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Responses of sugar beet (*Beta vulgaris* L.) to drought and nutrient deficiency stress

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Abstract

The responses of two sugar beet genotypes, 24367 (putative drought tolerant) and N6 (putative drought intolerant), to drought and nutrient deficiency stress were investigated in an attempt to identify reliable and sensitive indicators of stress tolerance. In glasshouse-grown plants of both genotypes, relative water content (RWC) of the leaves decreased and leaf temperature increased in response to drought stress. Genotype differences in response to drought included leaf RWC, glycine betaine accumulation, alteration of shoot/root ratio and production of fibrous roots. Thus, in comparison to N6, genotype 24367 lost less water from leaves, produced more fibrous roots, produced more glycine betaine in shoots and tap roots and had a much reduced shoot/root ratio in response to withholding water for up to 215 h. The hydraulic conductance and sap flow of sugar beet seedlings grown in nutrient culture decreased when subjected to nitrogen deficiency stress. Under nitrogen sufficient conditions sap flow was greater in 24367 than in N6. The results indicate that genotype 24367 is more tolerant to stresses induced by water and nitrogen deficiency and that increased fibrous root development may be a major factor in increasing sap flow via a concomitant enhancement of aquaporin activity.

Introduction

Crop plants rarely attain their full yield potential because of limitations imposed by the environment, such as water deficiency, adverse temperatures and nutrient imbalance (Kramer 1980). Drought stress is considered to be one of the largest, single causes of yield loss in UK sugar beet production (Clarke et al. 1993). The mean annual loss of sugar production due to water stress has been estimated to be as much as 141,000 tonnes per year, equivalent to £27.9 million revenue (Pidgeon and Jaggard 1998). A number of well-observed physiological and biochemical changes occur in a drought-stressed plant. Although sugar beet has a deep root system, leaf wilting occurs frequently under conditions of high evaporative demand (Clarke et al. 1993). Subsequent stomata closure can reduce leaf water potential thus maintaining water uptake, photosynthesis and growth for as long as possible (Clarke et al. 1993). However, stomatal closure is also coupled with an inhibition of carbon dioxide uptake and nutrient flow from the roots, the result being that, eventually, photosynthesis and subsequent carbohydrate production are reduced (Dunham and Clarke 1992). Closure of the stomata causes evapotranspiration to cease, which in turn raises the temperature of the leaf (Lourtie et al. 1995). Ehrler et al. (1978) argued that leaf temperature could be used as a reliable and amenable indicator of water stress and irrigation needs. The development of infrared thermometry to quantify differences in canopy temperature allowed a 3 °C difference in temperature between irrigated and unirrigated potatoes (*Solanum tuberosum*) to be observed (see Clawson and Blad 1982).

In dry soils, root growth is much less depressed than shoot growth and there is typically an increase in the root to shoot dry weight ratio in response to drought stress (Marschner 1995). The effect of water deficiency stress on sugar beet dry matter partitioning is unclear, though it seems that sugar beet has a great capacity to recover leaf area following drought and subsequent irrigation (Abdollahian-Noghabi and

Froud-Williams 1998). The greatest reduction in dry matter accumulation following drought stress usually occurs in the sugar beet storage root. Hostile environmental pressures such as predation, pathogen attack, chill injury and drought can also lead to chlorophyll degradation (Hendry et al. 1987), thus causing irreversible damage to photosynthesis from stress, including water deficiency (see Clarke et al. 1996).

Many plants are known to accumulate proline in response to drought stress. However, glycine betaine accumulation is characteristic of others, particularly members of the family Chenopodiaceae, including sugar beet (Wyn Jones and Storey 1978). The major role attributed to glycine betaine is as an osmoprotectant in water-stressed cells (see Clarke et al. 1993). Glycine betaine aids in the maintenance of enzyme activity under further environmental stresses (Mickelbart et al. 1997). Both glycine betaine and α-amino-N compounds accumulate in sugar beet roots following stress relief and, although the amino compounds are utilised readily during re-growth, glycine betaine is not (Clarke et al. 1993). Unfortunately, glycine betaine, along with sodium and potassium, are the principal impurities that reduce sugar beet quality for processing by inhibiting crystallisation during processing (Clarke et al. 1993).

Nitrogen is the most important element of those supplied to sugar beet because it is rare to find soils that contain sufficient amounts in an available form. Chlorophyll content and photosynthetic rates may also be reduced in plants lacking nitrogen (Draycott 1993). On the other hand, excess nitrogen can also have a detrimental effect on sugar beet. A delay in the initiation of storage processes, a reduction in both the growth rate and photosynthate accumulation of storage organs have all been identified in nitrogen-deficient sugar beet (Marschner 1995). Recent studies have shown that a plant's transpiration, stomatal conductance (G_s) and root hydraulic conductivity (Lp_r) are influenced strongly by its supply of certain mineral nutrients (Clarkson et al. 2000). Depriving plants of adequate supplies of the major nutrient anions has been shown to cause a reversible reduction of cell and root hydraulic conductivity. It seems likely that when a plant is subjected to nutrient deficiency, alterations in the aquaporins slow the movement of water through the plant. In this way, the plant is able to close its stomata and restrict leaf expansion with no detrimental effect on leaf water potential. When favourable conditions are re-instated, the plant is able to return to its fully functional state. A sugar beet genotype that can retain as near an optimum hydraulic conductance as possible while subjected to nitrogen deprivation would be of benefit to a reduced-input farming system.

The genetic capacity of sugar beet to overcome stress-induced problems needs to be assessed to aid in future breeding programmes. Thus the responses of two sugar beet genotypes, 24367 (putative stress tolerant) and N6 (putative stress sensitive), to drought and nutrient stress were investigated to determine whether a reliable indicator for the early recognition of stress in susceptible genotypes could be identified.

Materials and methods

Plant growth

Drought studies

Two sugar beet genotypes were used in the experiments. Genotype 24367 was tentatively designated as being 'stress tolerant' and N6 'stress intolerant', on the basis of yield results from drought stress field experiments conducted at IACR Broom's Barn in 1999 (E. Ober, unpublished results). Plants were raised in polystyrene modules with one plant per module from seed obtained from Broom's Barn. At the 6–8 true leaf stage, they were transferred to 10 cm diameter 0.4 l pots. Later, at the 12-leaf stage, they were transferred into 15 cm diameter 2 l pots containing John Innes compost mixture (by volume: 6 parts shredded/sterilised loam, 4 parts peat, 2 parts Cornish grit, 100 l perlite, 3.3 g l^{-1} Osmocote and 3.3 g l^{-1} magnesium limestone). There were 20 individuals per genotype. The plants were grown in a greenhouse with a minimum 16 h photoperiod at 15 °C to 20 °C and relative humidity of 60–70% over the 5 month growing period. The plants were arranged in a completely randomised formation on a greenhouse bench and watered twice daily for 75 s via capillary matting.

To induce drought stress, 3 randomly chosen individuals of each genotype were placed on a saucer so that no water could reach the roots. A further 3 individuals of each genotype were chosen randomly and remained fully watered. Water was withheld for approximately 200 h and the chlorophyll content, relative water content and leaf temperatures were sampled 7 times throughout that period. At the end of the stress period the plants were removed for biomass analysis and samples removed for glycine betaine determination. The whole procedure was repeated using

a further 6 individuals per genotype, 3 fully watered and 3 water stressed. In the final set, the remaining 8 individuals of each genotype were used.

Nutrient study
Plants were raised from seed in a 50:50 ratio of perlite and sand in a mist chamber until the first true leaves appeared. During this time they were watered 3 times a week with a weak nutrient solution. They were then carefully removed from the growth medium and the roots cleaned of any debris with distilled water. The seedlings were then grown in continuously aerated half-strength nutrient solution as described previously (Thomas 1993). Seedlings were allowed to acclimatise for 5 days on ½ strength +N nutrient solution. There were 2 trays per genotype and following the acclimatisation period, the +N solution from one tray per genotype was emptied and replaced with −N solution. The plants were then grown for a further 4 d before sap was collected for conductance and osmotic potential measurements.

Physical measurements

Relative water content
Relative water content of the leaves was determined using the methods of Weatherly (1949). One leaf disc (diameter 10 mm) was removed per plant from a mature, but not senescing leaf, making sure to avoid the main leaf vein. Each disc was placed into a separate glass-stoppered tube. Having ensured that there was no excess water on the discs, the fresh weight of each was recorded. The discs were then floated on 2 ml of distilled water for 24 h in natural daylight. At the end of this period the fully turgid leaf discs were rapidly surface dried with filter paper and re-weighed. These discs were then dried at 60 °C for 24 h and the dry weights established. The relative turgidity was calculated.

Leaf temperature
The temperature measurements were determined using a portable infrared thermometer (Linear Laboratories C-1600) held 1 cm away from the leaf. The youngest leaves emerging from the crown were chosen for measurement because they were easily identifiable and actively transpiring. The measurements were always taken between 9.30 and 10.00 a.m.

Plant growth
At the end of the drought studies each plant was harvested, the fresh and dry weights of the shoots, taproot and fibrous roots were determined. For dry wt determination the plant material was oven-dried at 80 °C for 48 h.

Biochemical methods

Chlorophyll determination
Chlorophyll was extracted using repeated acetone washings until the leaf was completely bleached. The absorbance at 645, 652 and 663 nm of combined extracts in 80% acetone was read on a spectrophotometer (SP8-100 UV/Vis) and the proportions of chlorophyll (total, *a* and *b*) were determined using standard equations.

Extraction and analysis of sap for glycine betaine
Sap was extracted using the methods of Bell et al. (1992). Firstly, tissue from either the taproot or the leaves (1 g) was placed into the barrel of a 2 ml plastic syringe with a disc of Whatman GF/A glass microfibre paper covering the outlet hole. The plunger was re-inserted and blue-tack was used to seal the hole. The whole syringe was stored at −20 °C until needed for analysis. Once the sample had thawed, the plunger was removed and the syringe barrel placed into a 15 ml centrifuge bucket with the finger-lugs resting on the rim of the bucket and centrifuged at 1500 *g* for 10 min. The sap was diluted 1:10 with deionised water and 250 μl aliquots were dispensed into 1.5 ml micro-centrifuge tubes containing 250 μl of 2N H_2SO_4 and cooled for 2 h on ice. Cold KI-I_2 reagent (200 μl; 17.5 g I_2 and 20 g KI in 100 ml of deionised water) was added and the contents were mixed thoroughly. The tubes were stored overnight at 4 °C, then spun in a microcentrifuge for 15 min. The supernatant was carefully removed using a 200 μl gilson pipette leaving the betaine periodide complex on the sides and bottom of the tube. The residue was re-suspended in 1,2-dichloroethane and transferred to a 10 ml graduated tube, diluting with washings to 9 ml. After leaving in the dark for 2 h the absorbance was read at 365 nm. Glycine betaine concentrations of unknown samples were calculated from a polynomial distribution curve derived from the absorbance of a serial dilution of a standard solution of glycine.

Hydraulic conductance

The stem of the plant was cut just below the first node, leaving a cylinder of stem and roots. A glass capillary tube was placed over the cut end of the stem and sealed with silicon grease. For a given period of time, the sap was collected in the capillary tube and then transferred to an Eppendorf tube. The volume of exuded sap was determined by weighing (ΔV_x). The roots were removed and weighed. Sap flow was expressed in mg.(g root FW)$^{-1}$. h^{-1}. The osmotic potential of the xylem sap samples (C_x) and nutrient medium (C_o) were used to measure osmotic pressure using a freezing-point osmometer. The hydraulic conductivity of the root system (L_{pr}, mg. (g root FW)$^{-1}$. h^{-1}.Mpa^{-1}) was calculated using the equation: $L_{pr} = J_v/RT.\Psi_\phi$, where R denotes the gas constant (0.0832), T the absolute temperature (K), J_v the flow rate (mg.(g root FW)$^{-1}$. h^{-1} of the sap through the roots, and the osmotic pressure difference between the xylem sap and external solution, Ψ_ϕ, ($C_x - C_o$) in mosm. The glycine betaine concentration of the exuded sap was determined using the method described previously.

Results

Drought study

Three separate experiments were done on successive weeks during the drought study. There was some inconsistency in the results from the different experiments but certain features were common to all three. Therefore the relevant results from experiment 2 are presented as being representative of the main findings.

A feature common to both genotypes was that % RWC decreased as a consequence of withholding water, particularly from 96 h onward, until by the end of the experiment (168 h) it was on average ca 20% less than in well-watered plants. However, the leaves of genotype 24367 contained more water (59%) than did those of N6 (44%) when drought stressed (data not presented). In both genotypes, the drought treatment had no significant effect on tap root water content. The reduction in RWC was correlated with an increase in leaf temperature of 2 °C from 96 h after the commencement of the drought treatment in both genotypes. Leaf chlorophyll content (total, *a* and *b*) was reduced by ca 38% as compared to that of watered plants by the end of the experiment, but there was no significant difference between both genotypes in this respect.

Table 1. Mean dry weights of plant parts and shoot:root ratios of two sugar beet genotypes in response to 168 h of drought stress.

Plant measurement	Genotype		l.s.d. at 5%
	24367	N6	
Watered plants			
D wt shoot (g)	13.59	10.40	2.76
D wt tap root (g)	9.40	14.90	4.66
D wt fibrous root (g)	3.40	7.60	0.74
Ratio total shoot/root	1.061	0.463	0.42
Droughted plants			
Ratio total shoot/root	0.541	0.438	0.42
% change* –shoot wt	−51.4	−39.8	8.2
–tap root wt	−6.5	−36.3	7.3
–fibrous root wt	+190	+60	21.3

* In comparison with fully watered plants.

Table 2. Effect of 168 h drought stress on the glycine betaine content (mg g^{-1} f wt) of shoots and tap roots of two sugar beet genotypes.

Genotype	Shoots		Roots	
	Watered	Droughted	Watered	Droughted
24367	1.93	8.18	2.82	6.37
N6	4.95	6.60	3.07	5.71
l.s.d. at 5% level	3.01		2.86	

Overall, the tap root dry matter percentage of genotype 24367 was greater than that of N6 but in well-watered conditions N6 had a more extensive root system, a smaller shoot system and hence a lower shoot/root ratio (Table 1). In response to drought, the major change in 24367 was a considerable reduction in shoot growth but this was compensated for by a large increase in fibrous root development. Genotype N6 showed a similar reduction in both shoot and tap root growth, but there was less change in fibrous root development than in genotype 24367.

Under well-watered conditions, 24367 had less glycine betaine in both shoots and roots as compared with N6 (Table 2). However, 24367 reacted to water-deficiency stress by increasing glycine betaine in both organs by between 2.5- and 4-fold. There was no statistically significant increase of glycine betaine in response to stress in N6.

Table 3. Changes in J_v and L_{pr} in two sugar beet genotypes without or supplied with nitrogen for 4 d.

Parameter	Genotype/treatment			
	24367 (+N)	24367 (–N)	N6 (+N)	N6 (–N)
J_v (mg g^{-1} h^{-1})	161.9 (17.30)[a]	18.9 (4.03)	88.5 (17.08)[a]	21.3 (1.52)
L_{pr} (mg g^{-1} h^{-1} Mpa^{-1})	1927 (126.3)[b]	238 (24.9)	1318 (181.6)[b]	226 (24.9)

Similar superscripts indicate a significant difference between values at the 5% level or better using the *t* test.

Nutrient study

Overall, there were no differences in either J_v or L_{pr}, between 24367 and N6 plants deprived of nitrogen. However, nitrogen deprivation resulted in a significant reduction of J_v and L_{pr} compared with plants maintained on a constant nitrogen supply (Table 3). The osmotic pressure of the xylem sap (C_x) was the same in 24367 and N6 and increased equally in both populations with nitrogen starvation (data not shown), indicating that the driving force for sap flow through the roots was similar for these genotypes. However, J_v and L_{pr} were greater in 24367 plants than in N6 plants supplied with nitrogen.

Discussion

Cessation of watering for a period of at least 6 days (144 h) imposed severe water stress on the two sugar beet genotypes and resulted in a reduction of the RWC of the leaves, as demonstrated previously by Clarke et al. (1993). However, the leaves of genotype 24367 contained more water than those of N6 when drought stressed, which suggests that RWC may be a good indicator of drought stress tolerance.

Withholding water for at least 72 h increased the temperature of the leaves, but there was no significant difference in the response of the genotypes. The usefulness of this technique as an indicator of drought stress in agriculture is limited because leaf temperature consistently changes with ambient temperature. To provide a baseline figure, from which to determine temperature differences, a fully watered control would need to be kept as close as possible to the crops of interest. A better method to employ would perhaps be the temperature difference between the crop canopy and the surrounding air. Previous studies have shown that before irrigation, the temperature of cotton leaves was 2 °C higher than that of the surrounding air (Ehrler et al. 1978).

The glycine betaine content of the shoots and tap roots was significantly different between genotypes, as was the response of the genotypes to drought. Withholding water increased the glycine betaine content of genotype 24367 only. The results from this experiment, and the RWC measurements, suggest that 24367 may be more tolerant to drought than N6. It could be that genotype N6 contains inherently higher levels of glycine betaine because even under well-watered conditions the solute potential of the cytoplasm and vacuole is low compared to that of 24367. Only under severe stress conditions is it necessary for genotype 24367 to accumulate significant quantities of 'compatible osmotic solutes'. The increase in glycine betaine content of the taproots of 24367 may well benefit the plant when subjected to drought stress, but would hinder the eventual extraction of sugar (Clarke et al. 1993). The usefulness of this genotype in a breeding programme is also limited because of its small taproot size. An ideal genotype to use would have a high yield, even when under stress, without accumulating large amounts of glycine betaine in the taproots.

Water stress has previously been shown to considerably lower the chlorophyll content of French and mung bean leaves (Upreti et al. 1998; Zayed and Zeid 1998). However, there was no indication from our experiments that chlorophyll measurements could be used as an indicator of genotype differences in drought stress tolerance in sugar beet. This is despite the fact that the effect of drought stress on chlorophyll fluorescence as an indicator of genotype differences in sugar beet has been demonstrated previously (Clarke et al. (1993, 1996)). Thus, it would seem that chlorophyll fluorescence remains the most effective method for measuring damage to the photosynthetic apparatus caused by drought stress and cannot be replaced by the less time consuming measurement of chlorophyll quantity.

A lack of water in the growth medium adversely affected every parameter of plant growth in at least one of the experiments. Abdollahian-Noghabi and

Froud-Williams (1998) noted an 84% reduction in the leaf area and a 46% reduction in the taproot d wt of sugar beet when subjected to drought stress. In our experiments, we noted significant decreases in shoot d wt in both genotypes but tap root d wt was significantly reduced only in the drought intolerant genotype.

Previously, it was shown that in maize there was a decrease in the shoot/root ratio following drought stress (see Marschner 1995). This was also observed in genotype 24367 but not in N6. The results also demonstrated that the former genotype had a large capacity for increasing fibrous root growth under water deficient conditions, which may well allow the plant to draw more moisture from the soil and hence reduce leaf wilting. Such ability would provide a favourable adaptation to confer some water deficiency stress tolerance.

A field trial of the two genotypes in 1999 indicated that when N6 was irrigated it was the more productive one (had a larger taproot) but showed the larger yield reduction when droughted (E. Ober, unpublished results). The results of our experiments indicated that N6 had, on average, 34.5% larger taproots than 24367. When water-deficiency stressed, the taproot d wts of 24367 and N6 were reduced by 6.5% and 36.3%, respectively. These results are consistent with the suggestion that 24367 may be drought 'tolerant' and N6 drought 'intolerant'.

In the nutrient deficiency stress experiments, the removal of nitrogen from the growth medium resulted in the reduction of hydraulic conductivity (expressed as L_{pr}) and sap flow (J_v) through the roots. In nitrogen supplied 24367 plants, the flow of sap through the roots was greater than in N6, although there was no difference between the osmotic potential of the xylem sap (C_x). This result suggests that the increased fibrous root development of the 24367 genotype may be an important factor in increasing sap flow via a concomitant increase in aquaporins. However, deprivation of nitrogen has been shown to reduce the density of aquaporins and so the transport of water through the plant becomes more dependent on diffusion (Carvajal et al. 1998). Therefore, the fact that in both genotypes J_v and L_{pr} were reduced to similar levels would tend to support the notion that a reduction in water channel density was the causative factor in the response to nitrogen deprivation. This is in agreement with the results of Carvajal et al. (1998), that information about the quality of the nutrient supply being received by a root can be transduced into a hydraulic response.

Conclusions

Sugar beet (*Beta vulgaris*) originates from the salt-tolerant *Beta maritima* that possesses special mechanisms to combat drought and is hence highly drought tolerant (Clarke et al. 1993). However, breeding for improved yield and quality may have inadvertently reduced the crop's ability to overcome drought stress. The results presented here have suggested some possible indicators of stress tolerance, particularly genotypic differences in RWC, glycine betaine content, shoot/root ratio and fibrous root growth in response to water deficiency.

In practice, the small tap root size of 24367, compared to N6, may limit its usefulness in a breeding programme for greater tolerance to water stress. In this respect, Abdollahian-Noghabi and Froud-Williams (1998) reported on the findings of numerous papers that had all observed that cultivars with a greater tolerance to water stress had reduced growth rates. This may prove to be a major impediment in the search for suitable germplasm to introduce into a breeding programme for stress tolerance.

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