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Research article

Fodder beet is a reservoir of drought tolerance alleles for sugar beet breeding

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ABSTRACT

Drought leads to serious yield losses and followed by increasing food prices. Thereby, drought tolerance is one of most important, pivotal issues for plant breeding and is determined by the very complex genetic architecture, which involves a lot of genes engaged in many cell processes. Within genomes of currently cultivated sugar beet forms, the number of favourable allelic variants is limited. However, there is a potential to identify genes related to drought tolerance deposited in genomes of wild or fodder relatives. Therefore, the goal of our study, was to identify the source of allelic variants involved in drought tolerance using a large spectrum of sugar or fodder beets and their wild relatives for analyses. Based on the drought tolerance index, calculated for morphophysiological traits, it was demonstrated that some of selected fodder beets showed the highest level of drought tolerance. The most drought tolerant fodder beet genotype did not show differences in the level of expression of genes engaged in osmoprotection and the antioxidative system, between control and drought condition, compared to sugar and wild beets. The genetic distance between selected beet forms was broad and ranged from 18 to 87%, however the most drought tolerant sugar, fodder and wild beets showed high genetic similarity and formed the common clade. Based on obtained results we propose that an adequate broad source of genes related to drought tolerance occurs in fodder beets, the crossing with which is easier, less time-consuming and more cost-effective than with wild forms of beets.

1. Introduction

The range of genetic diversity is of fundamental interest for plant breeders. On one hand, sugar beet species has a narrow range of genetic variability (Ober and Luterbacher, 2002). On the other side, economical traits (yield, biomass, stress tolerance and reproducibility) of sugar beet are very variable as they are determined by polygenes and are significantly altered by environmental factors. The 'White Silesian' beet is considered to be the ancestor of all present sugar beet forms. Presumably, it originated from crossing between fodder beet and chard (Francis, 2006). The concepts of gaining wider range of variability of important traits, by crossing sugar beet with another beet forms, appeared very early in sugar beet breeding history, along with the trials of re-synthesis of sugar beet from other *Beta* forms (Francis, 2006). Currently, one of the goals of obtaining new cultivars of sugar beet is the classification of differences between genotypes growing under drought conditions and the development of selection criteria based on morphological, physiological or molecular traits (Chołuj et al., 2014). It is noteworthy that so far no drought tolerance markers have been found for sugar beet, except for a decrease of root yield, which is inconvenient for breeders.

Inadequate water supply results in worldwide reductions in crop productivity (Farooq et al., 2012). Under significant water deficiency, loss in yield of sugar beet increases drastically and climate change is expected to enhance this effect in the future (Hoffmann and Kenter, 2018). Water deficiency induces morphological changes in beet leaves manifested as an increase of the specific leaf weight (SLW) and succulence index (Suc.I) (Chołuj et al., 2014). These xeromorphic effects of drought are related to the increase of leaf thickness, helping to protect leaves from an excessive heat load. Fewer and smaller leaves can withstand water deficit conditions by maintaining water budget by involving higher water uptake and reducing its loss. Even small changes in relative water content (RWC) and water potential in beet leaves substantially decrease their growth rate and leaf assimilatory expansion (Choluj et al., 2004). In field experiments, a water shortage markedly reduced the leaf area index (LAI), which is caused by drought induced leaf senescence (Chołuj et al., 2014, 2010). Sugar beet root growth is also inhibited by water deficits, but to a lesser extent than the growth of leaves. This difference results in an increase in the storage root to shoot dry matter ratio (Choluj et al., 2004). However, some opposed results have also been reported (Hoffmann, 2010).

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Drought is often accompanied by high levels of irradiation and heat. These conditions disturb the balance between the production of reactive oxygen species (ROS; which are necessary for the regulation different pathways during plant acclimation to abiotic stress) and antioxidant defence mechanisms, thereby causing oxidative stress (Choudhury et al., 2017; Farooq et al., 2012; Sayfzadeh and Rashidi, 2011). Sugar beet plants are characterized by a high capacity for osmotic adjustment (Chołuj et al., 2008). Under a reduced water supply, the accumulation of osmotically active inorganic and organic solutes is one of the most common acclimation mechanisms employed by plants (Farooq et al., 2012). The solute accumulation under water deficiency results in a reduction of the osmotic potential of sugar beet cells (Chołuj et al., 2014, 2008; Ober et al., 2005), which in turn attracts an influx of water and helps to maintain the pressure potential. Several types of compatible organic solutes, which protect cellular components from dehydration injury, are referred as osmoprotectants. They include proline, trehalose, quaternary ammonium compounds, such as glycine betaine and others (Ashraf and Foolad, 2007). Glycine betaine is known to be accumulated in response to dehydration stress and remains abundant mainly in chloroplast, where it plays a vital role in protection of thylakoid membrane securing photosynthetic processes. Glycine betaine is synthesized in chloroplast from serine via ethanolamine, choline and betaine aldehyde. Choline is converted to betaine aldehyde, by choline monooxygenase (CMO), which in turn is converted to glycine betaine by betaine aldehyde dehydrogenase (BADH) (Ashraf and Foolad, 2007). Tolerant genotypes of some crop species usually accumulate more glycine betaine than susceptible genotypes in response to stress (Ashraf and Foolad, 2007). Proline acts as an osmolyte for osmotic adjustment, contributes to stabilizing membranes and proteins by scavenging free radicals and buffering cellular redox potential under stress conditions (Ashraf and Foolad, 2007). In response to stress in plants, proline is accumulated in the cytosol, where it contributes to the cytoplasmic osmotic adjustment. In plants two enzymes, pyrroline-5-carboxylate synthetase (P5CS) and pyrroline-5-carboxylate reductase (P5CR), play major roles in proline biosynthetic pathway from l-glutamic acid. Probably, proline synthesis is mediated by both ABA-dependent and ABA-independent signalling pathways (Ashraf and Foolad, 2007). Trehalose is also engaged as a stress protector in responses to cold, salinity and in regulation of stomatal conductance and water-use efficiency. Trehalose is synthesized via a phosphorylated intermediate, trehalose 6-phosphate (Tre6P) by trehalose phosphate synthase (TPS) and trehalose phosphatases (TPPs) (Leyman et al., 2001).

Drought is considered to cause oxidative stress. Compatible solutes mentioned above are also responsible for the protection of cell membranes, enzymes and macromolecules from the damaging effects of ROS (Farooq et al., 2012), but negatively influence the quality of sugar beet roots by increasing the amount of sucrose lost to molasses (Bloch et al., 2006). Drought also affects the gene expression and activities of the major antioxidant/scavenging enzymes such as superoxide dismutase, catalase, ascorbate peroxidases and others (Noctor et al., 2014).

The information provided by aforementioned studies is potentially useful to understand the physiology underlying the response and/or as a marker in breeding programs. Breeders usually expect wild-type species to have broad sources of the genes responsible for tolerance or resistance to biotic or abiotic factors. The purpose of this work was to verify whether other *Beta* accessions might be potential sources of genes responsible for drought tolerance of sugar beet, which in future could be used for outcrossing in breeding programs. Another goal was to study the background of distinct physiological and molecular responses of different *Beta* accessions to drought conditions, with particular regard to genes engaged in osmoprotection and the antioxidative system.

2. Materials and methods

2.1. Plant materials

The preliminary experiments were performed on 12 double haploid (DH) sugar beet breeding genotypes (*Beta vulgaris* L. subsp. *vulgaris*) under polythene tunnels on the experimental field of Kutnowska Sugar Beet Breeding Company (KHBC Ltd.) in Straszków (Poland). The goal of field experiment was the selection of: two lines the most drought tolerant, two lines the least drought tolerant and one line with intermediate level of water stress tolerance. The seeds were kindly provided by KHBC. The tunnels imitated weather conditions (temperature, intensity of irradiance) similar to those experienced in an open field. Plants cultivated in the tunnel were protected from uncontrolled soaking with outside water by a polythene border of 1 m depth, installed around the tunnels. Plants were grown in randomized blocks in four plots with 60 plants of each genotype; the resultant plant density was of 10 plants per m^{-2}. The plots were surrounded by two border rows of plants that were not harvested. Initially, both the control and drought treated plants were automatically irrigated every day. The doses of water delivered to optimally irrigated plants were calculated and altered according to the requirements of each phase of beet plant development. Drought was initiated during the five-leaf phase (59 days after emergence, DAE) by completely withholding watering for approximately three months. At the end of vegetation (150 DAE) all plants were collected and their root yields were measured.

For pot experiments we used 5 out of aforementioned 12 genotypes selected in drought field conditions: two monogerm DH (double haploid) breeding genotypes (DH0726, DH0960) of sugar beet (*Beta vulgaris* L. subsp. *vulgaris*) less tolerant to drought, two monogerm DH genotypes (DH0734, DH0962) more tolerant to water stress and an analogue (NO0835, male-fertile) of male-sterile line O (Table 1). NO0835 line showed medium level of drought tolerance. Additionally, in pot experiments we analysed: two *Beta vulgaris* subsp. *maritima* (187786, 187794) and six fodder beet genotypes (188285, 188286, 188287, 188288, 188294 and 188655) (Table 1). The fodder beets differed in yield, sugar content, root shape and colour (Kużdowicz, 2013). The seeds of wild *Beta* species and fodder beet were kindly provided by National Centre for Plant Genetic Resources (NCPGR) at The Plant Breeding and Acclimatization Institute (IHAR) – National Research Institute (Radzików), Bydgoszcz Research Division (Poland).

Plants of those 13 accessions were grown on Wagner pots containing 9.5 kg substrate (mixture of soil with peat substrate and addition of macro- and microelements) in greenhouse of Warsaw University of Life Sciences – SGGW. After imbibition, 10 seeds were sown in each pot. Seedling were thinned after emergence to finally 4 plants per pot. Experiments were conducted as completely randomized for 3 replicates - Wagner pots per each genotype. At the beginning of plant vegetation both control and drought treated plants were irrigated to 60% of water-holding capacity (WC) on a weight basis every second day. Water supply was varied for drought treated plants at 61 DAE (3-leaf-stage) by reduction of watering to 30% WC, which is moderate stress for sugar beets. Illumination was provided under a 12 h light, 12 h dark cycle using sodium lamps. Estimation of most morpho-physiological traits were made in 12 replicates per genotype and both water regimes at 101 DAE.

2.2. Physiological methods

For measuring the specific leaf weight (SLW) and “succulence index” (Suc.I), ten leaf disks per every plant per genotype of 9 mm in diameter were collected using a cork borer, and their fresh (FM) and dry (DM) masses were determined. These parameters were calculated according to the formulas: SLW = DM/total sampled leaf disks area (mg DM cm^{-2}) and Suc.I = (FM-DM)/total sampled leaf disks area (mg H_2O cm^{-2}).

To determine osmotic potential (Ψ_s), fresh leaf samples (1 g FM) were harvested, frozen in liquid nitrogen and stored at −75 °C. Then, 0.5 g of each leaf FW sample was homogenized and the suspension was centrifuged. Filter paper disks were saturated with supernatant as a test solution and osmotic potential was measured using a HR-33T Microvoltometer (Wescor, USA) by hygrometric methods (i.e. dew point depression). Blades area were analysed by LI-3000A Portable Leaf Area Meter (Li-Cor, USA).

Table 1

List of accessions used in the study.

No.	Accession name	Taxonomic unit/common name	Gene bank or company/sample origin
1	DH0726[a]	*Beta vulgaris* subsp. *vulgaris*/sugar beet	KHBC Ltd., Poland
2	DH0734[b]	*Beta vulgaris* subsp. *vulgaris*/sugar beet	KHBC Ltd., Poland
3	DH0960[a]	*Beta vulgaris* subsp. *vulgaris*/sugar beet	KHBC Ltd., Poland
4	DH0962[b]	*Beta vulgaris* subsp. *vulgaris*/sugar beet	KHBC Ltd., Poland
5	NO0835[c]	*Beta vulgaris* subsp. *vulgaris*/sugar beet	KHBC Ltd., Poland
6	188285	*Beta vulgaris* subsp. *vulgaris*/fodder beet	NCPGR, PBAI-NRI Radzików, Poland/UK
7	188286	*Beta vulgaris* subsp. *vulgaris*/fodder beet	NCPGR, PBAI-NRI Radzików, Poland/UK
8	188287	*Beta vulgaris* subsp. *vulgaris*/fodder beet	NCPGR, PBAI-NRI Radzików, Poland/UK
9	188288	*Beta vulgaris* subsp. *vulgaris*/fodder beet	NCPGR, PBAI-NRI Radzików, Poland/UK
10	188294	*Beta vulgaris* subsp. *vulgaris*/fodder beet	NCPGR, PBAI-NRI Radzików, Poland/UK
11	188655	*Beta vulgaris* subsp. *vulgaris*/fodder beet	NCPGR, PBAI-NRI Radzików, Poland/Poland
12	187786	*Beta vulgaris* subsp. *maritima*/wild type	NCPGR, PBAI-NRI Radzików, Poland/Netherlands
13	187794	*Beta vulgaris* subsp. *maritima*/wild type	NCPGR, PBAI-NRI Radzików, Poland/Japan
14	187583[d]	*Patellifolia patellaris* Moq./wild type	NCPGR, PBAI-NRI Radzików, Poland/Canary Islands
15	01809[d]	*Beta macrocarpa*/wild type	NCPGR, PBAI-NRI Radzików, Poland/Portugal

[a] less drought tolerant line.

[b] more drought tolerant line.

[c] medium drought tolerant line.

[d] accessions used only in genetic diversity assay.

The relative chlorophyll and flavonoid contents in mature leaves were analysed by Dualex Scientific (Force-A, France). Six measurements were made for each leaf and their means were treated as a replicate. Chlorophyll *a* fluorescence parameters were measured on fully expanded leaves, which were the most photosynthetically active, with the fluorescence monitoring system FMS-2 (Hansatech Instruments Ltd, UK). In leaves adapted to ambient irradiation, the effective quantum efficiency of PSII (ΦPSII) was measured by FMS-2 and calculated by the manufacturer's software. During the measurement of chlorophyll *a* fluorescence in greenhouse, the photosynthetic active radiation (PAR) value oscillated between 1000 and 1400 μmol photons m^{-2} s^{-1} (PPFD), while the ambient temperature varied from 25 to 28 °C and air humidity 60–65%. At 101 DAE plants were harvested and the dry mass of select organs (i.e. blades, petioles and storage roots) were determined after drying at 105 °C for 24 h.

The drought tolerance index (DTI) was calculated for all measured traits using the following formula: DTI = $(Y_D/Y_I)/(\text{average } Y_D/\text{average } Y_I)$, where Y_D – value of trait under drought conditions for particular genotype; Y_I - value of trait under optimal conditions for particular genotype. Average Y_D/Average Y_I - average value for all genotypes under drought (D) or optimal (I) conditions.

2.3. RNA preparation and RT-PCR

Total RNA was isolated by acid guanidinium thiocyanate–phenol–-chloroform extraction (Chomczynski and Sacchi, 2006) from 100 mg of beet leaves from irrigated and drought treated plants (after 42 days of drought, 101 DAE). Prior to RT-PCR, the RNA preparations were treated with RNase-free DNase I (Thermo Scientific) to remove any genomic DNA contamination. First-strand cDNA was synthesized from 0.2 μg of DNase-treated RNA using a RevertAid™ First Strand cDNA Synthesis Kit (Thermo Scientific). For RT-PCR, 1 μl of the cDNA was used in final volume of 10 μl reaction with gene-specific primers. The primer sequences and annealing temperatures are listed in Table 2. The optimal number of PCR cycles was determined for each set of primers. A fragment of the constitutively expressed beet *BvAct7* gene was amplified as reference gene (Liu et al., 2008). To confirm a lack of DNA contamination in RNA samples, 0.2 μg of DNase-treated RNA were used in PCR with primers complementary to promoter region of *Beta vulgaris Bolting Time Control 1* (*BTC1*) gene.

2.4. DNA preparation and RAPD

Fresh leaves of greenhouse-grown 4-week-old plants were frozen in liquid nitrogen and stored at −75 °C. Genomic DNA extraction and preparation for RAPD was performed as described previously (Chołuj et al., 2014). 15 beet accessions were used for RAPD, aforementioned 13 and 2 additional *P. patellaris* (187583) and *B. macrocarpa* (01809) (Table 1), which did not survive in greenhouse conditions.

To screen beet accessions for DNA polymorphisms we employed the RAPD technique using 153 random decamer primers (Eurofins MWG Operon's RAPD 10-mer Kits), which generated clearly polymorphic amplification products and were used in our previous study (Chołuj et al., 2014). The distribution of polymorphic bands produced by randomly selected primers in reactions with the DNA of individuals representing part of the bulks was checked. DNA amplification, gel electrophoresis and visualisation was performed as previously described by Chołuj et al. (2014). All visible and unambiguously ‘scorable’ fragments were taken into account. Amplification profiles of all bulks were compared and DNA fragment bands were scored as present (1) or absent (0). A dissimilarity matrix was generated using Nei's measures of genetic identity/diversity and population relationships were inferred using the unweighted pair group method with the arithmetic mean (UPGMA) clustering method implemented in POPGENE as previously described by Chołuj et al. (2014).

2.5. Statistical analysis

Mean (± SD) of twelve replicates (plants) per genotype have been presented for each parameters in both watered and drought condition. Significance of data was tested by performing Fisher's Multiple Range Test using One Way ANOVA and least significant difference (LSD) was calculated at $p < 0.05$.

3. Results

3.1. Morphological and physiological traits

In this work, five sugar beet breeding genotypes (selected based on root yield measured in preliminary field experiments) along with six fodder and two wild beets were used for detailed analyses. Two genotypes, DH0962 and DH0734 (more drought tolerant sugar beet lines), were characterized by the lowest decrease of root yield in drought condition compared to irrigated plants (reduction of 11 and 21%, respectively). DH0726 and DH0960 genotypes (less drought tolerant sugar beet lines) reached greater reduction of yield 43 and 63%, respectively. NO0865 was a genotype with a medium reduction of yield of 27% (data not shown).

The detailed analyses on selected plant material were performed in

Table 2
Gene names, accession numbers, primer pairs and conditions used in RT-PCR assays.

Gene name	Gene	Acc. no	Forward primer (5′-3′) Reverse primer (5′-3′)	Product length (bp)	Ann. Temp. (°C)/no of cycles
Osmoprotection					
Choline Monooxygenase, chloroplastic	*BvCMO*	LOC104897072 XM_010683881	BvCMO_F1 - GCTGTTGCTTCCCCTGTTTT BvCMO_R1 - TTGATTTTTCTCCTTTACTTGCTCA	228	56/27
Betaine Aldehyde Dehydrogenase 1, chloroplastic	*BvBADH1*	LOC104894202 NM_001303094	BvBAD1_F1 – AGTTCTTGAATATATGTGA BvBAD1_R1 – CCTTACAATAATGAGCTTG	348	47/32
Betaine Aldehyde Dehydrogenase 2, chloroplastic	*BvBADH2*	LOC104908697 XM_010697841	BvBAD2_F1 - TAACTTGAGGATCCAATTTT BvBAD2_R1 - ATAAACCAACTATGCACATGGTAA	241	47/32
Delta-1-Pyrroline-5-Carboxylate Synthase1	*BvP5CR*	LOC104904817 XM_010693213	P5CR_F1 - TGTGATGGGGCTCGTTTTGG P5CR_R1 - GCGGGAGAGAGGACATGAAA	295	56/27
Proline Transporter 3-like (betaine/ proline transporter)	*BvBet/ProT1*	Bv2_025060[a]	BvBT1_F1 - CGGGTATGTGGTGAATGGTGA BvBT1_R1 - GGTTTTGTTGCCGTATGCCC	274	56/2
Proline Transporter 3-like (betaine/ proline transporter)	*BvBet/ProT2*	LOC104903897 Bv2_025070[a]	BvBT2_F1 - CCAGAAAATGCTTCACGCCC BvBT2_R1 - AATCCATCCAAGAGGCACCAT	204	56/27
Alpha,alpha-Trehalose-Phosphate Synthase [UDP-forming] 1	*BvTPS*	LOC104891294 XM_010676966	BvTPS_F1 - GACATGTGGTTGGCAGCAGA BvTPS_R1 – AGCCCTTTGTGACACCTACTG	334	56/27
Trehalose-Phosphate Phosphatase A	*BvTPP1*	LOC104906608 XM_010695382	BvTP1_F1 – AACAAGGGAAAAGCTGTGGAATTCTTA BvTP1_R1 - GTATATATTTCTGAAAGGTTCTG	353	56/32
Trehalose-Phosphate Phosphatase F	*BvTPP2*	LOC104892054 XM_010677870	BvTP2_F1 – AGTGATGATGTGCTCCCAATTTACA BvTP2_R1 – CTCTGATTCTGCTCTCTCCATTG	203	56/32
Antioxidant system					
Superoxide Dismutase [Cu–Zn]	*BvSD1*	LOC104891015 XM_010676650	BvSD1_F1 – CTCGGGTCTTAAGCCTGGGCTC BvSD1_R1 – CCAGCATTGCCTGTGGTCTTG	318	63/27
Superoxide Dismutase [Cu–Zn], chloroplastic	*BvSD2*	LOC104902979 XM_010690943	BvSD2_F1 – ACCAACTACAGTCAACGTTCGT BvSD2_R1 – CCAGCATTCCCAGTAGTCAGA	342	56/27
L-Ascorbate Peroxidase 3, peroxisomal	*BvAP3*	LOC104903932 XM_010692076	BvAP_F1 – GGACTATGCTGCCTCACACA BvAP_R1 – ACAGCACTCTGGGCCAATAC	102	56/27
Tocopherol cyclase, chloroplastic	*BvTC*	LOC104885180 XM_010669885	BvTCyc_F1 – GTAGAGGCGAAAACCGAGGA BvTCyc_R1 – GGCGTTGTGGTTCTTCCTTTC	233	56/27
Transcription factors					
Transcription factor MYB30	*BvMyb30*	LOC104906208 XM_010694935	BvMYB_F1 – ACTGAGAACATAGCGCGGTT BvMYB_R1 – CGGTGACACTGCTTGAGAGA	225	56/27
ABA responsive element binding protein 1 (ABA-Insensitive 5-like protein 5)	*BvAREB1 (BvABI5-like protein 5)*	LOC104899695 NM_001303075	BvARE_F1 – AGGAGCAATGGGGACTCATCCTCTGTATC BvARE_R1 – TTCAGCCTGTTTCTTCCGTAACTCTTG	243	63/27
Reference gene					
Bolting Time Control 1	*BTC1*	HQ709098.1	BTC1F - TGTTGACGTGTGAATGTGGG BTC1R – GTGGAGGAGTGAGGGGTAGA	302	54/30
Actin 7	*BvAct7*	LOC104888182 XM_010673076	BvAC1_F1 - ATCCAGGCCGTTCTTTCTCT BvAC1_R1 - ACGACCAGCAAGATCCAAAC	144	56/27

[a] http://bvseq.molgen.mpg.de/index.shtml.

greenhouse conditions. Water deficit caused a dramatical decrease of the dry mass (DM) of roots by over 30%, petioles and leaf blades by about 50% on average (Fig. 1A, B and C; see columns showing the means). The strongest decrease of storage root DM as a response to water stress was observed in one, less tolerant DH line out of all analysed accessions, while the smaller decrease was observed in more tolerant DH lines (this effect was similar to previously obtained field results), the least in *B. maritima* (187794) and fodder beet (188288). In other fodder beets (except 188286) this physiological trait was only slightly affected by drought (Fig. 1A), similar to more tolerant lines of DH. Similar differences between tolerant and susceptible DH lines were visible in blades DM and petioles DM. The highest decrease of petioles DM under drought conditions was denoted in less tolerant DH lines and *B. maritima* accessions, and the lowest decrease in most fodder beets (Fig. 1B). Similar results to the cessation of watering were observed in the case of blades DM (Fig. 1C). The blades area was drastically decreased by 40% in average under water shortage condition compared to the control ones (Fig. 1D). Less tolerant DH lines and *B. maritima* accessions were characterized by the highest decrease of this trait, but in three fodder beet genotypes (188288, 188294, 188655) blades area was slightly affected by drought (Fig. 1D).

Drought condition caused an increase of SLW by 24% and of Suc.I. by 12% only as compared to control (Fig. 1E and F). In response to drought stress, individual genotypes showed variation in morphological traits. The greatest increases of SLW as a result of water shortage were observed in NO0835 and *B. maritima* accessions, but in more tolerant DH lines and three fodder beet genotypes this trait was unaffected (Fig. 1E). In response to drought the significant, but slight increase of "succulence index" was shown only in DH0960, NO0835 line, *B. maritima* accessions and three fodder beet genotypes (188285, 188294, 188655, Fig. 1F). Osmotic potential decreased as a result of water shortage by 30% on average (Fig. 1G). This decrease was most visible in both tolerant DH lines, one *B. maritima* genotype (187786) and five fodder beet genotypes. The relative chlorophyll content of mature leaves (Fig. 1H) was strongest decreased under drought conditions compared to optimal ones in less tolerant DH0726 line, slightly decreased in other accessions or unimpaired in DH0962 and fodder beets (188288, 188655). Relative flavonoid content increased in response to drought in almost all accessions (Fig. 1I). This increase was higher in two more tolerant lines (DH0734 and DH0962) in comparison to less tolerant ones. In less tolerant DH0726, NO0835 lines and two fodder beet genotypes (188285, 188287) this trait did not change under water stress condition compared to control ones. Effective quantum yield of PSII in all accessions was slightly, but significantly affected by water shortage (Fig. 1J).

Taking into account morphological and physiological traits, the genotypic diversity in drought tolerance index (DTI) value was the highest in blade areas, DM of particular plant organs, SLW and relative flavonoid content, but the lowest in effective quantum yield of PSII (Table 3, shown as difference between maximal and minimal value of

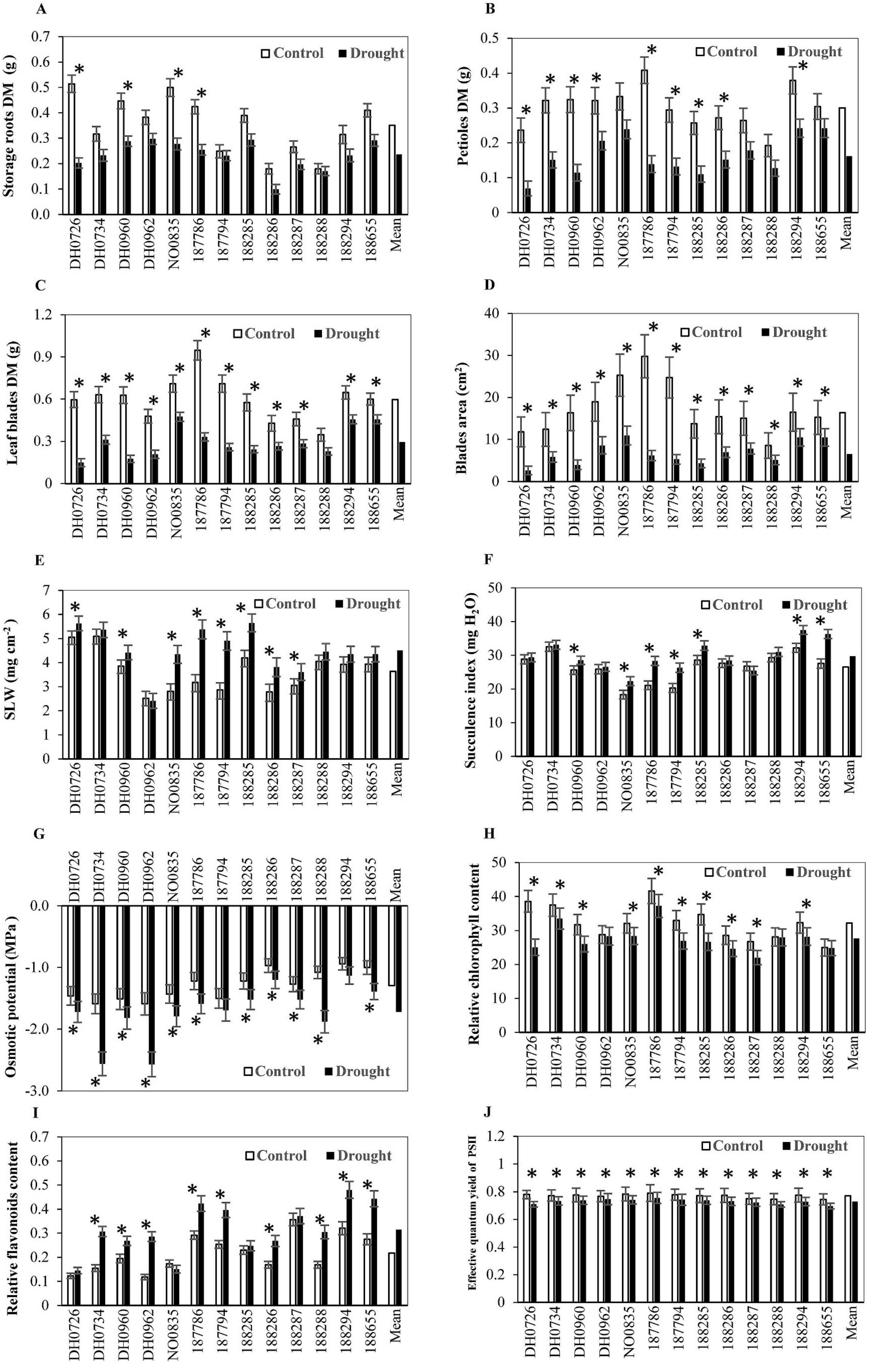

Fig. 1. Effects of drought treatment on morphology and physiology of different beet objects. Data are presented as means ± SD. *- statistically significant differences between drought and control conditions, $p < 0.05$ according to Fisher's multiple range test.

Table 3
Drought tolerance index (DTI) calculated for morphological and physiological traits on a basis of average for all accessions.

	Drought tolerance index (DTI)									
Accessions	Root DM	Petiole DM	Leaf blade DM	Blade area	SLW	Suc.I.	Ψs	Relative chlorophyll content	Relative flavonoids content	ϕPSII
DH0726	*0.58*	*0.54*	*0.50*	0.56	0.90	1.01	*0.88*	*0.76*	0.82	0.96
DH0734	1.08	0.87	1.00	1.17	0.85	1.01	1.20	1.04	1.37	1.00
DH0960	0.95	0.65	0.56	0.61	0.93	1.10	0.90	0.95	0.95	1.00
DH0962	1.15	1.19	0.88	1.13	*0.77*	1.02	1.21	1.14	**1.68**	1.03
NO0835	0.82	1.33	1.35	1.08	1.25	1.21	0.94	1.03	*0.60*	1.00
187786	0.89	0.63	0.71	*0.52*	1.37	**1.33**	0.97	1.04	1.00	1.01
187794	1.38	0.83	0.74	0.53	**1.38**	1.29	0.84	0.95	1.08	1.01
188285	1.11	0.80	0.85	0.78	1.09	1.14	0.93	0.89	0.75	1.01
188286	0,82	1.04	1.25	1.13	1.11	1.02	0.93	1.00	1.10	1.00
188287	1.09	1.25	1.25	1.31	0.96	*0.94*	0.90	0.96	0.72	1.02
188288	**1.40**	1.23	1.33	1.50	0.89	1.05	**1.30**	**1.16**	1.25	1.00
188294	1.09	1.19	1.43	1.59	0.89	1.15	0.90	1.02	1.04	0.99
188655	1.05	**1.48**	**1.54**	**1.72**	0.89	1.29	1.04	**1.16**	1.12	0.99
Max-Min*	0.81	0.94	1.04	1.20	0.61	0.39	0.42	0.40	1.08	0.07

Values bigger or equal to 1.05 were marked by dark grey colour and values lower or equal to 0.95 by light grey. The more dark fields are in the row, the more accession is drought tolerant. *- the differences between maximal (bold) and minimal (italics) values of drought tolerance index calculated for particular accessions. Accessions selected to RT-PCR are indicated by boldface.

DTI). Level of DTI also showed that two fodder beet genotypes (188288, 188655) and one DH line (DH0962) were the most drought tolerant, while two DH lines (DH0726, DH0960) and one *B. maritima* genotype (187786) were less tolerant.

3.2. Relative gene expression

We examined the level of expression of selected genes, encoding for components of the anti-oxidation system, osmoprotectants or transcription factors, to reveal which genes are differentially regulated during drought. For this analysis two most tolerant (DH0734, DH0962) and two most susceptible (DH0726, DH0960) double haploids of sugar beet where selected, based on their root yield reduction under drought condition in field, one an analogue (NO0835) of male-sterile line O. Additionally, one genotype of *B. maritima* (187794) and one fodder beet genotype (188655) were selected. All seven accessions were selected based on dry mass reduction of analysed plant organs under drought conditions and their drought tolerance index (Table 3).

The most tolerant double haploid sugar beet genotype DH0962 showed significant up-regulation of *BvBADH1* gene, encoding Betaine Aldehyde Dehydrogenase 1, under drought condition and surprisingly no expression of *BvBADH2* gene in both, under control and drought conditions compared to other accessions (Fig. 2). Subsequent gene engaged in response to drought of this genotype was *BvSD1*, in contrast to *BvSD2*. *BvSD1* gene encoding Superoxide Dismutase 1 was significantly down-regulated under drought conditions (Fig. 3). The second more tolerant DH0734 line showed the distinct pattern of transcription regulation of *BvBADH1*, *BvBADH2*, *BvTPP1* (encoding Trehalose-Phosphate Phosphatase A 1), *BvTPP2*, *BvSD1* and *BvAP3* (*Ascorbate Peroxidase 3*) genes than DH0962 line under drought conditions (Figs. 2 and 3).

The susceptible genotype DH0960 showed strong up-regulation of *BvBADH2* under drought conditions comparing to control conditions and to DH0962 genotype. Significant difference in expression was also observed for *BvBet/ProT2* gene encoding betaine/proline transporter. Inversely to DH0962, in DH0960 *BvSD2* and *BvAP3* genes were significantly up-regulated under drought conditions. Between these two genotypes are also differences in the expression level of *BvBet/ProT2*, *BvAP3* and *BvMyb30* genes under control condition (Fig. 2). The second less tolerant DH0726 line showed differences in expression level of *BvCMO* (*Choline Monooxygenase*) gene, *BvBet/ProT2*, *BvTPS* (*Trehalose-Phosphate Synthase 1*), *BvTPP1*, *BvTPP2* and *BvSD1* genes, compared with DH0960 line and under drought conditions (Figs. 2 and 3). Intermediate tolerant NO0835 line, similarly to other sugar beet genotypes, possessed its own pattern of transcript accumulation of analysed genes.

In 187794 genotype (*B. maritima*) most of analysed genes had changed their level of expression. Only in this genotype the expression level of *BvP5CR* (*Delta-1-Pyrroline-5-Carboxylate Synthase1*) and *BvBet/ProT1* genes were significantly altered between control and drought conditions. Similarly, the significant up-regulation of *BvAREB1* gene encoding transcription factor was confirmed only for this genotype (Fig. 3). In contrast to the wild type beet, most tolerant fodder beet genotype (188655) did not show differences in gene expression level between control and drought condition, with the exception of only one – *BvTPP2* gene, encoding Trehalose-Phosphate Phosphatase A, which was down-regulated under drought conditions (Fig. 2).

3.3. Genetic diversity

To screen 15 beet accessions for DNA polymorphisms, we employed the RAPD technique using 153 random decamer primers. Out of them, 91 yielded clearly polymorphic amplification products. The genetic distance based on RAPD data of 15 beet accessions ranged from 17.76 to 87.22% (Fig. 4, Table 4). The wild type *P. patellaris* showed the highest genetic distance from 73.13 to 87.22% to other accessions. *B. macrocarpa* showed less genetic distance than *P. patellaris* between 55.35 and 75.10%. The genetic diversity between sugar beet genotypes ranged from 28.69 to 34.80%. The smallest genetic distance was observed between fodder beets from 17.76 to 34.97%. The most drought tolerant sugar beet genotype DH0962 showed the highest genetic similarity to both *B. maritima* and to the fodder beet 188288. Genetic distance between two *B. maritima* (originated from Japan or Netherlands) was low and reached 26.07%. The genetic similarity between sugar and fodder beets was high and ranged from 68 to 79%. This results indicates a fairly low genetic diversity between them, and the range of variation between them coincides with the diversity of both accessions of *B. maritima*.

4. Discussion

Wild relatives of cultivated crops potentially possess important traits for agriculture, such as drought tolerance or pathogen resistance.

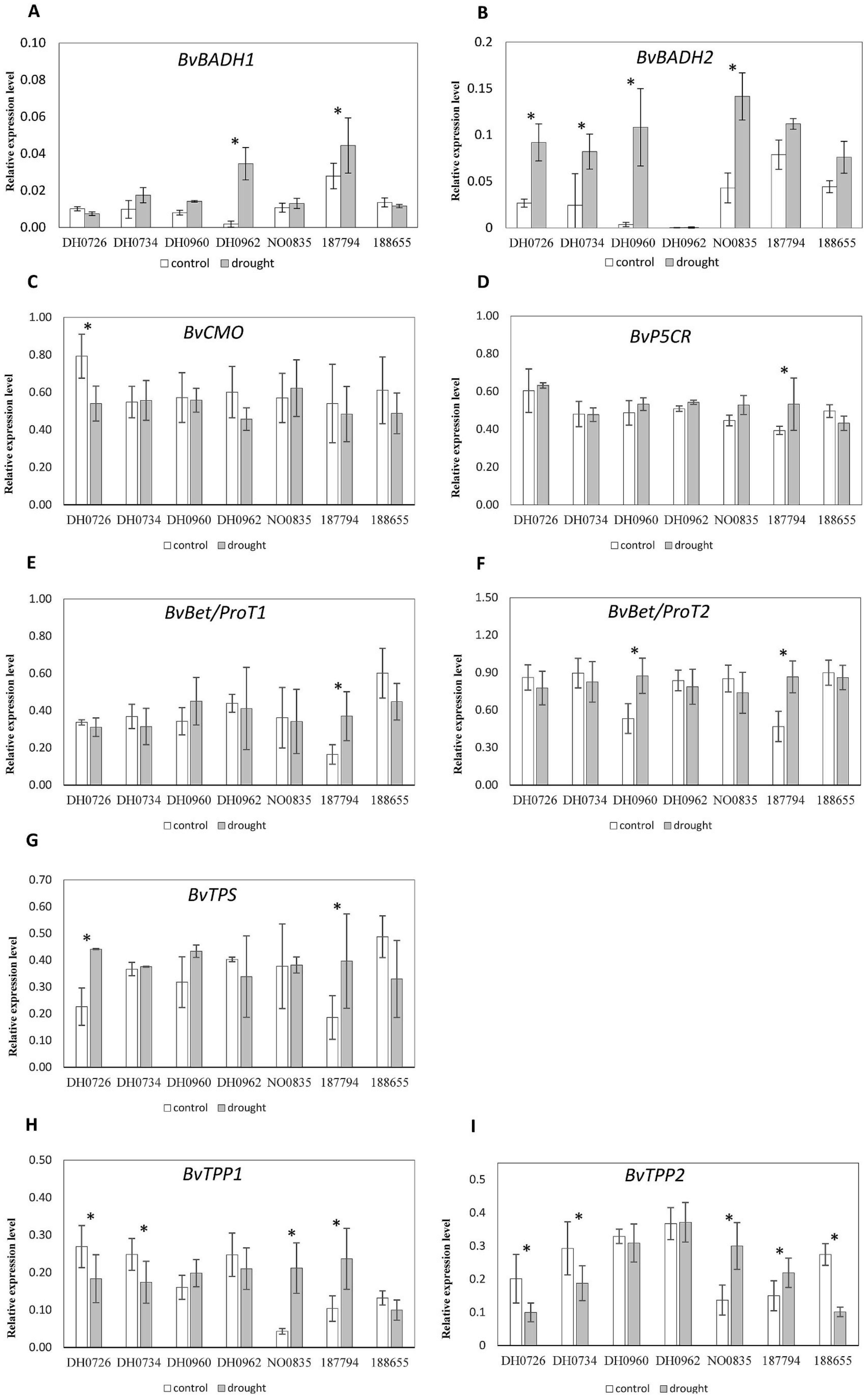

Fig. 2. Effects of drought treatment on the expression of genes involved in osmoprotection in leaves of different beet objects. Data are presented as means ± SD. Bar labelled with stars are significantly different at $p < 0.05$ between drought and control conditions, according to Fisher's multiple range test.

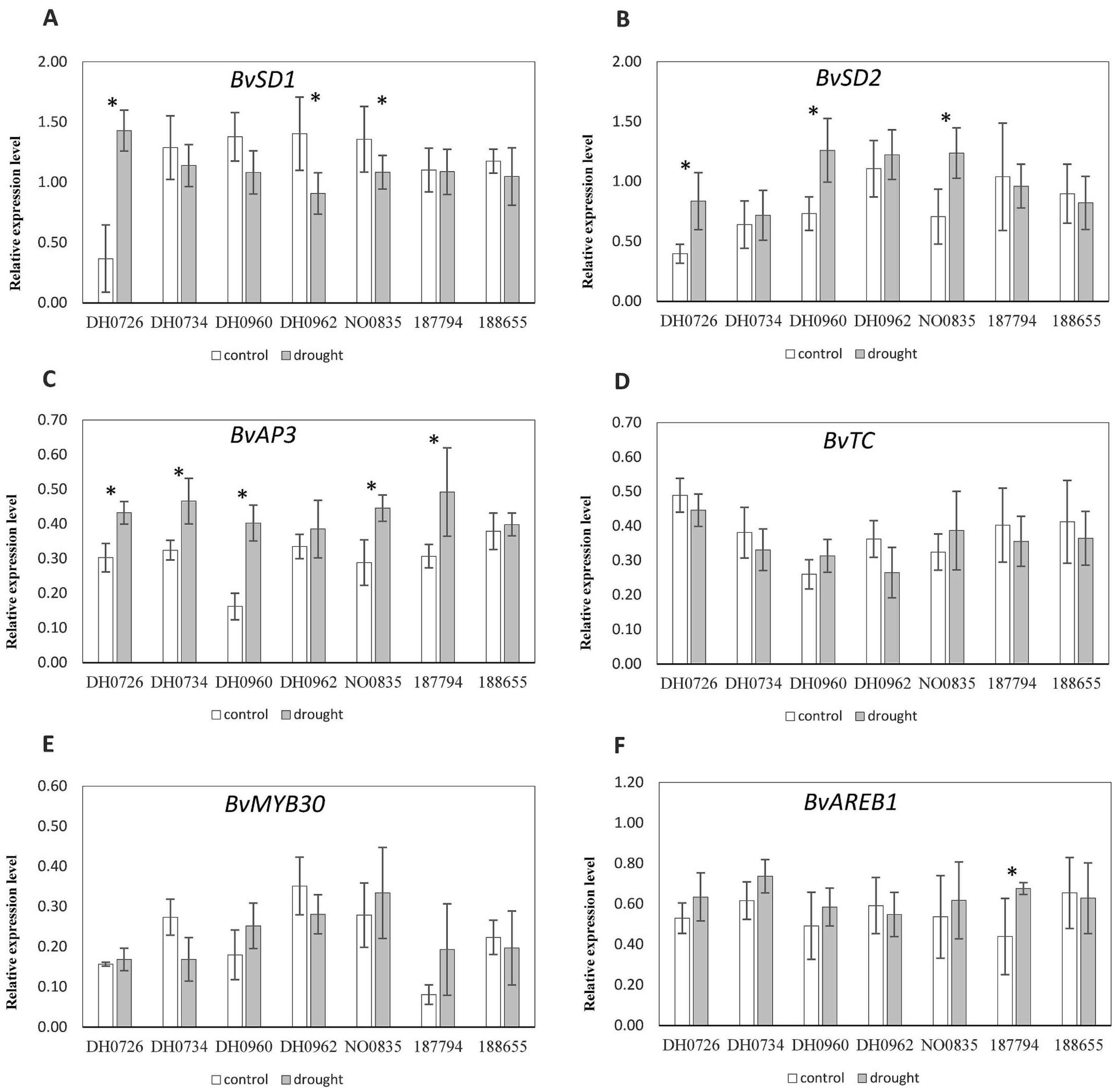

Fig. 3. Effects of drought treatment on the expression of genes involved in the antioxidant system and transcription regulation in leaves of different beet objects. Data are presented as means ± SD. Bar labelled with stars are significantly different at $p < 0.05$ between drought and control conditions, according to Fisher's multiple range test.

Genes responsible for these traits can be introduced to cultivated crops by crossing to produce better varieties. In this work, we present the physiological and molecular characterization of wild, fodder and sugar beet accessions. The purpose of this work was the evaluation, under drought condition, of some *Beta* objects as potential forms to out-crossing and the comparison of them to other breeding forms.

4.1. Changes of morpho- and physiological traits under drought stress

Based on our earlier field test, we selected five sugar beet genotypes for detailed analyses. Selection criterium was the reduction of root yield under drought condition and we selected genotypes with the extremely lowest and highest root yield decrease. To determine the potential genetic diversity in *Beta* we also analysed several wild beet species and fodder beets. Based on reduction of root yield under drought condition, two DH lines of sugar beets, DH0726 and DH0960, were the less drought tolerant from all of analysed genotypes in field condition (data not shown). Two wild species of beet had a highest reduction petiole DM or blade areas, but not root DM, out of studied objects. Many previous studies have shown that drought negatively influences root yield in sugar beet plants by 15–50% depending on stress duration and severity as well as plant genotype (Bloch and Hoffmann, 2005; Chołuj et al., 2014, 2008; Topak et al., 2011).

Drought induced changes in the morphological traits of beet leaves resulted in a higher specific leaf weight (SLW) as well as "succulence index" (Suc.I), especially in wild beets and some fodder beets. In our

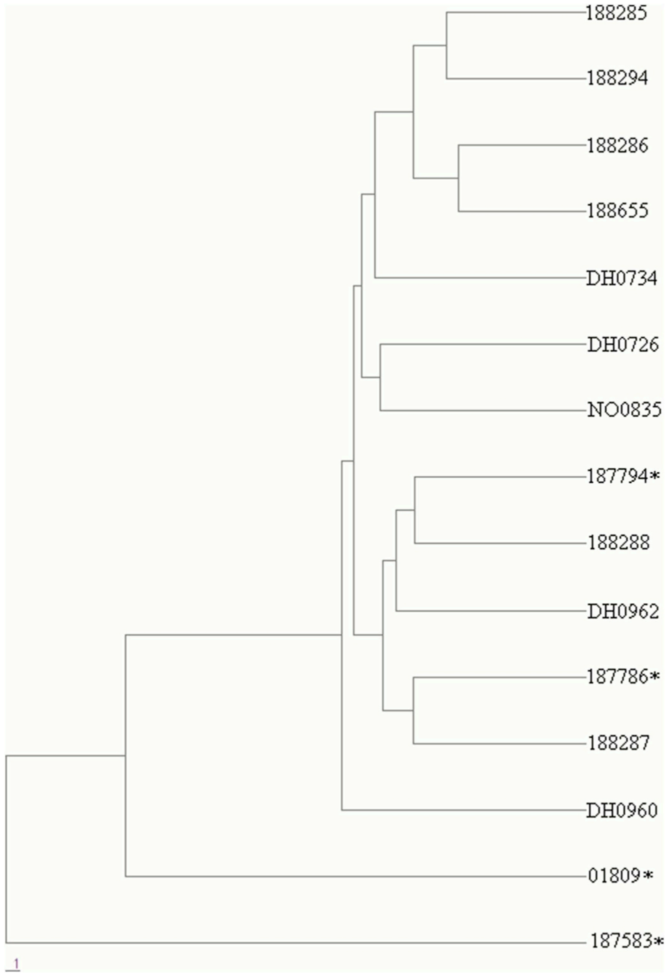

Fig. 4. Dendrogram of 15 beet accessions (Table 1) developed from RAPD data using unweighted pair group method with arithmetic mean (UPGMA) based on Nei's genetic distance. *- shows wild relatives of crop beets.

experiment we did not observe strong changes in SLW or Suc.I in sugar beet DH lines, in contrast to increased SLW demonstrated previously in sugar beets (Chołuj et al., 2014; Mohammadian et al., 2005; Ober et al., 2005).

Our results revealed a strong decrease in osmotic potential of leaves of two more tolerant DH sugar beet lines and one fodder beet, subjected to water stress, indicating a high capacity for osmotic adjustment. The reduction in Ψs in beet plant cells is due to the accumulation of potassium, sodium, soluble sugars and their derivatives, as well as nitrogen containing substances such as glycine betaine, proline and other free amino acids (Chołuj et al., 2008; Hoffmann, 2010; Mäck and Hoffmann, 2006; Shaw et al., 2002; Wedeking et al., 2017). The overproduction of osmolytes may help plants to tolerate dehydration by maintaining osmotic balance within the cell as well as by stabilizing membrane and protein structures (Farooq et al., 2012). Nevertheless, osmotic adjustment may be critical to survival rather than to the maintenance of plant growth - we observed a significant reduction in dry mass accumulation in drought treated plants.

The results of the present study clearly indicate that water shortage has no strong effect on the relative chlorophyll content, except the least tolerant DH0726 line. Relative flavonoid contents were strongly increased in two more tolerant DH lines DH0734 and DH0962 as well as in wild beets and some of fodder beets. Data in the literature remain controversial regarding the concentration of photosynthetic pigments. The stability of chlorophyll concentration in beet leaves under water stress conditions was reported (Vomacka and Pospisilova, 2003), but other authors showed an increase in chlorophyll $a + b$ content under drought conditions (Hussein et al., 2008; Shaw et al., 2002).

In this work, we observed a significant decrease of the quantum efficiency of PSII under drought conditions for all accessions, but no differences between the genotypes were identified. Reduction of the quantum yield of ΦPSII have been reported for sugar beet leaves in our previous studies (Chołuj et al., 2014, 2010), but this parameter cannot be selection criterium of drought tolerance of beets.

Based on analyses of selected beet accessions it was shown that morphology and physiological responses to drought condition are accession specific. We showed in this work that no morphological or physiological trait of beets could be useful for the selection of plants for drought tolerance, except for the reduction of the root yield in drought. Unfortunately, the root yield reduction can only be observed in drought conditions, which is time- and cost-consuming. The interest of researchers should be probably focused on the detailed investigations of seed germination rate or early seedling growth parameters, as it was studied in the case of other species (Channaoui et al., 2019; Thabet et al., 2018).

4.2. *Effect of drought treatment on expression level of genes engaged in osmoprotection, antioxidant system and transcription regulation*

Plant response to biotic or abiotic stresses is regulated by the signal transduction at the molecular level for protection of proteins and membranes of cells. Genes engaged in these processes can be divided into the following groups: involved in synthesis of osmoprotectants

Table 4
Genetic identity and genetic distance values obtained for 15 beet accessions using 153 RAPD markers.

accessions	DH0726	DH0734	DH0960	DH0962	NO0835	187583	187786	187794	188285	188286	188287	188288	188294	188655	01809
DH0726	***	0.73	0.72	0.71	0.75	0.42	0.67	0.72	0.77	0.71	0.70	0.72	0.76	0.69	0.53
DH0734	0.31	***	0.71	0.71	0.72	0.45	0.69	0.71	0.75	0.76	0.69	0.72	0.73	0.74	0.52
DH0960	0.33	0.34	***	0.71	0.73	0.45	0.70	0.73	0.71	0.68	0.72	0.74	0.70	0.68	0.52
DH0962	0.34	0.34	0.35	***	0.74	0.46	0.73	0.77	0.71	0.70	0.74	0.76	0.70	0.72	0.57
NO0835	0.29	0.33	0.31	0.31	***	0.44	0.69	0.71	0.79	0.71	0.68	0.73	0.75	0.69	0.51
187583	0.86	0.80	0.81	0.78	0.83	***	0.45	0.45	0.42	0.44	0.47	0.43	0.43	0.45	0.48
187786	0.39	0.38	0.36	0.31	0.37	0.79	***	0.77	0.70	0.72	0.79	0.75	0.72	0.74	0.57
187794	0.33	0.34	0.32	0.26	0.34	0.80	0.26	***	0.76	0.74	0.74	0.79	0.75	0.75	0.57
188285	0.26	0.29	0.34	0.35	0.24	0.87	0.36	0.28	***	0.78	0.70	0.76	0.82	0.75	0.49
188286	0.34	0.28	0.39	0.35	0.34	0.81	0.33	0.30	0.25	***	0.78	0.78	0.80	0.84	0.51
188287	0.36	0.37	0.33	0.29	0.38	0.76	0.24	0.30	0.35	0.25	***	0.78	0.74	0.76	0.56
188288	0.32	0.32	0.29	0.27	0.32	0.84	0.28	0.24	0.28	0.25	0.25	***	0.76	0.75	0.54
188294	0.28	0.31	0.36	0.36	0.28	0.84	0.33	0.28	0.19	0.23	0.31	0.27	***	0.82	0.47
188655	0.37	0.30	0.38	0.32	0.36	0.79	0.30	0.29	0.28	0.18	0.27	0.29	0.20	***	0.50
01809	0.64	0.65	0.65	0.56	0.67	0.73	0.55	0.57	0.71	0.68	0.58	0.61	0.75	0.69	***

Nei's genetic identity (above diagonal) and genetic distance (below diagonal).

(like glycine betaine, proline or trehalose), encoding ROS scavengers (such as superoxide dismutase, ascorbate peroxidase or tocopherol cyclase) or transcription factors belonging to different gene families.

We analysed the level of expression of genes engaged in synthesis and transport of glycine betaine, proline and synthesis of trehalose. Differential regulation of expression level of these genes under control and drought conditions was observed among tested beet accessions. Previous researches on osmoprotectants focused on their role in the maintenance of osmotic potential in plant cells under stress condition, however subsequent works proved that glycine betaine, proline or trehalose synthesis in transgenic plants increases drought and other stresses tolerance in plant (Iordachescu and Imai, 2008; Khan et al., 2009; Surekha et al., 2014; Yamada et al., 2005).

Strong up-regulation of *BvBADH1* (participated in glycine betaine synthesis) gene was observed only in leaves of drought tolerant sugar beet DH0962 line and in wild beet under drought conditions. At the same time, *BvBADH2* gene was up-regulated in all accessions with exception of drought tolerant sugar beet line DH0962, wild and fodder beets. The expression level of the *CMO* gene, responsible for the first step of glycine betaine synthesis, did not change between control and drought, except for DH0726 line. There are no scientific reports concerning the expression of genes engaged in osmoprotection during the drought stress in beets. However, the salt stress causes similar physiological response of plants, but as we showed in our work not at the molecular level. In other study, *CMO* gene expression level was lower in young leaves of sugar beet when compared to older leaves under control condition and was up-regulated only in young leaves under salt stress (Yamada et al., 2009). The same research showed that betaine is primarily synthesized in older leaves and translocated into young leaves. *CMO* gene expression on RNA and protein level was higher in older leaves than in young ones (Yamada et al., 2009). In our work, *BvBADHs* gene had greater meaning under drought stress than *CMO* gene, since *BvBADH* genes were strongly induced after drought treatment.

The induction of expressions of both *BvBet/ProT1* and *BvBet/ProT2* were distinctive in principle only for *B. maritima*. Little is known about the transport of glycine betaine in plant cells. It was demonstrated that *Bet/ProTs* from sugar beet are responsible for transport simultaneously proline, glycine betaine and choline (Yamada et al., 2015). In our study, we showed that two *Proline Transporter 3-like* (*betaine/proline transporter*) genes *BvBet/ProT1* and *BvBet/ProT2* were up-regulated only in wild beet (*BvBet/ProT1*) or in less drought tolerant sugar beet line (DH0960) and in wild beet (*BvBet/ProT2*). Transcript accumulation of betaine transporter *Bet/ProT* gene was reduced in young leaves of sugar beet in comparison to older ones and was slightly induced only in young leaves under salt stress (Yamada et al., 2009). It can be assumed, despite some similarities, the regulation of expression of aforementioned genes under salt stress and drought is different.

Proline accumulation has been reported in various plants during osmotic stress induced by salt and drought stresses and its primary function is to stabilize protein structures and scavenging free radicals in contrast to glycine betaine (Biedermannova et al., 2008; Signorelli et al., 2014). In this study, *BvP5CR* gene expression level, participating in proline synthesis, did not change in leaves in any accessions with exception of wild beet under drought condition. The expression level of *BvP5CR* in wild beet was slightly increased under drought stress. Some literature data showed that proline biosynthesis is regulated at the transcriptional level in response to drought (Dudziak et al., 2019).

The role of trehalose in protecting membranes and proteins in plant was established (Paul et al., 2008). The overexpression of genes engaged in trehalose synthesis led to increase of tolerance to various abiotic stresses in transgenic plants (Iordachescu and Imai, 2008). In our work the expression level of *BvTPS*, *BvTPP1* and *BvTPP2* gene varied depending on genotype. Under drought condition *BvTPS* was up-regulated in leaves of less tolerant sugar beet line DH0726 and wild beet. *BvTPP1* and *BvTPP2* gene expression level was down-regulated in DH0726 and DH0734 lines or up-regulated in NO0835 and wild beet and *BvTPP2* was additionally strong down-regulated in fodder beet.

Summarizing the analysis of expression level of selected genes related to osmoprotection, *BvBADHs* and *BvTPPs* were the most engaged genes in response of examined beet accessions to drought stress. The identification of stress-related metabolites and the genes engaged in their synthesis is crucial to successful molecular breeding of stress-tolerant plants. The description of specific drought related genes expression level can be helpful to extend this knowledge.

Drought is abiotic stress factor that induce oxidative stress in plants through the production of reactive oxygen species (ROS). ROS accumulated in the chloroplast result in lipid peroxidation and in consequent membrane injury, protein degradation and photosynthetic enzyme inactivation (Farooq et al., 2012). On the other hand, ROS are considered to be the important transduction signals. Plants have developed the antioxidant defence system that is comprised of both enzymatic (e.g. superoxide dismutase, ascorbate peroxidase or tocopherol cyclase) and non-enzymatic components. Several researches have reported that the ability of plant to balance between ROS production and scavenging is related to a higher tolerance to stress factors (Mittler, 2017).

Data regarding the effects of dehydration are controversial on antioxidant enzyme activities in sugar beet leaves. In our earlier research on sugar beets, catalase and guaiacol peroxidase activities increased only under long-term drought, leading to the accumulation of hydrogen superoxidase in leaves (Chołuj et al., 2010). In another study on various sugar beet lines growing in water or in the presence of polyethylene glycol, no changes in the activities of ascorbate and glutathione peroxidases and catalase were observed (Romano et al., 2013). In contrast, in plants growing in a field under different severities of drought, the activities of catalase, glutathione peroxidase and superoxide dismutase increased (Foroozesh et al., 2012; Sayfzadeh and Rashidi, 2011). In drought tolerant sugar beet mutants selected on medium containing polyethylene glycol, the activity of superoxide dismutase, guaiacol peroxidase and catalase was stimulated by osmotic stress in most genotypes, but ascorbate peroxidise activity was enhanced only in four out of ten mutants (Sen and Alikamanoglu, 2014). In our work *BvSD1* gene expression level was strongly increased in less tolerant DH0726 genotype, but slightly decreased in one more tolerant DH0962, whereas *BvSD2* gene was significantly increased in two less tolerant DH lines and NO0835 genotype. Drought condition also impaired on *BvAP3* gene expression level, but in more tolerant sugar beet DH0962 genotype there was no difference at the level of expression of this gene between control and drought condition, similarly as in the drought tolerant fodder beet. No significant differences in expression level of *BvTC* were shown in our work between control and drought conditions for particular accessions. Based on these results, it is suggested that long-term drought in tolerant accessions does not maintain oxidative stress and the induction of expression of genes encoding antioxidant enzymes is not required.

Drought stress induces defence response in plant, in which different transcription factors play a role. In this work we analysed gene expression of *BvMYB30*, a homolog of *A. thaliana MYB96*. *AtMYB96* regulates lateral root meristem activation under drought condition via an ABA-auxin signalling crosstalk and the *RD22* gene possibly in modulating stomatal movement. *myb96-1* mutant is susceptible to drought stress (Seo et al., 2009). *BvAREB1* was the second analysed gene encoding for transcription factor, which putatively binds to *ABRE* sequence in promoters of ABA responsive genes. *BvAREB1* was shown to be induced in leaves of sugar beet stressed by drought and control plants. The increase of *BvAREB1* gene expression level in drought stressed plant was correlated with the increase of ABA concentration (Schmidt et al., 2008). In our work these two genes had increased expression level in wild beet 187794, but only in the case of *BvAREB1* the difference was statistically significant. For other accessions analysed, drought stress did not modulated these gene expression.

Similarly to physiological responses to drought condition, responses on molecular level of different beet accessions also showed genotypic specificity.

4.3. Genetic diversity between analysed sugar, fodder beets and their wild relatives B. maritima

Natural populations related to crop plants can be potential source of novel tolerance genes for plant improvement. *B. maritima* (the wild sea beet) is the progenitor of all cultivated beets. Previously, the breeders concentrated on improvement of sugar yield and less on resistance to pathogens, nematodes and other pests (Panella and Lewellen, 2007). Selection to abiotic stress-tolerance was not of major interest and tolerance on them has been lost in the past breeding programs. Based on our results we can indicate some fodder beet accessions with low loss of root DM under drought condition and high DTI: 188286, 188288, 188655, which can be used to cross with tolerant DH0962 sugar beet line to improve drought tolerance. We showed that 188286 and 188655 are closely related and 188288 is most related with 'better' *B. maritima* (187794) and next with DH0962. Out crossing selected sugar beets with fodder beets possessing higher drought tolerance is undeniably easier than with *B. maritima* accessions because of the unfavourable root traits and other unwanted effects. However, *B. maritima* accessions collected across the world are under intensive exploration. Thirty-nine sea beets from Adriatic Sea coast were studied for root traits and abiotic tolerance. Analysis of genetic relationship between collected objects divides them on West and East Adriatic coast groups (Stevanato et al., 2013). Evaluation of genetic diversity and physiology of sea beets from different environments in Portugal revealed that wild beet populations exhibited physiological plasticity under drought and salinity conditions (Ribeiro et al., 2016). Wild relatives are also potential source of resistance genes for biotic factors e.g. rhizomania-resistance *locus Rz2* identified in *B. maritima* population from Kalundborg in Denmark (Capistrano-Gossmann et al., 2017) as well as within different sections of *Beta* genus: *Beta*, *Corollinae* and *Patellifolia* (Litwiniec et al., 2016).

5. Conclusions

Based on our results, breeding progress for the increase drought tolerance of sugar beets is still possible, but it seems that there is no need to cross sugar beets with their wild relatives. The studied accessions showed broad, but specific for each genotype, physiological and molecular response. Both fodder beets possessing the best DTI deserve particular attention for future to be the parent component for out crossing. Crossing sugar beets with more drought tolerant fodder beets will be sufficient to strengthen plant response to the water shortage and will reduce breeding time.

Author's contributions

A. Wiśniewska and D. Chołuj designed of the study. All authors performed the experiments. A. Wiśniewska and D. Chołuj analysed and interpreted the data and wrote the manuscript. All authors have read and approved the final manuscript.

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Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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