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Participation of Wheat and Rye Genome in Drought Induced Senescence in Winter Triticale (X *Triticosecale* Wittm.)

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Abstract: The aim of the study was to identify the regions of triticale genome responsible for senescence under drought induced during the generative stage. We performed quantitative analysis of chlorophylls (*a* and *b*), carotenoids, soluble carbohydrates, and phenolic compounds. QTL (Quantitative Trait Loci) calculations were based on a previously developed and characterized genetic map involving 92 lines of doubled haploid derived from F1 hybrid 'Hewo' × 'Magnat' and two DH parental lines ('Hewo' and 'Magnat'). We identified seven QTLs, including four on chromosome 2A, one on chromosome 1R, and two on chromosome 6R. Only three loci, QSPh.2A.1, QSC.2A.2 and QSC.2A.4 mapped single traits, i.e., the content of soluble phenolics and carbohydrates. Single QTL (QCSPh.1R) was responsible for changes in the levels of chlorophyll *a* and *b*, carotenoids and soluble phenolics. The remaining three loci, QCSPhC.2A.3, QCSPhC.6R.1 and QCSPhC.6R.2 controlled changes in the entire set of investigated traits. We also identified candidate genes for the investigated traits. The loci on chromosome 2A encoded proteins responsible for oligosaccharide transportation and mechanical properties of xylem and the genes regulating carbohydrate metabolism. The chromosomes 1R and 6R contained functional genes possibly associated with carbohydrate and phenolic metabolism.

Keywords: X *Triticosecale*; drought stress; senescence; QTLs; carbohydrates; chlorophyll; phenolics

1. Introduction

Senescence is a natural stage of plant ontogenesis manifested by the gradual decline of metabolic activity ultimately leading to cell death. The process may be markedly accelerated by unfavorable environmental conditions, such as e.g., soil drought [1]. Studies in transgenic tobacco showed that limitation of drought-induced leaf senescence improved plant tolerance to drought. In the transgenic plants, dehydration proceeded at a slower rate and the intensity of photosynthesis was less restricted than in control plants. After drought, their biomass growth was more intense and yield loss was smaller [2]. Similar results were also obtained for maize [3], wheat [4] and sorghum [5]. Gelang et al. [6] associates the decrease in plant yield with soil drought-induced accelerated senescence and a significant shortening of the grain filling stage.

In many species, the first visible sign of senescence is yellowing of leaves due to chlorophyll degradation and the appearance of other dominant pigments, mainly carotenoids, xanthophylls and

anthocyanins [7]. Possessing the stay green-trait closely correlates with delayed plant senescence, which is why leaf greenness index or green leaf area constitute reliable indicators of the process [8]. Kura-Hotta et al. [9] showed that chlorophyll *a* disappears more rapidly than chlorophyll *b* during senescence, which leads to a decrease in the chlorophyll *a*/*b* ratio. Degradation of the reaction centers was also observed to precede degradation of proteins forming the light-harvesting complex in PSII.

Another factor distinguishing the stay-green genotypes is an increase in carbohydrate content in green parts compared with genotypes aging at a normal rate. Enhanced accumulation of soluble carbohydrates in stay-green plants is often accompanied by increased leaf assimilation area during the grain filling stage [10]. Greater yield of the plants with delayed senescence may be due to e.g., maintaining a high level of leaf-soluble carbohydrates [11].

Carbohydrates are used up in the synthesis of phenolic compounds involved in plant defense during environmental stresses and may also serve as indicators of plant senescence. In aging plant organs, the levels of phenolic compounds increase at the cost of soluble carbohydrates [12,13]. This indicates an important role of sugars in integrating environmental signals during the regulation of leaf senescence [14].

Hexaploid triticale (X *Triticosecale* Wittm.), an intergeneric hybrid of wheat and rye with a genomic constitution of $2n = 6x = 42$ (AABBRR), is mainly used as animal feed, but also in the production of renewable energy and very little in food sector. Triticale combines favorable agronomic characteristics of wheat (high yield potential, good grain quality) and rye (abiotic stress tolerance, disease resistance) [15]. The mechanisms associated with plant senescence under soil drought may be controlled by wheat and/or rye genome [16]. However, triticale shows also specific responses to drought present neither in wheat nor in rye [17,18]. The genetic and molecular basis of triticale acclimation to drought have been so far poorly understood. It is not clear whether triticale responses to drought are specific to wheat or rye genome or result from the activity of both genomes.

There are genetic maps of triticale, wheat and rye that have been developed using SSR (simple sequence repeat), DArT (Diversity Arrays Technology), AFLP (Amplified Fragment Length Polymorphism), RAPD (Random Amplified Polymorphic DNA), RAMP (Random Amplified Microsatellite Polymorphic) and SNP (Single-Nucleotide Polymorphism) markers [19–25].

The aim of our research was to identify the regions of triticale genome associated with senescence under drought induced during the generative stage (reproductive stage). Studies on senescence in cereals usually focus on a flag leaf [6,26]. However, triticale senescence progresses from its lower parts towards the flag leaf. Therefore, our analyses involved the leaves located below the subflag leaf that the first reveal clear signs of drought-induced senescence.

2. Materials and Methods

2.1. Biparental Population and Genetic Map

The mapping population we used involved 92 lines of DH population 'Hewo' × 'Magnat' [27]. The genetic map composed of 1615 bin markers representing 13,402 DArTseq, 842 DArT, and 50 SSR markers used for QTL calculations [19]. The genetic map covered 4907 cM with a mean distance between two bins of 3.0 cM. For QTL calculations, the orientation of genetic positions of the markers on chromosomes 2R, 4R and 5R was inverted based on a comparison with a published whole-rye genome draft sequence of rye [15,28].

2.2. Plant Growth Conditions, Treatments, Leaf Sampling and Phenotypic Evaluation

Seeds of individual DH lines were sown into plastic pots with a capacity of 3.7 L filled with a mixture of soil and sand (1:3; v/v). Seedlings at the stage of two leaves were subjected to 8 weeks of vernalization in a cool chamber at +4 °C (±1 °C) and illumination of PPFD (photosynthetic photon flux density) at 150 µmol m^{-2} s^{-1}, photoperiod 10 h of light /14 h of darkness. After the vernalization, the plants at four-leaf stage were transferred into a greenhouse chamber (Department

of Plant Physiology, Faculty of Agriculture and Economics, Agricultural University, Kraków, south of Poland). The experiment was conducted during a 5-month period, from January to May. The air temperature in the greenhouse was about 26/18 °C (±2 °C) day/night, and relative air humidity reached 40%. Plants were additionally illuminated at PPFD about 150–200 μmol m^{-2} s^{-1} at the level of the top leaf. The plants were irrigated with nutrient solution once per week.

Soil drought at the stage of generative growth was applied individually when the flag leaf for each DH line was fully developed. Water content in the pots was gradually reduced down to 35% by not watering the plants for 7 days. Then it was maintained at that level for the next 2 weeks. The water content in the soil was controlled daily, using a gravimetric method [27].

Analyses were performed 14 days after water content in the pots reached 35%. They involved only the leaves located below the subflag leaf (the first two leaves), and we collected two adjacent leaves from each plant.

Lyophilized leaf samples (Freeze Dry System/Freezone® 4.5, LABCONCO, Kansas City, MO, USA) were ground in a mixer mill homogenizer (MM400, Retsch, Haan, Germany). Powdered plant material was then subjected to laboratory tests. Biochemical analyses were performed for nine biological replicates of each DH line and parental lines exposed to soil drought.

Chlorophyll and carotenoid contents were measured spectrophotometrically with a microplate reader (Synergy II; BioTek, Winooski, VT, USA). About 5 mg of dry weight was extracted in 95% ethanol (1.5 mL) as described by Czyczyło-Mysza et al. [29]. The ethanolic extract (100 μL) was added to a 96-well microplate, and absorbances at 470, 648 and 664 nm were measured. The concentration of chlorophyll and carotenoids was then calculated according to Lichtenthaler and Buschmann [30].

Soluble carbohydrate content was analyzed spectrophotometrically as described by Marcińska et al. [31]. All samples (5 mg) were extracted in 1.5 mL of 96% ethanol for 15 min. After that, 40 μL of supernatant was transferred to test tubes (10 mL) containing 400 μL of deionized water. Then 400 μL of 5% phenol and 2 mL of concentrated sulphuric acid were added. The reaction mixtures were incubated for 20 min and transferred to 96-well plates. The absorbance was read at 490 nm.

Total soluble phenolic content was analyzed spectrophotometrically with Folin-Ciocalteu reagent according to Bach et al. [32]. Absorbance at 765 nm was read on a microplate reader (Synergy II, BioTek, Winooski, VT, USA). Chlorogenic acid was used as a standard.

The analyses of phenolics, carbohydrates and plant pigments were performed for nine biological replicates of each DH line and parental lines exposed to drought at the generative stage (nine replicates mean nine plants from which the leaves were sampled).

2.3. Statistical Analysis

Statistical analysis was carried out using Statistica v. 12 (StatSoft Inc., Tulsa, OK, USA). Analysis of variance was used to determine the main effects of drought treatment on physiological and biochemical parameters of 'Hewo' and 'Magnat' cultivars. The data were checked for normality and homogeneity of variance. For all the traits, a normal distribution hypothesis (Shapiro–Wilk test, $p = 0.0005$) was not rejected. Broad-sense heritability estimates were made using the formula of Mahmud and Kramer [33] adapted to doubled haploid lines:

$$h^2 = (\{\sigma^2 DH - [(\sigma^2 P1 + \sigma^2 P2)/2]\}/\sigma^2 DH)$$

2.4. QTL Analysis

Composite interval mapping (CIM) analysis was performed using QTL Cartographer 2.5 software [34]. After performing a 1000-permutation test, a LOD threshold corresponding to genome-wide significance (p-value) of 0.05 was set individually for traits in the range 1.9–2.5 to declare a QTL as significant. A walk speed of 1.0 cM was chosen for all QTL detections. QTL effects were estimated as the proportion of phenotypic variance (R2) explained by the QTL. QTLs were

considered as minor or major by defining a major QTL as a QTL that explained more than 15% of the phenotypic variance in a primary genetic analysis [35].

2.5. In Silico Analysis

For all QTLs delimited by LOD values corresponding to a *p*-value of 0.05, redundant markers and corresponding DArTseq sequences were retrieved [19]. Selected sequences of rye DarT markers from the QTL regions were recently released [36] and obtained from the National Center for Biotechnology Information (NCBI). Wheat DarTseq sequences were employed for the identification of physical regions in wheat genome using Basic Local Alignment Search Tool (BLAST) for wheat sequence stored in URGI (Unité de Recherche Génomique Info, http://wheat-urgi.versailles.inra.fr/Seq-Repository/BLAST). Physical regions corresponding to ±2 cM were established based on data for 2A chromosome retrieved from URGI and [19] (1 cM = 2,872,695 bp) and then screened for candidate genes. Rye DarT and DarTseq sequences were used to identify contigs at IPK Database [28]. Selected contigs with verified localization were further used to search the database of rye transcripts. Sequences of predicted genes located within target physical regions were functionally annotated with Blast2GO [37]. The selected contigs were further used for BLASTx in NCBI database (https://blast.ncbi.nlm.nih.gov/) to identify homologs of known genes in collection of the non-redundant protein sequences (update 10 March 2019, 192,341,036 sequences).

3. Results and Discussion

Qualitative traits related to plant senescence have already been mapped for e.g., sorghum, maize, rice, wheat, potato, or cowpea [38–43]. Drought-induced plant senescence markedly limits crop productivity [44]. This is the first study that presents the results of QTL mapping in triticale, where drought-induced senescence may be controlled by wheat or/and rye genome [27].

The withholding of watering induced water stress in DH lines and revealed the differences in regards to well watered plants. Drought-stressed plants exhibited a decrease in leaf water content and dissipated a higher amount of energy from PSII (Figure S1). The triticale genotypes used for obtaining the populations of DH lines differed significantly in their content of chlorophyll *a* and *b*, carotenoids, and soluble carbohydrates and phenols. Cultivar 'Magnat' contained more investigated pigments and soluble sugars, while cv. 'Hewo' was richer in soluble phenols (Table 1).

Table 1. Mean, standard deviation (SD), distribution descriptors, and estimates of heritability for drought stress treatment. Means for parents differed significantly ($p < 0.00001$) for all the investigated traits. Chl*a*—chlorophyll *a*, Chl*b*—chlorophyll *b*, Chl*a* + *b*—chlorophyll *a* + *b*, CAR—carotenoids, SC—soluble carbohydrates, SPh—soluble phenolics.

Trait	Hewo	Magnat	DH			Heritability
	Mean ± SD	Mean ± SD	Mean ± SD	Min	Max	
Chl*a*	0.92 ± 0.12	3.24 ± 0.85	1.34 ± 0.82	0.37	2.99	0.464
Chl*b*	0.44 ± 0.05	1.36 ± 0.40	0.58 ± 0.32	0.20	1.20	0.220
Chl*a* + *b*	1.36 ± 0.16	4.60 ± 1.24	1.92 ± 1.14	0.58	4.15	0.400
CAR	0.41 ± 0.05	1.00 ± 0.20	0.52 ± 0.24	0.22	1.16	0.649
SC	31.0 ± 5.3	59.4 ± 7.8	45.9 ± 15.6	16.6	83.3	0.817
SPh	10.8 ± 1.0	5.4 ± 0.9	8.0 ± 1.43	5.8	11.4	0.542

Chlorophyll content over the entire cycle of plant growth and development is controlled by genetic programs [45]. Therefore, changes in photosynthetic pigments level are reliable indicators of plant senescence [46]. A decrease in chlorophyll content is a common plant response to drought [47]. Kar and Mishra [12] showed that an increase in phenolics levels may also be associated with senescence processes in rice. Phenolics are even supposed to play a role in plant senescence induction [48].

Our studies demonstrated high heritability of soluble carbohydrates (81.7%). Heritability of the content of carotenoids and soluble phenolics was moderately high (64.9% and 54.2%, respectively). In the case of chlorophyll content, the share of genetic variability in the total phenotypic variability was low (Table 1). Transgressive lines for all investigated traits were identified in DH population. In terms of carotenoids and soluble sugars content, the selected DH lines exceeded the values recorded for both parental lines. Some DH lines showed also lower content of chlorophyll and higher content of soluble phenolics than cv. 'Hewo' (Table 1).

Adding the content of soluble carbohydrates to the mapping process allowed us to investigate closer this key factor controlling plant senescence. Pourtau et al. [49] and Wingler et al. [50] demonstrated an essential role of soluble sugars in senescence regulation. The senescence-associated gene (*SAG12*), whose expression is highly specific, is induced several hundred-fold by plant growth on glucose [14].

We calculated QTL using a previously developed and characterized genetic map [19,27]. CIM analysis identified seven QTLs, including four on chromosome 2A, one on chromosome 1R, and two on chromosome 6R. Only three loci, QSPh.2A.2, QSC.2A.1 and QSC.2A.5 mapped single traits, i.e., the content of soluble phenolics and sugars. Single QTLs (QCSPh.1R) were responsible for changes in the levels of chlorophyll *a* and *b*, carotenoids, and soluble phenolics. The remaining three loci, QCSPhC.2A.3, QCSPhC.6R.1 and QCSPhC.6R.2 controlled changes in the entire set of investigated traits. With the threshold of 15%, a majority of identified QTLs can be classified as major with negative additive effects (Table 2)

Table 2. Characteristics of the loci for six quantitative traits in 'Hewo' × 'Magnat' DH population for drought stress treatment.

Chromosome	Locus	Region (cM)	Trait	Flanking Markers	LOD	R^2 (%)	Additive Effect [a]
2A	QSPh.2A.1	132.9–144.6	SPh	4215443	2.6	19.2	−14.8
	QSC.2A.2	146.9–153.2	SC	4362309	2.8	10.6	9.9
	QCSPhC.2A.3	169.8–184.5	Chl*a*	4200640–4201257	2.4	19.6	−13.9
		169.8–184.5	Chl*b*	4200640–4201257	2.4	17.1	−11.9
		168.8–185.5	Chl*a* + *b*	4200640–4201257	2.5	17.1	−12.0
		169.8–184.5	CAR	4200640–4201257	2.4	17.1	−11.9
		166.9–184.3	SC	3047278– 4201257	7.9	30.4	−40.2
		166.3–185.5	SPh	4343078– 4201257	2.8	21.5	−15.5
	QSC.2A.4	196.2	SC	3048208	2.8	17.2	−23.7
1R	QCSPh.1R	147.5–150.9	Chl*a*	3607029–3603565	4.4	23.3	−16.2
		147.5–150.9	Chl*b*	3607029–3603565	4.3	23.3	−16.1
		147.5–150.9	Chl*a* + *b*	3607029–3603565	4.4	23.3	−16.3
		147.5–150.9	CAR	3607029–3603565	4.3	23.3	−16.1
		147.5–150.9	SPh	3607029–3603565	4.2	23.4	−17.5
6R	QCSPhC.6R.1	339.1-345.9	Chl*a*	3618637–rPt–400935	3.5	14.6	15.9
		339.1–344.8	Chl*b*	3618637–rPt–399948	3.4	14.3	15.6
		338.1–345.9	Chl*a* + *b*	3618637–rPt-400935	3.5	14.8	16.1
		339.1–344.8	CAR	3618637–rPt–399948	3.4	14.3	15.6
		340.1–344.8	SC	3618637–rPt–399948	3.0	11.9	21.8
		339.1–345.9	SPh	3618637–rPt–505447	3.0	12.8	15.7
	QCSPhC.6R.2	356.6–382.5	Chl*a*	3613876–4215469	7.8	38.7	−21.7
		356.6–382.5	Chl*b*	3613876–4215469	7.7	38.7	−21.6
		356.6–382.5	Chl*a* + *b*	3613876–4215469	7.8	38.6	−21.8
		356.6–382.5	CAR	3613876–4215469	7.7	38.8	−21.6
		359.1–378.5	SC	4204839–rPt–505870	19.7	51.5	−66.2
		355.4–370.6	SPh	3604778–4215469	7.6	35.4	−24.8

[a] Additive effects of QTLs. Positive values of additive effects indicate that 'Hewo' allele has a positive effect on that trait.

In our earlier studies, the loci determining the content of soluble phenolics under drought were also located on wheat chromosome 2A in the vegetative stage of triticale growth (locus QVSPh.2A.1 and QVSPh.2A) and two rye chromosomes (1R and 6R) in the vegetative (loci QVSPh.1R.1 and QVSPh.1R.2 for chromosome 1R; locus QVCWPh.6R.3 for chromosome 6R) and generative (locus QGSPh.1R.2 for chromosome 1R; locus QGCWPh.6R.3 for chromosome 6R) stage [27]. Even though the current and

previous results are compatible, the earlier study involved leaves collected from the upper part of the plants, i.e., the first fully developed leaf from the vegetative stage and the flag leaf from the generative stage. However, the earliest signs of senescence are first visible on the leaves located in the lower part of a cereal stem. For this reason, the analyses presented in this study were performed for leaves located below the subflag leaf.

Under optimal growth conditions, the eight loci related to chlorophyll content on chromosomes 3A (3 loci), 4A (1 locus), 5A (3 loci), 7A (1 locus), and 12 loci on chromosomes 1B (2 loci), 2B (1 locus), 4B (5 loci), 5B (1 locus), and 6B (3 loci) of spring wheat were located. The greatest number of loci (27 in total) related to this trait were detected for wheat chromosomes D [29].

In another study on high temperature stress, as many as nine loci related to plant senescence were located on wheat chromosome 2A. The remaining chromosomes were shown to contain a single locus (3A, 4A) or from two (5A, 7A, 3B, 4B, 6B, 5D, 7D) to three (6A, 7B) loci. Contrary to that, a locus related to chlorophyll content was only identified on chromosome 7B [42].

To find out candidate genes, we obtained sequences of all DArTseq markers, including those of redundant markers, for four QTLs identified on chromosome 2A (Table S1). The loci QSPh.2A.1 and QSC.2A.2 were identified with single markers and the candidate genes were looked for within +/− 2cM that corresponds to 5.745 Mbp. These regions yielded 64 and 80 genes, respectively (Table S2). Five of the genes identified for QSPh.2A.1 region were responsible for carbohydrate metabolism, i.e., SBEIIa gene for starch branching enzyme IIa, invertase (alpha-glucosidase), an unnamed protein product (1,4-alpha-glucan branching enzyme), glucan endo-1,3-beta-glucosidase 14-like isoform X1, and putative cell wall invertase c. Locus QSC.2A.2 encoded sucrose synthase (Table 3, Table S3)

Table 3. Selected candidate genes identified (BLASTX) in physical regions corresponding to QTLs on wheat chromosome 2A (URGI database and HM genetic map, respectively).

Position of QTL		Number of Genes	Description	Max Score	Ident (%)	Accession
Genetic (cM)	Physical (Mbp)					
			QSPh.2A.1			
136.7	504.27	64	Starch branching enzyme IIa [*Zea mays* L.]	100	98	ONM17778.1
			alkaline/neutral invertase E, chloroplastic isoform X1 [*Prunus persica* L.]	312	86	XP_007221417.1
			unnamed protein product [*Triticum aestivum* L.]	523	93	SPT18309.1
			glucan endo-1,3-beta-glucosidase 14-like isoform X1 [*Aegilops tauschii* Coss. subsp. *tauschii*]	677	95	XP_020177221.1
			putative cell wall invertase c [*Secale cereale* L.]	597	98	AWT08285.1
			QSC.2A.2			
149.2	117.79	80	sucrose synthase type 2-1 [*Triticum polonicum* L.]	389	85	AIL88516.1
			QCSPhC.2A.3			
168.8–171.3	130.36/443.64	67/42	putative 6-phosphogluconolactonase 2 [*Triticum urartu* L.]	318	99	EMS47003.1
			predicted protein [*Hordeum vulgare* L. subsp. *vulgare*]	925	87	BAJ94765.1
			UDP-glycosyltransferase 85A3 [*Triticum urartu* L.]	935	91	EMS51148.1
177.7–184.5	103.37–105.63	21	[*Hordeum vulgare* L. subsp. *vulgare*] sphinganine C4-monooxygenase 1	370	99	BAK04950.1
			Anthocyanidin 5,3-O-glucosyltransferase [*Triticum urartu* L.]	921	99	EMS62298.1
			probable mannan synthase 7 [*Aegilops tauschii* Coss. subsp. *tauschii*]	202	71	XP_020168696.1
			hypothetical protein TRIUR3_18345 [*Triticum urartu* L.]	353	74	EMS68147.1
			predicted protein [*Hordeum vulgare* L. subsp. *vulgare*]	203	74	BAJ91161.1
			QSC.2A.4			
196.2	72.74–72.76	136	L-gulonolactone oxidase 2 [*Brachypodium distachyon* L.]	500	79	XP_003573784.1
			4-alpha-glucanotransferase DPE2 [*Triticum urartu* L.]	297	72	EMS56480.1

For QCSPhC.2A.3, as many as 180 redundant sequences were identified for two flanking markers (Table S1). The position of the selected 133 sequences with the best match indicated three physically remote locations in the wheat genome despite a small number of recombinations in the investigated triticale population. This is probably related to structural rearrangements within triticale chromosome 2A in relation to wheat as suggested in earlier works [15,20]. Considering

large physical distance of the markers flanking QCSPhC.2A.3 in wheat, we limited our search for candidate genes to +/− 2cM region around the markers. In this QTL, we identified 130 genes including putative 6-phosphogluconolactonase, 7-deoxyloganetin glucosyltransferase-like protein, and UDP-glycosyltransferase 85A3 (Table 3).

For five markers from the third region of QCSPhC.2A.3, we found an additional 28 redundant markers and identified a corresponding 2.26 cM physical region that contained 23 genes. Functional annotation of these genes revealed that four of them acted as glycosyltransferases (Table 3, Table S3).

For QSC.2A.4, we identified nine DArTseq sequences that indicated a narrow region in the physical position of 72 Mbp. An extended search revealed 137 genes in the region of +/− 2cM including L-gulonolactone oxidase 2 and 4-alpha-glucanotransferase DPE2 (Table 3, Table S3).

Plant senescence is a process controlled by multiple genes [51]. Some of them encode enzymes responsible for protein degradation [1], protein processing [52], N mobilization [53], lipid degradation [54], C mobilization [1], cell wall degradation [55], P mobilization [56], transport [57], transcriptional regulation [58], signaling pathways [59], antioxidants [7], metal binding [60], hormone biosynthesis [61], cell death [62], defense-related genes [52], translation [63] and some proteins of unknown functions [64]. These proteins involve also those related to carbohydrate metabolism, such as isocitrate lyase, malate synthase, pyruvate orthophosphate dikinase, sugar transporter, endoxyloglucan transferase or β glucosidase [51,65].

We used another approach to identify candidate genes (Table S3) located within the QTL from genome R of triticale. For three analyzed QTLs, we employed 16 marker sequences with unique segregation patterns and additionally 109 redundant sequences of DArTseq and 5 DArT (Table S1). BLAST for QCSPh.1R let us identify unique regions on Lo7 triticale map (Table S4). QCSPhC.6R.1 and QCSPhC.6R.2 included overlapping regions but contigs identified for both regions were unique. The next stage involved identification (Table S5) and functional annotation of genes in selected contigs (Table S6, Table 4). We were unable to identify potential transcripts for eight out of nine sequences of DArT markers from QCSPhC.6R.1 and QCSPhC.6R.2 regions, and the protein encoded by rPt-390,636 sequence was related to the regulation of photomorphogenesis. All genes were functionally annotated (Table S6) and some of them were found to be associated with carbohydrate and phenolic metabolism.

Table 4. Selected candidate genes identified (BLASTX) in rye contigs corresponding to QTLs on rye chromosomes 1R and 6R.

Locus	Genetic Position (cM) IPK Region	Description	*e*-Value	Mean Sim (%)
QCSPh.1R	137.80–127.42	sugar transporter ERD6-like 4	0.0	75.29
QCSPhC.6R.1	170.88–166.09	cytokinin dehydrogenase 11	0.0	86.45
QCSPhC.6R.2	171.27–165.28	14-alpha-glucan-branching enzyme 2-2 chloroplastic/amyloplastic	0.0	78.31
QCSPhC.6R.3	176.48–181.34	probable inorganic phosphate transporter 1–8	0.0	93.42
		protein FAR1-RELATED SEQUENCE 5-like	4.71918×10^{-110}	73.44

4. Conclusions

In conclusion, we found the senescence-related loci on one wheat chromosome 2A and two rye chromosomes (1R and 6R) in winter triticale exposed to drought. The identified loci were responsible for the levels of pigments and the content of soluble carbohydrates and phenolic compounds. We also identified candidate genes for the investigated traits. Further studies are necessary to identify genes within the obtained loci and to analyze their products.

Supplementary Materials: The following are available online at http://www.mdpi.com/2073-4395/9/4/195/s1, Table S1: Sequences of DArTseq and DArT markers from QTLs, Table S2: Sequences of genes identified in physical regions corresponding to QTLs located at chromosome 2A, Table S3: Genes identified in QTL region on triticale chromosome 2A, Table S4: Functional annotation of genes identified in rye contigs corresponding to four QTLs, Table S5: Sequences of rye genes indentified in contigs corresponding to the identified QTLs, Table S6: Annotations of genes identified in QTL region on triticale chromosomes 1R and 6R.

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Abbreviations

Chl*a*	chlorophyll *a*
Chl*b*	chlorophyll *b*
Chl*a*+*b*	chlorophyll *a* + *b*
CAR	carotenoids
SC	soluble carbohydrates
SPh	soluble phenolics

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