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PETITION FOR REGULATORY STATUS REVIEW

FOR WHEAT EVENT IND-00412-7 (HB4 WHEAT)

TRIGALL GENETICS SA.

PATRICIA V MIRANDA, PH.D.
DIRECTOR OF REGULATORY AFFAIRS

JULY 18, 2022

Certification

The undersigned certifies to the best of her/his knowledge that:

- All the information and data in this document are complete and correct
- All information known to be unfavorable is included in this document

July 18, 2022

Patricia V Miranda, Ph.D. Date

Director of Regulatory Affairs

1. Information about Requestor

First name: Patricia
Last name: Miranda
Position: Director of Regulatory Affairs
Organization name: Trigall Genetics SA
Contact information: patricia.miranda@bioceresgroup.com

2. Confidential Information

This application does not contain CBI

3. Description of the comparator plant

Scientific name: *Triticum aestivum*
Common name: Wheat
Subspecies*:* Not applicable
Cultivar: Cadenza

4. Genotype of the modified plant

The wheat event IND-00412-7 expresses two genes of interest (GOI): a variant of the sunflower *HaHB4* gene (*HaHB4v*) that codes for a transcription factor (TF) belonging to the HD-Zip protein family involved in the plant response to environmental stresses (Gago et al., 2002, Dezar et al., 2005; Manavella et al., 2006, 2008a, 2008b); and the *bar* gene from *Streptomyces hygroscopicus*, expressing the glufosinate-inactivating enzyme phosphinothricin N-acetyl transferase (PAT) (Thompson et al., 1987).

The commercial variety Cadenza (SASA, 2014) was used to generate the transgenic event via particle bombardment.

The molecular characterization of wheat event IND-00412-7 was carried out using Next Generation Sequencing (NGS) techniques. Given the complexities of the wheat genome (~17 Gpb size, hexaploidy, and ~80% of repetitive sequences) (Mayer et al., 2014), including the lack of a reference genome sequence until recently, a stepwise procedure was followed to reduce the size and complexity of the material being sequenced. First, the DArT platform (Diversity Arrays Technology, Jaccoud et al., 2001) was used to find an association between the *HaHB4v* gene and a particular chromosomal locus. The results indicated that the genetic modification was located in chromosome 2D.

Additional confirmation of the location of the genetic modification in chromosome 2D was achieved by analyzing the segregation of a set of 432 single nucleotide polymorphism markers distributed all along the wheat genome in 17 populations obtained from genotyping data during breeding. Genetic linkage with *HaHB4v* and glufosinate tolerance was only observed for those markers located in the 2D chromosome.

Then, the 2D chromosome from wheat event IND-00412-7 (T7 generation) was successfully isolated by flow cytometry (Doležel et al., 1989; Vrána et al., 2000; Suchánková et al., 2006; Šimková et al., 2008) and prepared for NGS. The sequencing strategy consisted of the combination of Illumina HiSeq (Illumina Inc.) and PacBio (Pacific Biosciences of California, Inc.) technologies, a powerful tool for the generation of high-accuracy long sequence fragments (Mahmoud et al., 2019).

The presence of four junction points (JPs) containing both insert and wheat sequences was supported by Illumina and PacBio reads, as well as by PCR amplification products obtained by Sanger sequencing. This revealed the existence of two inserts in wheat event IND-00412-7. The final assembly of the insertions revealed the presence of one complete copy of the *HaHB4v* gene and three complete copies of the *bar* gene, with their respective regulatory elements required for transcription in the correct positions. The inserts also contain incomplete and/or otherwise non-functional copies of other genetic elements. Completeness was evaluated by sequence comparison with the corresponding genetic element in the vectors used for transformation or the NCBI deposited sequence. Functionality was concluded based on the presence of promoter and termination elements located upstream and downstream, respectively, of a coding sequence, when all complete and oriented in the same direction. Taking into consideration that non-functional elements would not be expressed, they could not confer any trait. Even in the case these elements were expressed, the bioinformatic analysis of the insert indicates that the hypothetical products are not related to any plant pest's risk. The nucleotide sequence of the two inserts, named Short and Long, and their annotations, are provided in Appendix 1. A schematic representation of the genetic elements within each insert and their directionality is shown in Figure 1, Appendix 1. The insertion of new elements and complex structures with rearrangements is not unusual in transformations using particle bombardment methods (Altpeter et al., 2005) and is even common to occur during conventional breeding (Doebley et al., 2006; Batista et al., 2008; Sang, 2009; Lenser and Theißen, 2013; Koenig et al., 2013; Flint-García, 2013).

5. Description of new trait

Intended trait

The expected trait conferred by the *HaHB4v* gene introduced in transgenic wheat event IND-00412-7 is the differential response under low water availability conditions. In addition, there is a second trait for herbicide tolerance conferred by the *bar* gene.

Intended phenotype

The phenotype expected on wheat containing the event IND-00412-7, as a consequence of the expression of the *HaHB4v* gene, is the potential to minimize the yield losses caused by challenging water availability conditions when compared to wheat plants not containing the *HaHB4v* gene. Furthermore, the *bar* gene confers tolerance to ammonium glufosinate-based herbicides.

Description of the MOA

The studies that led to elucidating the mechanism of action of the *HaHB4* gene were carried out in *Helianthus annuus* (Gago et al., 2002, Manavella et al., 2006), the plant of origin of the gene, and by heterologous expression in *Arabidopsis thaliana* (Dezar et al., 2005, Manavella et al., 2006, 2008a). These studies showed that *HaHB4* is transcriptionally activated by environmental stress signals and provides tolerance to water stress by the regulation of the expression of genes implicated in the complex natural plant response to stress.

In particular, the observed water-stress tolerance has been linked to the ethylene signaling pathways, specifically with the inhibition of ethylene-induced senescence. This senescence delay maintains active photosynthesis, allowing plants to synthesize osmoprotectants, among other protecting metabolites (Manavella et al., 2006). It also has been shown that *HaHB4* participates in the transcriptional down-regulation of a large group of photosynthesis-related genes, although with no effect on CO_2 fixation (Manavella et al., 2008a). Reduced transcription of the main photosynthetic genes involved in light harvesting in transgenic plants would reduce the formation of reactive oxygen species, protecting the plant from photooxidative stress under water deficit.

Based on the mechanism described above in *Helianthus annuus* and Arabidopsis and considering the existence of endogenous HD-Zip proteins in wheat (Yue et al., 2018), it is reasonable to assume that the phenotype observed in wheat IND-00412-7 (greater yield than the conventional counterpart under challenging water availability conditions) is associated with the participation of *HaHB4v* in the signal transduction pathways involved in the wheat natural response to drought. The action of *HaHB4v* in wheat event IND-00412-7 would follow the same metabolic pathways involved in the wheat endogenous response to water deficit (Itam et al., 2020).

Concerning wheat physiology and development, HB4 wheat showed a reduced loss of fixed kernels and yield than the conventional counterpart in environments where water availability was limiting. While no changes in the cycle were observed, the results would be attributed to better maintenance of photosynthetic rates under stressed conditions in comparison with the conventional counterpart (González et al., 2019).

The evaluation of any physiologically relevant alteration in a genetically modified crop is carried out by measuring the different endpoints associated to crop development and physiology. No unintended effects by the action of *HaHB4v* were observed in wheat event IND-00412-7. The agronomic and phenotypic parameters measured in HB4 wheat in several field trials did not reveal any trait that may represent a plant pest risk (Gonzalez et al., 2019).

The mechanism of action of the phosphinothricin N-acetyl transferase (PAT) enzyme, encoded by the *bar* gene, is to inactivate glufosinate by acetylation (Thompson et al., 1987), conferring tolerance to glufosinate-based herbicides to IND-00412-7 wheat.

Other information on MOA (when relevant)

The same MOA has been evaluated in a different plant taxon namely a genetically engineered soybean variety containing the *HaHB4v* gene (soybean event IND-00410-5), which was deregulated by USDA-APHIS in August 2019 (USDA, 2019).

Table 1. Annotation of the genetic elements present in the Short and Long Inserts of IND-ØØ412-7 wheat event

Genetic element	Construct component donor	Size (bp)	Nucleotide position	Orientation	Accession	Description	Function of the inserted material	References
Short insert								
bla (i)	*Escherichia coli*	183	1-183	R	AAB59737.1	β-lactamase encoding gene	NA[a]	Olesen et al., 2004
bla (i)	*Escherichia coli*	98	184-281	R	AAB59737.1	β-lactamase encoding gene	NA[a]	Olesen et al., 2004
T35s (i)	Cauliflower Mosaic Virus	148	282-429	F	NC_001497.2 LC482137.1	Cauliflower Mosaic Virus terminator sequence	NA[a]	Sanfaçon et al. 1991
Intervening sequence	Synthetic sequence[#]	263	430 - 692	NA	L08959.1 L08752.1	DNA sequence used for cloning	NA[a]	Norrander et al.,1983
Promoter Ubi-1 (c)	*Zea mays*	898	693-1590	F	S94464.1	*Ubi-1* gene promoter	NA[b]	Christensen et al., 1992; Christensen and Quail, 1996
Exon Ubi-1 (c)	*Zea mays*	83	1591-1673	F	S94464.1	*Ubi-1* gene 5' untranslated exon	NA[b]	Christensen et al., 1992; Christensen and Quail, 1996
Intron Ubi-1 (i)	*Zea mays*	362	1674-2035	F	S94464.1	*Ubi-1* gene first intron	NA[b]	Christensen et al., 1992; Christensen and Quail, 1996, Vasil et al., 1993
bla (i)	*Escherichia coli*	522	2036-2557	F	AAB59737.1	β-lactamase encoding gene	NA[a]	Olesen et al., 2004

c: complete copy
i: incomplete copy
*: last 3 nucleotides missing
F: forward, R: reverse, as indicated from left to right in Figure 1
NA: not applicable
[#]: sequence from pUC8/pUC18 plasmids derived from Phague M13
a: unintended element, not expressed
b: not functional
Colored rows show the functional genetic elements that allow the expression of the GOIs

Table 1. Annotation of the genetic elements present in the Short and Long Inserts of IND-ØØ412-7 wheat event

Genetic element	Construct component donor	Size (bp)	Nucleotide position	Orientation	Accession	Description	Function of the inserted material	References
gus (i)	*Escherichia coli*	68	2558-2625	F	S69414.1	β-D-glucuronidase enzyme	NA[a]	Jefferson et al, 1987
Intron Ubi-1 (i)	*Zea mays*	496	2626-3121	R	S94464.1	*Ubi-1* gene first intron	NA[b]	Christensen et al., 1992; Christensen and Quail, 1996, Vasil et al., 1993
Promoter Ubi-1 (i)	*Zea mays*	185	3122-3306	R	S94464.1	*Ubi-1* gene promoter	NA[b]	Christensen et al., 1992; Christensen and Quail, 1996
Intervening sequence	Synthetic sequence[#]	528	3307-3834	NA	L08959.1 L08752.1	DNA sequence used for cloning	NA[a]	Norrander et al.,1983
bla (c)	*Escherichia coli*	931	3835-4765	R	AAB59737.1	β-lactamase encoding gene	NA[a]	Olesen et al., 2004
Intervening sequence	Synthetic sequence[#]	198	4766-4963	NA	L08959.1 L08752.1	DNA sequence used for cloning	NA[a]	Norrander et al.,1983
Promoter Gbl1-1 (i)	*Triticum aestivum*	545	4964-5508	F	M81719.1	*Gbl1* gene promoter	NA[a]	Shutov et al., 1995
gus (i)	*Escherichia coli*	525	5509-6033	F	S69414.1	β-D-glucuronidase enzyme	NA[a]	Jefferson et al, 1987
bla (c)	*Escherichia coli*	931	6034-6964	R	AAB59737.1	β-lactamase encoding gene	NA[a]	Olesen et al., 2004

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Table 1. Annotation of the genetic elements present in the Short and Long Inserts of IND-ØØ412-7 wheat event

Genetic element	Construct component donor	Size (bp)	Nucleotide position	Orientation	Accession	Description	Function of the inserted material	References
Intervening sequence	Synthetic sequence[#]	527	6965-7491	NA	L08959.1 L08752.1	DNA sequence used for cloning	NA[a]	Norrander et al.,1983
Promoter Ubi-1 (i)	*Zea mays*	690	7492-8181	F	S94464.1	*Ubi-1* gene promoter	NA[b]	Christensen et al., 1992; Christensen and Quail, 1996
Promoter Gbl1-1 (i)	*Triticum aestivum*	592	8182-8773	F	M81719.1	*Gbl1* gene promoter	NA[a]	Shutov et al., 1995
gus (i)	*Escherichia coli*	328	8774-9101	R	S69414.1	β -D-glucuronidase enzyme	NA[a]	Jefferson et al, 1987
bla (i)	*Escherichia coli*	245	9102-9346	R	AAB59737.1	β-lactamase encoding gene	NA[a]	Olesen et al., 2004
Intervening sequence	Synthetic sequence[#]	155	9348-9502	NA	L08959.1 L08752.1	DNA sequence used for cloning	NA[a]	Norrander et al.,1983
pBR322 origin (c)	*Escherichia coli*	620	9503-10122	NA	J01749.1	Plasmid replication origin	NA[a]	Yanisch Perron et al.,1985
Intervening sequence	Synthetic sequence[#]	403	10123-10525	NA	L08959.1 L08752.1	DNA sequence used for cloning	NA[a]	Norrander et al.,1983
Tnos (c)	*Agrobacterium tumefaciens*	253	10526-10778	R	V00087.1	Nopaline synthase gene Terminator sequence	NA[b]	Depicker et al., 1982

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Table 1. Annotation of the genetic elements present in the Short and Long Inserts of IND-ØØ412-7 wheat event

Genetic element	Construct component donor	Size (bp)	Nucleotide position	Orientation	Accession	Description	Function of the inserted material	References
Intervening sequence	Synthetic sequence	14	10779-10792	NA	NA	DNA sequence used for cloning	NA[b]	-
bar (c)	*Streptomyces hygroscopicus*	552	10793-11344	R	X17220.1	Gene coding for the enzyme phosphinothricin N-acetyl transferase (PAT)	NA[b]	Thompson et al., 1987
Intervening sequence	Synthetic sequence[#]	6	11345-11350	NA	L08959.1 L08752.1	DNA sequence used for cloning	NA[a]	Norrander et al.,1983
Promoter Gbl1-1 (i)	*Triticum aestivum*	76	11351-11426	F	M81719.1	*Gbl1* gene promoter	NA[a]	Shutov et al., 1995
pBR322 origin (i)	*Escherichia coli*	234	11427-11660	NA	J01749.1	Plasmid replication origin	NA[a]	Yanisch Perron et al.,1985
Intervening sequence	Synthetic sequence[#]	154	11661-11814	NA	L08959.1 L08752.1	DNA sequence used for cloning	NA[a]	Norrander et al.,1983
bla (c)	*Escherichia coli*	931	11815-12745	R	AAB59737.1	β-lactamase encoding gene	NA[a]	Olesen et al., 2004
Intervening sequence	Synthetic sequence[#]	527	12746-13272	NA	L08959.1 L08752.1	DNA sequence used for cloning	NA[a]	Norrander et al.,1983
Promoter Ubi-1(c)	*Zea mays*	898	13273-14170	F	S94464.1	*Ubi-1* gene promoter	NA[b]	Christensen et al., 1992; Christensen and Quail, 1996

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Table 1. Annotation of the genetic elements present in the Short and Long Inserts of IND-ØØ412-7 wheat event

Genetic element	Construct component donor	Size (bp)	Nucleotide position	Orientation	Accession	Description	Function of the inserted material	References
Exon Ubi-1 (c)	*Zea mays*	83	14171-14253	F	S94464.1	*Ubi-1* gene 5' untranslated exon	NA[b]	Christensen et al., 1992; Christensen and Quail, 1996
Intron Ubi-1 (i)	*Zea mays*	414	14254-14667	F	S94464.1	*Ubi-1* gene first intron	NA[b]	Christensen et al., 1992; Christensen and Quail, 1996, Vasil et al., 1993
Intervening sequence	Synthetic sequence	22	14668-14689	NA	NA	DNA sequence used for cloning	NA[b]	-
Tnos (c)	*Agrobacterium tumefaciens*	253	14690-14942	R	V00087.1	Nopaline synthase gene Terminator sequence	NA[b]	Depicker et al., 1982
HaHB4v (c)	*Helianthus annuus*	531	14943-15492	R	AF339748.1	Sequence coding for the transcription factor HAHB4v.	NA[b]	Dezar et al., 2005; Manavella et al., 2006
Intervening sequence	Synthetic sequence	14	15493-15506	NA	NA	DNA sequence used for cloning	NA[a]	-
Intron Ubi-1 (i)	*Zea mays*	616	15508-16122	R	S94464.1	*Ubi-1* gene first intron	NA[b]	Christensen et al., 1992; Christensen and Quail, 1996, Vasil et al., 1993
Intervening sequence	Synthetic sequence[#]	383	16123-16505	NA	L08959.1 L08752.1	DNA sequence used for cloning	NA[a]	Norrander et al.,1983

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Table 1. Annotation of the genetic elements present in the Short and Long Inserts of IND-ØØ412-7 wheat event

Genetic element	Construct component donor	Size (bp)	Nucleotide position	Orientation	Accession	Description	Function of the inserted material	References
pBR322 origin (c)	*Escherichia coli*	620	16506-17125	NA	J01749.1	Plasmid replication origin	NA[a]	Yanisch Perron et al.,1985
Intervening sequence	Synthetic sequence[#]	36	17126-17161	NA	L08959.1 L08752.1	DNA sequence used for cloning	NA[a]	Norrander et al.,1983
Intervening sequence	Synthetic sequence[#]	156	17162-17317	NA	L08959.1 L08752.1	DNA sequence used for cloning	NA[a]	Norrander et al.,1983
bla (c)	*Escherichia coli*	931	17318-18248	R	AAB59737.1	β-lactamase encoding gene	NA[a]	Olesen et al., 2004
Intervening sequence	Synthetic sequence[#]	528	18249-18776	NA	L08959.1 L08752.1	DNA sequence used for cloning	NA[a]	Norrander et al.,1983
Promoter Ubi-1 (i)	*Zea mays*	822	18777-19598	F	S94464.1	*Ubi-1* gene promoter	NA[b]	Christensen et al., 1992; Christensen and Quail, 1996
Long insert								
bla (i)	*Escherichia coli*	193	1-193	R	AAB59737.1	β-lactamase encoding gene	NA[a]	Olesen et al., 2004
Intervening sequence	Synthetic sequence[#]	528	194-721	NA	L08959.1 L08752.1	DNA sequence used for cloning	NA[a]	Norrander et al.,1983

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Genetic element	Construct component donor	Size (bp)	Nucleotide position	Orientation	Accession	Description	Function of the inserted material	References
Promoter Ubi-1 (c)	*Zea mays*	898	722-1619	F	S94464.1	*Ubi-1* gene promoter	NA[b]	Christensen et al., 1992; Christensen and Quail, 1996
Exon Ubi-1 (c)	*Zea mays*	83	1620-1702	F	S94464.1	*Ubi-1* gene 5' untranslated exon	NA[b]	Christensen et al., 1992; Christensen and Quail, 1996
Intron Ubi-1 (i)	*Zea mays*	174	1703-1876	F	S94464.1	*Ubi-1* gene first intron	NA[b]	Christensen et al., 1992; Christensen and Quail, 1996, Vasil et al., 1993
HaHB4v (i)	*Helianthus annuus*	414	1877-2290	R	AF339748.1	Sequence coding for the transcription factor HAHB4v.	NA[b]	Dezar et al., 2005; Manavella et al., 2006
Intron Ubi-1 (i)	*Zea mays*	600	2320-2919	R	S94464.1	*Ubi-1* gene first intron	NA[b]	Christensen et al., 1992; Christensen and Quail, 1996, Vasil et al., 1993
Intervening sequence	Synthetic sequence[#]	403	2920-3323	NA	L08959.1 L08752.1	DNA sequence used for cloning	NA[a]	Norrander et al.,1983
pBR322 origin (c)	*Escherichia coli*	620	3324-3944	NA	J01749.1	Plasmid replication origin	NA[a]	Yanisch Perron et al.,1985

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Genetic element	Construct component donor	Size (bp)	Nucleotide position	Orientation	Accession	Description	Function of the inserted material	References
Intervening sequence	Synthetic sequence[#]	154	3945-4098	NA	L08959.1 L08752.1	DNA sequence used for cloning	NA[a]	Norrander et al.,1983
bla (c)	*Escherichia coli*	931	4099-5029	F	AAB59737.1	β-lactamase encoding gene	NA[a]	Olesen et al., 2004
Intervening sequence	Synthetic sequence[#]	156	5030-5185	NA	L08959.1 L08752.1	DNA sequence used for cloning	NA[a]	Norrander et al.,1983
Tnos (c)	*Agrobacterium tumefaciens*	253	5186-5438	F	V00087.1	Nopaline synthase gene Terminator sequence	NA[b]	Depicker et al., 1982
Intervening sequence	Synthetic sequence	6	5439-5444	NA	NA	DNA sequence used for cloning	NA[b]	-
bar (c*)	*Streptomyces hygroscopicus*	549	5445-5993	F	X17220.1	Gene coding for the enzyme phosphinothricin N-acetyl transferase (PAT)	NA[b]	Thompson et al., 1987
Tnos (c)	*Agrobacterium tumefaciens*	253	5994-6246	F	V00087.1	Nopaline synthase gene Terminator sequence	NA[b]	Depicker et al., 1982
Intervening sequence	Synthetic sequence[#]	403	6247-6649	NA	L08959.1 L08752.1	DNA sequence used for cloning	NA[a]	Norrander et al.,1983
pBR322 origin (c)	*Escherichia coli*	620	6650-7269	NA	J01749.1	Plasmid replication origin	NA[a]	Yanisch Perron et al.,1985

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Genetic element	Construct component donor	Size (bp)	Nucleotide position	Orientation	Accession	Description	Function of the inserted material	References
Intervening sequence	Synthetic sequence[#]	154	7270-7423	NA	L08959.1 L08752.1	DNA sequence used for cloning	NA[a]	Norrander et al.,1983
bla (c)	*Escherichia coli*	931	7424-8354	R	AAB59737.1	β-lactamase encoding gene	NA[a]	Olesen et al., 2004
Intervening sequence	Synthetic sequence[#]	527	8355-8881	NA	L08959.1 L08752.1	DNA sequence used for cloning	NA[a]	Norrander et al.,1983
Promoter Ubi-1 (c)	*Zea mays*	898	8882-9779	F	S94464.1	*Ubi-1* gene promoter	Promoter sequence for *bar* expression	Christensen et al., 1992; Christensen and Quail, 1996
Exon Ubi-1 (c)	*Zea mays*	83	9780-9862	F	S94464.1	*Ubi-1* gene 5' untranslated exon		Christensen et al., 1992; Christensen and Quail, 1996
Intervening sequence	Synthetic sequence	14	9863-9878	NA	NA	DNA sequence used for cloning		-
Intron Ubi-1 (c)	*Zea mays*	1010	9877-10886	F	S94464.1	*Ubi-1* gene first intron		Christensen et al., 1992; Christensen and Quail, 1996, Vasil et al., 1993

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Genetic element	Construct component donor	Size (bp)	Nucleotide position	Orientation	Accession	Description	Function of the inserted material	References
Intervening sequence	Synthetic sequence	6	10887-10892	NA	NA	DNA sequence used for cloning		-
bar (c*)	*Streptomyces hygroscopicus*	549	10893-11441	F	X17220.1 P16426	Gene coding for the enzyme phosphinothricin N-acetyl transferase (PAT)	Sequence coding for PAT which confers glufosinate tolerance	Thompson et al., 1987
Tnos (c)	*Agrobacterium tumefaciens*	253	11442-11694	F	V00087.1	Nopaline synthase gene Terminator sequence	Transcriptional terminator of *bar* gene	Depicker et al., 1982
bla (i)	*Escherichia coli*	549	11695-12243	R	AAB59737.1	β-lactamase encoding gene	NA[a]	Olesen et al., 2004
Intervening sequence	Synthetic sequence[#]	598	12244-12841	NA	L08959.1 L08752.1	DNA sequence used for cloning	NA[a]	Norrander et al.,1983
Promoter Ubi-1 (i)	*Zea mays*	608	12842-13449	F	S94464.1	*Ubi-1* gene promoter	NA[b]	Christensen et al., 1992; Christensen and Quail, 1996
Promoter Gbl1-1 (i)	*Triticum aestivum*	308	13450-13757	F	M81719.1	*Gbl1* gene promoter	NA[a]	Shutov et al., 1995

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Colored rows show the functional genetic elements that allow the expression of the GOIs

Table 1. Annotation of the genetic elements present in the Short and Long Inserts of IND-ØØ412-7 wheat event

Genetic element	Construct component donor	Size (bp)	Nucleotide position	Orientation	Accession	Description	Function of the inserted material	References
Intervening sequence	Synthetic sequence[#]	6	13758-13763	NA	L08959.1 L08752.1	DNA sequence used for cloning	NA[a]	Norrander et al.,1983
gus (i)	*Escherichia coli*	1197	13764-14960	F	S69414.1	β -D-glucuronidase enzyme	NA[a]	Jefferson et al, 1987
Promoter Gbl1-1 (i)	*Triticum aestivum*	535	14961-15495	R	M81719.1	*Gbl1* gene promoter	NA[a]	Shutov et al., 1995
pBR322 origin (i)	*Escherichia coli*	43	15498-15540	NA	J01749.1	Plasmid replication origin	NA[a]	Yanisch Perron et al.,1985
Intervening sequence	Synthetic sequence[#]	156	15541-15696	NA	L08959.1 L08752.1	DNA sequence used for cloning	NA[a]	Norrander et al.,1983
bla (c)	*Escherichia coli*	931	15697-16627	R	AAB59737.1	β-lactamase encoding gene	NA[a]	Olesen et al., 2004
Intervening sequence	Synthetic sequence[#]	528	16628-17155	NA	L08959.1 L08752.1	DNA sequence used for cloning	NA[a]	Norrander et al.,1983
Promoter Ubi-1 (i)	*Zea mays*	190	17156-17345	F	S94464.1	*Ubi-1* gene promoter	NA[b]	Christensen et al., 1992; Christensen and Quail, 1996
Intron Ubi-1 (c)	*Zea mays*	1010	17346-18355	F	S94464.1	*Ubi-1* gene first intron	NA[b]	Christensen et al., 1992; Christensen and Quail, 1996, Vasil et al., 1993

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Colored rows show the functional genetic elements that allow the expression of the GOIs

Table 1. Annotation of the genetic elements present in the Short and Long Inserts of IND-ØØ412-7 wheat event

Genetic element	Construct component donor	Size (bp)	Nucleotide position	Orientation	Accession	Description	Function of the inserted material	References
Intervening sequence	Synthetic sequence[#]	40	18356-18395	NA	NA	DNA sequence used for cloning	NA[a]	Norrander et al.,1983
bar (c*)	*Streptomyces hygroscopicus*	549	18396-18944	F	X17220.1	Gene coding for the enzyme phosphinothricin N-acetyl transferase (PAT)	NA[b]	Thompson et al., 1987
Intron Ubi-1 (i)	*Zea mays*	815	18945-19759	R	S94464.1	*Ubi-1* gene first intron	NA[b]	Christensen et al., 1992; Christensen and Quail, 1996, Vasil et al., 1993
Exon Ubi-1 (c)	*Zea mays*	83	19760-19842	R	S94464.1	*Ubi-1* gene 5' untranslated exon	NA[b]	Christensen et al., 1992; Christensen and Quail, 1996
Promoter Ubi-1 (c)	*Zea mays*	898	19843-20740	R	S94464.1	*Ubi-1* gene promoter	NA[b]	Christensen et al., 1992; Christensen and Quail, 1996
Intervening sequence	Synthetic sequence[#]	527	20741-21267	NA	L08959.1 L08752.1	DNA sequence used for cloning	NA[a]	Norrander et al.,1983
bla (c)	*Escherichia coli*	931	21268-22198	F	AAB59737.1	β-lactamase encoding gene	NA[a]	Olesen et al., 2004
Intervening sequence	Synthetic sequence[#]	154	22199-22352	NA	L08959.1 L08752.1	DNA sequence used for cloning	NA[a]	Norrander et al.,1983

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Table 1. Annotation of the genetic elements present in the Short and Long Inserts of IND-ØØ412-7 wheat event

Genetic element	Construct component donor	Size (bp)	Nucleotide position	Orientation	Accession	Description	Function of the inserted material	References
pBR322 origin (c)	*Escherichia coli*	620	22353-22972	NA	J01749.1	Plasmid replication origin	NA[a]	Yanisch Perron et al.,1985
Intervening sequence	Synthetic sequence[#]	338	22973-23310	NA	L08959.1 L08752.1	DNA sequence used for cloning	NA[a]	Norrander et al.,1983
Promoter Ubi-1 (c)	*Zea mays*	898	23311-24208	F	S94464.1	*Ubi-1* gene promoter	Promoter sequence for *HaHB4v* expression	Christensen et al., 1992; Christensen and Quail, 1996
Intron Ubi-1 (i)	*Zea mays*	333	24209-24541	F	S94464.1	*Ubi-1* gene first intron		Christensen et al., 1992; Christensen and Quail, 1996, Vasil et al., 1993
Intervening sequence	Synthetic sequence	6	24542-24547	NA	NA	DNA sequence used for cloning		-
HaHB4v (c*)	*Helianthus annuus*	531	24548-25078	F	AF339748.1 AAA63768.2	Sequence coding for the transcription factor HAHB4v, a variant with a 4 amino acid deletion and 3 substitutions (US Patent 9,035,132 B2, 2015).	Sequence coding for the transcription factor HAHB4v. Its expression confers drought tolerance	Dezar et al., 2005; Manavella et al., 2006 Gonzalez et al., 2019

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Colored rows show the functional genetic elements that allow the expression of the GOIs

Table 1. Annotation of the genetic elements present in the Short and Long Inserts of IND-ØØ412-7 wheat event

Genetic element	Construct component donor	Size (bp)	Nucleotide position	Orientation	Accession	Description	Function of the inserted material	References
Tnos (c)	*Agrobacterium tumefaciens*	253	25079-25331	F	V00087.1	Nopaline synthase gene Terminator sequence	Transcriptional terminator of *HaHB4v* gene	Depicker et al., 1982
Intervening sequence	Synthetic sequence[#]	403	25332-25734	NA	L08959.1 L08752.1	DNA sequence used for cloning	NA[a]	Norrander et al.,1983
pBR322 origin (c)	*Escherichia coli*	620	25735-26354	NA	J01749.1	Plasmid replication origin	NA[a]	Yanisch Perron et al.,1985
Intervening sequence	Synthetic sequence[#]	154	26355-26508	NA	L08959.1 L08752.1	DNA sequence used for cloning	NA[a]	Norrander et al.,1983
bla (i)	*Escherichia coli*	357	26509-26865	F	AAB59737.1	β-lactamase encoding gene	NA[a]	Olesen et al., 2004
Intervening sequence	Synthetic sequence[#]	156	26866-27021	NA	NA	DNA sequence used for cloning	NA[a]	Norrander et al.,1983
pBR322 origin (c)	*Escherichia coli*	620	27022-27641	NA	J01749.1	Plasmid replication origin	NA[a]	Yanisch Perron et al.,1985
Intervening sequence	Synthetic sequence[#]	119	27642-27760	NA	L08959.1 L08752.1	DNA sequence used for cloning	NA[a]	Norrander et al.,1983
Promoter Gbl1-1 (i)	*Triticum aestivum*	650	27761-28410	R	M81719.1	*Gbl1* gene promoter	NA[a]	Shutov et al., 1995

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Table 1. Annotation of the genetic elements present in the Short and Long Inserts of IND-ØØ412-7 wheat event

Genetic element	Construct component donor	Size (bp)	Nucleotide position	Orientation	Accession	Description	Function of the inserted material	References
Promoter Ubi-1 (i)	*Zea mays*	326	28411-28736	R	S94464.1	*Ubi-1* gene promoter	NA[b]	Christensen et al., 1992; Christensen and Quail, 1996
Intervening sequence	Synthetic sequence[#]	528	28737-29264	NA	L08959.1 L08752.1	DNA sequence used for cloning	NA[a]	Norrander et al.,1983
bla (i)	*Escherichia coli*	748	29265-30012	R	AAB59737.1	β-lactamase encoding gene	NA[a]	Olesen et al., 2004
bar (i)	*Streptomyces hygroscopicus*	356	30013-30368	R	X17220.1	Gene coding for the enzyme phosphinothricin N-acetyl transferase (PAT)	NA[b]	Thompson et al., 1987
Promoter Ubi-1 (i)	*Zea mays*	350	30369-30718	R	S94464.1	*Ubi-1* gene promoter	NA[b]	Christensen et al., 1992; Christensen and Quail, 1996
Intron Ubi-1 (c)	*Zea mays*	1010	30719-31728	R	S94464.1	*Ubi-1* gene first intron	NA[b]	Christensen et al., 1992; Christensen and Quail, 1996, Vasil et al., 1993
Intervening sequence	Synthetic sequence	14	31729-31742	NA	NA	DNA sequence used for cloning	NA[b]	-

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Table 1. Annotation of the genetic elements present in the Short and Long Inserts of IND-ØØ412-7 wheat event

Genetic element	Construct component donor	Size (bp)	Nucleotide position	Orientation	Accession	Description	Function of the inserted material	References
Exon Ubi-1 (c)	*Zea mays*	83	31743-31825	R	S94464.1	*Ubi-1* gene 5' untranslated exon	NA[b]	Christensen et al., 1992; Christensen and Quail, 1996
Promoter Ubi-1 (c)	*Zea mays*	898	31826-32723	R	S94464.1	*Ubi-1* gene promoter	NA[b]	Christensen et al., 1992; Christensen and Quail, 1996
Intervening sequence	Synthetic sequence[#]	527	32724-33250	NA	L08959.1 L08752.1	DNA sequence used for cloning	NA[a]	Norrander et al.,1983
bla (c)	*Escherichia coli*	931	33251-34181	F	AAB59737.1	β-lactamase encoding gene	NA[a]	Olesen et al., 2004
Intervening sequence	Synthetic sequence[#]	154	34182-34335	NA	L08959.1 L08752.1	DNA sequence used for cloning	NA[a]	Norrander et al.,1983
pBR322 origin (c)	*Escherichia coli*	620	34336-34955	NA	J01749.1	Plasmid replication origin	NA[a]	Yanisch Perron et al.,1985
Intervening sequence	Synthetic sequence[#]	403	34956-35358	NA	NA	DNA sequence used for cloning	NA[a]	Norrander et al.,1983
Tnos (c)	*Agrobacterium tumefaciens*	253	35359-35611	F	V00087.1	Nopaline synthase gene Terminator sequence	NA[b]	Depicker et al., 1982

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Table 1. Annotation of the genetic elements present in the Short and Long Inserts of IND-ØØ412-7 wheat event

Genetic element	Construct component donor	Size (bp)	Nucleotide position	Orientation	Accession	Description	Function of the inserted material	References
bar (c*)	*Streptomyces hygroscopicus*	549	35612-36160	R	X17220.1	Gene coding for the enzyme phosphinothricin N-acetyl transferase (PAT)	NA[b]	Thompson et al., 1987
Intervening sequence	Synthetic sequence	6	36161-36166	NA	NA	DNA sequence used for cloning	NA[b]	-
Promoter Ubi-1 (c)	*Zea mays*	898	36167-37064	F	S94464.1	*Ubi-1* gene promoter	Promoter sequence for *bar* expression	Christensen et al., 1992; Christensen and Quail, 1996
Exon Ubi-1 (c)	*Zea mays*	83	37065-37147	F	S94464.1	*Ubi-1* gene 5' untranslated exon		Christensen et al., 1992; Christensen and Quail, 1996
Intervening sequence	Synthetic sequence	14	37148-37161	NA	NA	DNA sequence used for cloning		-
Intron Ubi-1 (c)	*Zea mays*	1010	37162-38171	F	S94464.1	*Ubi-1* gene first intron		Christensen et al., 1992; Christensen and Quail, 1996, Vasil et al., 1993
Intervening sequence	Synthetic sequence	6	38172-38177	NA	NA	DNA sequence used for cloning		Norrander et al.,1983

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Table 1. Annotation of the genetic elements present in the Short and Long Inserts of IND-ØØ412-7 wheat event

Genetic element	Construct component donor	Size (bp)	Nucleotide position	Orientation	Accession	Description	Function of the inserted material	References
bar (c)	*Streptomyces hygroscopicus*	552	38178-38729	F	X17220.1 P16426	Gene coding for the enzyme phosphinothricin N-acetyl transferase (PAT)	Gene coding for PAT which confers glufosinate tolerance	Thompson et al., 1987
Intervening sequence	Synthetic sequence	14	38729-38743	NA	NA	DNA sequence used for cloning	NA[b]	-
Tnos (c)	*Agrobacterium tumefaciens*	253	38744-38996	F	V00087.1	Nopaline synthase gene Terminator sequence	Transcriptional terminator of *bar* gene	Depicker et al., 1982
Intervening sequence	Synthetic sequence[#]	403	38997-39399	NA	L08959.1 L08752.1	DNA sequence used for cloning	NA[a]	Norrander et al.,1983
pBR322 origin (c)	*Escherichia coli*	620	39400-40019	NA	J01749.1	Plasmid replication origin	NA[a]	Yanisch Perron et al.,1985
Intervening sequence	Synthetic sequence[#]	154	40020-40173	NA	L08959.1 L08752.1	DNA sequence used for cloning	NA[a]	Norrander et al.,1983
bla (c)	*Escherichia coli*	931	40174-41104	R	AAB59737.1	β-lactamase encoding gene	NA[a]	Olesen et al., 2004
Intervening sequence	Synthetic sequence[#]	527	41105-41631	NA	L08959.1 L08752.1	DNA sequence used for cloning	NA[a]	Norrander et al.,1983

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Table 1. Annotation of the genetic elements present in the Short and Long Inserts of IND-ØØ412-7 wheat event

Genetic element	Construct component donor	Size (bp)	Nucleotide position	Orientation	Accession	Description	Function of the inserted material	References
Promoter Ubi-1 (c)	*Zea mays*	898	41632-42529	F	S94464.1	*Ubi-1* gene promoter	Promoter sequence for *bar* expression	Christensen et al., 1992; Christensen and Quail, 1996
Exon Ubi-1 (c)	*Zea mays*	83	42530-42612	F	S94464.1	*Ubi-1* gene 5' untranslated exon		Christensen et al., 1992; Christensen and Quail, 1996
Intervening sequence	Synthetic sequence	14	42613-42626	NA	NA	DNA sequence used for cloning		-
Intron Ubi-1 (c)	*Zea mays*	1010	42627-43636	F	S94464.1	*Ubi-1* gene first intron		Christensen et al., 1992; Christensen and Quail, 1996, Vasil et al., 1993
Intervening sequence	Synthetic sequence	36	43637-43672	NA	NA	DNA sequence used for cloning		-
bar (c)	*Streptomyces hygroscopicus*	552	43673-44224	F	X17220.1 P16426	Gene coding for the enzyme phosphinothricin N-acetyl transferase (PAT)	Gene coding for PAT which confers glufosinate tolerance	Thompson et al., 1987
Intervening sequence	Synthetic sequence	14	44225-44238	NA	NA	DNA sequence used for cloning	NA[b]	-

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Table 1. Annotation of the genetic elements present in the Short and Long Inserts of IND-ØØ412-7 wheat event

Genetic element	Construct component donor	Size (bp)	Nucleotide position	Orientation	Accession	Description	Function of the inserted material	References
Tnos (c)	*Agrobacterium tumefaciens*	253	44239-44491	F	V00087.1	Nopaline synthase gene Terminator sequence	Transcriptional terminator of *bar* gene	Depicker et al., 1982
Intervening sequence	Synthetic sequence[#]	403	44492-44894	NA	L08959.1 L08752.1	DNA sequence used for cloning	NA[a]	Norrander et al.,1983
pBR322 origin (c)	*Escherichia coli*	620	44895-45514	NA	J01749.1	Plasmid replication origin	NA[a]	Yanisch Perron et al.,1985
Intervening sequence	Synthetic sequence[#]	154	44515-45668	NA	L08959.1 L08752.1	DNA sequence used for cloning	NA[a]	Norrander et al.,1983
bla (c)	*Escherichia coli*	931	45669-46599	R	AAB59737.1	β-lactamase encoding gene	NA[a]	Olesen et al., 2004
Intervening sequence	Synthetic sequence[#]	527	46600-47126	NA	L08959.1 L08752.1	DNA sequence used for cloning	NA[a]	Norrander et al.,1983

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>Wheat_IND-00412-7_Short_Insert

ttggaacaccacgatgcctgtagcaatggcaacaacgttgcgcaaactattaactggcgaactacttactctagcttccc
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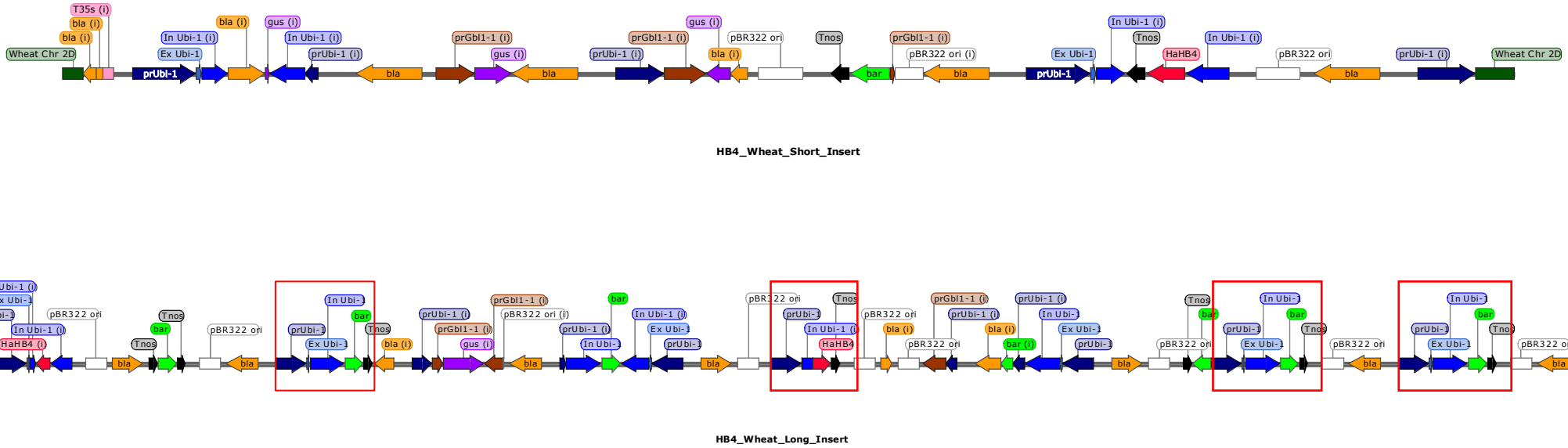

Figure 1. Wheat IND-00412-7 short insert (upper panel) and long insert (bottom panel). The different genetic elements listed in Table 1 are shown in different colors: HaHB4 in red, bar in bright green, Ubi-1 promoter (prUbi-1) in dark blue, Exon Ubi-1 (Ex Ubi-1) in light blue, Intron Ubi-1 (In Ubi-1) in blue, tnos in black, bla in orange, prGbl1-1 in brown, gus in violet, T35s in pink, pBR322 ori in white. Arrows represent the directionality (5'→3') of the genetic elements. Completeness was determined by sequence comparison with the corresponding genetic element in the transformation vector (for HaHB4, bar, prUbi-1, Ex Ubi-1, In Ubi-1, tnos, bla and pBR322 ori), or sequences deposited in NCBI (for T35s, gus and prGbl1-1). Those elements lacking a full match with their comparators are indicated as incomplete (i). The functional genetic elements that allow gene expression are highlighted with a red rectangle.

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