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Drought avoidance adaptive traits in seed germination and seedling growth of *Citrullus amarus* landraces

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ABSTRACT

Citrullus lanatus cultivation is affected by drought stress. *Citrullus* species that grow wild and domesticated in arid areas, are considered potential useful donors of drought tolerance traits. Here, we evaluated the response of seed germination, seedling establishment and growth to different water availabilities in eleven landraces of *C. amarus*, from most of their cultivation range and in one *C. lanatus* commercial cultivar ('Sugar Baby'). 'Sugar Baby' germinated to lower water potential better than all *C. amarus* landraces, while seedling establishment was much higher in *C. amarus* than in *C. lanatus*. Finally, seedling growth of *C. amarus* landraces and the *C. lanatus* cultivar followed different patterns depending on water availability, showing enhanced biomass growth under wet conditions and no changes between dry and wet growing treatments, respectively. The different water use strategies in seed germination and seedling growth found in the two crops highlight drought avoidance strategies linked to the species growing environment in *C. amarus*, not present in the *C. lanatus* cultivar. The high seedling establishment, the plastic responses to water availabilities and the strong root system, indicate that genetic resources of *C. amarus* may have important applications in breeding programmes and in the selection of water-use efficient rootstock lines.

1. Introduction

In 2012, 3.5 million hectares of agricultural land were used to cultivate watermelons (*Citrullus* Schrad). The annual production reached 105 million metric tons, which is 9.5% of the global vegetable production on 6% of the area used globally for the cultivation of vegetables (FAOSTAT, 2014). In particular in arid and semi-arid regions, the cultivation of *Citrullus lanatus* (Thunb.) Matsum. & Nakai, the sweet dessert watermelon, relies heavily on irrigation (Cohen et al., 2007) and is affected by water stress. In recent years, severe drought events caused a sharp decline of watermelon production in some areas (Zhang et al., 2011a). The selection of drought tolerant cultivars or rootstock lines with a more efficient water use might offer solutions to cope with water scarcity (Cohen et al., 2007; Davis et al., 2008; Zhang et al., 2011a). Despite the economic importance of *Citrullus lanatus*, so far only few research studies and breeding efforts dealt with the water and other abiotic stress resistances in watermelons (Wehner, 2008). Other species in the genus *Citrullus* which grow in arid areas, either as native, undomesticated crop wild relatives or as domesticated crops, are considered potential donors of useful traits to improve abiotic and also biotic stress resistances of modern *C. lanatus* cultivars (Hwang et al., 2011; Jarret, 2014). Underutilised crops and crop landraces, in particular, are known to be adapted to a cultivation in marginal environments and to tolerate high levels of abiotic stress (Zeven, 1998; Camacho Villa et al., 2006; Mabhaudhi et al., 2016). Landraces are also considered important resources to broaden the reduced crop gene pool in a climate change scenario, and to enhance low-input agricultural systems (e.g. organic farming) (Veteläinen et al., 2009).

All over the world, different landraces of *Citrullus amarus* Schrad., commonly known as citron watermelon, are locally cultivated as a minor crop (Laghetti and Hammer, 2007; Paris, 2015). Its fruits are used to produce jams, mustards and preserves, pectin for citron peel, and as livestock fodder (Laghetti and Hammer, 2007). For a long time, *C. amarus* had been classified as a botanical variety of *Citrullus lanatus* as *Citrullus lanatus var. citroides* Bailey or as *Citrullus lanatus* (Thunb.) Matsum. et Nakai subsp. *lanatus* var. *citroides* (Bailey) Mansf. ex Greb. It had often been considered the progenitor or an early domestication form of the sweet dessert watermelon (Vavilov, 1987; Jarret et al., 1997; Rubatsky, 2001). However, a recent molecular study revealed that citron watermelon is a different species from *C. lanatus*, which had been independently domesticated. The natural area of distribution and its centre of domestication lie in southern Africa (Chomiki and Renner, 2015). However, the two species are very similar, in fact *C. amarus*

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crosses readily with *C. lanatus* in the field (Wehner, 2008), even if reduced pollen fertility and massive preferential segregation were found in the hybrids (Levi et al., 2003; McGregor and Waters, 2013). *C. amarus* is also being used as rootstock for *C. lanatus* cultivars (Huitrón et al., 2007; King et al., 2008; Thies et al., 2015b). McGregor (2012) accounted for the different traditional uses of *Citrullus amarus* in southern Africa where the fruits are a source of water in arid regions and where the pulp, seeds and leaves are used as food, therefore this species also holds a certain importance for food security in those areas (Mujaju et al., 2011; Modi and Zulu, 2012).

C. amarus landraces show three distinct traits which are interesting for *Citrullus lanatus* breeding and crop improvement, namely pest resistances (Levi et al., 2001b; Davis et al., 2007; Zhang et al., 2011b; Wechter et al., 2012; Thies et al., 2015a) a high genetic diversity (Levi et al., 2001a; Hwang et al., 2011) and adaptations to arid conditions. Regarding this latter important characteristic, it is known that wild accessions of *C. amarus* have higher tolerance to drought stress than cultivated accessions of *C. lanatus* (Kawasaki et al., 2000; Yoshimura et al., 2008). Interestingly, Zhang et al. (2011a) screened 820 *C. lanatus* and *C. amarus* accessions as well as sweet dessert watermelon breeding lines for drought tolerance at seedling stage using a mean drought tolerance rating and ranking scale. Among the 25 accessions that showed the highest drought tolerance, 12 were *C. amarus* from southern Africa. Mo et al. (2016) confirmed that wild watermelon (wild *C. amarus*, referred to as *C. lanatus* var. *citroides*) is more drought tolerant than a *C. lanatus* inbred line. In particular, under drought conditions, *C. amarus* showed higher root/shoot ratio and higher root length, when compared to the domesticated *C. lanatus* lines. In other words, the wild accession showed a greater biomass allocation to the root system when exposed to drought than the inbred line.

The study presented here is the first work to evaluate different landraces, covering most of the wide production area of *C. amarus*, for drought resistance in the first key stages of the life cycle, and comparing them with a commercial high-yield cultivar of *C. lanatus*. In particular, we evaluated the drought responses in seeds and seedlings, in 11 landraces of *C. amarus* compared to a commercial cultivar of *C. lanatus*. The aim of the study was to 1) understand the degree and the mechanisms of drought stress tolerance in the tested *Citrullus* accessions in the first stages of plant development; 2) screen if any of the tested *C. amarus* landraces show interesting traits that could be used for a crop improvement programme of *C. lanatus* cultivars, aiming for drought resistance in the early stages of the life cycle or for the selection of water use efficient rootstock lines; 3) compare the drought tolerance strategies in *C. lanatus* and *C. amarus*.

2. Materials and methods

2.1. Seed accessions and germination study

Twelve *Citrullus* accessions were used in this study (Table 1). Seeds of six landraces (RSA, RUS, UKR, USA, UZB, CAN) of *C. amarus* were provided by the Vavilov Centre of Plant Industry (Saint Petersburg, Russia), five seed accessions of northern Italian *C. amarus* landraces (ITA2, ITA3, ITA4, ITA5, ITA6) were provided by the Plant Germplasm Bank of the University of Pavia (Italy). One commercial cultivar of *C. lanatus* called 'Sugar Baby' was purchased from a French seed company (B&T World Seeds). All germination experiments were carried out in the laboratories of the Millennium Seed Bank (Royal Botanic Gardens, Kew, United Kingdom). Seed of all accessions were weighted and the area estimated. Seed weight was determined by weighting 25 individual seeds from each accession using a microbalance (UMT2 Mettler Toledo), while the area was measured on 20 seeds per accession, using a microscope (Zeiss Stemi SV 11) with camera (Zeiss AxioCam). To evaluate the seed area, a two dimensional projection of the length was used with width as a size metric. Seed germination tests at five osmotic potentials (0, −0.1, −0.2, −0.3, −0.4 MPa), involved sowing seeds in 90 mm diameter Petri dishes containing two layers of germination paper soaked with 10 ml polyethylene glycol (PEG) 8000 solution (Fisher BioReagents, UK), at a concentration appropriate to the intended treatment (Michel, 1983; Hardegree and Emmerich, 1989). Five replicates of 15 seeds per population and osmotic treatment were incubated at 30 °C in temperature and controlled incubators (LMS Ltd., Sevenoaks, UK). The treatment at full hydration (0 MPa) was performed by soaking germination paper with 10 ml of distilled water. To prevent changes in water potential, Petri dishes were refilled with the appropriate solution when needed. Since *Citrullus* species are known to be dark germinators (Thanos and Mitrakos, 1992), the Petri dishes were covered with aluminium foil and put in black plastic bags to avoid any contact with light. Black plastic bags were randomly interspersed within the germinator. The germination scoring was performed in a dark room using green safe light. Seeds were daily checked for germination for four weeks. Germination was defined as a radicle emergence of, at least, 2 mm. We calculated the mean germination time (MGT) using the formula (Ellis and Roberts, 1980):

$$\mathrm{MGT} = \Sigma\,(\mathrm{nT})/\mathrm{N}$$

where:n = number of seeds that germinated at time T; T = the days between the beginning of the test and the measurement; N = the total number of seeds that germinated.

2.2. Growth experiment

Eight accessions (CAN, ITA6, RSA, RUS, S.Baby, UKR, USA, UZB), chosen to consider the widest geographical gradient possible, were used to perform a plant growth experiment lasting three weeks. Eighty seeds per accession, which germinated after two days of incubation (in the dark at 30 °C on 90 mm diameter Petri dishes with 1% agar), were sown in 350 ml pots filled with fine (1.00–3.00 mm) vermiculite and sand (1:1). The germinated seeds were sown under a thin soil layer. The experiment was performed in a glasshouse of the Millennium Seed Bank. Two data loggers checked temperature and humidity homogeneity (mean temperature ± st. dev.: 23.5 °C ± 5.6; mean relative humidity ± st.dev.: 69% ± 20.8). Soil moisture was kept at full saturation during the first week, putting the pots in water-filled trays, so that a water table was always available under the pots. At the sixth day of the experiment, a water-soluble fertiliser mixture at the injection rate of 1:200 (19% nitrogen, 19% phosphorus pentoxide, 19% potassium oxide and 0.15% magnesium oxide with iron, copper, boron, manganese and molybdenum as trace elements) was supplied for 24 h. During the remaining two weeks of the experiment, two different water treatments were used. In the control treatment (=wet treatment), full water saturation was continued as described above. In the dry treatment, the water in the trays was removed and plants left without water until the end of the experiment two weeks later. The pots were put in a randomised order in the glasshouse and their position was changed randomly twice a week. The seedling establishment was checked every 12 h for the first week. We considered a seedling established when the two cotyledons were fully unfolded and opened. We calculated the mean establishment time (MET) following the formula cited by Ellis and Roberts (1980) for MGT. After three weeks at the end of the experiment, 15 plants per accession and treatment were harvested. The soil was carefully removed from the roots by washing them. The roots were then separated from the shoots. The lengths of the harvested seedling shoots and the third leaves (=first true leaves) were measured. Both roots and shoots were put in aluminium foils and oven-dried at 103 °C for 24 h to evaluate the dry biomass weight. From these data we computed the root/shoot ratio. Three samples of soil for both the treatments were collected and then oven-dried at 103 °C for 24 h to measure the water content of the soil. At the end of the experiment, the water content (in percent) in the control

Table 1
The twelve *Citrullus* accessions tested. UNIPV = Plant Germplasm Bank of the University of Pavia (Italy), VIR = Vavilov Centre of Plant Industry (Saint Petersburg). Seed weight is the mean of the weights of 25 individual seeds, Seed area is the mean of the areas of 20 individual seeds.

Species	Year of production	Origin	Name	Provenience	Seeds colour	Code	Seed weight (g)	Seed weight st.dev. (g)	Seed area mm^2	Seed area st.dev. mm^2
Citrullus amarus	2013	Italy (Reggio Emilia)	Anguria da marmellata/mostarda	UNIPV	Red	ITA2	1.34083	0.10666	66.80	4.68
Citrullus amarus	2014	Italy (San Pancrazio-PR)	Anguria da mostarda	UNIPV	Red	ITA3	1.40650	0.10098	68.62	3.02
Citrullus amarus	2011	Italy (Cremona)	Anguria da mostarda da semi verdi	UNIPV	Green	ITA4	1.41619	0.09471	76.98	3.67
Citrullus amarus	2013	Italy (Reggio Emilia)	Cocomero da marmellata/mostarda seme rosso di Colorno (PR)	UNIPV	Red	ITA5	1.51823	0.08822	70.76	3.89
Citrullus amarus	2014	Italy (Parma)	Anguria da mostarda da semi rossi	UNIPV	Red	ITA6	1.41023	0.15959	67.93	3.87
Citrullus amarus	2014	Ukraine	Kormovoy 4–73	VIR	Light Grey	UKR	1.81772	0.09644	80.72	5.76
Citrullus amarus	2014	South Africa	Bethulie	VIR	Red	RSA	1.21717	0.10956	60.61	5.49
Citrullus amarus	2014	Uzbekistan	Bogarnyi 112	VIR	Light Grey	UZB	1.71796	0.15351	77.74	5.44
Citrullus amarus	2014	United States	Colorado green seeds	VIR	Light Grey	USA	1.43519	0.13945	76.89	4.08
Citrullus amarus	2014	Russia	Saratovskyi	VIR	Light Grey	RUS	1.77699	0.12946	82.62	2.22
Citrullus amarus	2014	Canada	Colorado Prosor	VIR	Dark Grey	CAN	1.58921	0.09547	73.6	4.19
Citrullus lanatus			Sugar Baby	Private seed company	Brown	S.Baby	0.40302	0.08614	30.71	2.85

(wet) treatment was 55.35% ± 10.07 (mean ± st. dev.), while in the dry treatment it was 4.84% ± 0.5.

2.3. Data analysis

Seed germination and seedling establishment were analysed by means of Generalized Linear Models (GLMs), using a binomial probability distribution and a logit link function. MGT and MET were analysed using univariate ANOVA. All other data (dry biomass, shoot length, leaf length, root/shoot ratio) were analysed through two-ways ANOVAs. Bonferroni post hoc tests were performed to identify differences between accessions. Mann-Whitney U-tests were performed to identify differences between treatments. Data were checked for normality and homogeneity of variance. Data were analysed in SPSS 21.0.

3. Results

3.1. Germination

Final germination percentages were significantly affected by treatment with a significant interaction between accession and treatment (Table 2). Highest germination at the two lowest osmotic potentials was found in the standard *C. lanatus* cultivar 'Sugar Baby'. Among the *C. amarus* landraces, the South African accession (RSA) achieved the best final germination at −0.3 MPa and −0.4 MPa (Fig. 1). Osmotic treatments had a significant effect on MGT (df = 3, F = 194.637, $P < 0.001$). The osmotic treatment slowed the MGT significantly in all populations ($P < 0.001$) except for ITA2 (P = 0.259). The population that showed the fastest MGT was 'Sugar Baby' (Table 3).

3.2. Seedling establishment

The difference in seedlings establishment among cultivars was significant (Wald Chi-square = 75.788, df = 7, $P < 0.001$). 'Sugar Baby' and RUS had a final seedling establishment significantly lower than the other landraces (Fig. 2), in which seedling establishment reached almost 100%. In particular, in 'Sugar Baby' seedling establishment was less than half than in the other accessions (Fig. 2). Similarly, MET was significantly different among accessions (df = 7, F = 22.406, $P < 0.001$). In 'Sugar Baby' the establishment occurred faster than in the *C. amarus* landraces (Table 3).

Table 2
Results of the Generalized Linear Model (GLM) for germination.

Factor	Wald Chi-Square	df	Sig.
Osmotic potential	125.769	4	0.000*
Accession	18.894	11	0.063
OsmPotential*Acc	162.139	44	0.000*

* $P < 0.05$.

3.3. Final dry biomass

Total dry biomass was significantly affected by treatment, accession and the interaction between these two factors (Table 4). The biomass was always greater in landraces of *C. amarus* than in 'Sugar Baby', with values about threefold higher in both dry and wet treatment. The response of *C. amarus* landraces in terms of final biomass significantly differed in wet and dry treatments, while in 'Sugar Baby' there were no statistical differences (P = 0.611; Fig. 3). Considering the shoot and the root dry biomass separately in all *C. amarus* landraces both, the root and the shoot were heavier in the control (wet) treatment than in the dry treatment ($P < 0.05$). At the same time, there was no statistically significant difference between the two water treatments for 'Sugar Baby', both in the shoot dry weight (P = 0.137) or in the root dry weight (P = 0.503).

3.4. Shoot length, leaf length and root/shoot ratio (RSR)

Treatment had no statistically significant effects on the shoot length (df = 1, F = 2.167, P = 0.142). However for the leaf length, all accessions had statistically significant higher values in the control (wet) treatment than in the dry treatment (Table 4, Fig. 4). Accession and the interaction between accession and treatment had significant effect on RSR, while the treatment had not (Table 4). There was a statistically significant difference between wet and dry treatments for RSR in 'Sugar Baby', ITA6, RSA, and UZB. In 'Sugar Baby' the mean RSR was higher in the dry treatment than in the wet treatment while, on the contrary, in ITA6, RSA and UZB the mean RSR was higher in the control (wet) than in dry treatment (Fig. 5).

4. Discussion

In this study, we investigated the response of seed germination and seedling growth of *C. amarus* landraces under different levels of water availability compared to a *C. lanatus* standard line. The aims of this study were to understand the drought responses in the two crops and to determine if *C. amarus* landraces can be useful donors of

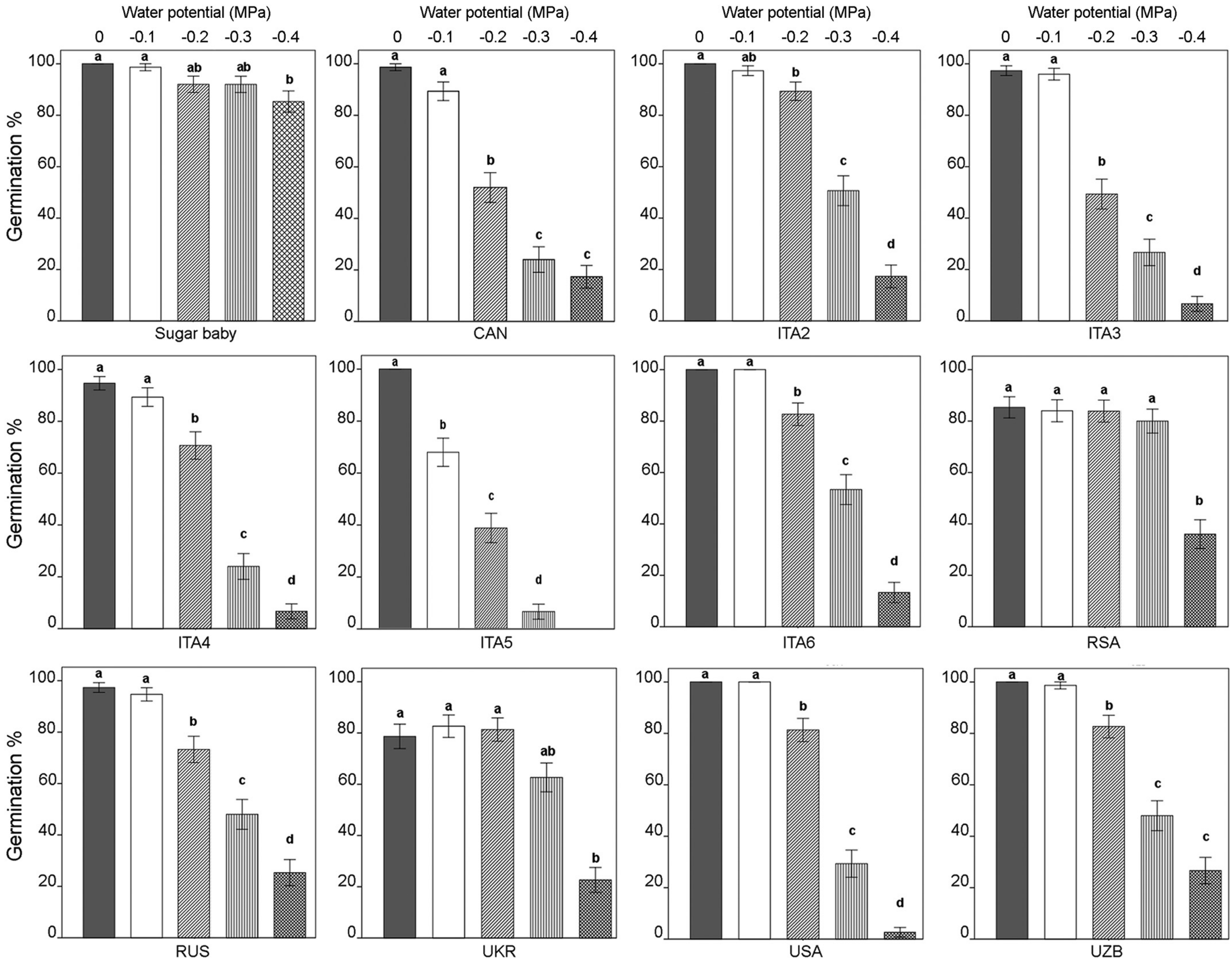

Fig. 1. Final germination percentages (mean ± st.err.) at the five water potentials tested for the twelve accessions. Lowercase letters indicate statistically significant differences between different osmotic potentials; same letters indicate non-significant differences; different letters indicate differences significant at P < 0.05.

resistance traits for improving drought tolerance in *C. lanatus* cultivars in the earliest and crucial stages of plant development (i.e. seed germination and seedling establishment). 'Sugar Baby' seeds are smaller than all *C. amarus* accessions (Table 1). This confirms that watermelons intended for confectionery food production produce big seeds while those watermelon cultivars intended for consumption possess small or medium sized seeds (Wehner, 2008). Our results suggest that

Table 3
Mean Germination Time (MGT) for the twelve accession used in the germination study (data from 0 MPa to −0.3 MPa pooled together) and Mean Establishment Time (MET) for the eight accession used in the growth study.

Accession	MGT (days, mean ± st.dev.)	MET (hours, mean ± st.dev.)
CAN	3.72 ± 1.82	67.12 ± 4.61
ITA2	4.28 ± 1.98	–
ITA3	3.76 ± 1.07	–
ITA4	4.40 ± 1.67	–
ITA5	5.97 ± 2.15	–
ITA6	4.77 ± 2.40	74.1 ± 8.01
S.Baby	2.31 ± 0.39	45.85 ± 6.30
RSA	2.44 ± 0.63	59.1 ± 2.70
RUS	4.21 ± 2,32	67.67 ± 6.53
UKR	3.36 ± 1.43	74.15 ± 7.07
USA	6.1 ± 2.53	62.85 ± 3.45
UZB	3.71 ± 1.88	69 ± 1.92

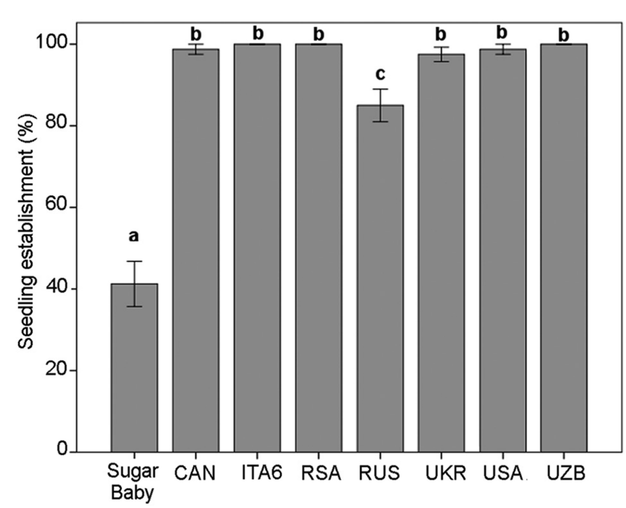

Fig. 2. Final seedling establishment percentages (mean ± st.err.) for the eight accessions tested. Lowercase letters indicate statistically significant differences between accessions; same letters indicate non-significant differences; different letters indicate differences significant at P < 0.05.

Table 4
Results of the two-ways ANOVAs for mean total biomass, mean leaf length and mean root/shoot ratio (RSR) at the end of the experiment. Levels of significance: *** P < 0.001; ** P < 0.01; * P < 0.05.

Factors	df	F		
		Biomass	Leaf length	RSR
Treatment	1	315.258***	250.888***	0.142
Accession	7	177.032***	34.059***	12.583***
Acc*Treatment	7	8.426***	2.750**	6.744***

germination in ‘Sugar Baby’ is less affected by low osmotic potentials than in *C. amarus* landraces, achieving high germination percentages also at the lowest potential (−0.4 MPa) (Fig. 1). The citron watermelon landraces tested in this study behaved in a similar fashion as the seeds of feral *C. amarus* from Florida tested by Ramirez et al. (2014), which showed a final germination percentage of 37% at −0.3 MPa. Interestingly, the *C. amarus* landrace from South Africa (RSA), native to the centre of domestication of this crop, reached the highest germination at −0.4 MPa (36%). Considering the germination percentages, MGT and MET, the behaviour of ‘Sugar Baby’ is typical of a crop, which had been artificially bred to achieve a prompt, uniform germination after sowing and even under non-optimal environmental conditions (Fuller and Allaby, 2009). On the contrary, the higher water requirement for germination observed in *C. amarus* landraces could be related to patterns of wild species, which tend to germinate only under optimal conditions. Indeed, seed germination of the wild *C. amarus* growing in arid areas of southern Africa occurs during the short rainy season (McGregor, 2012) and similarly *C. amarus* landraces are watered only during sowing (Jensen et al., 2011; *M. Fontana* personal communication). Hence, the scarce germination of *C. amarus* at low water availability could be considered as a drought avoidance strategy to prevent seedlings emergence during dry conditions, unfavourable for establishment, a common phenomenon in dryland plants (Evans and Etherington, 1990; Gutterman, 2000). This is in accordance with the fact that *C. amarus* seedling emergence does not occur at the soil surface (Ramirez et al., 2014), which is a typical response of semi-desert plants that prevent seedlings from drying out (Ren et al., 2002). Further studies are needed to clarify whether germination behaviours of citron watermelon landraces are the result of a less advanced level of domestication compared to sweet dessert watermelon cultivars.

MGT and MET were lower in the *C. lanatus* accession than in *C. amarus* landraces, with a high variability among the latter. It is of crucial importance for grafting, and therefore for the utilisation of *C. amarus* landraces as rootstock, to characterise the timing of germination and emergence, since grafting is performed at seedling stage and the uniformity in germination and growth of the rootstock and scion are critical for the survival rates of grafted material (Davis et al., 2008). In line with the findings of Mavi et al. (2010), the accession that showed the fastest MGT (‘Sugar Baby’), showed also the fastest MET. However, in contrast to the results presented by Mavi et al. (2010), there was no positive correlation

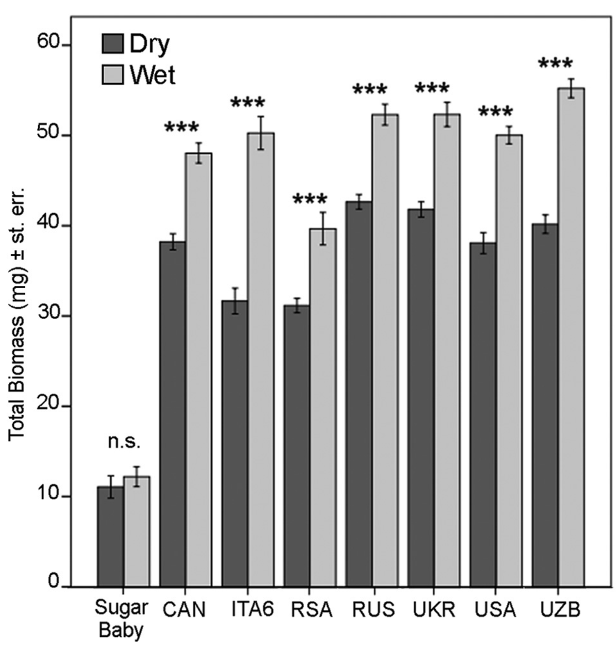

Fig. 3. Final total dry biomass (mean ± st.err.) for the eight accessions tested. Levels of significance of the treatment effect for each accession: *** P < 0.001; ** P < 0.01; * P < 0.05; n.s. = not significant.

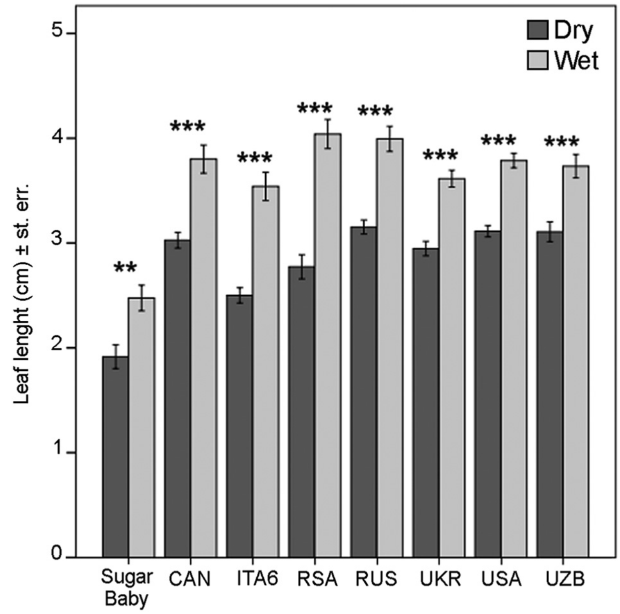

Fig. 4. Length of the first true leaf at the end of the experiment (mean ± st.err.) for the eight accessions tested. Levels of significance of the treatment effect for each accession: *** P < 0.001; ** P < 0.01; * P < 0.05; n.s. = not significant.

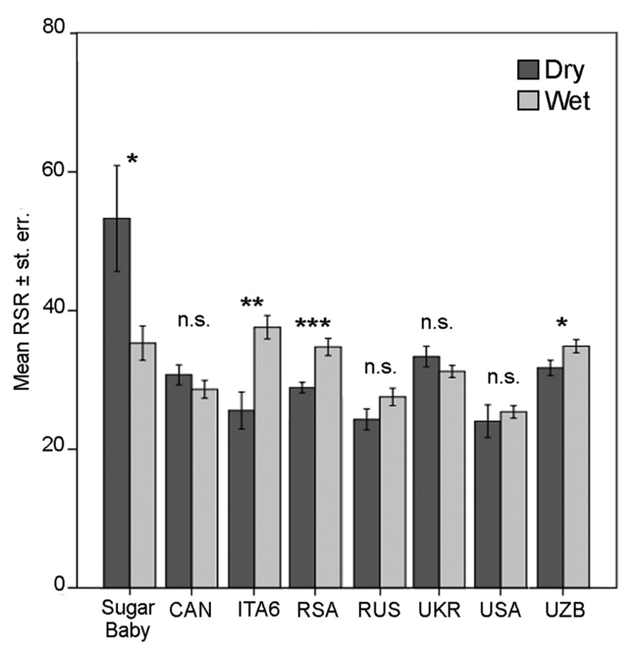

Fig. 5. Root/Shoot ratio (RSR) at the end of the experiment (mean ± st.err.) for the eight accessions tested. Levels of significance of the treatment effect for each accession: *** P < 0.001; ** P < 0.01; * P < 0.05; n.s. = not significant.

between MGT and final establishment. In fact, seed germination was the highest and fastest in 'Sugar Baby', though it had the lowest seedling establishment. *C. amarus* landraces, on the other hand, showed a high seedling establishment rate (close to 100% for most accessions, Fig. 2). The very high seedlings establishment percentages in *C. amarus* accessions can therefore be considered a useful trait to improve *C. lanatus* lines in order to decrease the high seedling mortality observed in commercial cultivars.

'Sugar Baby' demonstrated to be a drought tolerant cultivar. There were not significant differences between the treatments in relation to biomass, which demonstrates the capability of this cultivar to maintain the overall growth under drought stress conditions (Fig. 3). The size of the first true leaf of 'Sugar Baby' was smaller in the dry treatment than in the wet treatment (Fig. 4), showing a typical response to drought intended to limit the water loss by reducing the leaf area and therefore the transpiration rate (Farooq et al., 2009). Regardless the treatment, *C. amarus* plants were consistently bigger than the *C. lanatus* cultivar 'Sugar Baby', for all biomass and growth parameters. In *C. amarus*, the biomass was higher in the wet treatment for all accessions (Fig. 3), also the shoot biomass was significantly different between treatments. At the same time, shoot length did not change significantly between wet and dry treatments, while the first leaf length was always significantly different among treatments (Fig. 4). This means that the variation of shoot biomass in *C. amarus* is due to a different investment in the dimension of the first true leaf between the treatments, to cope with the necessity to decrease the transpiration rate during periods of dry weather. In 'Sugar Baby' the root/shoot ratio was higher in the dry treatment than in the wet treatment (Fig. 5), suggesting that when exposed to drought conditions, plants invest more biomass in the root than in the shoot. The development of the root system is a strategy to increase the water uptake in many plant species under water stress (Jaleel et al., 2009). On the contrary the root/shoot ratio was not different between treatments in some *C. amarus* accessions (CAN, UKR, RUS, USA), while it increased significantly in the wet treatment in others (ITA6, RSA, UZB), meaning that the biomass allocation to the root system was higher in the full hydration (wet) treatment (Fig. 5). This seems in contrast with a previous study on drought effects in wild *C. amarus* compared to commercial *C. lanatus* (Mo et al., 2016), in which *C. amarus*, under drought stress, showed a higher increase of RSR than commercial *C. lanatus*. The apparently opposite response, detected in this study, can be explained by a simple methodological difference. While in Mo et al. (2016) the plants were watered once a day until reaching the $75 \pm 5\%$ field capacity, in our experiment the plants had in the wet treatment a water table below the pots which kept the soil always at water saturation during the entire three weeks of the experiment. Indeed, the constant availability of water stimulated a continuous biomass increase in *C. amarus* landraces in terms of root biomass, shoot biomass and leaf length. Considering that rain is the most important source of water in *C. amarus* cultivation, the difference in biomass between wet and dry treatments can be considered as adaptive traits. That is, the citron watermelon plant is able to maximise its growth in periods of good water availability. The *C. lanatus* cultivar 'Sugar Baby' and the *C. amarus* landraces have clearly different responses to different moisture levels during germination and seedling phases. Nevertheless, given the high variability in the landraces responses, it was not possible to find groups of *C. amarus* accessions exhibiting the same behaviour, considering the different parameters, under the water treatments tested. Referring to the specific aims of this paper, we were not able to find a *C. amarus* accession that showed undoubtedly a better performance under the drought conditions. Nevertheless, the high plasticity of *C. amarus* landraces in response to different water availabilities is worthwhile of further investigations. The high vigour of *C. amarus* seedlings and their strong root systems are all parameters of interest in a rootstock line selection (Davis et al., 2008). This study confirms the assumption that landraces and neglected crops often show adaptive traits towards marginal conditions and abiotic stresses (Camacho Villa et al., 2006). The finding of these interesting adaptive features towards drought, reinforce the urgent need to continue the pre-breeding characterisation of landraces and minor crops in the genus *Citrullus*.

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