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Transcriptional profiling of genes encoding GABA-transaminases in *Brassica napus* reveals their regulation by water deficit

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ABSTRACT

In plants, GABA (γ-aminobutyric acid) accumulates in response to a wide range of environmental stresses. The metabolism of this non-protein amino acid occurs in two distinct cellular compartments: it is synthesized in the cytosol and degraded in mitochondria. Although many studies have reported its involvement in development, the role of stress-induced GABA accumulation remains unclear. The effects of GABA accumulation have been examined in plants defective in GABA catabolism. In *Arabidopsis* mutants deficient for GABA transaminase (GABA-T), the first step in GABA degradation, it was shown to be involved in salt stress tolerance. Here, we investigated *GABA-T* genes in the Brassicaceae crop species *Brassica napus* at the molecular structure and transcriptional levels. We show that several copies of *GABA-T* are ubiquitously expressed in many organs of oilseed rape. Analysis of *BnaGABA-T* promoters using the GUS reporter gene found no difference in localization between *GABA-T* family members. Additionally, we show that their expression profiles are modified during development and in response to water stress depending on leaf rank. The role of *GABA-T* regulation in water stressed *B. napus* plants is discussed.

1. Introduction

GABA (γ-aminobutyric acid), a non-protein amino acid first identified in potato (Steward et al., 1949), is well known as an important neurotransmitter in animal nervous systems (Represa and Ben-Ari, 2005). In other eukaryotes and prokaryotes it is reported as a molecule involved in developmental regulation (Bouche et al., 2003b; Chevrot et al., 2006). Plants can use GABA as a source of carbon and nitrogen (N) for growth (Breitkreuz et al., 1999) and accumulate it in response to a wide range of environmental stresses including drought, oxygen deficiency, mechanical stimulation, low temperature and pathogen attack (Bown et al., 2006; Kinnersley and Turano, 2000). GABA was also recently described as a metabolite regulator of nitrate uptake in *Brassica napus* and *Arabidopsis thaliana* (Barbosa et al., 2010; Beuve et al., 2004) and of the expression of several *14-3-3* genes (Lancien and Roberts, 2006). Its involvement in developmental processes was also demonstrated by the aberrant phenotypes observed in rice and tobacco plants over-expressing enzymes from its biosynthesis pathway (Akama and Takaiwa, 2007; Baum et al., 1996). In plants, GABA is mainly synthesized by the decarboxylation of L-glutamate (Fig. 1), catalyzed by the cytosolic enzyme glutamate decarboxylase (GAD; EC 1.4.1.15) (Baum et al., 1996), and/or the catabolism of polyamine (Bouchereau et al., 1999). Two mitochondrial enzymes are involved in GABA degradation. GABA-transaminase (GABA-T; EC 2.6.1.19) catalyzes the first reaction which converts GABA to succinic semialdehyde (SSA) (Fig. 1) (Van Cauwenberghe et al., 2002). As glyoxylate and pyruvate are used as potential amino acceptors, leading to the production of either glycine or alanine, respectively (Fig. 1), it was suggested that there is an interaction between GABA metabolism and photorespiration (Clark et al., 2009a,b,b). SSA is in turn oxidized to succinate by succinic semialdehyde dehydrogenase (SSADH; EC 1.2.1.24) (Fig. 1), (Busch and Fromm, 1999). SSA can be also reduced to gamma-hydroxybutyrate (GHB) by SSA reductase (SSAR or gamma-hydroxybutyrate dehydrogenase; EC 1.1.1.61) (Fig. 1) (Breitkreuz et al., 2003) although, the cytosolic and plastidial SSAR isoforms, renamed GLYR1 and GLYR2, show higher substrate specificity for glyoxylate than SSA (Allan et al., 2008; Shelp et al., 2012b; Simpson et al., 2008). Shelp and co-authors hypothesize that GABA-T and

Abbreviation: GABA, γ-aminobutyric acid; GABA-T, γ-aminobutyric acid transaminase; GAD, glutamate decarboxylase; GDH, glutamate dehydrogenase; GHB, gamma-hydroxybutyrate; GLYR, glyoxylate reductase (SSA reductase); NRE, nitrogen remobilization efficiency; SSA, succinic semialdehyde; CDS, coding sequence; GSA, glutamate-γ-semialdehyde; qRT-PCR, quantitative reverse transcription PCR; TFAA, total free amino acids; WGD, whole genome duplication; WGT, whole genome triplication.

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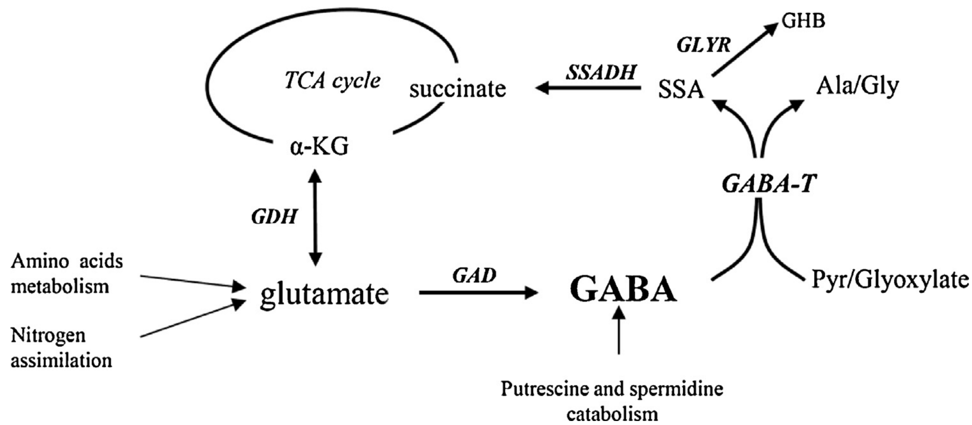

Fig. 1. Schematic representation of GABA metabolic pathways. Ala alanine; GABA, 4-aminobutyric acid; GABA-T, 4-aminobutyric acid transaminase; GAD, glutamate decarboxylase; GDH, glutamate dehydrogenase; GHB, gamma-hydroxybutyrate; GLYR, glyoxylate reductase (SSA reductase); Gly glycine; α-KG, alpha ketoglutarate; Pyr pyruvate; SSA, succinic semialdehyde; SSADH, succinic semialdehyde dehydrogenase.

GLYR could be involved in the rapid detoxification of glyoxylate and SSA produced in response to stress (Shelp et al., 2012a). The intriguing questions of the role of GABA and compartmentalization of its metabolism have led to significant interest in this metabolic pathway in the last few years, as shown by the high number of reports in *Arabidopsis*. Thus, studies of *gabp, pop2* and *ssadh* mutants deficient in mitochondrial GABA transport, GABA-T and SSADH, respectively, confirmed its major role in carbon metabolism and plant growth *via* its incorporation into the TCA cycle and then contribution to mitochondrial respiration, a pathway also known as the GABA shunt (Michaeli et al., 2011; Tuin and Shelp, 1996). *ssadh* mutants are dwarfed and have necrotic lesions (Bouche et al., 2003a) and pollen tube orientation and guidance are seriously affected in *pop2* mutants, resulting in sterility (Palanivelu et al., 2003). We have previously shown that GABA accumulation in GABA-T deficient lines (*pop2* mutants) in *A. thaliana* leads to elongation defects which inhibit primary root growth and this is associated with decreased expression of genes encoding secreted and cell-related proteins (Renault et al., 2011). Interestingly, studies of these *pop2* mutants also confirmed the involvement of GABA-T in salt stress tolerance (Renault et al., 2013, 2010), which was previously reported in *Nicotiana sylvestris* (Akçay et al., 2012). In tomato, RNAi suppression of GABA-T induces dwarfism and infertility associated with GABA accumulation (Koike et al., 2013). Our recent studies in *B. napus* seedlings demonstrated that inhibition of GABA-T activity affects root growth and induces metabolic disorders, especially in roots (Deleu et al., 2013). In the context of oilseed rape breeding, improvement of remobilization of nitrogen from vegetative to reproductive parts of the plant provides a promising lever for nitrogen use efficiency of this crop (Avice and Etienne, 2014). The particular role of glutamate metabolism in nitrogen remobilization is investigated (Orsel et al., 2014). As GABA is directly linked to the glutamate pathway, the role of GABA-T in the *B. napus* N management must be questioned.

The aim of the present study was to characterize the *GABA-T* gene family in *B. napus* both structurally and functionally at the molecular level with a specific focus on water stress. Although a single copy of the *GABA-T* gene is present in the *Arabidopsis* genome, several copies of *GABA-T* genes are expected in the genome of *B. napus*, according to its evolutionary history, as recently shown for other N-metabolism related gene families, i.e., glutamine synthetase (Orsel et al., 2014) and proline dehydrogenase (Faës et al., 2015). We thus identified the different *BnaGABA-T* gene copies from the *B. napus* genome sequence database (Chalhoub et al., 2014). The molecular structure and phylogeny of these copies were then established. Expression of all *BnaGABA-T* homeologues in different organs of flowering plants and in the leaves of water-stressed plants was also examined. GUS-promoter fusions were used to further explore expression patterns in different tissues. Our findings contribute to a better understanding of the regulation of GABA metabolism in oilseed rape plants, especially under water-stressed conditions, and give insight into possible adaptive roles of GABA catabolism in N and carbon recycling and utilization efficiency.

2. 2. Materials and methods

2.1. Brassica GABA-T *gene identification, cloning and sequencing*

The *Arabidopsis* GABA-T (*AT3G22200.1*) amino acid and nucleotide sequences were used as queries to identify *BnaGABA-T* genes in the *B. napus* cultivar Darmor-*bzh* genome (Chalhoub et al., 2014) using the BLAST algorithm (Altschul et al., 1990). The *B. napus GABA-T* gene sequences identified *in silico* were used to design oligonucleotide primers (Supplementary File 1) to amplify the *GABA-T* genes from the *B. napus* Tenor cultivar using high fidelity enzyme KOD Hot Start DNA polymerase (Novagen, Madison, WI, USA) according to the manufacturer's instructions. PCR products were cloned using the StrataClone PCR Cloning Kit (Agilent Technologies Inc., Santa Clara, CA, USA) and at least ten colonies per clone were sequenced (Genoscreen, Lille, France). Based on the gene sequences obtained, oligonucleotide primers were defined for amplifying, cloning and sequencing the corresponding coding sequences (CDS) from ATG to stop codon. Using BLAST, *BnaGABA-T* gene sequences were used to identify orthologs in the *B. rapa* accession Chiifu-401-42 draft genome in the BRAD database (http://brassicadb.org/brad) (Cheng et al., 2011) and in the *B. oleracea* variety capitata draft genome in the Bolbase database (http://ocri-genomics.org/bolbase) (Yu et al., 2013). All *in silico* sequence manipulations including primer design, alignments and contig assembly, were performed with CLC Main Workbench 6.9.1 software (Qiagen, Venlo, Netherlands).

2.2. Analysis of molecular phylogeny and evolution

Predicted Brassicaceae *GABA-T* gene sequences were retrieved from Phytozome (Goodstein et al., 2012). Sequences were aligned using MEGA6 software (Tamura et al., 2013) and the MUSCLE algorithm (Edgar, 2004). A phylogeny of different versions of the gene was reconstructed using the maximum likelihood method based on the best evolution model determined with MEGA6. The