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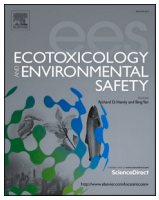

Antifungal triazoles affect key non-target metabolic pathways in *Solanum lycopersicum* L. plants

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ABSTRACT

Several 1,2,4-triazoles are widely used as systemic fungicides in agriculture because they inhibit fungal 14α-demethylase. However, they can also act on many non-target plant enzymes, thereby affecting phytohormonal balance, free amino acid content, and adaptation to stress. In this study, tomato plants (*Solanum lycopersicum* L. var. 'Cherrola') were exposed to penconazole, tebuconazole, or their combination, either by foliar spraying or soil drenching, every week, as an ecotoxicological model. All triazole-exposed plants showed a higher content (1.7–8.8 ×) of total free amino acids than the control, especially free glutamine and asparagine were increased most likely in relation to the increase in active cytokinin metabolites 15 days after the first application. Conversely, the Trp content decreased in comparison with control (0.2–0.7 ×), suggesting depletion by auxin biosynthesis. Both triazole application methods slightly affected the antioxidant system (antioxidant enzyme activity, antioxidant capacity, and phenolic content) in tomato leaves. These results indicated that the tomato plants adapted to triazoles over time. Therefore, increasing the abscisic and chlorogenic acid content in triazole-exposed plants may promote resistance to abiotic stress.

1. Introduction

1,2,4-triazoles are a group of biologically active compounds with a five-membered aromatic ring substituted with three nitrogen atoms. Since they were commercially developed in the late 1960s, they have been extensively applied as fungicides in various crops (wheat, barley, rapeseed, corn, and rice, among others), fruits and vegetables, and in horticultural and forestry industries against powdery mildew, rust, rots, leaf blotches, and other spot diseases (Fletcher et al., 2000; Lv et al., 2016; Strzelecka and Swiatek, 2021; Volova et al., 2017; Zhang et al., 2019). Similarly, triazoles are also used as antimycotics to treat human fungal infections (Bhagat et al., 2021). The primary antifungal effect of triazoles consists of inhibiting the fungal cytochrome P450 (CYP) dependent enzyme sterol 14α-demethylase (CYP51A and B), thus blocking the synthesis of ergosterol, a membrane component that is crucial for membrane integrity and whose deficit arrests fungal growth (Fera et al., 2009).

Triazole phytotoxicity is associated with sterol biosynthesis inhibition, oxidative stress induction, and mineral nutrition disruption. Triazoles disturb plant sterol demethylase activity and sterol-dependent signalling, thereby affecting cell proliferation and differentiation and inducing the production of reactive oxygen species (ROS). Their toxic effects lead to hormonal and nitrogen imbalance, reduced seed germination, root growth and development disorders, and even chromosomal abnormalities (Shishatskaya et al., 2018). In particular, tebuconazole and its photoproducts are reportedly toxic to lettuce and onion seeds

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(Arsand et al., 2021) and decrease germination, root growth, and mitotic index of *Allium cepa* roots, concomitantly increasing the incidence of chromosome and nuclear aberrations (Bernardes et al., 2015). These negative effects of tebuconazole on root growth have also been detected in germinated seeds of *Lactuca sativa*, causing chromosome alterations and DNA fragmentation at higher concentrations (Barroso Aragão et al., 2021). Tebuconazole rapidly penetrates plants through their vegetative organs and roots, as does penconazole (Volova et al., 2017). Tebuconazole and penconazole are classified as both slightly toxic to mammalians (toxicity class III), based on evidence supporting their role in disrupting endocrine activity and a possible mode of action in carcinogenesis, and very dangerous to the environment (environmental risk class II) (Arsand et al., 2021; Zhang et al., 2019). Moreover, 1,2,4-triazole, a major degradation product of several triazole fungicides, including tebuconazole, may leach into groundwater in concentrations exceeding the threshold limit of the European Union (Albers et al., 2022).

Triazoles may interact with other CYP-dependent enzymes in non-target organisms, including plants, where they potentially alter phytohormonal levels and the content of secondary metabolites, in particular. Triazoles interfere with the first three steps in the pathway of ent-kaurene oxidation to ent-kaurenoic acid catalysed by CYP701 leading to the biosynthesis of gibberellins. Gibberellin biosynthesis inhibition is related to retarded shoot growth as treated plants are greener and more compact. For this reason, triazoles are classified as plant growth regulators (Fletcher et al., 2000; Lee et al., 2023; Ohnishi, 2018). In addition, the synthesis of brassinosteroids involves several steps catalysed by CYP, which can be inhibited by triazoles and thus act as growth regulators (Ohnishi, 2018).

By interfering with the degradation pathway, triazole inhibition may increase the levels of phytohormones such as abscisic acid (ABA). Triazole derivatives inhibit CYP707A, which mediates ABA catabolism to phaseic acid. Since ABA induces not only seed dormancy but also stomata closure, this triazole effect can be beneficial in plant responses to environmental stresses, including drought and high salinity (Kitahata et al., 2005). Penconazole was also identified as a plant growth regulator and a protectant against abiotic stresses, such as drought, salt, and ozone stress (Chandra and Roychoudhury, 2020). Triazole treatment increases the chlorophyll and glutamate content and the photosynthetic rate of plants (Chandra and Roychoudhury, 2020; Desta and Amare, 2021; Rezayian et al., 2018). This trait may be related to the role of cytokinins (CK) involved in nitrate foraging through the preferential development of lateral roots in nitrate-rich areas, thereby maximising nitrate acquisition (Pavlu et al., 2018).

Structurally similar to the five-membered heterocycle of triazoles, benzothiadiazoles are considered priming molecules because they activate or enhance plant defence-associated genes and biologically active signals or molecules in response to biotic and abiotic stress factors (Yang et al., 2022). Based on the partial structural similarity between benzothiazoles and triazoles, the response of plants to triazole application may improve their defence. Moreover, some triazoles were developed as safeners, which are chemical agents that can improve the tolerance of crops to herbicides by upregulating the biotransformation enzymes glutathione-S-transferase (GST) and CYP and by increasing the content of glutathione and ATP binding cassette transporter (Jia et al., 2022). Therefore, triazoles can act as (i) abiotic stressors, (ii) elicitors, activating the plant defence system and inducing priming, and/or (iii) safeners, improving tolerance to herbicides (Barroso Aragao et al., 2021; Bernardes et al., 2015; Desta and Amare, 2021; Fletcher et al., 2000; Shaki et al., 2022).

Tomato (*Solanum lycopersicum* L.) is globally recognised as a major crop for the nutritional profile of this fruit, such as dietary fibre, minerals, vitamins, and antioxidants (Liu et al., 2022; Rodrigues and Furlong, 2022). As a result, tomato plants are regularly treated with pesticides to maintain high levels of fruit production. However, their exposure to pesticides may induce oxidative stress and increase the activity of antioxidant enzymes (Shakir et al., 2018; Singh et al., 2023). Given the economic importance of this crop, the mass use of triazole fungicides and their potentially contradictory effects on plants, further research must be conducted to clarify the effects of triazoles on tomato plants.

Considering the above, the aim of this study was to analyse in depth the metabolism of tomato plants exposed to penconazole, tebuconazole or their combination, applied either foliarly or to the soil. Using tomato plants as an ecotoxicological model, we tested the following hypotheses: namely whether i) triazoles damage plants, reduce the activity of the antioxidant system, and negatively affect phytohormonal levels, and ii) triazoles activate the plant defence system and increase the synthesis of defence substances and the activity of antioxidant enzymes.

2. Material and methods

2.1. Plant growth and treatment

Six-week-old tomato (*Solanum lycopersicum* L. var. 'Cherrola') seedlings were grown in 4 L-pots containing 500 g DW of the substrate "Horticultural Substrate with active Humus" and mineral nutrients (AGRO CS a.s., Czech Republic). The experiment encompasses six triazole treatments and a control group (Table 1), with six replicates each, i.e., six pots, with one plant in each pot. The triazoles tebuconazole (1-(4-chlorophenyl)− 4,4-dimethyl-3-(1 H-1,2,4-triazol-1-ylmethyl)pentan-3-ol) and penconazole (1-[2-(2,4-dichlorophenyl)pentyl]1,2,4-triazole) were of analytical-grade purity (Pestanal®, Supelco® Analytical Products) and purchased from Sigma-Aldrich (Czech Republic). Triazoles were applied either foliarly, i.e., by precisely and gently spraying the above-ground biomass to prevent losses, or directly into the soil, every week (i.e., five times) during tomato growth. For each application, the triazoles were dosed as recommended by the manufacturer for tomato spraying, i.e., the total triazole concentration was $3.52{\cdot}10^{-6}$ mol per plant (approximately 10 $mg{\cdot}m^{-2}$). The control variant sprayed or watered with deionised water was used for comparison. The tomato plants were grown for 35 days in a vegetation hall at ambient temperature and under light and root irrigation with demineralised water. Every three weeks, a liquid fertiliser mixed with mineral macronutrients (Lovoflor NPK 4–2.5–3, Lovochemie a.s., Czech Republic) was added to the soil in an optional fertiliser dose (0.75 $mL{\cdot}kg^{-1}$). Leaves were collected on the 15th and 35th day of tomato growth. Plant biomass samples were immediately frozen in liquid nitrogen and stored at − 80 °C until analysis.

2.2. Determination of free proteinogenic amino acids and proteins

The content of free amino acids in freeze-dried (Lyovac GT 2, FinnAqua, Finnland) leaves was analysed by capillary chromatography (Hodek et al., 2017). Protein content in plant extracts was analysed with the Bradford reagent (Sigma-Aldrich, USA) (Bradford, 1976).

Table 1
Experimental groups of tomato plants treated with triazoles. In all treatments, the total concentration of triazoles was the same (3.52 μmol per plant). The solution of penconazole and tebuconazole was mixed before application.

No.	Application	Triazole (abbreviation)	Triazole amount (μmol/plant)
1	-	none (control, C)	0
2	foliar	penconazole (fP)	3.52
3		tebuconazole (fT)	3.52
4		penconazole+tebuconazole (fPT)	1.76 + 1.76
5	soil	penconazole (sP)	3.52
6		tebuconazole (sT)	3.52
7		penconazole+tebuconazole (sPT)	1.76 + 1.76

2.3. Plant hormone analysis

Before analysis, samples of tomato leaves were freeze-dried and homogenised. The endogenous cytokinin content was quantified using 10 mg of plant tissue. The analytical procedure was performed as described previously (Holubova et al., 2018). In total, 3 mg of plant tissue was extracted and analysed as described previously (Danieli et al., 2023). All plant hormone and stable isotope-labelled internal standards were purchased from OlChemIm (Olomouc, Czechia). Both UHPLC-MS/MS analyses were performed on a Nexera X2 UHPLC modular system coupled with a MS-8050 mass spectrometer (Shimadzu, Japan).

2.4. Identification of secondary metabolites

UHPLC-MS/MS analysis of the tomato leaves was performed on a Nexera X2 UHPLC (Shimadzu Handels GmbH) system coupled with a MS-8050 mass spectrometer (Shimadzu Handels GmbH). Chromatographic separation was performed on a UHPLC Acquity BEH C18 (150 × 3.0 mm; 1.7 μm particle size) column (Waters Corp., Milford, MA, USA) at 40 °C. The mobile phase was composed of 10 mM formic acid in water (A) and pure acetonitrile (B). All analytes were identified and quantified in negative ionisation mode ESI via multiple reaction monitoring (MRM) mode. The spray voltage was 3 kV, and the vaporiser and ion transfer tube temperatures were set to 320 °C. Standard solutions of secondary metabolites (analytical grade purity, Sigma-Aldrich, Czech Republic) were prepared in methanol at a concentration of 1 mM. All solutions were gradually diluted in the mobile phase to working concentrations ranging from 0.01 to 50 μM. Quantification was performed using the isotope dilution method with *p*-coumaric acid-d6 and salicylic acid-d4 (Toronto Research Chemicals, Canada) as internal standards.

2.5. Antioxidant enzyme activity

Frozen leaf samples were ground in liquid N_2 with 1% (w/v) polyvinylpyrrolidone and mixed with extraction buffer (0.1 M tris(hydroxymethyl)aminomethane (Tris)–HCl, pH 7.8, 1 mM ethylenediaminetetraacetic acid tetrasodium salt dihydrate (EDTA), 10 mM dithiothreitol (DTT), 10 mM sodium ascorbate). The homogenate was centrifuged at 16,600 × *g* for 15 min at 4 °C. The resulting supernatant was immediately used in enzyme activity measurements. The remaining sediment was washed with the same extraction buffer containing 1 M NaCl and used to measure the total concentration of bound peroxidases.

The activity of total soluble and total bound peroxidases (POX, EC 1.11.1.7) was spectrophotometrically determined at 430 nm (Multiskan GO, Thermo Fisher Scientific Oy, Finland) using 3,3 ´-diaminobenzidine (DAB) as a substrate (Ryslava et al., 2015). Soluble cytosolic POX are found in the supernatant after centrifugation of the plant extract, and membrane-bound POX can be released from the sediment by increased ionic strength. The pattern of the soluble POX isozyme was assessed after separation by 10% native polyacrylamide gel electrophoresis (PAGE), loading 30 μg of leaf protein per lane. POX isozymes were detected by staining gels in 50 mM phosphate buffer, pH 7.0 with 0.04% w/v DAB (dissolved in 96%EtOH) and 10 mM H_2O_2 (Ryslava et al., 2015; Spoustova et al., 2015). Catalase (CAT, EC 1.11.1.6) was detected at 240 nm based on the H_2O_2 decomposition rate (Spoustova et al., 2015).

The total activity of ascorbate peroxidase (APX, EC1.11.1.11) was determined as the decrease in ascorbate absorbance at 298 nm. The reaction mixture (200 μL) contained 80 mM 2-[4-(2-hydroxyethyl)piperazin-1-yl]ethane-1-sulphonic acid (HEPES)-KOH buffer, pH 7.0; 80 μM EDTA, 3 mM ascorbate, 9 μL plant extract and was initiated by 0.7 mM H_2O_2; the extinction coefficient 2.80 $mM^{-1}{\cdot}cm^{-1}$ for reduced ascorbate was used to calculate enzyme activity (Ismaiel and Papenbrock, 2017). The APX isozyme pattern was detected by 10% native PAGE (Mittler and Zilinskas, 1993). Superoxide dismutase (SOD, EC 1.15.1.1) isozyme patterns and activities were assessed by 10% native PAGE (Synkova et al., 2006). The activity of GST (EC 2.5.1.18) was spectrophotometrically determined in 1 mL of reaction mixture containing 140 mM phosphate buffer (pH 7.2), 1 mM reduced glutathione, 1 mM 1-chloro-2, 4-dinitrobenzene (CDNB) and 250 μL of suitably diluted plant extract, and absorbance was measured at 340 nm and 35 °C for 10 min on a SPECORD 250 PLUS Double-Beam UV-Vis spectrophotometer (Analytik Jena, Germany). The blank was measured as the change in absorbance of the mixture with demineralised water replacing the plant extract, thus determining the non-enzymatic conjugation of glutathione and CDNB substrates. GST and glutathione peroxidase (GPX, EC 1.11.1.9) isozyme patterns were detected by 10% native PAGE (Lin et al., 2002). Individual isoforms of enzymes were characterised by relative molecular weight, according to Ferguson (1964).

2.6. Total phenolic, flavonoid content, and antioxidant capacity

Tomato leaf (grounded in liquid nitrogen) samples were treated with 50% EtOH under continuous shaking for one hour at room temperature to extract phenolic compounds. The total content of phenolic compounds was determined using the standard Folin-Ciocâlteu colorimetric method, with slight modifications, as previously reported (Tupec et al., 2017). The total flavonoids were assayed using a modified version of the Dowd colorimetric method (Herald et al., 2012). Antioxidant capacity was measured in three assays, namely the ferric ion reducing power (FRAP) assay and the trolox equivalent assay with 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulphonate) ($ABTS^{+}$) and with 2,2-diphenyl-1-picrylhydrazyl (DPPH) (Tupec et al., 2017).

2.7. Determination of glutathione content

Total glutathione content was detected using Ellman's reagent (5,5-dithio-bis-(2-nitrobenzoic acid, DTNB) and the Anderson cyclic method (Anderson, 1985). Tomato leaves were homogenised in liquid nitrogen, and 5% sulfosalicylic acid was added in a 2:1 ratio. Subsequently, the samples were extracted for 15 min and then centrifuged for 10 min at 4 °C, 12 000g. The supernatant (300 μL) was neutralised with 13 μL of 4.2 M triethanolamine. In a total volume of 1 mL, the reaction mixture contained 0.207 mM NADPH, 30 mM phosphate buffer, pH 6.5, 1 mM DTNB, and 1 (U/mL) glutathione reductase (from *Saccharomyces cerevisiae*, Sigma-Aldrich, USA). The reaction was initiated with 50 μL of neutralised plant extract and measured spectrophotometrically at 412 nm for 10 min on a SPECORD 250 PLUS Double-Beam UV-Vis spectrophotometer (Analytik Jena, Germany) calibrated with reduced glutathione in the range from 0.03 to 1.0 nmol.

2.8. Determination of residual triazoles in the tomato fruit

Triazoles were detected in tomato peel by micellar electrokinetic chromatography (Kovac et al., 2021). Briefly, triazole residues were extracted from tomato peel with chloroform (acidified with 0.1% acetic acid) and cleaned through the SPE cartridge, subsequently repeating sorbent rinsing with pure chloroform. Chloroform extracts were vacuum evaporated to dryness at 30 °C using Labconco CentriVap (Refrigerated CentriVap Benchtop Vacuum Concentrator; Kansas City, MO, USA). Upon evaporation, the samples were redissolved in 1.5 mL of 100 mM Tris, 100 mM phosphoric acid in mixed hydro-organic sol vent (80/20 v/v water/methanol) and analysed by capillary electrophoresis (CE) on a7100 CE system(Agilent Technologies 7100 CE, Waldbronn, Germany) equipped with a UV–VIS diode array spectrophotometric detection system. The measurements were performed on an internally uncoated fused silica capillary with outer polyimide coating (Polymicro Technologies, Phoenix, AZ, USA) of 425/500 mm (effective/total) lengths and 50/375 μm (I.D./O.D.) at a 200-nm detection wavelength with a reference at 350 nm and at a constant capillary cassette temperature of 22 °C. For more details, please refer to Kovac et al., 2021.

2.9. Statistical analysis

Experiments were prepared in six biological repeats. All measurements were performed in at least triplicates. Data were analysed in SigmaPlot 12.0 (Systat Software Inc.) by ANOVA (Holm-Sidak method) and t-test. Differences were considered significant at $p \leq 0.05$. Heatmaps and principal component analysis (PCA) were plotted using the Seaborn library in Python (Waskom, 2021).

3. Results

In this study, we assessed the effect of penconazole (P) and/or tebuconazole (T) on the metabolism of tomato plants. For this purpose, we tested two triazole application methods: (i) soil drenching (henceforth abbreviated as s), assuming plant root uptake and transport through the xylem, and ii) foliar spraying (henceforth abbreviated f), which is the usual fungicide application method. We used the concentrations recommended by the manufacturer, spraying and watering weekly. In the leaves of the tomato plants treated with P and/or T, we monitored the phytohormonal balance, free amino acid shift, and antioxidant system components at 15 (treated twice with triazoles) and 35 (treated 5 times) days after the first application. The fresh weight of the treatment tomato plants (and plant parts) slightly differed from that of control plants (Fig. S1). The lowest shoot-to-root ratio was found in the fPT group and the highest in the sPT and sT groups (Fig. S1C).

3.1. Triazole effect on free amino acids and proteins

On day 15, all triazole-treated plants, except for fP, showed a significantly higher leaf content of total free amino acids than control plants (Fig. 1A). Over time, the total free amino acid content decreased, as observed on day 35. Only the fPT, sP, and sPT groups significantly differed from the other experimental groups. No significant change in protein content was found, except for the sP group in which protein content transiently increased in leaves (Fig. 1B).

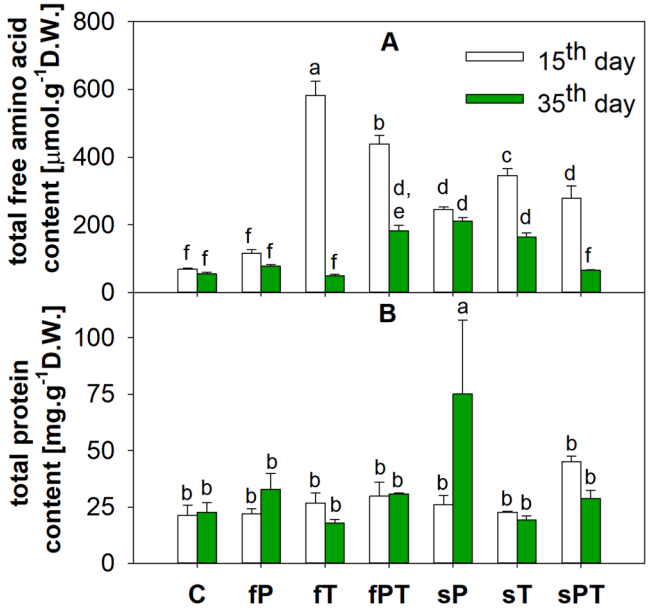

Fig. 1. Total free proteinogenic amino acid (A) and soluble protein (B) content in tomato leaves at 15 (white columns) and 35 (green columns) days after the first triazole application. Penconazole (P), tebuconazole (T), or their combination (PT) were applied foliarly (fP, fT, and fPT) or to the soil (sP, sT, and sPT) and compared with the control treatment without triazoles. The same letters above a bar indicate that no significant differences were found between groups. Each column bar represents the mean ± SD. All measurements were performed in triplicate, using mixed samples from 6 plants. Abbreviations: D.W., dry weight.

In leaves, some individual free amino acids, such as Gln and Asn (Fig. 2), substantially increased, especially in the sP, fPT, fP, and fT groups. As a case in point, the increase in Gln and Asn significantly contributed to the 9-fold increase in total free amino acids in the fT group on day 15 (Fig. 1). The sT group was rich in Arg and Lys on day 15, while the sPT group was rich in Gly on day 35 (Fig. 2).

On day 15, the relative content of Trp was significantly lower in all triazole-treated plants than in control plants (Fig. 2). Over time, in treated plants, the Trp concentration increased and was higher or at least similar to that of control plants, except for the fP group where the Trp content remained low; in the sP group, the Trp content decreased even further, below the detection limit. The Glu content also decreased (except for sPT, fP 35, and fT35), which may be related to the increased synthesis of Pro, as with other glucogenic amino acids (Fig. 2). On day 35, the highest content of Pro was detected in the leaves of the sP group.

3.2. Triazoles effect on phytohormonal balance

In tomato leaves, two bioactive phytohormones, namely *cis*-zeatin (cZ) and isopentenyl adenine (iP), were detected, as well as the cytokinin precursors *cis*- (cZR) and *trans*-zeatin riboside (tZR) and isopentenyl adenosine (IPR), in addition to the irreversibly modified cytokinin N-glucoside: isopentenyl adenine 9-glucoside (iP9G) (Fig. 3). The content of cytokinin (CK) precursors was significantly higher in all triazole-treated plants (except for sT) than in control plants on day 15. On day 35, only the sP and sPT groups showed a slightly higher content of CK precursors, albeit non-significant (Fig. 3A).

On day 15, the content of active CKs increased in all triazole-treated plants, except for sT (Fig. 3B), and the CK glucoside iP9G was significantly higher in fP, fPT, sP, and sPT than in control plants, remaining at least slightly higher on the next sampling day (Fig. 3C). The increased content of CK precursors (day 15) and iP9G (day 15 and 35) in triazole-treated groups suggests that triazoles elevate CK metabolism (Fig. 3).

The salicylic acid (SA) content was significantly higher in fP, sT, and fPT than in the control group on day 15. On day 35, the SA content was significantly higher in all groups, except for sPT, than in the control group (Fig. 3E). The ratio of salicylic to benzoic acid (BA) was also monitored for potential salicylic acid hydroxylase inhibition in treated plants (Fig. S2A). In control plants, the BA/SA ratio was approximately 0.5 on day 15; the ratio of triazole-treated plants was not significantly different. On day 35, the BA/SA ratio exceeded 2 in the control, fPT, and sPT groups but remained approximately 1 in the other groups (Fig. S2A). The ratio of salicylic acid 2-O-β-D-glucoside to SA was the same in all plants except for the sT group (Fig. S2B). Therefore, our results demonstrate the abundance of SA in triazole-treated plants, especially on day 15 (Fig. 3D, E).

On day 15, ABA was slightly higher in sP (1.5-fold), sPT (1.4-fold) and fP (1.3-fold) than in the control (Fig. 3F). However, on day 35, the concentration of ABA was (1.3–2.5-fold) higher in all groups treated with triazoles than in the control group (Fig. 3F).

3.3. Secondary metabolites

To assess whether triazoles stimulate the formation of phenylpropanoids, seventeen secondary metabolites were identified in tomato leaves by LC-MS, based on their appropriate standards, and expressed as g of dry weight (Table S1) or as % control (Fig. 4). In tomato leaves, chlorogenic acid was the most abundant secondary metabolite, followed by vanillic acid, gallic acid, and ferulic acid with caffeic acid (Table S1). On day 15, the chlorogenic acid content was significantly higher (1.3–1.7-fold) in all triazole-treated groups than in the control group. Furthermore, triazole treatment increased the content of vanillic acid in leaves on both sampling days.

After triazole treatment, changes in individual leaf phenylpropanoid compounds were apparent between day 15 and day 35. With some

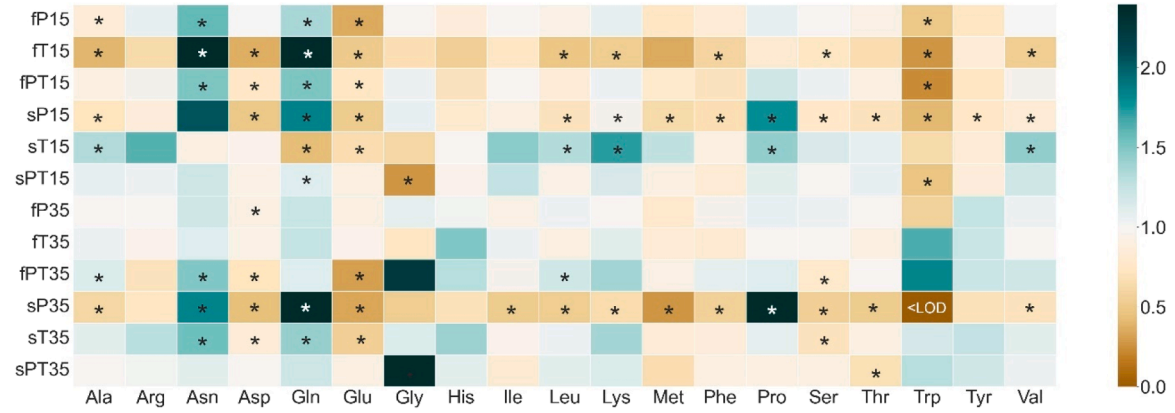

Fig. 2. The relative content of free amino acids determined in tomato leaves at 15 (label 15) and 35 (label 35) days after the first triazole application. Penconazole (P), tebuconazole (T), or their combination (PT) were applied foliarly (fP, fT and fPT) or to the soil (sP, sT, and sPT) and compared with the respective control without triazoles (C15 or C35). Asterisks denote significant differences ($p \leq 0.05$) between triazole-treated and control plants according to the *t*-test. The amount of individual free amino acids was related to the total free amino acid content (in μmol) and then compared with the control group. Trp content was below the limit of detection (<LOD) in the sP group on day 35. All measurements were performed in triplicates using mixed samples from 6 plants.

exceptions, abundance was lower on day 15 than on day 35 (especially in the sP group) (Fig. 4).

3.4. Antioxidant enzyme activities

To characterise the enzyme antioxidant system, we monitored the activity and isoform content of POX, APX, and SOD and determined the CAT activity. The activity of total soluble and membrane-bound POX was determined in tomato leaves (Fig. 5A,B). On day 15, the activity of total soluble POX was slightly higher in the leaves of triazole-treated plants than in control leaves from day 15, while the activity of membrane-bound POX was higher than that of soluble POX in all groups, including the control, suggesting a developmental effect rather than a triazole effect.

On day 35, soluble POX activities were mostly lower in triazole-treated plants than in control plants. Since no changes were observed in membrane-bound POX (Fig. 5A,B), we analysed individual POX isoforms by native electrophoresis (Fig. 6A,B), which showed both increases and decreases in the activity of some POX isoforms. Thus, the overall activity did not change significantly.

Six bands of POX isoforms were characterised based on their molecular mass, estimated using the Fergusson method. After day 15, the 240, 200, and 96 kDa POX isoforms showed decreased activity in fPT leaves. By contrast, on day 35, the activity of the 70 kDa POX isoform increased in the sT group (Fig. 6A).

APX regulates hydrogen peroxide concentration in cells. In tomato leaves, no significant changes in APX activity between treated and control plants were found on either experimental days (Fig. 5C). Two APX isoforms were identified, but the minor isoform of ~120 kDa was detected only on day 15 (Fig. S3A) and at higher activity levels in treatments groups, especially in fPT (2.4-fold), sT (2-fold), and fT (1.7-fold), than in the control group. In turn, the major isoform (~60 kDa) was slightly increased in sT, fP, and sPT on day 15 but decreased on day 35 (Fig. S3A,B).

SOD is an enzyme of the first-line antioxidant defence system. We detected three major SOD isoforms with ~70, 63, and 60 kDa (Fig. 6C, D). In leaves, in all triazole-exposed plants, the SOD activity of the ~70 and 60 kDa isoforms was only slightly increased or remained unchanged at 15 days after the first application, but the activity of the ~63 kDa isoform was rather lower, except in the sP and sT groups. After 35 days, the activity of all SOD isoforms decreased in the sP group; the isoforms of the other groups returned to control levels.

GPX is involved in the detoxification of H_2O_2 and organic hydroperoxides. GPX was detected in at least 5 isoforms, in tomato leaves (Fig. S3C,D). Most GPX isoforms were slightly decreased, especially the isoform with the lowest mobility or unchanged by triazole treatment in the sPT group on day 15.

The isoforms of GST, a conjugation enzyme of phase II of xenobiotic biotransformation, were also monitored by electrophoretic separation. We detected at least five GST isoforms (~ 90, 85, 77, 72, and 67 kDa) in leaves (Fig. 6E,F). By triazole treatment, GST activity either did not change or decreased. A decrease of ~90 kDa isoform was observed in sPT, sT, and fT plants. On day 35, the groups sP and sPT showed a decreased activity of all GST isoforms (Fig. 6F).

3.5. The effect of triazoles on total phenolics, flavonoids, antioxidant capacity, and glutathione

Total phenolics and flavonoids were correlated with antioxidant capacity using two radical quenching-based methods (DPPH and ABTS methods) and the FRAP method. The results from the determination of the antioxidant capacity by FRAP and ABTS correlated with each other, as did the findings of the ABTS and DPPH methods (Fig. S4). Moreover, the phenolic content correlated with the antioxidant capacity, as determined by ABTS (Fig. S4).

In leaves, the non-enzymatic antioxidant system, including total phenolics and antioxidant capacity, was downregulated on day 15. However, on day 35, the concentration of total phenolic compounds in the sP and sPT groups was significantly increased by at least 50% in relation to the control (Fig. 7).

Another important component of the antioxidant system, glutathione, was elevated on day 15, especially in sP and sPT leaves, but returned to control levels on day 35, except in fP leaves (Fig. 7). Despite the slight decrease in the sPT, sP and fT groups on day 15, the antioxidant capacity increased significantly in the sP group on day 35, as confirmed by ABTS and DPPH (Fig. 7).

The results from the PCA showed that all triazole-treated groups slightly differed from their control group 15 days after the first fungicide application but returned to control levels on day 35 (Fig. 8A). These findings indicate that plants likely adapted to triazole administration (Fig. 8B).

3.6. Triazoles remain in the peel of tomato fruit

Because tomato is a popular consumer product, the content of triazoles was determined on the 35th experimental day. In our experiment, the triazole concentration was under the limit of detection in tomato flesh. However, in tomato peel, residual triazoles were detected in all

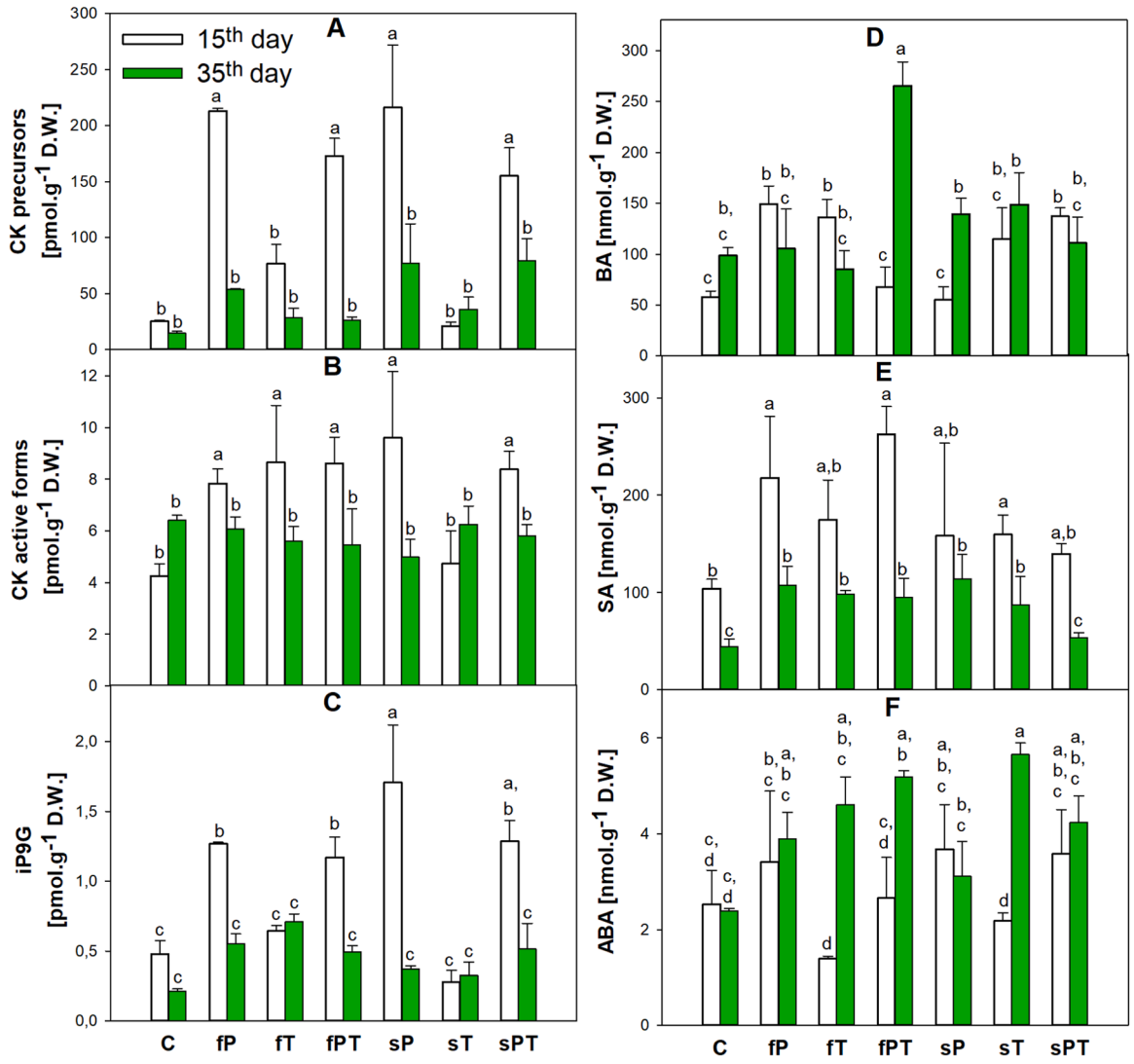

Fig. 3. Phytohormonal analysis of tomato leaves collected at 15 (white columns) and 35 (green columns) days after the first triazole application. Cytokinin precursors (A), active forms (B), or the irreversible modified N-glucoside iP9G (C) were determined with benzoic (D), salicylic (E), and abscisic (F) acid. Penconazole (P), tebuconazole (T), or their combination (PT) were applied foliarly (fP, fT, and fPT) or to the soil (sP, sT, and sPT) and compared with control without triazoles. The same letters above a bar indicate that no significant differences were found between groups. Each column bar represents the mean ± SD. All measurements were performed in triplicates using mixed samples from 6 plants. Abbreviations: ABA, abscisic acid; BA, benzoic acid; CK, cytokinins; D.W. dry weight; SA, salicylic acid.

groups after foliar application (Table 2). Interestingly, lower values were found in the case of a combination of triazoles than when a single substance was applied. When triazoles were applied directly to the soil, the presence of triazole fungicides was not detected even in the peel of tomato fruit. The highest amount was found for fT, which could suggest that tebuconazole is more persistent and less prone to metabolization than penconazole.

4. Discussion

Triazole fungicides play an ambivalent role in plants because they can both adversely affect plants as abiotic stressors and have a positive effect by activating the defence system, inducing priming, and/or regulating growth (Barroso Aragao et al., 2021; Bernardes et al., 2015; Desta and Amare, 2021; Fletcher et al., 2000; Shaki et al., 2022).

4.1. Plant response to triazoles can be divided into reaction and adaptation phases

To identify the prevailing role of penconazole and tebuconazole, we monitored tomato (*Solanum lycopersicum* L.) leaves at multiple levels, including the content of free amino acids and proteins (Figs. 1, 2), phytohormonal balance (Fig. 3), and antioxidant system (Figs. 4–7), after applying both triazoles and their combination weekly, either by foliar spraying or directly to the soil. Based on the results from all methods and PCA analysis, we can divide the overall reactions of the tomato plant into two phases, namely the reaction phase and the adaptation phase (Fig. 8B). The triazole-treated groups differed from the control on the 15th day (reaction phase) but were rather similar to both controls on the 35th day (adaptation phase).

Spraying tomato leaves with both penconazole and tebuconazole significantly reduced the fresh weight of shoots (Fig. S1A), thus reducing the shoot-to-root ratios. However, direct soil application of tebuconazole alone and in combination with penconazole significantly decreased the fresh weight of roots (Fig. S1A), increasing the shoot-to-root ratio (Fig. S1C). Therefore, direct watering with triazoles can negatively affect tomato roots.

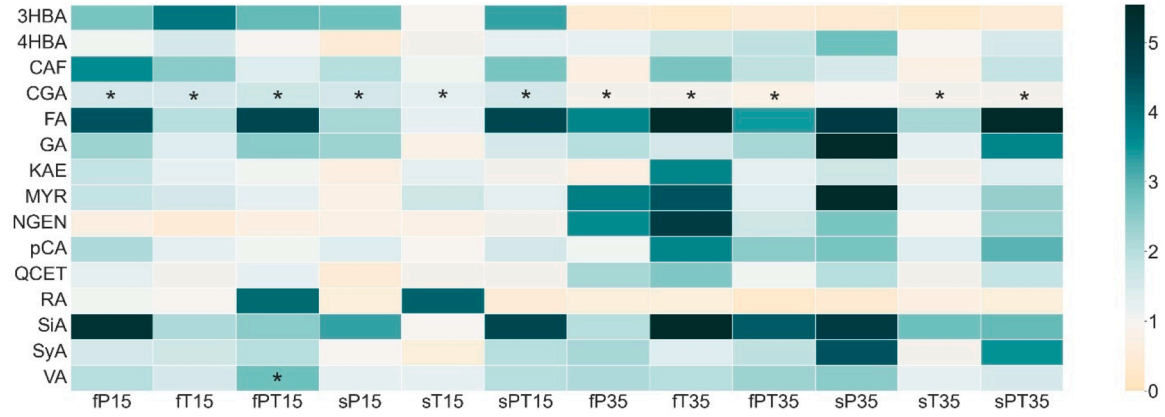

Fig. 4. Heatmap of the 17 secondary metabolites identified in tomato leaves at 15 (label 15) and 35 (label 35) days after the first triazole application. Penconazole (P), tebuconazole (T), or their combination (PT) were applied foliarly (fP, fT, and fPT) or to the soil (sP, sT, and sPT) and compared with the respective control without triazoles (C15 or C35). Asterisks denote significant differences ($p \leq 0.05$) between the triazole-treated and control plants according to the *t*-test. All measurements were performed in triplicates using mixed samples from 6 plants. Abbreviations: 3HBA, 3-hydroxybenzoic acid; 4HBA, 4-hydroxybenzoic acid; CAF, caffeic acid; CGA, chlorogenic acid; FA, ferulic acid; GA, gallic acid; KAE, kaempferol; LUT, luteolin; MYR, myricetin; NGEN, naringenin; NGIN, naringin; pCA, *p*-coumaric acid; QCET, quercetin, RA, rosmarinic acid; SiA, sinapic acid; SyA, syringic acid; VA, vanillic acid.

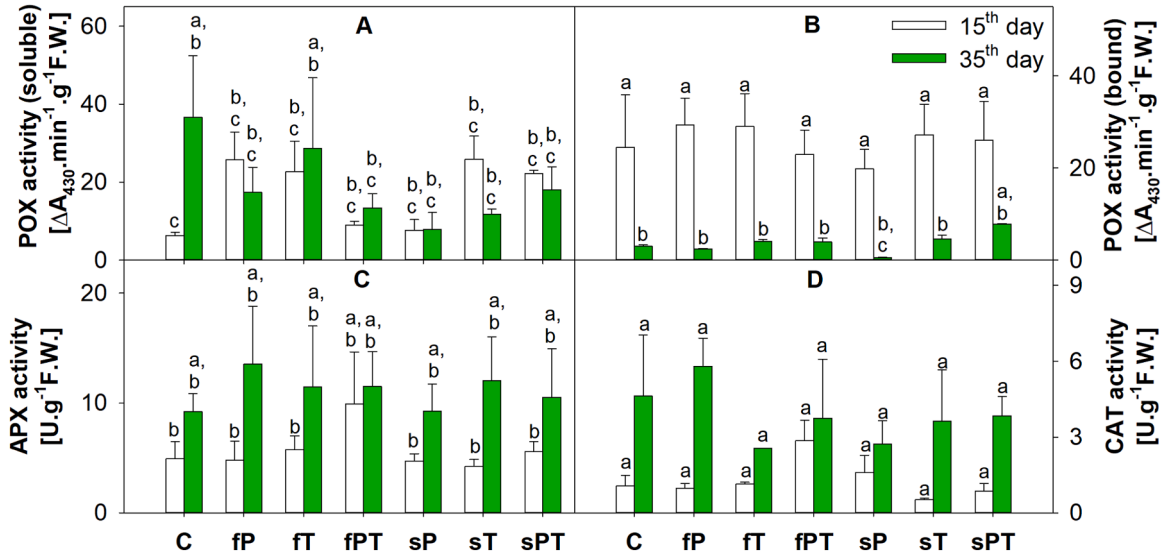

Fig. 5. Fresh weight activity of total soluble POX (A) and membrane-bound POX (B), APX (C), and CAT (D) at 15 (white columns) and 35 (grey columns) days after the first triazole application in leaves. Penconazole (P), tebuconazole (T), or their combination (PT) were applied foliarly (fP, fT, and fPT) or to the soil (sP, sT, and sPT) and compared with the control without triazoles. Different letters above each bar denote significant differences ($p \leq 0.05$) between plant groups according to a one-way analysis of variance (ANOVA; Holm-Sidak test). The same letters above a bar indicate that no significant differences were found between groups. Each column bar represents the mean ± SD. All measurements were performed in triplicates using mixed samples from 6 plants. Abbreviations: APX, ascorbate peroxidase; CAT, catalase; POX, peroxidases; F.W., fresh weight.

4.2. High content of cytokinin active forms and abscisic acid initiate plant defence

In fungi, the main target of action of triazoles is the inhibition of CYP51, thereby blocking ergosterol synthesis (Fera et al., 2009). However, triazoles can also bind to the active sites of other CYP, including non-target CYP in plants, particularly in the synthesis (or degradation) of phytohormones or the synthesis of phenolic or terpenoid compounds. Accordingly, through CYP inhibition, triazoles can affect phytohormone levels and thus increase plant tolerance to other stressors and influence plant growth and development (Fletcher et al., 2000; Kitahata et al., 2005; Ohnishi, 2018).

Several triazoles are regarded as plant growth regulators (Desta and Amare, 2021). Triazoles may regulate plant growth by altering the balance of key phytohormones, including gibberellins and ABA (Fletcher et al., 2000). ABA, in particular, is involved in plant acclimation and protection against various abiotic stresses, such as heat, cold, drought, and flooding (Fletcher et al., 2000). After 35 days of weekly exposure to penconazole and tebuconazole, ABA concentration increased in tomato plants (Fig. 3F). This finding is in line with the increased ABA and even CK levels induced by the triazoles triadimefon and hexaconazole and the resulting increased tuber growth previously observed in cassava (Gomathinayagam et al., 2007). Triazoles could prevent ABA catabolism to phaseic acid, an enzymatic step that is catalysed by the CYP-dependent monooxygenase CYP707A (Fletcher et al., 2000). CYP707A is inhibited by some triazoles, such as uniconazole and diniconazole, providing plants with drought stress resistance (Kitahata et al., 2005). For example, penconazole enhanced *Brassica napus* L. growth under drought stress (Rezayian et al., 2018). Increasing the ABA concentration helps plants to survive under water stress, as shown in wheat

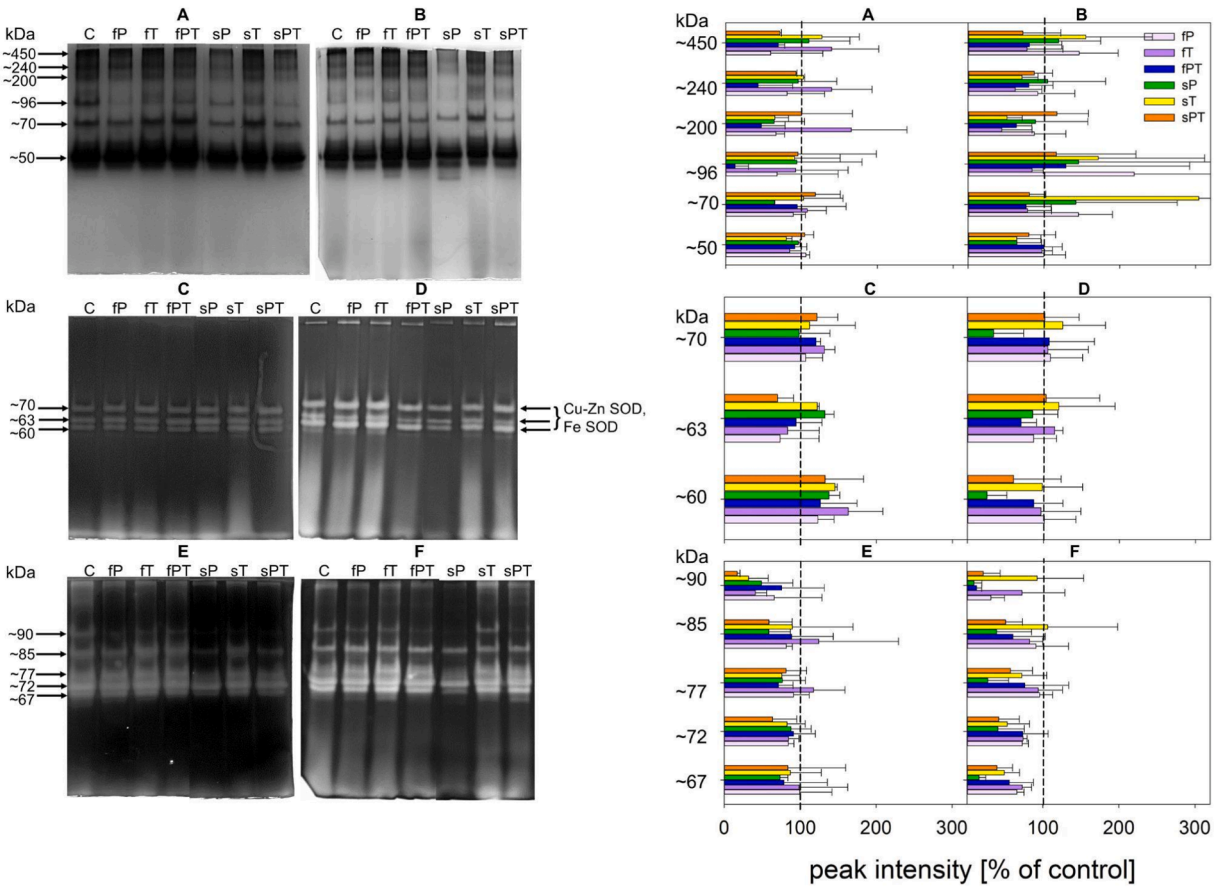

Fig. 6. Tomato leaf isoforms of soluble POX (A, B), SOD (C, D), and GST (E, F) detected after native electrophoresis in 10% polyacrylamide gels on day 15 (A, C, E) or day 35 (B, D, E) by densitometric evaluation (3 repetitions) of the band intensity (relative to the control) represented by a bar graph. The same amount of protein (30 μg) was applied in each lane. Similarly, the analysis of five polyacrylamide gel concentrations (5–10%) with appropriate native protein standards (not shown) enabled us to estimate the relative weight using the Ferguson method. Abbreviations: POX, peroxidases; SOD, superoxide dismutase; GST, glutathione-S-transferase.

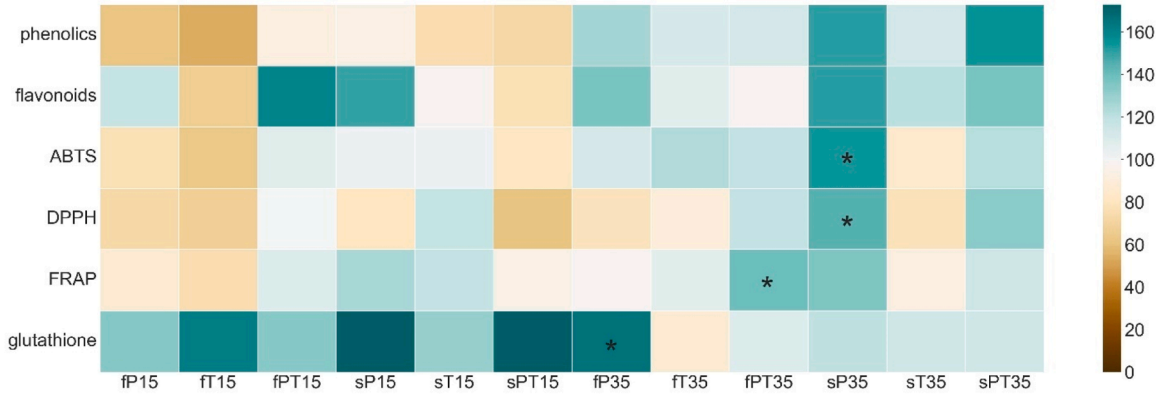

Fig. 7. Total phenolics, flavonoids, and antioxidant capacity were measured using three different methods (ABTS, DPPH, and FRAP), and the content of glutathione was determined at 15 (label 15) and 35 (label 35) days after the first triazole application in tomato leaves. Penconazole (P), tebuconazole (T), or their combination (PT) were applied foliarly (fP, fT, fPT) or to the soil (sP, sT, sPT) and compared with the control without triazoles. The values are expressed as % of control (C15 or C35). Asterisks denote significant differences ($p \leq 0.05$) between treated and control plants according to the one-way ANOVA (Holm-Sidak test). All measurements were performed in triplicates using mixed samples from 6 plants.

cultivars, mangos, apples, snap beans, rapeseed, and rice varieties (Maheshwari et al., 2022).

IAA is another phytohormone important for plant growth and development. Here, we detected a depletion of its precursor Trp on day 15 in leaves (Fig. 2), which could be related to the production of this auxin after exposure to triazoles. As a case in point, the foliar application of paclobutrazol on *Marubakaido* apple increased the endogenous levels of IAA in a previous study (Opio et al., 2020). Similarly, tebuconazole application in combination with strobilurin significantly increased the activity of IAA oxidase, enhancing auxin content, growth, and development (Punitha et al., 2016). Other pathways can, nevertheless, deplete Trp, including the formation of the indoleamines serotonin and

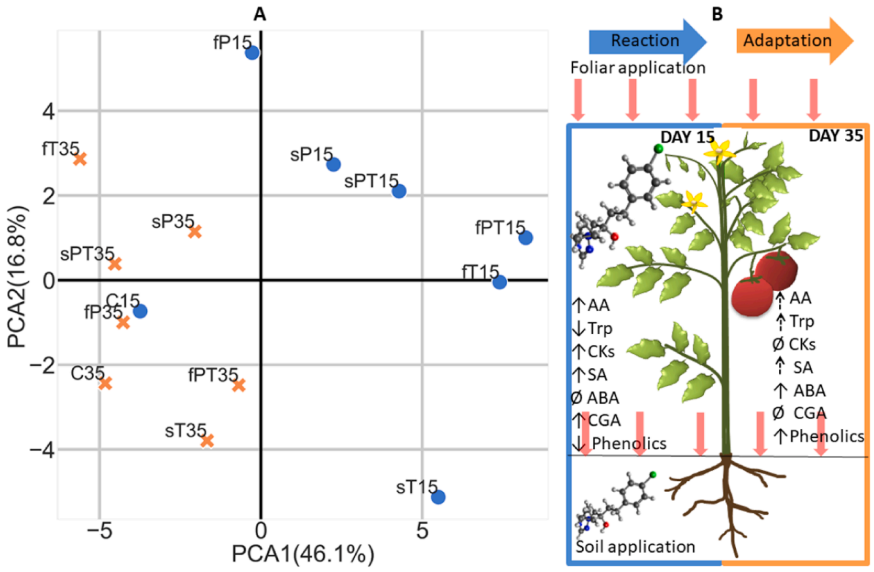

Fig. 8. The PCA results of all experimental groups based on metabolism analysis in leaves at 15 (label 15, blue dots) and 35 (label 35, orange crosses) days after the first triazole application (A). Penconazole (P), tebuconazole (T), or their combination (PT) were applied foliarly (fP, fT, fPT) or into the soil (sP, sT, sPT). In the control group, triazoles were substituted with deionised water (C15 or C35). Graphical illustration highlighting the main results (B). The plant response to triazoles can be divided into the reaction phase and the adaptation phase. The symbols ↑↓ Ø correspond to increased, decreased, or unchanged values, respectively, in comparison with control plants, and a dashed arrow indicates that only some treated groups responded to the triazole application. Abbreviations: AA, amino acids; ABA, abscisic acid; CGA, chlorogenic acid; CKs, cytokinins; SA, salicylic acid; Trp, tryptophan.

Table 2
Detection of residual triazoles in tomato peel on the 35th experimental day. Penconazole (P), tebuconazole (T), or their combination (PT) were applied foliarly (fP, fT, fPT) or to the soil (sP, sT, sPT). In the control group (C), triazoles were substituted with deionised water. Abbreviations: <LOD, under the limit of detection.

Group	Triazole in tomato peel [μmol.kg^{-1}]
C	0
fP	1.74 ± 0.08
fT	2.34 ± 0.11
fPT	0.28 ± 0.03 (P) 0.15 ± 0.02 (T)
sP	<LOD
sT	<LOD
sPT	<LOD

melatonin, which are involved in plant growth and development, as well as nicotinamide, a component of NAD and NADP, which are crucial for energy metabolism (Corpas et al., 2021; Kaur et al., 2015).

As expected, considering that CKs play a key role in cell proliferation, shoot growth and differentiation, promoting lateral growth and delaying senescence (Fletcher et al., 2000; Pavlu et al., 2018), the content of CK precursors, active forms and the irreversibly modified N-glucoside iP9G (except for sT group) increased on day 15, indicating active CK metabolism (Fig. 3A-C). CYP is also involved in CK metabolism; more specifically, CYP735A catalyses the hydroxylation of iPRTP (isopentenyl adenine riboside 5´-triphosphate), iPRDP (iPR 5´- diphosphate) and iPRMP (iPR 5´-monophoshate), forming trans-zeatin (tZ) (Takei et al., 2004). Biosynthesis of isopentenyl adenine (iP) and tZ takes place in roots, and these active CKs are transported by the xylem to shoots, where cis-zeatin is produced via a different prenyl-tRNA pathway (Sakakibara, 2021). Due to the complexity of CK metabolism, we have not been able to assess the effect of CYP735A on the hydroxylation of the CK side chain from our results thus far; moreover, iP with a non-hydroxylated side chain is also active, so the physiological significance of this modification remains unclear.

Some triazoles delay leaf senescence in many plant species by increasing CK levels (Fletcher et al., 2000). A well-known connection between nitrogen metabolism and CKs is nitrate supplementation-induced CK biosynthesis in roots (Pavlu et al., 2018). Triazoles stimulate CK synthesis, enhance chloroplast differentiation and chlorophyll biosynthesis and prevent chlorophyll degradation (Desta and Amare, 2021; Fletcher et al., 2000). Triazole-treated *Brassica napus* L. showed increased photosynthetic ability and delayed senescence, which improved the morphological characters and yield components (Ijaz et al., 2015). Foliar treatment of rapeseed plants (*Brassica napus* L. ssp. napus cv. Linetta) with triazole (BAS 111) markedly increased CK content, delaying senescence (Grossmann et al., 1994). In *Camelina sativa* L. Crantz, paclobutrazol enhanced the endogenous levels of CKs, promoted chlorophyll formation, and increased the activity of antioxidant enzymes (Kumar et al., 2012).

Among the phytohormones, SA and its precursor BA were also investigated for the possible negative effects of penconazole and tebuconazole or their combination on this biosynthetic pathway with benzoic acid 2-hydroxylase (also CYP). At the fungicide concentrations used in this study, however, BA hydroxylation did not appear to be inhibited by tebuconazole or penconazole (Fig. 3D, E, S2). Although the biosynthesis of salicylic acid from isochorismate or cinnamate via o-coumarate without the involvement of hydroxylation must be taken into account (Chen et al., 2009). These results contradict previous findings showing that paclobutrazol inhibits SA biosynthesis from BA (Li et al., 2015).

4.3. Role of low aspartate and glutamate concentrations in the triazole-treated plants

In addition to phytohormones, we also monitored free amino acids, many of which play active roles in plant development and contribute to plant responses to environmental stresses (Trovato et al., 2021). The triazole-treated groups stood out, especially for their reduced Asp and Glu levels on both day 15 and 35 (Fig. 2). One of the reasons for the relatively reduced Glu content in triazole-treated tomato leaves is the synthesis of glutathione (Fig. 7), which is an important substance for detoxification and an agent against oxidative stress. Gly is also required for glutathione synthesis, and its content varies with photorespiration (Timm and Hagemann, 2020). The increase in Gln and Asn content may also be related to the conversion of Glu to Gln and Asp to Asn during

nitrogen mobilisation (Fig. 2). In turn, Asp and Glu are used in the synthesis of pyrimidine and purine nucleotides used in energy metabolism and in nucleic acid synthesis (Noctor et al., 2006).

4.4. Penconazole and tebuconazole can induce lignification of the plant cell wall

Triazoles may cause severe abiotic stress by producing ROS, significantly altering plant quality and productivity. In response to stress, plants increase the levels of defence compounds, such as phenolics, which maintain redox homoeostasis, chelate metal ions, and participate in plant defence (Sambangi, 2022). For this reason, we analysed the antioxidant system of plants, monitoring differences in antioxidant enzyme activities (Figs. 5, 6) and antioxidant capacity (Fig. 7) and in the content of phenylpropanoid compounds (Fig. 4).

The results highlighted differences in phenylpropanoid content due to penconazole and tebuconazole, which were highly correlated with the antioxidant capacity (Figs. 4, 7).

Even though triazole compounds induced stress tolerance in plants and enhanced the antioxidant potential in previous studies (Kraus and Fletcher, 1994; Senaratna et al., 1988; Senaratna et al., 2003), neither spraying nor watering with triazoles caused marked changes in the plant antioxidant system. But several differences were identified between control and treated plants (Figs. 6,7). The total content of phenolics and flavonoids initially decreased on day 15 (Fig. 7). Spraying penconazole has been shown to increase the activity of polyphenol oxidase in sesame, which may decrease the content of phenolic compounds and thus enhance cell wall growth through lignification (Heydari et al., 2019).

In tomato leaves, chlorogenic acid was the most abundant secondary metabolite among the 17 phenylpropanoids identified in this study and significantly increased upon triazole exposure (Fig. 4, Table S1). Chlorogenic acid is known as a prominent antioxidant with a higher antioxidant capacity than other common antioxidants, such as vitamins C and E, protecting cells against the damaging effects of ROS (Lu and Yeap Foo, 2000).

Ferulic acid and sinapic acid were the most abundant at 35 days after weekly exposure to triazole. Since lignin can form a physical barrier to the penetration of xenobiotics, some lignin biosynthesis precursors may be involved in this process. Lignin is typically polymerised from three phenylpropanoid monomers, *p*-coumaryl, coniferyl, and sinapyl alcohols. Lignification may also be sensitive to triazoles because cinnamic acid 4-hydroxylase is CYP, which catalyses aromatic ring 4-hydroxylation of cinnamic acid into *p*-coumaric acid, the most direct precursor of *p*-coumaryl alcohol (Chen et al., 2011). Here, the content of *p*-coumaric acid was only slightly increased or unchanged after applying triazoles. These results suggest that this P450-monooxygenase was not inhibited (Fig. 4). Such inhibition could also have caused feedback inhibition of phenylalanine ammonia-lyase, a key enzyme of the phenylpropanoid pathway, triggering considerable problems in these plants (Blount et al., 2000). The levels of individual phenylpropanoids differed between day 15, when they were much lower in triazole-treated leaves than in control leaves, and day 35, when the opposite was observed (Fig. 4). This result matches the total content of phenolic compounds, flavonoids, and antioxidant capacity (Fig. 7).

4.5. Activities of antioxidant enzymes returned to the control level after 35 days of triazole application

No major differences were found in the activity or the abundance of individual isoforms of antioxidant enzymes (POX, APOD, CAT, and GPX), in leaves (Figs. 5, 6), demonstrating that none of the concentrations and application intervals of tebuconazole and penconazole significantly affected the tomato antioxidant enzymes. Upon exposure to triazoles, the 70- and 60-kDa isoforms of SOD slightly increased, while the approximately 63 kDa isoform decreased (Fig. 6C,D). The isoforms with molecular masses of 60 and 63 kDa could correspond to Cu/Zn tetramers with molecular masses of 15.3 and 15.9 kDa, respectively, given the known tomato genome (Feng et al., 2016). By contrast, under severe abiotic stress, applying triazoles should elevate both enzymatic and non-enzymatic components of the antioxidant system, based on the literature (Heydari et al., 2019; Shaki et al., 2022). For example, triazole application increased APX, CAT, SOD and POX activity under severe abiotic stress, such as drought (Rabert et al., 2014; Sofy et al., 2020; Wang et al., 2022) and high salinity (Heydari et al., 2019; Tuna, 2014). These findings suggest short-term changes in the antioxidant system in response to triazoles soon after their application. Therefore, results likely show a return to antioxidant system balance.

4.6. Penconazole and tebuconazole did not significantly influence the detoxification potential

GSTs are key biotransformation enzymes that catalyse conjugation with glutathione and can thus detoxify xenobiotics. In addition, triazole derivatives, e.g., fenchlorazol-ethyl, are considered safeners, protecting the crop from the effects of herbicides. These substances may promote herbicide metabolism and increase GST expression and activity and the amount of glutathione and transporters for conjugated xenobiotics (Jia et al., 2022). In Arabidopsis plants, some safeners increased GST activity, and some types of abiotic stress increased GST expression (DeRidder et al., 2002). In tomato plants, at least five GST isoforms were identified in leaves, but no isoform responded to triazole application with increased activity (Fig. 6E, F). The activity of some isoforms was even reduced, the concentrations of penconazole and/or tebuconazole used in this study did not increase the conjugation of xenobiotics with glutathione, and hence the detoxification potential of the plant remained unchanged.

4.7. Issues concerning triazole residues in plants

For tomato consumers, the main danger may lie in the residual content of triazoles, which can adversely affect the human biotransformation system (Jaklova Dytrtova et al., 2023; Zarn et al., 2003). In addition to increased environmental pollution, extensive triazole use has caused many food safety problems (Li et al., 2022). For example, tebuconazole residues were detected in various fruits and vegetables, such as peaches, passion fruits, tomatoes, pepper, and others (Li et al., 2022; Kovac et al., 2021; Sharma et al., 2019). In a similar experiment with penconazole, tebuconazole, and cyproconazole, residual triazole fungicide concentrations higher than the recommended maximum were detected in tomato peels (Kovac et al., 2021). Here, the triazole residues were detected only upon foliar application (Table 2). Although the combination showed the lowest residual values, various triazole metabolites (degradation products) may persist on the tomato surface.

Nutritional parameters, especially the proteinogenic amino acid content, antioxidant capacity and phenolic compound content, were also affected by tebuconazole, penconazole, and their combination in tomato fruits (Hyskova et al., 2023). Although triazoles play a key role in antifungal protection and show several beneficial effects on plant metabolic pathways, their residues raise some concern because they may accumulate in edible plant parts, soil, and underground water.

5. Conclusion

Most metabolic changes, including increases in free amino acid, CK, salicylic acid, and chlorogenic acid content, occur 15 days after the first treatment in tomato plants. As shown by principal component analysis, all triazole-treated groups significantly differ from the control on the 15th day, suggesting a reaction phase to the triazole exposure and, thus, enabling the tomato plants to respond to stress more quickly and vigorously. These findings support our second hypothesis according to which triazoles activate the plant defence system in the reaction phase. However, after repeated triazole application, the tomato plants show

increased concentrations of phenolic compounds and ABA, with no significant change in the antioxidant system. Accordingly, after 35 days, tomato plants reach the phase of adaptation to abiotic (chemical) stressors. As shown by principal component analysis as well, triazole-treated groups cluster together with both controls from the 15th to the 35th day, demonstrating that many metabolic parameters return to their initial values.

Ethical approval

The research presented in this study has not involved any human or animal testing.

CRediT authorship contribution statement

Conceptualization: MJ, JJD, HR; Data curation: VH, KB; Funding acquisition KB, JJD, SCZ; Investigation KB, DK, TK, AŠ, MV, ARZ, IK, OV, SCZ; Methodology: SCZ, TK, VH, JJD; Project administration MJ, JJD; Resources MJ, JJD; Supervision VH; HR, TK, MJ; Writing - original draft VH, KB, HR; Writing - review & editing MJ, OV, JJD, SCZ.

Declaration of Competing Interest

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

Data availability

Data will be made available on request.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.ecoenv.2023.115729.

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