Drought tolerance in potential oilseed plants for biofuel production

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Abstract

Raphanus sativus L. and *Crambe abyssinica* Hochst. are promising crops for supplying oil to chemical industries. They are not used for human consumption and can be grown off-season due to their short life cycle. However, drought may limit their growth, photosynthesis and productivity. Thus, the aim of this research was to evaluate the morphological and physiological responses of *R. sativus* and *C. abyssinica* subjected to water deficit to identify the strategies used by these species to tolerate the effects of water stress. Two independent experiments for *R. sativus* and *C. abyssinica* were conducted with different water replacements: 100% (control), 66% and 33% of the field capacity. The characteristics of water relations, gas exchange, chlorophyll fluorescence, chloroplastidic pigments, morphology and root distribution in the soil profile were evaluated separately in both crops. The results indicate the ability of both species to control water loss. In *R. sativus* and *C. abyssinica*, the cell turgor and consequently photosynthesis and growth were maintained even under drought conditions. The maintenance of water status was achieved by osmotic adjustment in *R. sativus*, and also the root distribution and deepening in both species. Such responses allowed *R. sativus* and *C. abyssinica* to be classified as tolerant to drought conditions.

Keywords: abiotic stress; crambe; forage turnip; root system; water deficit.
Abbreviations: FC_field capacity; A_net photosynthetic assimilation rate; C_i/C_a_ratio between internal and external CO_2 concentration; DAP_days after planting; DAT_days after treatment imposition; E_transpiration rate; ETR_electron transport rate; F_v/F_m_potential quantum yield of photosystem II; g_s_stomatal conductance; LDM_leaf dry matter; NL_number os leaves; NPQ_non-photochemical quenching coefficient; PSII_photosystem II; RWC_l_leaf relative water content; RWC_r_root relative water content; SDM_stem dry matter; WR_water replacement; WUE_instantaneous water use efficiency; WUE_water use efficiency; $\Delta F/Fm'$_effective quantum yield of PSII; Ψ_{sl}_leaf osmotic potential; Ψ_{sr}_root osmotic potential; Ψ_{wl}_leaf water potential.

Introduction

Raphanus sativus L. var. *oleiferus* Metzg. (turnip forage) and *Crambe abyssinica* Hochst. (crambe) are oilseed crops with great potential for commercial exploitation as oil sources (Lalas et al., 2012; Shah et al., 2013). The oil of both species is rich in erucic acid, which is not suitable for use in food applications but important as industrial crops to produce lubricants, polymers, pharmaceuticals items (Lalas et al., 2012; Chammoun et al., 2013) and biodiesel (Wazilewski et al., 2013). The biodiesel produced of the crambe and turnip forage oils have higher composition of fatty acids and fatty alcohols which confer a higher oxidative stability (Oliveira et al., 2015; Zhu et al., 2016) and a similar energetic conversion, compared to soybean oil (Wazilewski et al., 2013), demonstrating its great potential for biodiesel feedstock production. In addition, the use of vegetable oils instead of diesel is of great environmental importance for ensuring power generation with reduced gas emission (Rosa et al., 2014).

In Brazil, *R. sativus* and *C. abyssinica* are winter crops and can be cultivated in the off-season of soybean and corn crops (Carlsson et al., 2014; Oliveira et al., 2015), which corresponds to the dry period. The frequent exposure to water deficits compromises the growth and development of plants and, consequently, the crop production (Liu et al., 2011). Under field condition, water deficits in the soil and atmosphere limits growth rates due to loss of cell turgor (Bartlett et al., 2012) and it may restrict photosynthesis (Warren, 2006). To minimize water loss, the reduction of transpiration and CO_2 assimilation is caused by stomata and mesophyll diffusion limitations (Flexas et al. 2004; Chaves et al. 2009), mainly at moderate stress condition (Chaves and Oliveira, 2004). Under severe and long-term drought, reductions in photosynthetic rate can also be associated with biochemical limitations (Lawlor 2002; Lawlor and Cornic, 2002) resulting from damage in the ribulose bisphosphate carboxylase Rubisco (Bota et al., 2004) or in

the regeneration of ribulose-1.5-bisphosphate (RuBP). These limitations due to oxidative stress usually occur as a secondary stress by reducing the carbon assimilation and hence plant growth. To survive under stress conditions, the plants have developed mechanisms that adapt to drought and attempt to maintain water status and photosynthetic capacity of the leaves (Rahbarian et al., 2011). The main defensive strategies used by drought tolerant plants are stomatal control (Pinheiro and Chaves, 2011; Campos et al., 2014), the accumulation of osmotically compatible solutes (Silva et al., 2010), non-photochemical energy dissipation (Eskling et al., 1997; Asada, 2000; Heber, 2002; Krause and Jahns, 2004), antioxidant defense (Javed et al., 2013) and architectural changes in the root system (Gowda et al., 2011; Vadez, 2014; Königshofer and Löppert, 2015).

Considering the increasing interest in *R. sativus* and *C. abyssinica* cultivation as an alternative for biodiesel production and their use in crop rotation, it becomes essential to perform studies that generate data for establishing management strategies for these cultures. This also consolidates the use of these non-food oilseed crops for industrial oil production.

Here, we investigated the hypothesis that morpho-physiological adjustability of *R. sativus* and *C. abyssinica* confers tolerance to water deficit. Thus, the aim of this study was to evaluate the morphological and physiological responses of *R. sativus* and *C. abyssinica* to identify the mechanisms used by these species to tolerate drought stress.

Results

Effects of drought on water status, photosynthesis and growth

In general, the water deficit did not cause major changes in the physiology and morphology of *R. sativus* and *C. abyssinica*. In *R. sativus*, the water deficit increased the leaf osmotic potential (Ψ_{sl}) at 10, 15 and 21 DAT (Table 1). In contrast, the leaf water potential (Ψ_{wl}) and the relative water content (RWC_l) were not affected by drought condition (Table 1). The photosynthesis rate and stomatal conductance were higher in the treatment that replenished 33% of FC at 21 DAT (Table 2). The water use efficiency (WUE) was also higher at 21 DAT compared to 15 DAT in plants exposed to water stress (Table 2). The drought did not significantly affect the chlorophyll fluorescence or the pigment content, independent of the water treatment (Table 3). However, the number of leaves (NL) and the leaves dry matter (LDM) were smaller in plants subjected to 66% FC (Table 4).

In *C. abyssinica*, the Ψ_{wl} was increased when the plants were irrigated with 66% FC at 13 DAT (Table 1). The Ψ_{sl}, RWC_l (Table 1), gas exchange (Table 2) and chlorophyll fluorescence parameters (Table 3) had no significant changes in plants subjected to a water deficit. The concentration of total chlorophyll and carotenoids were reduced in plants subjected to 66% FC at 13, 17 and 24 DAT. Of note, the reduction in total chlorophyll (Table 3) concentration was compared to the 33% FC. The stem diameter, number of leaves, leaves and stem dry matter decreased in plants under 33% FC (Table 4).

The Ψ_{sr} and RWC_r have not significantly changed in both *R. sativus* and *C. abyssinica* under water stress (Table 5). Regardless of the species, most characteristics were affected by the days of sampling, indicating that the differences were due to the development of plants and not because of the water stress.

Effects of water stress on the root distribution pattern in the soil profile

Digital images of the roots were used to analyze the root system in plants of *R. sativus* and *C. abyssinica* (Supplementary Fig. 1) that were subjected to three different soil water replacement treatments (100%, 66% and 33% of FC). Overall, in both species, the roots from control plants (100% FC) developed fewer secondary roots and more shallow roots (Supplementary Fig. 1A and 1D). The *R. sativus* plants grown under 66% (Supplementary Fig. 1B) and 33% FC (Supplementary Fig. 1C) showed a deep root system because of the increase in the root length. This increases the root exploration area for water uptake and storage.

In water replacements of 66% FC (Supplementary Fig. 1E) and 33% FC (Supplementary Fig. 1F), the plants of *C. abyssinica* increased the growth of surface roots and resulted in a larger root distribution reaching the deeper soil layers.

Discussion

The physiological and morphological characteristics of *R. sativus* and *C. abyssinica* were not markedly affected by water restriction. The morpho-physiological responses suggest that both species have strategies to minimize water loss and to mitigate the deleterious effects of drought.

Usually, in plants sensitive to drought, reductions are expected in CO_2 assimilation, stomatal conductance and transpiration rate due to stomatal limitations (Chaves et al., 2009; Campos et al., 2014). The stomatal closure is one of the first responses to drought since it reduces the loss of water through transpiration (Pinheiro and Chaves, 2011). A reduction in the leaf water potential and relative water content are also described in the literature as a consequence of reduced water content in the soil (Rahbarian et al., 2011; Fini et al., 2013). To avoid excessive water loss, some osmo-regulatory compounds are produced to maintain the water status of the plant, as observed in the leaves of *R. sativus*, with a longer exposure to water stress. The reduction in osmotic potential (to more negative values) in *R. sativus* retained the cell turgor as observed in others oilseed plants such as *Ricinus communis* (Babita et al., 2010) and *Jatropha curcas* (Silva et al., 2010). Among the compatible osmolytes, proline is described as increasing in several species of Brassicaceae (Phutela et al. 2000; Khan et al., 2010) in response to water stress. Askari and Ehsanzadeh (2015) compared twelve fennel genotypes and observed that proline content is related with leaf water potential, dry matter and grain yield of drought tolerant genotypes. This is an important physiological adaptation strategy that increases drought tolerance.

In this study, the adjustment of leaf osmotic capacity in *R. sativus* and the high water content in both species allowed

Table 1. Predawn leaf water potential (Ψ_{wl}, MPa), leaf osmotic potential (Ψ_{sl}, MPa), and relative water content of leaves (RWC_l, %) in *Raphanus sativus* and *Crambe abyssinica* subjected to three levels of water replacement (WR): 100% of field capacity (FC; control), 66% FC and 33% FC at three different days after start the treatments (DAT).

DAT	WR	Ψ_{wl}	Ψ_{sl}	RWC_l
		R. sativus		
10	Control	-0.21±0.04 Aab	-0.86±0.02 Ba	85.87±3.76 Ab
	66	-0.17±0.02 Aab	-0.90±0.00 Aa	86.88±1.58 Ab
	33	-0.19±0.02 Aab	-0.87±0.02 Aa	84.37±3.10 Ab
15	Control	-0.17±0.05 Ab	-0.81±0.03 Ba	91.04±3.09 Aa
	66	-0.18±0.05 Ab	-0.86±0.02 Aa	92.04±2.07 Aa
	33	-0.18±0.06 Ab	-0.86±0.02 Aa	92.62±3.73 Aa
21	Control	-0.24±0.03 Aa	-0.47±0.02 Bb	87.33±2.38 Ab
	66	-0.20±0.03 Aa	-0.57±0.05 Ab	87.34±3.98 Ab
	33	-0.21±0.04 Aa	-0.62±0.10 Ab	86.74±2.26 Ab
WR		n.s.	*	n.s.
DAT		*	**	**
WR ^{X} DAT		n.s.	n.s.	n.s.
Block		n.s.	n.s.	n.s.
C.V. WR (%)		29.43	6.25	3.21
C.V. DAT (%)		17.74	12.30	3.34
		C. abyssinica		
13	Control	-0.26±0.07 ABa	-0.76±0.07 Aa	95.05±4.76 Aa
	66	-0.22±0.05 Ba	-0.77±0.06 Aa	94.20±3.60 Aa
	33	-0.30±0.04 Aa	-0.74±0.11 Aa	95.49±2.16 Aa
17	Control	-0.20±0.04 Aa	-0.64±0.05 Ab	88.10±2.82 Ab
	66	-0.16±0.04 Aa	-0.65±0.06 Ab	92.93±3.18 Ab
	33	-0.13±0.02 Ab	-0.64±0.08 Ab	89.73±1.07 Ab
24	Control	-0.20±0.05 Aa	-0.78±0.04 Aa	91.81±1.66 Aab
	66	-0.18±0.04 Aa	-0.77±0.07 Aa	94.34±1.28 Aab
	33	-0.17±0.02 Ab	-0.85±0.04 Aa	91.26±2.09 Aab
WR		n.s.	n.s.	n.s.
DAT		**	**	**
WR ^{X} DAT		*	n.s.	n.s.
Block		n.s.	n.s.	n.s.
C.V. WR (%)		22.74	6.30	2.73
C.V. DAT (%)		18.14	8.86	3.13

*Significant by factorial analysis (p ≤ 0.05).Mean ± SD (*n*=4) followed by the same letter, uppercase between water replacement levels and lowercase between DAT, do not differ significantly from each other as determined by Tukey's test (*p* ≥ 0.05).

Table 2. Net photosynthetic assimilation rate (*A*, $\mu mol\ m^{-2}\ s^{-1}$), stomatal conductance (g_s, $mol\ m^{-2}\ s^{-1}$), transpiration rate (*E*, $mmol\ m^{-2}\ s^{-1}$), ratio between internal and external CO_2 concentration (C_i/C_a) and the instantaneous water use efficiency (*WUE*) in *Raphanus sativus* and *Crambe abyssinica* subjected to three levels of water replacement (WR): 100% of field capacity (FC; control), 66% FC and 33% FC at three different days after the start of treatments (DAT).

DAT	WR	A	g_S	E	C_i/C_a	WUE
		R. sativus				
10	Control	26.26±0.57 Aa	1.30±0.06 Aa	5.31±0.16 Ab	0.86±0.01 Aa	4.95±0.23 Aa
	66	25.28±1.43 Aa	1.30±0.19 Aa	5.28±0.25 Ab	0.86±0.02 Aa	4.79±0.24 Aa
	33	25.98±1.90 Aa	1.09±0.21 Aa	5.09±0.36 Ab	0.85±0.01 Aa	5.11±0.07 Aa
15	Control	24.66±2.34 Aab	0.97±0.18 Aa	6.04±0.63 Aa	0.85±0.03 Aa	4.12±0.58 Ab
	66	23.23±0.72 Aa	1.20±0.12 Aa	6.56±0.34 Aa	0.86±0.01 Aa	3.58±0.18 Bb
	33	23.02±1.33 Ab	1.18±0.15 Aa	6.34±0.67 Aa	0.87±0.01 Aa	3.66±0.32 Bb
21	Control	23.58±0.42 Bb	1.03±0.21 Ba	6.49±0.66 Aa	0.85±0.02 Aa	3.66±0.38 Ac
	66	25.53±1.51 ABa	1.10±0.10 ABa	6.52±0.51 Aa	0.85±0.01 Aa	3.90±0.17 Ab
	33	26.68±2.18 Aa	1.36±0.46 Aa	6.75±0.93 Aa	0.86±0.02 Aa	3.99±0.38 Ab
WR		n.s.	n.s.	n.s.	n.s.	n.s.
DAT		**	n.s.	**	n.s.	**
WR ^{X} DAT		*	*	n.s.	n.s.	**
Block		n.s.	n.s.	n.s.	n.s.	n.s.
C.V. WR (%)		7.22	22.56	7.15	1.62	4.83
C.V. DAT (%)		5.27	15.56	6.11	1.86	5.28
		C. abyssinica				
13	Control	21.05±2.76 Aa	0.83±0.18 Aa	4.90±0.26 Ab	0.85±0.02 Aa	4.30±0.47 Aa
	66	20.20±1.00 Aa	0.76±0.39 Aa	4.76±0.41 Ab	0.82±0.07 Aa	4.25±0.23 Aa

DAT	WR					
	33	20.96±1.18 Aa	0.80±0.14 Aa	5.01±0.40 Ab	0.84±0.03 Aa	4.23±0.53 Aa
17	Control	22.69±2.10 Aa	0.82±0.13 Aa	4.85±0.30 Ab	0.84±0.02 Aa	4.68±0.20 Aa
	66	21.39±1.77 Aa	0.80±0.06 Aa	4.81±0.20 Ab	0.84±0.01 Aa	4.46±0.50 Aa
	33	23.48±2.21 Aa	0.85±0.09 Aa	5.00±0.37 Ab	0.83±0.02 Aa	4.70±0.42 Aa
24	Control	21.96±1.63 Aa	0.79±0.10 Aa	6.34±0.84 Aa	0.83±0.01 Aa	3.52±0.63 Ab
	66	20.75±2.77 Aa	0.76±0.15 Aa	5.95±0.31 Aa	0.84±0.02 Aa	3.50±0.51 Ab
	33	20.63±2.96 Aa	0.94±0.26 Aa	6.52±0.20 Aa	0.86±0.03 Aa	3.18±0.54 Ab
WR		n.s.	n.s.	n.s.	n.s.	n.s.
DAT		n.s.	n.s.	**	n.s.	**
WR ^{X} DAT		n.s.	n.s.	n.s.	n.s.	n.s.
Block		n.s.	n.s.	n.s.	n.s.	n.s.
C.V. WR (%)		10.13	13.13	4.96	2.10	8.63
C.V. DAT (%)		8.89	21.36	8.70	3.35	11.08

*Significant by factorial analysis (p ≤ 0.05). Mean ± SD (*n*=4) followed by the same letter, uppercase between water replacement levels and lowercase between DAT, do not differ significantly from each other as determined by Tukey's test (*p* ≥ 0.05).

Table 3. Potential quantum yield of photosystem (PS) II (F_v/F_m), effective quantum yield of PSII ($\Delta F/F_m'$), apparent electron transport rate (*ETR*), non-photochemical quenching coefficient (*NPQ*) and total chlorophyll (µg g^{-1} FM) concentration in *Raphanus sativus* and *Crambe abyssinica* subjected to three levels of water replacement (WR): 100% of field capacity (FC; control), 66% FC and 33% FC at three different days after start the treatments (DAT).

DAT	WR	F_v/F_m	$\Delta F/F_m'$	ETR	NPQ	Total chlorophyll
		R. sativus				
10	Control	0.80±0.01 Ab	0.37±0.03 Ab	280±22.8 Aa	0.91±0.08 Aa	976.5±105 Ab
	66	0.80±0.01 Ab	0.38±0.02 Ab	275±17.0 Aa	0.89±0.16 Aa	1016±152 Ab
	33	0.80±0.02 Ab	0.40±0.08 Ab	268±56.2 Aa	0.75±0.12 Aa	894.5±168 Ab
15	Control	0.81±0.01 Aa	0.57±0.01 Aa	137±10.1 Ab	0.38±0.07 Ab	1168±154 Ab
	66	0.82±0.01 Aa	0.58±0.01 Aa	152±10.0 Ab	0.34±0.05 Ab	1042±163 Ab
	33	0.82±0.01 Aa	0.57±0.02 Aa	138±8.21 Ab	0.37±0.09 Ab	1000±124 Ab
21	Control	0.82±0.01 Aa	0.55±0.01 Aa	150±23.7 Ab	0.44±0.05 Ab	1264±157 Aa
	66	0.82±0.01 Aa	0.57±0.04 Aa	163±5.62 Ab	0.44±0.10 Ab	1206±129 Aa
	33	0.82±0.02 Aa	0.59±0.02 Aa	172±5.47 Ab	0.32±0.07 Ab	1420±341 Aa
WR		n.s.	n.s.	n.s.	n.s.	n.s.
DAT		**	**	**	**	**
WR ^{X} DAT		n.s.	n.s.	n.s.	n.s.	n.s.
Block		n.s.	n.s.	n.s.	n.s.	n.s.
C.V. WR (%)		2.29	6.97	10.90	16.06	13.74
C.V. DAT (%)		1.37	7.27	12.09	18.25	17.15
		C. abyssinica				
13	Control	0.81±0.005 Ac	0.40±0.07 Ac	152±29.0 Ab	1.16±0.37 Aa	840.5±96.1 ABb
	66	0.81±0.003 Ac	0.39±0.07 Ac	140±15.0 Ab	1.28±0.29 Aa	796.2±179 Bb
	33	0.81±0.005 Ac	0.44±0.02 Ac	152±16.0 Ab	0.93±0.13 Aa	878.4±129 Ab
17	Control	0.83±0.005 Ab	0.47±0.09 Ab	167±17.4 Aa	1.00±0.46 Aa	1099±121 ABa
	66	0.83±0.001 Ab	0.49±0.06 Ab	177±23.6 Aa	1.00±0.33 Aa	956.4±61.4 Ba
	33	0.82±0.006 Ab	0.50±0.06 Ab	177±20.9 Aa	0.79±0.39 Aa	1144±57.7 Aa
24	Control	0.82±0.011 Aa	0.57±0.03 Aa	133±14.6 Ab	0.44±0.15 Ab	1083±189 ABa
	66	0.82±0.008 Aa	0.58±0.02 Aa	138±16.7 Ab	0.42±0.07 Ab	946.5±96.5 Ba
	33	0.81±0.008 Aa	0.55±0.02 Aa	131±2.58 Ab	0.55±0.26 Ab	1204±122 Aa
WR		n.s.	n.s.	n.s.	n.s.	*
DAT		**	**	**	**	**
WR ^{X} DAT		n.s.	n.s.	n.s.	n.s.	n.s.
Block		n.s.	n.s.	n.s.	n.s.	n.s.
C.V. WR (%)		1.42	14.78	12.07	41.04	13.25
C.V. DAT (%)		0.72	8.96	13.29	29.30	11.57

*Significant by factorial analysis (p ≤ 0.05). Mean ± SD (*n*=4) followed by the same letter, uppercase between water replacement levels and lowercase between DAT, do not differ significantly from each other as determined by Tukey's test (*p* ≥ 0.05).

Table 4. Plant height (H, cm), stem diameter (SD, mm), number of leaves (NL), leaves dry matter (LDM, g) and stem dry matter (SDM, g) in *Raphanus sativus* and *Crambe abyssinica* subjected to three levels of water replacement (WR): 100% of field capacity (FC; control), 66% FC and 33% FC at 42 and 43 days after start the treatments (DAT).

DAT	WR	H	SD	NL	LDM	SDM
		R. sativus				
	Control	110.1±8.35 A	17.57±1.67 A	27.00±6.26 A	12.18±1.93 A	22.53±6.41 A
42	66	111.5±9.14 A	15.41±1.86 A	19.25±4.40 B	7.53±1.25 B	16.63±4.50 A
	33	108.2±18.1 A	16.29±3.66 A	28.13±8.68 A	10.65±3.06 A	21.15±4.97 A
WR		n.s.	n.s.	**	**	n.s.
Block		n.s.	n.s.	n.s.	n.s.	n.s.
C.V. (%)		11.53	17.09	22.56	20.81	26.99
		C. abyssinica				
	Control	107.0±9.38 A	11.30±1.65 A	28.13±12.8 AB	4.97±2.27 A	8.76±3.35 AB
43	66	97.83±7.24 A	10.83±1.62 AB	34.86±6.71 A	4.91±1.91 A	11.00±3.74 A
	33	105.9±10.8 A	9.16±1.53 B	19.88±8.95 B	1.94±0.37 B	6.56±2.78 B
WR		n.s.	*	*	**	*
Block		n.s.	n.s.	n.s.	n.s.	n.s.
C.V. (%)		7.79	14.67	34.59	47.21	37.06

* Significant by factorial analysis (p ≤ 0.05).Mean ± SD (*n*=8) followed by the same letter, in the column, do not differ significantly from each other as determined by Tukey's test (p ≥ 0.05)

Table 5. Root osmotic potential (Ψ_{sr}, MPa) and relative water content of roots (RWC_r, %) in *Raphanus sativus* and *Crambe abyssinica* subjected to three levels of water replacement (WR): 100% of field capacity (FC; control), 66% FC and 33% FC at 42 and 43 days after start the treatments (DAT).

DAT	WR	Ψ_{sr}	RWCr
		R. sativus	
	Control	-0.86±-0.10 A	88.64±3.37 A
42	66	-0.86±-0.06 A	88.15±2.58 A
	33	-0.86±-0.11 A	91.07±3.60 A
WR		n.s.	n.s.
Block		n.s.	n.s.
C.V. (%)		9.12	3.09
		C. abyssinica	
	Control	-0.92±-0.10 A	94.60±2.48 A
43	66	-0.93±-0.10 A	93.35±2.08 A
	33	-0.95±-0.08 A	92.64±2.89 A
WR		n.s.	n.s.
Block		n.s.	n.s.
C.V. (%)		6.37	2.01

*Significant by factorial analysis (p ≤ 0.05).Mean ± SD (*n*=8) followed by the same letter, in the column, do not differ significantly from each other as determined by Tukey's test (p ≥ 0.05).

Table 6. Monthly averages of climatic variables recorded during the experimental period. Rio Verde, Goiás, Brazil, 2015.

Month	Temperature			RU*	Precipitation	VPD▲
	Maximum (°C)	Minimum (°C)	Average (°C)	(%)	(mm)	(kPa)
May	28.4	15.5	21.3	78	133.7	0.56
June	27.7	15.3	20.6	71	3.0	0.70
July	28.4	14.2	20.5	54	19.0	1.11

*RU – Relative air humidity; ▲VPD – pressure deficit. Vapor.

Table 7. Chemical characteristics of the experimental area soil. Rio Verde, GO, Brazil. 2015.

Depth	pH	O.M.	P	K	Ca	Mg	Al	H+Al	SB	CEC	V
(m)	$CaCl_2$	(g dm^{-3})	(mg dm^{-3})	............................(mmol$_c$ dm^{-3})........................							(%)
0.00 – 0.20	5.0	49.5	2.05	3.7	15.5	13.1	0.04	31.4	32.3	63.7	50.7
0.20 – 0.40	5.0	44.3	1.43	3.2	12.1	10.1	0.04	29.5	25.4	54.9	46.3

*pH values in CaCl$_2$; P: phosphorus and K: potassium, Melhich^{-1} extractor; O.M.: organic matter; H+Al: total acidity; SB: sum of bases; CEC: cation exchange capacity; V: base saturation (SB/CEC ratio).

to keep the stomatal aperture. Thus, even under water restriction, the photosynthetic metabolism of *R. sativus* and *C. abyssinica* were not committed. This was verified by the results of gas exchange and chlorophyll fluorescence, which showed the absence of damage in the photosynthetic apparatus. The crop tolerance to water stress requires the plant ability to maintain the vital functions of the cellular metabolism as well as rapid recovery after drought stress (Waseem et al., 2011). It was observed in two crambe genotypes, in which with a significant reduction in the photosynthetic activity without irrigation, they showed total recovery after 3 days of rehydration (Martins et al., 2017), characterizing these plants as tolerant to water stress, as observed in the present study.

Drought stress inevitably leads to oxidative stress in the plant cells due to higher leakage of electrons during photosynthetic and respiratory processes, increasing the reactive oxygen species (ROS) generation (Asada, 2006). ROS degrades photosynthetic pigments as chlorophylls and carotenoids in species sensitive to drought (Chakraborty and Pradhan, 2012). On the other hand, drought tolerant plants maintain chlorophyll contents, as observed in the present study, which is associated with light capture and photosynthetic efficiency (Khanna-Chopra and Selote, 2007). Also the maintenance of the carotenoid concentration is important since it acts as antioxidants with a key role in protecting the photochemical processes against oxidative damage under stress conditions (Caser et al., 2016). Thus, the photosynthetic efficiency as well as the plant's adaptive capacity for environmental conditions might be explained by the photosynthetic pigment concentrations (Chen et al., 2016).

To maintain plant water status, changes in root system in response to drought were observed in both species. The high leaf water content in *C. abyssinica* probably resulted from the increased capacity for soil water uptake. Maintaining water status at appropriate levels provides a continuity of metabolic activity for long periods of stress (Babita et al., 2010; Fioreze et al., 2011; Pinheiro and Chaves, 2011). Thereby, the deepening of the root system and its distribution in the soil are important morphological adaptations to drought tolerance (Vadez, 2014) as observed in *R. sativus* and *C. abyssinica*. The large volume of soil explored by the roots promotes water uptake and water retention even under low soil water availability which keeps the plant hydrated (Jaleel et al., 2009; Gowda et al., 2011; Henry et al., 2011). In this case, the reduction observed in the NL and DML in *R. sativus* and SD, DMS and DML in *C. abyssinica* can be the effect of assimilated re-distribution of root growth in detriment to the shoot under drought. The absence of expressive drought effects on morpho-physiological responses in *R. sativus* and *C. abyssinica* is due to water uptake capacity from the soil. A more robust root system can ensure the growth and survival of plants subjected to long periods of water restriction (Henry et al., 2011; Pinheiro e Chaves, 2011). The drought signalization from the roots stimulates the activation of numerous morphological and physiological responses in the plant and ensures that key processes such as photosynthesis and growth are not adversely affected by drought (Jensen et al., 2010).

Under the conditions of this experiment, the high root efficiency in *R. sativus* and *C. abyssinica* were sufficient to attend the water demands of these plants. This avoids drought damage on the photosynthetic machinery. The ability to tolerate water stress enhances the cultivation of these species in areas with low precipitation during the off-season. In addition, high adaptability, low incidence of pests and diseases, and fully mechanized cultivation reduces production costs compared with other winter crops that are more susceptible to biotic and abiotic stresses (Nagaoka and Silveira, 2012).

This study offers information that will be useful for drought models that monitor the growth and physiology of *R. sativus* and *C. abyssinica*. These models can optimize the cropping conditions and consolidate these species into the agricultural production system.

Materials and Methods

Experimental conditions

Independent field experiments were conducted at the Experimental Station of The Goiano Federal Institute of Science and Technology– Campus Rio Verde, Goiás, Brazil from May to July 2015. Temperature and relative air humidity were monitored with an automatic meteorological station (Table 6) located at Rio Verde city. Precipitation data were obtained from pluviometers installed at the experimental area (Table 6).

The climate in the region is classified as Tropical Savana (Aw) (Köppen, 1931), with a rainfall season from November to April and dry months from May to October. The annual average temperature ranges from 20 to 35 ºC and precipitation range, from 1500 to 1800 mm per year. The soil was classified as Red Latosol (LVdf) with a medium texture (Embrapa, 2006). The soil chemical composition was analyzed in the layers 0.00 - 0.20 and 0.20 - 0.40 m (Table 7). The correction and soil fertilization were based on chemical analysis of the soil and the nutrient requirement for each species (Souza and Lobato, 2004; Pitol, 2010).

Plant material, experimental design and treatments

The cultivars *R. sativus* and *C. abyssinica* were CATI AL 1000 and FMS Brilhante, respectively. The sowing was made by hand and 20 seeds were distributed per meter of furrow at a depth of 0.02 m. The experimental plots had an area of 19.5 m^2 (3.0 x 6.5 m) and were composed of six sowing rows, of which only the four central were considered for measurements to avoid border effects.

After sowing, the plants were irrigated daily until the establishment of crops. Subsequently, the irrigation was suspended until the water pressure in the soil reached a critical tension of 25 kPa. For both species, the treatments consisted of the application of water replacements of 100% (control), 66% and 33% soil moisture at field capacity (FC). The treatments were imposed during the vegetative growth stage, 22 days after planting (DAP) and maintained throughout the experiment.

The water replacement was conducted by the surface drip irrigation method using a drip line model with a nominal flow rate of 1.0 L h^{-1}, 17 mm nominal diameter, maximum working pressure of 0.09 MPa and drip spacing of 0.50 m. The tensiometers were installed at a depth of 0.20 m, parallel to the row, with a reading of the soil matrix potential

(Ψ_m) recorded every two days. The effective depth of the root system was considered 0.20 m (Marquelli and Calbo, 2009). The physical and hydraulic characteristics of the soil were determined by the soil water retention curve according to Van Genuchten (1980).

The experimental design included randomized blocks with a split-plot design with three experimental plots (water replacements), three subplots (evaluated days) and four replications for each crop/species. *R. sativus* were evaluated during the vegetative stage, at 10 and 15 days after start the treatment (DAT), and at the beginning of the reproductive stage (21 DAT). The *C. abyssinica* were evaluated only during the vegetative stage, 13, 17 and 24 DAT. The biometric analyzes were conducted at 42 DAT (*R. sativus*) and 43 DAT (*C. abyssinica*) with two plants per plot.

Water relations

The predawn leaf water potential (Ψ_{wl}) was measured using a Scholander pressure chamber (model 3005-1412, Soilmoisture Equipment Corp., Goleta, CA, USA). The relative water content in leaves (RWC_l) and roots (RWC_r) were evaluated according Barrs and Weatherley (1962) with the formula:

$$RWC\ (\%) = \frac{FM - DM}{TD - DM} \times 100$$

Where; FM is the fresh matter, TM is the turgid matter and DM is the dry matter. Measurements of Ψ_{wl} and RWC_l were conducted between 04:00 and 06:00 am.

The leaf (Ψ_{sl}) and root (Ψ_{sr}) osmotic potential were evaluated according to Pask et al. (2012) using a vapor pressure osmometer (model 5600, VAPRO, Wescor, Logan, Utah, USA). The osmotic potential values were obtained using the Van't Hoff's 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 Kelvin (*T* ºK) and *Cs* the solute concentration (M), usually expressed in atmospheres and converted to MPa (0.987 ≈ 1 atm = 0.1 MPa). The leaves were collected between 09:00 and 10:00 am, and the roots between 08:00 and 11:30 am.

Gas exchange parameters

Gas exchange from *R. sativus* and *C. abyssinica* were measured in fully expanded leaves to determine the net photosynthetic assimilation rate (*A*, µmol CO_2 m^{-2} s^{-1}), stomatal conductance (*g*$_s$, mol H_2O m^{-2} s^{-1}), transpiration rate (*E*, mmol H_2O m^{-2} s^{-1}), as well as the ratio between internal and external CO_2 concentration (*C*$_i$/*C*$_a$). These were done with an infrared gas analyzer (IRGA, model LI-6400xt, Licor, Lincoln, Nebraska, USA). The instantaneous water use efficiency (WUE) was calculated as the ratio between *A* and *E*. The measurements were performed in the field between 8:00 am and 11:00 am under constant photosynthetically active radiation (PAR, 1000 µmol photons m^{-2} s^{-1}), and at environmental atmospheric CO_2 concentration (*C*$_a$) (~430 µmol mol^{-1}), temperature (~25 ºC) and relative air humidity (48 – 65%).

Chlorophyll a fluorescence analysis

Variables of chlorophyll fluorescence were measured in the same leaf as the gas exchange using a modulated portable fluorometer (model MINI-PAM, Walz, Effeltrich, GE) equipped with a special leaf clip (model 2030-B, Walz, Effeltrich, GE) (Bilger et al., 1995; Rascher et al., 2000). The potential quantum yield of photosystem II (PSII) (F_v/F_m) was calculated after 30 minutes of a dark adaptation (Van Kooten and Snel, 1990). The effective quantum yield of PSII ($\Delta F/Fm'$) was determined by superimposing a saturation pulse on the leaves' previously ambient light as adapted from the literature (Genty et al., 1989). The $\Delta F/Fm'$ was also used to estimate the apparent electron transport rate according Bilger et al. (1995), using the equation:

$$ETR = \frac{\Delta F}{Fm'} \times PAR \times 0.84 \times 0.5$$

Where; PAR defines the photon flow (µmol m^{-2} s^{-1}) on the leaves, 0.84 is the fraction of incident light that is absorbed by the leaves (Ehleringer, 1981), and 0.5 is the excitation energy fraction directed to the PSII (Laisk e Loreto, 1996). The non-photochemical quenching coefficient (NPQ) was calculated according to Bilger and Björkman, (1990) using the formula:

$$NPQ = \frac{(Fm - Fm')}{Fm'}$$

The measurements were performed between 07:00 and 11:00 am.

Chloroplastidic pigments analysis

The concentration of pigments was determined by extraction with dimethylsulfoxide (DMSO) saturated with calcium carbonate ($CaCO_3$) via method described by Ronen and Galun (1984). Three 5 mm diameter leaf discs were incubated in 5 mL of DMSO solution, and the pigments were extracted for 24 h in a 65 ºC water bath. The spectrophotometric readings were later obtained at 480, 649.1 and 665.1 nm using a UV-VIS spectrophotometer (model Evolution 60S, Thermo Fisher Scientific Inc., MA, EUA). The chlorophyll *a*, chlorophyll *b* and total carotenoids concentration were calculated according to Wellburn (1994). The pigment concentrations were expressed on a fresh matter base.

Plant growth parameters

To evaluate *R. sativus* and *C. abyssinica* growth, we collected biometric data of plant height (H, cm), stem diameter (SD, mm), number of leaves (NL) and leaf (LDM), stem (SDM) dry matter. The plant material was dried in a forced air circulation oven at 65 ºC until a constant weight was achieved. The LDM and SDM were expressed by g plant^{-1}.

Root system architecture

To evaluate the root system, trenches were opened in the three soil water replacements treatments at 40 DAT (*R. sativus*) and 41 DAT (*C. abyssinica*). The trenches were

opened perpendicular to the furrow, between rows, and totaling 150 dm^3 (0.50 x 0.50 x 0.60 m). After the trenches were opened, the roots were manually scarified using nails and brushes. Images were taken with a digital camera. The architecture of the root system was analyzed on these images.

Statistical analysis

The physiological and biometric data were submitted to analysis of variance by F test, and the means were compared by Tukey test ($p \leq 0.05$) using the Analysis System Program Variance (SISVAR®, version 5.3).

Conclusion

The cell turgor maintenance mitigates the negative effects of drought on the growth and photosynthesis in *Raphanus sativus* L. and *Crambe abyssinica* Hochst. The main mechanisms of drought tolerance in *R. sativus* are adjustment in leaf osmotic capacity and a deepening of the root system in the soil. *C. abyssinica* shows changes in the root architecture due to a higher distribution of roots in the soil. The physiological and morphological data suggest that *R. sativus* and *C. abyssinica* plants have strategies that allow them to be classified as tolerant to water stress.

Acknowledgments

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