

REPORT NO.: MSL-17561

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TITLE: DNA Sequences Flanking the 3' End of the Functional Insert of Roundup Ready Soybean Event 40-3-2 are Identical to DNA Sequences from the Wild-type Soybean Lines A5403 and A3244

AUTHORS:

ABSTRACT: Recently, an independent report by Windels *et al.*, confirmed previous Monsanto findings that described the soybean genomic DNA sequences flanking the functional insert of Roundup Ready[®] soybean event 40-3-2. Windels *et al.* described a 534 base pair segment of genomic DNA sequence flanking the 3' end of the functional insert immediately after the partial CP4 EPSPS element. Their comparison of this genomic DNA sequence with the public DNA database did not identify any homology between the 534 base pair DNA segment and DNA sequences in public DNA sequence databases. In addition to describing these flanking DNA sequences, Windels *et al.* performed PCR experiments using oligonucleotide primers designed to the genomic DNA sequences flanking the 3' and 5' end of the functional insert to assess whether the sequences flanking the functional insert in Roundup Ready soybean event 40-3-2 were present in the parent soybean line, A5403. No PCR product was obtained in the parental soybean line using the primer pair that spanned the insert. These results are consistent with the results previously reported by Monsanto (Lirette, *et al.*, 2000).

To confirm that the 534 base pairs of genomic DNA sequence flanking the 3' end of the functional insert in event 40-3-2 is soybean genomic DNA, we compared this 534 base pair DNA sequence against Monsanto's proprietary soybean genomic DNA sequence collection. This comparison identified a soybean BAC clone, 104c16, that contains large portions of sequence identity to the 534 base pair region. Additionally, PCR assays were performed on both Roundup Ready soybean event 40-3-2 and the parental line A5403 in order to confirm that the DNA sequence flanking the 3' end of the functional insert is soybean genomic DNA. These PCR analyses resulted in the predicted products in both 40-3-2 and A5403. Based upon these findings, it can be concluded that the DNA sequence is soybean genomic DNA.

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Recently, an independent report by Windels *et al.*, confirmed previous Monsanto findings that described the soybean genomic DNA sequences flanking the functional insert of Roundup Ready® soybean event 40-3-2 (Lirette, *et al.*, 2000). Windels *et al.* described a 534 base pair segment of genomic DNA sequence flanking the 3' end of the functional insert immediately after the partial CP4 EPSPS element (Lirette, *et al.*, 2000). Their comparison of this genomic DNA sequence with sequences in the public DNA database did not identify any homology between the 534 base pair DNA segment and DNA sequences known or publicly available. In addition to describing these DNA sequences, Windels *et al.* performed PCR experiments using oligonucleotide primers designed to the genomic DNA sequences flanking the 3' and 5' end of the functional insert to assess whether the sequences flanking the functional insert in Roundup Ready soybean event 40-3-2 were present in the parent soybean line, A5403. No PCR product was obtained in the parental soybean line using the primer pair that spanned the insert. These results are consistent with the results previously reported by Monsanto (Lirette, *et al.*, 2000).

To confirm that the 534 base pairs of genomic DNA sequence flanking the 3' end of the functional insert in event 40-3-2 is soybean genomic DNA, we compared this 534 base pair DNA sequence against Monsanto's proprietary soybean genomic DNA sequence collection. Additionally, PCR assays were conducted on both Roundup Ready soybean event 40-3-2 and the parental line A5403 in order to confirm that the DNA sequence flanking the 3' end of the functional insert is soybean genomic DNA.

Using BLASTN analysis (which determines DNA sequence homologies in complex databases; Altschul, *et al.*, 1990), DNA sequences in Monsanto's proprietary soybean genomic DNA sequence collection that contain sequences identical to the majority of the 534 base pair region were identified. This analysis identified a clone from a BAC (Bacterial Artificial Chromosome) library designated 104c16 that contains sequence identical to the 534 base pair DNA sequence flanking the 3' end of the functional insert. This BAC clone was derived from genomic DNA of the conventional non-transgenic soybean variety A3244, and thus represents a small portion of the genome of that variety. A BestFit alignment (Wisconsin Version 10.0, Genetics Computer Group (GCG), Madison, WI) was performed between the 534 base pair DNA sequence from event 40-3-2 and the BAC clone 104c16. Base pair positions 1-28 and 108-528 of the 534 base pair DNA sequence have 100% sequence identity to sequence from clone 104c16. These regions are underlined in Figure 1. While these two regions share 100% sequence identity to clone 104c16, they do not represent a continuous sequence on this clone; these two regions of sequence are present on separate sections of the BAC clone. Previous Monsanto reports, as well as Windels *et al.*, suggested that the soybean genomic DNA flanking the 3' end of the functional insert in Roundup Ready event 40-3-2 is rearranged relative to these sequences in conventional soybeans. These data confirm this hypothesis.

To confirm that the DNA sequence flanking the 3' end of the functional insert of Roundup Ready soybean event 40-3-2 is soybean genomic DNA, PCR analysis was performed using oligonucleotide primers designed to the DNA sequence flanking the 3' end of the functional insert (Figure 1). Three different DNA sources were used as templates including genomic DNA from Roundup Ready soybean event 40-3-2, genomic DNA prepared from the parental line A5403, and purified BAC DNA of the clone 104c16, which was derived from the conventional variety A3244. In all three experimental samples the amplification resulted in the predicted size PCR product of 295 base pairs (Figure 2). These three PCR products were subsequently gel excised and submitted for DNA sequencing. Comparison of the DNA sequences of these three independent products demonstrated that they were 100% identical to each other. Comparison of the resulting consensus sequence from these products to the DNA sequence reported by Windels, *et al.* demonstrated ~99% sequence similarity. The few mismatched bases that were observed, relative to the sequence reported by Windels, *et al.*, are in lower case letters in Figure 1. These mismatched bases could be the result of either sequencing errors or amplification artifacts, since in some cases, the DNA sequence from the PCR products reported by Windels, *et al.* was based on DNA that was subjected to three rounds of PCR amplification.

Based upon the findings that the majority of the 534 base pairs in the 3' region flanking the functional insert in Roundup Ready soybeans previously described is also present in both the wild-type soybean lines A5403 and A3244, it can be concluded that this sequence is genomic soybean DNA.

References:

Windels, P., Taverniers, I., Depicker, A., Van Bockstaele, E., and M. De Loose. 2001. Characterization of the Roundup Ready soybean insert. *Eur. Food Res. Technol.* 213,107-112.

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Altschul, S. F., Gish, W., Miller, W., Myers, E. W., & Lipman, D.J. Basic local alignment search tool. *J. Mol. Biol.* 215, 403-410 (1990).

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1 GCATCATGCT GGGAAATTTT AGCGAGATTA TAAGTATCTT CCTGGGGATC TCTGCTGTTA
61 CTGGTGAATA GTGAGACAGA GTCTTCTGAG CTCATAGGAT AAAATAAATT ATAATTAGTA
121 AATTTTTTTAA TTAAATAAAT CAATTACTTC ATAAATAAATT TTTTTTATAG AATATGTTGA
181 CATTCTAGCT GGATATAGAA CTAATaTAAa GAAACCTTAA AAATTTTGTT TGGAAGAATA
241 TGTATTGAA AGACAAATCT AATTAAGTTT ATCAGGGTCA TTTGTTGAAG ATAGGAAACC
301 TTCAGCAATT TGAATATTAA GTAACCTGCTT CTCCCAGAAT GATCGGAGTT TCTCCTCCTG
361 CTATTACATG AaaAAAAATA AAAAAATAAa AAAAGATAAG ATTAAGCTTC AACATGTGAA
421 GGAGTAGTAC ACTCACCAGT GACCCTAATA GGCAACAGCA TGAAAAAAAA TAAAAAGAA
481 TAAAAATAGC ATCTACATAT AGCTTCTCGT TGTTAGAAAA ACAAACCTAT TTGG
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Figure 1. Regions of Sequence Similarity Between the 534 Base Pairs Flanking the 3' End of the Functional Insert of Roundup Ready Soybean Event 40-3-2 and BAC Clone 104c16.

The DNA sequence shows 534 base pairs flanking the 3' end of the functional insert of Roundup Ready soybean event 40-3-2. The underlined regions have 100% sequence identity to the soybean BAC clone 104c16, prepared from conventional soybean line A3244, as well as the conventional soybean parental line A5403. Oligonucleotide primers used for PCR are highlighted as bold, double underlined text. Amplification with these primers produced the predicted size amplicon (Figure 2) from genomic DNA templates prepared from Roundup Ready soybean event 40-3-2 and conventional soybean line A5403, as well as DNA prepared from the BAC clone 104c16. Nucleotide bases in lower case were observed not to be consistent with the DNA sequence of the region as reported by Windels *et al.*

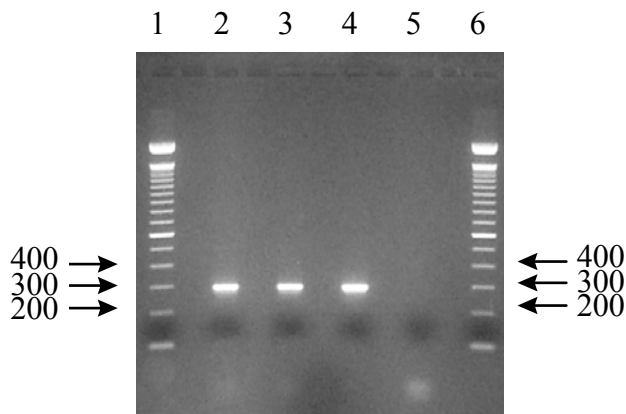


Figure 2. Demonstration that the Genomic DNA Flanking the 3' End of the Functional Insert of Roundup Ready Soybean Event 40-3-2 is Soybean Genomic DNA.

PCR amplification using the primers highlighted in Figure 2 and genomic DNA template from Roundup Ready soybean event 40-3-2 (Lane 2) as well as the conventional soybean line A5403 (Lane 3) and soybean genomic BAC clone 104c16 (Lane 4). Lane 5 contains no DNA template and serves as the negative control. Lanes 1 and 6 contain Gibco BRL 100 bp DNA Ladders. All PCR analyses were conducted using 100 ng of genomic DNA template or 10 ng of BAC template in a 50 μ L reaction volume containing a final concentration of 1.5 mM Mg^{2+} , 0.2 μ M of each primer, 200 μ M of each dNTP, and 2.5 units of Platinum *Taq* DNA polymerase. The reactions were performed under the following cycling conditions: 1 cycle of 94°C for 3 minutes; 35 cycles of 94°C for 30 seconds, 60°C for 30 seconds, 72°C for 30 seconds; 1 cycle of 72°C for 10 minutes. Twenty microliters of each PCR reaction were loaded on the gel.

→ Symbol denotes sizes obtained from MW markers on ethidium bromide stained gel.