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RESEARCH ARTICLE

Characterization of Ribulose-1,5-bisphosphate carboxylase-oxygenase activase (*Rca*) genes in durum wheat

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Abstract Durum wheat *(T. turgidum L. var. durum)* is one of the most widely cultivated cereal crop in the Mediterranean area. Its production has been triggered by drought and rising temperature, both affecting the photosynthetic machinery. Rubisco is one of the most important enzymes in plants. Despite its major role in the control of carbon cycle it has a very low efficiency, which is restored by the action of Ribulose-1,5-bisphosphate carboxylase/oxygenase activase (Rca), a protein belonging to the AAA^+ family. The main objective of our work was to isolate and characterize *Rca* genes in durum wheat and determine their phylogeny with other main crops and model species. Besides a genetic and physical position of *Rca1* gene was allowed in a RIL mapping population previously developed. In silico analysis, performed in order to understand whether *Rca1* gene was differentially expressed under stress condition, highlighted that homoeologous *Rca1* genes have different expression levels especially after infections by *Zymoseptoria*, powdrey mildew and fusarium. A deeper knowledge of *Rca* genes structures as well as a better understanding of their physiological role in durum wheat might be of greater importance in panning future modern breeding programs to improve crop yield in adverse environmental condition.

Keywords Rubisco · Rubisco activase genes · Durum wheat · In silico expression

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Introduction

The world's population is expected to reach over 9 billion by 2050, as the latest FAO forecast reports (OECD/FAO 2020), determining a 70% increase in the global food demand, especially on cereals (York 2016; Chen et al. 2017; FAO 2017). To satisfy this demand, the development of improved cultivars along with the adoption of new and optimized management practices will be essential. As reported by Beres et al. (2020), the accomplishment of this goal will necessitate a significant yield boost for crops such as wheat, which rate of gain needs to be improved by 30–40% (FAO 2017; Cassman et al. 2020). Wheat is one of the most globally grown crop, supplying about 20% of the human daily calories and protein intake (CRP-WHEAT 2016). Most of the cultivated wheats belong

either to the hexaploid *Triticum aestivum* L. or to the tetraploid *T. durum* species, the latter representing only the 5% of the total wheat production. Durum wheat is an élite crop in dry lands, such as the Mediterranean basin, with a yearly production average of 40 million tonnes (MT) on a planting area of 17 million hectares globally (IGC 2020). Durum wheat is mostly grown under rain-fed conditions, so its productivity is severely influenced by rainfall as well as biotic (pests and diseases) and abiotic (drought, cold, salinity) stresses. So far, breeding programs aiming at durum wheat yield improvement, will also have to deal with the variable effects induced by the climate changes we are witnessing (Reynolds et al. 2020). The increasing atmospheric CO_2 is affecting global temperatures, water availability and weather events, all having strong impacts on plant productivity and carbon assimilation, as well as on agriculture and food production.

So far, bigger efforts will be required to face and deal with these issues and guarantee sustainable crop production and provide climate-smart agriculture, especially from a genetic perspective. Indeed, the identification of candidate genes having a major role in the control of central metabolic pathways is of crucial importance in underpinning and revealing the genomic region actively involved in those traits' control.

Ribulose-1,5-bisphosphate carboxylase/oxygenase (Rubisco, EC 4.1.1.39) is responsible for the photosynthetic assimilation of CO_2 into organic compounds, the rate limiting step in photosynthesis (Spreitzer et al. 2002). As reported by Perdomo et al. (2021), Rubisco is a quite complex enzyme, consisting of eight large subunits, encoded by a single plastidial gene (*RbcL*), and eight small subunits which are instead encoded by a nuclear multigene family (*RbcS*), (Schmidt et al. 1986; Roy 1989; Carmo-Silva et al. 2015; Bracher et al. 2021).

Despite being the most abundant protein in plants (and on Earth) (Raven 2013), Rubisco has been reported as one of the most inefficient enzymes ever, both because of its very low catalytic turnover rate and the predisposition to be inhibited by sugar-phosphate derivatives which latches the active (Portis 1995; Carmo-Silva et al. 2015; Bracher 2021). Enhancing Rubisco functionality has significant repercussions concerning the improvement of plant productivity and resource use efficiency (Parry et al. 2007; Whitney et al. 2011). It has been demonstrated that its activity depends on interaction with the Rubisco activase (Rca), a chloroplast ATPases associated with diverse cellular aActivities (AAA + protein family) encoded by a nuclear gene (Bhat 2017).

Rca plays a significant role in photosynthesis, as catalyzes the Rubisco activation in the photosynthetic light-independent Calvin-Benson-Basham cycle. Rca utilizes the energy derived by ATP hydrolysis to release the sugar-phosphates from Rubisco active sites, thus reestablishing its catalytic competence (Bhat et al. 2017; Shivhare et al. 2017).

Bread wheat (*T.* aestivum) contains two *Rca* genes, as most of flowering plant species, including grasses. Carmo-Silva et al. (2015) reported the presence of two *Rca* sequences, in tandem, on chromosome group 4 in bread wheat (specifically, long arm for A genome and short arm for B and D genomes). *TaRca1* has a simpler structure, consisting of only two exons, and originates a single short mature protein named TaRca1-*β*. *TaRca2* is a longer gene, with a total of six exons; as alternative splicing has been demonstrated to occur at the end of exon 5, two isoforms might be obtained: a shorter one, *TaRca2-β,* and a complete one, *TaRca2-α,* 37 aa longer than the previous. The two *β* isoforms different just for 0.5 kDa (TaRca1-β and TaRca2-β being 42.7 and 42.2 kDa, respectively), while TaRca2-α isoform has a predicted molecular weight of 46.0 kDa (Carmo-Silva et al. 2015).

Little is known about Rca detailed mechanism(s) in plants, since only recently plant Rubiscos have been recombinantly expressed (Ng et al. 2020).

Some specific conditions might affect its efficiency, such as abiotic or biotic stresses, especially light, temperature and pathogen infection. Some studies have been carried out to determine the expression of Rca specific isoforms during the 24 h diel cycle (Perdomo et al. 2021), and heat-stress condition (Degen et al. 2020). Nevertheless, several proteomic studies have highlighted a differential expression of Rca enzymes after pathogen infection. Interestingly, *Fusarium graminearum* seemed to determine a change in Rca protein abundance after infection both in wheat and barley (Zhou et al. 2006; Geddes et al. 2008). The recently published bread and durum wheat genome sequences (Appels et al. 2018; Maccaferri et al. 2019), might be considerably valuable in disclosing the genetic complexity of those species. By exploiting these and other publicly available data, we

focused on the isolation and characterization of *Rca* genes in durum wheat, as well as on the depiction of their phylogeny with other main crops and model species. Also, a RIL mapping population previously developed to study *Fusarium graminearum* resistance (Giancaspro et al. 2016) was used to genetically and physically map the genes, and in silico analysis was performed in order to identify differential expressions under different environmental condition. A deeper knowledge of *Rca* genes structures as well as their physical and genetic mapping might be of greater importance in planning future breeding programs based on the most recent and precise genetic techniques, such as the genome editing approaches, to improve both crop yield and/or pathogen resistance, such as to *Fusarium*.

Materials and methods

Ribulose-1,5-bisphosphate carboxylase-oxygenase activase (*Rca*) genes isolation and characterization in durum wheat

Bread wheat *Rca* gene sequences (accession numbers LM992844 (*TaRca1*) and LM992846 (*TaRca2*) (Carmo-Silva et al. 2015) were used as queries and launched against the publicly available durum wheat cv Svevo genome browser (https://d-gbrowse.interomics.eu/). The retrieved gene sequences were further analyzed for gene structure detection and used to primer pairs design for further investigation in different wheat genotypes.

Phylogenetic analysis of *Rca* genes

Rca1 and *Rca2* orthologous genes for *T. aestivum, T. durum, Triticum turgidum L. subsp. dicoccoides, Triticum urartu, Aegilops tauschii, Hordeum vulgare, Brachypodium distachyon, Oryza sativa, Sorghum bicolor* and *Zea mays* were retrieved from the EnsemblePlant database (http://plants.ensembl.org/) by blasting the two genes of *T. aestivum* against each of the considered species' genome.

For ease of phylogenetic tree reading, abbreviations of common names and genus names will be used as follows. Each plant species considered in this paper was indicated with a two-letter prefix (followed by each gene symbol): *Ta* for *T. aestivum, Tt* for *T. durum, Tdic* for *T. dicoccoides, Tu* for *T. Urartu, Ae* for *Ae. tauschii, Hv* for *H. vulgare, Bd* for *B. distachyon*, *Os* for *O. sativa*, *Sb* for *S. bicolor* and *Zm* for *Z. mays*.

All retrieved genes' cDNAs were aligned by using the ClustalW method via MegaX software (Kumar et al. 2018). Phylogenetic analysis was carried out on 28 CDS sequences using the *Maximum Likelihood based on the Tamura-Nei model* (Tamura et al. 1993).

The tree was drawn to scale, with branch lengths measured in the number of substitutions per site. The tree was generated with MegaX as well and modified with the FigTree program (http://tree.bio.ed.ac.uk/software/figtree/).

Plant material and polymorphism detection through ecotilling analysis

Two wheat genotypes, 02-5B-318 and Saragolla, parents of a mapping population consisting of a set of 135 recombinant inbred lines (RILs) segregant for FHB resistance (Giancaspro et al. 2016), were used to eventually identify polymorphisms within *Rca* genes sequence to genetically and physically map them. 02-5B-318, a breeding line derived from the resistant Chinese cv. Sumai-3, is a FHB-resistant bread wheat accession, while durum wheat cv. Saragolla is FHB-susceptible. Primer combination for were designed. By using Oligo Explorer (http://www.genelink.com/tools/gl-oe.asp) and Primer3 (http://frodo.wi.mit.edu/primer3/) software, a set of genome specific primer pairs were designed based on the retrieved Svevo *Rca1* and *Rca2* gene sequences and used to amplify target DNA from both 02 and Saragolla parental lines (Table 1). The RIL population was also used for mapping yield and Protein content QTLs (Giancaspro et al. 2019).

DNA amplifications were carried out in 20 µL reaction mixtures, each containing 50 ng DNA template, 200 M of dNTP, each primer in 0.5 µM concentrations, 1 × buffer, 0.02 U/µL Taq polymerase. The following PCR protocol in a BIORAD thermo cyclers was used: initial denaturation at 95 °C for 3 min, followed by 35 cycles of 94 °C for 30'', 60.5 °C/63 °C for 30'', 72 °C for 1 min with a final extension at 72 °C for 10 min (Nigro et al. 2014).

In order to discover and map eventual polymorphism between the genomic sequences of the two varieties previously described, Single Nucleotide

Table 1 Primer sequences used to amplify *Rca* genes from 02-5B-318 and Saragolla parental lines

Primer name	Primer sequences		Annealing temperature (°C)
	Forward (5'-3')	Reverse (5'-3')	
Rca-1	AAACAAGATAGTATATACGGGCGA	CCTCTGGTGATGTCCTGCTG	60.5
Rca-2	TGGCTAATAAACAAGACGATCCG	AGGCAAGACCCTTCCACTTG	62.5
Rca-3	AGGCCAACAGGTTCACAGTC	ATTGGGCTGTGGTGAAAAGC	63
Rca-4	CATACTGTCACACAGTCATAGATGC	TGTGCGTAGTTCACCTCCTC	62
Rca-5	ATGCTAACCAGGATGCGATGA	AAAGCAGAAGCAGTCTCCACT	62

Polymorphisms (SNPs) were detected using the Surveyor nuclease kit (Transgenomic, Inc.), following manufacture's instruction. The heteroduplex formation, *CELI* digestion and gel analysis were carried out as reported by Nigro et al. (2014, 2017).

To confirm the polymorphisms within genome specific genes, the heteroduplex hybridization digestion pattern was compared to the ones obtained in each parental lines. Furthermore, PCR products giving a digestion pattern after *CEL1* treatment were re-amplified and sequenced by 96 capillary 3100 Avant (Life Technologies) (Nigro et al. 2014, 2017).

Development of *Rca1* specific markers

The *Rca1* sequences of the two wheat genotypes 02-5B-318 and Saragolla were aligned using ClustalΩ (https://www.ebi.ac.uk/Tools/msa/clustalo/) from EBI website to identify polymorphisms. The polymorphic markers were mapped in the 'Saragolla' and'02-5B-318' mapping population. The observed segregation ratio for the marker was tested by Chi-square analysis for deviation from the expected 1:1 ratio. The linkage analysis was performed by JoinMap v. 4.0 (Ooijen et al. 2006) and the Kosambi mapping function was used to calculate map distances (1943).

Rca genes in-silico expression analysis

In silico expression analysis and the identification of upregulated gene models was carried out using the RNAseq data available at http://www.wheat-expression.com/ using gene models from 'Chinese spring'. All experimental conditions were considered. Additionally, the homologous genes from 'Svevo' were picked in durum wheat.

Results

Isolation of genomic sequences of Rca genes in wheat

The bread wheat *TaRca1* and *TaRca2* sequences were used as queries by blast analysis in the Svevo portal (https://d-gbrowse.interomics.eu/gb2/gbrowse/Svevo/), and several different durum wheat sequences annotated as the Ribulose-1,5-bisphosphate carboxylase-oxygenase activase, were retrieved. Specifically, 79 different splicing isoforms were identified on 4A chromosome, while 74 different ones were identified for 4B homoeologous.

Indeed, differently from bread wheat and other species, the two genes were not separately and individually annotated, but reported in tandem as different splicing form of the same gene: TRITD4Av1G139700 on minus strand of 4A chromosome, and TRITD4Bv1G060980 on plus strand of 4B. By sequence analysis and comparison of CDS and predicted aminoacidic sequence, it was possible to determine the most similar and more likely to be the durum wheat *Rca1* and *Rca2* sequences for both A and B genome: *Rca1-4A*: TRITD4Av1G139700.79 (chr4A:442,229,641.0.442,232,675); *Rca2-4A*: TRITD4Av1G139700.4; *Rca1-4B*: TRITD4Bv1G060980.1; *Rca2-4B*: TRITD4Bv1G060980.64. Both *Rca1* homoeologous genes comprise 2 exons, a complete CDS of 1299 bp and a predicted protein of 432 aa.

Rca2 genes have 6 exons, a CDS of 1404 bp and a protein of 467 aa. It should be reminded that in this case, an alternative splicing at exon 5 might induce a shorter isoform (Fig. 1).

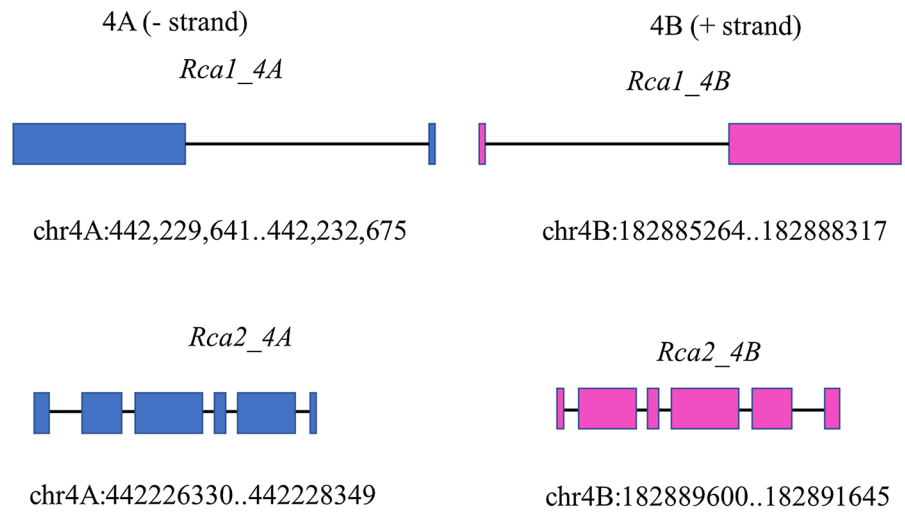

Fig. 1 Durum wheat *Rca1* and *Rca2* sequences for A and B genome and physical position on reference Svevo genome

Phylogenetic analysis

Orthologues *Rca1* and *Rca2* gene sequences were retrieved for ten species from the EnsemblePlant database (http://plants.ensembl.org/), including *T. aestivum*, *T. durum*, *T. dicoccoides*, *T. urartu*, *Ae. tauschii*, *H. vulgare*, *B. distachyon*, *O. sativa*, *S. bicolor* and *Z. mays*. The above reported species were chosen as either sequenced wheat genome progenitor and related species, or model plants which genome sequences have been fully sequenced and annotated. Furthermore, two C4 species were chosen in order to compare the evolutive distance of *Rca1* genes with C3 ones.

The identified CDS were checked for sequence structure and similarity. A total of 28 gene sequences were retained to build a phylogenetic tree comprising the ten considered species (Table 2), which were firstly aligned by using the ClustalW method via MegaX software (sequences and alignment are reported in Supplementary file 1).

The evolutionary history was inferred by using the Maximum Likelihood method based on the Tamura-Nei model (Tamura et al. 1993). The tree with the highest log likelihood (−8623.3812) is shown (Fig. 2). Initial tree(s) for the heuristic search were obtained automatically by applying Neighbor-Join and BioNJ algorithms to a matrix of pairwise distances estimated using the Maximum Composite Likelihood (MCL) approach, and then selecting the topology with superior log likelihood value. The tree is drawn to scale, with branch lengths measured in the number of substitutions per site. Codon positions included were 1st + 2nd + 3rd + Noncoding. There were a total of 1774 positions in the final dataset. Evolutionary analyses were conducted in MEGAX (Kumar et al. 2018).

As shown by the tree in Fig. 2, two main clades, and a third smaller one, were generated. The first one, grouped 11 *Rca1* sequences (reported in pink) belonging to the 7 of the analyzed species; as expected, sequences belonging to A genome from *Triticum* species clustered altogether with the *Urartu* one, and the same situation was observed for orthologous *Rca1* belonging to B and D genome, the latter clustering with the *Rca1* of *Ae. tauschii* as well. *H. vulgare* and *B. distachyon* had the more dissimilar sequences, as expected. The same situation was observed for the second cluster, which grouped 12 *Rca2* sequences. *Triticum* orthologous belonging to the same genome clustered together, as previously observed for *Rca1* CDS. Interestingly, a third smaller cluster formed, which grouped both *Rca1* and *Rca2* from *Sorghum* and *Maize*, the only C4 species included in the analysis. A divergent sequence was also found out, a *Rca2* CDS sequence of rice.

Genetic and physical mapping of wheat Rca genes sequences

ECOTILLING approach requires a treatment of the amplified DNA with *CELI* endonuclease, or any of a number of single strand endonucleases, after heteroduplex formation between the lines to be

Table 2 Ensembl entries of the *Rca1* and *Rca2* genes retrieved in *T. aestivum*, *T. durum*, *T. dicoccoides*, *T. urartu*, *Ae. tauschii*, *H. vulgare*, *B. distachyon*, *O. sativa*, *S. bicolor* and *Z. mays*. (EnsemblPlants website: http://plants.ensembl.org/)

Gene	*T. aestivum*	*T. durum*	*T. dicoccoides*	*T. urartu*	*A. tauschii*	*H. vulgare*	*B. distachyon*	*O. sativa*	*S.bicolor*	*Z. mais*
Rca1	TraesCS4A02G177600.1	TRITD4Av1G139700.79	TRIDC4AG028530.14	TRIUR3_23730-T1	AET4Gv20287100.8	HOR-VU4Hr1G027260.19	BdPNT62864	Os11t0707100-00	SbEES10316	Zm00001eb164380_T001
	TraesCS4B02G140200.1	TRITD4Bv1G060980.1	TRIDC4BG021940.1							
	TraesCS4D02G134900.1									
Rca2	TraesCS4A02G177500.1	TRITD4Av1G139700.4	TRIDC4AG028530.4	TRIUR3_23731-T1	AET4Gv20287100.2	HORVU4Hr1G027260.8	BdKQJ87035	Os11t0707000-02	SbEES10317	Zm00001eb164390_T001
	TraesCS4B02G140300.1	TRITD4Bv1G060980.64	TRIDC4BG021940.8							
	TraesCS4D02G135000.9									

investigated. Surveyor nuclease cleaves with high specificity at the 3′ side of any mismatch site in both DNA strands, including all base substitutions and insertion/deletions up to at least 12 nucleotides. The treatment of all amplicons for each *Rca* gene allowed the identification of a mismatch in Rca1 sequence, and specifically, a SNP was identified within the second exon of *Rca1-4A* gene. Precisely, the T/C SNP identified between the two parental line, 02-5B-318 (C) and Saragolla (T), was mapped in the RIL mapping population, and was localized at 123.9 cM (Fig. 3). Analysis of SNP in the predicted mature protein showed that the polymorphism resulted in amino acid substitution in position 260, a leucine to phenylalanine switch L-F (C/T). Unfortunately, no polymorphism was detected within *Rca2* gene sequence**.** The projected SNP of *Rca1* in the Svevo genome mapped at 37.7 cM, at physical position 442,230,162 bp. The metaQTL analysis conducted by Maccaferri et al. (2019), identified 14 different QTLs underlying the *Rca* genes region, most of which related to yield traits, but two of them found to be involved for *Fusarium graminearium* and leaf rust resistance. On these bases a new QTL analysis for Fusarium resistance was contacted using the same phenotypic data and genetic map of Giancaspro et al. (2016) adding data from *Rca1*. QTL analysis conformed the presences of a QTL for FHB resistance coincident with *Rca* gene with a LOD of 3.

Expression profile of Rca genes in wheat

Using the genome browser for 'Svevo' (https://d-gbrowse.interomics.eu/gb2/gbrowse/Svevo/) reference genomes and the RNAseq data available at http://www.wheat-expression.com/ (Borrill et al. 2016), we carried out an in silico gene expression analysis to identify in which tissue and phenological stage *Rca1* gene transcripts were more abundant.

In addition, the analysis was conducted to correlate the gene expression with biotic stress conditions and detected where the expression was higher during plant development.

The *Rca1-4A* gene expression was detected in leaves (including flag leaf), followed by roots and spikes. Considering the developmental stages and types of leaf, the higher level of *Rca1-4A* gene

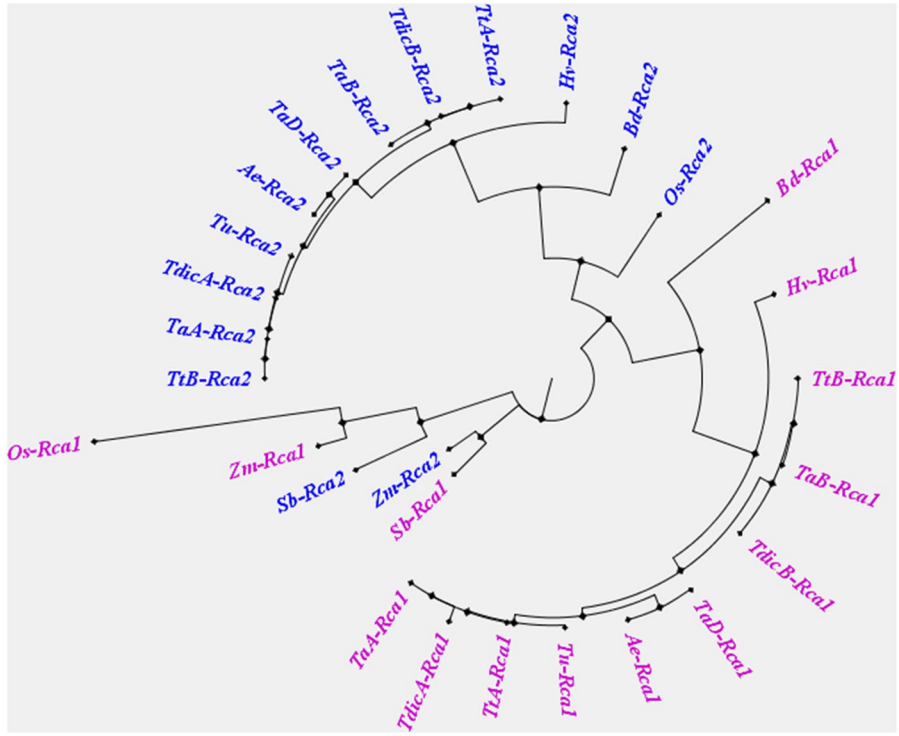

Fig. 2 Molecular Phylogenetic analysis by Maximum Likelihood method of *Rca1* and *Rca2* genes retrieved from *T. aestivum*, *T. durum*, *T. dicoccoides*, *T. urartu*, *Ae. tauschii*, *H. vulgare*, *B. distachyon*, *O. sativa*, *S. bicolor* and *Z. mays*

expression was reported at seedling, three leaf and reproductive stages and during grain filling.

According to stress response, the wheat *Rca1-4A* gene showed to be particularly expressed during *Zymoseptoria tritici*, Stripe rust, *Powdery mildew* and *Fusarium* infections (Fig. 4), conforming what obtained with QTL analysis for FHB resistance.

Expression analysis of *Rca1-4A* under abiotic stress included: drought stress, heat stress, combined drought and heat stresses, water stress, chitin addition and PEG 6000 treatment to simulate drought and cold stress.

The homeologus gene *Rca1-4B* was highly expressed in leaves, while lower levels were detected in roots and spikes. Comparing the expression of the *Rca1-4A and Rca1-4B genes* under stress conditions, the latter one showed a higher expression level under the *Powdery mildew* infection. Overall, the expression data reported for the *Rca* genes located on chromosome 4B appeared to be more abundant compared to the ones on the 4A homoeologues.

Discussion

The rise in atmospheric temperatures has affected the length of cereal growing season in large areas of Europe. This, together with water scarcity, are two main constraining factors for crop productivity, as reported by Perdomo et al. (2015, 2016).

Over the past 150 years, the concentration of carbon dioxide in the atmosphere increased by 32%, going from 280 to 370 parts for million in volume. As known, CO_2 concentration directly affects photosynthesis, a process extremely susceptible to both drought and heat stress (Chaves et al. 2009; Carmo-Silva et al. 2012; Vile et al. 2012; Mathur et al. 2014; Singh et al. 2014).

Rubisco, the key enzyme of CO_2 carboxylation in the Calvin–Benson cycle, represent 50% or more of total proteins in leaves. Its deactivation represents a major element in decreased CO_2 assimilation rate at high temperatures (Parry et al. 2013; Bracher et al. 2017). Rubisco activase (*Rca*) facilitates the dissociation of inhibitory sugar phosphates from the active

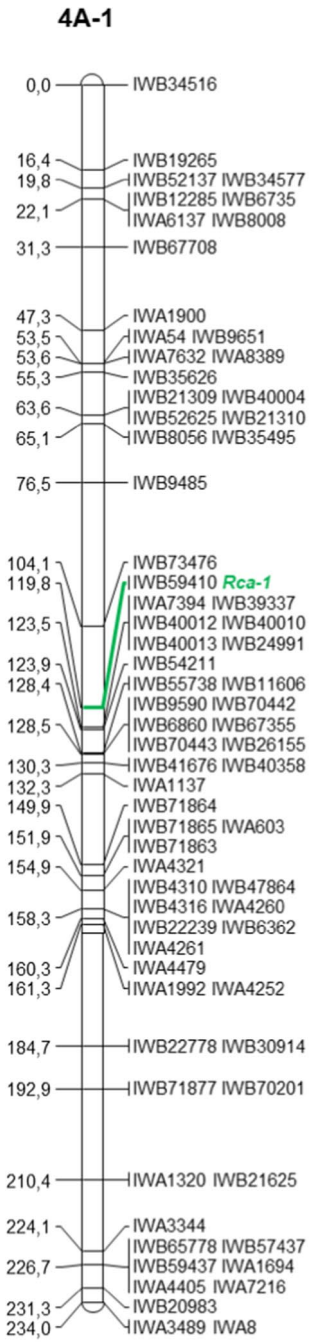

Fig. 3 Graphic representation of chromosome 4A and *Rca-1* gene location

site of Rubisco (Spreitzer et al. 2002), affecting the efficiency of photosynthetic activity.

Due to its pivotal role in photosynthetic processes, the enhancement of Rubisco activation by Rca may be a potential strategy for improving a photosynthesis-driven increase in crop yield.

In this paper, we focused on the gene structure characterization of *Rca* genes in durum wheat, and their mapping on a RIL population previously developed and used to map QTL involved in *Fusarium graminearum* resistance.

Previous proteomic studies have indeed highlighted a differential expression of Rca enzymes after *Fusarium graminearum* infection. Interestingly, the pathogen seemed to determine a change in Rca protein abundance after infection both in wheat and barley (Zhou et al. 2006). Zhou et al. (2006), identified a down-regulation of Rubisco activase (2,sevenfold change detected) along with glyceraldehyde 3-phosphate dehydrogenase (GAPDH) and degradation of Rubisco, suggesting that photosynthesis was disrupted or at least decreased after *F. graminearum* infection in wheat spikes. Recently, a study of Kosovà et al. (2017) analyzed the effect of an artificial infection with *Fusarium culmorum* and application of deoxynivalenol (DON) on barley spikes at flowering by proteomic analysis (2D-DIGE technique combined with LC–MS/MS). They found out a decrease in photosynthesis-related proteins in *Fusarium*- and DON-treated plants with respect to control indicating an adverse effect of stress on photosynthetic apparatus. Rubisco activase small isoform A were detected only in *Fusarium*- and DON-treated plants while they were ab sent in control plants of both analyzed genotypes. According to Geddes et al. (2008), enhanced level of Rubisco activase isoform1was found in *Fusarium*-treated susceptible and intermediate-resistant barley cultivars, while no change was found in resistant ones, suggesting indicating that they Rca were latterly induced as a consequence of both pathogen infection and mycotoxin application.

We mapped *Rca* gene on 4A chromosome in 02-5b-318 × Saragolla RIL population, and the SNP was projected on the reference Svevo genome (Macaferri et al. 2019). MetaQTL analysis previously performed highlighted a very interesting situation. Indeed, the region on *Rca1*-4A gene was surrounded by 14 different QTL. Despite 12 of them were involved in yield and yield-related traits control, two of them were a FHB resistance QTL and a LR resistance one (Prat et al. 2014; Aoun et al. 2016). This confirm our data that identified a QTL coincident with the *Rca1* gene location on 4A chromosome.

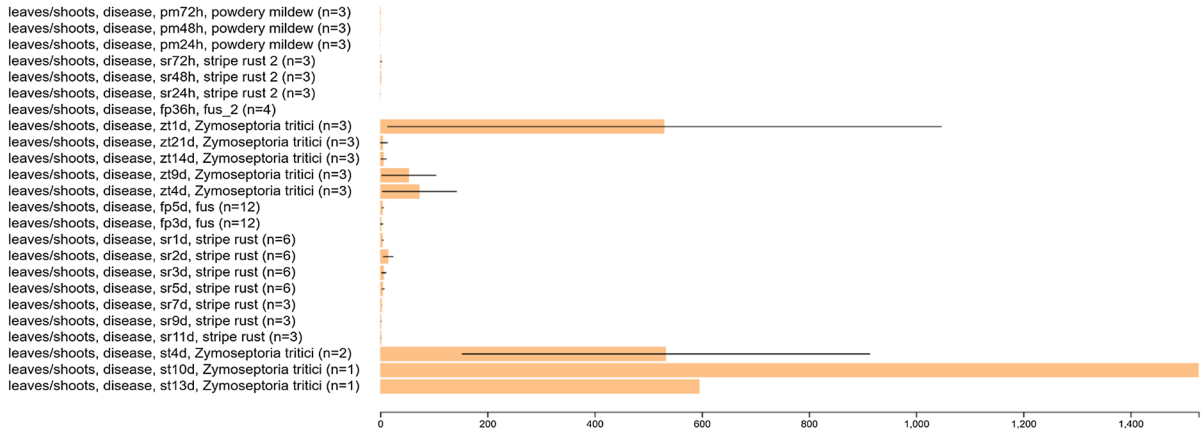

Fig. 4 In silico expression analysis of durum *Rca1_4A* gene in leaves/shoots under stress disease, in particular in response to *Zymoseptoria tritici*, Stripe rust pathogen, *Powdery mildew* and *Fusarium* infections

The same investigation carried out on the 4B homoeologous genes region, showed similar result, with 35 different QTL underlying the region, and specifically two involved in FHB resistance QTL and a LR resistance (Marone et al. 2009; Ruan et al. 2012), suggesting that this region and its homoeologous are actively involved in both yield and pathogen resistance.

Furthermore, the SNP (C/T) identified between the two parental lines determined an amino acidic change. Specifically, the amino acid substitution is located at position 260, at the beginning of the α helical subdomain of the AAA + module, and determines a leucine to phenylalanine switch L-F. Previous studies have reported single amino acid substitution in Rca proteins, and in some cases, they have been shown having a solid effect on the enzyme thermostability.

Scafaro et al. (2019) found that two mutant wheat Rca2b proteins showed 5–7 °C increases in thermo stability. Those mutants were found in warm adapted species and presented eight and 11 residue substitutions, respectively, and both including the mutation M159I. Also, Scafaro et al. (2019) reported that a single lysine residue in the C-terminal extension of Rca was responsible for ADP sensitivity decrease.

Degen et al. (2020) demonstrated that a single amino acid residue substitution (methionine/ isoleucine at position 159) in the wheat Rca2b isoform (Scafaro et al. 2016) extended the temperature optimum while maintaining the efficiency of Rubisco activation by Rca. It was supposed that this substitution altered the regulatory properties of Rca which was more thermostable, resulting in a more efficient activation of Rubisco. The occurrence of an isoleucine at position 159 in Rca2 might indicate an adaptation to warm environments, and some authors hypothesized that the ancestral monocots Rcas had a methionine residue at this position.

Further investigation will be carried out to determine its involvement in FHB resistance and whether also the single substitution we reported here might have similar effects on the Rca activity, as a thermal and regulatory switch that can be exploited to improve the efficiency and climate resilience of wheat carbon fixation, besides with.

Ultimately, the importance of targeting Rca in future breeding programs is also enhanced considering its pivotal role in photosynthetic processes, which will be adapting to always more environmental changes. The strong correlation and interaction between C and N metabolisms have been widely studied, and recent reports pointed their attention on how Rubisco and Rca might change their expression whether different N supplement were given to plants. Tetard-Jones et al. (2013), compare the effect of contrasting components (fertilization and crop protection regime) of organic and conventional cropping systems, and found out an up-regulation of both *GS2* and *Rca* when wheat plants were supplemented with mineral fertilizers, as also reported by other authors (Fortunato et al. 2019; Lacolla et al. 2019). Another study from Yousuf et al. (2017) analyzed the proteomic pattern in two wheat cultivars with contrasting

nitrogen efficiencies when both high temperature and low nitrogen stresses were combined. The authors found the Rca significantly increased in abundance under stressful conditions, suggesting its potential to regulate Rubisco activity.

Rca has been widely demonstrated to be a valuable candidate gene to enhance Rubisco activity and CO_2 assimilation under mutable environmental conditions, especially water and nutrient availability which are predicted to be occurring even more frequently due to climate change scenarios. So far, developing new traditional as well as innovative breeding programs, such as with specific genome editing experiment to induce new and useful amino acid substitutions, might be a promising strategy for the improvement of yield capacity and sustainability of crops under global warming.

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Data availability The datasets generated during and/or analysed during the current study are available from the corresponding author on reasonable request.

Declarations

Conflict of interest The authors have no relevant financial or non-financial interests to disclose.

References

Aoun M, Breiland M, Turner MK, Loladze A, Chao S, Xu S, Acevedo M (2016) Genome-wide association mapping of leaf rust response in a durum wheat worldwide germplasm collection. Plant Genome. https://doi.org/10.3835/plantgenome2016.01.0008

Appels R, Eversole K, Feuillet C, Keller B, Rogers J, Stein N, Pozniak CJ, Choulet F, Distelfeld A, Poland J et al (2018) International Wheat Genome Sequencing Consortium (IWGSC) Shifting the limits in wheat research and breeding through a fully annotated and anchored reference genome sequence. Science 361:6403

Beres BL, Rahmani E, Clarke JM, Grassini P, Pozniak CJ, Geddes CM, Porker KD, May WE, Ransom JK (2020) A systematic review of durum wheat: enhancing production systems by exploring genotype, environment, and management (G × E × M) Synergies. Front Plant Sci 11:568–657. https://doi.org/10.3389/fpls.2020.568657

Bhat JY, Thieulin-Pardo G, Hartl FU, Hayer-Hartl M (2017) Rubisco activases: AAA+ chaperones adapted to enzyme repair. Front Mol Biosci 4:20. https://doi.org/10.3389/fmolb.2017.00020

Borrill P, Ramirez-Gonzalez R, Uauy C (2016) expVIP: a customizable RNA-seq data analysis and visualization platform. Plant Physiol 170(4):2172–2186

Bracher A, Whitney SM, Hartl FU, Hayer-Hartl M (2017) Biogenesis and metabolic maintenance of Rubisco. Annu Rev Plant Biol 68:29–60

Bracher A, Spencer MW et al (2021) Biogenesis and metabolic maintenance of Rubisco. Annu Rev Plant Biol 68:29–60. https://doi.org/10.1146/annurev-arplant-043015-111633

Carmo-Silva AE, Gore MA, Andrade-Sanchez P, French AN, Hunsaker DJ, Salvucci ME (2012) Decreased CO2 availability and inactivation of Rubisco limit photosynthesis in cotton plants under heat and drought stress in the field. Environ Exp Bot 83:1–11. https://doi.org/10.1016/j.envexpbot.2012.04.001

Carmo-Silva E, Scales JC, Madgwick PJ, Parry MAJ (2015) Optimizing Rubisco and its regulation for greater resource use efficiency. Plant Cell Environ 38:1817–1832. https://doi.org/10.1111/pce.12425

Cassman KG, Grassini PA (2020) Global perspective on sustainable intensification research. Nat Sustain 3:262–268. https://doi.org/10.1038/s41893-020-0507-8

Chaves MM, Flexas J, Pinheiro C (2009) Photosynthesis under drought and salt stress: regulation mechanisms from whole plant to cell. Ann Bot 103(4):551–560

Chen L, Liao H (2017) Engineering crop nutrient efficiency for sustainable agriculture. J Integr Plant Biol 59(10):710–735

CRP-WHEAT (2016) Wheat Agri-Food Systems Proposal 2017–2022. Research Program on Wheat, (CGIAR).

WHEAT Full Proposal Draft 1.93. https://www.google.com/url?sa=t&rct=j&q=&esrc=s&source=web&cd=&cad=rja&uact=8&ved=2ahUKEwjekLTsr7v2AhUxQvEDHSqBAGQQFnoECAIQAQ&url=https%3A%2F%2Fcgspace.cgiar.org%2Fbitstream%2Fhandle%2F10947%2F4419%2F2.%2520WHEAT%2520-%2520CRP%2520and%2520FP%2520Narratives%2520Proposal%25202017-2022.pdf%3Fsequence%3D1%26isAllowed%3Dy&usg=AOvVaw3j9GFEyVlcOFtePbyKhIxm

Degen GE, Orr DJ, Carmo-Silva E (2020) Heat-induced changes in the abundance of wheat Rubisco activase isoforms. New Phytol 229(3):1298–1311

FAO. The Future of Food and Agriculture (2017) Trends and challenges. Food and Agriculture of the United Nations, Rome

Fortunato S, Nigro D, Paradiso A, Cucci G, Lacolla G, Trani R, Gadaleta A (2019) Nitrogen metabolism at tillering stage differently affects the grain yield and grain protein content in two Durum wheat cultivars. Diversity 11(10):186

Geddes J, Eudes F, Laroche A, Selinger LB (2008) Differential expression of proteins in response to the, interaction between the pathogen Fusarium graminearum and its host, Hordeum Vulgare. Proteomics 8(3):545–554

Giancaspro A, Giove SL, Zito D, Blanco A, Gadaleta A (2016) Mapping QTLs for Fusarium head blight resistance in an interspecific wheat population. Front Plant Sci 7:1381

Giancaspro A, Giove SL, Zacheo SA, Blanco A, Gadaleta A (2019) Genetic variation for protein content and yield-related traits in a durum population derived from an inter-specific cross between hexaploid and tetraploid wheat cultivars. Front Plant Sci 10:1509

International Grains Council [IGC] (2020) World Grain Statistics 2016, https://www.igc.int/en/default.aspx

Kosambi DD (1943) The estimation of map distances from recombination values. Ann Eugenic 12:172–175

Kosová K, Chrpová J, Šantrůček J, Hynek R, Štěrbová L, Vítámvás P, Bradová J, Prášil IT (2017) The effect of Fusarium culmorum infection and deoxynivalenol (DON) application on proteome response in barley cultivars Chevron and Pedant. J Proteom 169(3):112–124. https://doi.org/10.1016/j.jprot.2017.07.005

Kumar S, Stecher G, Li M, Knyaz C, Tamura K (2018) MEGA X: molecular evolutionary genetics analysis across computing platforms. Mol Biol Evol 35(6):1547–1549. https://doi.org/10.1093/molbev/msy096

Lacolla G, Fortunato S, Nigro D, de Pinto MC, Mastro MA, Caranfa D, Cucci G (2019) Effects of mineral and organic fertilization with the use of wet olive pomace on durum wheat performance. Int J Recycl Org Waste Agric 8(1):245–254

Maccaferri M, Harris NS, Twardziok SO, Pasam RK, Gundlach H, Spannagl M, Ormanbekova D, Lux T, Prade VM, Milner SG et al (2019) Durum wheat genome highlights past domestication signatures and future improvement targets. Nat Genet 51:885

Marone D, Del Olmo AI, Laidò G, Sillero JC, Emeran AA, Russo MA, Mastrangelo AM (2009) Genetic analysis of durable resistance against leaf rust in durum wheat. Mol Breed 24(1):25–39

Mathur S, Agrawal D, Jajoo A (2014) Photosynthesis: response to high temperature stress. J Photochem Photobiol B Biol 137:116–126

Ng J, Guo Z, Mueller-Cajar O (2020) Rubisco activase requires residues in the large subunit N terminus to remodel inhibited plant Rubisco. J Biol Chem 295(48):16427–16435

Nigro D, Blanco A, Anderson OD, Gadaleta A (2014a) Characterization of ferredoxin-dependent glutamine-oxoglutarate amidotransferase (Fd-GOGAT) genes and their relationship with grain protein content QTL in wheat. PLoS ONE 9(8):e103869. https://doi.org/10.1371/journal.pone.0103869

Nigro D, Giove SL, Fortunato S, Incerti O, Zacheo SA, Blanco A, Gadaleta A (2014b) Allelic variation of wheat flour allergens in a collection of wheat genotypes. J Chem 2014.

Nigro D, Fortunato S, Giove SL, Mangini G, Yacoubi I, Simeone R, Gadaleta A (2017) Allelic variants of glutamine synthetase and glutamate synthase genes in a collection of durum wheat and association with grain protein content. Diversity 9(4):52

OECD/FAO (2020) OECD-FAO Agricultural Outlook 2020–2029. OECD/FAO. OECD-FAO Agricultural Outlook 2020–2029. FAO, Rome/OECD Publishing Paris. https://doi.org/10.1787/1112c23b-en

Ooijen, J.W. (2006) JoinMap 4: Software for the Calculation of Genetic Linkage Maps in Experimental Populations. Kyazma B.V., Wageningen.

Parry MAJ, Madgwick PJ, Carvalho JFC, Andralojc PJ (2007) Prospects for increasing Photosynthesis by overcoming the limitations of Rubisco. J Agric Sci 145:31–43

Parry MAJ, Andralojc PJ, Scales JC, Salvucci ME, Carmo-Silva AE, Alonso H, Whitney SM (2013) Rubisco activity and regulation as targets for crop improvement. J Exp Bot 64(3):717–730

Perdomo JA (2015) Acclimation of photosynthesis to water deficit and high temperature: physiological and biochemical aspects. Ph.D. thesis, University of the Balearic Islands, Palma de Mallorca. Availabe from: https://www.google.com/url?sa=t&rct=j&q=&esrc=s&source=web&cd=&ved=2ahUKEwjzrvqe56byAhVYtqQKHULgAwEQFnoECAIQAQ&url=http%3A%2F%2Fibdigital.uib.es%2Fgreenstone%2Fsites%2Flocalsite%2Fcollect%2FtesisUIB%2Findex%2Fassoc%2FPerdomo_.dir%2FPerdomo_Lopez_JAlejandro.pdf&usg=AOvVaw17UVv4mXmh8RjAFLQdbhPC

Perdomo JA, Carmo-Silva E, Hermida-Carrera C, Flexas J, Galmés J (2016) Acclimation of biochemical and diffusive components of photosynthesis in rice, wheat and maize to heat and water deficit: implications for modeling photosynthesis. Front Plant Sci 7:1719. https://doi.org/10.3389/fpls.2016.01719

Perdomo JA, Buchner P, Carmo-Silva E (2021) The relative abundance of wheat Rubisco activase isoforms is post-transcriptionally regulated. Photosynth Res 148(1):47–56

Portis AR Jr (1995) The regulation of Rubisco by Rubisco activase. J Exp Bot 46:1285–1291

Prat N, Buerstmayr M, Steiner B, Robert O, Buerstmayr H (2014) Current knowledge on resistance to Fusarium head blight in tetraploid wheat. Mol Breed 34:1689–1699

Raven JA (2013) Rubisco: still the most abundant protein of Earth? New Phytol 198(1):1–3

Reynolds MP, Quilligan E, Aggarwal PK, Bansal KC, Cavalieri AJ, Chapman SC et al (2020) An integrated approach to maintaining cereal productivity under climate change. Glob Food Sec 8:9–18

Roy H (1989) Rubisco assembly: a model system for studying the mechanism of chaperonin action. Plant Cell 1:1035–1042

Ruan Y, Comeau A, Langevin F, Hucl P, Clarke JM, Brule-Babel A, Pozniak CJ (2012) Identification of novel QTL for resistance to Fusarium head blight in a tetraploid wheat population. Genome 55(12):853–864

Scafaro AP, Gallé A, Van Rie J, Carmo-Silva E, Salvucci ME, Atwell BJ (2016) Heat tolerance in a wild Oryza species is attributed to maintenance of Rubisco activation by a thermally stable Rubisco activase ortholog. New Phytol 211:899–911

Scafaro AP, Bautsoens N, den Boer B, Van Rie J, Gallé AA (2019a) conserved sequence from heat-adapted species improves Rubisco activase thermostability in wheat. Plant Physiol 181:43–54

Scafaro AP, De Vleesschauwer D, Bautsoens N, Hannah MA, den Boer B, Gallé A, van Rie J (2019b) A single point mutation in the C-terminal extension of wheat Rubisco activase dramatically reduces ADP inhibition via enhanced ATP binding affinity. J Biol Chem 294:17931–17940

Schmidt GW, Mishkind ML (1986) The transport of proteins into chloroplasts. Annu Rev Biochem 55:879–912

Shivhare D, Mueller-Cajar O (2017) In vitro characterization of thermostable CAM Rubisco activase reveals a Rubisco interacting surface loop. Plant Physiol 174:1505–1516

Singh J, Pandey P, James D, Chandrasekhar K, Achary VMM, Kaul T et al (2014) Enhancing C3 photosynthesis: an outlook on feasible interventions for crop improvement. Plant Biotechnol J 12:1217–1230. https://doi.org/10.1111/pbi.12246

Spreitzer RJ, Salvucci ME (2002) Rubisco: structure, regulatory interactions, and possibilities for a better enzyme. Annu Rev Plant Biol 53:449–475. https://doi.org/10.1146/annurev.arplant.53.100301.135233

Tamura K, Nei M (1993) Estimation of the number of nucleotide substitutions in the control region of mitochondrial DNA in humans and chimpanzees. Mol Biol Evol 10(3):512–526. https://doi.org/10.1093/oxfordjournals.molbev.a040023

Tétard-Jones C, Shotton PN, Rempelos L, Cooper J, Eyre M, Orr CH, Gatehouse AM (2013) Quantitative proteomics to study the response of wheat to contrasting fertilization regimes. Mol Breeding 31(2):379–393

Vile D, Pervent M, Belluau M, Vasseur F, Bresson J, Muller B et al (2012) Arabidopsis growth under prolonged high temperature and water deficit: independent or interactive effects? Plant Cell Environ 35:702–718. https://doi.org/10.1111/j.1365-3040.2011.02445.x

Whitney SM, Houtz RL, Alonso H (2011) Advancing our understanding and capacity to engineer nature's CO2-sequestering enzyme, Rubisco. Plant Physiol 155(1):27–35

York R (2016) Decarbonizing the energy supply may increase energy demand. Sociol Dev 2(3):265–272

Yousuf PY, Abd-Allah EF, Nauman M, Asif A, Hashem A, Alqarawi AA, Ahmad A (2017) Responsive proteins in wheat cultivars with contrasting nitrogen efficiencies under the combined stress of high temperature and low nitrogen. Genes 8(12):356

Zhou W, Eudes F, Laroche A (2006) Identification of differentially regulated proteins in response to a compatible interaction between the pathogen Fusarium graminearum and its host, Triticum Aestivum. Proteomics 6(16):4599–4609