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Bt CORN HAS A HIGHER LIGNIN CONTENT THAN NON-*Bt* CORN[1]

DEEPAK SAXENA AND G. STOTZKY[2]

Laboratory of Microbial Ecology, Department of Biology, New York University, New York, New York 10003 USA

Bt corn has been genetically modified to express the Cry1Ab protein of *Bacillus thuringiensis* to kill lepidopteran pests. Fluorescence microscopy and staining with toluidine blue indicated a higher content of lignin in the vascular bundle sheaths and in the sclerenchyma cells surrounding the vascular bundle in all ten *Bt* corn hybrids, representing three different transformation events, studied than of their respective non-*Bt* isolines. Chemical analysis confirmed that the lignin content of all hybrids of *Bt* corn, whether grown in a plant growth room or in the field, was significantly higher (33–97% higher) than that of their respective non-*Bt* isolines. As lignin is a major structural component of plant cells, modifications in lignin content may have ecological implications.

Key words: *Bacillus thuringiensis*; *Bt* corn; Cry1Ab protein; lignin; pleiotropic effects; transgenic plants.

Bt corn is maize (*Zea mays* L.) that has been genetically modified to express the *cry1Ab* gene from *Bacillus thuringiensis* (*Bt*) and produce a larvicidal toxin to kill lepidopteran pests, especially the European corn borer (ECB; *Ostrinia nubilalis*). There is concern that genetically engineered crops may pose risks to natural and agricultural ecosystems (e.g., Rissler and Mellon, 1996; Conway, 2000; Hails, 2000; Stotzky, 2000). Transformation with the *cry1Ab* gene may lead to pleiotropic effects that could have ecological implications. Here we show that *Bt* corn has a higher lignin content than isogenic non-*Bt* corn.

MATERIALS AND METHODS

A freshly collected Riverhead sandy loam soil from East Marion, Long Island, New York, USA, was sieved through a 15-mm screen, then through a 5-mm screen, and mixed thoroughly; 4.5 kg was placed in each of 20 plastic pots (18 cm diameter, 21 cm deep). Some physicochemical characteristics of the soil are the following: pH 5.2; 0.92% carbon and 0.07% nitrogen; 58% sand, 41% silt, and 1% clay. Seeds of ten different *Bt* corn hybrids (Table 1), representing three transformation events (Bt11, MON810, and 176), and their respective non-*Bt* isolines were planted (3 seeds/pot), and the pots were kept in a plant-growth room at 26 ± 2°C, with a 12-h light-dark cycle (light intensity of ~110 μmol from Cool-White, Sylvania F96T12, 215W fluorescent lamps; Ontario, Canada). Seeds of eight *Bt* hybrids (Table 2) and their non-*Bt* isolines were also planted in a Haven sandy soil in another site in East Marion, Long Island, New York, USA. Some features of the soil are the following: pH 7.1; 5.24% carbon and 0.25% nitrogen; 94% sand, 5% silt, and 1% clay. Both soils are classified as mesic typic dystochrepts. Plants were irrigated but not fertilized. The plants (*Bt* hybrids or isolines) were harvested after 97 d of growth in the plant growth room and after 90 d of growth in the field. Uniform free-hand sections of fresh corn stems between the 3rd and 4th node from the surface of the soil (thickness was ~11 mm for plants grown in the plant growth room and ~18 mm for field-grown plants) were examined for lignin by fluorescence microscopy at 400 nm (Hu et al., 1999) and by staining with 0.01% toluidine blue (Sylvester and Ruzin, 1994). The content of lignin of the same portion of the stems (oven-dried, ground, and passed through an 80-mesh sieve) was determined by the acetyl bromide method (Hatfield et al., 1999).

RESULTS AND DISCUSSION

A higher content of lignin was observed by fluorescence microscopy in the vascular bundle sheath and in the sclerenchyma cells surrounding the vascular bundle of all *Bt* corn hybrids than of their respective non-*Bt* isolines grown in the

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[2] Author for reprint requests (tel: 01-212-998-8268; FAX: 01-212-995-4015; e-mail: gs5@nyu.edu).

TABLE 1. Lignin content (% ± SEM) in different hybrids of corn, grown in a plant growth room, with (*Bt*+) and without (*Bt*−) the *cry1Ab* gene.

		Bt+		*Bt*−		
Company	Event[a]	Hybrid	% Lignin	Hybrid	% Lignin	*P*
Novartis[b]	Bt11	N7590Bt	7.2 ± 0.10	N7590	4.8 ± 0.14	0.00001
Novartis	Bt11	N67-T4	6.3 ± 0.25	N67-H6	3.9 ± 0.15	0.00175
Novartis	Bt11	N3030Bt	7.0 ± 0.22	N3030	4.4 ± 0.22	0.00003
Novartis	Bt11	NC4990Bt	6.6 ± 0.18	NC4880	3.4 ± 0.27	0.00020
Novartis	Bt11	NK4640Bt	6.3 ± 0.14	NK4640	3.2 ± 0.12	0.00001
Novartis	176	Maximizer	4.0 ± 0.15	—	—	—
Pioneer[c]	MON810	P31B13	6.0 ± 0.24	P3223	3.2 ± 0.18	0.00032
DeKalb[d]	MON810	DK647Bty	6.2 ± 0.25	DK647	4.4 ± 0.22	0.00174
DeKalb	MON810	DK679Bty	6.6 ± 0.11	DK679	3.8 ± 0.10	0.00005
DeKalb	MON810	DK626Bty	6.1 ± 0.20	DK626	3.2 ± 0.18	0.00006

[a] Insertion of the *cry1Ab* gene by transformation. Site of insertion differed.
[b] Novartis AG., 4002 Basel, Switzerland.
[c] Pioneer Hi-Bred USA, Des Moines, Iowa 50306 USA.
[d] DeKalb ℅ Monsanto Company, St. Louis, Missouri 63198 USA.

TABLE 2. Lignin content (% ± SEM) in different hybrids of corn, grown in the field, with (*Bt*+) and without (*Bt*−) the *cry1Ab* gene.

		Bt+		*Bt*−		
Company	Event[a]	Hybrid	% Lignin	Hybrid	% Lignin	*P*
Novartis[b]	Bt11	N7590Bt	7.4 ± 0.15	N7590	5.2 ± 0.11	0.00007
Novartis	Bt11	N67-T4	7.1 ± 0.22	N67-H6	4.5 ± 0.14	0.00082
Novartis	Bt11	NC4990Bt	7.7 ± 0.06	NC4880	4.8 ± 0.22	0.00033
Novartis	Bt11	NK4640Bt	7.9 ± 0.10	NK4640	4.9 ± 0.09	0.00001
Novartis	176	Maximizer	5.9 ± 0.13	—	—	—
Pioneer[c]	MON810	P32P76	6.8 ± 0.14	P32P75	4.8 ± 0.22	0.00016
Pioneer	MON810	P31B13	7.0 ± 0.04	P3223	4.9 ± 0.13	0.00007
DeKalb[d]	MON810	DK626Bty	6.8 ± 0.15	DK626	5.1 ± 0.12	0.00085

[a] Insertion of the *cry1Ab* gene by transformation. Site of insertion differed.
[b] Novartis AG., 4002 Basel, Switzerland.
[c] Pioneer Hi-Bred USA, Des Moines, Iowa 50306 USA.
[d] DeKalb % Monsanto Company, St. Louis, Missouri 63198 USA.

plant growth room (Fig. 1A, B), which was confirmed by staining the sections with toluidine blue (Fig. 1C, D). The average diameter of the vascular bundle and surrounding lignified cells in *Bt* corn was 21.5 ± 0.84 μm, whereas that of non-*Bt* corn was 12.4 ± 1.14 μm (Fig. 1C, D). Similar results were obtained with plants grown in the field.

The content of lignin of the same portion of the stems, determined by the acetyl bromide method, was significantly higher (33–97% higher) in all hybrids of *Bt* corn than in their respective non-*Bt* isolines, whether grown in the plant growth room (Table 1) or in the field (Table 2). The lignin content of field-grown plants was higher than that of plants grown in the plant growth room, which were smaller. There was a significantly higher lignin content ($P < 0.002$) in plants transformed by event Bt11 (7.4 ± 0.10 and 6.7 ± 0.12% for field- and growth room-grown plants, respectively) than by event MON810 (6.9 ± 0.07 and 6.2 ± 0.10% for field- and growth room-grown plants, respectively). There were no significant differences in the lignin content of isogenic non-*Bt* plants ($P > 0.67$ for field-grown plants and $P > 0.30$ for growth room-grown plants). The lignin content of the only available hybrid transformed by event 176 was lower than that of hybrids transformed by events Bt11 and MON810. These results differ from those reported by Faust (1999), which indicated no significant differences in lignin content between the dried biomass of whole plants of *Bt* (event MON810) and non-*Bt* corn but which indicated that *Bt* corn had a higher moisture content and a lower level of ammonia than non-*Bt* corn ($P < 0.05$). However, Masoero et al. (1999) reported a 16% higher lignin content in *Bt* than in non-*Bt* corn.

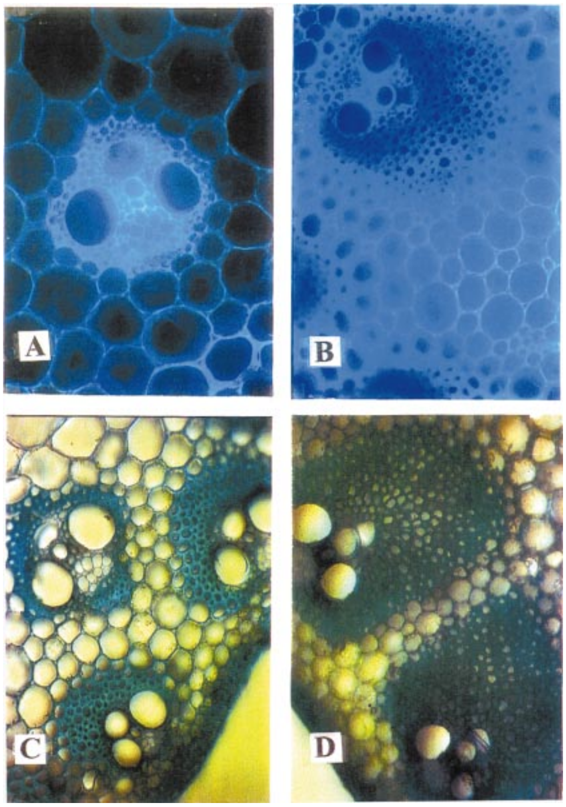

Fig. 1. Fluorescence microscopy of a transverse stem section of the third internode from the soil surface from (A) non-*Bt* and (B) *Bt* corn grown in a plant growth room. Lignin autofluorescence was visualized following ultraviolet excitation at 400 nm (×250). The same transverse sections from (C) non-*Bt* and (D) *Bt* corn were stained with toluidine blue. More lignified cells and large vascular bundles were observed in *Bt* corn than in non-*Bt* corn (×250).

Lignin is a major structural component of plant cells that confers strength, rigidity, and impermeability to water. Any modifications in lignin content could have ecological implications (Halpin et al., 1994). For example, the increase in lignin content in *Bt* corn may be beneficial, as it can provide greater resistance to attack by second-generation ECB (Ostrander and Coors, 1997), reduce susceptibility to molds (Masoero et al., 1999), and retard litter degradation and decomposition by microbes (Reddy, 1984; Tovar-Gomez et al., 1997). The addition of biomass from *Bt* corn to soil resulted in a significantly lower gross metabolic activity (i.e., CO_2 evolution) in soil than did the addition of non-*Bt* corn (S. Flores, D. Saxena, and G. Stotzky, New York University, unpublished data; Stotzky, 2000), which may be beneficial, as the organic matter derived from *Bt* corn may persist and accumulate longer and at higher levels in soil, thereby improving soil structure and reducing erosion (James et al., 1998), or it may be detrimental, as the longer persistence of the biomass of *Bt* corn may extend the time that the toxin is present in soil and, thereby, may enhance the hazard to nontarget organisms and result in the selection and enrichment of toxin-resistant target insects. Moreover, lignin is relatively indigestible and reduces the ability of herbivores to digest plant material, and its increase in forages might affect rates of feeding and population dynamics of defoliators (Barriere and Argillier, 1993; Jung and Allen, 1995; Gardner et al., 1999). Additional studies are necessary to clarify the environmental impacts of a higher lignin content, especially in *Bt* corn, as about 8.1 million hectares (20 million acres) of *Bt* corn (26% of total corn acreage) were planted in the United States alone in 1999 (U.S. Environmental Protection Agency, 2000).

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