Root traits in *Crambe abyssinica* Hochst and *Raphanus sativus* L. plants are associated with differential tolerance to water deficit and post-stress recovery

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ABSTRACT

Background: Previous studies have shown that *Crambe abyssinica* and *Raphanus sativus* are physiologically tolerant to water deficits; however, there is a lack of information on the mechanisms responsible for their tolerance regarding root morphological characteristics. This study aimed to characterize morphological changes in the root system of *C. abyssinica* and *R. sativus* subjected water deficit, as well as to identify the responses that improve tolerance and post-stress recovery capacity of these plants.

Methods: Independent experiments for each specieswere performed in a controlled greenhouse, where plants were randomly set in a randomized block design with five replicates. Plants of *C. abyssinica* and *R. sativus* were cultivated in pots and exposed to well-watered treatment (WW; 90% water holding capacity–WHC of the substrate) or water deficit (WD; 40% WHC) conditions, at 28 days after planting. The plants were kept under WD for 7, 14, or 21 days with rehydration soon after each episode of water deficit. Assessment of water relations, biomass allocation, leaf and root system morphological characteristics and gas exchange were performed after each period of water deficit and 48 h after rehydration.

Results: The water deficit reduced the water status of both species, and morphological and biomass allocation were not recovered after rehydration. Photosynthesis of *C. abyssinica* decreased with prolonged water deficit, which was also not recovered after rehydration. In *R. sativus*, photosynthesis was not altered by WD for 21 days, and a higher WUE was recorded. Root morphology of *R. sativus* was mainly affected at 14 days of WD, while the traits related to very fine roots increased at 21 days of WD, when compared to WW plants. Thus, *R. sativus* has shown greater tolerance to water deficits mainly due to the presence of very fine roots throughout the period of stress, when compared to *C. abyssinica* in which the fine roots predominated.

INTRODUCTION

Crambe abyssinica Hochst and *Raphanus sativus* L. var. *oleiferus* are non-food crops considered potential oil source for the chemical industry (*Vasconcelos et al., 2018*; *Samarappuli et al., 2020*). The seeds of these species contain 20% (*R. sativus*) to 60% (*C. abyssinica*) of erucic acid in oil content, that makes them raw materials with high manufacturing value for biodiesel production (*Donadon et al., 2015*; *Oliveira et al., 2015*; *Silveira et al., 2019*). The use of renewable energy sources constitutes an environmental advantage by reducing the emission of polluting gases (*Zhu, 2016*).

As observed in other crops, grain yields of *C. abyssinica* and *R. sativus* are subject to the severity of water constraints (*Boiago et al., 2018*; *Stagnari et al., 2018*). The physiological tolerance of these plants to drought stress has already been observed (*de Moura et al., 2018*), and longer periods of water deficit may cause significant reductions in plant growth and development (*Basu et al., 2016*; *Abid et al., 2018*), and consequently, in grain yield (*Rivas et al., 2016*). Plants ability to maintain its physiological functions during and after water deficit relies on adaptive mechanisms of the aerial part and/or root system (*Chen et al., 2016*; *Bristiel et al., 2019*). The severity and duration of the stresses are also important factors (*Basu et al., 2016*).

Physiological and biochemical responses related to drought tolerance in the shoot of plants have already been widely reported, where (*Osmolovskaya et al., 2018*; *Zhu et al., 2020*; *Dhansu et al., 2021*; *Mthembu et al., 2022*) most of these responses are found to be directly related to the ability of the root system to efficiently obtain water and nutrients. However, when it comes to adaptive mechanisms involving root morphology, there are few studies related to drought tolerance compared to plant shoot responses (*Wasaya et al., 2018*). The study of the roots of plants grown in soil is still challenging, either because of the technical difficulties to evaluate this system in the plant *in vivo*, or because of the limitations in collecting the entire root system (*Li et al., 2021*). Nevertheless, understanding the roles of root system architecture and functions are crucial for choosing the best adapted species for cultivation in regions subjected to drought (*Lynch, 2015*).

In addition to the crucial role of water and nutrient absorption, roots are organs that signal drought in the soil (*Vadez, 2014*; *Takahashi et al., 2020*). The drought avoidance strategy in plants maintain an adequate water status through continuous water absorption. Usually this can be noticed on a deeper root system, with greater soil exploration capacity (*Belachew et al., 2018*; *Katuwal, Schwartz & Jespersen, 2020*). This response increases the efficiency of water and nutrient used by plants, reducing the impact of stresses. Thus, a robust and well-developed root system can determine survival and productivity of crops under limiting water conditions (*Koevoets et al., 2016*; *Ramamoorthy et al., 2017*).

Roots traits such specific length, specific surface area, root area-to-leaf area ratio, tissue density, length density, and a smaller root diameter are attributes of the root system involved in adaptation to water deficit (*Comas et al., 2013*; *Wasaya et al., 2018*; *Li et al., 2021*). In this regard, recent studies have focused attention on traits of the root system as

targets for breeding strategies for greater drought tolerance in crops (*White, 2019*; *Xiong et al., 2021*; *Maqbool et al., 2022*).

Several authors claim that the root system bestow drought tolerance to *C. abyssinica* and *R. sativus* (*Falasca et al., 2010*; *Cremonez et al., 2013*; *Dias et al., 2015*; *Bassegio et al., 2016*; *Zanetti et al., 2016*), but few studies have identified which root traits are involved in this response in both species. In this study, we tested two hypotheses: (i) adaptive adjustments in the root morphology of *C. abyssinica* and *R. sativus* increase the tolerance to water deficit and the capacity of plants to recover after stress; and (ii) whether the proportion of thinner fine roots is decisive in differentiating the level of tolerance to water deficit between these species. Thus, this study aimed to characterize morphological changes in the root system of *C. abyssinica* and *R. sativus* subjected to water deficit, as well as to identify the responses which may improve the tolerance and post-stress recovery capacity of these plants.

MATERIAL AND METHODS

Plant material and growth conditions

Independent experiments were performed at the Laboratory of Ecophysiology and Plant Productivity at the Goiano Federal Institute–Rio Verde campus, Goiás, Brazil (17°48′ 07.9″S 50°54′20.7″W), from March to May 2018 for each species in a greenhouse with controlled temperature (~26 °C) and relative humidity (51–75%). Plants of *Crambe abyssinica* Hochst (FMS Brilhante, 2017/2017 crop) and *Raphanus sativus* L. var. oleiferus Mertzg (IPR 116, 2017/2017 crop) were cultivated in 4.65 dm^3 plastic pots (14.0 high diameter × 10.2 base diameter × 35.5 cm height) filled with 4.5 kg of a mixture of Red Latosol soil (LVdf) and sand (2:1). The substrate had the following chemical characteristics: P – 0.7 mg dm^{-3}; K – 13.0 mg dm^{-3}; Ca – 1.54 cmol$_c$ dm^{-3}; Mg – 0.22 cmol$_c$ dm^{-3}; Al – 0.05 cmol$_c$ dm^{-3}; H+Al – 1.3 cmol$_c$ dm^{-3}; S – 3.5 mg dm^{-3}; B – 0.8 mg dm^{-3}; Cu – 1.0 mg dm^{-3}; Fe – 37.8 mg dm^{-3}; Mn – 13.2 mg dm^{-3}; Zn – 0.1 mg dm^{-3}; Na – 6.0 mg dm^{-3}; pH CaCl$_2$ – 5.6; SB – 58%; cation exchange capacity – 3.1 cmol$_c$ dm^{-3}; organic matter – 10.9 g dm^{-3}. The total substrate (0.60 m^3) was fertilized with 82.8 g of formulated NPK 4 - 14 - 08 and 2.4 g of filler dolomitic limestone to increase base saturation to 60%. The water holding capacity (WHC) of the substrate was determined through the saturation of the pots and the moisture was monitored daily by the gravimetric method.

A total of 28 days after planting, plants of *C. abyssinica* and *R. sativus* having three of four pairs of expanded leaves were exposed to well-watered conditions (WW; 90% WHC) and water deficit (WD; 40% WHC) conditions. The plants were kept under WD for 7, 14, and 21 days with rehydration for 48 h soon after each episode of water deficit. Treatments consisted of well-watered plants at 7 (WW7), 14 (WW14) and 21 (WW21) days; water deficit for 7 (WD7), 14 (WD14) and 21 (WD21) days; and rehydrated plants after WD for 7 (RH7), 14 (RH14) and 21 (RH21) days.

The experiments were performed with a randomized block design including five replicates. Each experimental unit consisted of one plant per pot.
Water relations

Predawn leaf water potential (ψ_w) was measured between 04:00 and 06:00 am using a Scholander pressure chamber (model 3005-1412, Soilmoisture Equipment Corp., Goleta, CA, USA). Leaf osmotic potential (ψ_{sl}) and root osmotic potential (ψ_{sr}) were evaluated using a vapor pressure osmometer (VAPRO 5600, VAPRO®, Wescor, UT, USA) according to *Pierre & Arce (2012)*. Osmotic potential values were calculated using the Van't Hoff equation: ($\psi_s = -R \times T \times Cs$, where R is the universal gas constant (0.08205 L atm mol^{-1} K^{-1}), T is the temperature in °K (T °K = T °C + 273) and Cs the solute concentration (M), usually expressed in atmospheres and converted to MPa ($0.987 \approx 0.1$ MPa). Relative water content (RWC) was determined from fresh matter (FM), turgid matter (TM) and dry matter (DM) data obtained by weighing leaf discs according to *Barrs & Weatherley (1962)*, and calculated as RWC (%) = (FM − DM)/ (TM − DM) × 100.

Morphological analysis and biomass allocation

Plants were measured to determine the plant height (PH, cm), stem diameter (SD, mm), nodes number (NN), leaf number (LN), and the main root length (RL, cm). The leaf area (LA, m^2) was calculated using the ImageJ software (Image Processing and Analysis in Java, v. 1.52d, USA). Leaves, stem and roots were separated into paper bags and dried in an air circulation oven at 65 °C until constant weight to obtain the leaf dry matter (LDM, g), stem dry matter (StDM, g) and root dry matter (MSR, g). Shoot dry matter (SDM = LDM + StDM), total dry matter (TDM = SDM + RDM), specific leaf area (SLA), leaf area ratio (LAR) and RDM/SDM were calculated.

The root system was evaluated with digital images obtained by digital camera (6MP; 3264 × 1836 px) using the Safira v1.1 software (Fiber and Root Analysis System; Embrapa Agricultural Instrumentation, São Paulo, Brazil). The roots were classified into three diameter classes: very fine roots (Ø < 0.5 mm), fine roots (0.5 < Ø < 2.0 mm) and large roots (Ø > 2.0 mm), according to the criterion proposed by *Böhm (1979)*. From the data obtained by image processing, the morphological characteristics were determined: mean root diameter (MRD, mm), very fine root length (VFRL, cm), fine root length (FRL, cm), large root length (LRL, cm), total root length (TRL, cm), very fine root surface area (VFRA, cm^2), fine root surface area (FRA, cm^2), large root area (LRA, cm^2), total root surface area (TRA, cm^2), very fine root volume (VFRV, cm^3), fine root volume (FRV, cm^3), large root volume (LRV, cm^3), total root volume (TRV, cm^3), TRV/SDM ratio (cm g^{-1}), specific root length (SRL = TRL/RDM, cm g^{-1}), root thickness, (RT = TRV/VTR, cm cm^{-3}), root tissue density (RTD = RDM/TRV, g cm^{-3}), total root area and leaf area ratio (TRA/LA, m^2 m^{-2}), specific surface area (SSA = TRA/RDM, cm^2 g^{-1}) and root length density (RLD = TRL/V$_{soil}$, cm cm^{-3}).

Gas exchange

Gas exchange analyses were carried out with an infrared gas analyzer (LI-6400XTR, Licor®, Lincoln, Nebraska, EUA). Photosynthetic rate (*A*, μmol CO$_2$ m^{-2} s^{-1}), stomatal conductance (*g*$_s$, mol H$_2$O m^{-2} s^{-1}), transpiration (*E*, mmol H$_2$O m^{-2} s^{-1}) and the relation

between internal and external CO_2 concentration (C_i/C_a) were measured in fully expanded leaves in the middle third of the plant, obtained under constant photosynthetic photon flux density (PPFD, 1000 μmol photons m^{-2} s^{-1}), atmospheric CO_2 concentration (C_a) (~384 μmol mol^{-1}), temperature (~26 °C), and relative humidity (~64%). From these data, the instantaneous water use efficiency (WUE = A/E) was calculated.

Statistical analysis

The data obtained were subjected to ANOVA ($p \leq 0.05$) and treatment means were compared by the Scott-Knott clustering method ($p \leq 0.05$) using the Analysis of Variance System software (SISVAR®, version 5.3). For multivariate analyses, data were scaled bg the means of mean-centering and scaled by the root-mean-square $\sqrt{\sum(x^2/n-1)}$, using the *scale* function in R (*R Core Team, 2021*). Principal Component Analyses (PCAs) were modelled using the package pcaMethods (*Stacklies et al., 2007*) and graphs were generated using FactoMineR and factoextra packages, respectively (*Kassambara & Mundt, 2020*; *Lê, Josse & Husson, 2008*). Initial models were built using data from all period in order to assess an optimal number of Principal Components. By assessing the eigenvalues, the best number of Principal Components (PCs) was #4 for both species (Fig. S1). The R script and data are available at the public repository: https://github.com/candidosobrinhosa/MOURA_LMF_2022.

RESULTS

Water relations

The water deficit decreased the Ψ_w in *C. abyssinica*, which was intensified according to the stress duration (Fig. 1A). The relative water content decreased with increasing in water deficit duration, but the values remained around 80% (Fig. 1D). With rehydration, all characteristics recovered to equal well-watered plants. The exception was the root osmotic potential which decreased in rehydrated plants after 7 days of stress (RH7) *vs*. WW-treated plants (WW7) and WD-treated plants (WD7) (Fig. 1C).

In general, as expected, the duration of water restriction reduced the water status of *R. sativus* plants, which were observed through leaf water potential, leaf osmotic potential, root osmotic potential, and relative water content at 21 days after the onset of water deficit (WD21). Upon rehydration, these characteristics reached values similar to those of the WW plants regardless of the evaluation time. On the other hand, root osmotic potential decreased after rehydration in 7-days WD-treated *R. sativus* plants (RH7) (Fig. 1G).

Morphological traits of the aerial part

The prolonged duration of the water deficit inhibited the growth of *C. abyssinica* plants as seen by reductions in plant height, stem diameter, and node number (Fig. 2). After 7 (WD7) and 14 days (WD14) from the water deficit imposition, reductions in leaf area were observed (Fig. 2E), and the leaf number decreased only after the 14-day stress period (Fig. 2D). Upon rehydration, the *C. abyssinica* plants did not recover their morphological traits (Fig. 2).
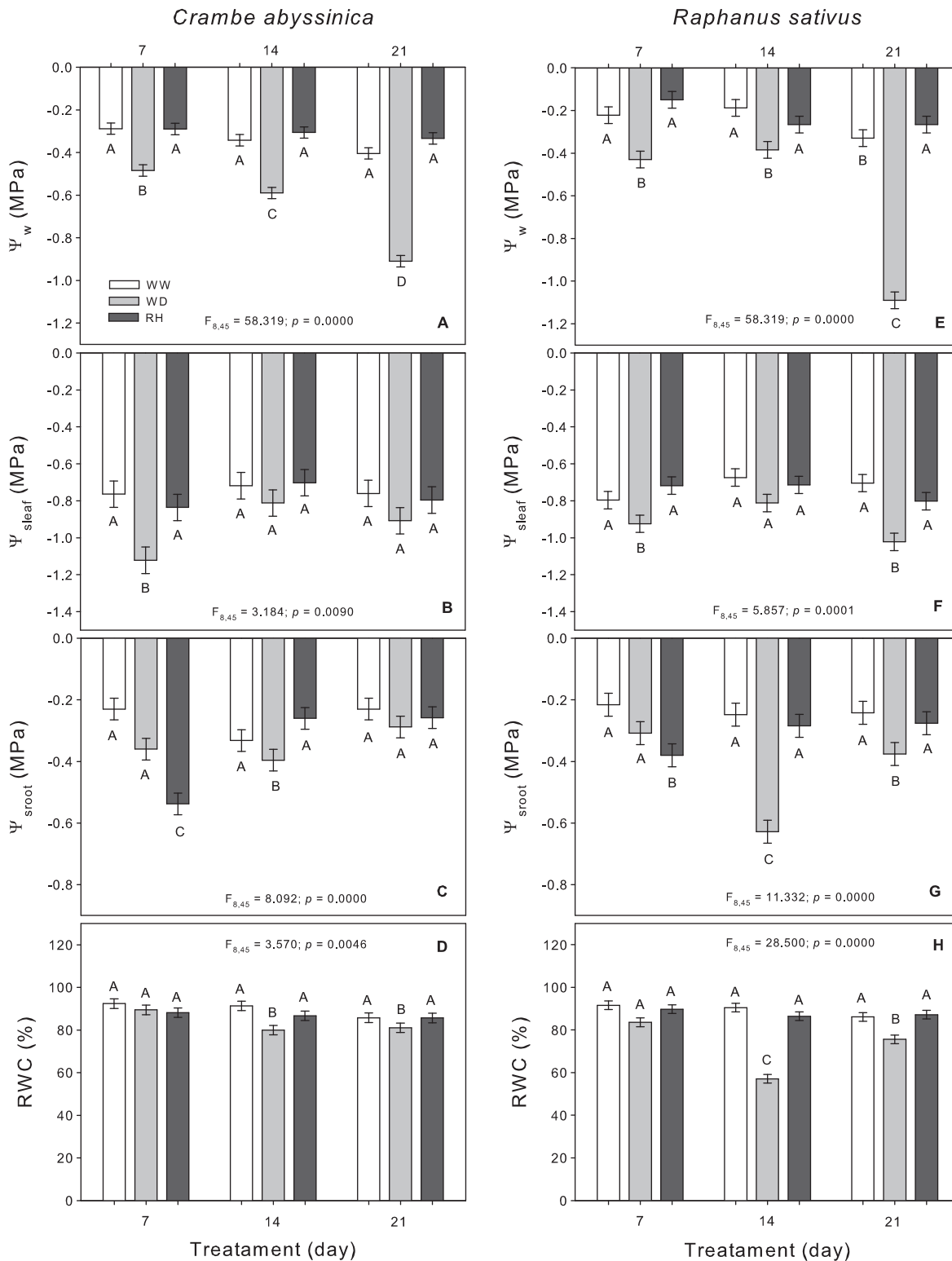
Figure 1 **Water relations in _Crambe abyssinica_ and _Raphanus sativus_.** Leaf water potential (Ψ_w), leaf osmotic potential (Ψ_{sleaf}) (B; F), root osmotic potential (Ψ_{sroot}) (C; G) and relative leaf water content (RWC) (D; H) in _Crambe abyssinica_ (A–D) and _Raphanus sativus_ (E–F) plants under well-watered (WW) and water deficit (WD) for 7, 14 or 21 days, and after rehydration (RH) condition. Bars represent means ± SE (n = 5). Means followed by different letters between treatments differ by the Scott-Knott test (p < 0.05).
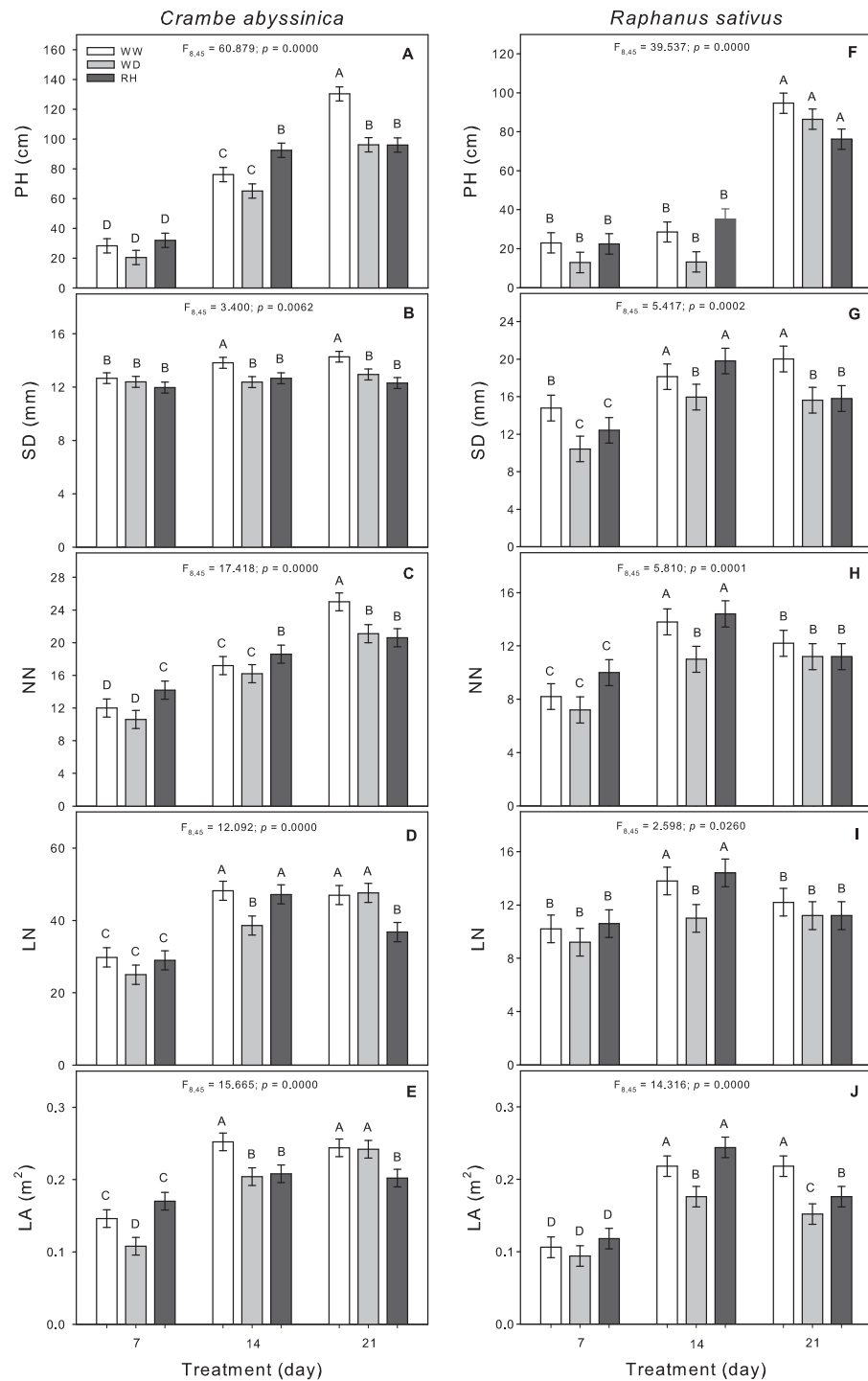
Figure 2 Morphological traits in *Crambe abyssinica* and *Raphanus sativus*. Plant height (PH) (A; F), stem diameter (SD) (B; G), node number (NN) (C; H), leaf number (LN) (D; I), and leaf area (LA) (E; J) in *Crambe abyssinica* (A–E) and *Raphanus sativus* (F–J) plants under well-watered (WW) and water deficit (WD) for 7, 14 or 21 days, and after rehydration (RH) condition. Bars represent means ± SE (*n* = 5). Means followed by different letters between treatments differ by the Scott-Knott test (*p* < 0.05).

The 14-day water deficit caused reductions in stem diameter, node number, leaf number and leaf area in *R. sativus* plants (Figs. 2G–2J). The permanency of stress led to a reduction in stem diameter; but leaf area was maintained in the WD plants. After rehydration (RH21), the effects of the 21-day stress (WD21) on stem diameter and leaf area were not reversed (Figs. 2G and 2J).

Biomass allocation

Changes in the biomass allocation of *C. abyssinica* plants were observed during the initial phase of stress. As the duration of water deficit increased, the shoot dry matter, and total dry matter were reduced in WD-treated plants (Fig. 3). Upon rehydration after a 21-day stress period (RH21), the dry matter of *C. abyssinica* was lower compared to WW plants (WW21) (Fig. 3).

In *R. sativus*, the prolonged duration of the WD inhibited the allocation of biomass in leaves, shoots, and thus, reduced the total dry matter. Shoot and total dry matter of *R. sativus* did not recover in RH21 (Fig. 3).

Morphological traits of the root system

The water deficit caused changes in the architecture of the root system of *C. abyssinica* and *R. sativus* (Tables 1 and 2). In *C. abyssinica* at WD7 treatment there was an increase in the specific surface area (SSA) and very fine roots volume (VFRV) as well as in the rehydrated plants (RH7). Reductions in mean root diameter and increase in fine root length were observed after 14 days of stress. At 21 days, root depth decreased in *C. abyssinica* plants under water deficit. The mean root diameter, and fine and total root volume increased in the rehydrated plants after 14 days of water deficit (RH14). The root length, root surface area, and volume of large roots increased with rehydration after 21 days of water deficit (RH21) (Tables 1 and 2).

The water deficit reduced the mean root diameter at 7 (WD7) and 14 days (WD14) of water deficit in *R. sativus* plants. The length of fine and large roots as well as the surface area and volumes of very fine, fine, large, and total roots were reduced 14 days after the onset of water deficit (WD14). The length of very fine, fine, and total roots; surface area of very fine roots; surface area of total roots; and the volume of very fine roots increased at 21 days in plants under water deficit (WD21) (Table 1). After rehydration, the length, surface area, and volume of fine and large roots, as well as the total root volume increased in the rehydrated plants after 7 days of water deficit (RH7). During rehydration after 14 days of WD (RH14), there was an increase in the mean root diameter and volume of very fine, fine, and total roots, compared to WW plants. Rehydration after 21 days of stress (RH21) caused reductions in fine root length; fine root surface area; and very fine, fine, large, and total root volume, compared to WW plants (Table 1).

Proportion of roots by diameter classes in length, surface area, and volume

A higher proportion of fine roots was observed in *C. abyssinica* after 7 days, regardless of the treatment (Figs. 4A–4C). On the other hand, *R. sativus* showed a greater proportion of
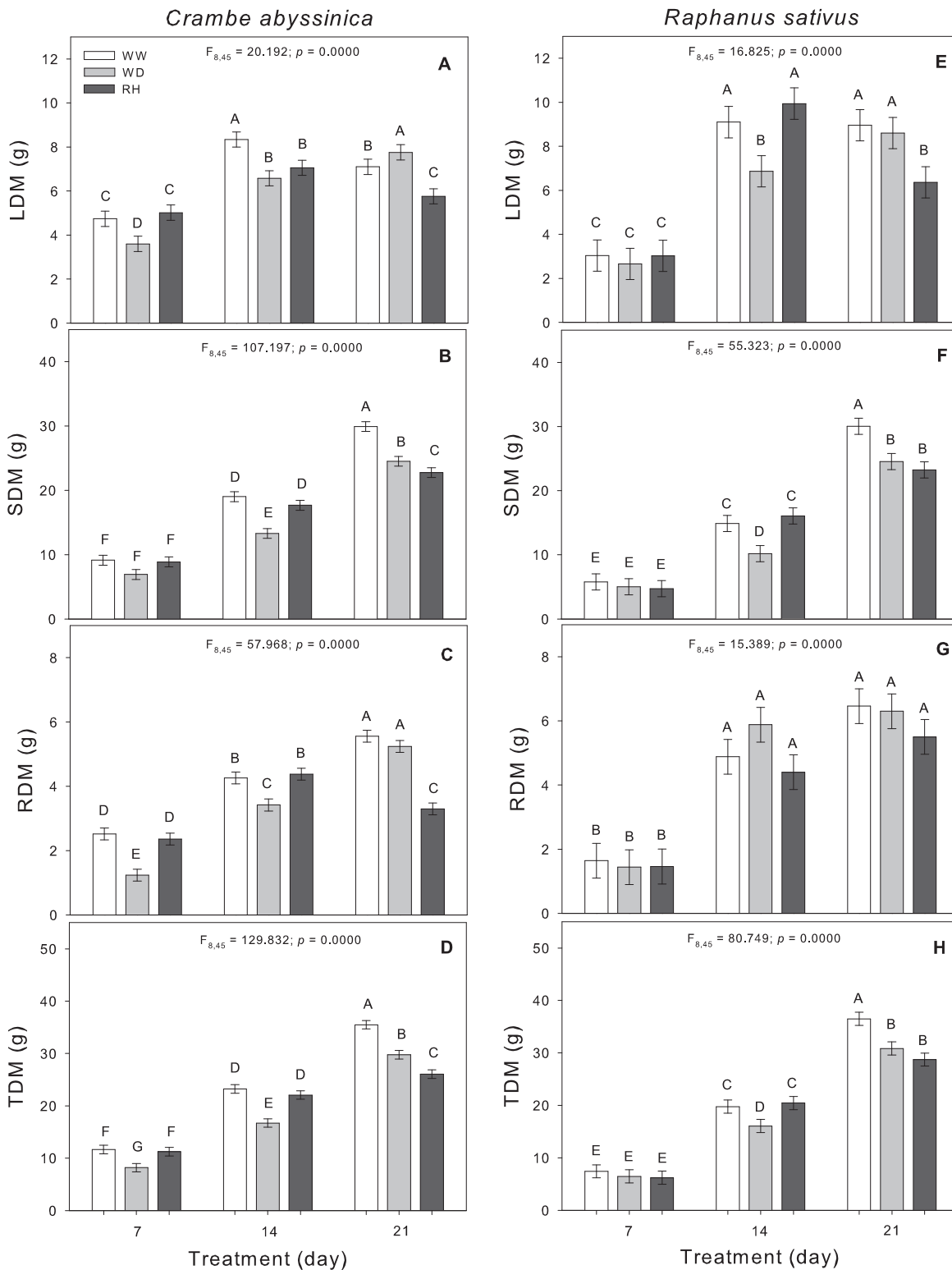
Figure 3 Biomass allocation in *Crambe abyssinica* and *Raphanus sativus*. Leaf dry matter (LDM) (A; E), shoot dry matter (SDM) (B; F), root dry matter (RDM) (C; G), and total dry matter (TDM) (D; H) in *Crambe abyssinica* (A–D) and *Raphanus sativus* (E–H) plants under well-watered (WW) and water deficit (WD) for 7, 14 or 21 days, and after rehydration (RH) condition. Bars represent means ± SE (*n* = 5). Means followed by different letters between treatments differ by the Scott-Knott test (*p* < 0.05).

Table 1 Morphological traits of the root system.

	TREAT	RL	MRD	VFRL	FRL	LRL	TRL	VFRA	FRA	LRA	TRA	VFRV	FRV	LRV	TRV
Crambe abyssinica															
7	WW	50.8 A	0.58 D	266 A	56.6 C	2.2 C	325 B	44.3 B	19.4 B	1. 57 C	65.3 B	0.62 B	0.61 C	0.09 B	1.31 D
	WD	50.3 A	0.59 D	301 A	64.4 C	2.4 C	367 B	53.8 A	22.6 B	1.79 C	78.3 B	0.77 A	0.72 C	0.08 B	1.57 D
	RH	51.2 A	0.57 D	297 A	54.7 C	2.3 C	354 B	51.8 A	20.9 B	1.77 C	74.4 B	0.71 A	0.57 C	0.08 B	1.37 D
14	WW	54.1 A	0.83 B	0.00 B	511 B	5.9 A	517 A	0.00 C	135 A	3.92 A	139 A	0.00 C	2.95 B	0.19 A	3.15 B
	WD	49.0 A	0.72 C	0.00 B	668 A	1.0 D	669 A	0.00 C	158 A	0.65 D	158 A	0.00 C	3.10 B	0.04 C	3.13 B
	RH	51.5 A	0.91 A	0.00 B	503 B	3.8 B	507 A	0.00 C	150 A	2.90 B	152 A	0.00 C	3.61 A	0.17 A	3.78 A
21	WW	50.9 A	0.70 C	0.00 B	596 A	0.5 D	597 A	0.00 C	133 A	0.35 D	133 A	0.00 C	2.35 B	0.02 C	2.37 C
	WD	43.7 B	0.69 C	0.00 B	587 A	0.5 D	587 A	0.00 C	144 A	0.32 D	144 A	0.00 C	2.67 B	0.02 C	2.69 C
	RH	43.2 B	0.71 C	0.00 B	615 A	1.8 C	617 A	0.00 C	144 A	1.23 C	145 A	0.00 C	2.81 B	0.07 B	2.88 C
$F_{8,45}$ value		2.512	36.040	101.139	58.585	27.693	10.469	94.992	46.208	27.224	14.141	92.530	38.835	24.669	3.356
p value		0.0305	0.0003	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	0.0067
Raphanus sativus															
7	WW	39.1 B	0.62 D	401.9 C	70.5 C	3.2 D	475.6 C	71.1 D	27.5 C	2.59 C	101 D	1.32 F	0.89 D	0.15 D	2.36 E
	WD	28.8 C	0.67 C	329.9 C	77.3 C	3.1 D	410.4 C	62.4 D	31.2 C	2.38 C	96.0 D	0.92 F	1.03 D	0.16 D	2.10 E
	RH	45.0 A	0.58 D	402.1 C	165 A	14 B	580.9 C	70.5 D	66.1 B	12.5 B	149 D	0.98 F	2.18 C	0.64 B	3.81 D
14	WW	51.7 A	0.79 B	1,287 A	157 A	24 A	1,468 A	318 A	77.3 A	20.7 A	416 A	6.34 B	3.49 B	1.53 A	11.4 B
	WD	48.2 A	0.68 C	1,243 A	85.1 C	8.1 C	1,336 A	243 B	32.6 C	6.38 C	282 B	3.99 C	1.28 D	0.35 C	5.62 C
	RH	48.1 A	0.91 A	1,220 A	156 A	5.5 D	1,382 A	344 A	91.7 A	5.05 C	441 A	7.74 A	4.85 A	0.66 B	13.3 A
21	WW	49.3 A	0.72 C	640.9 B	115 B	5.3 D	760.7 B	170 C	53.1 B	5.27 C	229 C	2.81 D	2.19 C	0.41 C	5.41 C
	WD	54.7 A	0.66 C	1,230 A	147 A	3.4 D	1,381 A	242 B	61.4 B	3.70 C	307 B	3.92 C	2.53 C	0.22 D	6.68 C
	RH	43.0 A	0.68 C	627.8 B	80.2 C	4.6 D	712.6 B	141 C	36.2 C	3.43 C	180 C	2.20 E	1.44 D	0.22 D	3.87 D
$F_{8,45}$ value		5.302	10.681	27.571	13.713	69.390	26.080	37.880	13.100	31.556	34.358	56.498	18.047	43.371	15.971
p value		0.0003	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001

Notes:

Main root length (RL, cm), mean root diameter (MRD, mm), very fine root length (VFRL, cm), fine root length (FRL, cm), large root length (LRL, cm), total root length (TRL, cm), very fine root surface area (VFRA, cm^2), fine root surface area (FRA, cm^2), large root surface area (LRA, cm^2), total root surface area (TRA, cm^2), very fine root volume (VFRV, cm^3), fine root volume (FRV, cm^3), large root volume (LRV, cm^3), and total root volume (TRV, cm^3) in *Crambe abyssinica* and *Raphanus sativus* plants under well-watered (WW) and water deficit (WD) for 7, 14 or 21 days, and after rehydration (RH) condition. Very fine root (Ø < 0.5 mm), fine root (0.5 < Ø < 2.0 mm) and large root (Ø > 2.0 mm).

Means followed by different letters between treatments, in the columns, differ from each other by the Scott-Knott test (*p* < 0.05).

very fine roots *vs.* fine and large roots over all periods evaluated (Figs. 4D–4F). In this species, there was an increase in very fine roots under conditions of water deficit and rehydration at 14 and 21 days. Low proportions of large roots were observed in both species (Fig. 4).

Morphological analyses of shoot and root system

In *C. abyssinica*, water deficits promoted an increase in the total root length/shoot dry matter ratio (TRL/SDM), specific root length, total root area/leaf area ratio (TRA/LA), and specific surface area. Reductions in root thickness and root tissue density were observed 7 days after the beginning of the WD period. There was an increase in the ratio RDM/SDM, TRL/SDM, specific root length, root thickness, TRA/LA, and specific surface area in a 14-day water deficit (WD14). The increase in the RDM/SDM ratio and the

Table 2 Morphological analyzes of shoot and root system.

DAT	TREAT	SLA	LAR	RDM/SDM	TRL/SDM	SRL	RT	RTD	TRA/LA	SSA	RLD
Crambe abyssinica											
7	WW	31.3 A	12.7 B	0.28 A	35.7 C	133 C	256 A	2.05 A	0.04 B	27.0 C	0.15 B
	WD	29.8 A	13.4 B	0.18 C	52.8 A	307 A	232 B	0.79 B	0.07 A	65.5 A	0.16 B
	RH	33.9 A	15.2 A	0.26 A	39.9 B	151 C	259 A	1.76 A	0.05 B	31.7 C	0.16 B
14	WW	30.6 A	10.9 B	0.23 B	27.2 C	122 C	165 C	1.38 B	0.06 B	32.7 C	0.23 A
	WD	31.0 A	12.3 B	0.26 A	51.5 A	196 B	215 B	1.13 B	0.08 A	46.4 B	0.30 A
	RH	29.7 A	9.53 C	0.25 A	29.0 C	116 C	134 C	1.16 B	0.07 A	34.8 C	0.23 A
21	WW	35.1 A	6.93 C	0.19 C	20.2 C	108 C	256 A	2.42 A	0.05 B	24.1 C	0.27 A
	WD	31.3 A	8.13 C	0.21 B	24.0 C	114 C	222 B	2.02 A	0.06 B	27.8 C	0.26 A
	RH	35.2 A	7.70 C	0.15 C	27.2 C	189 B	214 B	1.15 B	0.07 A	44.3 B	0.27 A
$F_{8,45}$ value		1.712	14.647	9.786	10.687	14.158	12.528	8.379	3.913	12.599	10.662
p value		0.1337	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	0.0026	<0.0001	<0.0001
Raphanus sativus											
7	WW	37.5 A	14.2 B	0.29 B	86.0 B	292 B	203 B	0.71 C	0.10 B	62.4 B	0.21 C
	WD	35.1 A	13.9 B	0.30 B	88.3 B	290 B	195 B	0.69 C	0.12 B	67.9 B	0.18 C
	RH	40.3 A	19.1 A	0.33 B	129 A	401 A	153 C	0.39 C	0.13 B	103 A	0.26 C
14	WW	24.0 B	11.1 C	0.34 B	101 B	308 B	130 C	0.43 C	0.20 A	87.1 A	0.65 A
	WD	25.9 B	11.1 C	0.57 A	135 A	255 B	235 A	1.08 B	0.17 A	52.7 B	0.59 A
	RH	24.9 B	11.9 C	0.28 B	88.2 B	314 B	105 D	0.34 C	0.19 A	100 A	0.61 A
21	WW	24.7 B	5.98 D	0.22 B	25.3 C	125 C	145 C	1.28 A	0.10 B	37.8 C	0.34 B
	WD	18.3 B	4.91 D	0.27 B	57.7 C	227 B	210 B	0.97 B	0.20 A	50.4 B	0.61 A
	RH	28.6 B	6.25 D	0.24 B	30.7 C	131 C	184 B	1.50 A	0.10 B	33.2 C	0.32 B
$F_{8,45}$ value		10.312	31.169	5.641	8.350	10.143	23.226	9.632	4.096	13.688	25.854
p value		<0.0001	<0.0001	0.0002	<0.0001	<0.0001	<0.0001	<0.0001	0.0019	<0.0001	<0.0001

Notes:

Specific leaf area (SLA, $m^2\ kg^{-1}$), leaf area ratio (LAR, $m^2\ kg^{-1}$), root/shoot dry matter ratio (RDM/SDM), total root length/shoot dry matter ratio (TDL/SDM, $cm\ g^{-1}$), specific root length (SRL, $cm\ g^{-1}$), root thickness (RT, $cm\ cm^{-3}$), root tissue density (RTD, $g\ cm^{-3}$), total root area ratio/leaf area (TRA/LA, $m^2\ m^{-2}$), specific surface area (SSA, $cm^2\ g^{-1}$), and root length density (RLD, $cm\ cm^{-3}$) in *Crambe abyssinica* and *Raphanus sativus* plants under well-watered (WW) and water deficit (WD) for 7, 14 or 21 days, and after rehydration (RH) condition.
Means followed by different letters between treatments, in the columns, differ from each other by the Scott-Knott test ($p < 0.05$).

reduction in root thickness were observed at 21 days in plants under a water deficit. Upon rehydration after 14 days of stress, the RDM/SDM and TRA/LA ratios remained higher than the WW plants. The increase in the specific length of root, the TRA/LA, and the specific surface area as well as the reduction in the density of root tissue were observed in *C. abyssinica* rehydrated after 21 days of water deficit (Table 2).

In *R. sativus*, 14 days of water deficit increased the RDM/SDM ratio, TRL/SDM, root thickness, and root tissue density, but the specific surface area decreased in stressed plants. At 21 days of water deficit, there was an increase in the root-specific length, root thickness, TRA/LA, specific surface area, and root length density as well as a reduction in root tissue density. Rehydration after 7 days of water deficit led to an increase in the leaf area ratio, TRL/SDM, specific root length, and specific surface area compared to WW
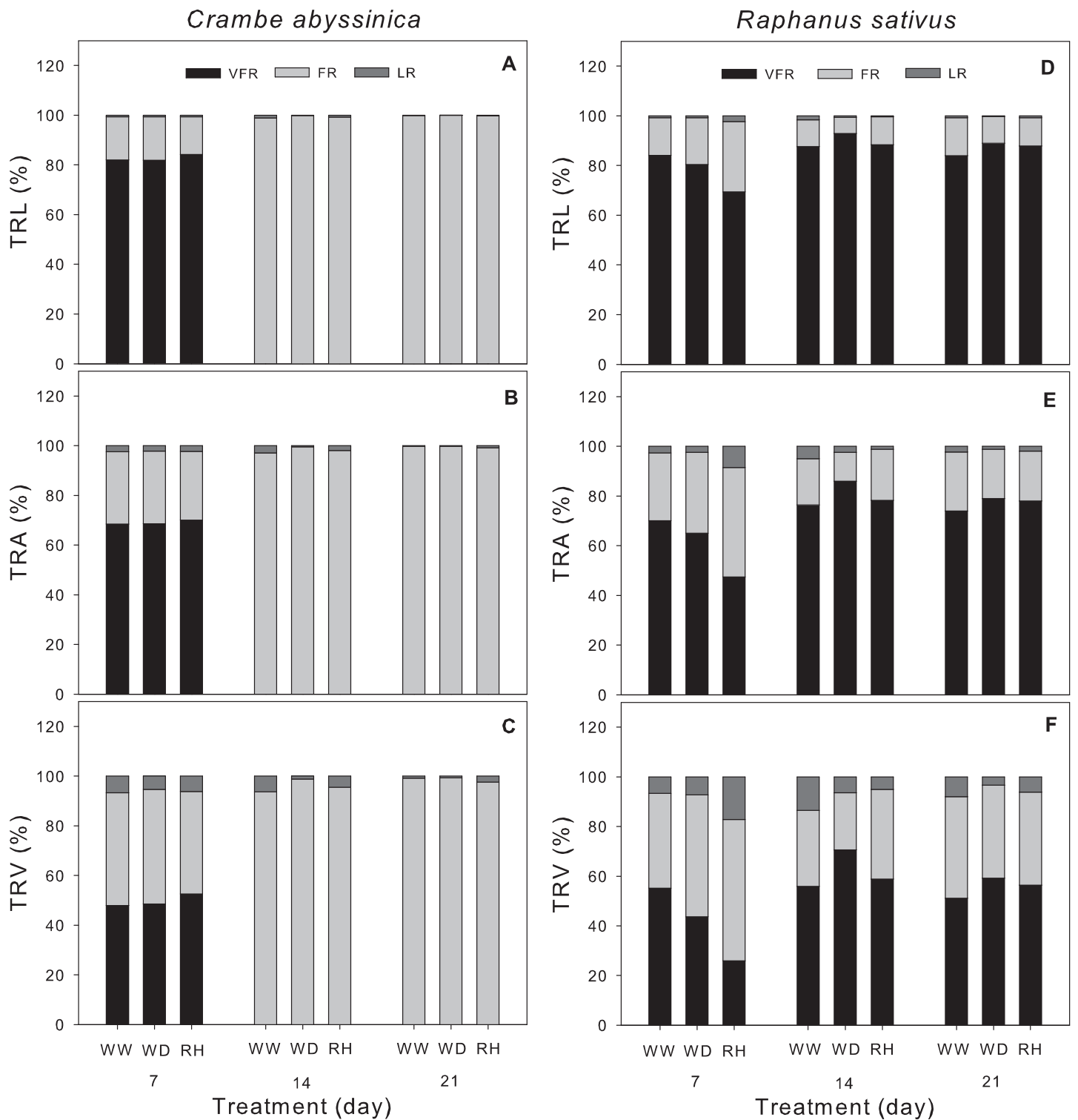
Figure 4 Morphological traits of the root system in *Crambe abyssinica* and *Raphanus sativus*. Proportion of very fine root (VFR), fine root (FR), and large roots (LR) in the total root length (TRL) (A; D), total root surface area (TRA) (B; E), and total root volume (TRV) (C; F) in *Crambe abyssinica* and *Raphanus sativus* plants under well-watered (WW) and water deficit (WD) for 7, 14 or 21 days, and after rehydration (RH) condition. Bars represent means ($n = 5$). Very fine root (Ø < 0.5 mm), fine root (0.5 < Ø < 2.0 mm) and large root (Ø > 2.0 mm).

plants. The effect of stress on other root characteristics was reversed after rehydration (Table 2).

Gas exchange

Reductions in the photosynthetic rate and C_i/C_a were observed in *C. abyssinica* plants as the water deficit persisted (Figs. 5A and 5D). The stomatal conductance decreased only in plants exposed to 14 days of water deficit (WW14) compared to WW plants (Fig. 5B). The reductions observed in *A* and g_S were not reversed after plants rehydration (Figs. 5A and 5B).

In *R. sativus*, the water deficit caused reductions in g_S, *E*, and C_i/C_a (Figs. 5G–5I), which in turn led to an increase in WUE at 21 days after WD imposition (Fig. 5J). Upon rehydration after a period of 14 days under water deficit, there was an increase in *A* but with lower g_S in *R. sativus* plants (Figs. 5F and 5G). The g_S was not fully recovered after the 21-day WD, but the *E* increased compared to WW plants (Figs. 5G and 5H).

Multivariate approach

Principal components (PCs) of both *C. abyssinica* and *R. sativus* were performed for 7, 14 and 21 days after treatments imposition. Based on scaled values, the first two PCs for *C. abyssinica* explained 57.7%, 64.5%, and 56.1% of the total variation for 7, 14 and 21 days after treatments imposition, respectively (Fig. 6). At 7 days, PC1 and PC2 had a higher contribution for SSA, Ψ_{sleaf}, SRL, RTD, and RDM/SDM. At 14 days, both PC1 and PC2 had a higher correlation with large roots, TRA/LA, TRV/SDM, and RWC; and at 21 days with Ψ_w, RTD, SD, WUE, RDM and RL (Fig. 6).

For *R. sativus*, the first two PCs explained 62.0%, 68.1%, and 65.3% of the total variation for 7, 14, and 21 days after treatments imposition (Fig. 7). At 7 days, fine root length and fine root area, TRV/SDM, LRA and RL contributed in greater proportion to PC1 and PC2 (Fig. 7). At 14 days, RWC, root thickness, large roots and fine root volume large provided the mains contributions; and at 21 days, PC1 and PC2 had a strong correlation with Ψ_w, Ψ_{sleaf}, Ci/Ca, WUE TRA/LA, g_S and FRL (Fig. 7).

DISCUSSION

The results of this study confirmed the hypothesis that root changes are involved in the responses of tolerance to water deficit and post-stress recovery in *C. abyssinica* and *R. sativus*. The results showed that *R. sativus* has greater tolerance to water deficit and showed faster recovery of physiological characteristics than in *C. abyssinica* due to the efficiency of its root system. Very fine (*R. sativus*) *vs.* fine roots (*C. abyssinica*) attributes were decisive to determine tolerance levels.

The duration of the water deficit affected the morphological characteristics of *C. abyssinica* and *R. sativus* at different levels. The recovery was determined by the adjustment capacity of these species to the stress. During the 7-days of water deficit, the maintenance of the water status of *C. abyssinica* and *R. sativus* can be mainly attributed to the formation of thinner and larger roots. Fine roots make up a large part of the root system and increase the surface area for water extraction (*McCormack et al., 2015*; *Wasaya*
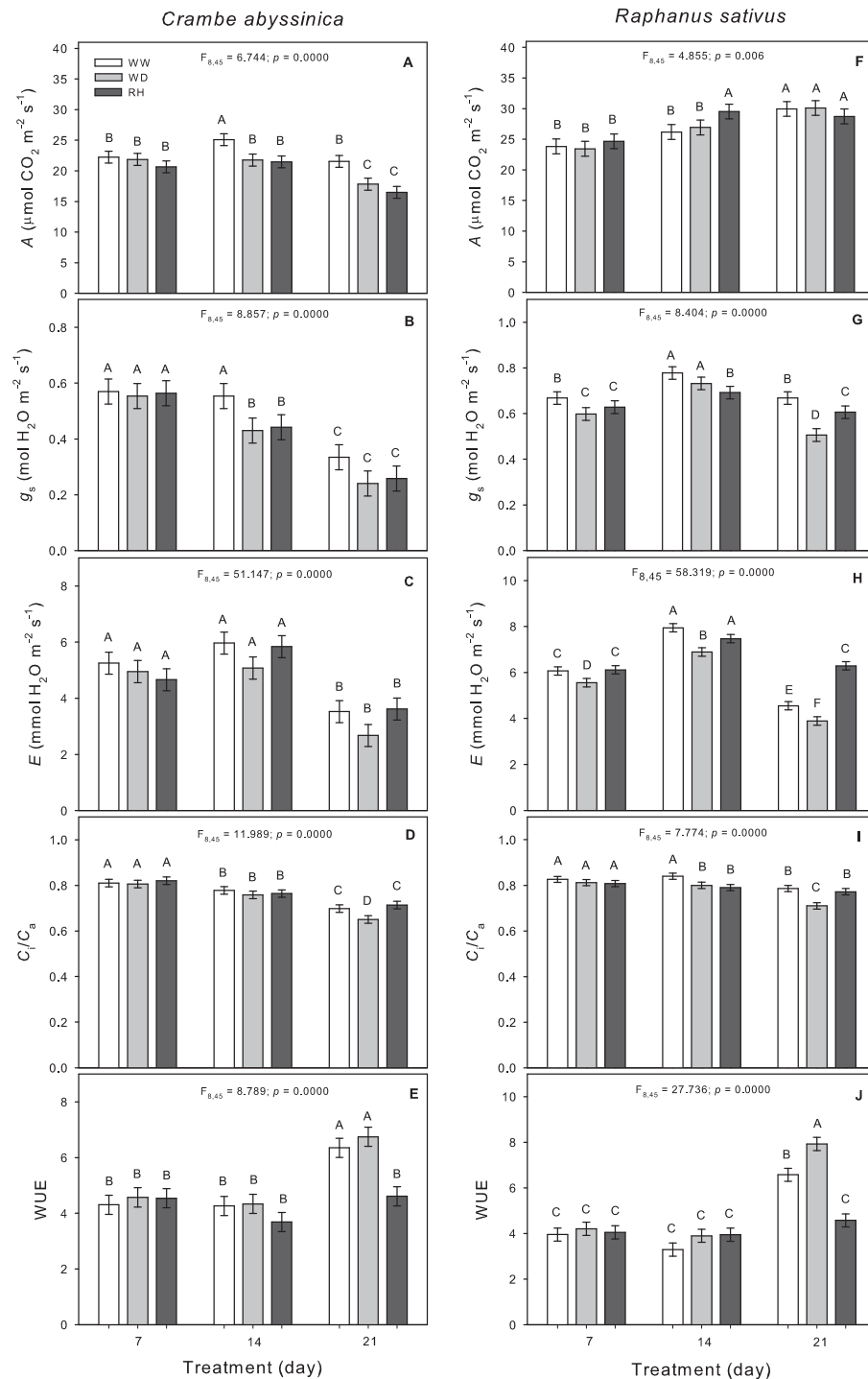
Figure 5 **Gas exchange in *Crambe abyssinica* and *Raphanus sativus*.** Photosynthetic rate (A) (A; F), stomatal conductance (g_s) (B; G), transpiration rate (E) (C; H), relation between internal and external CO_2 concentration (C_i/C_a) (D; I), and water use efficiency (WUE) (E; J) in *Crambe abyssinica* (A–E) and *Raphanus sativus* (F–J) plants under well-watered (WW) and water deficit (WD) for 7, 14 or 21 days, and after rehydration (RH) condition. Bars represent means ± SE ($n = 5$). Means followed by different letters between treatments differ by the Scott-Knott test ($p < 0.05$).
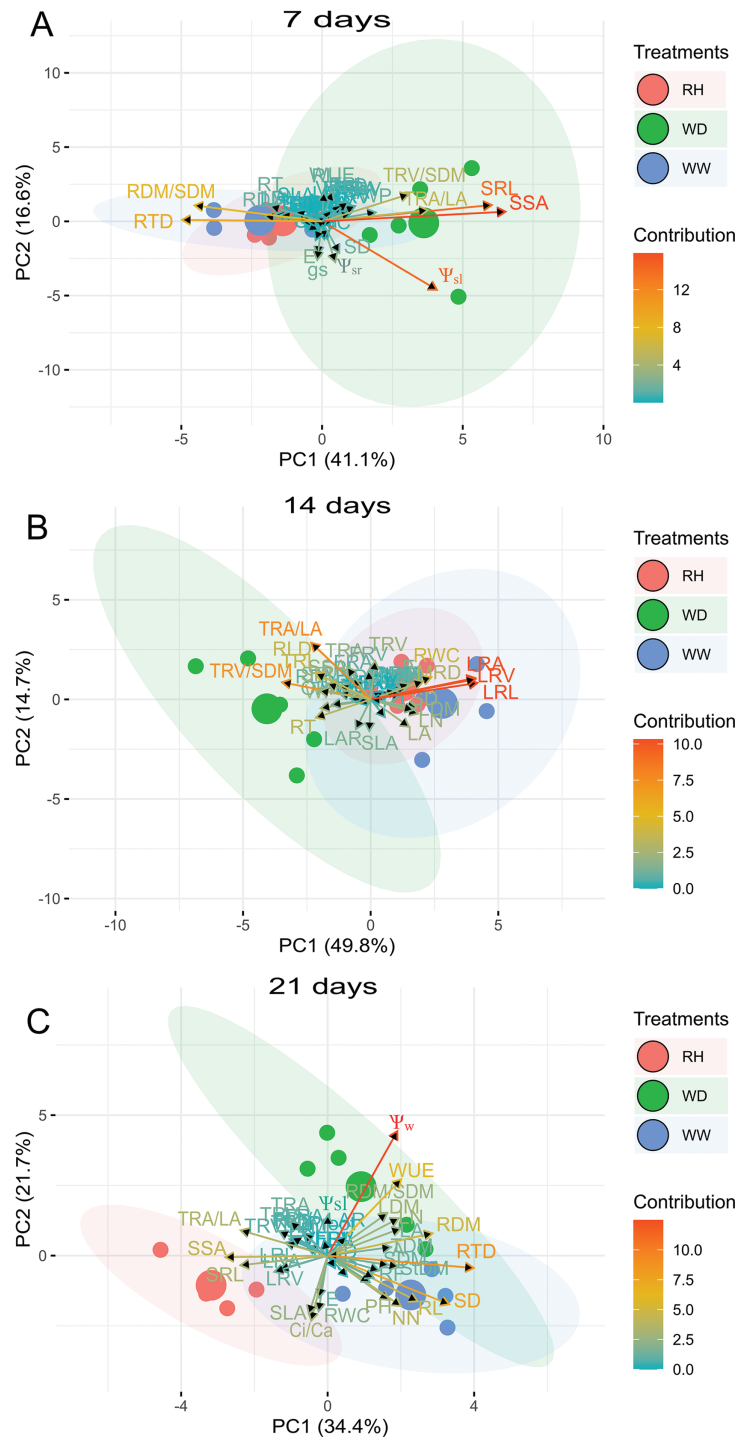
Figure 6 Principal Component Analysis (PCA) of *Crambe abyssinica*. Biplot between PC1 and PC2 showing the contribution of 43 morphological, water relation and gas exchange traits in variability and the segregation of *Crambe abyssinica* plants under well-watered (WW), water deficit (WD) and after rehydration (RH) condition, at 7 (A), 14 (B), and 21 (C) days.
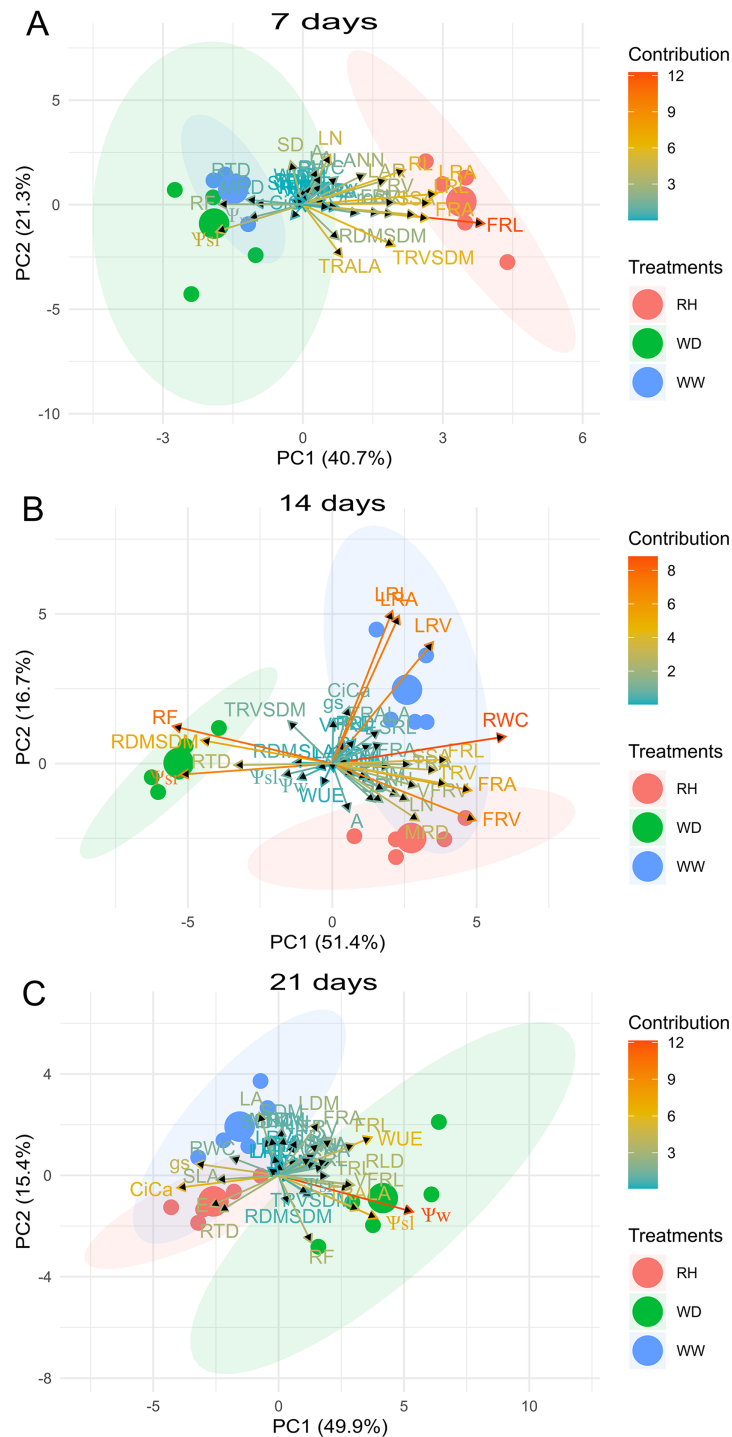
Figure 7 Principal Component Analysis (PCA) of *Raphanus sativus*. Biplot between PC1 and PC2 showing the contribution of 43 morphological, water relation and gas exchange traits in variability and the segregation of *Raphanus sativus* plants under well-watered (WW), water deficit (WD) and after rehydration (RH) condition, at 7 (A), 14 (B), and 21 (C) days.

_et al., 2018_). In _C. abyssinica_, a higher specific length and volume of fine roots were observed in addition to the increase in surface area after 7 days. These characteristics are associated with greater efficiency in water absorption and are reported as drought tolerance strategies in chickpea (_Ramamoorthy et al., 2016_) and wheat (_Awad et al., 2018_) plants. In addition, the investment of _C. abyssinica_ in thinner roots and longer root length associated with the maintenance of higher water content in the leaves suggests adjustment between root length and diameter as a mechanism to increase water uptake (_Comas et al., 2013_).

A higher TRA/LA in _C. abyssinica_ corroborates with an increase in water uptake. Plants capable of maintaining a balance between water uptake by the root system and water loss through transpiration are more tolerant to water deficit (_He et al., 2017_; _Tardieu, Draye & Javaux, 2017_). Furthermore, the maintenance of specific leaf area and leaf area ratio as observed in _C. abyssinica_ indicates that the plant's performance was not impaired even under water deficits. This response corroborates the maintenance of photosynthesis (_Wellstein et al., 2017_).

At the same time that the higher TRL/SDM point to roots as the main drain of photoassimilates produced during stress, the reduction in root tissue density demonstrates the rapid expansion of the root system but with a low accumulation of dry matter (_Birouste et al., 2014_). The root tissue density controls the specific root length and specific surface area, thus increasing water uptake under water deficit (_Wasaya et al., 2018_). After rehydration, _C. abyssinica_ reestablished physiological functions, which allowed the resumption of plant growth. Similar results were observed by _Braga et al. (2021)_ which reported that _C. abyssinica_ plants maintained the photosynthesis performance under drought by increasing its water conductivity and water use efficiency.

Photosynthesis was limited in _C. abyssinica_ during the 14 and 21-days of water deficit, associated with a reduction in leaf tissue hydration and constant water use efficiency. The maintenance of WUE in this case is associated with greater investment in roots as corroborated by a higher RDM/SDM and fine root length. There were also root morphological characteristics that contribute to tolerance to water deficit through efficiency in water acquisition and use as previously reported by _Koevoets et al. (2016)_. Furthermore, stomatal closure is an important strategy to prevent water loss by plants (_Chaves et al., 2016_; _Yan, Zhong & Shangguan, 2016_; _Braga et al., 2021_). This can also reduce shoot dry matter to minimize water loss (_He et al., 2017_; _da Cruz et al., 2019_).

In _R. sativus_, photosynthesis and growth were not compromised by the 7-day water deficit possibly due to the maintenance of leaf tissue hydration as observed by the higher relative water content—a characteristic of plants tolerant to drought (_Zegaoui et al., 2017_). With rehydration after water deficit for 7 days, the increase in length, surface area, and volume of fine roots demonstrated greater efficiency in water absorption in _R. sativus_ (_Bristiel et al., 2019_).

R. sativus maintained photosynthesis despite the reduction in the relative water content of the leaves. This suggests that the tissue water content was sufficient to support the
photosynthetic metabolism with the persistence of the water deficit, which was observed by the osmotic adjustment and increase in water use efficiency at 21 DAT. This led to changes in the anatomy of the leaves that are important indicators of the level of tolerance to water deficit (*Souza et al., 2018*). The specific leaf area and the leaf area ratio characteristics are indicative of drought-tolerant genotypes when maintained during 14 and 21 days of water deficit as observed in chickpea plants (*Wellstein et al., 2017*).

The mechanisms used by *R. sativus* for the maintenance of water status after 14 and 21 days of water deficits were also involved with an increase in dry matter and reductions in leaf area and root diameter. The maintenance of root dry mater seen in *R. sativus* (WD14 and WD21) and *C. abyssinica* (WD21) indicates the capacity of these plants to tolerate the stress due to the ability to develop roots and/or maintain them. This response is associated with greater adaptation to drought conditions (*Comas et al., 2013*; *Koevoets et al., 2016*). Furthermore, the increase in root tissue density during a 14-day of water deficit concomitant with the reduction in specific surface area and maintenance of the root length in *R. sativus*, demonstrate the occurrence of longer roots. Interestingly, these features are seen without an increase in volume of explored soil, which may be associated with water conservation as a strategy to tolerate drought (*Luke Mccormack et al., 2012*). In contrast, the reduction in the root tissue density and the increase in the specific surface area and root length density—together with the maintenance of the root length observed during the water deficit for 21 days in *R. sativus*—indicate the development of roots with a low metabolic cost, but with a greater volume of soil explored. This shows that water acquisition is a dehydration-prevention strategy (*White & Snow, 2012*; *Bristiel et al., 2019*). In this case, the root alterations of *R. sativus* demonstrated the use of different adaptive strategies in response to the duration of the water deficit.

The ability to fully recover photosynthesis after water deficit can be considered an indication of greater tolerance to drought (*Challabathula, Zhang & Bartels, 2018*; *Zhang et al., 2018*). *C. abyssinica* could not recover photosynthesis after reestablishing water conditions, despite the use of root strategies to increase water acquisition. A similar response was observed in bean plants exposed to prolonged water deficit and subsequent rehydration (*Widuri et al., 2018*). However, *R. sativus* showed a higher rate of plant recovery after stress in addition to tolerating the water deficit for 14 and 21 days. The growth was partially recovered in the two species studied.

The similarity between the adaptive mechanisms employed by *C. abyssinica* and *R. sativus* to control water use and consequently maintain physiological activity under water deficit, indicate that the characteristics of the roots were the most determinant to differentiate drought tolerance of plants—especially root diameter (*Koevoets et al., 2016*; *Awad et al., 2018*). Thus, the formation of fine roots in *C. abyssinica* and very fine roots in *R. sativus* constituted key points for the differentiation of physiological performance between the species (*McCormack et al., 2015*; *Ramamoorthy et al., 2016*).

Thinner roots are usually associated with greater volume of soil explored by the larger root surface (*Carminati et al., 2017*; *Ahmed, Passioura & Carminati, 2018*). In addition, thinner roots have greater permeability, thus leading to increased efficiency in water acquisition and use (*McCormack et al., 2015*; *Wasaya et al., 2018*). This favors better

adaptation of plants to drought conditions (*Pierret et al., 2007*; *Bristiel et al., 2019*) as observed in *R. sativus*.

CONCLUSIONS

Water restriction affected both *C. abyssinica* and *R. sativus* plants, causing changes in their water status and reduction in growth and biomass allocation. Both species have root morphological traits that improve the water uptake. In this sense, the main traits involved in the water deficit tolerance of the two species were the smallest root diameter, and the total length of fine roots (*C. abyssinica*) or very fine roots (*R. sativus*). The ability of plants to recover from stress was influenced by the duration of the water deficit and was less efficient after 21 days. Thus, the rehydration was not effective for recovering photosynthesis and growth of *C. abyssinica*. The lack of very fine roots, more efficient in absorbing water and nutrients, contributed to this response. On the other hand, *R. sativus* maintained very fine roots throughout the water deficit period and showed greater stress tolerance and faster recovery of physiological functions after rehydration.

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Competing Interests

The authors declare that they have no competing interests.

Author Contributions

- Luciana Minervina de Freitas Moura conceived and designed the experiments, performed the experiments, analyzed the data, prepared figures and/or tables, authored or reviewed drafts of the article, and approved the final draft.
- Alan Carlos da Costa conceived and designed the experiments, authored or reviewed drafts of the article, and approved the final draft.
- Roberto Gomes Vital performed the experiments, prepared figures and/or tables, and approved the final draft.
- Adinan Alves da Silva conceived and designed the experiments, analyzed the data, prepared figures and/or tables, authored or reviewed drafts of the article, and approved the final draft.
- Arthur de Almeida Rodrigues performed the experiments, prepared figures and/or tables, and approved the final draft.
- Silvio Alencar Cândido-Sobrinho analyzed the data, prepared figures and/or tables, and approved the final draft.
- Caroline Müller conceived and designed the experiments, analyzed the data, prepared figures and/or tables, authored or reviewed drafts of the article, and approved the final draft.

Data Availability

The raw measurements are available in the Supplemental Files.

The R script and data are available at GitHub: https://github.com/candidosobrinhosa/MOURA_LMF_2022.

Supplemental Information

Supplemental information for this article can be found online at http://dx.doi.org/10.7717/peerj.13595#supplemental-information.

REFERENCES

Abid M, Ali S, Qi LK, Zahoor R, Tian Z, Jiang D, Snider JL, Dai T. 2018. Physiological and biochemical changes during drought and recovery periods at tillering and jointing stages in wheat (*Triticum aestivum* L.). *Scientific Reports* **8(1)**:1–15 DOI 10.1038/s41598-018-21441-7.

Ahmed M, Passioura J, Carminati A. 2018. Hydraulic processes in roots and the rhizosphere pertinent to increasing yield of water-limited grain crops: a critical review. *Journal of Experimental Botany* **69(13)**:3255–3265 DOI 10.1093/jxb/ery183.

Awad W, Byrne PF, Reid SD, Comas LH, Haley SD. 2018. Great plains winter wheat varies for root length and diameter under drought stress. *Agronomy Journal* **110(1)**:226–235 DOI 10.2134/agronj2017.07.0377.

Barrs HD, Weatherley PE. 1962. A re-examination of the relative turgidity technique for estimating water deficits in leaves. *Australian Journal of Biological Sciences* **15(3)**:413–428 DOI 10.1071/BI9620413.

Bassegio D, Zanotto MD, Santos RF, Werncke I, Dias PP, Olivo M. 2016. Oilseed crop crambe as a source of renewable energy in Brazil. *Renewable and Sustainable Energy Reviews* **66(1)**:311–321 DOI 10.1016/j.rser.2016.08.010.

Basu S, Ramegowda V, Kumar A, Pereira A. 2016. Plant adaptation to drought stress. *F1000Research* **5**:1–10 DOI 10.12688/f1000research.

Belachew KY, Nagel KA, Fiorani F, Stoddard FL. 2018. Diversity in root growth responses to moisture deficit in young faba bean (*Vicia faba* L.) plants. *PeerJ* **6**:e4401 DOI 10.7717/peerj.4401.

Birouste M, Zamora-Ledezma E, Bossard C, Pérez-Ramos IM, Roumet C. 2014. Measurement of fine root tissue density: a comparison of three methods reveals the potential of root dry matter content. *Plant and Soil* **374(1–2)**:299–313 DOI 10.1007/s11104-013-1874-y.

Böhm W. 1979. *Methods of studying root systems*. New York: Springer-Verlag, 188.

Boiago NP, Fortes AMT, Coelho SRM, Cassol FDR, Pilatti DM. 2018. Morphophysiological and nutritional characteristics of *Crambe abyssinica* Hochst under hydric restriction in different phenological stages. *Acta Physiologiae Plantarum* **40(6)**:99 DOI 10.1007/s11738-018-2678-8.

Braga PCS, Martins JPR, Bonomo R, Falqueto AR. 2021. Morphophysiological responses of *Crambe abyssinica* Hochst. lineages submitted to water deficit during flowering. *Photosynthetica* **59(4)**:486–495 DOI 10.32615/ps.2021.039.

Bristiel P, Roumet C, Violle C, Volaire F. 2019. Coping with drought: root trait variability within the perennial grass *Dactylis glomerata* captures a trade-off between dehydration avoidance and dehydration tolerance. *Plant and Soil* **434(1–2)**:327–342 DOI 10.1007/s11104-018-3854-8.

Carminati A, Passioura JB, Zarebanadkouki M, Ahmed MA, Ryan PR, Watt M, Delhaize E. 2017. Root hairs enable high transpiration rates in drying soils. *New Phytologist* **216(3)**:771–781 DOI 10.1111/nph.14715.

Challabathula D, Zhang Q, Bartels D. 2018. Protection of photosynthesis in desiccation-tolerant resurrection plants. *Journal of Plant Physiology* **227**:84–92 DOI 10.1016/j.jplph.2018.05.002.

Chaves MM, Costa JM, Zarrouk O, Pinheiro C, Lopes CM, Pereira JS. 2016. Controlling stomatal aperture in semi-arid regions—The dilemma of saving water or being cool? *Plant Science* **251**:54–64 DOI 10.1016/j.plantsci.2016.06.015.

Chen D, Wang S, Cao B, Cao D, Leng G, Li H, Yin L, Shan L, Deng X. 2016. Genotypic variation in growth and physiological response to drought stress and re-watering reveals the critical role of recovery in drought adaptation in maize seedlings. *Frontiers in Plant Science* **6(151)**:1–15 DOI 10.3389/fpls.2015.01241.

Comas LH, Becker SR, Cruz VMV, Byrne PF, Dierig DA. 2013. Root traits contributing to plant productivity under drought. *Frontiers in Plant Science* **4**:1–16 DOI 10.3389/fpls.2013.00442.

Cremonez PA, Feiden A, Cremonez FE, De Rossi E, Antonelli J, Nadaleti WC, Tomassoni F. 2013. Nabo forrageiro: do cultivo a produção de biodiesel. *Acta Iguazu* **2**:64–72 DOI 10.48075/actaiguaz.v2i2.8392.

da Cruz YC, Scarpa ALM, Pereira MP, de Castro EM, Pereira FJ. 2019. Growth of Typha domingensis as related to leaf physiological and anatomical modifications under drought conditions. *Acta Physiologiae Plantarum* **41(5)**:1–9 DOI 10.1007/s11738-019-2858-1.

de Moura LMF, Costa AC, Müller C, de Filho ROS, Almeida GM, Vital RG, de Castro JN, Teixeira MB. 2018. Drought tolerance in potential oilseed plants for biofuel production. *Australian Journal of Crop Science* **12(2)**:289–298 DOI 10.21475/ajcs.18.12.02.pne836.

Dhansu P, Kulshreshtha N, Kumar R, Raja AK, Pandey SK, Goel V, Ram B. 2021. Identification of drought-tolerant co-canes based on physiological traits, yield attributes and drought tolerance indices. *Sugar Tech* **23(4)**:747–761 DOI 10.1007/s12355-021-00967-7.

Dias PP, Secco D, Santos RF, Bassegio D, Santos FS, Silva PRA, de Sousa SFG, da Correia TPS. 2015. Soil compaction and drought stress on shoot and root growth in crambe (*Crambe abyssinica*). *Australian Journal of Crop Science* **9**:378–383.

Donadon JR, Bessa JFV, Resende O, Alves RMV, de Sales JF, Costa LM. 2015. Storage of crambe seeds in different containers and environments: part I - physiological quality. *Revista Brasileira de Engenharia Agrícola e Ambiental* **19(3)**:224–230 DOI 10.1590/1807-1929/agriambi.v19n3p231-237.

Falasca SL, Flores N, Lamas MC, Carballo SM, Anschau A. 2010. *Crambe abyssinica*: an almost unknown crop with a promissory future to produce biodiesel in Argentina. *International Journal of Hydrogen Energy* **35(11)**:5808–5812 DOI 10.1016/j.ijhydene.2010.02.095.

He J, Du YL, Wang T, Turner NC, Yang RP, Jin Y, Xi Y, Zhang C, Cui T, Fang XW, Li FM. 2017. Conserved water use improves the yield performance of soybean (*Glycine max* (L.) Merr.) under drought. *Agricultural Water Management* **179(21)**:236–245 DOI 10.1016/j.agwat.2016.07.008.

Kassambara A, Mundt F. 2020. factoextraCRAN. *Available at* https://cran.r-project.org/web/packages/factoextra/index.html (accessed 14 September 2021).

Katuwal KB, Schwartz B, Jespersen D. 2020. Desiccation avoidance and drought tolerance strategies in bermudagrasses. *Environmental and Experimental Botany* **171**:103947 DOI 10.1016/j.envexpbot.2019.103947.

Koevoets IT, Venema JH, Elzenga JTM, Testerink C. 2016. Roots withstanding their environment: exploiting root system architecture responses to abiotic stress to improve crop tolerance. *Frontiers in Plant Science* **7(112)**:1–19 DOI 10.3389/fpls.2016.01335.

Li C, Li L, Reynolds MP, Wang J, Chang X, Mao X, Jing R. 2021. Recognizing the hidden half in wheat: root system attributes associated with drought tolerance. *Journal of Experimental Botany* **72(14)**:5117–5133 DOI 10.1093/jxb/erab124.

Luke Mccormack M, Adams TS, Smithwick EAH, Eissenstat DM. 2012. Predicting fine root lifespan from plant functional traits in temperate trees. *New Phytologist* **195(4)**:823–831 DOI 10.1111/j.1469-8137.2012.04198.x.

Lynch JP. 2015. Root phenes that reduce the metabolic costs of soil exploration: opportunities for 21st century agriculture. *Plant, Cell and Environment* **38(9)**:1775–1784 DOI 10.1111/pce.12451.

Lê S, Josse J, Husson F. 2008. FactoMineR: an R package for multivariate analysis. *Journal of Statistical Software* **25(1)**:253–258 DOI 10.18637/jss.v025.i01.

Maqbool S, Hassan MA, Xia X, York LM, Rasheed A, He Z. 2022. Root system architecture in cereals: progress, challenges, and perspective. *The Plant Journal* **110(1)**:23–42 DOI 10.1111/tpj.15669.

McCormack ML, Dickie IA, Eissenstat DM, Fahey TJ, Fernandez CW, Guo D, Helmisaari HS, Hobbie EA, Iversen CM, Jackson RB, Leppälammi-Kujansuu J, Norby RJ, Phillips RP, Pregitzer KS, Pritchard SG, Rewald B, Zadworny M. 2015. Redefining fine roots improves understanding of below-ground contributions to terrestrial biosphere processes. *New Phytologist* **207(3)**:505–518 DOI 10.1111/nph.13363.

Mthembu SG, Magwaza LS, Mashilo J, Mditshwa A, Odindo A. 2022. Drought tolerance assessment of potato (*Solanum tuberosum* L.) genotypes at different growth stages, based on morphological and physiological traits. *Agricultural Water Management* **261(4)**:107361 DOI 10.1016/j.agwat.2021.107361.

Oliveira RC, Reis ACCS, Aguiar CG, Viecelli CA, Primieri C, Tomasi GA, Bleil Junior HG, Andrade MAA, Viana OH. 2015. *Agroindustrialização do crambe*. Second Edition. Cascavel: Assoeste, 144.

Osmolovskaya N, Shumilina J, Kim A, Didio A, Grishina T, Bilova T, Keltsieva OA, Zhukov V, Tikhonovich I, Tarakhovskaya E, Frolov A, Wessjohann LA. 2018. Methodology of drought stress research: experimental setup and physiological characterization. *International Journal of Molecular Sciences* **19(12)**:4089 DOI 10.3390/ijms19124089.

Pierre CS, Arce VT. 2012. Osmotic adjustment. In: Pask AJD, Pietragalla J, Mullan DM, Reynolds MP, eds. *Physiological Breeding II: a Field Guide to Wheat Phenotyping D.F..* México: CIMMYT, 21–24.

Pierret A, Doussan C, Capowiez Y, Bastardie F, Pagès L. 2007. Root functional architecture: a framework for modeling the interplay between roots and soil. *Vadose Zone Journal* **6(2)**:269–281 DOI 10.2136/vzj2006.0067.

R Core Team. 2021. R: a language and environment for statistical computing. R foundation for statistical computing, Vienna, Austria. *Available at https://www.R-project.org/*.

Ramamoorthy P, Lakshmanan K, Upadhyaya HD, Vadez V, Varshney RK. 2016. Shoot traits and their relevance in terminal drought tolerance of chickpea (*Cicer arietinum* L.). *Field Crops Research* **197**:10–27 DOI 10.1016/j.fcr.2016.07.016.

Ramamoorthy P, Lakshmanan K, Upadhyaya HD, Vadez V, Varshney RK. 2017. Root traits confer grain yield advantages under terminal drought in chickpea (*Cicer arietinum* L.). *Field Crops Research* **201**:146–161 DOI 10.1016/j.fcr.2016.11.004.

Rivas R, Falcão HM, Ribeiro RV, Machado EC, Pimentel C, Santos MG. 2016. Drought tolerance in cowpea species is driven by less sensitivity of leaf gas exchange to water deficit and rapid recovery of photosynthesis after rehydration. *South African Journal of Botany* **103**:101–107 DOI 10.1016/j.sajb.2015.08.008.

Samarappuli D, Zanetti F, Berzuini S, Berti MT. 2020. Crambe (*Crambe abyssinica* Hochst): a non-food oilseed crop with great potential: a review. *Agronomy* **10(9)**:1–18 DOI 10.3390/agronomy10091380.

Silveira EG, Barcelos LFT, Perez VH, Justo OR, Ramirez LC, de Rêgo Filho LM, de Castro MPP. 2019. Biodiesel production from non-edible forage turnip oil by extruded catalyst. *Industrial Crops and Products* **139**:111503 DOI 10.1016/j.indcrop.2019.111503.

Souza PU, Lima LKS, Soares TL, de Jesus ON, Coelho Filho MA, Girardi EA. 2018. Biometric, physiological and anatomical responses of *Passiflora* spp. to controlled water deficit. *Scientia Horticulturae* **229**:77–90 DOI 10.1016/j.scienta.2017.10.019.

Stacklies W, Redestig H, Scholz M, Walther D, Selbig J. 2007. pcaMethods - A bioconductor package providing PCA methods for incomplete data. *Bioinformatics* **23(9)**:1164–1167 DOI 10.1093/bioinformatics/btm069.

Stagnari F, Galieni A, D'Egidio S, Pagnani G, Pisante M. 2018. Responses of radish (*Raphanus sativus*) to drought stress. *Annals of Applied Biology* **172(2)**:170–186 DOI 10.1111/aab.12409.

Takahashi F, Kuromori T, Urano K, Yamaguchi-Shinozaki K, Shinozaki K. 2020. Drought stress responses and resistance in plants: from cellular responses to long-distance intercellular communication. *Frontiers in Plant Science* **11**:556972 DOI 10.3389/fpls.2020.556972.

Tardieu F, Draye X, Javaux M. 2017. Root water uptake and ideotypes of the root system: whole-plant controls matter. *Vadose Zone Journal* **16(9)**:1–10 DOI 10.2136/vzj2017.05.0107.

Vadez V. 2014. Root hydraulics: the forgotten side of roots in drought adaptation. *Field Crops Research* **165(2)**:15–24 DOI 10.1016/j.fcr.2014.03.017.

Vasconcelos MDS, Passos WE, Lescanos CH, Pires De Oliveira I, Trindade MAG, Caires ARL, Muzzi RM. 2018. Fluorescence spectroscopy applied to monitoring biodiesel degradation: correlation with acid value and UV absorption analyses. *Journal of Analytical Methods in Chemistry* **2018(5)**:1–11 DOI 10.1155/2018/4175843.

Wasaya A, Zhang X, Fang Q, Yan Z. 2018. Root phenotyping for drought tolerance: a review. *Agronomy* **8(11)**:1–19 DOI 10.3390/agronomy8110241.

Wellstein C, Poschlod P, Gohlke A, Chelli S, Campetella G, Rosbakh S, Canullo R, Kreyling J, Jentsch A, Beierkuhnlein C. 2017. Effects of extreme drought on specific leaf area of grassland species: a meta-analysis of experimental studies in temperate and sub-Mediterranean systems. *Global Change Biology* **23(6)**:2473–2481 DOI 10.1111/gcb.13662.

White PJ. 2019. Root traits benefitting crop production in environments with limited water and nutrient availability. *Annals of Botany* **124(6)**:883–890 DOI 10.1093/aob/mcz162.

White TA, Snow VO. 2012. A modelling analysis to identify plant traits for enhanced water-use efficiency of pasture. *Crop and Pasture Science* **63(1)**:63–76 DOI 10.1071/CP11250.

Widuri L, Lakitan B, Sodikin E, Hasmeda M, Meihana M, Kartika K, Siaga E. 2018. Shoot and root growth in Common bean (*Phaseolus vulgaris* L.) exposed to gradual drought stress. *Agrivita Journal of Agricultural Science* **40(3)**:442–452 DOI 10.17503/agrivita.v40i0.1716.

Xiong R, Liu S, Considine MJ, Siddique KH, Lam HM, Chen Y. 2021. Root system architecture, physiological and transcriptional traits of soybean (*Glycine max* L.) in response to water deficit: a review. *Physiologia Plantarum* **172(2)**:405–418 DOI 10.1111/ppl.13201.

Yan W, Zhong Y, Shangguan Z. 2016. A meta-analysis of leaf gas exchange and water status responses to drought. *Scientific Reports* **6(1)**:1–9 DOI 10.1038/srep20917.

Zanetti F, Scordia D, Vamerali T, Copani V, Dal Cortivo C, Mosca G. 2016. *Crambe abyssinica* a non-food crop with potential for the Mediterranean climate: insights on productive performances and root growth. *Industrial Crops and Products* **90(3)**:152–160 DOI 10.1016/j.indcrop.2016.06.023.

Zegaoui Z, Planchais S, Cabassa C, Djebbar R, Belbachir OA, Carol P. 2017. Variation in relative water content, proline accumulation and stress gene expression in two cowpea landraces under drought. *Journal of Plant Physiology* **218**:26–34 DOI 10.1016/j.jplph.2017.07.009.

Zhang X, Lei L, Lai J, Zhao H, Song W. 2018. Effects of drought stress and water recovery on physiological responses and gene expression in maize seedlings. *BMC Plant Biology* **18(1)**:1 DOI 10.1186/s12870-018-1281-x.

Zhu LH. 2016. *Crambe (Crambe abyssinica)*. Urbana: AOCS Press.

Zhu Y, Luo X, Nawaz G, Yin J, Yang J. 2020. Physiological and biochemical responses of four cassava cultivars to drought stress. *Scientific Reports* **10(1)**:1–12 DOI 10.1038/s41598-020-63809-8.