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Assessment of the physiological responses to drought in different sugar beet genotypes in connection with their genetic distance

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SUMMARY

Drought affects many physiological processes, which influences plant productivity. The aim of this study was to evaluate the degree of genotypic diversity in drought tolerance of sugar beet genotypes (*Beta vulgaris* L.) in connection with their genetic distance. Three hybrid genotypes produced by crossing double haploid genotype (P-pollinator) with cytoplasmic male-sterile female part (MS), as well as with two parent lines, were examined. Drought conditions were imposed by the cessation of watering at the 3–4 leaf stage for about three months, after which irrigation was resumed. Control plants were optimally irrigated throughout the entire vegetation period. Long-term drought significantly increased the wilting of leaves (Wilt.), specific leaf weight (SLW), the succulence index (Suc.I), leaf senescence and membrane damage (El-l). Simultaneously, the osmotic potential (ψ_s), leaf area index (LAI), absorption of photosynthetic active radiation (PAR) and the efficiency of the photosynthetic apparatus (Φ PSII) declined under water deficit conditions. The examined genotypes demonstrated a clear diversity in their physiological response to drought. Based on these findings, we suggest that traits that are strongly correlated with root and sugar yield, e.g. Φ PSII, LAI, PAR absorption and ψ_s, could be used as potential selection criteria in physiological-associated breeding strategies to improve drought tolerance in sugar beet. There was not a significant correlation between the genetic distance separating different sugar beet genotypes and the observed heterotic effect of root or sugar yields, with the exception of heterosis of root yield under optimal conditions, where the correlation was negative.

Introduction

World agriculture is greatly affected by the lack of a sufficient amount of water, which can seriously reduce crop yield. The productivity of sugar beet is currently decreasing under conditions of intermittent water deficiency. Estimates of potential sugar beet yield losses in Europe, due to insufficient water resources, vary between 5% and 30% (Pidgeon et al., 2001). One solution is to breed beet varieties that are tolerant to water stress, based upon morphological, physiological biochemical and molecular criteria. Appropriate criteria may be identified by detailed study of the effects of water stress on this crop and its drought tolerance.

Even small changes in the water potential in beet leaves (0.2–0.3 MPa) can substantially decrease the growth rate of this organ, with a complete cessation of growth at a Ψ_w of −1.5 MPa (Milford and Lawlor, 1976). Water deficiency imposed at an early stage of beet development was found to not only dramatically hamper leaf assimilatory surface expansion (Chołuj et al., 2004), but also to reduce, to a lesser extent, taproot growth. Drought decreases the leaf area index (LAI) in stressed sugar beet plants (Clover et al.,

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Abbreviations: DAE, days after emergence; El-l, membrane damage; F'_m, maximum fluorescence; F_t, steady-state fluorescence; NPQ, non-photochemical quenching; LAI, leaf area index; ΦPSII, effective quantum efficiency of photosystem II; ψ_s, osmotic potential; PAR, photosynthetic active radiation; RAPD, random amplification of polymorphic DNA; ROS, reactive oxygen species; RUE, radiation use efficiency; SLW, specific leaf weight; Suc.I, succulence index; Wilt., wilting of leaves; WUE, water use efficiency.

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1999; Chołuj et al., 2004; Mohammadian et al., 2005). Thus, the determination of leaf area and/or LAI represents a useful tool for assessing the drought tolerance of sugar beet cultivars (Ober and Luterbacher, 2002; Ober et al., 2005; Tsialtas and Maslaris, 2007; Chołuj et al., 2010).

Under drought conditions, the wilting of beet leaves (Wilt.) in response to dehydration varies according to their age; mature leaves lose turgor earlier than the youngest ones (Milford and Lawlor, 1976). Reduced water availability may cause "xenomorphic" changes in beet leaves, such as increased specific leaf weight (SLW) and succulence index (Suc.I) resulting from increases in leaf thickness (Mohammadian et al., 2005; Ober et al., 2005). Simultaneously, the senescence of the oldest leaves may be accelerated by water shortage (Mohammadian et al., 2005; Ober et al., 2005).

Sugar beet plants subjected to drought show high osmotic adaptation ability (Gzik, 1996; Ober et al., 2005; Chołuj et al., 2008). The lowering of the osmotic potential (ψ_s) in the cells of sugar beet occurs mainly due to the accumulation of osmoprotectants such as: potassium or sodium, soluble sugars and their derivatives as well as nitrogen-containing substances such as glycine betaine, proline or other free amino acids (Shaw et al., 2002; Bloch and Hoffman, 2005; Bloch et al., 2006; Chołuj et al., 2008). Osmoprotectants, which are beneficial for the acclimation response of the plant to dry conditions, are melassigenic and so negatively influence the quality of the sugar beet root (Clarke et al., 1993; Campbell, 2002). Moreover, these substances may be rapidly reduced to ammonia, which causes juice alkalization.

It is thought that the initial stages of photosynthesis, connected with energy absorption and the rate of electron transport, are relatively resistant to dehydration. Under field conditions, it is difficult to separate the effects of insufficient moisture, high irradiation and/or heat. Therefore, in times of severe water deficiency, the carboxylation capacity is lowered and the demand for energy dissipation increases (Wilhelm and Selmar, 2011). Under these conditions, the protective mechanisms against the deleterious effects of free radicals become insufficient, as they are overwhelmed by the production of reactive oxygen species (ROS) (Møller and Sweetlove, 2010). Thus, damage to photosystem II (PSII) and thylakoid membrane disintegration are well recognized effects of severe dehydration. Chlorophyll a fluorescence parameters can be used to show the influence of stress factors on the activity of the primary photosynthetic reaction and in sugar beet, such measurements have been employed to detect the photoinhibitory effects of drought (Clarke et al., 1993; Clover et al., 1999; Chołuj et al., 2001; Ober et al., 2005; Bloch et al., 2006; Mohammadian et al., 2003).

There is a lack of reported data characterizing the reactions of currently grown varieties of sugar beet to drought. Nevertheless, recent research has demonstrated that there is considerable genotypic variability in drought tolerance in the available sugar beet germplasm (Ober and Rajabi, 2010). Under drought conditions, the examined sugar beet genotypes exhibited a clear diversity in the root yield and stability of yielding, harvest index and radiation use efficiency (RUE) (Ober et al., 2004). Genotypic diversity in water use efficiency (WUE), the accumulation of sodium cations (Sadeghian et al., 2004), as well as variation in chlorophyll fluorescence parameters depending on water deficiency tolerance (Mohammadian et al., 2003), have also been demonstrated. Furthermore, Ober et al. (2005) observed genotypic diversity in drought-related physiological traits such as stomatal conductance, succulence index, SLW and osmotic adjustment. Recently, the results of Romano et al. (2013) showed a wide genetic variation in root morphological parameters in sugar beet genotypes.

Many plant breeding programs are based on heterosis, in which hybrids exceed their parents with respect to yield, biomass, stress tolerance and reproducibility. Despite considerable research effort over the last century, the genetic mechanisms underlying this natural phenomenon are still largely unknown (Birchler et al., 2003). A greater knowledge of heterosis would permit its more effective utilization for practical breeding. One important unanswered question is how the degree of heterosis in F1 progeny correlates with the level of differentiation between parental lines. Previously, there have been indications that the degree of heterosis for some characters is related to the genetic divergence between the parents. The genetic distance between parents can be measured by PCR-based techniques such as AFLP (amplified fragment length polymorphic DNA), SSR (simple sequence repeats) or RAPD (random amplification of polymorphic DNA). The RAPD strategy has been used in genetic distance studies as a cost-effective tool to screen random genome sites of many crop species and their wild relatives, including *Beta* species (Reamon-Büttner et al., 1996; Shen et al., 1996).

The aim of the present study was to analyze and select morphological and physiological traits that describe the degree of diversity in sugar beet genotypes in their response to drought. Another goal was to evaluate the genetic distance between the studied genotypes and to correlate this with the effects of heterosis in the root and sugar yield.

Materials and methods

Plant materials

Experiments were conducted on eight monogerm diploid ($2n = 18$) sugar beet breeding genotypes (*Beta vulgaris* L. subsp. *vulgaris*) that were hybrids and their parents. Hybrids were produced by crossing a cytoplasmic male-sterile seed-bearing partially inbred parent (MS) with a pollen fertile double haploid parent (P, pollinator). Two of the hybrids (MS2 × P2 and MS2 × P3) came from the same male-sterile maternal form using different pollinators. All seeds used here were kindly provided by Kutnowska Sugar Beet Breeding Company (KHBC Ltd., Poland) and represent part of an ongoing breeding program.

Plants were grown in 8 polythene tunnels (8 m long, 3.6 m wide and 4 m high) on the experimental field of KHBC in Straszków, Poland. The tunnels had a minor effect on the microclimate, and did not create any combinations of weather factors that are unlikely to occur in the open field. The experiment was performed during 2008 and 2009. In 2008, we performed screening experiment and examined 24 different genotypes. Three hybrids with the greatest effects of heterosis in the root and sugar yield and their parents were selected for the next investigation. Records for the outdoor temperature and rainfall during the period of plant vegetation are shown in Table 1S. In both years, the months with the highest temperatures were July and August, while those with the greatest rainfall were May, July and September or June and July in 2008 and 2009, respectively. Plants were grown in light, leached, sandy-clayey soil. This soil belongs to quality IIIb or IVa and to the "very good rye complex." In the tunnels, the plant roots were protected from uncontrolled soaking from the outside by a surrounding polythene border of 1 m depth. Plants were grown in randomized blocks with one (2008) or four (2009) replicates – plots with 60 plants per genotype and their final density constituted 10 plants per m^2 (six rows per plot). To avoid border effects, the plots were surrounded two border rows, which were not harvested.

In the spring before the sowing, the field was fertilized with pure nutrients ha^{-1} as following: in 2008 – 68 kg N; 50 kg P; 85 kg K but in 2009 – 75 kg N; 60 kg P and 90 kg K, according to the requirements calculated based on the analysis of soil samples.