Increased benzoxazinoid (Bx) levels in wheat seedlings via jasmonic acid treatment and etiolation and their effects on Bx genes including Bx6

Masayuki Sue*, Miha Fujii, Takahiro Fujimaki

Department of Agricultural Chemistry, Faculty of Applied Biosciences, Tokyo University of Agriculture, Sakuragaoka 1-1-1, Setagaya, Tokyo, 156-8502, Japan

ARTICLE INFO

Keywords:
Benzoxazinoid
Bx6
Etiolation
Jasmonic acid
Tritium aestivum

ABSTRACT

Wheat accumulates benzoxazinoid (Bx) as a defensive compound. While Bx occurs at high concentrations, particularly in the early growth stages, its mechanism of regulation remains unclear. In the present study, we first examined the effects of several plant hormones on Bx concentrations in wheat seedlings. Among the compounds tested, jasmonate (JA) elevated the concentrations of DIMBOA-Glc (2-β-D-glucoside of 2,4-dihydroxy-7-methoxy-1,4-benzoxazin-3-one), the primary Bx species in intact wheat seedlings, without a significant increase in HDMBOA-Glc (4-O-methyl-DIMBOA-Glc), which is known to be upregulated by stresses. In addition, growing the plants in the dark increased DIMBOA-Glc levels. Quantification of the Bx-biosynthetic genes showed that TaBx8 (UDP-Glc:Gx glucosyltransferase) was influenced by neither JA nor etiolation, indicating that TaBx8 is under the regulation mechanism distinct from the mechanisms influencing the others. In addition, none of the other gene expression patterns exhibited considerable correlation with DIMBOA-Glc accumulation. Since there was no correlation between transcript levels of the genes involved in Bx biosynthesis and Bx accumulation, other factors may control the levels of Bx in wheat. In the course of gene analyses, we isolated TaBx6, one of the last two genes that had not been identified in wheat in the DIMBOA-Glc biosynthetic pathway. All the four TaBx6 genes cloned in the present study were expressed in Escherichia coli and characterized their activity.

1. Introduction

Benzoxazinoids (Bx) represent one of the most predominant families of defensive secondary metabolites in wheat (Triticum aestivum), maize (Zea mays), and rye (Secale cereale). The structural variations of Bx and their physiological functions have been explored extensively in several reviews [1–3]. The major Bx species vary across plants, tissues, and growth stages. In rye, for example, most of the Bx species in the shoots is DIBOA-Glc (2-O-glucoside of 2,4-dihydroxy-1,4-benzoxazin-3-one), while DIMBOA-Glc (7-methoxy-DIBOA-Glc) and DIBOA-Glc exist at comparable concentrations in the roots [4]. In wheat and maize, DIMBOA-Glc is the predominant Bx species both in the aerial and underground plant parts [5]. Generally, the concentrations of Bx are higher in young plants (a few days after germination) and gradually decrease as plants grow, suggesting that Bx are involved in defense systems particularly in the early growth stages.

Enhancing the Bx production capacity of plants could minimize the amount of pesticides applied in the field. However, the mechanisms that regulate Bx-biosynthesis and accumulation have not yet been elucidated. The DIBOA biosynthetic pathway was first determined in maize [6], in which ZmBx1–ZmBx5 convert indole-3-glycerolphosphate into DIBOA (Fig. 1). Later, their orthologs in wheat (TaBx1–TaBx5) were isolated [7,8]. Subsequently, DIBOA undergoes glucosylation by UDP-Glc:Gx glucosyltransferase (Bx8) [9–11], followed by two steps including oxidation and methylation by Bx6 and Bx7, respectively, to yield DIMBOA-Glc [12,13]. Bx6 and Bx7 have been identified in maize (ZmBx6 and ZmBx7) but not yet in wheat. Recent reports on the isolation of rye Bx-related genes (ScBx1–ScBx6 and ScBx8) [11,14,15] indicate that the pathway is also conserved between maize and rye. More recently, rye Bx7 (ScBx7) was isolated [16] although it has not been characterized enzymatically. Bx1–Bx8 loci in maize are located close to each other on the short arm of chromosome 4, and are proposed to form a gene cluster [6,9,12,13] that facilitates the coordinated expression of the members. However, this is not the case in wheat and rye, in which the loci are dispersed across several chromosomes [7,11,17].

Some stress factors could influence Bx production and its composition [18–20]. One of the most notable changes reported is increasing HDMBOA-Glc accumulation. HDMBOA, the aglycone of HDMBOA-Glc,
Biochemistry and Biophysics Reports 27 (2021) 101059

2. Materials and methods

2.1. Plant growth conditions

Wheat (T. aestivum cv. Asakazekomugi) seeds were washed in 70\%(v/v) ethanol/H\textsubscript{2}O for a minute and sterilized further by treatment with sodium hypochlorite solution for 15 min. Subsequently, the seeds were rinsed with distilled water and imbibed in water for 2 h at room temperature. The seeds were placed on 0.8\%(w/v) agar containing a plant hormone in a plant culture box (6 seeds/box) and grown at 25°C under a 12-h photoperiod. Plant hormones (abscisic acid, gibberelline A\textsubscript{3}, salicylic acid, jasmonic acid, indole acetate, and 1-aminocyclopropane-1-carboxylate) were purchased from Sigma-Aldrich Corp. (St. Louis, MO, USA) and FujiFilm-Wako pure chemical corporation (Osaka, Japan) and solved in either ethanol or water.

2.2. Analyses of Bx concentrations and Bx-gene expression levels in wheat shoots

To quantify the Bx concentrations in wheat shoots, each plant was separated into a coleoptile and each leaf, and the tissues were weighed. The tissues were frozen in liquid nitrogen and ground into powder. Thereafter, the Bx was extracted using 10–20 times of volume of methanol. The extracts were diluted by adding equal volume of water and the mixtures were chilled at –25°C for 20 min. The resultant precipitate was removed by centrifugation, and the Bx in the supernatant was quantified using a HPLC system (LC-10ADvp, Shimadzu, Kyoto, Japan) equipped with SPD-M20A photodiode array detector (Shimadzu). Chromatography was performed on a Kinetex XB-C18 column (4.6 × 150 mm, 5 μm; Phenomenex, Torrance, CA, USA) under the following conditions: a linear gradient from A:B = 91:9 to 85:15 for 15 min (where A = H\textsubscript{2}O with 0.1% trifluoroacetic acid and B = acetonitrile) at 40°C and a flow rate of 1.0 mL/min. The concentrations of Bx were evaluated by monitoring the absorption at a wavelength of 280 nm.

For the analyses of the transcript levels of Bx-related genes (TaBx1–TaBx6, TaBx8), total RNA was prepared using RNaseasy Plant mini kit (QIAGEN, Germany), and reverse transcribed using PrimeScript RT Master Mix (TaKaRa Bio Inc., Tokyo, Japan) with a mixture of random hexamers and oligo dT as primers. Real-time PCR was carried out using SYBR Green PremixEX Taq II (TaKaRa Bio) and sets of gene-specific primers (Table S1). A two-step PCR protocol was employed for the amplification where denaturation and annealing-elongation were carried out at 98°C and 60°C, respectively. Other qPCR conditions were set according to the manufacturer’s instructions. The gene expression levels were normalized by RNase inhibitor-like protein (RLJ) as a housekeeping gene [23].

2.3. Western blot

Wheat shoots were ground in 5 vol of an extraction buffer (100 mM Tris-HCl [pH 7.5], 25\%(w/v) sucrose, 5\%(v/v) glycerol, 10 mM EDTA, 5 mM KCl, and 1 mM DTT) and the supernatant was recovered from centrifugation at 15,000 g for 15 min. Microsomal fraction was obtained as the pellet of a further centrifugation at 150,000 g for 60 min. After resuspension of the pellet in the extraction buffer, the protein was subjected to SDS-PAGE and then transferred to Immobilon-P PVDF membrane (Merck Millipore). TaBx5 was probed using a polyclonal antibody raised against a TaBx5 fragment as a primary antibody and anti-rabbit IgG HRP conjugate (Promega, Madison, WI, USA) as a secondary antibody followed by detection with ECL Prime Western Blotting Detection Reagent (GE Healthcare UK Ltd., Buckinghamshire, UK) as a substrate.

2.4. Isolation of wheat Bx6 gene (TaBx6)

To obtain the wheat Bx6 (TaBx6) sequence using 3′-RACE and 5′-RACE, cDNA that could be used for both RACE reactions was synthesized using Gene Racer kit (Thermo Fisher Scientific, Waltham, MA, USA) using total RNA from 72-h-old wheat shoots (cv. Chinese Spring) as a template. To isolate the TaBx6 fragment, we first designed a set of primers based on the rye Bx6 (ScBx6; GenBank accession number...
reaction was conducted in 50 mM HEPES (pH 7.2) containing 1 mM action product, TRIBOA-Glc, using HPLC as described above. The His-tagged TaBx6 was purified by affinity chromatography using supernatant of centrifugation (15,000 rpm for 15 min). The N-terminal cDNA, including the coding sequence of TaBx6 (TaBx6-1), was amplified using a set of primers, one of which corresponds to the 5′-untranslated region (forward primer) and the other is the adaptor primer for 3′-RACE (reverse primer).

For the isolation of the other TaBx6s, the fragments of genomic DNA were first amplified using a set of primers supposed to amplify regions containing introns as well as exons (the forward and reverse primers were similar to those used for 3′-RACE and 5′-RACE, respectively) that was estimated from an alignment of the maize (ZmBx6) and ScBx6. Several fragments with different lengths were sequenced, and specific forward primers were designed based on intron sequences. A second PCR was performed using genomic DNA (template), the designed forward primers, and reverse primers (designed from exons) to obtain exons (including intron) sequences that were different from TaBx6-1. After the isolation of new exon sequences, we designed new specific primers and performed 3′-RACE and 5′-RACE followed by PCR to amplify their full-length coding sequences. During the procedures, pBluescript SK(−) and DH5α were used as cloning vectors and an E. coli strain, respectively. All the primers used are listed in Table S1. PrimeStar max DNA polymerase (TaKaRa Bio) was used in all the reactions. The nucleotide sequences of TaBx6-1, TaBx6-2, TaBx6-3, and TaBx6-4 have been submitted to the GenBank/EMBL/DDBJ databases with accession numbers LC519324, LC519325, LC519326, and LC519327, respectively.

2.5. Heterologous expression of TaBx6 and validation of its activity

The TaBx6s were amplified by PCR using the cloned TaBx6 as a template to yield fragments flanked by recognition sites of Ndel and HindIII (TaBx6-1 and TaBx6-4) or Ndel and EcoRI (TaBx6-2 and TaBx6-3). After digestion using the restriction enzymes, the fragment was ligated into the Ndel-HindIII or Ndel-EcoRI site of pET28. After sequence verification, the resulting plasmid was further transferred into E. coli, BL21CodonPlus (DE3)-RIL. Subsequently, the cells were cultured in 150 mL of Luria-Bertani broth containing kanamycin and chloramphenicol at 37 °C till the OD600 value reached 0.7, and protein expression was achieved by the addition of IPTG (1 mM) followed by a 16-h culture at 20 °C. The cells were harvested by centrifugation (5000g, 5 min) and resuspended in 5 mL of Tris-HCl (pH 7.2). After cell disruption by sonication (20 s × 6), the soluble enzyme fraction was recovered as the supernatant of centrifugation (15,000 rpm for 15 min). The N-terminal His-tagged TaBx6 was purified by affinity chromatography using TALON metal affinity resin (Clontech, USA). The 2-oxoglutarate-dependent dioxygenase activity of TaBx6 was evaluated by detecting the reaction product, TRIBOA-Glc, using HPLC as described above. The reaction was conducted in 50 mM HEPES (pH 7.2) containing 1 mM FeSO4, 10 mM 2-oxoglutarate, and 10 mM ascorbate with various concentrations of DIBOA-Glc at 30 °C. Kinetic parameters (kcat and Km) were calculated by fitting the data to Michaelis-Menten equation using SigmaPlot (Systat Software Inc., San Jose, CA, USA).

3. Results and discussions

3.1. Effects of plant hormones on DIMBOA-Glc concentrations in wheat shoots

In the present study, we first intended to determine conditions that can up- or downregulate Bx concentrations without converting DIMBOA-Glc into HDMBOA-Glc. We attempted to evaluate the effects of plant hormones on Bx concentrations in wheat. The time courses of Bx concentrations were recorded from 72 to 144 h after imbibition. Among the hormones examined, abscisic acid inhibited germination or growth extremely at concentrations higher than 1 μM, and lower concentrations (0.1 and 0.01 μM) did not influence Bx concentrations. The other compounds, excluding JA, also had no significant effect on Bx concentrations at concentrations between 0.1 and 100 μM (data not shown).

JA administered at 100 μM elevated DIMBOA-Glc and HDMBOA-Glc concentrations in 72-h-old plants more than 4- and 10-fold, respectively (Fig. 2). However, the treatment reduced the fresh weight of the shoots to about 50% that of the untreated plants. Considering plant growth influences Bx concentrations considerably, the finding was potentially an indirect effect of JA. While there was no significant difference in the plant growth at concentration of JA below 10 μM, the concentration of HDMBOA-Glc was 2.5 times as high as that of the control when JA was administered at 10 μM. For our purposes, it is essential to exclude the influence of growth retardation and to avoid conversion of DIMBOA-Glc into HDMBOA-Glc. Therefore, we conducted further analyses using 5 μM JA, at which the plants retained growth comparable to growth in the untreated individuals, and less conversion of DIMBOA-Glc to HDMBOA-Glc was observed (Fig. 2). The results indicate that JA could be a useful tool for distinguishing between constitutive DIMBOA-Glc production and inducible HDMBOA-Glc production. In the following experiments, the DIMBOA-Glc concentrations in wheat are referred to as Bx concentrations because DIMBOA-Glc accounted for more than 90% of all the Bx species detected.

3.2. DIMBOA-Glc concentrations in JA-treated and etiolated wheat

For further evaluation of the effects of JA, each plant was separated into three parts, i.e., coleoptile, 1st leaf, and 2nd leaf (96 h and after), and the Bx (DIMBOA-Glc) concentrations in each plant part were quantified (Fig. 3). In untreated (control) plants, Bx concentrations peaked 96 h after imbibition and decreased slightly depending on the rate of growth. The concentrations in the coleoptiles were the highest among the three plant parts throughout the experimental period (72-144 h). In addition, the 2nd leaves had higher concentrations than the 1st leaves (about 2-fold at 144 h).

While treatment with JA (5 μM) did not alter Bx concentrations in the coleoptile remarkably, the Bx concentrations in the 1st (except for 96 h) and the 2nd leaves increased about 2-fold. In the 1st leaves, Bx concentrations increased to a level comparable with the levels in the untreated coleoptiles. Notably, in the 2nd leaves, Bx concentrations increased to the highest value in the control and the JA-treated wheat shoots. The insensitivity of the coleoptiles to the JA treatments may be

Fig. 2. Effect of jasmonate treatment on Bx accumulation and plant weight. The Bx (DIMBOA-Glc and HDMBOA-Glc) concentrations and fresh weights of each wheat shoot are shown. The concentrations of Bx are described as concentrations relative to the DIMBOA-Glc concentrations in the untreated sample.
correlated with the minor time-dependent fluctuation in their weight. The fresh weights of coleoptiles decreased slightly in the course of the experiments (control:15.9 to 10.7 mg; JA-treated: 15.8 to 10.6 mg), and those of the 1st and 2nd leaves increased about 2.5–3-fold (control 1st leaf: 19.8–50.7 mg; control 2nd leaf: 9.0–22.3 mg; JA-treated 1st leaf: 16.8–37.5 mg; JA-treated 2nd leaf: 5.5–16.2 mg). A potential explanation of the observations is that plant growth and DIMBOA-Glc accumulation in the coleoptiles are already close to peaking at 72 h and exogenous stimulation would not alter the amount of a compound stored.

Subsequently, we investigated the effects of light on Bx concentrations, considering light influences plant metabolism and a considerable part of the 2nd leaf that contained higher Bx concentration than the 1st leaves were shielded from light by being wrapped by a coleoptile or a sheath. When we grew wheat seedlings in the dark, as expected, the coleoptiles became longer and the fresh weights were approximately 2.6-fold the fresh weight of the control plants at all growth stages (data not shown). However, the DIMBOA-Glc concentrations in them did not vary considerably and was at most 150% of the control plants (144 h) (Fig. 3). In contrast, the exclusion of light enhanced Bx accumulation in leaves. In particular, the increase in the 1st leaves was marked (2.5–4.7 fold) and the concentration was as high as in the JA-treated 2nd leaves.

3.3. Isolation and heterologous expression of wheat Bx6 (TaBx6)

To elucidate the mechanism by which JA treatment and etiolation enhance Bx accumulation, it is critical to determine the genes that the treatments influence. In the present study, we attempted cloning the TaBx6 responsible for the conversion of DIMBOA-Glc into 7-hydroxy-DIBOA-Glc (TRIBOA-Glc). We first isolated TaBx6 fragments by 3′-RACE and 5′-RACE using primers designed based on SxB6x and then amplified the coding sequences using the specific primers based on the results of RACE. The cloned sequence contained a 1167-bp open reading frame encoding a polypeptide of 388 amino acids (designated as TaBx6-1) whose primary structure shared 94% identity with ScBx6 (Fig. 4). Since wheat is a hexaploid plant consisting of three genomes (2n = 6x = 42), it was assumed that there were at least three homoeologs of Bx6. The transcript levels of wheat Bx genes identified so far tend to be unbalanced among the homoeologous genes [31,17]. Therefore, we attempted to amplify other Bx6 fragments using genomic DNA as a template. The PCR isolated three other DNA fragments, which finally yielded three Bx6 cDNAs (TaBx6-2, TaBx6-3, and TaBx6-4) encode polypeptides consisting of 388, 373, and 373 amino acids, respectively, and they shared more than 93% identity. TaBx6 genes are located on chromosome 2 as per the analysis using EnsemblPlants (https://plants.ensembl.org/).

According to the cDNA alignment analyses, all the four TaBx6s possess two ATG sequences in their 5′-terminal regions (Fig. 4B). When they are translated from the first ATG, TaBx6-1 and TaBx6-2 encode polypeptides with 388 residues, while TaBx6-3 and TaBx6-4 encode much shorter polypeptides. Translation from the second ATG yielded polypeptides with 373 residues across all the four TaBx6s. The primary structure of the rye orthologue, ScBx6, which was found in a database, corresponds to the deduced sequences of TaBx6 translated from the second ATG. We performed 5′-RACE using RNA from rye root to confirm the 5′-UTR sequence of ScBx6. According to the results, all the sequences possessed ATGG sequences at the position corresponding to the first ATG of TaBx6 (Fig. 4B). Starting from the ATG codon, subsequent to translation, ScBx6 should be a polypeptide consisting only of 28 amino acids. Rye possesses an enzyme that catalyzes sequential 7-hydroxylation of DIBOA-Glc, because rye produces DIMBOA-Glc mainly in the roots. In the present study, therefore, we consider the second ATG as the initiation codon for TaBx6 genes, namely, TaBx6-1–TaBx6-4, which consist of similar residue numbers, 373 residues, although the N-terminal residue of the naturally occurring TaBx6 is not confirmed. Tanwir et al. [14] reported a TaBx6-like gene with 86% identity with ScBx6 at the amino acid level by searching for Bx6-like homologs in a wheat sequence database. However, the TaBx6 isolated in the present study share higher identity with ScBx6 at both nucleotide and amino acid levels. Considering the high identity (>90%) between the known wheat and rye Bx-related genes, TaBx6 genes reported here have to be the orthologs of rye and maize Bx6s.

All the four recombinant TaBx6s exhibited a 2-oxoglutarate-dependent dioxygenase activity that converted DIBOA-Glc into TRIBOA-Glc (Table 1). While their kinetic parameters did not vary considerably among the isozymes, TaBx6-4 exhibited the highest efficiency (kcat/Km), which was more than three times higher than in the others. Since their primary structures share high sequence identities, a detailed activity evaluation based on the primary-structure differences would offer some insights on the structural factors involved in their substrate recognition and activity.

3.4. Quantification of Bx-genes in JA-treated and etiolated wheat shoots

The transcript levels of the Bx-related genes (TaBx1–TaBx6 and TaBx8) in individual plants (n = 4) were quantified using real-time PCR (Fig. 5). Notably, TaBx8 was the only gene that was not influenced by any treatments. Although TaBx6 expression in coleoptile was affected by JA and etiolation, its transcript levels in the 1st and 2nd leaves were

Fig. 3. Time course (72–144 h) of DIMBOA-Glc concentration in jasmonate (JA)-treated and etiolated wheat shoot. A wheat shoot was separated into coleoptile, 1st leaf, and 2nd leaf (after 96 h), and the concentrations of DIMBOA-Glc analyzed using HPLC. JA was administered to agarose at a concentration of 5 μM. Asterisks indicate statistically significant difference from the value of the control (*p < 0.01, **p < 0.001; Student’s t-test).
remarkably lower than the coleoptiles throughout the experimental period regardless of the treatments. These expression profiles did not obviously reflect the Bx concentrations in wheat, suggesting that TaBx6 and TaBx8 were not the factors influencing Bx concentration.

In the coleoptiles, the expression levels of all the genes decreased significantly in a time-dependent manner, although the accumulation of DIMBOA-Glc did not change so largely (Fig. 3). The discrepancy between Bx concentration and gene expression levels was also notable in the effects of JA on the 72-h-old coleoptiles; TaBx1–TaBx6 were upregulated by JA, although the effect on Bx concentration was not considerable. The effects of etiolation on 72-h-old coleoptiles varied depending on the genes; TaBx2, TaBx5, and TaBx6 were upregulated Fig. 4.

Fig. 4. Amino-acid sequence alignment of Bx6. The primary structures of Bx6 in wheat (TaBx6–1–TaBx6–4), rye (ScBx6; MG516219), and maize (ZmBx6; AF540907) were aligned using ClustalOmega. A, Alignment of the amino acid sequences that were deduced from the longest reading frames. The sequences of TaBx6-1 and TaBx6-2 used for heterologous expression in Escherichia coli start from the methionine indicated with closed triangle. B, Alignment of the nucleotide sequences corresponding to the N-terminal regions of Bx6. The sequences from the most upstream ATG in cDNA are shown. ATG sequence regarded as the initiation codon of each gene in this study is indicated in a red box. Note that translation from the most upstream ATG in TaBx6-3, TaBx6-4, and ScBx6 (green box) results in production of short peptides. Their corresponding termination codons are indicated in green boxes. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)
more than 2-fold, which did not correspond with the rates of accumulation of the compound, while the others were not affected. In the 1st leaves, etiolation had greater effects on gene expression than JA at all growth stages, except for TaBx6 and TaBx8. Such trends in TaBx1–TaBx5 were consistent with the Bx accumulation levels. In the 2nd leaves, however, none of the genes exhibited correlative expression profiles with the Bx accumulation levels.

To examine whether the transcript levels corresponded with the amounts of enzymes, one of the Bx-associated enzymes, TaBx5, was detected by Western blot analysis. TaBx5 is the enzyme responsible for the last step of the four sequential oxygenation by cytochrome P450 enzymes (CYPs) required for the formation of the 2,4-dihydroxy-1,4-benzoxazin-3-one skeleton (Fig. 1) and whose activity was the lowest among the four CYPs [17]. Treatment with JA increased TaBx5 in both coleoptiles and leaves at 72 h (Fig. 6). In the 120-h old plants, the amounts in both tissues were lower and treatment with JA did not influence them. The data are consistent with the results of quantitative-PCR.

In the present study, the Bx concentration in wheat leaves were

| Table 1 | Kinetic parameters of TaBx6s. |
|---------|-----------------------------|
| K_m (μM) | k_cat (s^-1) | k_cat/K_m (s^-1/mM) |
| TaBx6-1 | 217 | 0.433 | 2.00 |
| TaBx6-2 | 318 | 0.508 | 1.60 |
| TaBx6-3 | 282 | 0.524 | 1.86 |
| TaBx6-4 | 104 | 0.715 | 6.86 |

Fig. 5. Time course (72–120 h) of the transcript levels in jasmonate (JA)-treated and etiolated wheat shoot. RNase inhibitor-like protein (RLI) was used as a housekeeping gene. JA was administered to agarose at a concentration of 5 μM.
Microsomal fraction (30 pathways into complexes of multiple enzymes, which are referred to as secondary metabolites such as cyanogenic glucosides [25]. This could also apply in the case of Bx biosynthesis. Further analyses, including investigation of enzyme organization in cells and the transport of the metabolites through membrane systems, are required for the comprehensive understanding of Bx accumulation mechanisms.

Credit author statement

Masayuki Sue: Conceptualization, Investigation, Writing – review & editing; Miha Fuji: Investigation, Validation; Takahiro Fujimaki: Investigation, Validation.

Funding

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit organizations.

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.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.bbrep.2021.101059.

References

[1] W.J.C. de Bruijn, H. Gruppen, J.-P. Vincken, Structure and biosynthesis of benzoxazinoids: plant defence metabolites with potential as antimicrobial scaffolds, Phytochemistry 155 (2018) 233–243.

[2] H.M. Niemeyer, Hydroxamic acids derived from 2-hydroxy-2H-1,4-benzoxazin-3 (4H)-one: key defense chemicals of cereals, J. Agric. Food Chem. 57 (2009) 1677–1696.

[3] D. Sicker, M. Frey, M. Schulz, A. Gierl, Role of natural benzoxazinones in the survival strategy of plants, Int. Rev. Cytol. 198 (2000) 319–346.

[4] M. Sue, A. Ishihara, H. Iwamura, Purification and characterization of a β-glucosidase from rye (Secale cereale L.) seedlings, Plant Sci. 155 (2000) 67–74.

[5] V. Cambiere, T. Hance, E. De Hoffmann, Effects of 1,4-benzoxazin-3-one derivatives from maize on survival and fecundity of Mepolopodium dibrum (Walker) on artificial diet, J. Chem. Ecol. 27 (2001) 359–370.

[6] M. Frey, P. Chomet, E. Glawschign, C. Stettner, S. Grun, A. Winklmair, W. Eisenreich, A. Bacher, B.R. Meeley, S.P. Briggs, K. Simcox, A. Gierl, Analysis of a chemical plant defense mechanism in grasses, Science 277 (1997) 696–699.

[7] T. Nomura, A. Ishihara, H. Imaishi, H. Ohkawa, T.R. Endo, H. Iwamura, Rearrangement of the genes for the biosynthesis of benzoxazinones in the evolution of Triticeae species, Planta 217 (2003) 776–782.

[8] T. Nomura, A. Ishihara, H. Imaishi, T.R. Endo, H. Ohkawa, H. Iwamura, Molecular characterization and chromosomal localization of cytochrome P450 genes involved in the biosynthesis of cyclic hydroxamic acids in hexaploid wheat, Mol. Genet. Genom. 267 (2002) 210–217.

[9] U. von Rad, R. Huntl, F. Lottepeich, A. Gierl, M. Frey, Two glucosyltransferases are involved in detoxification of benzoxazinoids in maize, Plant J. 28 (2001) 633–642.

[10] M. Sue, A. Ishihara, H. Iwamura, Occurrence and characterization of a UDP-glucose:hydroxamic acid glucosyltransferase isolated from wheat (Tritium aestivum) seedlings, Z. Naturforsch. C Biosci. 55 (2000) 701–707.

[11] M. Sue, N. Nakamura, T. Nomura, Dispersed benzoxazinone gene cluster: molecular characterization and chromosomal localization of glucosyltransferase and glucosidase genes in wheat and rye, Plant Physiol 157 (2011) 985–997.

[12] R. Jorsczuk, H. Schmidt, A. Osterrieder, A. Frieselmeier, K. Schuhleber, M. Haslebeck, D. Sicker, D. Hofmann, N. Yalpani, C. Simmons, M. Frey, A. Gierl, Elucidation of the final reactions of DIMBOA-Glucoze biosynthesis in maize: characterization of βxβ and βzxβ, Plant Physiol 146 (2008) 1053–1063.

[13] M. Frey, K. Huber, W.J. Park, D. Sicker, P. Lindberg, R.B. Meeley, C.R. Simmons, N. Yalpani, A. Gierl, A 2-oxoglutarate-dependent dioxygenase is integrated in DIMBOA-biosynthesis, Phytochemistry 62 (2003) 371–376.

[14] F. Tanwir, G. Dionisio, K.B. Adhikari, I.S. Fomsgaard, P.L. Gregersen, Biosynthesis and chemical transformation of benzoxazinoids in rye during seed germination and the identification of a rye-like gene, Phytochemistry 140 (2017) 95–107.

[15] B. Bakera, B. Makowska, J. Groszyk, M. Niziolek, W. Orzecz, H. Bolbok-Bragowszewska, A. Hromada-Judycka, M. Rakocy-Trojanowska, Structural characteristics of Scβ genes controlling the biosynthesis of hydroxamic acids in rye (Secale cereale L.), J. Appl. Genet. 56 (2015) 287–298.

[16] B. Bakera, M. Swiącicka, A. Stochmal, M. Kowalczyk, L. Bolbok, M. Rakocy-Trojanowska, Benzoxazinoids biosynthesis in rye (Secale cereale L.) is affected by low temperature, Agronomy 10 (2020) 1260.
[17] T. Nomura, A. Ishihara, R.C. Yanagita, T.R. Endo, H. Iwamura, Three genomes differentially contribute to the biosynthesis of benzoxazinones in hexaploid wheat, Proc. Natl. Acad. Sci. U.S.A. 102 (2005) 16490–16495.

[18] Y. Tzin, Y. Hojo, S.R. Strickler, L.J. Bartsch, C.M. Archer, K.R. Ahern, S. Zhou, S. A. Christensen, I. Galis, L.A. Mueller, G. Jander, Rapid defense responses in maize leaves induced by Spodoptera exigua caterpillar feeding, J. Exp. Bot. 68 (2017) 4709–4723.

[19] A. Huffaker, N.J. Dafoe, E.A. Schmelz, ZmPep1, an ortholog of Arabidopsis elicitor peptide 1, regulates maize innate immunity and enhances disease resistance, Plant Physiol 155 (2011) 1325–1338.

[20] A. Oikawa, A. Ishihara, C. Tanaka, N. Mori, M. Tsuda, H. Iwamura, Accumulation of HDMBOA-Glc is induced by biotic stresses prior to the release of MBOA in maize leaves, Phytochemistry 65 (2004) 2995–3001.

[21] A. Oikawa, A. Ishihara, H. Iwamura, Induction of HDMBOA-Glc accumulation and DIMBOA-Glc 4-O-methyltransferase by jasmonic acid in poaceous plants, Phytochemistry 61 (2002) 331–337.

[22] A. Oikawa, A. Ishihara, M. Hasegawa, O. Kodama, H. Iwamura, Induced accumulation of 2-hydroxy-4,7-dimethoxy-1,4-benzoxazin-3-one glucoside (HDMBOA-Glc) in maize leaves, Phytochemistry 56 (2001) 669–675.

[23] M.J. Gimenez, F. Piston, S.G. Atienza, Identification of suitable reference genes for normalization of qPCR data in comparative transcriptomics analyses in the Triticeae, Planta 233 (2011) 163–173.

[24] Q. Xiong, B. Ma, X. Lu, Y.-H. Huang, S.-J. He, C. Yang, et al., Ethylene-inhibited jasmonic acid biosynthesis promotes mesocotyl/coleoptile elongation of etiolated rice seedlings, Plant Cell 29 (2017) 1053–1072.

[25] K. Jørgensen, A.V. Rasmussen, M. Morant, A.H. Nielsen, N. Bjarnholt, M. Zagrobelny, S. Bak, B.L. Møller, Metabolon formation and metabolic channeling in the biosynthesis of plant natural products, Curr. Opin. Plant Biol. 8 (2005) 280–291.