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Restricted transpiration may not result in improved drought tolerance in a competitive environment for water

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Abstract

We have investigated how mutants with enhanced stomatal closure behave in a competitive situation with wild type plants for water. The abscisic acid oversensitive *cbp20* and *era1 Arabidopsis* mutants retain more water when subjected to limited water supply due to restricted gas exchange, resulting in improved drought tolerance. This phenotype, however, was greatly reduced or disappeared when the root systems of neighboring wild type *Arabidopsis* plants competed for water in the soil around the mutants. These findings have implications in the potential use of this mutant class in agronomy as well as in designing genetic screens for drought tolerance.

Keywords: Abscisic acid; Drought resistance; Competition for water; *Arabidopsis*

1. Introduction

As more molecular details become available on abscisic acid and drought signal transduction it emerges that adaptation to water stress is a highly regulated process [1,2]. Plants may fine tune their responses by finding optimal trade-offs between responses to different environmental stresses. A major control point for water loss is stomatal regulation [3,4], although protection against dehydration may also be a result of activation of different stress response pathways [5,6]. It is noteworthy that stomatal closure may be up or down regulated by modifying the action of different regulatory proteins. A growing number of mutants are being discovered with improved drought resistance phenotype; *gcr1* [7], *mrp5* [8], *cbp20* (Cap Binding Protein 20 [9]) are a few examples. Pei et al (1998) and Hugouvieux et al (2001) [10,11] reported reduced leaf wilting due to fast stomatal closure of the *era1* (enhanced response to abscisic acid) and *abh1* mutants, respectively. Strikingly none of these mutants has been discovered as a result of direct conventional selective screen for drought resistance in mutagenized populations. Instead, ABA oversensitivity at germination (*era1, abh1*), reverse genetic approach (*gcr1, mrp5*) or screen for pleiotropic traits (*cbp20*) have led to the isolation of the mutants. Here we propose an explanation why direct screens apparently fail to yield "closed stomata" mutants by their drought resistance phenotype. We have simulated direct selective screening conditions by using two of the aforementioned *Arabidopsis* mutants, *cbp20* and *era1*. In a screen one would expect a mutant to appear among a number of wild type plants. This was simulated by sowing grids of mutant and wild type plants, followed by monitoring the plants responses to drought.

2. Materials and methods

2.1. Plant material and growth conditions

Arabidopsis thaliana cv. Columbia was used as wild type control for the *cbp20* and *era1-1* mutants. Plants were grown under short day light conditions (10-h light:14-h dark periods) for 4 weeks after sowing. From the fifth week on long day illumination was applied (16-h light:8-h dark). 8-week-old wild type and 9-week-old mutant plants were used in the experiments to compensate for the mutants' somewhat slower growth rate. Relative humidity was kept at 65%, temperature was 21 °C and photon fluence rate was 120 μEinstein m^{-2} s^{-1}. Plants were sown in pots according to the patterns in Fig. 1. To

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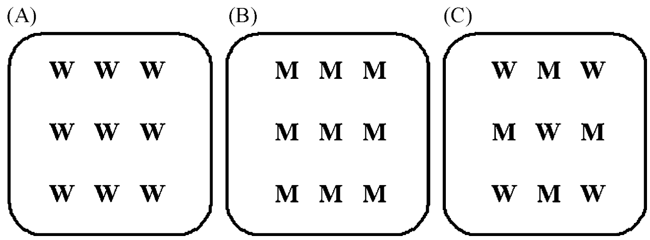

Fig. 1. Sowing grids of wild type (W) and *cbp20* mutant (M) plants. 9 plants were grown in each pots at the patterns indicated with an average distance of 6.5 cm. The weights of pots ranged between 900 and 1100 g at field capacity.

estimate the root systems of the plants they were de-earthed and soaked overnight in water. The following day the attached soil particles were carefully washed away and the fresh weights of the roots recorded.

2.2. Watering regime

On the first day of the measurements the soil was saturated with water to field capacity. This was followed by lack of irrigation or reduced watering for the duration of the experiment. Limited watering comprised supplying all pots with water equivalent to half of evapotranspiration of "pattern B" pots on the previous day. This amount of water was distributed individually to the plants, injected directly to the vicinity of the root systems of each plant by a syringe.

2.3. Characterization of the water status of the plants and soil

Soil water content was monitored by a gravimetric method. Pots were weighed in the early afternoon at the days indicated during the measurement period. At the end of the experiment the pots' contents were baked for 24 h at 80 °C to achieve total desiccation of the soil. Gravimetric water content (GWC) was calculated as a ratio between the water content of pots at field capacity and at the actual time point of the measurements by using the equation below:

$$\mathrm{GWC}\,(\%) = \left[\frac{(W - \mathrm{DW})}{(\mathrm{FW} - \mathrm{DW})}\right] \times 100$$

where W is the weight of pot at the time point indicated, DW is the weight of pot after desiccation, and FW is the weight of pot at field capacity.

Plant water status was characterized by water potential measured by a pressure bomb (PMS610, PMS Instrument Co.). In the pressure chamber increasing external gas pressure was applied to a leaf of the plant investigated, pressure was recorded at the time when the first droplet of sap appeared from the petiole.

Relative water content (RWC) of the leaves was determined as follows: leaves were floated on water for 4 h at room temperature in a closed Petri dish to obtain full hydration, then "turgid weight" was measured. At the appropriate time points leaf samples were taken and their "fresh weight" recorded. Dry weight of the leaves was determined after baking the samples for 24 h at 80 °C. RWC was calculated by the following equation:

$$\mathrm{RWC}\,(\%) = \left[\frac{(W - \mathrm{DW})}{(\mathrm{TW} - \mathrm{DW})}\right] \times 100$$

where W is the fresh weight, DW is the dry weight, and TW is the turgid weight.

All measurements were done in the early afternoon of the day indicated. Five leaves from two different pots were measured for one data point. All experiments were repeated three times unless otherwise stated, with one representative result shown.

3. Results

3.1. Phenotypic differences between plants in different environments in response to drought

Wild type, *cbp20* and *era1* mutant plants behaved as expected when grown in separate pots, mutants remained greener and more turgid than wild type by the end of the approx. 1-week period of water deprivation. The non-wilting phenotype of the mutants, however, was lost among wilt type neighbors (Fig. 2). At the phenotypic level "pattern C" mutants did show wilting and characteristics of water shortage very similar or indiscernible from the other (wild type) plants in the same pot.

The development of the root system of "pattern A, B and C" wild type and *cbp20* mutant plants was measured as described

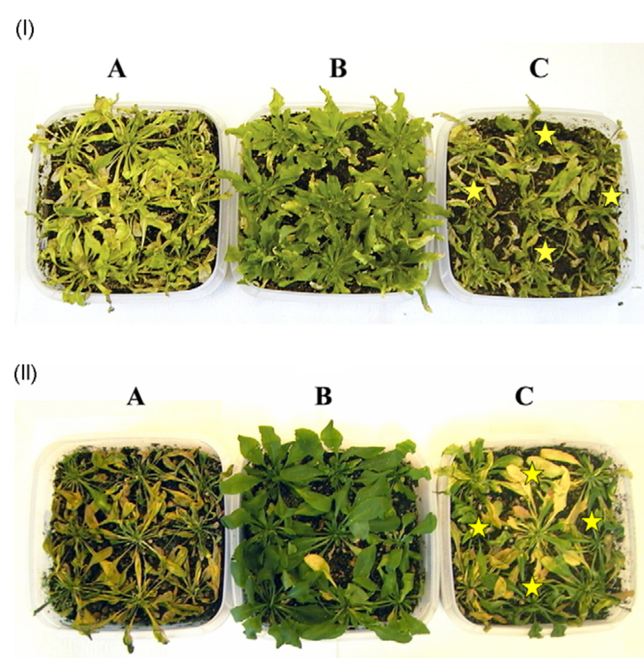

Fig. 2. Rosette leaves of wild type and *cbp20* mutant as well as wild type and *era1* mutant plants (panel I and II, respectively) in the sowing grids after 7 days of water deprivation, flowering stems removed. A—wild type plants ("pattern A"), B—mutants ("pattern B"), and C—mixed plants ("pattern C", yellow asterisks mark mutant plants). The experiment was repeated five times for *cbp20* and three times for *era1* with similar results, one representative is shown.