

Quantitative analysis of the seasonal and tissue-specific expression of Cry1Ab in transgenic maize Mon810

Quantitative Analyse der saisonalen und gewebespezifischen Expression von Cry1Ab in transgenen Mon810-Mais

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Summary

The tissue-specific expression and seasonal abundance of Cry1Ab protein were determined in transgenic maize plants (Mon810, variety 'Novelis') from two field trials located near Bonn and Halle, Germany. A total of 1085 samples were analysed by using Double Antiserum-Enzyme Linked Immunosorbent Assay (DAS-ELISA). The Cry1Ab contents of various plant tissues (root, stem, upper leaf, lower leaf, anther, pollen and kernel) were determined at four different growth stages (BBCH19, BBCH30, BBCH61 and BBCH83) collected in 2001, 2002 and 2003. Mon810 showed the highest Cry1Ab contents in the leaves ($5.5 - 6.4 \mu\text{g g}^{-1}$ fresh weight [fw]) at BBCH83, whereas the lowest Cry1Ab contents were detected in the pollen ($1 - 97 \text{ ng g}^{-1}$ fw). Cry1Ab content of residual root stocks collected in the field nine months after harvest was $15 - 17 \text{ ng g}^{-1}$ fw. This demonstrated that the Cry1Ab concentration in residual root stocks was reduced to about one-hundredth of the fresh roots. The monitoring of Cry1Ab expression showed that the Cry1Ab contents varied strongly between different plant individuals.

Key words: Cry1Ab expression, ELISA, European corn borer, Mon810, transgenic maize

Zusammenfassung

Die gewebespezifische Expression und die saisonale Verbreitung des Cry1Ab-Proteins in transgenen Maispflanzen (Mon810, Sorte „Novelis“) wurde in zwei Feldversuchen bei Bonn und Halle untersucht. Insgesamt wurden 1085 Proben mit Hilfe des Double Antiserum-Enzyme Linked Immunosorbent Assay (DAS-ELISA) untersucht. Der Cry1Ab-Gehalt verschiedener Pflanzengewebe (Wurzel, Stängel, oberes Blatt, unteres Blatt, Staubbeutel, Pollen und Korn) wurde in vier verschiedenen Entwicklungsstadien (BBCH19, BBCH30, BBCH61 and BBCH83) in den Jahren 2001, 2002 und 2003 bestimmt. Die Blätter von Mon810 zeigten den höchsten Cry1Ab-Gehalt ($5.5 - 6.4 \mu\text{g g}^{-1}$ Frischgewicht [FG] bei BBCH83), während die Pollen mit $1 - 97 \text{ ng g}^{-1}$ FG den geringsten Gehalt aufwiesen. Der Cry1Ab-Gehalt auf dem Feld verbliebener Wurzeln betrug neun Monate nach der Ernte $15 - 17 \text{ ng g}^{-1}$ FG, also nur etwa ein Hundertstel des Gehalts frischer Maiswurzeln. Die Expression des Cry1Ab-Proteins variierte gravierend zwischen einzelnen Maispflanzen.

Stichwörter: Cry1Ab-Expression, ELISA, Maiszünsler, Mon810, transgener Mais

1 Introduction

The soil bacterium *Bacillus thuringiensis* (*Bt*) *kurstaki* strain HD1 produces a number of insect toxins during bacterial sporulation, including the delta endotoxin Cry1Ab (WHITELEY

and SCHNEPF 1986). For decades, the potential of *Bt* insecticidal proteins for control of certain insects has been known and since its registration in the USA in 1961 *Bt* has been a widely used biopesticide (KRATTIGER 1996). In recent years, a modified bacterial Cry1Ab gene was introduced into maize plants (*Zea mays*) by genetic engineering (PERLAK et al. 1991; KOZIEL et al. 1993). *Bt* maize expressing the Cry1Ab protein is a new approach to provide protection against corn borers including the European corn borer (*Ostrinia nubilalis*) (ECB) and Mediterranean corn borer (*Sesamia nonagrioides*) (SHELTON et al. 2002). Several transgenic maize events, e. g. Mon810, Bt11, Bt176, have been developed and commercialised (MENDELSON et al. 2003). In 2005, more than 21 Mio hectares were grown world wide (TRANSGEN 2005). These events express the Cry1Ab toxin in different truncated forms. Mon810 contains a truncated *cry1Ab* coding sequence, that expresses the 92 kDa N-terminal fragment of the full length Cry1Ab protein (130 kDa) of *B. thuringiensis* ssp. *kurstaki* strain HD1 (ESSENTIAL BIOSAFETY 2001). In the United States, Mon810 was tested at field scale in 1994 and 1995 and it was registered in 1996 (MENDELSON et al. 2003). Since then, Mon810 has been cultivated in many countries world-wide. In 1998, the Mon810 varieties also became registered in the European Union.

Large-scale plantation of *Bt* crops raised scientific concerns about a rapid selection for resistance in field population of the target insects (ALSTAD and ANDOW 1995; GOULD 1998). Also the potential effect on non-target organisms and potential persistence of recombinant Cry1Ab in soil became an issue of ecological debate (TAPP and STOTZKY 1998; HELLMICH et al. 2001; DUTTON et al. 2003; ROMEIS et al. 2004).

One of the important parameters to assess the potential of development of resistance by the European corn borer and the toxicity of *Bt* maize to the exposed non-target insects is the level of Cry1Ab toxin expression in *Bt* maize crops. There is an almost complete lack of peer-reviewed literature on the Cry1Ab expression of Mon810 at different plant growth stages, tissue types and seasons, despite of the world-wide use of Mon810 varieties (CLARK et al. 2005). Most of the published data on Cry1Ab expression levels derived from a very few studies and are limited to a few tissue types, such as root, stalk and anther (EPA BRAD 2001; AGBIOS 2001). In order to close this gap in knowledge, we conducted a systematic investigation on Cry1Ab contents of event Mon810 (variety 'Novelis') and examined Cry1Ab expression in different plant tissues at four growth stages. Data from two different geographical locations in Germany and from three successive growing seasons are compared.

2 Materials and methods

2.1 Plant variety and collection of plant samples

The transgenic maize variety 'Novelis' (event Mon810) and the corresponding non-transgenic variety 'Nobilis' were grown on two field sites, which were located near Bonn

(North Rhine-Westphalia) (N 50°38', E 006°54') and Halle (Saxony-Anhalt) (N51°40', E 012°07') in Germany over a period of 3 years (2001–2003). These field sites were for many years under usual agricultural cultivation. The “Bonn” field was 7.8 ha in size and included two fields, containing eight plots of each ‘Novelis’ (Mon810) and ‘Nobilis’. Each plot was 2,430 m² in size. The “Halle” field was 27 ha in size and included six repetitive plots of the transgenic and the control varieties, respectively. Each plot was 3,650 m² in size. The soils at the field sites Bonn and Halle were a Stagnic Luvisol and Luvic Phaeozem, respectively. All clay fractions from these soils showed nearly the same mineralogical composition (PAGEL-WIEDER et al. 2004).

Maize plants were collected at four growth stages following the BBCH scale of MEIER (2001): leaf development (BBCH19); stem elongation (BBCH30); flowering, anthesis (BBCH61) and ripening (BBCH83). In 2001 and 2003, one *Bt* maize plant was randomly sampled per plot at each of the four BBCH stages, whereas in 2002 two *Bt* maize plants per plot were harvested. Non-transgenic control plants were collected from a single control plot of each field experiment in “Bonn” and in “Halle”. They were used as negative controls.

In 2002 and 2003, pollen samples were collected from different *Bt* plants at BBCH61. The samples were sifted through a 0.5-mm mesh screen to remove anthers and tassel material and poured into 2.0-ml Eppendorf tube. Maize ear shanks were collected at BBCH83 in 2003. In addition, decaying root stocks, which remained in/on the soil from growing seasons 2001 and 2002, were collected in June 2002 (two samples) and June 2003 (16 samples), respectively, at the field site “Bonn”.

For BBCH19 and BBCH30, the whole plants were harvested and transported at 4°C to the laboratory. For BBCH61 and BBCH83, the plant was first cut into small segments on the field, placed in a cool polystyrene box and then transported to the laboratory. The root samples and rotten root stocks were washed with water to remove soil particles and dried on paper towels. The leaf samples were collected from the middle of the second upper leaf and the second lower leaf, respectively. The selected plant tissues including root, stalk, upper leaf, lower leaf, anthers, pollen, kernel, maize ear shank and root residues were immediately stored at -80°C until further analysis.

2.2 Quantification of Cry1Ab using ELISA

Cry1Ab protein contents of transgenic maize plant tissues were quantified by the sandwich ELISA using the Cry1Ab/Cry1Ac Quantiplate® kit (Envirologix, Portland, OR, USA). The Cry1Ab extraction protocols for different plant tissues were slightly modified from manufacturer’s instructions. One-hundred mg of each plant sample, except for pollen, kernel and root residues, were grinded in 2.5 volumes of extraction buffer (Cry1Ab/Cry1Ac Quantiplate® kit) in universal homogenization bags (Bioreba, Reinach, Switzerland) using a Bioreba homogenizer. Then, the plant extracts were transferred into a 1.5-ml Eppendorf tube and kept on ice. The pollen samples were weighted in a 2-ml Eppendorf tube. The pollen was grinded in one volume of extraction buffer by rotating the pestle against the sides of the tube with twisting motions and incubated over night at 4°C with slightly shaking (100 rpm). Cry1Ab was extracted from kernels by putting them into universal homogenization bags (Bioreba) and crushing single seeds with a hammer. One-hundred mg of this powder and one volume of 1x grain extraction solution (Cry1Ab/Cry1Ac Quantiplate® kit) were then transferred into a 2-ml Eppendorf tube. The samples were extracted in the buffer for at least 4 h at 4°C with shaking (100 rpm). The rotten root stocks were grinded in liquid nitrogen using a mortar and a pestle.

All plant extracts were centrifuged at 2700 × *g* (Eppendorf centrifuge 5417C) for 5 min at 4°C. The supernatants were

diluted and used for the ELISA test. The extracts of the non-transgenic plants were used as a blank control. The absorbance was measured at 450 nm with a BioRad model 550 microplate reader (BioRad, Hercules, CA, USA) and data were analysed using the Microplate Manager 4.0 program (BioRad). The limit of detection of the ELISA quantification was determined according to KAISER (1965) and was 0.14 ng Cry1Ab. Cry1Ab concentrations were determined by calculating the mean optical density reading against the standard curve. Cry1Ab expression was quantified as µg or ng per g fresh weight after multiplication with the dilution factors. The means of the duplicate measurement in ELISA was used for the analyses.

2.3 Statistical analysis

The Mon810 plants were grown in two geographic locations which differed in their environmental factors (e.g. soil, weather). Therefore, the obtained data were separately evaluated for each location. Statistical analysis was performed using software package SAS (SAS Institute 2003). The means of Cry1Ab levels of three crop seasons and their standard errors were computed with PROC MEANS. The dataset was tested for normality of the residuals by Shapiro-Wilk *W* statistics (PROC UNIVARIATE) and for homogeneity of variances among treatment groups with Levene test (PROC GLM). Log-transformed Cry1Ab concentrations satisfied both assumptions, so LOG10(Cry1Ab+1) data were used in all analysis of variances. Therefore, covariance parameters were estimated using PROC MIXED with REML and the denominator degrees of freedom for the tests of fixed effects were computed with KENWADROGER option (SPILKE and TUCHSCHERER 2001; SPILKE et al. 2002;). The effect of growth stages on Cry1Ab levels were analyzed separately within each tissue type, with crop seasons (three year 2001–2003) as fixed effect (PIEPHO et al. 2003). Not all plant tissues were available in each growth stage. The kernel only exists in BBCH83 and anthers only appear in BBCH61. Therefore, the analyses were separated for the four growth stages BBCH19, BBCH30, BBCH61 and BBCH83, in order not to violate the MCAR assumption (missing completely at random) for the analysis of variances to compare plant tissues (PIEPHO et al. 2003). Furthermore, the whole plants were collected in each growth stage. Hence, the four stages were disregarded in the statistical model as repeated measures over time. To evaluate the difference of Cry1Ab levels in various tissues within each growth stage, crop seasons were considered as fixed effect and growth stages were analyzed separately. For pairwise comparison of differences between the means among growth stages and between plant tissues least square means (PROC MIXED, LSMEANS statement) were performed.

3 Results and discussion

In total, the Cry1Ab contents of 1085 plant tissue samples of Mon810 were determined during three growing seasons 2001, 2002 and 2003. The Cry1Ab levels at four growth stages and different tissues differed significantly ($F = 46.26$, $df = 801$, $P < 0.001$) among the three growing seasons, which might have been caused by differing weather conditions. Since it was the aim of the study to provide a comprehensive overview on the Cry1Ab expression levels in various maize tissues at different growth stages, only the means of the three year study are presented and discussed in the following.

3.1 Cry1Ab levels of Mon810 at the field site “Bonn”

3.1.1 Comparison of Cry1Ab levels among different plant tissues. The analysis of the Cry1Ab contents of Mon810 at the field site “Bonn” revealed significant differences among the

four growth stages (BBCH19: $F = 159.99$, $df = 85$, $P < 0.0001$; BBCH30: $F = 311.55$, $df = 115$, $P < 0.0001$; BBCH61: $F = 45.75$, $df = 90$, $P < 0.0001$; BBCH83: $F = 344.50$, $df = 95$, $P < 0.0001$) (Table 1). The Cry1Ab levels were the highest in the leaves, followed by anther, root and the stalk tissues. However, the mean Cry1Ab levels in upper leaves and in the lower leaves did not differ significantly ($t = 0.43$, $df = 90$, $P = 0.6663$) at BBCH61. A low Cry1Ab level ($0.268 \mu\text{g g}^{-1}$ fw) was detected in the kernels, where a strong variation was observed (Table 1). This result is similar to previous observations in the US in 1994 (AGBIOS 2001) but it is about 1.5 to 2.0-fold lower than reported from European field trials in 1995 (AGBIOS 2001). Cry1Ab levels in anthers were $0.48 - 4.65 \mu\text{g g}^{-1}$ fw. In addition, single pollen samples were collected in 2002 and 2003 and their Cry1Ab contents were 1 ng g^{-1} fw and 3 ng g^{-1} fw, respectively. This low level of Cry1Ab in pollen is consistent with a previous study (EPA 1999).

3.1.2 Comparison of Cry1Ab levels among different growth stages. The expression of Cry1Ab in the roots was relatively constant throughout the growing seasons in all three years. The mean Cry1Ab levels did not show significant differences among the four growth stages ($F = 0.95$, $df = 95$, $P = 0.4213$). In the stalks, the levels of Cry1Ab increased steadily during the growing season. The mean Cry1Ab level at BBCH83 was 2-3-fold higher than at BBCH19 ($t = -9.45$, $df = 93$, $P < 0.0001$) and BBCH30 ($t = -11.94$, $df = 93$, $P < 0.0001$). No significant difference was found between BBCH61 and BBCH83 ($t = -1.50$, $df = 93$, $P = 0.1382$) (Table 1). In the lower leaves, the mean Cry1Ab level at BBCH 61 was significantly lower than at BBCH30 ($t = 6.66$, $df = 66$, $P < 0.0001$) and BBCH83 ($t = -5.49$,

$df = 66$, $P < 0.0001$) (Table 1). A significant increase of the Cry1Ab levels at BBCH83 was determined in the upper leaves. The Cry1Ab content was approximately 2-fold higher at BBCH83 than at BBCH19 ($t = -10.92$, $df = 94$, $P < 0.0001$) (Table 1). The Cry1Ab content in the individual upper leaf samples varied similar strongly as in the lower leaf at BBCH83.

3.2 Cry1Ab levels of Mon810 at the field site "Halle"

3.2.1 Comparison of Cry1Ab levels among different plant tissues. At the field site "Halle", a similar picture of Cry1Ab expression patterns to that of "Bonn" was observed. The Cry1Ab levels were found significantly different among various plant tissues in each developmental stage (BBCH19: $F = 116.95$, $df = 54$, $P < 0.0001$; BBCH30: $F = 255.44$, $df = 80$, $P < 0.0001$; BBCH61: $F = 71.07$, $df = 100$, $P < 0.0001$; BBCH83: $F = 153.04$, $df = 99$, $P < 0.0001$) (Table 2). No difference in the Cry1Ab level was observed between the lower leaves and the upper leaves at BBCH61 ($t = 1.61$, $df = 100$, $P = 0.1106$) and at BBCH83 ($t = 0.92$, $df = 99$, $P = 0.3617$) and between the roots and the stalks at BBCH83 ($t = -1.57$, $df = 99$, $P = 0.1203$) (Table 2). The highest Cry1Ab contents were found in the leaf samples, followed by the anthers, roots and the stalks. However, the Cry1Ab content of leaves varied strongly between $1.88 - 11.07 \mu\text{g g}^{-1}$ fw (BBCH83). These values corroborate previous studies in the US (1994) and in the European field trials (1995), when $7.9 - 10.3 \mu\text{g g}^{-1}$ fw and $7.59 - 9.39 \mu\text{g g}^{-1}$ fw leaves were determined, respectively (AGBIOS 2001). Low levels of Cry1Ab were detected in the kernels. Cry1Ab contents in the pollen were 6 ng g^{-1} fw and 97 ng g^{-1} fw, respectively.

Table 1: Mean Cry1Ab levels in various tissues of event Mon810 (variety Novellis) sampled during three growing seasons (2001–2003) at the field site "Bonn"*

Tissues		Cry1Ab ($\mu\text{g/g}$ fresh weight)			
		Growth stage			
		BBCH19	BBCH30	BBCH61	BBCH83
Root	Mean (SE)	1.449 (0.098) aA	1.386 (0.112) aA	1.421 (0.108) aA	1.419 (0.087) aA
	Range	0.471 – 2.389	0.266 – 2.402	0.589 – 2.429	0.748 – 2.139
	n	32	32	21	22
Stalk	Mean (SE)	0.404 (0.038) aB	0.333 (0.028) bB	0.988 (0.100) cB	1.127 (0.073) cB
	Range	0.133 – 1.096	0.078 – 0.621	0.511 – 2.402	0.354 – 1.910
	n	31	31	21	22
Lower leaf	Mean (SE)	nd	4.373 (0.267) aC	2.541 (0.188) bC	3.946 (0.204) aC
	Range		1.138 – 7.759	1.265 – 4.750	2.227 – 5.868
	n		32	21	22
Upper leaf	Mean (SE)	2.451 (0.149) aC	3.236 (0.244) bD	2.718 (0.212) aC	5.521 (0.242) cD
	Range	0.316 – 4.620	0.699 – 6.591	1.241 – 4.518	3.589 – 8.597
	n	31	32	21	22
Anther	Mean (SE)	–	–	2.050 (0.268) AD	–
	Range			0.485 – 4.658	
	n			21	
Kernel	Mean (SE)	–	–	–	0.268 (0.023) E
	Range				0.057 – 0.509
	n				22

* SE = standard error, n = number of samples, nd = not determined. Range gives the minimum and the maximum value during the survey of three years. Means within a row followed by the same lowercase letter are not significantly different. Means within a column followed by the same capital letter are not significantly different ($P > 0.05$, PROC MIXED, LSMEANS, SAS Institute 2003).

Table 2: Mean Cry1Ab levels in various tissues of event Mon810 (variety Novelis) sampled during three growing seasons (2001–2003) at the field site “Halle”*

Tissues		Cry1Ab ($\mu\text{g/g}$ fresh weight)			
		Growth stage			
		BBCH19	BBCH30	BBCH61	BBCH83
Root	Mean (SE)	1.594 (0.229) aA	1.683 (0.200) aA	1.606 (0.105) aA	1.583 (0.137) aA
	Range	0.279 – 3.947	0.466 – 4.174	0.608 – 2.690	0.336 – 2.789
	n	21	23	21	22
Stalk	Mean (SE)	0.463 (0.047) aB	0.433 (0.046) aB	1.017 (0.108) bB	1.238 (0.127) cA
	Range	0.174 – 1.034	0.180 – 0.850	0.356 – 1.982	0.467 – 2.605
	n	21	23	23	23
Lower leaf	Mean (SE)	nd	4.618 (0.298) abC	4.205 (0.295) aC	5.779 (0.504) bB
	Range		2.553 – 6.976	2.026 – 7.043	1.359 – 9.603
	n		23	23	23
Upper leaf	Mean (SE)	3.333 (0.188) aC	2.911 (0.311) bD	5.060 (0.365) cC	6.367 (0.436) dB
	Range	1.964 – 4.707		1.960 – 8.580	1.878 – 11.072
	n	21	23	23	23
Anther	Mean (SE)	–	–	2.808 (0.294) D	–
	Range			0.301 – 6.650	
	n			23	
Kernel	Mean (SE)	–	–	–	0.235 (0.026) C
	Range				0.008 – 0.461
	n				23

* SE = standard error, n = number of samples, nd = not determined. Range gives the minimum and the maximum value during the survey of three years. Means within a row followed by the same lowercase letter are not significantly different. Means within a column followed by the same capital letter are not significantly different ($P > 0.05$, PROC MIXED, LSMEANS, SAS Institute 2003).

3.2.2 Comparison of Cry1Ab levels among different growth stages. In the root, Cry1Ab was stably expressed and no statistical difference in Cry1Ab levels was observed among the four growth stages ($F = 0.53$, $df = 77$, $P = 0.6617$). In the stalks, mean levels of Cry1Ab increased significantly from BBCH30 to BBCH83 ($F = 51.31$, $df = 78$, $P < 0.0001$). However, there was no significant difference in Cry1Ab levels between BBCH19 and BBCH30 ($t = 0.67$, $df = 78$, $P = 0.5028$). In the lower leaves, significant differences in Cry1Ab levels were found among the different growth stages ($F = 3.19$, $df = 60$, $P = 0.0483$) and the mean Cry1Ab level in BBCH83 was significantly higher than in BBCH61 ($t = -2.51$, $df = 60$, $P = 0.015$). For the upper leaves, a steady and significant increase of the Cry1Ab content was observed throughout the four tested growth stages ($F = 28.16$, $df = 78$, $P < 0.0001$). The lowest was in BBCH30 ($2.911 \mu\text{g g}^{-1}$ fw) and the highest was in BBCH83 ($6.367 \mu\text{g g}^{-1}$ fw).

3.3 Cry1Ab levels in maize ears and residual roots

During the field season 2001 we observed that ECB occasionally infested either the stalks or the ears of some *Bt* maize plants. Therefore, it was important to survey the levels of Cry1Ab in these tissues. Samples of the maize ear shank were also collected in 2003 and their Cry1Ab levels were quantified. The average Cry1Ab contents were $0.79 \mu\text{g g}^{-1}$ fw (“Bonn”) and $0.90 \mu\text{g g}^{-1}$ fw (“Halle”) in the maize ear shank samples (Fig. 2A) and $0.26 \mu\text{g g}^{-1}$ fw (“Bonn”) and $0.34 \mu\text{g g}^{-1}$ fw (“Halle”) in the kernel samples, respectively. Hence, their Cry1Ab levels were obviously lower than those in the leaves at

BBCH83, which probably contributes to the survival of ECB on transgenic maize by tunnelling in the ear shank.

Since Mon810 was cultivated during three subsequent seasons in the same field, numerous root stocks residues remaining from the previous year could be observed on the field surface after soil tillage. The residual roots stocks were collected in June 2002 and June 2003, respectively, from the field “Bonn”. Their Cry1Ab level was $15\text{--}17 \text{ ng g}^{-1}$ fw (Fig. 3). This level was about 100 times lower than that in fresh root samples. BAUMGARTE and TEBBE (2005) reported higher Cry1Ab contents (183 ng g^{-1} fw) in root residues from the field trial “Halle”, which were collected two months earlier than in our investigations. These findings suggest that Cry1Ab or ELISA detectable breakdown products can persist at low level in the residual plant in the field for a relatively long time. However, it is not clear whether these ELISA detectable Cry1Ab residues still retain their bioactivity. Bioassays with highly susceptible neonate ECB larvae did not provide conclusive results whether Cry1Ab still had insecticidal activity after 200 days of decay (ZWÄHLEN et al. 2003). Hence, the observation of some long-term persistence of Cry1Ab in the remainder of the *Bt* maize plant on the field requests more extensive studies on the potential effects of these products on non-target organisms in or on the soil.

3.4 Comparison of Cry1Ab levels between the field sites “Bonn” and “Halle”

The comparison of Cry1Ab levels between the two field sites “Bonn” and “Halle” showed significant differences ($F = 47.58$,

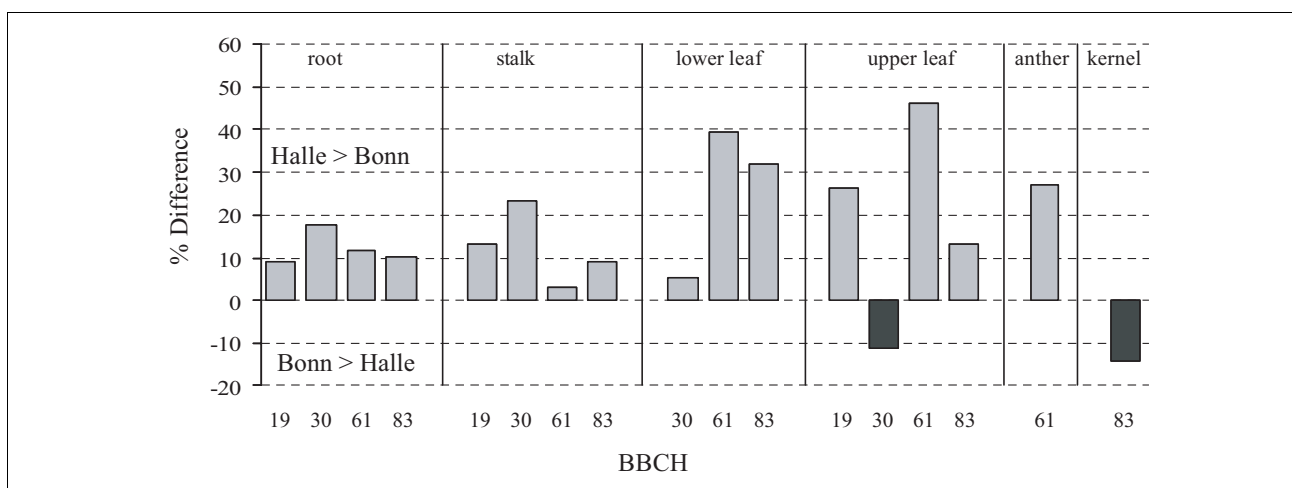


Fig. 1: Percentage of differences in expression levels of Cry1Ab in various plant tissues of Mon810 (variety Novellis) between two field sites “Halle” and “Bonn” over three seasons (2001-2003). The percentage difference was calculated as following:

$$\% \text{ difference} = \frac{\text{Cry1Ab level in Halle} - \text{Cry1Ab level in Bonn}}{\text{Cry1Ab level in Halle}} \times 100\% .$$

Positive differences indicate that Cry1Ab levels in “Halle” were higher than in “Bonn”, negative differences mean that Cry1Ab levels in “Halle” were lower than in “Bonn”.

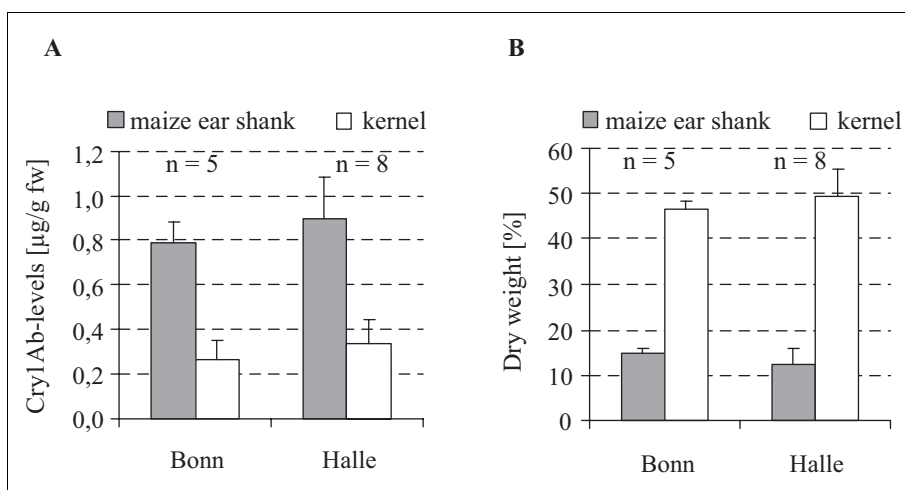


Fig. 2: A. Mean expression levels of Cry1Ab (± SD, standard deviation) in maize ear of event Mon810 at field sites “Bonn” and “Halle”. B. Dry weight of maize ear shank and kernel of Mon810, n = number of samples.

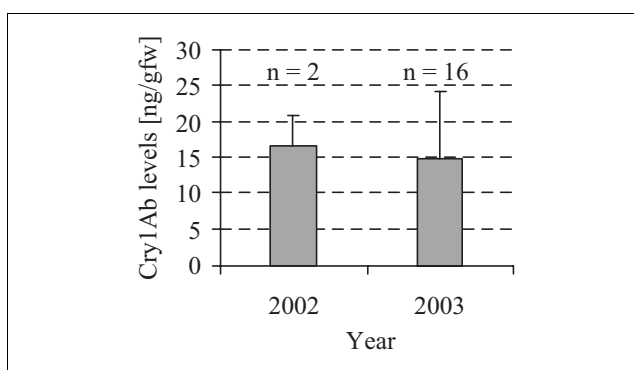


Fig. 3: Mean Cry1Ab levels (± SD, standard deviation) in residual roots of Mon810 that were collected eight months after harvest, n = number of samples.

df = 801, $P < 0.001$). The Cry1Ab levels in plants of the “Halle” field were predominantly higher than those from the “Bonn” field. In 15 out of 17 tissue samples the mean Cry1Ab contents of plants growing in “Halle” were higher than those detected from “Bonn” (Fig. 1). The highest difference in Cry1Ab contents was in the upper leaf (46%). Field site effects on mean Cry1Ac levels of tissue samples were also observed for trans-

genic cotton Bollgard I531 variety when sensitive quantitative bioassays were applied (GREENPLATE 1999).

In conclusion, our analyses are the first large-scale expression monitoring of Cry1Ab under European field conditions and provide a comprehensive data set of the temporal distribution of Cry1Ab in transgenic maize Mon810. Cry1Ab expression was lowest in pollen, very low in the stalks, low in roots, but highest in the leaves. Although our studies corroborate the tendencies of reported Cry1Ab contents of Mon810 (AGBIOS 2001, MENDELSON et al. 2003), a considerable variation in the expression levels of Cry1Ab was observed. The observed variation exceeds variation levels reported previously and may be due to the large number of analysed samples and different growing years. They suggest a certain plant to plant variation in Cry1Ab expression. Cry1Ab levels also varied in different plant tissues of Mon810 at different growth stages. The overall small differences but similar patterns of Cry1Ab levels at the two field sites “Bonn” and “Halle” clearly indicate that plant tissue and plant development are the main parameters affecting the Cry1Ab contents of transgenic Mon810.

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