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Molecular characterization of benzoxazinone-deficient mutation in diploid wheat

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Abstract

Benzoxazinones (Bxs) are representative defensive compounds in gramineous plants, including wheat (genus *Triticum*) and its wild relative species (genus *Aegilops*). Bx production was found to be variable among three diploid wheat species with the same A genome as hexaploid wheat ($2n = 6x = 42$, genomes AABBDD). All accessions of *Triticum monococcum* ($2n = 2x = 14$, AA) and *Triticum urartu* ($2n = 2x = 14$, AA) accumulated Bxs, but 18 out of 28 accessions of *Triticum boeoticum* ($2n = 2x = 14$, AA) were Bx-deficient. Bx-deficient accessions were grouped into two types by genomic PCR analysis of the five Bx biosynthetic loci (*TbBx1–TbBx5*): those retaining all five loci (type I) and those lacking *TbBx3* and *TbBx4* loci (type II). Despite the Bx-deficient phenotype, all five *TbBx* genes were transcribed in the type-I accessions. The Bx deficiency in one accession of type I was due to the disintegration of the *TbBx1*, *TbBx4* and *TbBx5* genes due to insertions or deletions in their coding sequences. The *TbBx2* and *TbBx3* genes of those accessions had the complete sequences of the functional enzymes. In the type-II accessions, the remaining three genes, *TbBx1*, *TbBx2* and *TbBx5*, were all transcribed, with the exception of two accessions in which either *TbBx1* or *TbBx5* was not transcribed. The *TbBx1* coding sequence of the type-II accessions was also disintegrated, like that of the type-I accessions. These findings suggest that the Bx deficiency in *T. boeoticum* first resulted from disintegration of the *TbBx1* coding sequence, followed by transcription failure, disintegration of the coding sequences and elimination of the *TbBx1–TbBx5* genes.

Keywords: *Triticum boeoticum*; Gramineae; Wheat; Secondary metabolism; Biosynthesis; Benzoxazinone; DIBOA; DIMBOA; Mutant

1. Introduction

The benzoxazinones (Bxs), the major secondary metabolites in gramineous plants, including hexaploid wheat (*Triticum aestivum*), rye (*Secale cereale*) and maize (*Zea mays*), are involved in their defense against pathogens and insects (Niemeyer, 1988; Sicker et al., 2000). Two representative Bxs are 2,4-dihydroxy-1,4-benzoxazin-3-one (**1**) (DIBOA) and its 7-methoxy derivative **2** (DIMBOA), which are constitutively present in the vacuole in the form of glucosides (DIBOA-Glc **3** and DIMBOA-Glc **4**). Their amounts reach a maximum soon after germination, and then decrease gradually to constant, low levels. Thus, the Bxs are considered to be important to the defense of plants during the juvenile stage of growth (Nakagawa et al., 1995; Ebisui et al., 1998).

The biosynthetic pathway of Bxs branches off from that of tryptophan at indole-3-glycerol phosphate **5** (Frey et al., 1997, 2000; Melanson et al., 1997; Gierl and Frey, 2001). Five consecutive reactions lead from indole-3-glycerol phosphate **5** to the formation of DIBOA **1** (Fig. 1). The

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Abbreviations: Bx, benzoxazinone; CS, Chinese Spring; DIBOA, 2,4-dihydroxy-1,4-benzoxazin-3-one; DIMBOA, 2,4-dihydroxy-7-methoxy-1,4-benzoxazin-3-one; MITE, miniature inverted-repeat transposable element; TIR, terminal inverted repeat; TSD, target site duplication.

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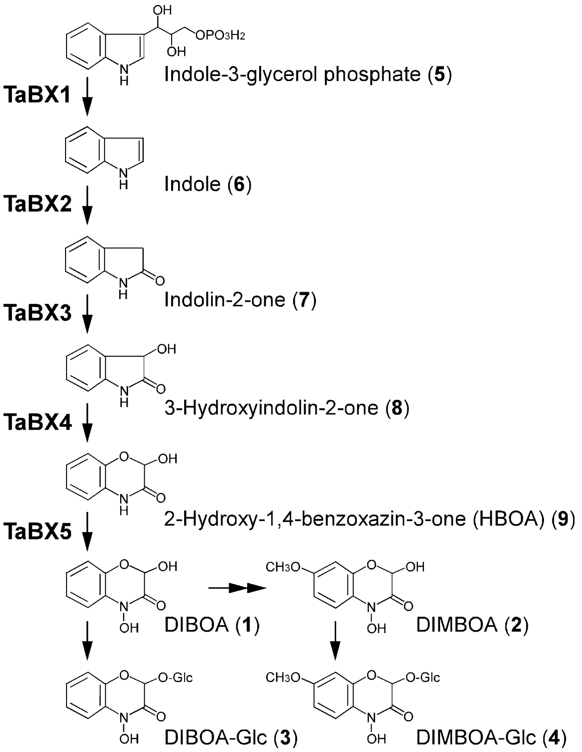

Fig. 1. Bx biosynthetic pathway showing the enzymatic reactions controlled by the *TaBx1*–*TaBx5* genes of hexaploid wheat.

genes involved have been isolated from maize (*ZmBx1*-*ZmBx5* genes; Frey et al., 1995, 1997), wild barley (*Hordeum lechleri*, *HlBx1*–*HlBx5*; Grün et al., 2005), and hexaploid wheat (*TaBx1A*–*TaBx5A*, *TaBx1B*–*TaBx5B*, and *TaBx1D*–*TaBx5D*; Nomura et al., 2002, 2003, 2005).

Hexaploid wheat ($2n = 6x = 42$, genome formula AABBDD) arose from two hybridization events followed by chromosome doubling of three ancestral diploid species ($2n = 2x = 14$) with different genomes A, S($\approx$B) and D. There are three A-genome diploid species, *Triticum boeoticum*, *Triticum urartu* and *Triticum monococcum*. *T. monococcum* is a domesticated form of the wild species *T. boeoticum*, and *T. urartu* is the donor of the A genome of hexaploid wheat (Dvorák et al., 1988, 1993; Dvorák and Zhang, 1992; Takumi et al., 1993). The donors of the B and D genomes of hexaploid wheat are *Aegilops speltoides* (SS) and *Ae. tauschii* (DD), respectively (Huang et al., 2002a). Nomura et al. (2002, 2003) demonstrated that the five Bx biosynthetic genes are all present in those three diploid progenitors.

Wheat, rye ($2n = 2x = 14$, RR) and barley (*Hordeum vulgare*, $2n = 2x = 14$, HH), which belong to the tribe Triticeae, are considered to be derived from a common ancestor (Huang et al., 2002b). Nevertheless, the occurrence of Bxs is not ubiquitous in Triticeae. Although some wild barley species biosynthesize the Bxs (Barria et al., 1992; Grün et al., 2005), cultivated barley is devoid of all five Bx biosynthetic genes (Gierl and Frey, 2001; Nomura et al., 2003); instead it accumulates another defensive secondary metabolite 'gramine' (Grün et al., 2005). Recently, we have found a Bx-deficient variant of *T. boeoticum* (unpublished data). The Triticeae species seem to have been developing different defense system utilizing secondary metabolites. This raised other questions: whether the deficiency in Bxs is found only in *T. boeoticum* or is also found in the other A-genome species, i.e. *T. monococcum* and *T. urartu*, and what happened to the Bx biosynthetic genes in such variants? In the present study, we first surveyed the Bx production in three A-genome species, *T. boeoticum*, *T. monococcum* and *T. urartu*, and found that some of the *T. boeoticum* accessions were deficient in Bxs. Then we investigated the Bx biosynthetic genes in detail to clarify the cause of Bx deficiency in those accessions.

2. Results

2.1. Variation of Bx production in A-genome diploid wheat species

Bxs were detected in 48-h-old shoots and roots of all accessions of *T. monococcum* (24 accessions) and *T. urartu* (15 accessions). However, Bxs were found only in 10 of the 28 accessions of *T. boeoticum* (Table 1, Fig. 2). The

Table 1
Accessions of A-genome diploid species used in this study. (+) and (−) after the accession numbers indicate the presence and absence of Bxs, respectively

T. boeoticum	*T. urartu*	*T. monococcum*
KU1501 (+)[a]	KU199-1 (+)	KU104-1 (+)
KU1519 (+)	KU199-2 (+)	KU104-2 (+)
KU8417 (+)	KU199-3 (+)	KU104-3 (+)
KU10603 (+)	KU199-4 (+)	KU105 (+)
KU10730 (+)	KU199-5 (+)	KU106 (+)
KU10760 (+)	KU199-6 (+)	KU1404 (+)
KU10782 (+)	KU199-7 (+)	KU1420 (+)
KU10812 (+)	KU199-8 (+)	KU1427 (+)
KU10833 (+)	KU199-9 (+)	KU1428 (+)
KU10859 (+)	KU199-11 (+)	KU1431 (+)
KU102 (−)	KU199-12 (+)	KU1432 (+)
KU8026 (−)	KU199-13 (+)	KU3636 (+)
KU8103 (−)	KU199-14 (+)	KU3637 (+)
KU8116 (−)	KU199-15 (+)	KU3640 (+)
KU8128 (−)	KU199-16 (+)	KU3641A (+)
KU8134 (−)		KU3641B (+)
KU8150 (−)		KU11040 (+)
KU8221 (−)		KU11047 (+)
KU8235 (−)		KU11070 (+)
KU8259 (−)		KU11072 (+)
KU8266 (−)		KU11083 (+)
KU8397 (−)		KU11084 (+)
KU8404 (−)		KU11357 (+)
KU8407 (−)		KT3-5 (+)
KU8412 (−)		
KU10653 (−)		
KU10867 (−)		
KT1-1 (−)[b]		

[a] KU numbers are the accession numbers in the Graduate School of Agriculture, Kyoto University, Japan.

[b] KT numbers are the accession numbers in the Kihara Institute for Biological Research, Yokohama City University, Japan.