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Flagellin Induced GABA-shunt improves Drought stress tolerance in *Brassica napus* L

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Abstract

Background High GABA levels and its conversion to succinate via the GABA shunt are known to be associated with abiotic and biotic stress tolerance in plants. The exact mode of action is still under debate and it is not yet clear whether GABA is a common component of the plant stress defense process or not. We hypothesized that if it is a common route for stress tolerance, activation of GABA-shunt by a biotic stressor might also function in increased abiotic stress tolerance. To test this, *Brassica napus* plants treated with Flagellin-22 (Flg-22) were exposed to drought stress and the differences in GABA levels along with GABA-shunt components (biosynthetic and catabolic enzyme activities) in the leaf and root samples were compared. In order to provide a better outlook, *MYC2*, *MPK6* and *ZAT12*, expression profiles were also analyzed since these genes were recently proposed to function in abiotic and biotic stress tolerance.

Results Briefly, we found that Flg treatment increased drought stress tolerance in *B. napus* via GABA-shunt and the MAPK cascade was involved while the onset was different between leaves and roots. Flg treatment promoted GABA biosynthesis with increased GABA content and GAD activity in the leaves. Better performance of the Flg treated plants under drought stress might be dependent on the activation of GABA-shunt which provides succinate to TCA since GABA-T and SSADH activities were highly induced in the leaves and roots. In the transcript analysis, Flg + drought stressed groups had higher *MYC2* transcript abundances correlated well with the GABA content and GABA-shunt while, *MPK6* expression was induced only in the roots of the Flg + drought stressed groups. *ZAT12* was also induced both in leaves and roots as a result of Flg-22 treatment. However, correlation with GABA and GABA-shunt could be proposed only in Flg + drought stressed group.

Conclusion We provided solid data on how GABA-shunt and Fgl-22 are interacting against abiotic stress in leaf and root tissues. Fgl-22 induced ETI activated GABA-shunt with a plausible cross talk between *MYC2* and *ZAT12* transcription factors for drought stress tolerance in *B. napus*.

Keywords Effector molecule, Flg-22, GABA, ETI, Proline, MAPK

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Introduction

Plants are exposed to a wide range of abiotic (drought, heat, freezing, radiation, salt and heavy metal) and biotic (herbivore, pathogen, fungus, virus and parasite) stresses that negatively affect growth and development leading to significant decline in the yield and productivity [1]. Common routes, pathways and signaling networks are promising candidates for accommodating the impact of different environmental stressors therefore, the use of priming and pretreatments with defense activating molecules have long been studied in order to improve stress tolerance [1]. In the case of biotic stress tolerance, several components of plant immunity related to perception, recognition and defense are well defined. However, we still lack the exact mode of action in these processes [2, 3].

The non-protein amino acid, gamma amino butyric acid (GABA) is among the intriguing molecules involved in stress responses [4]. GABA biosynthesis takes place through the conversion of glutamate to GABA by the activity of glutamate decarboxylase (GAD). The follow-up steps of the GABA-shunt is the transport of GABA to the mitochondria, where it is deaminated to succinic semialdehyde (SSA) via GABA-transaminase (GABA-T) activity and the oxidation of SSA to succinate by succinic semialdehyde dehydrogenase (SSADH) activity which provides a by-pass to TCA cycle [4–7]. As indicated by Seifikalhor et al. [4], GABA might function in adaptation of plants to abiotic stress by altering their physiological and biochemical processes however, the exact mode of action including signaling is yet to be defined. Under stress conditions, increased Ca^{2+} levels activate GAD through the Ca^{2+}/calmodulin interaction which is usually associated with increased GABA levels. However, GABA accumulation also occurs as a result of an inefficient GABA-shunt as GABA-T function is sensitive to induced oxidative stress which reduces the flow of GABA through the GABA-shunt [8].

GABA levels increase in response to biotic stress in plants and the first evidence came from studies on soybean plants infested by leaf curler (*Choristoneura rosaceana*) larvae [9]. Species and developmental differences, the degree and duration of the stress also influences this interaction since there is a discrepancy in the literature for GABA being beneficial and damaging under biotic stress conditions. When plants such as *Phaseolus vulgaris* L., *Solanum lycopersicon* L., soybean and tobacco recognize the presence of pathogens GABA accumulation is triggered and is usually associated with increased GAD activity [10]. On the other hand, low levels of GABA might serve as nutrient while, elevated levels can prevent the colonization and development of bacteria. In infected tomato plants *Pseudomonas syringae* DC3000 used apoplastic GABA when aspartate and glutamate sources in the nutrient media was scarce [11]. On the other hand, GABA-shunt functions as a by-pass to the TCA for providing succinate which becomes more remarkable under stress conditions. It was reported that, to maintain the different energy demands of shoots and roots, the TCA cycle and amino acid metabolism related enzymes were altered without changing the components of the electron transport chain under various stress conditions [12, 13]. Accordingly, GABA accumulation can increase the host's resistance to fungal pathogens by maintaining the TCA cycle and reducing oxidative damage [14].

GABA plays a critical role in both pattern triggered immunity (PTI) and effector triggered immunity (ETI) along with increased GAD activity. However, over-accumulation of GABA leads to impaired growth and development [15]. In a previous study, the ETI-inducing protein-harpin, activated defense responses through the GABA-shunt without inducing H_2O_2 accumulation [16]. Recently, chemotaxis of plant pathogens for infection was reported to be associated with GABA sensing [17]. Since GABA plays an interactive role in plant and plant pathogens inducing general defense responses by exogenous treatments of these effector molecules may be important not only in terms of biotic stresses but also in coping with other stresses. Flagellin-22 (Flg-22) is a well-known bacterial effector molecule with highly conserved 22 amino acid long peptide chain in the N-terminal region of bacterial flagellin. It promotes immune reactions via hypersensitive response in *S. lycopersicum*, *S. tuberosum*, *A. thaliana* and *Saccharina japonica* and not much is known how it interacts with GABA-shunt [18].

In the light of these knowledge, we hypothesized that activating general defense by an effector molecule might increase abiotic stress tolerance via GABA-shunt. To assess this, we treated *Brassica napus* plants with the bacterial effector, Flg-22 and then subjected them to PEG-induced drought stress. We assayed GDH and GAD enzyme activities related to GABA biosynthesis, and GABA-T and SSADH enzyme activities related to GABA catabolism in the leaf and root samples taken on the 1st, 3rd and 7th days after the drought stress. We also analyzed the transcript profiles of *MYC2*, *MPK6* and *ZAT12*, which function in common defense routes in abiotic and biotic stress responses. According to our results in relation to the responses of the solely drought stressed and Flg+drought stressed plants, we found that Flg-22 regulates tolerance to drought stress by activating the GABA-shunt.

Materials and methods

Plant growth and treatments

Rapeseed (*Brassica napus* L.) seeds were sterilized with 10% hypochlorite solution for 10 min and washed with

deionized water (dH_2O) three times. The seeds were kept in sterile dH_2O at dark overnight and then sown on sterilized sponges. After germination they were transferred to hydroponic culture boxes containing ½ strength Hoagland nutrient solution. Plants were grown under controlled conditions with a light intensity of 350 µmolm^{-2} s^{-1}, a photoperiod of 16 h light / 8 h dark, a temperature of 25° C and a humidity rate of 75–80%. When plants were 40 days old, equal numbers of plants were divided into four groups as; control, effector treated (Flg), drought stressed and drought stressed after effector treatment (Flg+Drought). For effector treatment 1µM Flg-22 was applied by pulverization on the leaves (Fig. 1). Drought stress was performed by adding 10% Polyethylene glycol (PEG) 6000 which was equivalent to an osmotic potential of −0.41 MPa to the Hoagland nutrient solution. In the Flg+Drought stressed group; drought stress treatment started 24 h after the effector treatment. Leaf and root samples were taken on the first 12 h and 1st, 3rd, and 7th days of the treatments. Analysis with fresh samples was done immediately on the sampling days and for other analysis samples were frozen in liquid nitrogen and stored at -20 °C until the analysis. The experiments were repeated in two independent series with random sampling ($n=6$).

Relative Growth Rate (RGR) Relative Water Content (RWC) Chlorophyll Fluorescence (Fv/Fm) and Osmotic Potential Measurements

Differences in the growth of control, Flg-treated, Drought and Flg+Drought stressed plants were compared by their RGR according to Hunt et al. [19] by measuring leaf and root fresh (FW) and dry weights (DW). RWC of the leaves was measured according to Smart and Bingham [20]. FW was recorded immediately after sampling and for turgid weights (TW) leaves were submerged in dH_2O for 5 h at room temperature. TW of the leaves were measured and then dried at 70°C for 72 h for determination of DW. RWC was calculated according to formula; $RWC\% = [(FW-DW)/(TW-DW)] \times 100$. Leaf chlorophyll fluorescence was measured using the Plant Efficiency Analyser (Hansatech). Leaves were kept in the dark for 30 min and basal chlorophyll fluorescence (F0), maximum fluorescence excitation (Fm), variable fluorescence (Fv) were measured for the determination of *Fv/Fm* rates. The osmotic potential of leaf samples was measured using WESCOR Vapro Vapor Pressure Osmometer 5520.

Determination of GABA content

GABA content of the leaf and root samples was determined by high performance liquid chromatography (HPLC), Shimadzu-VP101, Japan. GABA extraction was performed according to Baum et al. [21] and HPLC analysis was done according to Bor et al. [22]. Leaf and root samples (0.5 g) were crushed into powder in liquid nitrogen and then homogenized with 1 mL of water: chloroform: methanol (3:5:12 v/v/v) solution. Samples were then centrifuged at 10.000 rpm and 4 °C for 2 min and supernatants were transferred to new tubes and dried. The dried samples were first dissolved in dH_2O and then the derivatization process was carried out by adding borax buffer (pH 8.0) and 2-Hydroxynaphthaldehyde (0.3% w/v dissolved in methanol). The mixture was then incubated at 80 °C for 20 min and cooled to room temperature. The derivatized samples were diluted with methanol and filtered through 0.45 µm sample filters. In HPLC analysis isocratic elution was used with methanol: water (62:38 v/v) mobile phase at a flow rate of 0.5 mL min^{-1} for 20 min. Samples (20 µL) were loaded on

Fig. 1 *B. napus* plants (40-days old) the day after Flg-22 treatment. Plants were grown in hydroponic culture boxes containing ½ strength Hoagland nutrient solution and 1µM Flg-22 was pulverized on to the leaves

to a Supelco LC18 (250×4.6 mm, 2. 5 µm) column and UV detection was performed at 330 nm. GABA contents (µmol g FW^{-1}) of the samples were quantified by using a standard curve ($n=6$).

Determination of lipid peroxidation and proline contents

Lipid peroxidation levels were determined as malondialdehyde (MDA) contents according to Madhava Rao and Sresty [23]. Fresh leaf and root samples (1 g) were homogenized in 0.1% trichloroacetic acid (TCA) and centrifuged at 10.000 g at 4 °C for 5 min. Supernatants were transferred to a new tube and 0.5% thiobarbituric acid (TBA) in 20% TCA was added to the supernatant. The mixture was incubated at 95 °C for 30 min and then cooled in an ice bath. Then samples were centrifuged at 10,000 g at 4 °C for 15 min. The absorbance values of the samples were measured at 532 and 600 nm. MDA content of the samples was calculated using extinction coefficient of 155 mM^{-1} cm^{-1} and expressed as nmol MDA g FW^{-1}. Determination of proline contents of the leaf and root samples were performed according to Quamar and Kumar [24]. The samples (0.1 g) were homogenized with 1% sulphosalicylic acid (SSA). The extract was centrifuged at 12.000 rpm for at +4℃ for 10 min. Supernatants were taken and 1.25% ninhydrin solution was added before incubation at 95 ℃ for 30 min. Samples were then cooled and pipetted into the plate-wells. The absorbances were measured at 520 nm with Thermofisher Multiscan Go plate-reader. Proline contents (µmol g FW^{-1}) of the samples were quantified by using a standard curve ($n=6$).

Enzyme assays

Leaf (0.5 g) and root samples (1 g) were macerated in liquid nitrogen and two different extraction buffers were used for enzyme assays. For protein content, GAD and GABA-T enzyme activity determination samples were extracted in buffer containing 100 mM Tris-HCl (pH 8.0), 1.5 mM dithiothreitol (DTT), 5 mM ethylene diamine tetra acetic acid (EDTA), 0.5 mM phenylmethylsulfonyl fluoride (PMSF), 10% glycerol and 1% polyvinylpyrrolidone (PVP). Protein content was assayed according to the Bradford [25] method using bovine serum albumin as the standard. For GDH and SSADH enzyme activity determination, samples were homogenized in buffer containing 5 mM EDTA, 0.1 mM Tris-HCl (pH 7.8), 10 mM dithiothreitol (DTT), 1 mM pyridoxal-5-phosphate hydrate and 1% PVPP. After the extraction samples were centrifuged at 10,000 g at 4°C for 40 min and the supernatants were transferred and used for enzyme analysis. Enzyme analysis was performed with the Shimadzu UV-1600 (Shimadzu, Japan) spectrophotometer. GDH enzyme activity was done according to Akhiro et al. [26]. The reaction mixture contained 50 mM $(NH_4)_2SO_4$, 13 mM α-ketoglutarate, 0.25 mM NADPH and 1 mM $CaCl_2$ in 100 mM Tris-HCl buffer (pH 8.0). After addition of the sample to the reaction mixture the change in the absorbance was determined and recorded at 340 nm. GAD enzyme activity was determined according to Bartyzel et al. [27] with slight modifications. For the decarboxylation process, a reaction mixture containing 20 µM pyridoxal phosphate (PLP) and 50 mM (pH 5.8) K-phosphate buffer was prepared. Samples were first incubated with the glutamate-free reaction mixture at 30 °C for 10 min, and then the reaction was initiated by adding 3 mM L-glutamate. After one hour, the reaction was stopped by adding 0.5 M HCl to the tubes. Samples were centrifuged at 12.500 rpm for 10 min. For derivatization, ninhydrin was added to the mixture at a ratio of 1:5 and then incubated at 80 °C for 10 min. The absorbance was measured at 570 nm and GAD activity was calculated using a GABA standard curve. Determination of GABA-T activity was performed as pyruvate dependent GABA-T towards alanine/SSA direction according to Miyashita and Good [28] and Jalil et al. [29] with slight modifications. In the first step, samples were mixed with a reaction mixture containing 50 mM Tris-HCl (pH 8.0), 0.75 mM EDTA, 1.5 mM DTT, 0.1 mM pyridoxal 5-phosphate, 10% glycerol, 16 mM GABA and 4 mM pyruvic acid and kept at 30°C for 1 h. The reaction was stopped by incubating the mixture at 97°C for 7 min. In the second step; for the alanine dehydrogenase assay, samples from the previous step was mixed with a reaction mixture containing 50 mM sodium carbonate buffer (pH 10), 1 mM β-NAD, and 0.02 units of *Bacillus subtilis* AlaDH (Sigma-Aldrich) and the absorbance difference was measured at 340 nm for 5 min. This second step was repeated using alanine standards to produce a standard curve. GABA-T activity was expressed as specific activity (unit mg $protein^{-1}$); where one unit corresponded to µmol NADH produced during alanine conversion per minute. SSADH enzyme activity was assayed according to Busch and Fromm [30]. The reaction mixture contained 0.1mM succinic semialdehyde (SSA), 0.5mM NAD, and Na-phosphate buffer (pH 9.0). After the addition of the sample to the reaction mixture, the differences in the absorbance values were measured at 340 nm for 2 min. SSADH activity was expressed as specific activity (unit mg $protein^{-1}$); where one unit corresponded to µmol NADH produced during the conversion of SSA per minute.

qPCR analysis

The differences in the transcript abundances of *MYC2*, *MPK6* and *ZAT12* encoding genes were analyzed by real-time PCR and normalized to the reference gene *ACTIN*. Total RNA from the leaf and root samples (100 mg) of control, Flg-treated, drought stressed and Flg+drought stressed plants was extracted by using the Norgen Plant/Fungi RNA Purification Kit, (Canada) according to the

manufacturer's instructions. Before cDNA synthesis, the quality and integrity of RNAs were verified on EtBr gels and quantified by BioPhotometer UV/Vis Spectrophotometer (Eppendorf). RNA from the samples (10 µg) was used for cDNA synthesis by Xpert cDNA Synthesis Kit (Grisp Research Solutions) and cDNA diluted 10-fold from each sample was used for qPCR analysis. Gene-specific primers for *MYC2*, *MPK6*, *ZAT12* and *Actin* were designed by Primer-Blast (http://www.ncbi.nlm.nih.gov/tools/primer-blast/) and their sequences presented in Table 1. qPCR reactions were performed using the Applied Biosystems (ABI) Step One Plus Real time PCR system with the Roche FastStart Essential DNA SYBR Green PCR master mix. The total volume of the reaction mixture was 10 µL (2 µL of cDNA, 8 µL PCR master mix). Reactions were initiated by the activation of the enzyme at 95 °C for 10 min, followed by 40 cycles of: 95 °C for 15 s, 60 °C for 1 min and final extension at 72 °C for 1 min. The resulting Ct values were used to calculate the relative transcript abundance according to the standard curve method.

Statistical analysis

All analyses were done on a completely randomized design and the data obtained were subjected to one-way analysis of variance (ANOVA). The mean differences were compared by the least standard difference (LSD) test between the control and Flg-treated, drought-stressed and Flg+drought stressed groups. Each data point was the mean of six replicates ($n=6$) except that of qPCR values ($n=3$). Comparisons with P values <0.05 and <0.01 were considered significantly different. In all the figures the bars represent the standard errors of the means.

Results

Flg treatment alleviated the negative impact of drought on growth and physiological parameters

In general, *B.napus* L. plants treated with Flg before drought stress application performed better as compared to drought only ones (Table 1). RGR values were not different between any treatment group on the 1st and 3rd days while drought stress decreased RGR significantly on the 7th day. The *Fv/Fm* values did not show any statistical differences in the control and treatment groups on 1st and 3rd days however, drought stressed plants had the lowest *Fv/Fm* value on the 7th day. The fact that photosynthetic efficiency did not change in the Flg treated plants may also indicate that Flg alone did not induce any stress effects. RWC is an important parameter related to the leaf water status and did not change between the control and Flg treated groups. Drought and Flg+drought stressed groups had lower RWC values, with the former was lowest on days 3 and 7 (Table 1). Osmotic potential values were higher in drought and Flg+drought treated groups compared to the control and Flg treated groups. The drought stressed groups either with or without Flg had similar values on the 1st and 3rd days while drought stressed group had the highest leaf osmotic potential on 7th day (Table 1).

The effects of drought stress were also reduced by Flg-treatment in terms of lipid peroxidation and proline content

Lipid peroxidation is an indicator of stress that is induced by oxidative stress leading to impairment in the membrane integrity. The lipid peroxidation levels were higher in the leaves of Flg-treated, drought stressed and Flg+drought stressed groups compared to the control group. Flg-treated and Flg+drought stressed groups had similar levels while the drought only stressed groups

Table 1 Relative growth rate (RGR), relative water content (RWC), osmotic potential and photosynthetic efficiency (*Fv/Fm*) in *B. Napus* plants treated with Flg22, drought and Flg22+drought for 7 days. Means ± SD of $n=6$ plants are presented. Letters near the mean values indicate significant differences at $p^{<}$ 0.05 and the ones with a common letter are not significantly different

		RGR	RWC (%)	Osmotic Potential (mmol kg^{-1})	Fv/Fm
D1	**Control**	$0.20^{a} \pm 0.009$	$89.38^{a} \pm 1.34$	$283.5^{c} \pm 4.89$	$0.865^{a} \pm 0.025$
	Flg	$0.22^{a} \pm 0.001$	$86.00^{a} \pm 5.90$	$383.5^{b} \pm 5.45$	$0.858^{a} \pm 0.003$
	Drought	$0.20^{a} \pm 0.003$	$82.10^{b} \pm 1.90$	$528.7^{a} \pm 3.30$	$0.845^{a} \pm 0.002$
	Flg + Drought	$0.19^{a} \pm 0.005$	$81.97^{b} \pm 1.20$	$531.1^{a} \pm 1.67$	$0.853^{a} \pm 0.003$
D3	**Control**	$0.22^{a} \pm 0.002$	$84.30^{b} \pm 3.70$	$316.5^{b} \pm 1.00$	$0.864^{a} \pm 0.007$
	Flg	$0.20^{a} \pm 0.007$	$87.25^{a} \pm 3.08$	$317.3^{b} \pm 2.92$	$0.858^{a} \pm 0.009$
	Drought	$0.21^{a} \pm 0.001$	$79.93^{c} \pm 2.09$	$510.3^{a} \pm 2.39$	$0.850^{a} \pm 0.006$
	Flg + Drought	$0.19^{a} \pm 0.001$	$83.65^{b} \pm 2.10$	$510.9^{a} \pm 4.87$	$0.856^{a} \pm 0.003$
D7	**Control**	$0.25^{a} \pm 0.008$	$86.05^{a} \pm 5.17$	$318.5^{d} \pm 9.60$	$0.859^{a} \pm 0.015$
	Flg	$0.23^{a} \pm 0.004$	$85.02^{a} \pm 5.52$	$340.2^{c} \pm 5.87$	$0.850^{a} \pm 0.004$
	Drought	$0.16^{b} \pm 0.003$	$79.05^{c} \pm 3.50$	$551.4^{a} \pm 2.91$	$0.820^{b} \pm 0.006$
	Flg + Drought	$0.24^{a} \pm 0.002$	$82.99^{b} \pm 1.80$	$491.3^{b} \pm 3.76$	$0.856^{a} \pm 0.005$

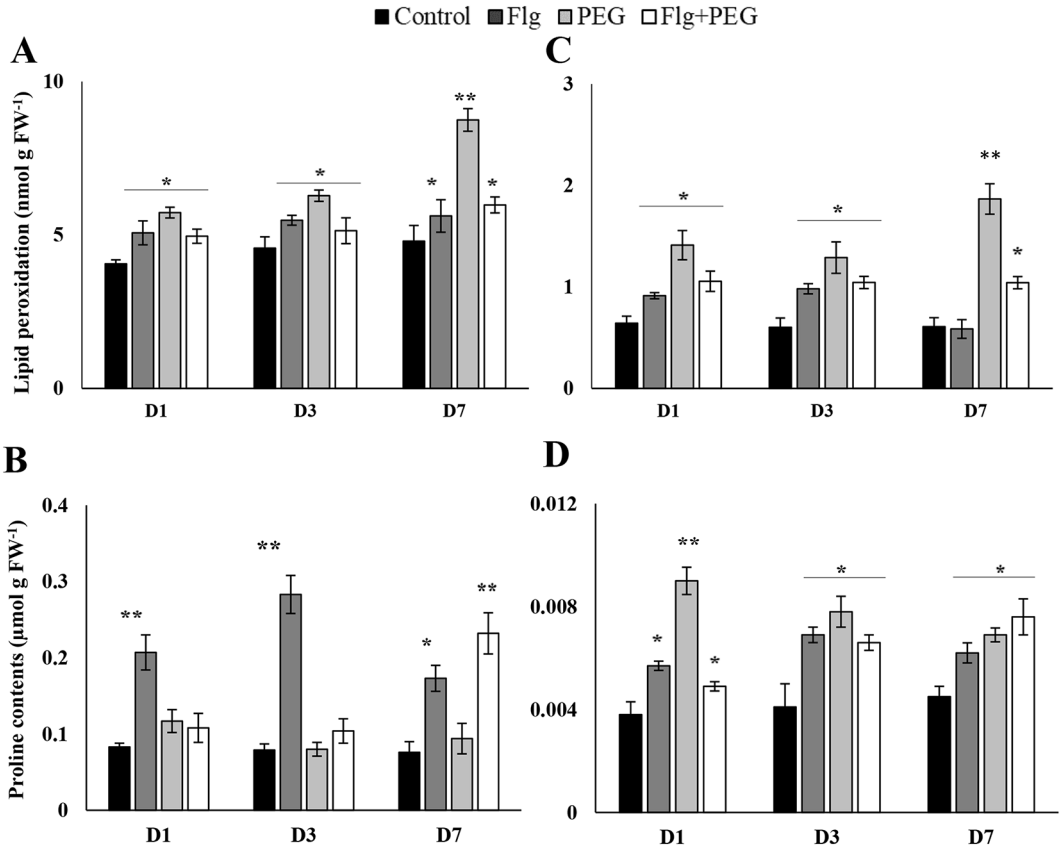

Fig. 2 Lipid peroxidation levels (nmol g FW-1) (**A** and **C**) and proline content (µmol g FW-1) (**B** and **D**) in the leaves and roots of B. napus control, Flg-treated, drought stressed and Flg+drought stressed plants for 7 days. Means ±SE of n=6 plants are presented and values with asterisks indicate significant differences at *p< 0.05 and **p< 0.01 as compared to the control group values

had the highest lipid peroxidation (Fig. 2A and C). The highest level of lipid peroxidation was recorded in the drought stressed group on the 7th day which was almost 2-folds higher than the control (Fig. 2A). Parallel to leaf lipid peroxidation results, in the root samples Flg-treated and Flg+drought stressed groups had similar levels and, the drought only groups had the highest levels (Fig. 2C). Accordingly, the highest lipid peroxidation level was recorded in the drought only group on the 7th day at almost 3-fold higher than the control group. Although, Flg-treated groups had higher lipid peroxidation on the 1st and 3rd days, it was similar to the control group's level on the 7th day.

The differences in the leaf proline contents in all groups were examined and the Flg-treated groups had higher values compared to the control groups (Fig. 2B and D). In the Flg-treated groups, proline increased approximately 2- and 3-fold on the 1st and 3rd days, respectively. Although non-significant increase was detected in the drought only group, the highest proline content was found in the Flg+drought stressed group on the 7th day (Fig. 2B). Root proline contents increased in Flg-treated, drought and Flg+drought stressed groups (Fig. 2D). The highest levels were recorded in the drought only groups at almost 2-fold higher than that of control group on the 1st day. In the Flg-treated and Flg+drought stressed groups proline content was also higher than the control levels with slight differences from the drought only ones.

GABA and GABA-shunt induced by flg treatment promoted drought stress tolerance

GDH enzyme is the main enzyme that provides glutamate to the free amino acid pool and is essential for GABA biosynthesis via GAD. Leaf GDH activity was not significantly different in any group on the first three days but was its highest level in Flg+drought group on the 7th day (Fig. 3A). Root GDH activity was higher in both Flg-treated groups, with the highest activity in Flg+drought stress group on the 3rd day (Fig. 3B). GAD is the most important enzyme of GABA biosynthesis and differences in the GAD activity were examined in *Brassica napus* L. root and leaf samples. In the leaves, generally GAD activity did not change in control groups while in Flg-treated, drought only and Flg+drought stressed groups its activity was higher (Fig. 3A). Drought with or without the Flg-treatment induced GAD activity on the 1st day however,

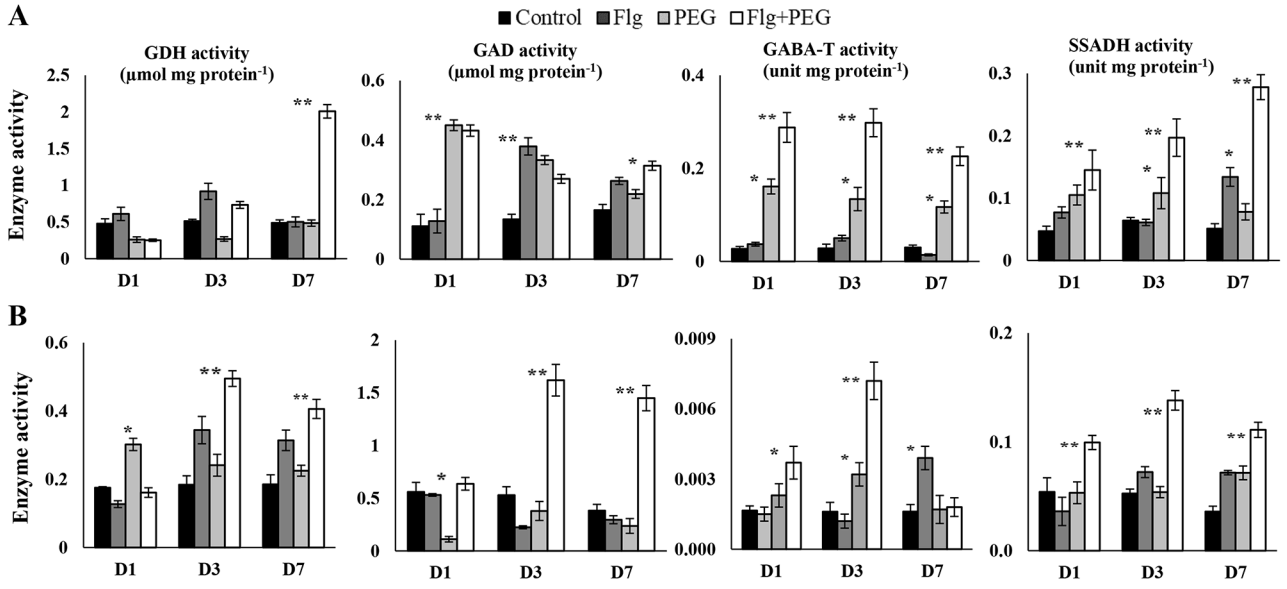

Fig. 3 GDH (µmol mg protein^{-1}), GAD (µmol mg protein^{-1}), SSADH (unit mg protein^{-1}) and GABA-T (unit mg protein^{-1}) enzyme activities in the leaves (**A**) and roots (**B**) of control, Flg-treated, drought stressed and Flg + drought stressed *B. napus* plants. Means ± SE of $n = 6$ plants are presented and values with asterisks indicate significant differences at *p< 0.05 and **p< 0.01 as compared to the control group values

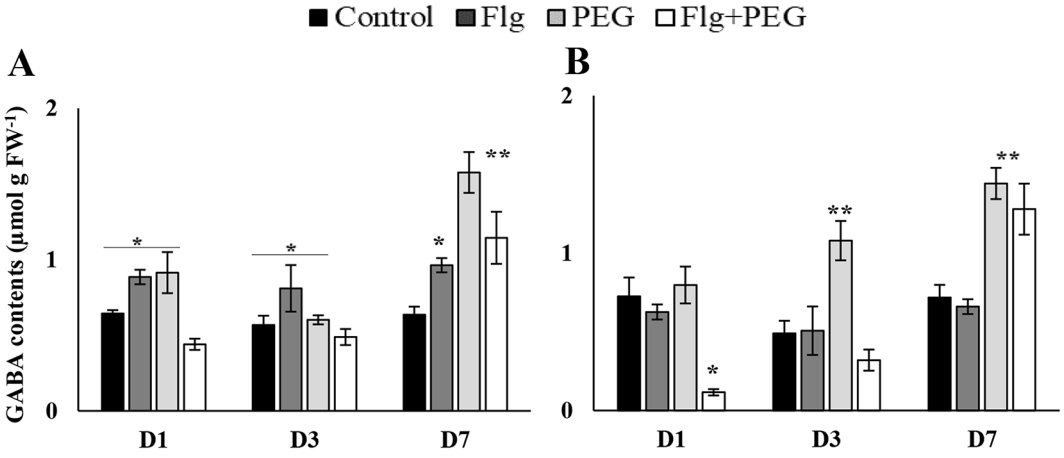

Fig. 4 GABA contents (µmol g FW-1) in the leaves (**A**) and roots (**B**) of B. napus control, Flg-treated, drought stressed and Flg+drought stressed plants for 7 days. Means ±SE of n=6 plants are presented and values with asterisks indicate significant differences at *p< 0.05 and **p< 0.01 as compared to the control group values

in the following days Flg + drought stressed plants had the highest GAD activity. GAD activity in drought only plants decreased to control levels on the 7th day. In root samples, GAD activity was significantly higher in the Flg + drought stressed group on the 3rd and 7th days while, it did not change in the other groups except in the drought only group on the 1st day (Fig. 3B). The lowest GAD activity was found in this group and it increased to control levels in the following days. Differences in the GABA contents of leaf and root samples were also examined (Fig. 4A and B). GABA content showed significant differences in the control and treatment groups in the first seven days. GABA levels increased in the Flg treated and drought only groups on the first day while, the highest GABA content was recorded in drought only leaves on the 7th day (Fig. 4A). In the root samples, Flg treated groups had similar GABA levels to that of control groups through the experimental period (Fig. 4B). GABA content increased in drought only roots which was significantly higher on the 3rd day. The lowest GABA content was recorded on the 1st day in Flg + drought stressed group while it increased to the level of drought only group on the 7th day which was more than 5-fold higher than on the 1st day. In leaves, along with the difference in the GABA content and the increased GAD activity, we can predict that Flg treatment promoted GABA biosynthesis

and that constitutive GDH activity was adequate to provide glutamate for GAD.

In order to understand whether the differences in GABA levels were related to the induction of GABA biosynthesis or to the inhibition of GABA catabolism, we also assayed the activities of two GABA-shunt enzymes: GABA-T and SSADH in both leaves and roots (Fig. 3A and B). Control and Flg treated leaves had similar levels while, drought only and Flg+drought stressed groups had significantly higher GABA-T activities. However, by far the most significant response GABA-T activity was seen in the Flg+drought stressed leaves at all sample times being 10-fold higher than the control on the 3rd day. (Fig. 3A). GABA-T activities exhibited a similar trend in the root samples on the 3rd day. GABA-T activities were only significantly different in control and Flg-treated groups on the 7th day (Fig. 3B). Along with the induced GABA-T activity, the induction of SSADH activity was also examined in Flg+drought stressed leaf and root samples (Fig. 3A and B). In leaves, almost 6-fold higher SSADH activity was found in this group as compared to that of control group on the 7th day. SSADH activity was also high in the drought only group on the 3rd day but not as high as the Flg+drought stressed group's level (Fig. 3A). In the roots, SSADH activity was higher in Flg+drought stressed groups as compared to all other groups over 7 days. The highest activity was on the 3rd day which was 4 and 2.5-fold higher than in the control and drought only groups' values respectively (Fig. 3B). The better performance of the Flg treated plants under drought stress might be dependent on GABA-shunt activation which provides succinate to TCA as GABA-T and SSADH activities were highly induced in the leaves and roots of these groups.

Flg-22 induced transcription of MYC2 MPK6 and ZAT12 might modulate GABA-shunt for drought stress tolerance

The transcript profiles of *MYC2*, *MPK6* and *ZAT12* were analyzed in the leaves and roots of drought stressed plants with or without Flg treatment and compared to that of control group values over 12 h (Figs. 5A-B and 6A-B). There was no significant difference in the transcript abundance of the *MYC2* in the leaves of the Flg-treated and control groups until it increased in the Flg-treated leaves in the 12 h. However, in the drought stressed groups with or without the Flg treatment *MYC2* transcript abundance was highly induced within the first three hours and particularly high in the drought only group after 6 h (Fig. 5A). Following the decline at 12th h, Flg+drought stressed groups exhibited higher *MYC2* transcript levels on the 1st and 3rd days as compared to that of control levels. Nevertheless, this induction was at the same level on day 3 and lower on day 7 as compared to the drought only group. *MYC2* transcript abundance exhibited a different trend in the roots (Fig. 6A-B). Flg-treated groups had the highest rate of induction within the first 12 h and on the first day while the drought only groups had similar levels both in the short and long term. The highest *MYC2* transcript levels were detected in Flg+drought stressed groups in the long term which was almost 10-fold higher than in the control (Fig. 6B).

The *MPK6* transcript abundance was highest of all groups in the Flg-treated leaves within the first twelve hours (Fig. 5A). Moreover, the drought only group had the same transcript abundance as the Flg-treated group after 12th h. And this was almost 3.5-folds higher than in the control group. In the long term, *MPK6* transcript levels were also high in the Flg-treated leaves however it declined to control levels on the 7th day. A slight induction in the *MPK6* transcript level was seen in the Flg+drought stressed group which was significantly higher than the control and drought only groups on the 7th day (Fig. 5B). In the roots, *MPK6* transcript levels were highly induced by Flg treatment in the 1st and 12th h and, the highest level was almost 4-fold higher than the control levels (Fig. 6A). In the long term, *MPK6* transcript abundances were not significantly different in all groups on the 1st day. However, the highest *MPK6* transcript levels were seen in the Flg+drought stressed group on the 7th day (Fig. 6B).

In the drought only groups *ZAT12* transcript abundances was low in the first three hours but it was highly induced in the 6th and 12th h (Figs. 5A-B and 6A-B); and was 4-fold higher than the control at 6 h. Flg-treated groups also had induced *ZAT12* transcript levels in the 6th and 12th h (Fig. 5A). In the long term, *ZAT12* transcript abundances were highest of all in the Flg+drought stressed groups and it was 2.5-folds higher as compared to control group on the 7th day (Fig. 5B). In the roots, Flg treatment highly induced *ZAT12* transcript levels in the first three hours compared to the control. The transcript levels increased significantly again at 12 h (Fig. 6A). There was a clear discrepancy in the induction of the *ZAT12* transcript levels between the short and long term. The Flg+drought stressed group had the lowest *ZAT12* transcript levels in the short term, but in the long term it was siginificantly induced up to more than 5-fold higher than that of the control groups (Fig. 6B). The *ZAT12* transcript abundances were also high in Flg-treated and drought only groups which had similar levels. These levels were almost 2.5-fold higher as compared to the control groups.

Discussion

GABA is an amino acid found in all organisms and it functions in signaling, growth and development, regulation of cell pH, maintenance of C: N balance and protection against environmental stresses in plants [31, 32]. As a common stress responsive molecule, communication

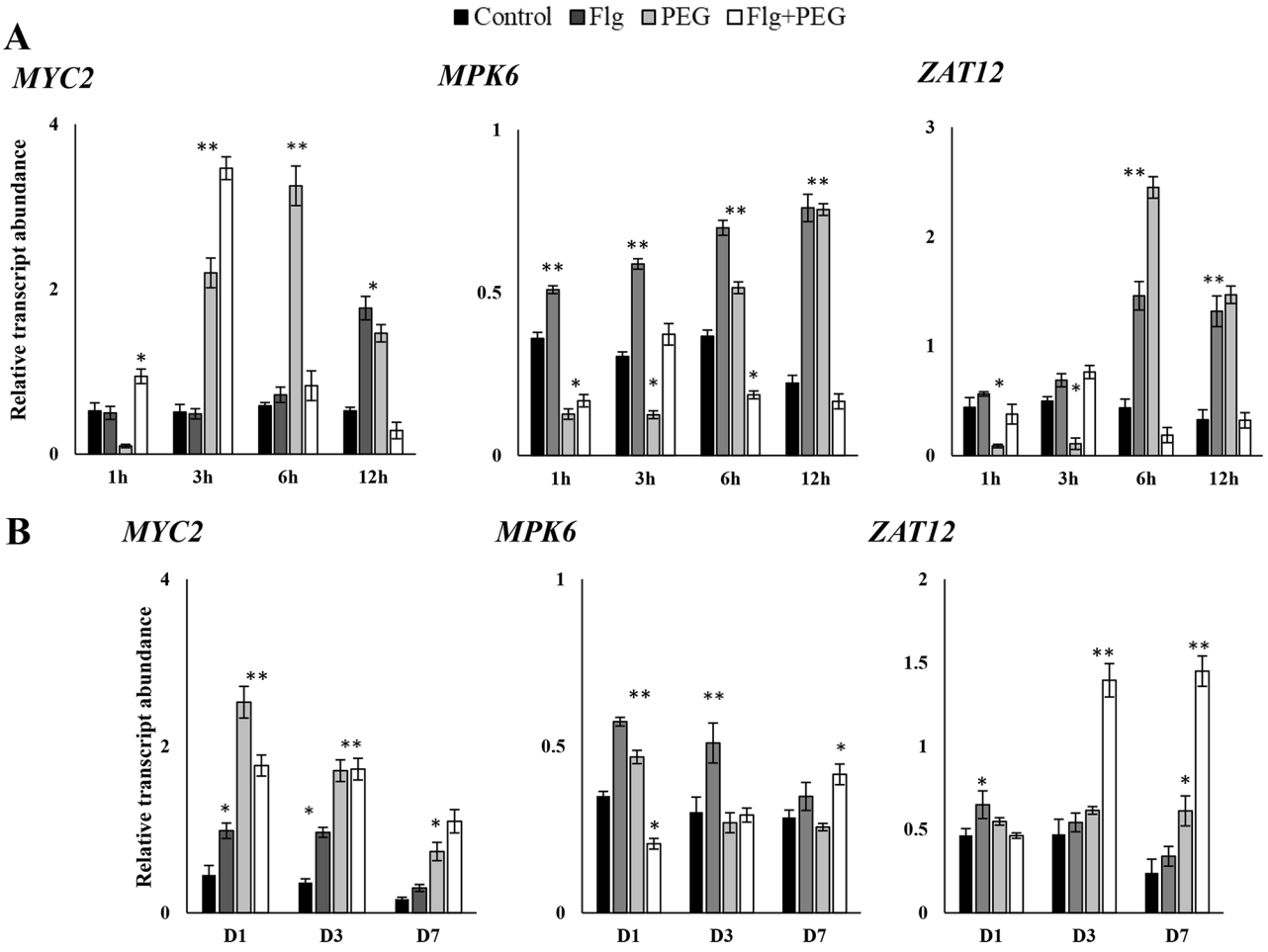

Fig. 5 The relative transcript abundances of MYC2, MPK6 and ZAT12 genes in the leaf samples of B. napus control, Flg-treated, drought stressed and Flg+drought stressed plants in the short term-1 to12h (**A**) and in the long term-1 to7 days (**B**) Relative expressions were analyzed by qPCR and the data was normalized by actin. Values presented are the mean ± SE of (n=3), significant differences were assigned as *p< 0.05 and **p< 0.01

through this molecule might reveal a common route to manipulate stress tolerance in plants. Under various abiotic and biotic stress conditions, exogenous phytohormone and metabolite treatments GABA levels rapidly increases in plant cells [8, 16, 33, 34]. Although increases in the GABA content seems to be a general response, it is necessary to distinguish whether this high level is due to the activation of GABA biosynthesis or inhibition of the GABA catabolic pathway. On the other hand, if activation of GABA-shunt is a common route for stress tolerance then inducing it by a biotic stress factor might also function in increased tolerance against abiotic stresses. In this context, we conducted experiments to understand whether the GABA-shunt is activated through ETI and if so, would this be advantageous for plants to cope with drought stress. Therefore, we treated *B. napus* plants with Flg-22 and then exposed them to PEG-induced drought stress. Leaf and root samples were analyzed for physiological and growth parameters along with GABA-shunt components and transcript profiles of general modulators of stress responses.

In general, under drought stress water content decreases in plant cells due to increased osmotic potential and disruption of membrane integrity, damage to photosynthetic systems took place due to excessive ROS accumulation [35]. We found similar osmotic potential values in drought only and Flg+drought stressed groups on the first three days however, the osmotic potential was significantly higher in the former on the seventh day. Accordingly, along with a lower osmotic potential the Flg+drought stressed groups had higher RWC values which indicated better water use efficiency under drought conditions. Besides that, Flg+drought stressed groups had RGR and *Fv/Fm* values similar to control groups while this was not the case in the drought only ones. Flg-treatment also had a priming effect without creating a stress effect in relation to growth and physiological analysis. This is seen when we compare the drought only group with the Flg+drought stressed group, the latter

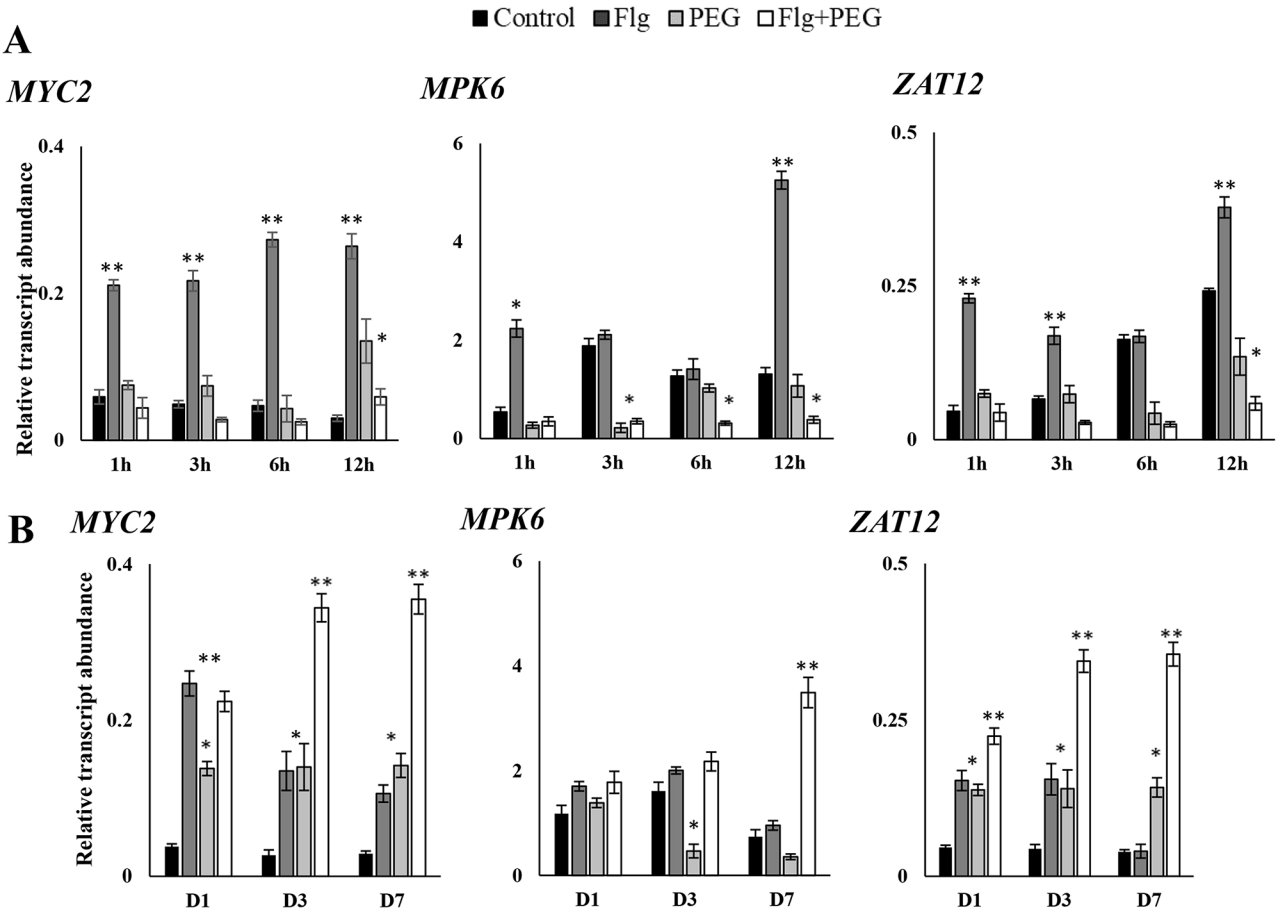

Fig. 6 The relative transcript abundances of MYC2, MPK6 and ZAT12 genes in the root samples of B. napus control, Flg-treated, drought stressed and Flg+drought stressed plants in the short term-1 to12h (**A**) and in the long term-1 to7 days (**B**) Relative expressions were analyzed by qPCR and the data was normalized by actin. Values presented are the mean ± SE of (n=3), significant differences were assigned as *p < 0.05 and **p < 0.01

had lower lipid peroxidation, higher proline content, better RGR and *Fv/Fm* values which indicated the ameliorative effect of Flg treatment. Seifikalhor et al. [35] reported that drought stress affected growth and photosynthesis in chickpea plants and, exogenous GABA treatment alleviated these negative impacts to a higher extent in the sensitive cultivar. Parallel to this study, we think that the better drought stress coping of the Flg+drought stressed group may also be related to GABA accumulation.

Drought stress induced GABA accumulation hence, the highest levels were observed in the leaf and root samples of the drought only group during the experimental period. We analyzed all GABA-shunt components to understand whether the increased GABA levels were related to enhanced biosynthesis or inhibited catabolism. In plants main route for GABA synthesis is through GAD activity in the cytosol [4, 36]. In our study, GABA contents were affected as a result of effector recognition. However, there was a discrepancy between Flg-treated leaf and roots in terms of GDH, GAD activity and GABA accumulation, the former was affected to a higher extend while the latter did not except that of GAD activity. GABA accumulation was also increased in *Phaseolus vulgaris* L., *Solanum lycopersicon* L. and *Glycine max* L upon pathogen recognition or infection [37–39]. Deng et al. [15] reported that GABA levels were increased along with high GAD1, GAD2, and GAD4 activities when plants were subjected to *Pseudomonas syringae* pv tomato DC3000 (Pst) which expressed the avrRpt2 effector.

Increased GDH and GAD activities were correlated to the GABA content and GABA-shunt components only in Flg+drought stressed leaf and roots. Similar to our study, Dimlioğlu et al. [16], found that GDH, GAD activities and GABA contents were increased after harpin treatment (another bacterial effector molecule). In a study on tobacco plants, under moderate Zn (10 µmol) stress conditions, GDH and GAD activities and the GABA content were increased [40]. GABA-T is the enzyme of the GABA-pathway that is most affected and inhibited by increased reactive oxygen species under stress conditions [7, 41]. When GABA-shunt is efficiently functioning, the

synthesized GABA might quickly enter to the catabolic process and be used to provide succinate to the TCA. This might be the case for explaining the better performance of Flg+drought stressed groups. We assayed the activities of GABA-shunt catabolic enzymes; GABA-T and SSADH in the leaves and roots. GABA accumulation under stress conditions is reported to be dependent on the decreased activities of these catabolic enzymes. We found GABA-T and SSADH activities were induced in all groups however, only in the Flg+drought stressed groups there was an accompanying increase in GABA contents and biosynthesis in both leaves and roots which indicated that the entire GABA shunt was activated to cope with drought stress.

Resistance to *Agrobacterium* and *Botrytis cinerea* was associated to increased GABA shunt activity [15, 42]. In addition, there was a direct correlation with high GABA levels and reduced infection of *Pseudomonas syringae* in tomato [15, 43]. These findings indicated that effector recognition and/or infection activated the GABA-shunt but the exact mode of action was not clearly defined. In the light of our findings, we propose that Flg induced ETI positively affected drought stress tolerance in *B. napus* via GABA-shunt and the connection might be the activation of transcription factors with multiple tasks in biotic and abiotic stress tolerance. To clarify this, we also analyzed the expression profiles of *MYC2*, *MPK6* and *ZAT12*. *MYC2* is among the key regulators of the jasmonic acid signaling pathway participating in plant development and multiple stresses [44]. *MYC2* transcription enables the activation of defense processes against insects by promoting flavonoid biosynthesis. *MPK6* is transiently activated during PTI but, it is persistent and long-lasting activated during ETI [45, 46]. *MPK6* has shown to have an important role in programmed cell death and hypersensitivity responses, as well as defense responses against salt stress and low temperature stress [47]. Besides these it was reported to be involved in root, stomata behavior and seed development processes. The expression of the zinc-finger protein encoding gene *ZAT12* is highly induced in response to injury, bacterial, fungal and nematode infection, H_2O_2, high temperature, cold, drought, elicitor applications, heavy metal and UV stress [48, 49].

Although mitogen-activated protein kinase (MAPK) signaling via MPK3/MPK6 is among the first events for sensing pathogens, they also play important functions in plant growth and development [15, 50]. In *A. thaliana*, BRI1-associated receptor kinase 1 (BAK1) mutants were insensitive to Flg-22 and it was also shown that stable resistance to *Pseudomonas syringae* (DC3000) infection was related to increased *MPK3* and *MPK6* transcript abundances which were plausibly activated by *BAK-1* mediated MAP kinase signaling [3]. There is also a discrepancy between the magnitude and time-course of MAPK activation in PTI and ETI hence the defense response depends on the downstream processing along with the transcription factors. Induction of *WRKY33* transcription is an example of these processes by which increased phytoalexin and ethylene biosynthesis facilitated immunity in *(A) thaliana* [15, 51]. Here we propose a plausible interaction between GABA-shunt and MAPK signaling in stress tolerance via *MPK6* in *(B) napus*. Previously, Deng et al. [15] found that along with induced GAD1 and GAD4 expression, GABA content was increased in ETI via high *MPK3*/*MPK6* expression in *A. thaliana*. In our study, we observed a significant relationship between the activation of GABA-shunt and *MPK6* expression only in the roots of the Flg+drought stressed groups which might indicate the importance of root signaling for early onset of drought tolerance. On the other hand, highest *MPK6 t*ranscript abundances were observed in the leaves and roots of Flg-treated groups that is plausibly related to activation of ETI. Similar to our findings an interaction with GABA and MAPK cascade was reported by Ding et al. [52]. Exogenous GABA treatment increased resistance in Pitaya (*Hylocereus costaricensis*) plants along with increased levels of HcMAPKKK1; HcMAPKK2/4; HcMAPK1/7 transcripts. However, the resistance was diminished when MAPK inhibitor (PD98059) and GABA were applied together to the plants [52].

In plants *MYC2* is the key regulator of plant defense against biotic stress via JA-signaling and it also controls JA-induced root growth inhibition and leaf senescence [53–55]. Recent evidence indicate the contribution of JA-signaling to drought stress tolerance through *MYC2* by regulating the stomatal movements [56]. In our study, *MYC2* transcript abundance was highly induced in all treatment groups and there was a discrepancy between leaf and root levels in the short and long term. Nevertheless, when we consider drought stress tolerance related parameters and GABA-shunt the only rational relationship was found in the Flg+drought stressed groups. We found that in Flg+drought stressed groups higher *MYC2* transcript abundances correlated well with the GABA content and other GABA-shunt components therefore, we propose that effector triggered *MYC2* might be associated with the activation of GABA-shunt for drought tolerance. Accordingly, Xu et al. [57] found that, in guard cells GABA mediated stomatal behavior was dependent on ROS and Ca^{2+} levels which was proposed to be connected to other signaling routes in *(A) thaliana*. Recently, *MYC2* reported to be related to dehydration stress memory and regulation of stomatal closure [57–59]. Moreover, *MYC2*-silenced leaves in *(B) napus* had decreased drought stress tolerance due to reduced stomatal closure [60]. Besides these we can also speculate that the function of *MYC2* in drought stress tolerance might also be

related to polyamine metabolism and GABA-shunt. Ding et al. [52] demonstrated that *arginine decarboxylase 1* (*ADC1*) expression was affected by *MYC2* hence, decreased putrescine (Put) content was found when *MYC2* expression was downregulated. Put can be converted to GABA by the activity of diamine oxidase (DAO) [7, 8]. In our study, some but not in all drought only groups we found that increased GABA content was followed by high *MYC2* expression without enhanced GAD activity. This might also be related to the differentiation of signaling routes when plant cells encounter abiotic or biotic stressors.

In *Arabidopsis*, abiotic and biotic stress-responsive transcription factors are different and act differentially but *ZAT12* is a common transcription factor that is activated by drought, heavy metal, high temperature, UV, wounding, herbivory, fungal and bacterial infection and acts in a similar way. Analysis of *ZAT12* mutants and transcriptomic data provided information that rather than being directly involved in stress tolerance its increased expression might be necessary for inducing other stress tolerance mechanisms [61]. GABA and GABA-shunt might be among these stress tolerance mechanisms since they are also multi-stress responsive and directly affect the energy status through the TCA which is extremely important under stress conditions. In our study, the transcript abundances of *ZAT12* was induced both in leaves and roots as a result of Flg22 treatment however, correlation with GABA and GABA-shunt could be proposed only in Flg+drought stressed group. Although early onset and the highest level was recorded in the drought only group, it would not be possible to correlate this level neither with GABA nor with stress tolerance. Contrary to our results, tomato *ZAT12* transgenic plants had increased drought stress tolerance in relation to high proline accumulation and increased antioxidative enzyme activities [62]. Accordingly, Kaur et al. [63] found that wheat *ZAT12* transgenic plants cope well with temperature stress by enhanced osmotic adjustment, lower lipid peroxidation and higher antioxidative capacity. This discrepancy might indicate the threshold level for stress tolerance is higher for *ZAT12* and its high transcript abundance was responsible for activation of other stress tolerance mechanisms. We also found a nice correlation between *MYC2* and *ZAT12* indicating a plausible cross talk between different stress coping processes.

Conclusion

Plant cells are energy efficient systems, and this provides a critical advantage under stress conditions. Although the activation of stress defense, starting from sensing, reprograming of transcription and biosynthesis of defense molecules are all demanding a vast amount of energy, plants also maintain growth and development under stress conditions. Therefore, by pass of two steps in the TCA with the succinate provided from the GABA-shunt might also be an important strategy for efficient use of energy under stress conditions. Not only the high levels of GABA but also the activation of GABA shunt is usually associated by abiotic and biotic stress tolerance as this was the case in our study. We found a clear discrepancy between the drought only and Flg+drought stressed groups by means of GABA-shunt and the latter has increased stress tolerance. This is the first report on how GABA-shunt and Fgl-22 are interacting against abiotic stress, and how these processes are differentiated between leaf and root tissues. Here we provided solid data that; drought stress tolerance was maintained by Fgl-22 induced ETI through the activation of GABA-shunt by a plausible cross talk between *MYC2* and *ZAT12* transcription factors in the leaves and roots of *B. napus*.

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Author contributions
M.B. conceived the research plans and designed the experiments, S.P., I.C. and T.A.O. performed the experiments, M.B. wrote the manuscript.

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Data availability
Data is provided within the manuscript.

Declarations

Ethics approval and consent to participate
All of the material is owned by the authors and no permissions are required.

Consent for publication
Not applicable.

Competing interests
The authors declare no competing interests.

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References

1. Kahraman M, Sevim G, Bor M. The role of Proline, glycinebetaine, and Trehalose in stress-responsive gene expression. In: Hossain M, Kumar V, Burritt D, Fujita M, Mäkelä P, editors. Osmoprotectant-mediated abiotic stress tolerance in plants. Cham: Springer; 2019.
2. Jones JD, Dangl JL. The plant immune system. Nature. 2006;444:323–9.
3. Bhar A, Chakraborty A, Roy A. Plant Responses to Biotic Stress: Old Memories Matter, Plants. 2021; 11(1):84.
4. Seifikalhor M, Aliniaeifard S, Hassani B, NiknamV, Lastochkina O. Diverse role of γ-aminobutyric acid in dynamic plant cell responses. Plant Cell Rep. 2019;38:847–67.
5. Michaeli S, Fait A, Lagor K, Nunes-Nesi A, Grillich N, Yellin A. A mitochondrial GABA permease connects the GABA shunt and the TCA cycle, and is essential for normal carbon metabolism. Plant J. 2011;67:485–98.
6. Shelp BJ, Zarei A. Subcellular compartmentation of 4-aminobutyrate metabolism in Arabidopsis: an update. Plant Signal Behav. 2017;12(5):e1322244.

7. Bor M, Turkan I. Is there a room for GABA in Ros ve RNS signalling? Environ. Exp Bot. 2019;161:67–73.
8. Shelp BJ, Mullen RT, Waller JC. Compartmentation of GABA metabolism raises intriguing questions. Trend. Plant Sci. 2012;17(2):57–9.
9. Ramputh A, Shelp AW. Rapid y-aminobutyric acid synthesis and the inhibition of the growth and development of oblique banded leaf roller larvae. Plant Physiol. 1996;111:1349–52.
10. Li L, Dou N, Zhang H, Wu C. The versatile GABA in plants. Plant Signal Bhv. 2021;16(3):1862565.
11. Mc Craw SL, Park DH, Jones R, Bentley MA, Rico A, Ratcliffe RG, Kruger NJ, Collmer A, Preston GM. GABA (γ-aminobutyric acid) uptake via the GABA permease GabP represses virulence gene expression in Pseudomonas syringae Pv. Tomato DC3000. Mol Plant Mic Interact. 2016;29(12):938–49.
12. Lee CP, Eubel H, O'Toole N, Millar AH. Combining proteomics of root and shoot mitochondria and transcript analysis to define constitutive and variable components in plant mitochondria. Phytochem. 2011;72:1092–108.
13. Zhang Y, Jaime SM, Bulut M, Graf A, Fernie AR. The conditional mitochondrial protein complexome in the *Arabidopsis thaliana* root and shoot. Plant Comm. 2003;4:100635.
14. Gupta K, Sengupta A, Chakraborty M, Gupta B. Hydrogen peroxide and polyamines act as double edged swords in plant abiotic stress responses. Front Plant Sci. 2016;7:1343.
15. Deng X, Xu X, Liu Y, Zhang Y, Yang L, Zhang S, Xu J. Induction of γ-aminobutyric acid plays a positive role to *Arabidopsis* resistance against *Pseudomonas syringae*. J Integ Plant Biol. 2020;62:1797–812.
16. Dimlioğlu G, Daş ZA, Bor M, Özdemir F, Türkan İ. The impact of GABA in Harpin-elicited biotic stress responses in *Nicotiana tabaccum*. J Plant Physiol. 2015;188:51–7.
17. Tumewu SA, Matsui H, Yamamoto M, Noutoshi Y, Toyoda K, Ichinose Y. Requirement of γ-Aminobutyric acid Chemotaxis for Virulence of Pseudomonas syringae Pv. Tabaci 6605. Microbes Environ. 2020;35:ME20114.
18. Lu B, Li D, Zhang R, Shuai L, Schulze B, Kroth PG, Zhan D, Wang G. Defense responses in female gametophytes of Saccharina Japonica (Phaeophyta) induced by flg22-derived peptides. J Appl Phycol. 2016;28:1793–801.
19. Hunt R, Causton DR, Shipley B, Askew AP. A modern tool for classical plant growth analysis. Ann Bot. 2002;90:485–8.
20. Smart RE, Bingham GE. Rapid estimates of relative water content. Plant Physiol. 1974;53(2):258–60.
21. Baum G, Lev-Yadun S, Fridmann Y, Arazi T, Katsnelson H, Zik M, Fromm H. Calmodulin binding to glutamate decarboxylase is required for regulation of glutamate and GABA metabolism and normal development in plants. EMBO J. 1996;15(12):2988–96.
22. Bor M, Seckin B, Ozgur R, Yılmaz O, Ozdemir F, Turkan I. Comparative effects of drought, salt, heavy metal ve heat stresses on gamma-aminobutryric acid levels of sesame (*Sesamum indicum* L). Acta Physiol Plant. 2009;31:655–9.
23. Madhava Rao KV, Sresty TVS. Antioxidative parameters in the seedlings of pigeonpea (*Cajanus cajan* L. Millspaugh) in response to Zn and Ni stresses. Plant Sci. 2000;157:113–28.
24. Quamar A, Kumar MS. *Arabidopsis* exhibits differential response in basal immunity and proline metabolism during defense against host and non-host pathogen infection. Plant Physiol Rep. 2019;24(4):496–506.
25. Bradford MM. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal Biochem. 1976;72(1–2):248–54.
26. Akihiro T, Koike S, Tani R, Tominaga T, Watanabe S, Iijima Y, Aoki K, Shibata D, Ashihara H, Matsukura C. Biochemical mechanism on GABA accumulation during fruit development in tomato. Plant Cell Physiol. 2008;49(9):1378–89.
27. Bartyzel I, Pelczar K, Paszkowski A. Functioning of the γ-aminobutyrate pathway in wheat seedlings affected by osmotic stress. Biol Plant. 2003;46(2):221–5.
28. Miyashita Y, Good AG. Contribution of the GABA shunt to hypoxia-induced alanine accumulation in roots of *Arabidopsis thaliana*. Plant Cell Physiol. 2008;49(1):92–102.
29. Jalil US, Khan MI, Ansari MI. Role of GABA transaminase in the regulation of development and senescence in *Arabidopsis thaliana*. Curr Plant Biol. 2019;19:100119.
30. Busch KB, Fromm H. Plant succinic semialdehyde dehydrogenase. Cloning, purification, localization in mitochondria, and regulation by adenine nucleotides. Plant Physiol. 1999;121:589–97.
31. Bouché N, Fait A, Bouchez D, Møller SG, Fromm H. Mitochondrial succinic-semialdehyde dehydrogenase of the γ-aminobutyrate shunt is required to restrict levels of reactive oxygen intermediates in plants. PNAS. 2003;100(11):6843–8.
32. Khan MI, Jalil SU, Chopra P, Chillar H, Ferrante A, Khan NA, Ansari MI. Role of GABA in plant growth, development and senescence. Plant Gene. 2021;26:100283.
33. Akçay N, Bor M, Karabudak T, Özdemir F, Türkan İ. Contribution of gamma amino butyric acid (GABA) to salt stress responses of *Nicotiana sylvestris* CMSII mutant and wild type plants. J Plant Physiol. 2012;169(5):452–8.
34. Yang R, Guo Q, Gu Z. GABA shunt and polyamine degradation pathway on gamma aminobutyric acid accumulation in germinating fava bean (*Vicia faba* L.) under hypoxia. Food Chem. 2013;136:152–9.
35. Seifikalhor M, Niknam V, Aliniaeifard S, Didaran F, Tsaniklidis G, Fanourakis D, Teymoorzadeh M, Mousavi SH, Bosacchi M, Li T. The regulatory role of γ-aminobutyric acid in chickpea plants depends on drought tolerance and water scarcity level. Sci Rep. 2022;12:7034.
36. Forde BG, Lea PJ. Glutamate in plants: metabolism, regulation, and signaling. JXB. 2007;58:2339–58.
37. Copley TR, Aliferis KA, Kliebenstein DJ, Jabaji SH. An integrated RNAseq-1H NMR metabolomics approach to understand soybean primary metabolism regulation in response to Rhizoctonia foliar blight disease. BMC Plant Biol. 2017;17:84.
38. O'Leary BM, Neale HC, Geilfus CM, Jackson RW, Arnold DL, Preston GM. Early changes in apoplast composition associated with defence and disease in interactions between *Phaseolus vulgaris* and the halo blight pathogen *Pseudomonas syringae Pv. Phaseolicola*. Plant Cell Environ. 2016;39:2172–84.
39. Wang G, Kong J, Cui D, Zhao H, Niu Y, Xu M, Jiang G, Zhao Y, Wang W. Resistance against *Ralstonia solanacearum* in tomato depends on the methionine cycle and the γ-aminobutyric acid metabolic pathway. Plant J. 2019;97:1032–47.
40. Akcan Daş Z, Dimlioğlu G, Bor M, Özdemir F. Zinc Induced activation of GABA-Shunt in Tobacco (*Nicotiana tabaccum* L). Environ Exp Bot. 2016;122:78–84.
41. Shelp BJ, Bown AW, Zarei A. 4-Aminobutyrate (GABA): a metabolite and signal with practical significance. Bot. 2017;95:1015–32.
42. Chevrot R, Rosen R, Haudecoeur E, Cirou A, Shelp BJ, Ron E, Faure D. GABA controls the level of quorum sensing signal in *Agrobacterium tumefaciens*. PNAS. 2006;103:7460–4.
43. Park DH, Mirabella R, Bronstein PA, Preston GM, Haring MA, Lim CK, Collmer A, Schuurink RC. Mutations in gamma-aminobutyric acid (GABA) transaminase genes in plants or *Pseudomonas syringae* reduce bacterial virulence. Plant J. 2010;64:318–30.
44. Cheng H, Zha S, Luo Y, Li L, Wang S, Wu S, Cheng S, Li L. JAZ1-3 and MYC2-1 synergistically regulate the Transformation from Completely Mixed Flower Buds to Female Flower Buds in *Castanea Mollisima*. Int J Mol Sci. 2022;23:6452.
45. Tsuda K, Mine A, Bethke G, Igarashi D, Botanga CJ, Tsuda Y, Glazebrook J, Sato M, Katagiri F. Dual regulation of gene expression mediated by extended MAPK activation and salicylic acid contributes to robust innate immunity in *Arabidopsis thaliana*. PLoS Genet. 2013;9:e1004015.
46. Guan R, Su J, Meng X, Li S, Liu Y, Xu J, Zhang S. Multilayered regulation of ethylene induction plays a positive role in *Arabidopsis* resistance against Pseudomonas syringae. Plant Physiol. 2015;169:299–312.
47. Su J, Yang L, Zhu Q, Wu H, He Y, Liu Y. Active photosynthetic inhibition mediated by MPK3/MPK6 is critical to effector-triggered immunity. PLoS Biol. 2018;16(5):e2004122.
48. Iida A, Kazuoka T, Torikai S, Kikuchi H, Oeda K. A zinc finger protein RHL41 mediates the light acclimatization response in *Arabidopsis*. Plant J. 2000;24:191–203.
49. Cheong YH, Chang HS, Gupta R, Wang X, Zhu T, Luan S. Transcriptional profiling reveals novel interactions between wounding, pathogen, abiotic stress, and hormonal responses in *Arabidopsis*. Plant Physiol. 2002;129:661–77.
50. Zhang M, Su J, Zhang Y, Xu J, Zhang S. Conveying endogenous and exogenous signals: MAPK cascades in plant growth and defense. Curr Opin Plant Biol. 2018;45:1–10.
51. Li G, Meng X, Wang R, Mao G, Han L, Liu Y, Zhang S. Dual-level regulation of ACC synthase activity by MPK3/MPK6 cascade and its downstream WRKY transcription factor during ethylene induction in *Arabidopsis*. PLoS Gene. 2012;8:e1002767.
52. Ding X, Liu S, Duan X, Pan X, Dong B. MAPK cascade and ROS metabolism are involved in GABA-induced disease resistance in red pitaya fruit. Postharvest Biol Tech. 2023;200:112324.

53. Creelman RA, Mullet JE. Oligosaccharins, brassinolides, and jasmonates: nontraditional regulators of plant growth, development, and gene expression. Plant Cell. 1997;9(7):1211–23.
54. Kazan K, Manners JM. Jasmonate signaling: toward an integrated view. Plant Physiol. 2008;146:1459–68.
55. Zhang C, Lei Y, Lu C, Wang L, Wu J. MYC2, MYC3, and MYC4 function additively in wounding-induced jasmonic acid biosynthesis and catabolism. J Integr Plant Biol. 2020;62:1159–75.
56. Xing Q, Liao J, Cao S, Li M, Lv T, Qi H. CmLOX10 positively regulates drought tolerance through jasmonic acid-mediated stomatal closure in oriental melon (Cucumis melo var. makuwa Makino) Sci Rep. 2020;10:1–14.
57. Xu B, Feng X, Piechatzek A, Zhang S, Konrad KR, Kromdijk J, Hedrich R, Gilliham M. The GABA shunt contributes to ROS homeostasis in guard cells of *Arabidopsis*. New Phytol. 2024;241:73–81.
58. Avramova Z. Defence-related priming and responses to recurring drought: two manifestations of plant transcriptional memory mediated by the ABA and JA signalling pathways. Plant Cell Environ. 2019;42:983–97.
59. Zhao W, Huang H, Wang J, Wang X, Bingqin X, Yao X, Sun L, Yang R, Wang J, Sun A, Wang S. Jasmonic acid enhances osmotic stress responses by MYC2-mediated inhibition of protein phosphatase 2C1 and response regulators 26 transcription factor in tomato. Plant J. 2023;113:546–61.
60. Wang W, Shi X, Chen D, Wang F, Zhang H. The *Brassica napus* MYC2 regulates drought tolerance by monitoring stomatal closure. Eur J Hort Sci. 2020;85:226–31.
61. Davletova S, Schlauch K, Coutu J, Mittler R. The zinc-finger protein Zat12 plays a central role in reactive oxygen and abiotic stress signalling in *Arabidopsis*. Plant Physiol. 2005;139:847–56.
62. Rai AC, Singh M, Shah K. Engineering drought tolerant tomato plant overexpressing BcZAT12 gene encoding a C2H2 zinc finger transcription factor. Phytochem. 2013;85:44–50.
63. Kaur M, Asthir B, Kaur R, Chaudhary A. Zat12 gene ameliorates temperature stress in Wheat Transgenics by modulating the antioxidant Defense System. Stress. 2023;3:316–30.

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