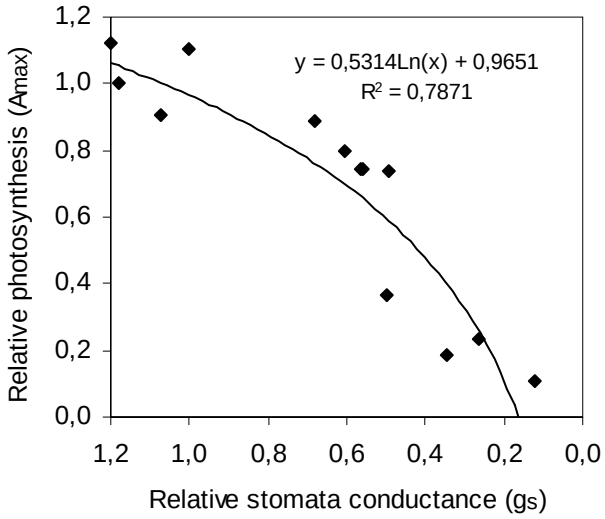
y = 0,5314Ln(x) + 0,9651
R2 = 0,7871
Relative photosynthesis (Amax)
Relative stomata conductance (gs)
1,2
1,0
0,8
0,6
0,4
0,2
0,0

Fig. 3

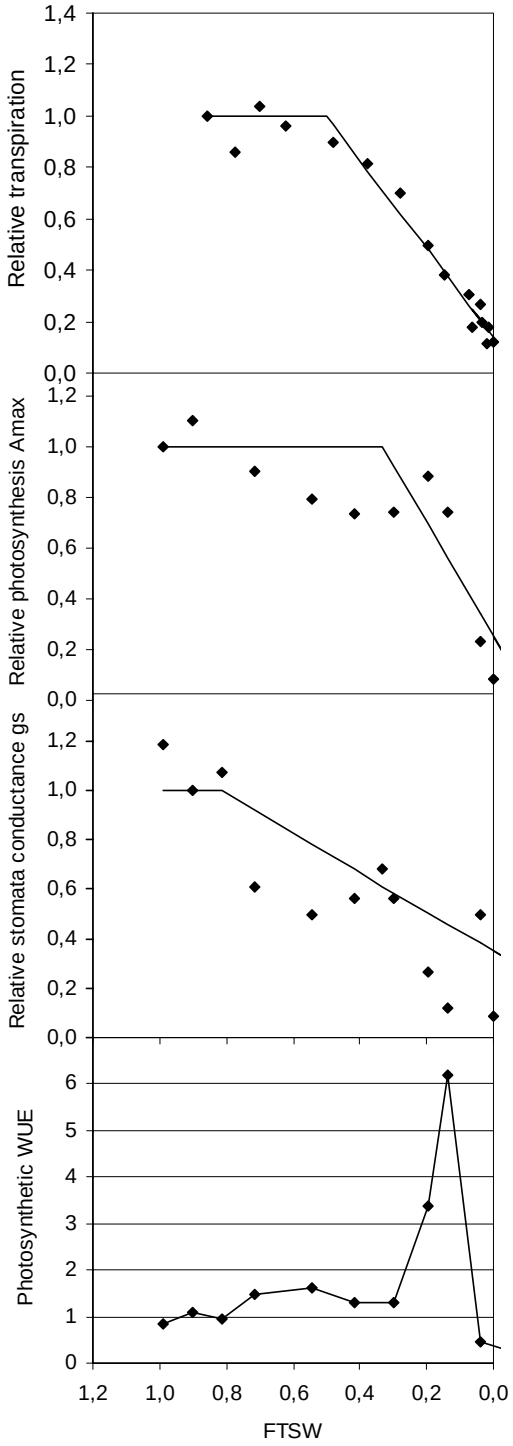
Relative transpiration
1,4
1,2
1,0
0,8
0,6
0,4
0,2
0,0
Relative photosynthesis Amax
1,2
1,0
0,8
0,6
0,4
0,2
0,0
Relative stomata conductance gs
1,2
1,0
0,8
0,6
0,4
0,2
0,0
Photosynthetic WUE
6
5
4
3
2
1
0
1,2
1,0
0,8
0,6
0,4
0,2
0,0
FTSW

Fig. 4

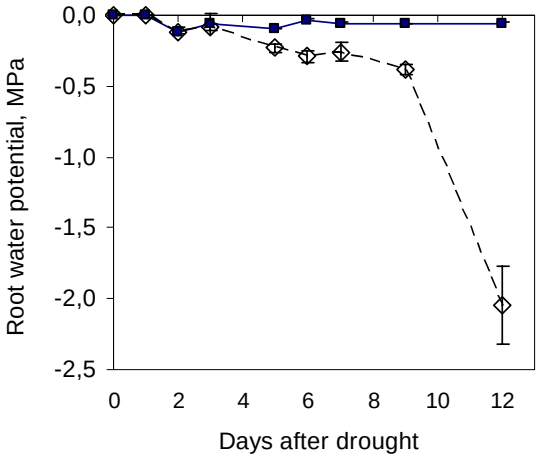

Fig. 5a

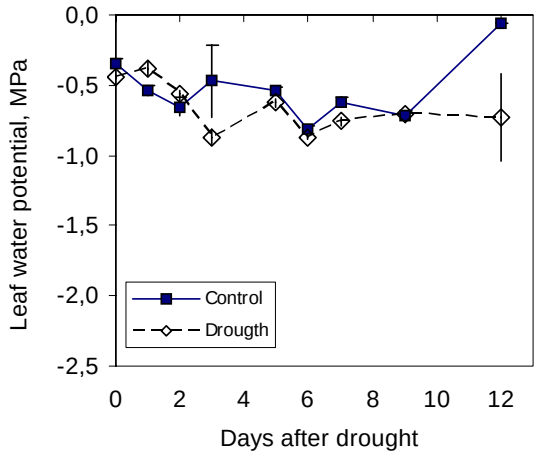

Fig. 5b

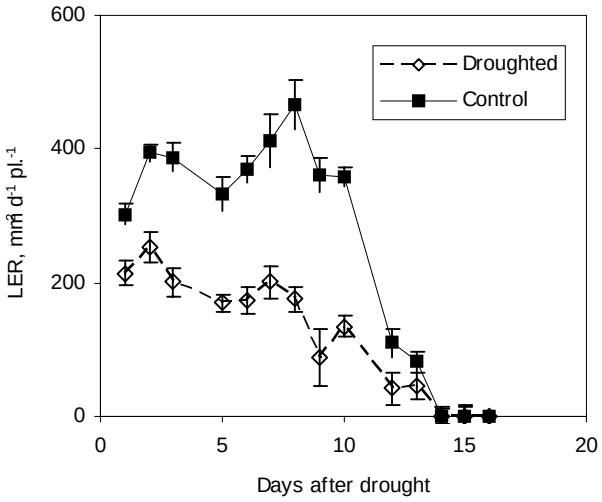

Fig. 6

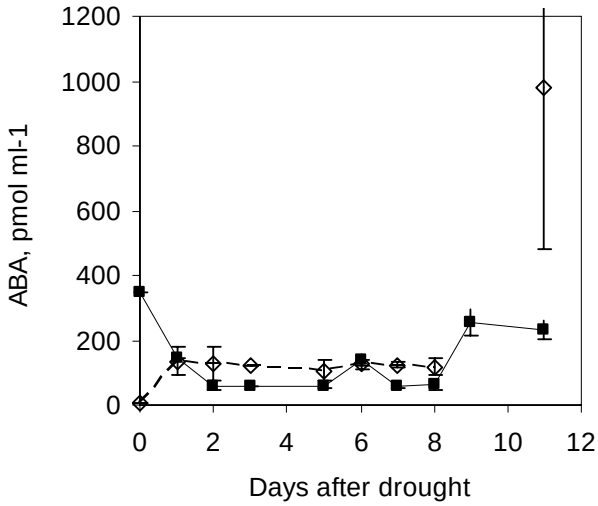
ABA, pmol ml-1
1200
1000
800
600
400
200
0
0
2
4
6
8
10
12
Days after drought

Fig. 7a

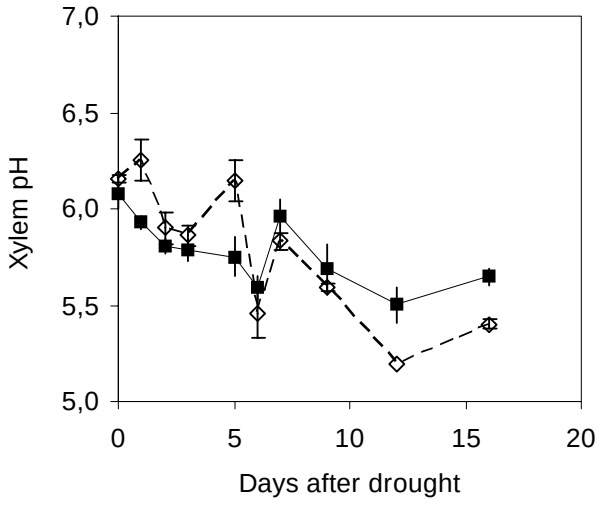
Xylem pH
7,0
6,5
6,0
5,5
5,0
0
5
10
15
20
Days after drought

Fig. 7b