Enhanced transpiration rate in the high pigment 1 tomato mutant and its physiological significance

R. F. Carvalho1, S. T. Aidar1, R. A. Azevedo2, I. C. Dodd3 & L. E. P. Peres1

1 Departamento de Ciências Biológicas, Escola Superior de Agricultura “Luiz de Queiroz”, Universidade de São Paulo, São Paulo, Brazil
2 Departamento de Genética, Escola Superior de Agricultura “Luiz de Queiroz”, Universidade de São Paulo, São Paulo, Brazil
3 The Lancaster Environment Centre, Lancaster University, Lancaster, UK

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Correspondence
L. E. P. Peres, Departamento de Ciências Biológicas, Escola Superior de Agricultura “Luiz de Queiroz”, Universidade de São Paulo, Av. Pádua Dias, 11, CP 09, CEP 13418-900, Piracicaba, SP, Brazil. E-mail: lazaropp@esalq.usp.br

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ABSTRACT

Tomato high pigment (hp) mutants represent an interesting horticultural resource due to their enhanced accumulation of carotenoids, flavonoids and vitamin C. Since hp mutants are known for their exaggerated light responses, the molecules accumulated are likely to be antioxidants, recruited to deal with light and others stresses. Further phenotypes displayed by hp mutations are reduced growth and an apparent disturbance in water loss. Here, we examined the impact of the hp1 mutation and its near isogenic line cv Micro-Tom (MT) on stomatal conductance (gs), transpiration (E), CO2 assimilation (A) and water use efficiency (WUE). Detached hp1 leaves lost water more rapidly than control leaves, but this behaviour was reversed by exogenous abscisic acid (ABA), indicating the ability of hp1 to respond to this hormone. Although attached hp1 leaves had enhanced gs, E and A compared to control leaves, genotypic differences were lost when water was withheld. Both instantaneous leaf-level WUE and long-term whole plant WUE did not differ between hp1 and MT. Our results indicate a link between exaggerated light response and water loss in hp1, which has important implications for the use of this mutant in both basic and horticultural research.

INTRODUCTION

In tomato, the photomorphogenic mutants high pigment1 (hp1) (Lieberman et al. 2004; Liu et al. 2004) and hp2gs (Mustilli et al. 1999; Levin et al. 2003) are characterised by an exaggerated light response (Bino et al. 2005). These mutants have shorter hypocotyls and higher anthocyanin levels in seedlings (Peters et al. 1989; Kerckhoffs et al. 1997), as well as darker pigmentation of leaves and fruits compared to the wild-type plants (Jarret et al. 1984; Wann et al. 1985). In addition, metabolic analysis of mutant fruits revealed an increased content of carotenoids, flavonoids and vitamin C (Jarret et al. 1984). Such molecules, which are likely to be antioxidants recruited by hp plants to deal with light (Bino et al. 2005) and other stresses (Gratão et al. 2005; Kellos et al. 2008), suggest that these mutants may represent interesting genotypes for plant improvement (Liu et al. 2004; Carvalho et al. 2010a).

The pleiotropic nature of the hp mutations suggests that basic processes affecting plant response to light and morphogenesis are affected (Peters et al. 1989). The molecular cloning of hp1 revealed its homology to the human and Arabidopsis thaliana gene encoding the UV-DAMAGED DNA-BINDING PROTEIN 1 (DDB1) (Lieberman et al. 2004; Liu et al. 2004). DDB1 interacts with the Arabidopsis nuclear protein DEETIOLATED1 (DET1), which is encoded in tomato by hp2gs (Mustilli et al. 1999; Levin et al. 2003). Both DDB1 and DET1 are negative regulators of photomorphogenesis (Pepper et al. 1994; Lieberman et al. 2004). In accordance with the predicted physiological disturbances, transgenic tomato plants with reduced transcript levels of DDB1 displayed, among phenotypes that are major characteristics of hp mutants, enhanced leaf senescence (Azari et al. 2010). Other previously reported detrimental effects of hp mutants are reduced seed germination and increased seedling mortality (Thompson 1962; Peters et al. 1989), as well as reduced leaf area, fruit size and plant dry weight (Jarret et al. 1984). All these responses suggest that hp1 and hp2 mutants may have their growth and capacity to exploit environmental resources (e.g. water and nutrients) compromised by light stress.

Recently, a new tomato mutant, named high-pigment 3 (hp3), was isolated (Galpaz et al. 2008) that displayed some characteristics of hp mutants. Unlike hp1 and hp2, hp3 is not a photomorphogenic mutant, but harbours a mutation in the zeaxanthin epoxidase (ZEP) gene. The loss of function in the ZEP gene, which converts zeaxanthin to violaxanthin, resulted in low abscisic acid (ABA) levels in hp3. As a consequence, field-grown hp3 plants were more susceptible to water stress, leading to decreased biomass and yield. Furthermore, these authors also showed that the ABA-deficient tomato mutants flaccus (flc) and sitiens (sit) had darker green fruit and approximately 35% higher carotenoid concentrations than wild-type plants, but they were not fully comparable to hp mutants. Although light and hormones are known to coincide in the coordination of different plant responses (Carvalho et al.
it is not clear whether the photomorphogenic mutants hp1 and hp2, which also have an elevated carotenoid content, show disturbances in ABA levels or physiological responses (e.g. stomatal conductance) mediated by this hormone.

Here, we examined the impact of the hp1 mutation and its near isogenic line cv Micro-Tom (MT) on stomatal conductance (gs), transpiration (E), CO2 assimilation (A) and water use efficiency (WUE). Our results indicate a link between exaggerated light response and water loss in hp1.

MATERIAL AND METHODS

Plant material and growth conditions

The photomorphogenic mutant hp1 and the hormonal mutant notabilis (not), which is deficient in ABA, were kindly provided by R. Chetelat (The C.M. Rick Tomato Genetics Resource Center, Davis, CA, USA). These genotypes were introgressed into the cultivar Micro-Tom (kindly provide by A. Levy, Weizmann Institute of Science, Israel) through successive backcrosses (BCs), resulting in near-isogenic lines after the BC6F2 generation (Reid 1993). The cultivar Micro-Tom (MT) allows cultivation of a large number of plants in a reduced space using small pots (50–200 ml) that produce seeds 70 days after sowing. The crosses, backcrosses and phenotype screening procedures used in the introgression of mutations were as described previously (Lima et al. 2004, 2009; Zsögön et al. 2008). All plants used in the experiments were grown in a glasshouse with automatic irrigation (four times a day), average mean temperature of 28 °C, 11 h/13 h (winter/summer) photoperiod, and 250–350 μmol·m−2·s−1 PAR (natural radiation reduced with a reflecting mesh (Aluminet – Polysack Industrias Ltda, Leme, Brazil). Seeds were sown in trays containing a 1:1 mixture of commercial substrate (Plantmax HT, Eucatex, Brazil) and expanded vermiculite, supplemented with 1 g l−1 10:10:10 NPK and 4 g l−1 lime (MgCO3 + CaCO3). Ten days after germination, plants were transferred to 150 ml pots (for short-term WUE, gs, A and E measurements) or 350-ml capillary pots (for long-term WUE) containing the described soil mix and fertiliser.

Water loss in detached leaves

Water loss was determined using fully expanded third leaves of three 40-day-old plants per treatment (n = 3). The petiole was placed in 1.5-ml microcentrifuge tubes containing a solution of artificial xylem sap (AX) at 0, 1, 10 or 100 μM abscisic acid (ABA). The AX solution consisted of 3 mM KNO3, 1 mM CaCl2, 1 mM KH2PO4, 1 mM K2HPO4, 0.1 mM MnSO4 and 0.1 mM MgSO4. Detached leaves were maintained overnight at room temperature and leaves were weighed on an analytical balance on the following morning (06:00 h to 18:00 h) at 2-h intervals. At the end of the experiment, leaf areas were determined using a standard scanner with image analysis and the Quant software (Vale et al. 2003).

Measurement of instantaneous and long-term water status

In 40-day-old plants irrigation was interrupted for 4 days, then the plants were rehydrated over the course of the fifth day. As controls, three plants per treatment remained well-watered. On a daily basis, CO2 assimilation (A), stomatal conductance (gs), transpiration (E) and instantaneous water use efficiency (WUE) were measured in the morning (08:00 h) with a porta-
ble infrared gas analyser (IRGA, LI-6400; LiCor, Lincoln, NE, USA). All measurements were performed on the third fully expanded leaf. For long-term measurements, seedlings showing the first true leaves were transplanted into 350 ml Leonard pots (Grata˜o et al. 2008) filled with the same substrate as described above. Pots were weighed daily and water was supplied to the roots via capillary action for 40 days. WUE was calculated by dividing the total dry weight (shoots and roots dried at 50 °C until constant weight) by the total water used [weightgP∑H2Okg(system)].

RESULTS AND DISCUSSION

Detached leaves of hp1 lost water faster than the non-mutant plants (Fig. 1A). Since the genotypes had a similar leaf area (Fig. 1A, insert), the water spending behaviour of hp1 is likely to be due to poor control of stomatal movement. To verify a possible relationship between hp1 water loss and ABA, detached leaves were treated with progressively higher ABA concentrations (Fig. 1B–D). ABA application clearly reduced the differences in water loss between hp1 and the control MT, giving the same values for the two genotypes at 100 μM ABA (Fig. 1D). This indicates that hp1 plants can respond to ABA and that this hormone limits transpiration of this mutant.

Intact leaves from whole plants growing in the glasshouse were used for IRGA measurements. The hp1 and MT plants were subjected to two water regimes: (i) 4 days of withholding water, with rewatering from day 4 to day 5, and (ii) well watered throughout the entire experiment. Consistent with the high water loss observed in hp1 detached leaves (Fig. 1), well hydrated hp1 plants tended to have increased E and gs during the period observed (Fig. 2A,B). Since A and gs were coordinately regulated, there were no differences in WUE between hp1 and control MT plants under the same conditions (Fig. 2D). Withholding water resulted in similar values of E, gs, A and instantaneous WUE in hp1 and MT plants from day 3 of stress (Fig. 2). Interestingly, this reversion of the water-spending behaviour of hp1 upon imposition of water stress is somewhat similar to that obtained after ABA application to detached leaves (Fig. 1).

Since water stress minimised differences in leaf gas exchange between hp1 and MT plants (Fig. 2), a third set of plants were grown with capillary watering (Leonard pots) to maintain both genotypes at a high substrate water potential. This capillary system allows control and direct measurement of water use during growth. Total water loss (WL) did not significantly differ between hp1, MT and the ABA-deficient not mutant (Fig. 3A), even though not shoots still exhibited a characteristic wilty phenotype. Total dry weight (DW) was significantly reduced in both hp1 and not when compared to MT (Fig. 3A). Thus a low DW significantly decreased whole plant WUE of not, but not of the hp1 mutant (Fig. 3A). The reduction of DW in the mutants may have diverse causes. Excessive transpiration of well-watered hp1 (Figs 1 and 2) and not mutants may have decreased shoot turgor thus limiting leaf area of these mutants (Fig. 3B). Alternatively, other known features of the mutants such as constitutive light stress in hp1 (Levin et al. 2006) and ethylene accumulation in tomato ABA-deficient mutants (Sharp et al. 2000) may have led to the observed reduction in leaf area and DW.

![Fig. 2. Transpiration (A), stomatal conductance (B), CO2 assimilation (C) and instantaneous water use efficiency (D) of hp1 and MT plants subjected to water stress (4 days of withholding water, followed by rewatering from day 4 to day 5) and well-watered plants (hydrated) during the same period. Data are means ± SE (n = 3 plants).](image-url)
research will be still necessary to elucidate this result and the mechanisms involved in control of transpiration of this mutant. This is because a subset of photoreceptors can be involved in light signal transduction in hp mutants, e.g. the phytochromes. Recent evidence shows that phytochromes can exert strong control on transpiration. For instance, phyB mutants of Arabidopsis have reduced rates of transpiration per unit leaf area (Boccalandro et al. 2009).

In this study, the different experiments used supported the hypothesis that the hp1 mutant has enhanced stomatal conductance per unit leaf area. The results provide evidence for the further use of this mutant in the study of integrative interactions between light and the diverse endogenous molecules involved in signal transduction controlling water status. In addition, although hp1 has enhanced stomatal conductance, our results show that WUE, which is critical in determining the capacity for adaptation and the productivity of plants (Parry & Lea 2009), did not alter. This reinforces current expectations of using this mutation to enhance antioxidant content of edible fruits (Azari et al. 2010).

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