
Evaluation of the Osmotic Adjustment Response within the Genus *Beta*

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ABSTRACT

Beta genus includes both industrial and horticultural species, and wild species and subspecies, which are possible reservoirs of agronomically important characters. Among the traits for which *Beta* has been recently studied, drought tolerance or avoidance is one of the most important. In this work, relative water content and the osmotic potential in well-watered and stressed conditions of three beet types, one *B. vulgaris* subspecies and one species other than *B. vulgaris*, all belonging to the *Beta* genus, were analysed. In addition, relative water content, succulence index and osmotic potential were measured during a three-week water deprivation period, and the osmotic adjustment was estimated for each *Beta* accession. The results showed that succulence was higher for *B. vulgaris* ssp. *maritima*. It was also shown that all *Beta* accessions were capable of adjusting osmotically, but that the *B. vulgaris* *maritima* accession examined had a higher osmotic adjustment value compared to the accessions belonging to cultivated *Beta* types, and that the accession of the wild species *Beta webbiana* had a comparatively limited capacity to adjust osmotically.

Additional key words: Sugarbeet, sea beet, germplasm, drought, osmotic adjustment

Drought is one of the greatest limitations for agriculture and crop expansion (Boyer, 1982). Sugarbeet (*Beta vulgaris* ssp. *vulgaris*) is a deep-rooting crop, more adapted to withstand water shortage or nutritional deprivation than many other crops (Doorenbos and Kassam, 1979; Biancardi et al., 1998); however, drought stress is becoming a major

cause of sugar yield losses (Pidgeon et al., 2001). Water deficit is growing increasingly important in regions where it was previously negligible; estimates of how global climate change will affect sugarbeet crop production have been developed for some European areas (Donatelli et al., 2002).

The development of more drought-tolerant sugarbeet genotypes is therefore an essential breeding objective, and large-scale screening programs for this trait have also been undertaken under the auspices of the EU (Germeier and Frese, 2002). It is in fact known that, within the different taxa of the genus *Beta*, there are subspecies particularly well adapted to arid or semi arid environments, though there is little direct knowledge of the mechanisms underlying this adaptability.

The *Beta* genus is divided by taxonomists into four sections: *Procumbentes* (including *B. patellaris*, *B. procumbens* and *B. webbiana*), *Nanae* (including *B. nana*), *Corollinae* (e.g. *B. trygina* and *B. lomatomogona*) and *Beta*, the only section including cultivated forms. Different types of cultivated beets were described, defined by their morphology, physiology and end use; all these forms were classified as *Beta vulgaris* ssp. *vulgaris* and include sugarbeet, garden or red beet, forage beet and leaf beet or Swiss chard. The Section *Beta*, however, also comprises wild subspecies, like the sea beet (*B. vulgaris* ssp. *maritima*), and the ssp. *adanensis* (Lange et al., 1999). The sea beet is widespread in the Mediterranean region, and although the modern sugarbeet probably originated from a single cultivar (the "White Silesian"), hybridization with sea beet has contributed to the genetic diversity of sugarbeet and the introgression of resistance traits (de Bock, 1986; Bartsch et al., 2002). The other sections of the genus include only wild species. Among these, *Beta webbiana* belongs to the *Procumbentes* section, a taxon often used in the past as source of valuable traits (Coons, 1954; Ford-Lloyd and Williams, 1975).

The ability to withstand water shortage can be conferred to a plant by many anatomical and physiological traits (Clarke et al., 1993). Roots reaching deeper layers of soil are likely to confer an advantage to the species; as for beet, there are studies suggesting that sugarbeet roots can explore soil depths up to three meters (Biancardi et al., 1997).

Another trait for which variability within the *Beta* genus is possible, associated with the ability to withstand water shortage, is stomata density. There are reports indicating a large variability for this trait, from 70 to 150 stomata per mm² (Thomas and Clarke, 1995). Recently, changes in lipid peroxidation and antioxidant systems in response to salt stress have been shown to be higher in wild sea beet than sugarbeet (Bor et al., 2003).

Drought avoidance can be obtained by plants through osmotic adjustment (OA), a mechanism involving the active accumulation of solutes within the symplast in response to water deficit. The conse-

quent lowering of osmotic potential contributes to the maintenance of water within cells, delaying dehydration and maintaining turgor for longer periods (Morgan et al., 1984; Munns, 1988; Zhang et al., 1999; Verslues and Bray, 2004). Sugarbeet, similar to sorghum, was reported to keep stomata open and to maintain turgor mainly by osmotic adjustment mechanisms (Biscoe, 1972). For some crops, there is a critical leaf water potential at which stomata close (Turner, 1974); in sugarbeet, stomata were reported to close gradually with the decline of leaf water potential from -0.5 to $-1.5/-2.0$ MPa (Milford and Lawlor, 1975).

In this work, in order to compare OA in *Beta* accessions, and to clarify whether it could be a factor contributing to genotype-specific responses during the induction of drought, OA was quantified and compared in five *Beta vulgaris* species, subspecies or types.

MATERIALS AND METHODS

The following *Beta* types and species were selected (IDBB numbers refer to the International Data Base for *Beta* [www.genres.de/idb/beta/] accession code): *Beta vulgaris* subsp. *vulgaris* IDBBNR 6309 (leaf beet); *Beta vulgaris* subsp. *vulgaris* IDBBNR 7580 (garden beet); *B. vulgaris* ssp. *maritima* IDBBNR 7268 (wild sea beet); *B. webbiana* IDBBNR 3244 (wild); and *B. vulgaris* ssp. *vulgaris* cv. Bianca, a commercial sugarbeet variety (KWS Saat AG, Einbeck, Germany). Seed samples were obtained from Dr. L. Frese, Federal Centre for Breeding Research on Cultivated Plants, BAZ Genbank, Braunschweig (Germany), except seed of cv. Bianca which was provided by Dr. E. Biancardi, C.R.A.-CIN Rovigo (Italy).

Seeds of each *Beta* accession were planted in 6 L pots, filled with a mixture of peat and sand (2:1), with one plant per pot. Plants were raised in a growth chamber (Sanyo Gallenkamp model SGC 097.CFX), under a 16/8 photoperiod and a 23°C/19° C day/night temperature. Relative humidity was set constant at about 60%, and light intensity was approximately 150 $\mu\text{mol sec}^{-1} \text{m}^{-2}$.

All plants were regularly irrigated with tap water to field capacity. Their midday water potential at full turgor, measured by a pressure chamber, was always in the range of -0.35 to -0.45 MPa, with limited variability among plants and accessions. Sixty days after seedling emergence, when the plants were approximately at the 12 to 15 leaf stage, watering was suspended on a group of six out of 12 plants and at 2 or 3 days intervals, two leaf discs were taken from all plants for osmotic potential and RWC measurements.

Measurements were taken at the beginning of the light period, from the first day of water withdrawal, on both groups of plants (watered and stressed).

For each plant, two leaf discs, 10 mm in diameter, were collected and immediately weighed (FW); the same discs were then floated on 20 mL of sterile distilled water in Petri dishes, at 4°C for 16 h in the dark. After this period, the discs were quickly blotted onto filter paper and weighed to determine their turgid weight (TW). The discs were then dried overnight at 80°C for 24 h, and again weighed for dry weight (DW) determination. Relative water content (RWC) was calculated after Barr and Weatherley (1962):

$$\text{RWC} = (\text{FW} - \text{DW})/(\text{TW} - \text{DW}) \times 100$$

Leaf succulence index (LS) of the same discs was calculated as water content (FW - DW) per leaf unit area (considering that a leaf disc had an area of 0.79 cm² and that the areas of the leaf discs were the same for all samples).

Osmotic pressure (OP) was measured on two additional, 15 mm leaf discs excised from the same plants used for RWC determinations, immediately frozen in liquid nitrogen and stored at -80°C until used. After thawing and centrifuging at 3000 x g for 5 min. in a 0.2 mL microcentrifuge tube with a hole in the bottom which was placed inside a 0.5 mL microcentrifuge tube, osmolarity was measured on 10 µL aliquots of the collected liquid using a vapour pressure osmometer (Wescor 5520 VAPRO, Logan, USA). The conversion from mosmol kg⁻¹ to MPa was made as:

$$\text{OP (MPa)} = -c (\text{mosmol/kg}) \times 2.58 \times 10^{-3}$$

where c is the osmolarity of the sap (Bajji et al., 2001).

Osmotic adjustment (OA) was calculated according to method 1 of Chandra Babu et al. (1999), as the difference between two regression lines, one of RWC as a function of the measured OP and the other of RWC as a function of OP corrected for the concentration effects (OP₀), due to passive water loss, according to the equation:

$$\text{OP}_0 = \text{OP}_i (\text{RWC}_i/\text{RWC})$$

where OP_i is the initial OP in watered plants with a given RWC_i value, and RWC the measured RWC at the different times. OA was determined graphically as the difference in OP between the two regression lines at RWC = 60%. This method has the advantage of correcting the OP values measured for the simple passive water loss due to dehydration and not to active accumulation mechanisms (see Discussion). The method is a graphical one because it is the direct measure, from RWC vs. OP plots, of the distance, taken at RWC = 60%, between the two regression lines (Chandr Babu et al., 1999).

RESULTS

In well watered plants, the RWC measured on leaf discs was fairly constant and very similar in the five *Beta* types, under the light, humidity and temperature conditions described in the Materials and Methods section. The measured average values were around 90% throughout the entire duration of the experiment (up to 23 days, Fig. 1a). Under the same conditions, the leaf succulence index was higher in the *maritima* ssp. (sea beet) and in *B. webbiana*, compared to the other *Beta vulgaris* types, but slightly decreased with plant growth in all accessions (Fig. 1c). Also OP values were very similar in all control *Beta* plants throughout 23 days of measurements (Fig. 1e), and in stressed plants at the beginning of the experiment (up to 1 week from water withdrawal). The range of average OP values in control plants during the experiments was between -0.81 MPa in cv. Bianca, and -0.97 MPa in the wild *B. maritima*.

Assuming an average value of -0.35 MPa for foliar water potential, according to initial status measurements in fully hydrated conditions, the turgor pressure in the *Beta* accessions analysed can be estimated to be approximately 0.50 to 0.60 MPa.

When plants of *Beta* accessions were subjected to water withdrawal, a progressive stress was induced, causing a decline of RWC and, to a lesser extent, of LS, during the three weeks of water deprivation. On average, relative water content dropped to 55 to 60% after two to three weeks, but leaf beet lost water content earlier than the other types (Fig. 1b). The leaf succulence index was less sensitive to water shortage, being apparently already influenced by growth stage, as suggested by its decline also in control plants (Fig. 1c). LS values in stressed conditions, after two to three weeks of water deprivation, averaged about 30% less than found at full hydration in all the *Beta* types. Succulence of the leaf beet was the earliest to decrease, while the LS of sea beet, despite its decrease in water shortage conditions, remained higher than the other accessions until 20 days after stress initiation (Fig. 1d).

Upon water stress, OP recorded in leaf discs declined in a differentiated way, depending on the *Beta* accession. The leaf beet OP dropped rapidly after the first week, reaching a value more than twice as negative as the initial OP, in only 15 days of water deprivation (Fig. 1f). This rapid decrease of RWC and OP was also accompanied by early and significant wilting of the wide leaves of this beet type. As for the other *Beta* accessions, the decrease in OP was more gradual, although after 23 days of water withdrawal, OP values for these accessions approached those found for leaf beet (Fig. 1f). After 23 days of water withdrawal, it became very difficult to excise discs from wilted leaves, and no data were taken. The lowest RWC recorded was 52.7% (Fig. 1b; average of measurements on six different

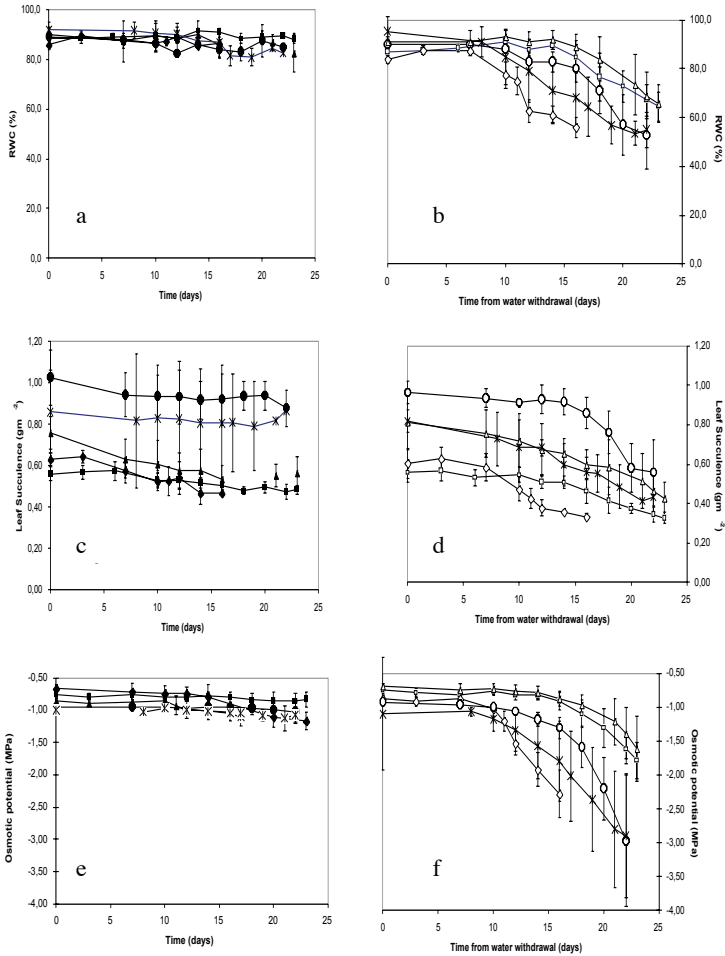


Fig. 1 a,b & c: Time course of relative water content (RWC, a and b), leaf succulence index (LS, c and d) and osmotic potential (OP, e and f) throughout the duration of the experiments in plants kept at full water field capacity (close symbols, left panels) and after water withdrawal (open symbols, right panels), in the different *Beta* accessions. -■- and -□-, sugarbeet (cv. Bianca); -▲- and -△-, garden beet; -◆- and -◇-, leaf beet; -●- and -○-, sea beet; -*- and -x-, *B. webbiana*.

plants), measured at day 22 in *Beta vulgaris* ssp. *maritima* (sea beet), when also the lowest average OP was recorded (-2.98 MPa; Fig. 1f).

On the basis of RWC and OP data obtained during long term water deprivation, regression lines of measured OP, and of OP corrected for passive water loss (OP_0) vs. RWC were derived for each *Beta* type, to estimate their osmotic adjustment (OA), according to Morgan (1992) and method 1 of Chandra Babu et al. (1999). The plots of RWC vs. OP and RWC vs. OP_0 for the five different *Beta* types are shown in Fig. 2, while the R^2 values of the regressions and the calculated OA values are shown in Table 1. At RWC = 60% (Table 1), the highest OA value was obtained for *Beta vulgaris* ssp. *maritima*, the sea beet (0.95 MPa), while sugarbeet had an OA substantially lower (0.81 MPa); the lowest value in the cultivated group was found for the leaf beet (0.70 MPa), while among all accessions, the wild *B. webbiana* had a comparatively low OA value of 0.53 MPa. The dependence of OA on water content is also expressed in Fig. 3, where OA was calculated at different RWC values from the data of Fig. 2. Sea beet showed the highest OA in conditions of limiting water (for RWCs approaching 75%). In addition, OA of sea beet increased more rapidly than OA of the other *Beta* types (as indicated by the steeper slope of the line).

DISCUSSION

Beta is a genus that includes species described as tolerating low water potential environments, compared to other genera of economic importance (Greenway and Munns, 1980). It has also been considered a typical example of a genus containing species that cope with water shortage by adjusting osmotically, rather than closing stomata (McCree and Richardson, 1987). The capacity to adjust osmotically, can be only identified by analysing OP and RWC reciprocal dependence in plants under slow-developing water stress. Therefore, stress experiments were carried out, in which OP was measured with an osmometer, and the plants were subjected to a slow stress, developed through water supply suspension, rather than measured by, e.g., detached leaf tests, during which the dehydration rates are very high. The OP values measured under slow developing stress are not only affected by possible osmoregulative mechanisms, but also by passive water loss which is affected by leaf area, stomata density, and differences in the native habitats of the accessions analysed. The calculation of OP_0 from the experimental OP and RWC values was shown to be a reliable method to take into account passive water losses (Chandra Babu et al., 1999), and it was therefore applied to the data obtained. However, it should be kept in mind that RWC, calculated according to Barr and Weatherley (1962),

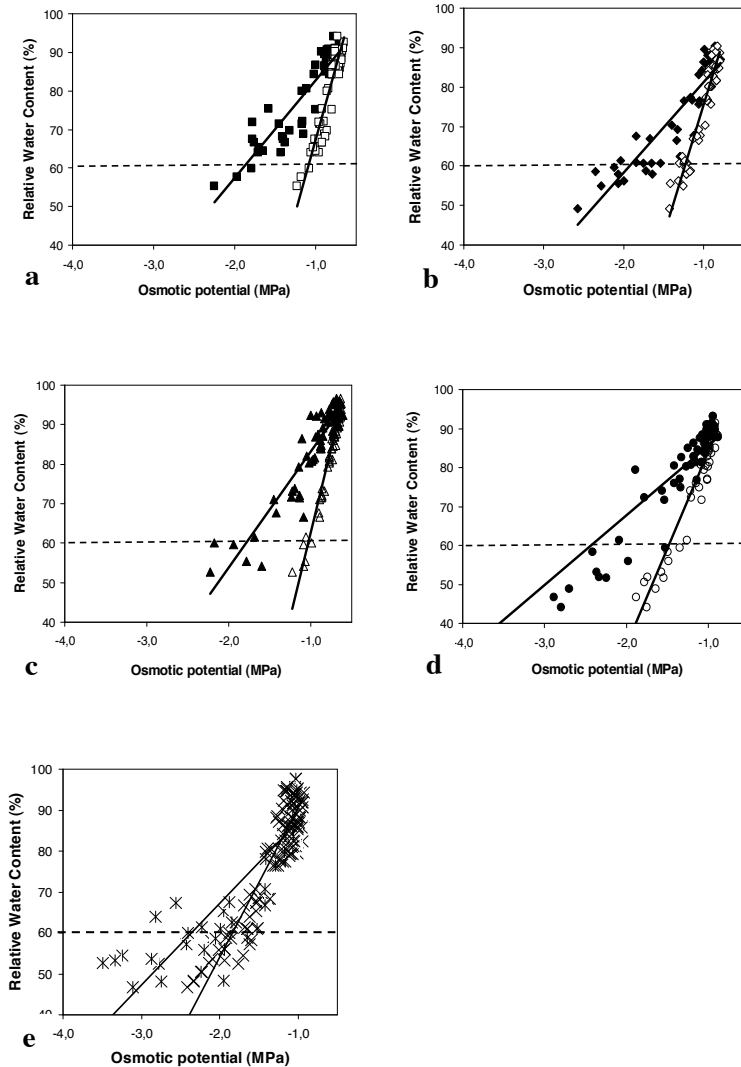


Fig. 2 a & b: Linear regressions of relative water content (RWC) as a function of measured osmotic potential (OP; solid symbols) and as a function of calculated OP (OP_0) corrected for passive water loss (open symbols) for the five Beta types under drought stress. The horizontal dotted line indicates the 60% RWC, at which the difference between the two regression lines was calculated to estimate OA. a, sugarbeet cv. Bianca; b, leaf beet; c, garden beet; d, sea beet; e, *B. webbiana*.

can in some conditions show ambiguities, as recently showed by Boyer et al. (2008). These authors noticed that the occurrence of osmotic adjustment, and hence of high amounts of solutes in adjusting tissues, can lead to exceedingly high turgors upon the rehydration step used for RWC determination, with a consequent underestimation of RWC values due to an excessively high turgid weight factor. However, a time course analysis of turgid weight during the onset of stress (and therefore of OA), suggests that in our experimental system such an effect can be considered negligible, as no differences between TW vs. time dependence plots were evident in control and stressed plants (data not shown), and the TW values remained relatively stable over the 3-week period of the experiments.

The results indicated that sea beet has the highest OA capacity (Fig. 2), and a higher succulence under both well-watered and droughted conditions (Fig. 1c and d). In our growth conditions, however, this marked difference in succulence between accessions declined after 20 days of water withdrawal (Fig. 1d). The loss in LS at low RWCs was similar for all *Beta* types, and is in contrast with the finding of Ober et al. (2005) who reported an increase in specific leaf weight and succulence index in sugarbeet plants under drought. It should however be noted that Ober et al. recorded their data in a much more advanced growth stage, and the plants subjected to the stress were grown in field conditions.

The OP values measured in our experiments are consistent with similar data obtained by other authors within the *Beta* genus (Biscoe, 1972; McCree and Richardson, 1987; Katerij et al., 1997). Biscoe (1972) measured OP and leaf water potential Ψ_1 by thermocouple psychrometry in sugarbeet plants undergoing a 7-day stress. He found values between -0.80 to -1.40 MPa for OP and -0.50 to -1.40 MPa for Ψ_1 . These values compare well with the decrease found in the present paper from -0.85 to -1.75 MPa in OP (upon three weeks of water withdrawal)

Table 1. R² values for the linear regressions of RWC as a function of OP and as a function of OP₀, and the OA values calculated graphically in Fig. 2.

	R ²		OA at RWC=60%,
	RWC vs. OP	RWC vs. OP ₀	(MPa)
sugarbeet	0.87	0.87	0.81
leaf beet	0.85	0.92	0.70
garden beet	0.86	0.94	0.76
sea beet	0.80	0.93	0.95
<i>B. webbiana</i>	0.76	0.85	0.53

in sugarbeet plants during stress experiments, and the initial overall Ψ_1 found in our conditions ranging from -0.30 to -0.40 MPa, as measured by pressure chamber (data not shown). Therefore, the turgor pressure, P , of 60 day-old sugarbeet plants can be estimated to be around 0.50 to 0.60 MPa at full hydration. Similar values were also obtained for the other *Beta* types and species (data not shown).

However, for the purpose of comparing the ability of different *Beta* types to withstand drought, the relevant factor is OA rather than the simple measures of RWC and OP. *Beta* genus is in fact already known to react to water shortage by lowering its OP (McCree and Richardson, 1987; Ober et al., 2005). However, no data on OA had been so far obtained in different beet types, or in wild *Beta* species or subspecies, such as *Beta vulgaris* ssp. *maritima* or *Beta webbiana*. These species are potentially interesting, as they are known to be adapted to salty and/or semiarid environments (Frese, 2004). In fact, it has been demonstrated by other authors that there exists potentially useful genetic variation for traits related to drought tolerance (Ober and Lutherbacher, 2002; Ober et al., 2004). The OA values found at RWC equal to 60% for the different *Beta* types confirmed a higher capacity of sea beet (*Beta vulgaris* ssp. *maritima*) to adjust osmotically (OA=0.95 MPa, Table 1) compared to the other beet types, which nevertheless exhibited significant adjustment values (0.70 to 0.81 MPa range). OA values in *Beta vulgaris* ssp. *vulgaris* had been estimated by other authors who reported

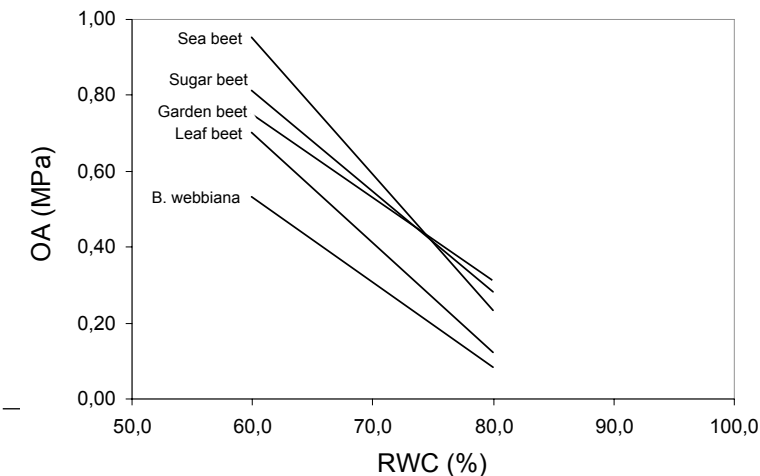


Fig. 3: Dependence of OA on water status of the plant, in the five *Beta* accessions. Water status is expressed as relative water content (RWC).

values of 0.40 MPa in sugarbeet plants adapted to irrigation with saline water (Katerij et al., 1997), or ranging from 0.22 to 0.50 MPa in different sugarbeet breeding lines grown in the field under drought conditions (Ober et al., 2005). However, both these authors estimated OA by different methods; the OA values found in the present work for the different *Beta* types were calculated taking into account the passive water loss, due to the drought conditions, according to the method of Chandra Babu et al. (1999). The 0.70 to 0.95 MPa overall range found for *Beta vulgaris* at RWC equal to 60%, was the graphically calculated difference between two regression lines, each having high R^2 values (Table 1), and with no need of logarithmic transformation of the data. When OA was plotted against RWC, the superior performance of sea beet in conditions of limiting water content was apparent, with this accession having the steepest slope (Fig. 3), indicating a more prompt and effective osmotically-adjusting response to RWC lowering. *Beta webbiana*, a wild species adapted to limited water conditions, seemed on the contrary, to rely less on OA (0.53 MPa) compared with the other *B. vulgaris* taxa. Probably different adaptive traits, such as leaf anatomy or limited leaf area (this species has small, lanceolate leaflets), are more important than OA in maintaining water content (Frese, 2004). However, OA values found in this work place all *Beta* types in the category of effectively adjusting species, compared to other crop plants. The variability within the genus, however, seems to be narrower for *Beta* relative to OA values for other species that were calculated using the same method (0.35 to 1.10 MPa at RWC equal to 60% for different rice cultivars, Chandra Babu et al., 1999; 0.13 to 0.30 MPa at RWC equal to 80% for different populations of maritime pine, Nguyen-Queyrens and Bouchet-Lannat, 2003). Nevertheless, if all *Beta* types exhibited fairly high OA values, sea beet had an OA exceeding by 15 to 25% those recorded for the three cultivated beet types, and almost twice that of *B. webbiana*.

In this study, only a specific *B. maritima* accession was examined, and therefore the superior OA value found for this wild accession is not necessarily a general feature of the entire subspecies. However, IDBBNR 7268, the *B. vulgaris* ssp. *maritima* accession used in this study, and other sea beets that might exhibit similarly effective water stress avoidance mechanisms in further studies, are suitable candidates for the study of the mechanism of OA onset at the biochemical and/or molecular level, with possible implications for breeding and sugar beet improvement.

The findings reported in this paper suggest specific and efficient mechanisms of solute accumulation mediating the slow onset of OA in *Beta*, particularly in the *maritima* ssp. accession. This research

provides information useful for future molecular analysis of this trait in wild and cultivated beets, and for the possible exploration of further variability in OA within the wild *Beta* germplasm.

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