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# Wound-induced systemic accumulation of a transcript coding for a Bowman–Birk trypsin inhibitor-related protein in maize (*Zea mays* L.) seedlings

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The wound-induced accumulation of a transcript coding for a polypeptide of 72 amino acid residues in maize (*Zea mays* L.) seedlings was investigated. Sequence comparison showed strong homology with Bowman–Birk trypsin inhibitors of cereal and legume species. The local accumulation pattern of this transcript demonstrated, after wounding various parts of the seedling, a local as well as a systemic response which implies the transmission of a wound signal specifically from the lower to the upper parts of the plant.

Amino acid sequence (trypsin inhibitor), Wound reponse (trypsin inhibitor); Bowman–Birk trypsin inhibitor; Systemic induction (gene expression); *Zea mays*

## 1. INTRODUCTION

Plant proteinase inhibitors are small proteins with a molecular mass of 8–20 kDa which are widespread in both monocotyledons and dicotyledons and appear to function as a part of the defense system against pathogen attack, e.g. by herbivorous insects [1,2]. In many cases these proteins have been found to be inducible by wounding locally at the site of injury and systemically in the whole plant [3,4]. In the latter case a 'wound signal' must be postulated which transmits the information from the damaged cells to the distant parts of the plant where specific expression of proteinase-inhibitor genes occurs [5]. In tomato a wound-induced small polypeptide (systemin) has been identified as a signal substance mediating systemic proteinase-inhibitor expression [6].

We report here the characterisation of a transcipt from maize (*Zea mays* L.) seedlings recently described as an auxin-induced mRNA [7]. We found that the induced expression of this transcript in isolated maize coleoptile segments is not elicited by auxin but by injury caused by cutting segments. This observation has led us to investigate the function of this transcript in the response of the plant to wounding.

## 2. MATERIALS AND METHODS

### 2.1. *Growth and treatment of plants*

Standard procedures for growing etiolated, 4-day-old maize seedlings (*Zea mays* L.; cv. Brio; from Asgrow) were used [8]. For experiments with indole acetic acid (IAA) subapical coleoptile segments (10 mm long, 3 mm below the tip) were incubated in aerated IAA solution (10 $\mu$M) or $H_2O$ as control at 25°C in normal laboratory light. For wounding experiments 1-cm segments of either coleoptiles, primary leaves, mescotyls and roots were cut and incubated on moist filter paper at 25°C in the dark. All other experiments were carried out with whole maize seedlings as described in the figure legends.

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### 2.2. *RNA extraction*

Total RNA was extracted as described by Wadsworth et al. [9]. Poly(A)$^+$ RNA was isolated according to Aviv and Leder[10].

### 2.3. *PCR and sequence analysis*

Two oligonucleotides (a) 5′-CTGACGACCAGCTGGTCGAT and (b) 5′-CACAAGACTGCAGTACGTATCGTAG, were synthesized as primers. The first strand cDNA was synthesized from 1 $\mu$g of poly (A)$^+$ RNA extracted from IAA-treated maize coleoptile segments primed with primer b, by AMV reverse transcriptase (Pharmacia) at 42°C for 60 min. PCR was carried out using the cDNA, 1 $\mu$M each of the primers a and b and 5 U *Taq* polymerase (BTS) according to the manufacturer's instructions. After 30 cycles the PCR product was separated from the remaining oligonucleotides on a 1.0% agarose gel, cut out, electroeluted, digested with *Pst*I and *Pvu*II and ligated into a *Eco*RV, *Pst*I-digested Bluescript KS$^+$ vector.

Sequence analysis was carried out on both strands by the dideoxy method of Sanger et al. [11].

### 2.4. *RNA dot blot and Northern hybridisation*

Experiments were performed with equal amounts of total RNA. Hybridisation was carried out using a *Hin*dIII, *Pst*I fragment of the Bluescript vector. Labeling was carried out using $^{32}$P (Amersham;110 TBq·mmol$^{-1}$) and a Multiprime labeling kit (Amersham).

Hybridisation was performed as described by Ehmann et al. [12], using a hybridisation buffer with 50% formamide.

### 2.5. *Comparison of amino-acid sequences*

The amino acid sequence of the BBI-M sequence was compared with those of other proteins stored in a databank (EMBL, Heidelberg)

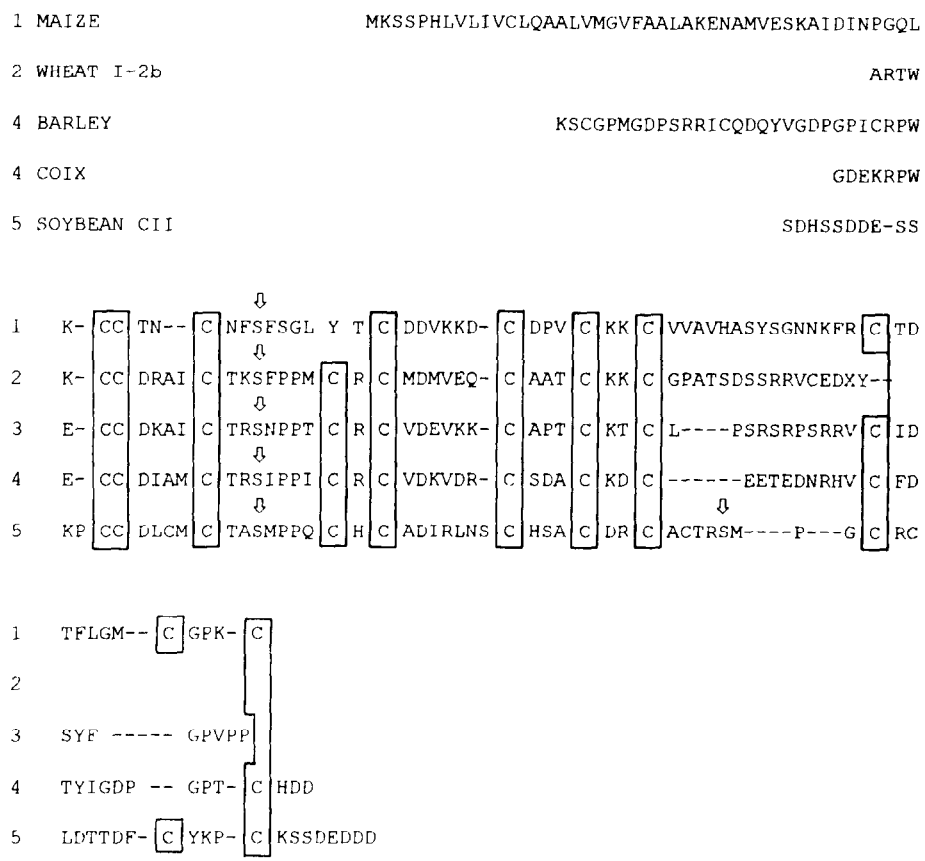


Fig. 1. Homologies between the deduced amino acid sequence of the isolated maize transcript (BBI-M) and the Bowman–Birk family of trypsin inhibitors reported for other cereals (wheat germ, *Triticum avestivum* [15]; barley, *Hordeum vulgare* [16]; Job's tears, *Coix lachryma-jobi* [17]) and a legume (soybean, *Glycine max* [18]). The sequences were aligned, for maximum homology, resulting in some gaps (- -) which may represent insertions/deletions. The reactive sites are indicated by arrows. The soybean sequence contains two reactive sites because of gene dublication [13]. The cysteine residues are boxed to facilitate comparison.

## 3. RESULTS AND DISCUSSION

Using the polymerase chain reaction (PCR) we obtained the cDNA sequence recently reported by Rohrmeier and Lehle [7]. The amino acid sequence deduced from the nucleotide sequence was 72 residues in length with a molecular mass estimated to be 9 kDa. Sequence comparison (Fig.1) shows significant homology between the cloned maize sequence and the Bowman–Birk trypsin inhibitor (BBI) family [13,14]. The maize sequence (BBI-M) demonstrates strongest homology with the cereal BBIs (*Triticum* [15], *Hordeum* [16], *Coix* [17]) but there is also significant homology with the legume BBI (*Glycine* [18]). Comparison of BBI- M with the BBIs from other plants demonstrates at least 14 corresponding amino-acid residues. Nine of ten cystein residues, which are characteristic for BBIs, are conserved (one homologous exchange at position 17). Homology is high in the region around the reactive site (arrows in Fig. 1). As BBI-M displays only one reactive site, it apparently encodes a 'single-headed' BBI similar to the type II inhibitor in wheat [15].

Using the BBI-M cDNA as a hybridisation probe, strongly increased amounts of BBI-M transcript can be detected in the RNA fraction from isolated maize coleoptile segments treated with IAA (Fig. 2, bottom). However, a quantitative comparison between induced transcript levels in IAA-treated and water control segments revealed no detectable differences (Fig. 2, top). These results indicate that the induction of BBI-M transcript formation results from wounding the coleoptile by cutting segments rather than from an effect of IAA. Wound-induced transcript accumulation at comparable levels independent of IAA treatment could also be observed in isolated segments from mesocotyls, primary leaves and roots of maize seedlings (Fig. 3). We conclude from these experiments that transcription of the BBI-M gene is not mediated by IAA, as reported [7], but by a signal resulting from wounding the tissue, as described for BBIs of other plants [4,19].

The signal which is elicited by wounding is translocated between the different organs of the maize seedling

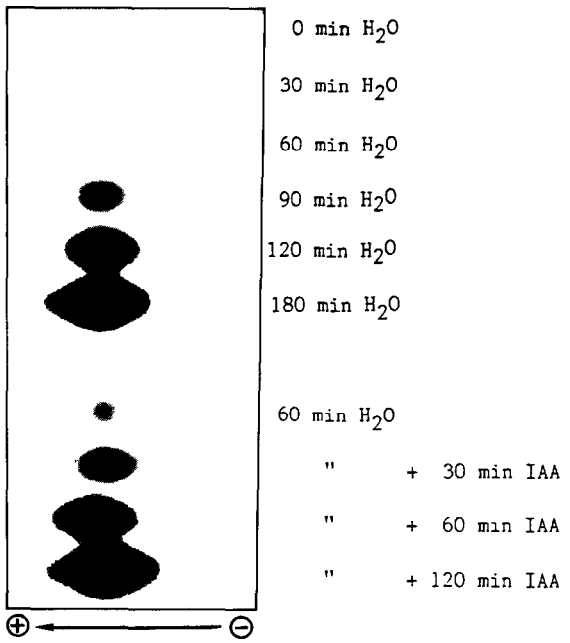


Fig. 2. Accumulation of BBI-M transcript in isolated maize coleoptile segments (1 cm long, 3 mm below the tip) incubated in water with (bottom) or without (top) IAA (10 $\mu$M) for the indicated times. The autoradiogram was obtained after electrophoresis and gel-blot hybridisation of total RNA (20 $\mu$g per slot) against $^{32}$P-labeled BBI-M cDNA probe.

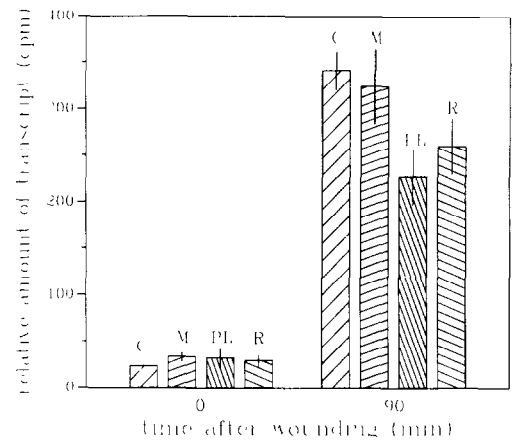


Fig. 3. Induced expression of BBI-M transcript in different organs of the maize seedling upon wounding. Total RNA was extracted from coleoptiles (C), mesocotyls (M), primary leaves (PL) and roots (R) of maize seedlings cut into 1-cm segments and incubated on moist filter paper for 0 or 90 min. Relative amounts of transcript were determined after dot-blot hybridisation of total RNA with $^{32}$P-labeled BBI-M cDNA probe and scintillation counting (means of 3 independent experiments ± S.E.M.

in a polar manner. Wounding the coleoptile results in strong BBI-M transcript accumulation in the tissue above the wound (segment C1) while a clearly reduced response is induced in the coleoptile tissue below the wound (segment C2), although both include a wound site. The mesocotyl and the root show no significant response (Fig. 4). Similarly, wounding the mesocotyl results in strong BBI-M transcript accumulation only in the tissue above the wound (segments M2, M1, C2, C1) and no significant response in the root (Fig. 5). No response was observed in the primary leaf (PL) in these experiments, indicating that the signal released from the wounded mesocotyl does not reach the leaf or the leaf shows a lower sensitivity towards the systemic signal. Taken together, these data show that the induced accumulation of BBI-M accumulation is not limited to the wound site but can occur as a response to a signal which is transmitted along the seedling axis to the undamaged parts of the plant, preferentially in the direction from the bottom to the top. This systemic transcript accumulation starts within 30–90 min in all segments affected by the wound signal. The kinetics reveal no temporal differences in the time courses of responses between segments close to or more distant from the wound site. Thus, the distribution of the wound signal appears to be rather rapid and homogeneous, except for a higher level close to the wounded cells.

Systemic wound responses have recently been observed in several plants, including systemic formation of proteinase inhibitors [1,6]. In the systemic expression of proteinase inhibitor II in potato, the signal has been shown to move equally in both directions from the site of wounding [19]. Etiolated maize seedlings appear to represent the first example for a unidirectionally transmitted wound signal which may be involved in confer-

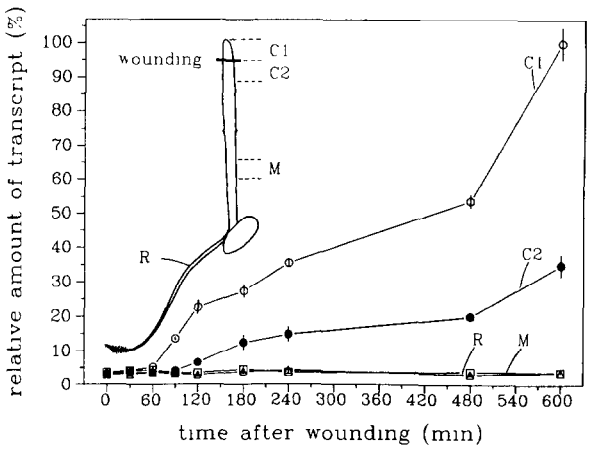


Fig. 4. Accumulation of BBI-M transcript in different parts of the maize seedling upon wounding the coleoptile tip. Coleoptiles were wounded by cutting off an 1-cm segment including the tip (C1) which was incubated on moist filter paper. Rest-seedlings were kept in a moist atmosphere. C1 segments or 1-cm segments isolated at various times from the coleoptiles (C2) and mesocotyls (M) or whole roots (R) of the decapitated rest-seedlings were analysed for BBI-M transcript as described in Fig. 3 and expressed as percentage of the transcript level at 600 min after wounding (means of 3 independent experiments ± S.E.M.

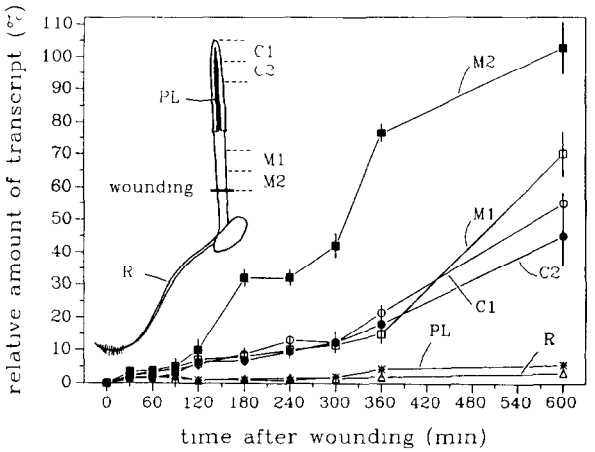


Fig. 5. Accumulation of BBI-M transcript in different parts of the maize seedling upon wounding the mesocotyl. Mesocotyls were wounded by cutting off the upper part of the seedling including the coleoptile and part of the mesocotyl. This section was incubated on on moist filter paper while rest seedlings were kept in a moist atmosphere. 1-cm segments from the coleoptile (C1, C2), mesocotyl (M1, M2) and whole primary leaves and roots were isolated at various times and analysed for BBI-M transcript as in Fig. 4.

ring resistance against insect attack to the plant specifically elicited by tissue damage in the underground parts of the plant.

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*Note added in proof*

After completion of this work a paper by Rohrmeyer and Lehle, the original antecedents (Plant Mol. Biol., in press), was brought to our attention where they describe cloning, sequencing and functional identification of the same gene (designated as WIP 1 by these authors; EMBL data bank accession number X 71396 ZMWIP 1).