Plant Soil (2021) 463:249–263
https://doi.org/10.1007/s11104-021-04912-1

REGULAR ARTICLE

Root system of *Medicago sativa* and *Medicago truncatula*: drought effects on carbon metabolism

Andres Echeverria · Esther M. Gonzalez

Received: 4 October 2020 / Accepted: 7 March 2021 / Published online: 18 March 2021

Abstract

Aims Here, we assess the differential impact of drought on root carbon metabolism in the widely cultivated alfalfa (*Medicago sativa*, *Ms*) and the model legume *Medicago truncatula* (*Mt*). Understanding how carbon allocation is regulated under drought stress conditions is a central issue to improving alfalfa productivity under future climate change scenarios.

Methods Alfalfa and *Medicago truncatula* were compared under water deficit conditions. Root carbon metabolism of the taproot and fibrous roots was analysed. *M. truncatula* drought tolerance variability was compared to that of alfalfa using six accessions of the *Medicago* Hapmap project. The prominent taproot is much less developed in *M. truncatula* than in alfalfa with the former exhibiting an extensive fibrous root system.

Results In both examined *Medicago* species the taproot contained the major pools of soluble protein, sucrose and pinitol, whereas the major pools of hexoses and carbon metabolism enzymes appeared to be in the fibrous roots. Under water-deficit conditions, the response of *M. sativa* strongly differed from that of *M. truncatula* at the root level.

Conclusions Water deficit conditions differentially modulate the root carbon metabolism of *M. sativa* and *M. truncatula*. *Mt* maintained a more active carbon metabolism in the fibRs, as sucrose, myo-inositol and pinitol accumulated to cope with the water deficit (WD). Conversely, the root system of *Ms* did not accumulate cyclitols and carbon metabolism was more severely affected under water deficit conditions. This differentially exerted control may determine the drought response of these two close relatives.

Keywords Carbon metabolism · Fibrous roots · Taproots · Sucrose synthase · Water deficit

Abbreviations

C	control
DW	dry weight
G6PDH	glucose-6-phosphate dehydrogenase
fibRs	fibrous roots
FW	fresh weight
g_s	stomatal conductance
INV	alkaline invertase
MD	moderate deficit
Ms	*Medicago sativa*
Mt	*Medicago truncatula*
SuSy	sucrose synthase
T	transpiration
tapRs	taproots
WC	water content
WD	water deficit

Responsible Editor: Ian Dodd.

A. Echeverria · E. M. Gonzalez (✉)
Department of Science, Institute for Multidisciplinary Research in Applied Biology – IMAB, Public University of Navarra, E-31006 Pamplona, Spain
e-mail: esther.gonzalez@unavarra.es

Introduction

Drought is the main adverse environmental factor that affects crop productivity (Sinclair 2011; Lipiec et al. 2013). Climate change is predicted to aggravate the intensity and distribution of drought worldwide (Lobell et al. 2008; Dai 2011). Therefore, it is very important to select and develop tolerant crop varieties adapted to drought under future scenarios, as crop production needs to double over the next 50 years to meet food supply requirements for an increasing world population (Rothstein 2007; Foley et al. 2011).

The model plant barrel medic *Medicago truncatula* (*Mt*) is a cool-season legume originating from the Mediterranean basin and is now cultivated as an annual forage crop (~32 Mha) in several regions in the world, especially in Australia (Michaud 1988). In addition, this species is largely employed as a model legume for molecular studies (Barker et al. 1990; Young and Udvardi 2009). One of the most valuable resources generated for *Mt* is the Medicago Hapmap Project. Developed by an international consortium involving institutions in the USA and France, the project has sequenced 384 inbred lines spanning the range of *Medicago* diversity using Illumina next-generation technology (Stanton-geddes et al. 2013). Due to the Mediterranean-wide origin of the collection, a region experiencing annual cycles of drought, the *M. truncatula* Hapmap accessions provide a rich source of drought tolerance variability (Yoder et al. 2014). In this context, 220 drought-adaptive traits of the Hapmap collection were characterized by in vitro leaf dehydration assays using 25% polyethylene glycol (PEG) 8000, revealing significant differences among accessions (Kang et al. 2015).

Medicago sativa (*Ms*) is a common forage legume worldwide and is economically important in the temperate European region. Alfalfa may be grown as an irrigated or rainfed forage crop depending on the region, although as a rainfed crop, it faces environmental constraints and is marginally economical. Although alfalfa is a close relative of *Mt*, there are significant shoot and root anatomical differences between them, which may determine their differential drought tolerances (Castañeda et al. 2019). Regarding their underground organs, alfalfa has a large taproot (Radovic et al. 2009; Araújo et al. 2015; Quan et al. 2016), while *Mt* is defined by a well-developed fibrous root system (Zhang et al. 2014; Castañeda et al. 2019).

Drought tolerance mechanisms have been explored in different alfalfa varieties with a major focus on shoot strategies (Kang et al. 2011; Zhang et al. 2019) with attention given to the roots only recently (Soba et al. 2019; Zhang et al. 2019). However, the root system is essential for crop productivity (Paez-Garcia et al. 2015) and is the first organ to sense water deficit (WD) and, as such, has an essential signalling role. Tian et al. (2014) propose that differential growth dynamics between primary and lateral roots are crucial for plants to adapt to ever-changing environmental conditions. Lynch and Brown (2012) distinguished between taproots (tapRs) or primary roots, which are those that grow from the embryo directly downwards, and fibrous roots (fibRs) or secondary roots, which are formed post-embryonically, developing from the pericycle.

In situations where water is a limiting factor, metabolites that participate in energy production and growth are transported from shoots to roots (Gargallo-Garriga et al. 2014). Although effects on photosynthesis have received most of the attention (Chaves et al. 2009), it should be noted that upon the progressive imposition of WD stress, this process is affected long after cell growth (Hsiao and Acevedo 1974; Taiz and Zeiger 2010). At the whole-plant level, numerous studies have shown that, compared with aboveground organs, root systems show an earlier and more active response to drought (Gargallo-Garriga et al. 2014). Recently, modulation of carbon metabolism has been shown to markedly disturb the metabolism and development of *Arabidopsis* roots (Pignocchi et al. 2020). Castañeda et al. (2019) showed that carbon metabolism was differentially modulated in the different parts of the root system of *Mt* under WD conditions, sparking a new hypothesis about the role of root metabolism as a mechanism of drought tolerance. Roots use sucrose, which may be catabolized by the enzymes sucrose synthase (SuSy) and alkaline invertase (INV), as the main form of carbon transport (Koch 1996; Ciereszko and Kleczkowski 2002). SuSy is a cytosolic reversible glycosyltransferase that degrades sucrose into UDP-glucose and fructose in the presence of UPD, controlling the partitioning of sucrose into various metabolic, structural, and storage pools within plant cells (Zrenner et al. 1995; Dejardin et al. 1997; Sturm 1999; Haigler 2001). Conversely, INV irreversibly hydrolyses sucrose into glucose and fructose (Winter and Huber 2000) and is involved in sucrose catabolism in nitrogen-fixing root nodules (Morell and Copeland 1984; Barratt et al. 2009; Ruan et al. 2010).

Sucrose metabolism has been extensively analysed in the last decade, but the regulation of carbon reallocation is still not fully understood and is a central issue to improve plant productivity under abiotic stress such as drought (Ruan 2014).

Although the root is the first organ to sense drought, the role of root carbon metabolism on carbon partitioning at the whole plant level has been scarcely explored in forage plants. We hypothesize that root carbon metabolism plays a role in the drought tolerance of *Medicago* forage species. For this purpose, we first explore *Mt* drought tolerance variability in a collection of accessions of the Hapmap Project. Second, we discuss the existing differences between *Ms* and *Mt* in root system morphology and explore carbon metabolism in the root systems of both Medicago species exposed to moderate water deficit conditions.

Materials and methods

Plant material and growth conditions

M. sativa seeds of the commercial variety Sitel were used. *M. truncatula* seeds were obtained through the *M. truncatula* germplasm request service of the Hapmap Project (http://www.medicagohapmap.org/hapmap/germplasm). Kang et al. characterized 220 drought-adaptive traits in the *Medicago truncatula* Hapmap Project collection. These researchers performed in vitro leaf dehydration assays using 25% polyethylene glycol (PEG) 8000, revealing significant differences among accessions (Kang et al. 2015). We selected six *M. truncatula* (*Mt*) accessions based on the abovementioned PEG assays exhibiting from 2 to 41% leaf biomass reduction (*Mt. IDRA* (2%), *Mt. HM267* (10.4%), *Mt. HM290* (15%), *Mt. HM268* (20.2%), *Mt. HM287* (30%) and *Mt. HM307 (*41.4%). First, progressive water deficit conditions were applied to late-vegetative-stage (8-week-old) *M. truncatula* accessions to evaluate the tolerance to water deficit among them and in relation to that of *M. sativa* plants. Based on this screening, the *Mt. HM307* accession was selected for further comparisons with *M. sativa*, since they reported a similar response to WD in terms of growth and time required to reach −1.8 MPa water potential values.

Seeds were scarified with 98% sulfuric acid for 7 min, washed several times and then sterilized with 3.5% sodium hypochlorite for 90 s. After that, seeds were washed again approximately 10 times, soaked in water and shaked for 3 h. When the seeds were hydrated, they were transferred to 7% agar plates at 4 °C for one day in the dark and then incubated at 20 °C for two days. After germination, seeds were sown in 1-L pots containing a mixture of perlite:vermiculite (2:5, *v*/v) under controlled environmental conditions (14 h photoperiod; 400 μmol m^{-2} s^{-1} light intensity; 22 °C/16 °C day/night temperature; 60 to 70% relative humidity). Plants were irrigated to field capacity 3 times a week with Evans nutrient solution containing (values in mg L^{-1}) the following: $MgSO_4 \cdot 7H_2O$ (493), K_2SO_4 (279), K_2HPO_4 (145), $CaCl_2$ (56), KH_2PO_4 (23), EDTA-Fe (17), H_3BO_3 (1.43), $CaSO_4 \cdot 2H_2O$ (1.03), $MnSO_4 \cdot 7H_2O$ (0.77), $ZnSO_4 \cdot 7H_2O$ (0.22), $CoCl_2 \cdot 6H_2O$ (0.12), $CuSO4 \cdot 5H_2O$ (0.08), and $NaMoO_4 \cdot 2H_2O$ (0.05); (Evans, 1981). This nutrient solution was supplemented with 5 mM NH_4NO_3.

Eight-week-old plants were randomly separated into 2 treatments, control and water deficit (WD), containing 4 biological replicates each. Controls (C) were irrigated daily with Evans nutrient solution to field capacity, and WD was imposed by withholding water. The hydric status of water-deprived and well-watered plants was monitored daily by measuring the water potential of the leaves of different plants. Water-stressed and control plants were harvested simultaneously when the desired water potential of the leaves was reached (≈ −1.8 MPa). The harvesting period lasted for 7 to 19 days if we considered all *Mt* species (Fig. 1) and for 7 days if we considered the *Ms* and *Mt. HM307* species comparison (Figs. 2, 3, 4 and 5). The whole tapRs and fibRs were harvested, immediately frozen in liquid nitrogen and stored at −80 °C for various analyses. A tissue aliquot of each root type was used for dry weight (DW) determinations after drying for 48 h at 70 °C. Water content was calculated using the following equation: WC (%) = (FW-DW) /(FW*100) where FW represents the fresh weight (FW).

Plant water status

Leaf water potential was monitored daily from the 5th day of the water stress period in the first fully expanded leaf of three randomly selected plants at 10:00 h using a pressure chamber (Scholander et al.

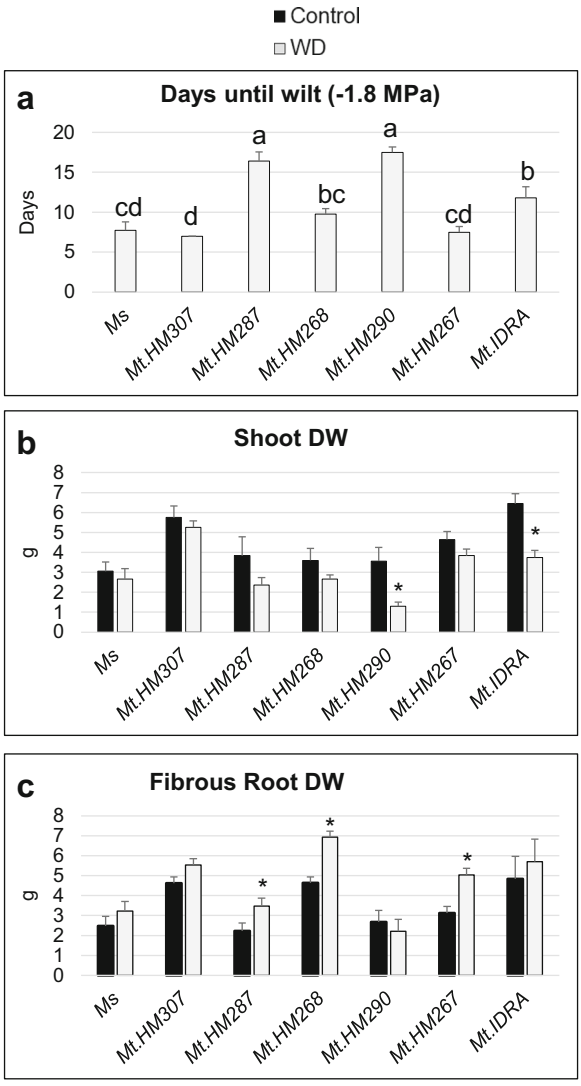

Fig. 1 (**a**) Days until wilt in water deficit-treated plants (defined at −1.8 MPa). (**b**) Shoot and (c) fibR dry weight biomasses in control and water deficit-treated plants in the Hapmap Project accession experiment. Bars represent the mean ± SE ($n = 4$). Different letters indicate differences according to Duncan's test ($P \leq 0.05$). Significant differences between the control and water deficit treatments are indicated with an asterisk (Student's t test; $p < 0.05$)

1965). Control plants showed a leaf Ψ_w value of -1.16 ± 0.06 for *Ms* and -1.20 ± 0.15 for *Mt. HM307* and were harvested randomly during the WD experiment. Moderate water deficit (MD)-stressed plants were collected when the leaf Ψ_w reached a value of -1.92 ± 0.06 for *Ms* and -1.8 ± 0.09 for *Mt. HM307*. Water stress was monitored by daily measurements of stomatal conductance (mmol m^{-2} s^{-1}) using a delta AP4 cyclic porometer. Plant transpiration (T) was measured gravimetrically daily.

Determination of soluble sugars

Ethanol extraction was performed for soluble sugar determination (Gálvez et al. 2005). Frozen aliquots of root tissue (100 mg FW) were extracted three times in 1.5 mL 80% (*v*/v) boiling ethanol for 30 s and once more at room temperature. All the collected supernatants were dried in a Turbovap® LV Evaporator (Zymark, Hopkinton, MA, USA) at 40 °C and 1.2 bar. After total evaporation the dry residue was resuspended in 1 mL deionized water in two steps, with ultrasonication for 10 min between both steps. After that, 2 mL of the suspension was centrifuged for 10 min (2300 g, 4 °C) and the supernatants were stored at −20 °C for future determinations. Sucrose, fructose and glucose were determined by ionic chromatography using a 940 Professional IC Vario Metrohm system (Metrosep Carb2 guard and Metrosep Carb2 150/4.0 Metrohm columns; 0.5 ml/min; 30 °C; 300 mM NaOH, 1 mM sodium acetate).

Determination of total soluble protein and enzymatic activities

Aliquots of frozen tapRs and fibRs (≈0.35 g FW) were homogenized into a fine powder with liquid nitrogen. Extraction buffer (50 mM MOPS pH 7.5, 0.1% (v/v) Triton X-100, 10 mM $MgCl_2$, 1 mM EDTA, 20 mM KCl, 10 mM DTT β-mercaptoethanol, 2.5% PVPP, 2 mM PMSF and protease inhibition cocktail tablet) was added, and samples were centrifuged at 24,000 *g* (4 °C, 30 min). The protein content was determined in the crude extract per Bradford (Bradford 1976) using BSA as the protein standard.

The crude extract was desalted through a BioGel P-6 DG desalting gel (BioRad) equilibrated with desalting buffer (250 mM MOPS pH 7.5, 50 mM $MgCl_2$, 100 mM KCl) to determine the activity of UDP-sucrose synthase (SuSy, EC 2.4.1.13), alkaline invertase (INV, EC 3.2.1.26) and glucose-6-phosphate dehydrogenase (G6PDH, EC 1.1.1.49). SuSy and INV were assayed per Gonzalez et al. (1998), whereas G6PDH was determined per Gibon et al. (2004). All the enzyme activities were assayed spectrophotometrically at 30 °C and 340 nm for

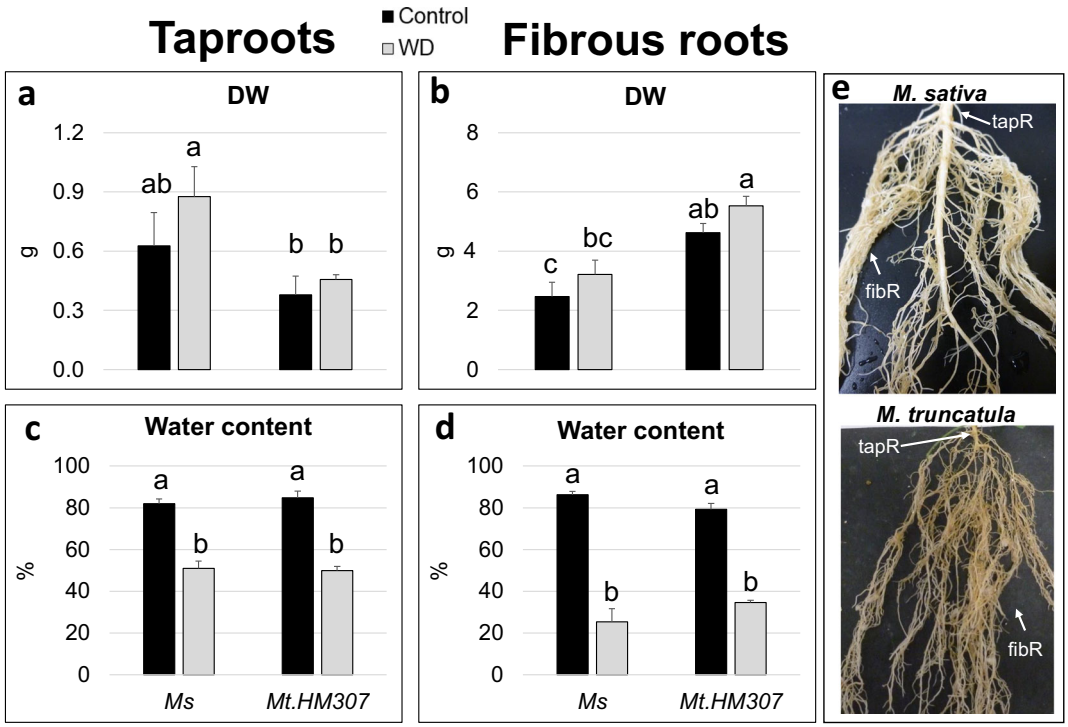

Fig. 2 Dry weight (DW) biomass and water content of (**a**,**c**) taproots and (**b**,**d**) fibrous roots of *Medicago sativa* (*Ms*) and *Medicago truncatula HM307* (*Mt. HM307*) under control (C) and water deficit (WD) conditions. Control and WD-treated plants were harvested when WD-treated plants reached MD conditions (Ψ_w, −1.8 MPa). Bars represent the mean ± SE (n = 4). Different letters indicate differences according to Duncan's test ($P \leq 0.05$). (**e**) Visual appearance of *Medicago sativa* and *Medicago truncatula* taproots (tapR) and fibrous roots (fibR) in control conditions

10 min in their respective assay media. Enzymatic activities were expressed on a protein basis.

Statistical analysis

The results were examined using two-factor analysis of variance ($P \leq 0.05$) and Duncan's multiple range test. Student's *t* test was employed when a single comparison was needed. All data are shown as the mean ± standard error of $n = 4$–5 independent measurements. Each figure legend shows the different biological replicates used in each analysis. Two-way ANOVA was performed to analyse the interactions of the accessions (*Ms*, *Mt. HM307*) and organs (tapRs and fibRs) with the WD treatment. The ANOVA results are presented in Supplementary Table 1.

Results

The WD effect on shoot and root dry weight and the days until wilt were determined to confirm the differential tolerance of Hapmap *M. truncatula* accessions and *M. sativa* under MD conditions ($\Psi_w \approx -1.8$ MPa) (Fig. 1). On one side, *Mt. IDRA*, which exhibited higher tolerance in the in vitro PEG assays (2% leaf DW reduction), reached MD conditions 11.80 ± 1.40 days after irrigation was withheld. On the other side, *Mt. HM307,* which exhibited lower tolerance in the in vitro PEG assays (40% leaf DW reduction), reached MD conditions 7.00 ± 0.01 days after irrigation was withheld (Fig. 1a). However, regarding the shoot biomass reduction induced by the WD conditions, *Mt. IDRA* was markedly affected by WD stress while *Mt. HM307* maintained biomass production at control levels (Fig. 1b). The *Mt* accessions exhibiting an intermediate response to water deficit in the in vitro PEG assays (*Mt. HM287, Mt. HM268, Mt. HM290, Mt. HM267*) and, showed a variable response in biomass production and days until wilt, which did not correlate with the tolerance range established by Kang et al. (2015). The response of Ms was similar to that of *Mt. HM307,* as it reported 7.75 ± 1.06 days to reach MD conditions and stable

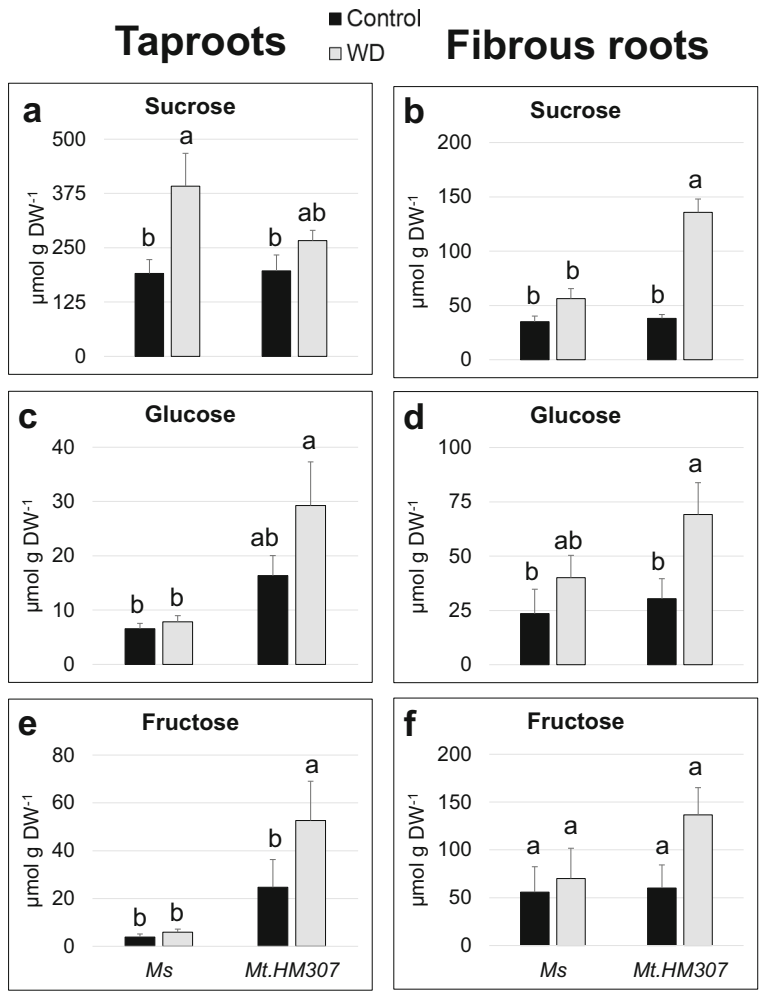

Fig. 3 Concentrations of (**a**-**b**) sucrose, (**c**-**d**) glucose and (**e**-**f**) fructose contents (µmol g DW^{-1}) *of Medicago sativa* (*Ms*) and *Medicago truncatula HM307* (*Mt. HM307*) taproots and fibrous roots under control and moderate water deficit conditions. Control and WD-treated plants were harvested when WD-treated plants reached MD conditions (Ψ_w, −1.8 MPa). Bars represent the mean ± SE (n = 4). Different letters indicate differences according to Duncan's test ($P \leq 0.05$). WD*S indicates the interactive effect between the water deficit treatment and species

shoot biomass under WD conditions (Fig. 1b). Therefore, the *Mt. HM307* accession was employed for further comparative studies with *Ms*.

Transpiration (T) and stomatal conductance (*gs*) were monitored throughout the time-course study. Figure S1 represents the reduction of both parameters in the WD-treated plants compared to the controls (percentage) during the first four days of WD treatment. Stomatal conductance decreased progressively in both species along with the establishment of WD stress during the initial days of the treatment. On day 2, *Mt. HM307* showed a substantial reduction in *gs* and T, while the *Ms* plants maintained mostly open stomata and were only significantly affected after 3 days (Fig. S1). During the following days of the WD time-course, T and *gs* significantly declined in both *Ms* and *Mt. HM307* species without any significant differences among them (Fig. S1).

As mentioned above, MD-stressed plants entailed a decline of approximately 0.6–0.8 MPa with respect to control plants. Days to reach this WD level were monitored and similar values were observed between species i.e., periods of 7.75 ± 1.05 for *Ms* and 7.00 ± 0.01 days for *Mt. HM307* (Fig. 1a).

Under MD, the shoot biomass of *Ms* and *Mt. HM307* was unaltered (Fig. 1). In the roots, although an increasing trend was observed in both *Ms* and *Mt. HM307*, no significant difference was detected between the control and MD treatments in any root type (Fig. 2a, b). The fibR:tapR DW ratio was significantly higher in *Mt. HM307* (12.21 ± 0.83) than in *Ms* (3.94 ± 0.77). The relevance of the *Ms* tapR is well known while this part of the root is less developed in *Mt*, with fibRs being the main organ type (Fig. 2e).

Water content was significantly affected in all the organs examined with a similar pattern in the two

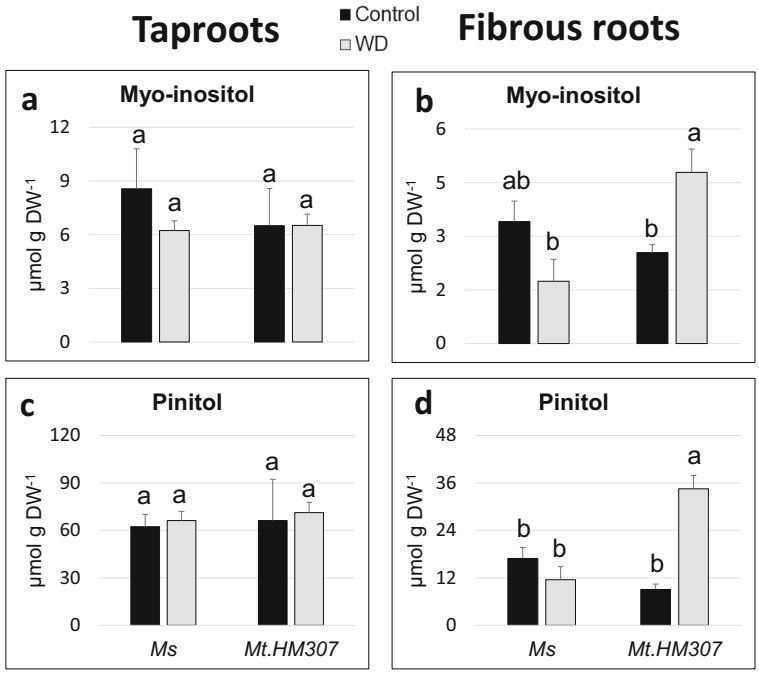

Fig. 4 Changes in (**a**-**b**) myo-inositol and (**c**-**d**) pinitol contents (µmol g DW^{-1}) *of Medicago sativa* (*Ms*) and *Medicago truncatula HM307* (*Mt. HM307*) taproots and fibrous roots under control and moderate conditions. Control and WD-treated plants were harvested when WD-treated plants reached MD conditions (Ψ_w, −1.8 MPa). Bars represent the mean ± SE (n = 4). Different letters indicate differences according to Duncan's test ($P \leq 0.05$). WD*S indicates the interactive effect between the water deficit treatment and species

species (Fig. 2c, d). However, tapRs maintained a higher water content under MD (close to 50%), while fibRs showed a water content decline of approximately 30% in both species (Fig. 2c, d). Two-way ANOVA showed a significant interaction between WD treatment and organs in *Ms* (Supplementary Table 1).

Important differences were observed when comparing the tapRs with the fibRs in terms of the concentration of neutral sugars in well-watered plants. Regardless of the species, the sucrose concentration was up to four times higher in tapRs than in fibRs, while conversely, the glucose and fructose concentrations tended to be higher in fibRs (Fig. 3). MD stress induced the accumulation of sucrose in the roots, but a differential pattern was observed in *Ms* compared to that in *Mt. HM307*. In *Ms*, sucrose accumulation occurred mainly in the tapRs where the concentration increased 100% in MD plants compared to controls. Conversely, in *Mt. HM307*, the main accumulation occurred in the fibRs, where the concentration of sucrose in WD-treated plants was triple the values of the controls (Fig. 3a, b). Two-way ANOVA indicated a significant interaction between the WD treatment and species in terms of the sucrose concentration of fibRs (Supplementary Table 1). For hexoses, glucose and fructose were significantly accumulated in the fibRs and tapRs of *Mt. HM307*, respectively.

The concentrations of polyalcohols in roots of well-watered plants tended to be higher in the tapRs than in fibRs, with the major difference being the pinitol concentration (Fig. 4c, d). The concentration of pinitol in *Ms* and *Mt. HM307* tapRs was similar, but it significantly increased in the fibRs of *Mt. HM307* under WD conditions. *Mt. HM307* showed a significant accumulation of myo-inositol and pinitol in the fibRs, whereas *Ms* did not show any response. Two-way ANOVA showed a significant interaction between WD treatment and species in terms of myo-inositol and pinitol at the fibR level (Supplementary Table 1). Soluble protein was usually higher in the tapRs than in the fibRs, with this difference particularly marked for *Ms,* with a value 12 mg g DW^{-1} (Fig. 5a, b). *Mt. HM307* showed a lower soluble protein content in the tapRs at approximately 8 mg g DW^{-1} than did *Ms*, but even so, it was higher than that of the fibRs (Fig. 5a, b). MD only modulated the total soluble protein content in the *Ms* roots, with significant differences only at the tapR level. Conversely, the root soluble protein content of *Mt. HM307* was completely unaltered under MD stress (Fig. 5a, b).

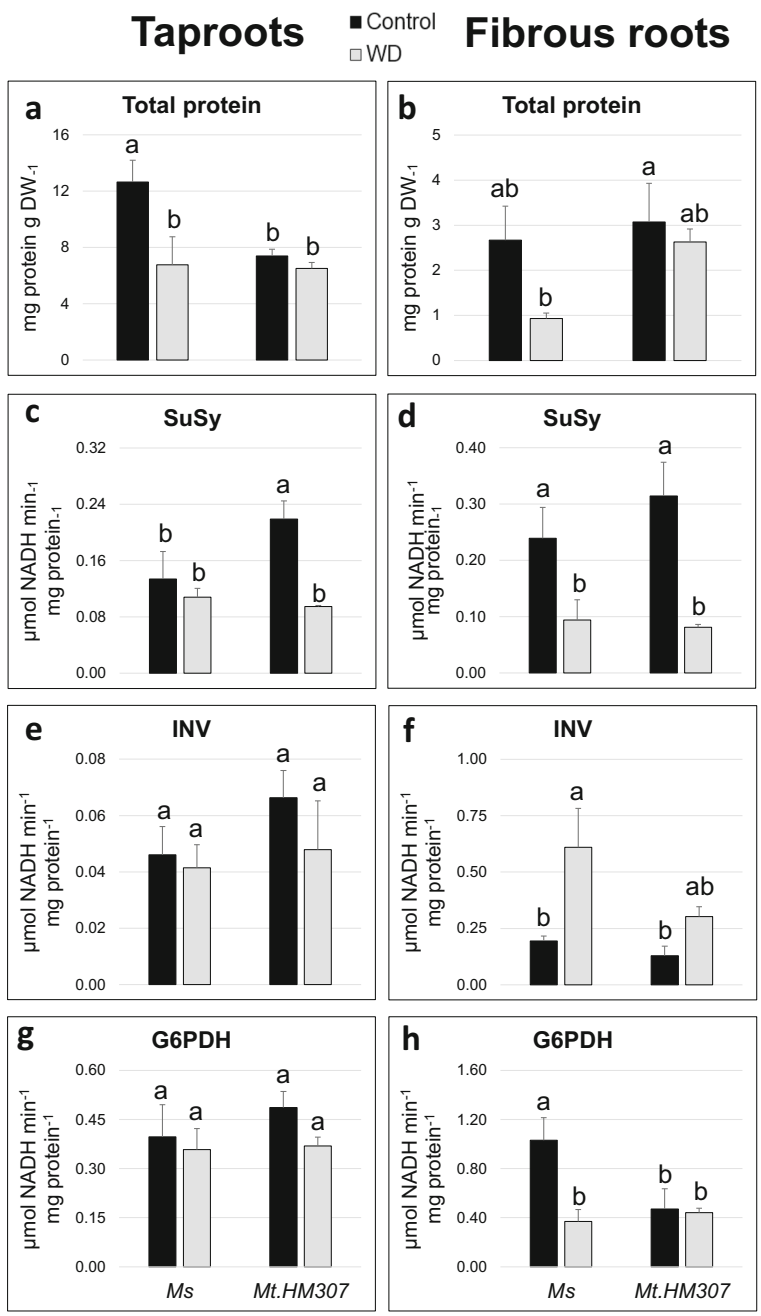

Fig. 5 Total soluble protein of the (**a**) taproots and (**b**) fibrous roots of *Medicago sativa* (*Ms*) and *Medicago truncatula HM307* (*Mt. HM307*) under control and moderate water deficit conditions. Control and WD-treated plants were harvested when WD-treated plants reached MD conditions (Ψ_w, −1.8 MPa). Carbon metabolism-related enzymatic activities (measured as nmol NADH min^{-1} µg $prot^{-1}$) of UDP-SuSy (**c**-**d**), INV (**e**-**f**), and G6PDH (**g**-**h**) measured in the taproots and fibrous roots of *Medicago sativa* and *Medicago truncatula HM307* under control and moderate water deficit conditions. Bars represent the mean ± SE (n = 4). Different letters indicate differences according to Duncan's test ($P \leq 0.05$). UDP-SuSy, UDP-glucose synthase; INV, alkaline invertase; G6PDH, glucose-6-phosphate dehydrogenase. WD*O indicates the interactive effect of the organ and water deficit treatment for each species defined in brackets

With regard to carbohydrate metabolization at the root level, *Ms* and *Mt. HM307*, showed similar sucrose-degrading activities in the tapRs, except for UDP-SuSy, which significantly decreased in *Mt. HM307* (Fig. 5c, e). In the fibRs, sucrose degradation by UDP-SuSy significantly decreased in both *Ms* and *Mt. HM307* (Fig. 5d). Conversely, INV activity increased significantly in *Ms* fibRs (Fig. 5f). Furthermore, INV levels in the fibRs were significantly higher than those in the tapRs under control conditions (Fig. 5e, f); therefore, ANOVA showed a significant interaction between WD and organs in both *Ms* and *Mt. HM307* (Supplementary Table 1). G6PDH, a crucial enzyme in the pentose phosphate pathway, was

significantly higher in *Ms* fibRs under control conditions (Fig. 5g, h). Thus, G6PDH significantly decreased in *Ms* fibRs under MD conditions (Fig. 5h), showing a significant interaction between the WD treatment and organs (Supplementary Table 1).

Discussion

Alfalfa (*Medicago sativa L.*) is a major perennial forage legume crop of agronomical importance in temperate regions and is widely grown in arid and semiarid regions. Although compared to other crops, alfalfa exhibits a substantial drought avoidance strategy linked to its capacity to explore deep soil layers, crop productivity is still affected by drought (Quan et al. 2016; Huang et al. 2018). *M. truncatula* (*Mt*) species far exceed *M. sativa* (*Ms*) in terms of drought tolerance capacity, being naturally present in the Mediterranean basin and cultivated annually in several regions worldwide (Aubert et al. 2006; Phan et al. 2007). The *M. truncatula* Hapmap project provides a rich source of drought tolerance variability (Yoder et al. 2014) that needs to be explored. Based on PEG in vitro assays, Kang et al. (2015) established the drought tolerance of 220 accessions of the Hapmap Project. We selected six of these accessions with differential drought tolerances to test their response to progressively applied and moderate WD conditions in late vegetative stage plants to simulate field conditions. Our results showed a lack of correlation with the in vitro studies performed by Kang et al. (2015) which may be related to the assay system employed in each study. Therefore, *Mt. IDRA,* which was unaffected under in vitro PEG assays, exhibited a 50% reduction in shoot biomass when moderate WD was applied progressively at the late vegetative stage in a growth chamber (Fig. 1b). Conversely, *Mt. HM307*, which was characterized as nontolerant based on in vitro PEG studies (Kang et al. 2015), maintained stable biomass production under moderate WD conditions (Fig. 1b). In addition, *Mt. HM307* and *Ms* exhibited a similar WD response pattern at the physiological level (Figs. S1, 1 and 2). Based on this screening, we compared the WD response of root metabolism in *Mt. HM307* and *Ms* to explore the differential strategies of these closely related forage legumes at the root system level.

Ms is a perennial forage legume and, therefore, possesses a deep taproot with limited fibrous roots (Humphries and Auricht 2001; Radovic et al. 2009; Araújo et al. 2015; Quan et al. 2016). Conversely, *Mt. HM307* develops branched tapRs in the upper part of the root system with abundant fibRs that explore the surrounding soil (Zhang et al. 2014; Castañeda et al. 2019). These size differences are observable in the present comparison, wherein *Ms* showed significantly higher tapRs, and *Mt. HM307* showed more fibRs biomass (Fig. 2a, b, e). MD conditions did not significantly promote root growth, but a slight increase was observed in both species and root types (Fig. 2a, b). Although an increase in the root elongation rate has been widely described in response to drought stress (Chaves et al. 2003; Kang et al. 2011; Araújo et al. 2015; Zhang et al. 2015; Quan et al. 2016), it must be noted that a moderate water deficit over a short time period was assayed in the present study.

The tapRs of *Ms* have been extensively studied and are described as the main storage organ containing the most important soluble nitrogen pools (Erice et al. 2007). The annual life cycle of *Mt. HM307* may explain why these tapRs are only 50% of the size of those of alfalfa (Fig. 2a) and have much less soluble protein (Fig. 5a). Although taproot proteins are known to be used for the regrowth of aerial parts under optimal conditions, drought induced a marked reduction in this nitrogen reserve in alfalfa (Fig. 5a; Erice et al. 2007) being unaffected in *Mt*, which supports the higher drought tolerance of this latter species. Overall, a comparison between the different root types has not been undertaken in alfalfa, and studies on forage legumes are generally scarce. Concerning the different root types of *Mt*, Castañeda et al. (2019) remarked that tapRs showed a higher resilience than fibRs towards water deficit stress, reporting a key role of this primary root in carbon partitioning. Our data reported similar differences in WC in the tapRs and fibRs of both species under WD conditions (Fig. 2c, d). TapRs of *Ms* and *Mt. HM307* was less affected than fibRs in terms of the WC, showing values of approximately 50% in the tapRs that decreased to values of approximately 30% in the fibRs (Fig. 2c, d). Several factors may play a role in this response, as the higher surface to volume ratio of the fibRs favours the dehydration of this tissue under soil water restrictions. On the one hand, the thickness of the root cortex and the suberization characteristics of the exodermis determine the tissue hydraulic conductivity (Rieger and Litvin 1999) favouring water retention by tapRs compared to fibRs. In addition, the dispersion of fibRs in the soil exposes them to dehydrating conditions, creating a protective environment around the main

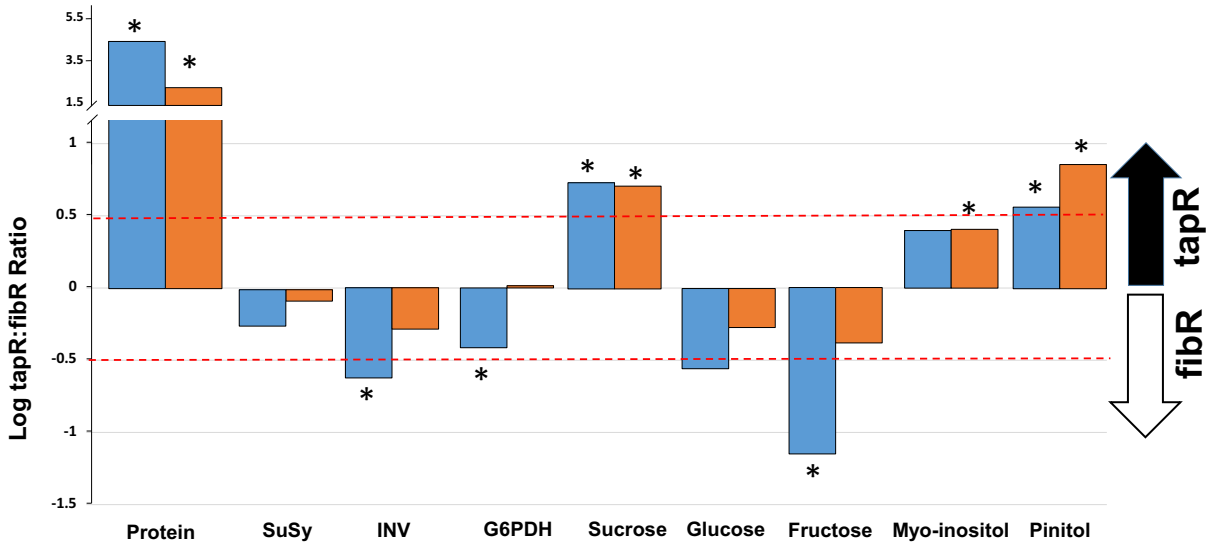

Fig. 6 Distribution of enzymatic activities, and metabolite and protein contents between both root types. Bars represent the log2 of the taproot:fibrous root ratio for each parameter under control conditions. Those parameters more abundant in the taproots (taproots:fibrous root ratio higher than 1.5) were marked as positive, and those more abundant in the fibrous roots (taproots:fibrous root ratio lower than −1.5) were marked as negative. *Medicago sativa* (*Ms*) and *Medicago truncatula* HM307 (*Mt. HM307*) are labelled with blue and orange, respectively. Control and WD-treated plants were harvested when WD-treated plants reached MD conditions (Ψw, −1.8 MPa). Asterisks indicate significant differences for a given parameter between the values of the taproots and the fibrous roots (Student's t test, $P \leq 0.05$)

tapR (Ye et al. 2018). This differential resilience throughout the root system together with the particular anatomy of alfalfa compared to that of *Mt. HM307*, makes it particularly important to examine how primary root metabolism is modulated by WD stress. Maize genotypes with reduced cortex thickness showed improved yield and growth under water stress, opening new strategies in breeding to improve the drought tolerance of cereals (Chimungu et al. 2014).

Figure 6 summarizes the distribution of carbon metabolites and enzymes between the tapRs and fibRs in *Ms* and *Mt. HM307*. In agreement with Castañeda et al. (2019), tapRs contain the main pool of sucrose, whereas hexoses such as glucose and fructose are mainly located in fibRs (Fig. 6), suggesting a high rate of sucrose metabolization in this root type. Overall, this pattern is in agreement with the distribution of carbon metabolism enzyme activities, which were mostly concentrated in fibRs. In root tissue, sucrose is rapidly hydrolysed by invertase into hexoses or cleaved by SuSy into UDP-glucose and fructose to power and support the growth of the roots (Ruan 2014). Although, the role of sucrose cleavage has been traditionally assigned to SuSy, emerging evidence indicates that INV is required for root growth and cell development (Barratt et al. 2009; Welham et al. 2009). Indeed, comparing the *Medicago* species, the hexose content seems to be well correlated with INV and G6PDH enzyme activities in the fibRs. In the A17 *Mt* accession, Castañeda et al. (2019) linked the high sucrose content of tapRs to the higher SuSy activity in this root type. However, SuSy seems to be equally distributed among tapRs and fibRs in alfalfa as it was in *Mt. HM307* in the present study, suggesting a similar role in carbon partitioning for both root types.

Under WD conditions, the carbon metabolism of the root system significantly differed among the studied species (Fig. 7). *Ms* accumulated sucrose mainly at the tapRs whereas *Mt. HM307* restricted its accumulation to fibRs (Fig. 3b). In other genotypes of *Mt*, Castañeda et al. (2019) observed sucrose accumulation in both root types, and linked this to a blockage of SuSy activity in both organs. In addition, the increase in INV activity observed in the fibRs of *Ms* may suggest that carbon metabolism of this species is more affected than that of *Mt* (Fig. 5f). Indeed, a maintenance role has been assigned to INV in those cases when the activities of other sucrose metabolizing enzymes, such as SuSy, are low (Winter and Huber 2000; Barratt et al. 2009). Additionally, cytosolic invertases have been demonstrated to play a role in stress responses involved in reactive

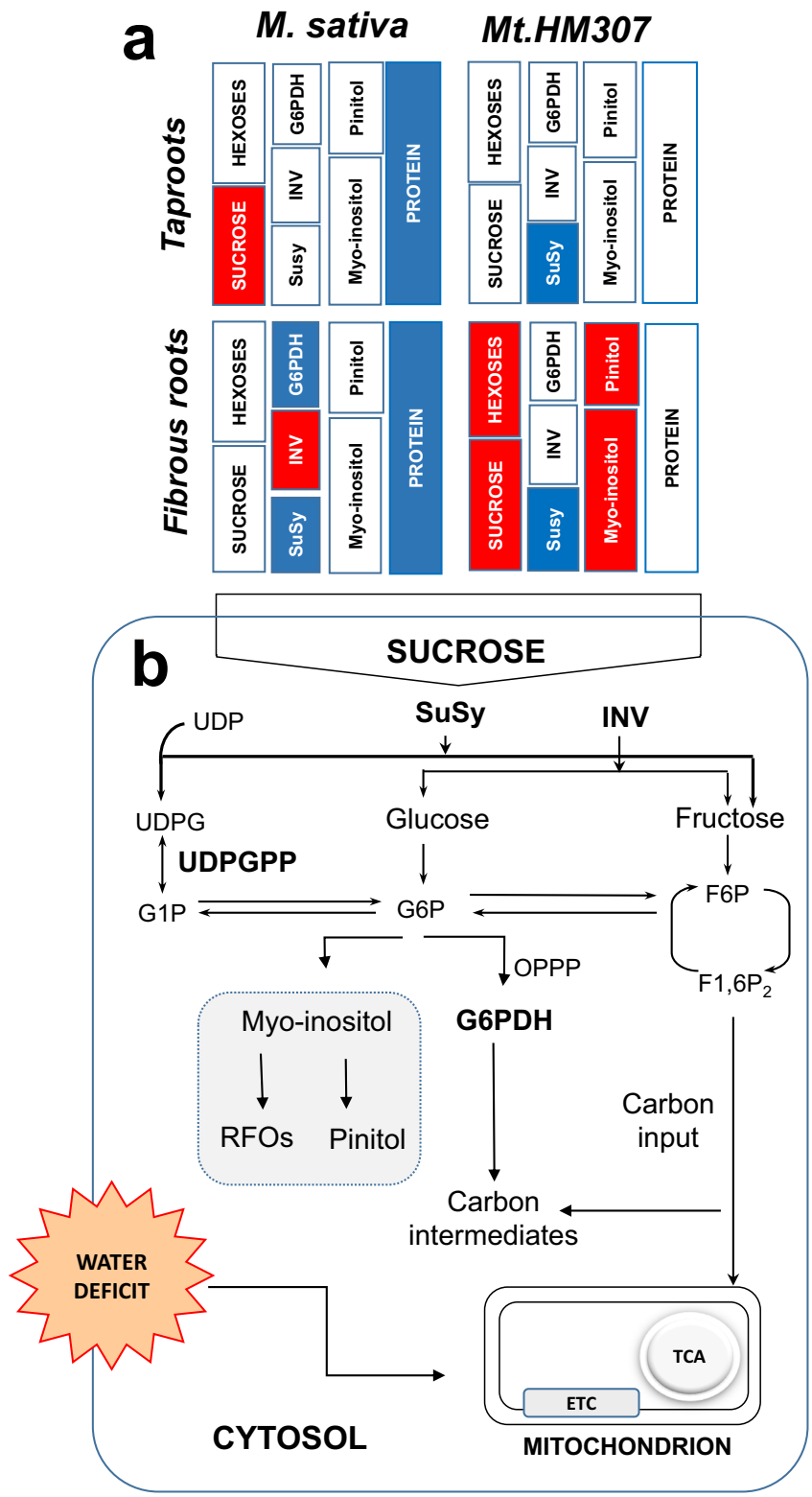

Fig. 7 (**a**) Graphical overview of the water deficit response at the different root systems in *Medicago sativa, Mt.HM307* and *Mt.IDRA*. (**b**) A general overview of carbon metabolism in the root. INV, alkaline invertase; F6P, fructose-6-phosphate; $F1,6P_2$, fructose-1,6-biphosphate; G1P, glucose-1-phosphate; G6P, glucose-6-phosphate; G6PDH, glucose-6-phosphate dehydrogenase; RFOs, raffinose family oligosaccharides; Susy, sucrose synthase; UDP, uridine diphosphate; UDPG, UDP-glucose; UDPGPP, UDP-glucose pyrophosphorylase; 2-OG, 2-oxoglutarate. ETC, Electron Transport Chain; TCA, Tricarboxylic acid cycle

oxygen species homeostasis (Xiang et al. 2011). On the other hand, the decrease in G6PDH, a key enzyme of the pentose phosphate pathway, suggests that the carbon supply to fibRs in *Ms* is affected at these downstream steps of catabolism (Fig. 5h). In *Mt. HM307*, carbon metabolism decreased markedly, as represented by the SuSy activity level in the whole root system, but sucrose accumulation was limited to fibRs (Fig. 5c, d, 3a, b). In nitrogen-fixing legumes, the presence of nodules may provoke a sink effect modulating carbon allocation in the whole root system and explaining the better performance under water deficit stress observed in different species (Lodeiro et al. 2000; Frechilla et al. 2000; Kirova et al. 2008). Carbon metabolism has been shown to be rapidly blocked in nodules (Gonzalez et al. 1998; Gálvez et al. 2005) and carbon competition between roots and nodules may determine root development under water deficit stress. Indeed, the carbon metabolism of alfalfa nodules seems to be less affected by drought than that in other legumes (Naya et al. 2007), which may limit carbon availability for the root system.

D-pinitol and its precursor, myo-inositol, are natural compounds commonly found in most plants (Al-Suod et al. 2017), although legumes are the major natural source (Smith and Phillips 1982; Lahuta et al. 2018). These compounds play a particularly important role in cell functioning, osmoregulation and antioxidation (Loewus and Murthy 2000; Al-Suod et al. 2017). Thus, myo-inositol-derived galactinol and raffinose family oligosaccharides (RFOs), can also function as antioxidants or stress-signalling response molecules that are important in stress tolerance (Valluru and Van den Ende 2011). Indeed, it is known that myo-inositol, sucrose and RFOs, are intimately connected in a regulatory circuit under sugar starvation (Valluru and Van den Ende 2011). This sugar regulatory complex may help plants face different stresses when sugars are limited, contributing to plant growth and homeostasis (Valluru and Van den Ende 2011). In *Medicago* roots, tapRs are the main reservoir of myo-inositol, and particularly pinitol (Figs. 4, 6). In addition, pinitol was found to be a good drought marker in *Mt. HM307*, accumulating significantly in the fibRs but remaining stable in alfalfa (Fig. 4d, 7). The differences in the activation of cyclitol metabolism in the roots between *Ms* and *Mt. HM307* may be explained by the nature of the species, as they are cultivated and wild species, respectively. Although in this study no relevant differences in drought tolerance were observed among *Mt. HM307* and *Ms* at moderate water stress levels, the accumulation of myo-inositol and pinitol in the fibRs of *Mt. HM307* under WD conditions may represent one of the mechanisms acquired over time by *Mt* that make this species more WD tolerant than Ms under more severe stress conditions.

Conclusions

The *M. truncatula* Hapmap project provides a rich source of drought tolerance variability that needs to be explored. The progressive water deficit applied under field-like growth conditions in the present study shows the complexity of the drought tolerance variability exhibited in the *M. truncatula* Hapmap accessions.

Carbon metabolism is finely modulated in *Medicago* species with sucrose catabolizing enzymes controlling carbon partitioning within the root system. This control is differentially exerted in alfalfa and *Mt* at the taproot and fibrous root levels (Fig. 7); therefore, it may determine the drought responses of these two close relatives. *Mt* maintained a more active carbon metabolism in the fibRs, as sucrose, myo-inositol and pinitol accumulated in response to the WD. Conversely, the root system of *Ms* did not accumulate cyclitols and carbon metabolism was more severely affected under water deficit conditions.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s11104-021-04912-1.

Acknowledgements Andres Echeverria was supported by a predoctoral fellowship from the Government of Navarra. We would like to thank Dr. Gustavo Garijo for technical assistance and the seed bank of the *Medicago truncatula* Hapmap Project consortium for supplying seeds.

Declarations

Conflict of interest The authors declare that they have no conflict of interest.

References

Al-Suod H, Ligor M, Raţiu IA et al (2017) A window on cyclitols: characterization and analytics of inositols. Phytochem Lett 20:507–519. https://doi.org/10.1016/j.phytol.2016.12.009

Araújo SS, Beebe S, Crespi M, Delbreil B, González EM, Gruber V, Lejeune-Henaut I, Link W, Monteros MJ, Prats E, Rao I, Vadez V, Patto MCV (2015) Abiotic stress responses in legumes: strategies used to cope with environmental challenges. CRC Crit Rev Plant Sci 34:237–280. https://doi.org/10.1080/07352689.2014.898450

Aubert G, Morin J, Jacquin F, Loridon K, Quillet MC, Petit A, Rameau C, Lejeune-Hénaut I, Huguet T, Burstin J (2006) Functional mapping in pea, as an aid to the candidate gene selection and for investigating synteny with the model legume *Medicago truncatula*. Theor Appl Genet 112:1024–1041. https://doi.org/10.1007/s00122-005-0205-y

Barker DG, Bianchl S, Blondon F et al (1990) *Medicago truncatula*, a model plant for studying the molecular genetics of the rhizobium-legume symbiosis. Plant Mol Biol Rep 8:40–49. https://doi.org/10.1007/BF02668879

Barratt DHP, Derbyshire P, Findlay K, Pike M, Wellner N, Lunn J, Feil R, Simpson C, Maule AJ, Smith AM (2009) Normal growth of Arabidopsis requires cytosolic invertase but not sucrose synthase. PNAS 106:13124–13129. https://doi.org/10.1073/pnas.0900689106

Bradford MM (1976) A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal Biochem 72:248–254. https://doi.org/10.1016/0003-2697(76)90527-3

Castañeda V, de la Peña M, Azcárate L, Aranjuelo I, Gonzalez EM (2019) Functional analysis of the taproot and fibrous roots of *Medicago truncatula*: sucrose and proline catabolism primary response to water deficit. Agric Water Manag 216:473–483. https://doi.org/10.1016/j.agwat.2018.07.018

Chaves MM, Flexas J, Pinheiro C (2009) Photosynthesis under drought and salt stress: regulation mechanisms from whole plant to cell. Ann Bot 103:551–560. https://doi.org/10.1093/aob/mcn125

Chaves MM, Maroco JP, Pereira JS (2003) Understanding plant responses to drought — from genes to the whole plant. Funct Plant Biol 30:239–264. https://doi.org/10.1071/fp02076

Chimungu JG, Brown KM, Lynch JP (2014) Reduced root cortical cell file number improves drought tolerance in maize. Plant Physiol 166:1943–1955. https://doi.org/10.1104/pp.114.249037

Ciereszko I, Kleczkowski LA (2002) Glucose and mannose regulate the expression of a major sucrose synthase gene in *Arabidopsis* via hexokinase-dependent mechanisms. Plant Physiol Biochem 40:907–911. https://doi.org/10.1016/S0981-9428(02)01452-3

Dai A (2011) Drought under global warming: a review. Wiley Interdiscip Rev Clim Chang 2:45–65. https://doi.org/10.1002/wcc.81

Dejardin A, Rochat C, Maugenest S, Boutin J-P (1997) Purification, characterization and physiological role of sucrose synthase in the pea seed coat (*Pisum sativum L.*). Planta 201:128–137. https://doi.org/10.1007/bf01007697

Erice G, Irigoyen JJ, Sánchez-Díaz M, Avice JC, Ourry A (2007) Effect of drought, elevated CO_2 and temperature on accumulation of N and vegetative storage proteins (VSP) in taproot of nodulated alfalfa before and after cutting. Plant Sci 172:903–912. https://doi.org/10.1016/j.plantsci.2006.12.013

Evans HJ (1981) Symbiotic nitrogen fixation in legume nodules. In TC Moore, ed, Research Experiences in Plant Physiology. Springer-Verlag, New York, pp 294–310

Foley JA, Ramankutty N, Brauman KA et al (2011) Solutions for a cultivated planet. https://doi.org/10.1038/nature10452

Frechilla S, Gonzalez EM, Royuela M et al (2000) Source of nitrogen nutrition (nitrogen fixation or nitrate assimilation) is a major factor involved in pea response to moderate water stress. J Plant Physiol 157:609–617. https://doi.org/10.1016/S0176-1617(00)80003-6

Gálvez L, González EM, Arrese-Igor C (2005) Evidence for carbon flux shortage and strong carbon/nitrogen interactions in pea nodules at early stages of water stress. J Exp Bot 56:2551–2561. https://doi.org/10.1093/jxb/eri249

Gargallo-Garriga A, Sardans J, Pérez-Trujillo M, Rivas-Ubach A, Oravec M, Vecerova K, Urban O, Jentsch A, Kreyling J, Beierkuhnlein C, Parella T, Peñuelas J (2014) Opposite metabolic responses of shoots and roots to drought. Sci Rep 4:1–7. https://doi.org/10.1038/srep06829

Gibon Y, Blaesing OE, Hannemann J, Carillo P, Höhne M, Hendriks JHM, Palacios N, Cross J, Selbig J, Stitt M (2004) A robot-based platform to measure multiple enzyme activities in Arabidopsis using a set of cycling assays: comparison of changes of enzyme activities and transcript levels during diurnal cycles and in prolonged darkness. Plant Cell 16:3304–3325. https://doi.org/10.1105/tpc.104.025973

Gonzalez EM, Aparicio-Tejo PM, Gordon AJ, Minchin FR, Royuela M, Arrese-Igor C (1998) Water-deficit effects on carbon and nitrogen metabolism of pea nodules. J Exp Bot 49:1705–1714. https://doi.org/10.1093/jxb/49.327.1705

Haigler C (2001) Carbon partitioning to cellulose synthesis. Plant Mol Biol 47:29–51. https://doi.org/10.1023/A:1010615027986

Hsiao TC, Acevedo E (1974) Plant responses to water deficits, water use efficiency, and drought resistance. Ag Meteorogical 14:59–84

Huang Z, Liu Y, Cui Z, Fang Y, He H, Liu BR, Wu GL (2018) Soil water storage deficit of alfalfa (*Medicago sativa*) grasslands along ages in arid area (China). F Crop Res 221:1–6. https://doi.org/10.1016/j.fcr.2018.02.013

Humphries AW, Auricht GC (2001) Breeding lucerne for Australia's southern dryland cropping environments. Aust J Agric Res 52:153–169. https://doi.org/10.1071/AR99171

Kang Y, Han Y, Torres-Jerez I, Wang M, Tang Y, Monteros M, Udvardi M (2011) System responses to long-term drought and re-watering of two contrasting alfalfa varieties. Plant J 68:871–889. https://doi.org/10.1111/j.1365-313X.2011.04738.x

Kang Y, Sakiroglu M, Krom N, Stanton-Geddes J, Wang M, Lee YC, Young ND, Udvardi M (2015) Genome-wide association of drought-related and biomass traits with HapMap SNPs in *Medicago truncatula*. Plant Cell Environ 38:1997–2011. https://doi.org/10.1111/pce.12520

Kirova E, Tzvetkova N, Vaseva I, Ignatov G (2008) Photosynthetic responses of nitrate-fed and nitrogen-fixing soybeans to progressive water stress. J Plant Nutr 31:445–458. https://doi.org/10.1080/01904160801894988

Koch KE (1996) Carbohydrate-modulated gene expression in plants. https://doi.org/10.1146/annurev.arplant.47.1.509

Lahuta LB, Ciak M, Rybiński W, Bocianowski J, Börner A (2018) Diversity of the composition and content of soluble carbohydrates in seeds of the genus Vicia (Leguminosae). Genet Resour Crop Evol 65:541–554. https://doi.org/10.1007/s10722-017-0552-y

Lipiec J, Doussan C, Nosalewicz A (2013) Effect of drought and heat stresses on plant growth and yield: a review. 2015–2020. https://doi.org/10.2478/intag-2013-0017

Lobell D, Burke M, Tebaldi C, et al (2008) Prioritizing climate change adaptation needs for food security in 2030. 319:607–611. https://doi.org/10.1126/science.1151194

Lodeiro AR, González P, Hernández A et al (2000) Comparison of drought tolerance in nitrogen-fixing and inorganic nitrogen-grown common beans. Plant Sci 154:31–41. https://doi.org/10.1016/S0168-9452(99)00246-0

Loewus FA, Murthy PPN (2000) Myo-inositol metabolism in plants. Plant Sci 150:1–19. https://doi.org/10.1016/S0168-9452(99)00150-8

Lynch JP, Brown KM (2012) New roots for agriculture: exploiting the root phenome. Philos Trans R Soc B Biol Sci 367:1598–1604. https://doi.org/10.1098/rstb.2011.0243

Michaud R (1988) World distribution and historical development. In: Hanson A (ed) Alfalfa and alfalfa improvement, vol. 29. American society of agronomy. Madison, Wisconsin, pp. 259–291

Morell M, Copeland L (1984) Enzymes of sucrose breakdown in soybean nodules. Plant Physiol 74:1030–1034. https://doi.org/10.1104/pp.74.4.1030

Naya L, Ladrera R, Ramos J, González EM, Arrese-Igor C, Minchin FR, Becana M (2007) The response of carbon metabolism and antioxidant defenses of alfalfa nodules to drought stress and to the subsequent recovery of plants. Plant Physiol 144:1104–1114. https://doi.org/10.1104/pp.107.099648

Paez-Garcia A, Motes CM, Scheible WR, Chen R, Blancaflor E, Monteros M (2015) Root traits and phenotyping strategies for plant improvement. Plants 4:334–355. https://doi.org/10.3390/plants4020334

Phan HTT, Ellwood SR, Hane JK, Ford R, Materne M, Oliver RP (2007) Extensive macrosynteny between *Medicago truncatula* and *Lens culinaris* ssp. culinaris. Theor Appl Genet 114:549–558. https://doi.org/10.1007/s00122-006-0455-3

Pignocchi C, Ivakov A, Feil R, Trick M, Pike M, Wang TL, Lunn JE, Smith AM (2020) Restriction of cytosolic sucrose hydrolysis profoundly alters development, metabolism, and gene expression in *Arabidopsis* roots. J Exp Bot 72:1850–1863. https://doi.org/10.1093/jxb/eraa581

Quan W, Liu X, Wang H, Chan Z (2016) Comparative physiological and transcriptional analyses of two contrasting drought tolerant alfalfa varieties. Front Plant Sci 6:1–16. https://doi.org/10.3389/fpls.2015.01256

Radovic J, Sokolovic D, Markovic J (2009) Alfalfa-most important perennial forage legume in animal husbandry. Biotechnol Anim Husb 25:465–475. https://doi.org/10.2298/bah0906465r

Rieger M, Litvin P (1999) Root system hydraulic conductivity in species with contrasting root anatomy. J Exp Bot 50:201–209. https://doi.org/10.1093/jxb/50.331.201

Rothstein SJ (2007) Returning to our roots: Making plant biology research relevant to future challenges in agriculture 19:2695–2699. https://doi.org/10.1105/tpc.107.053074

Ruan Y, Jin Y, Yang Y et al (2010) Sugar input , metabolism , and signaling mediated by invertase: Roles in development , yield potential , and response to drought and heat. Mol Plant 3:942–955. https://doi.org/10.1093/mp/ssq044

Ruan YL (2014) Sucrose metabolism: gateway to diverse carbon use and sugar signaling. Annu Rev Plant Biol 65:33–67. https://doi.org/10.1146/annurev-arplant-050213-040251

Scholander PF, Bradstreet ED, Hemmingsen EA, Hammel HT (1965) Sap pressure in vascular plants: negative hydrostatic pressure can be measured in plants. Science 148:339–346. https://doi.org/10.1126/science.148.3668.339

Sinclair TR (2011) Challenges in breeding for yield increase for drought. Trends Plant Sci 16:289–293. https://doi.org/10.1016/j.tplants.2011.02.008

Smith AE, Phillips DV (1982) Influence of sequential prolonged periods of dark and light on pinitol concentration in clover and soybean tissue. Physiol Plant 54:31–33. https://doi.org/10.1111/j.1399-3054.1982.tb00572.x

Soba D, Zhou B, Arrese-Igor C, Munné-Bosch S, Aranjuelo I (2019) Physiological, hormonal and metabolic responses of two alfalfa cultivars with contrasting responses to drought. Int J Mol Sci 20. https://doi.org/10.3390/ijms20205099

Stanton-geddes J, Paape T, Epstein B, et al (2013) Candidate genes and genetic architecture of symbiotic and agronomic traits revealed by whole-genome , Sequence-Based Association Genetics in *Medicago truncatula* 8:1–9. https://doi.org/10.1371/journal.pone.0065688

Sturm A (1999) Invertases: primary structures, functions and roles in plant development and sucrose partitioning. Plant Physiol 121:1–7. https://doi.org/10.1104/pp.121.1.1

Taiz L, Zeiger E (2010) Plant physiology. Sinauer Associates

Tian H, De Smet I, Ding Z (2014) Shaping a root system: regulating lateral versus primary root growth. Trends Plant Sci 19:426–431. https://doi.org/10.1016/j.tplants.2014.01.007

Valluru R, Van den Ende W (2011) Myo-inositol and beyond - emerging networks under stress. Plant Sci 181:387–400. https://doi.org/10.1016/j.plantsci.2011.07.009

Welham T, Pike J, Horst I, Flemetakis E, Katinakis P, Kaneko T, Sato S, Tabata S, Perry J, Parniske M, Wang TL (2009) A cytosolic invertase is required for normal growth and cell development in the model legume, *Lotus japonicus*. J Exp Bot 60:3353–3365. https://doi.org/10.1093/jxb/erp169

Winter H, Huber SC (2000) Regulation of sucrose metabolism in higher plants: localization and regulation of activity of key enzymes. Crit Rev Biochem Mol Biol 35:253–289. https://doi.org/10.1080/10409230008984165

Xiang L, Li Y, Rolland F, van den Ende W (2011) Neutral invertase, hexokinase and mitochondrial ROS homeostasis: emerging links between sugar metabolism, sugar signaling and ascorbate synthesis. Plant Signal Behav 6:1567–1573. https://doi.org/10.4161/psb.6.10.17036

Ye H, Roorkiwal M, Valliyodan B, Zhou L, Chen P, Varshney RK, Nguyen HT (2018) Genetic diversity of root system architecture in response to drought stress in grain legumes. J Exp Bot 69:3267–3277. https://doi.org/10.1093/jxb/ery082

Yoder JB, Stanton-Geddes J, Zhou P, Briskine R, Young ND, Tiffin P (2014) Genomic signature of adaptation to climate in

Medicago truncatula. Genetics 196:1263–1275. https://doi.org/10.1534/genetics.113.159319

Young ND, Udvardi M (2009) Translating *Medicago truncatula* genomics to crop legumes. Curr Opin Plant Biol 12:193–201. https://doi.org/10.1016/J.PBI.2008.11.005

Zhang C, Shi S, Liu Z, Yang F, Yin G (2019) Drought tolerance in alfalfa (*Medicago sativa* L.) varieties is associated with enhanced antioxidative protection and declined lipid peroxidation. J Plant Physiol 232:226–240. https://doi.org/10.1016/J.JPLPH.2018.10.023

Zhang JY, Cruz De Carvalho MH, Torres-Jerez I et al (2014) Global reprogramming of transcription and metabolism in *Medicago truncatula* during progressive drought and after rewatering. Plant Cell Environ 37:2553–2576. https://doi.org/10.1111/pce.12328

Zhang T, Yu L-X, Zheng P et al (2015) Identification of loci associated with drought resistance traits in heterozygous autotetraploid alfalfa (*Medicago sativa* L.) using genome-wide association studies with genotyping by sequencing. PLoS one 10:e0138931. https://doi.org/10.1371/journal.pone.0138931

Zrenner R, Salanoubat M, Willmitzer L, Sonnewald U (1995) Evidence of the crucial role of sucrose synthase for sink strength using transgenic potato plants (*Solanum tuberosum* L.). Plant J 7:97–107. https://doi.org/10.1046/j.1365-313x.1995.07010097.x