The genome of cultivated sweet potato contains *Agrobacterium* T-DNAs with expressed genes: An example of a naturally transgenic food crop

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*Agrobacterium rhizogenes* and *Agrobacterium tumefaciens* are plant pathogenic bacteria capable of transferring DNA fragments [transfer DNA (T-DNA)] bearing functional genes into the host plant genome. This naturally occurring mechanism has been adapted by plant biotechnologists to develop genetically modified crops that today are grown on more than 10% of the world’s arable land, although their use can result in considerable controversy. While assembling small interfering RNAs, or siRNAs, of sweet potato plants for metagenomic analysis, sequences homologous to T-DNA sequences from *Agrobacterium* spp. were discovered. Simple and quantitative PCR, Southern blotting, genome walking, and bacterial artificial chromosome library screening and sequencing unambiguously demonstrated that two different T-DNA regions (IbT-DNA1 and IbT-DNA2) are present in the cultivated sweet potato (*Ipomoea batatas* [L.] Lam.) genome and that these foreign genes are expressed at detectable levels in different tissues of the sweet potato plant. IbT-DNA1 was found to contain four open reading frames (ORFs) homologous to the tryptophan-2-monoxygenase (iaaM), indole-3-acetamide hydrolase (iaaH), C-protein (C-prof), and agrocinopine synthase (Aca) genes of *Agrobacterium* spp. IbT-DNA1 was detected in all 291 cultivars examined, but not in close wild relatives. IbT-DNA2 contained at least five ORFs with significant homology to the *orfF14*, *orfF17*, rooting locus (Ro)B/RoIC, *orfF13*, and *orfF18/orfF17* genes of *A. rhizogenes*. IbT-DNA2 was detected in 45 of 217 genotypes that included both cultivated and wild species. Our finding, that sweet potato is naturally transgenic while being a widely and traditionally consumed food crop, could affect the current consumer distrust of the safety of transgenic food crops.

Horizontal gene transfer | *Agrobacterium* spp. | food safety | sweet potato | transgenic crops

H

orizontal gene transfer (HGT) has long been recognized as a natural phenomenon, especially between bacteria, but it is also being increasingly detected in eukaryotic genomes (1). Many instances of HGT include the transfer of genes from various donors in bdelloid rotifers (2) or from the intracellular bacterium *Wolbachia* into various insect and nematode genomes (3, 4). Some of the transferred genes are not functional in the recipient organism, but others are transcribed, indicating that this phenomenon represents an operational mechanism for the acquisition of new genes. Several horizontally transferred genes have been shown to be correlated with the occurrence of a specific phenotype. Examples include the transfer of carotenoid biosynthetic genes from fungi to aphids that results in the red or green coloration of the aphids (5), or the transfer of genes from hornworms to ferns that results in a more efficient photoreceptor (6). HGT from microbes to plants is well documented. Perhaps the most familiar example is the transfer of the transfer DNA (T-DNA) from *Agrobacterium* spp. This HGT often results in the occurrence of crown galls. The mechanism of this transfer has been extensively studied and is well understood (7, 8).

Crown gall is a disease that afflicts orchards and vineyards in particular. It has long been known to be caused by a bacterial agent (9). In the late 1970s, it was shown that the disease resulted from the transfer of a part of the tumor-inducing (Ti) plasmid, the T-DNA, from *Agrobacterium tumefaciens* into the host plant genome (10). The transfer of the T-DNA from the root-inducing (Ri) plasmid in a related bacterium, *Agrobacterium rhizogenes*, induces abundant root proliferation (hairy roots) at the infection site (11). Once integrated, the genes of the T-DNA are expressed and are responsible for tumor (crown gall) or hairy root formation, as well as the production of opines, in the infected plant tissue. The types of opines synthesized have been used to classify Ti and Ri plasmids into octopine, nopaline, and agropine-type plasmids (12–14).

*Agrobacterium rhizogenes* agropine strains contain two physically separated T-DNA regions (the TR-DNA and the TL-DNA).

Significance

We communicate the rather remarkable observation that among 291 tested accessions of cultivated sweet potato, all contain one or more transfer DNA (T-DNA) sequences. These sequences, which are shown to be expressed in a cultivated sweet potato clone ("Huachano") that was analyzed in detail, suggest that an *Agrobacterium* infection occurred in evolutionary times. One of the T-DNAs is apparently present in all cultivated sweet potato clones, but not in the crop’s closely related wild relatives, suggesting the T-DNA provided a trait or traits that were selected for during domestication. This finding draws attention to the importance of plant-microbe interactions, and given that this crop has been eaten for millennia, it may change the paradigm governing the "unnatural" status of transgenic crops.


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Data deposition: The data reported in this paper have been deposited in the Gene Expression Omnibus (GEO) database, www.ncbi.nlm.nih.gov/geo [accession nos. KM052616 (IbT-DNA1 "Huachano"), KM052617 (IbT-DNA2 "Huachano"), KM113766 (IbT-DNA1 BAC clone "Xu781"), KM658934 (ORF13 CIP_A20065), KM658949 (ORF13 CIP_A40031), KM658950 (ORF13 CIP_A40016), KM658951 (ORF13 P1, S61255), KM658952 (ORF13 CIP_A40352), KM658953 (ORF13 CIP107665.9), KM658954 (aaM CIP_A40032), KM658955 (aaM CIP_A40016), KM658956 (aaM CIP_A400274), KM658957 (aaM CIP_A400398), KM658958 (aaM CIP_400450), KM658959 (aaM CIP_A41724), KM658960 (aaM CIP_A40016), KM658961 (aaM CIP_A40416), and KM658962 (aaM CIP_A400031)].

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on their Ri plasmid. These are independently integrated into the host genome (15). The TR-DNA generally encodes the proteins required for opine synthesis (16) in addition to the auxin biosynthesis genes tryptophan-2-monoxygenase (iaaM) and indole-3-acetamide hydrolase (iaaH) (15) and the less-studied open reading frame (ORF) encoding for C-protein (17). The TL-DNA contains ORFs encoding the rooting locus (Rol) genes RolA (ORF10), RolB (ORF11), RolC (ORF12), and RolD (ORF15), which are involved in auxin/cytokinin balance, in addition to other genes such as ORF8, ORF13, ORF14, and ORF18, the functions of which are not completely understood (18, 19). The RolB, RolC, S’ part of ORF8, S’ portion of iaaM, and other T-DNA genes belong to a family of highly diverged genes without detectable DNA similarity but with statistically significant similarity at the protein level (20).

Soon after the discovery of Agrobacterium-mediated gene transfer, the fixation of *A. rhizogenes* T-DNAs in the *Nicotiana* genome was reported (21, 22). These T-DNA sequences, originating from different *A. rhizogenes* strains, are present in *Nicotiana* spp., are transmitted to the progeny, and are expressed at low levels but are not associated with the hairy root or tumor-like symptoms that are usually induced by *Agrobacterium* infections (23–25). The Rol genes in *Nicotiana glauca* appear to play a role in various developmental processes and in the expression of morphologic characteristics that have contributed to the evolution of the genus (23, 26). A PCR and hybridization-based search for naturally transgenic species in the family *Solanaceae* was conducted without success (23). However, a subsequent, more extensive search resulted in the identification of *A. rhizogenes*-homologous T-DNA sequences in *Linaria vulgaris* (27, 28). Neither *Nicotiana nor Linaria* are food crops, and such findings have so far not been associated with domesticated edible crops. In the course of a high-throughput sequence analysis of small interfering RNAs, or siRNAs, of sweet potato plants (29), siRNAs homologous to T-DNA-like sequences from *Agrobacterium* spp. were discovered in the cultivated sweet potato (*Ipomoea batatas* [L.] Lam.) landrace “Huachano.” This observation prompted us to further investigate the presence of T-DNAs in the genome of this crop plant and its related wild species.

Sweet potato is one of the oldest domesticated crops in the Americas, with archeological remains from caves in the Chica Canyon in Peru dating back to 8,000–10,000 y ago (30). The crop and its relatives (*Ipomoea* [family *Convolvulaceae*]) and are confined to section *Eriospermum* Hallier f., series *Batatas* (Choisy) D.F. Austin. This group contains 13 species and two naturally occurring hybrids (31). All of them, with the exception of *Ipomoea littoralis*, are endemic to the Americas (32). The cultivated sweet potato is hexaploid (2n = 6x = 90), although wild tetraploid (2n = 4x = 60) specimens have been collected in Ecuador, Colombia, Guatemala, and Mexico (33). The other species in series *Batatas* are either diploid (2n = 2x = 30) or tetraploid. The identity of the wild ancestors of sweet potato has not been fully elucidated, although morphologic (34, 35), molecular (36–40), and cytogenetic (41) studies all support a close relationship with *Ipomoea trifida* (2x). Therefore, our search for T-DNA in section *Batatas* genomes included a large number of both domesticated and semidomesticated sweet potatoes (hereafter referred to as cultivars which includes landraces, varieties, and feral forms of *I. batatas*) and wild sweet potato-related species.

**Results**

The Sweet Potato Genome Contains *Agrobacterium* T-DNAs. In the process of performing small RNA sequencing of sweet potato landrace “Huachano” (29), a number of contigs were assembled that showed significant similarity to *Agrobacterium* genes: agrocinopine synthase (*Ac*), C-protein (*C-prot*), *iaaH*, *iaaM*, *RolB*, and *ORF18*. Sequencing of PCR products from these contigs confirmed the presence of these genes in sweet potato DNA. Genome walking techniques were subsequently used to identify two large T-DNA regions from *Agrobacterium* spp. inserted into the sweet potato “Huachano” genome (Fig. 1). Further analysis revealed that the first region, *Ipomoea batatas* T-DNA (**A**), had at least four successive intact ORFs homologous to the *Ac*, *C-prot*, *iaaH*, and *iaaM* sequences of *Agrobacterium* spp., as well as a truncated *iaaM* in an inverted orientation (Fig. 1A and Dataset S1). The second region, *Ib*-T-DNA2, contained at least five intact ORFs with significant homology to the *ORF14*, *ORF17n*, *RolB/RolC* family, *ORF13*, and *ORF18/ORF17n* family of *A. rhizogenes* (Fig. 1B and Dataset S1). The flanking sequences of *Ib*-T-DNA1 (Fig. 1A), obtained by genome walking, revealed at least 80% sequence identity (BlastN) to predicted F-box genes from *Nicotiana*, *Monos*, *Solanum*, and *Fragaria* spp. A tBLASTX of this region predicts orthologous F-box proteins across numerous other plant families (Dataset S2). This presumed sweet potato sequence was a 100% nucleotide match to several transcript sequences from the sweet potato gene index (https://research.cgiar.org/confluence/display/SPGI/Home; contig 02446) and a sweet potato transcriptome shotgun library at NCBI (JPI111314.1), which were predicted to encode F-box proteins (e-value = e-177). These observations suggest the “Huachano” *Ib*-T-DNA1 is inserted into an F-box gene intron.

Southern blot analyses were performed to confirm the insertion of both T-DNAs into the sweet potato (“Huachano”) genome (Fig. 2). An estimated four copies of each T-DNA were present (Fig. 2A and C). When comparing these copies with the two T-DNA sequences identified through genome walking (Fig. 1), it is not possible to determine whether the three homologous sequences of each T-DNA are the result of polymorphic *SpeI* sites, and thus allelic, or arose independently as the result of separate integration events or from rearrangements subsequent to a single integration event. Hybridization of total genomic DNA, digested with *SpeI*, with a probe for *Ib*-T-DNA1 (*C-prot* probe 1; Fig. 1A) produced several bands (Fig. 2A) that appeared (Fig. 2B) when hybridized to the probe corresponding to the flanking plant DNA (F-box; probe 2; Fig. 1A), confirming their physical linkage. Additional bands hybridizing to probe 2 (Fig. 2B) suggest that at least two additional DNA regions homologous to the F-box gene, unlinked to *Ib*-T-DNA1, are present.
IbT-DNA1 but Not IbT-DNA2 Is Ubiquitous in Sweet Potato, Whereas Closely Related Species Appear to Have None or Only One T-DNA. To examine the presence of the T-DNA sequences in the cultivated sweet potato gene pool, PCR analyses were performed for three or four of the ORFs of IbT-DNA1 and ORF13 of IbT-DNA2. The analyses included a total of 291 and 204 cultivars for IbT-DNA1 and IbT-DNA2, respectively, which represented a random sampling of the global hexaploid sweet potato gene pool. These analyzed plant materials were collected in South and Central America, Africa, Asia, and Oceania. Ten tetraploid plants (nine I. batatas and one Ipomoea tabacum) and three diploid plants (two I. trifida and one Ipomoea triloba), representing closely related taxa, were also included in this analysis. The results are shown in Dataset S3. IbT-DNA1 was detected in all 291 hexaploid sweet potato cultivars, but not in the wild relatives. In contrast, ORF13 from IbT-DNA2 was detected in 42 hexaploid cultivars, two of the nine tetraploid genotypes of I. batatas, and a single diploid I. trifida, among a total of 217 genotypes examined. The tested genotypes originated from different germplasm collections, and the analyses were conducted independently in two different laboratories.

IbT-DNA2 and Root Characteristics. In Nicotiana, Rol-containing genotypes are rooting-prone, whereas the others are shoot-producing
genomic region includes predicted plant genes shown in orange, and T-DNA genes in green. The T-DNA region, enlarged in B, bears an insertion, containing three segments with homology to Gypsy-2-LTRs and several SSRs (seven in total, with the motifs TAT<sub>60</sub>GA<sub>60</sub>GA<sub>60</sub>TAT<sub>60</sub>GA<sub>60</sub>TAT<sub>60</sub>TA<sub>60</sub> and TA<sub>60</sub> in one of the iaaM ORFs compared with the partial sequence determined for “Huachano” and a deletion in one copy of the C-prot gene. T-DNA1 of “Huachano” contains a deletion in one of the iaaM copies. Semitransparent ORFs indicate they are (partially) interrupted by indels or stop-codons (indicated by lines inside the ORF) compared with the full-length and uninterrupted ORFs.

(23). Because qRT-PCR analysis revealed expression of RolB/RolC and ORF13 in “Huachano” tissues, this prompted us to look at root parameters in sweet potato in relation to the presence or absence of IbT-DNA2 (as all clones tested positive for the presence of IbT-DNA1). Using the primers listed in Dataset S4, we observed IbT-DNA2 polymorphisms for two primer pairs corresponding to the genes RolB/RolC and ORF13 in cultivars “Beauregard” and “Tanzania” (positive). Hence, we tested by segregation analysis for the presence of IbT-DNA2 (using the RolB/RolC and ORF13 primers) in an existing biparental segregating population from these parents for possible association with root parameters (total root yield, dry matter content, and harvest index). Only four of 76 progeny were negative for both markers, and nine additional genotypes were negative only for RolB/RolC, indicating the presence of variants of IbT-DNA2. No association between IbT-DNA2 marker alleles and root characteristics was found with the exception of root yield at one location (Wilcoxon rank sum test with continuity correction, \( P = 0.04211; \) Dataset S5). This preliminary genetic analysis does not prove a role for the IbT-DNA in root development, and additional segregating populations will have to be generated and analyzed in dedicated experiments to detect potential associations.

Discussion

The acquisition of new genes that confer a selective advantage is an important factor in genome evolution. Significant parts of prokaryotic and eukaryotic genomes originated from the exchange of genetic material among related or unrelated species through HGT. This is considered to be a significant source of molecular variability and a driver of evolution (42–45). Our data provide evidence of an ancient HGT between an Agrobacterium spp. and an ancestor (or ancestral form) of the sweet potato. Our data indicate that the presence of IbT-DNA1 is a general feature of the domesticated sweet potato gene pool. The presence of IbT-DNA1 in all hexaploid cultivars examined, and the lack of segregation in the progeny of the analyzed cross, suggests this T-DNA fragment is fixed in the cultivated sweet potato genome, in contrast to its close wild relatives. The DNA polymorphisms between “Huachano” and “Xu781” IbT-DNA1 (Fig. 4) attest to an ancient transfer of the T-DNA into an Ipomoea species. It is therefore conceivable that one or more of the transferred genes contributed to the expression of a trait that was subsequently selected for during domestication. Our preliminary analysis of root parameters did not reveal a consistent role for the T-DNA genes in storage root development, which was a distinctive feature of domesticated forms in the examined cross, although the significant association of the presence of ORF13 with higher root yield at one location (Dataset S5) merits further follow-up. Additional IbT-DNA1 nucleotide sequence studies, IbT-DNA segregation studies using additional populations, and further examination of alterations of gene expression are required to more fully elucidate a functional role of the T-DNA genes in storage root development, if one exists.

Remarking on the occurrence of ORFs detected on IbT-DNA1 and IbT-DNA2 are intact, and expression of these genes was detected in different organs of “Huachano,” indicating their potential functionality. Future expression studies should be conducted using several biological replicates and genotypes with contrasting root characteristics to infer possible roles of the IbT-DNAs.

The gene sequences identified in IbT-DNA1 and IbT-DNA2 indicate that the transforming Agrobacterium most likely was A. rhizogenes (46), an ancestral form of A. rhizogenes or a species (perhaps extinct) closely related to A. rhizogenes. In this scenario, IbT-DNA1 corresponds to TR-DNA (typically containing the auxin biosynthesis genes iaaM and iaaH), and IbT-DNA2 corresponds to TL-DNA (harboring the Rol genes). The gene organization (Fig. 4) and DNA sequences (Dataset S1) of the T-DNAs are similar to, but distinctly different from, the ORFs of the Ri and Ti-plasmids in well-characterized Agrobacterium strains. The identified RolB/RolC region represents a new member of the RolB family (Fig. S1). This indicates that, unlike the T-DNA found in Nicotiana spp. (26), the Agrobacterium strain (or species) that transferred its T-DNA into the sweet potato genome is not one of the commonly studied strains.

Agrobacterium-mediated transformation has been the method of choice for the development of genetically modified crops. Despite their cultivation on more than 170 million ha, the growth and consumption of transgenic crops still faces societal opposition. This has impeded their use in efforts to contribute to a more sustainable agricultural future (47–52). Our data reveal that T-DNA integration, the interruption of an F-box gene, and the subsequent fixation of foreign T-DNA into the sweet potato genome occurred during the evolution and domestication of this crop, which is one of the world’s most consumed foods (53). This

![Fig. 5. Relative expression of four ORFs on IbT-DNA1 and two ORFs located on IbT-DNA2. The figure shows the relative presence of mRNA in different tissues of sweet potato, based on qRT-PCR. The expression levels are shown relative to the expression level of the Cox housekeeping gene in roots. Data were normalized using data from two reference genes (PLD and RPL).](image-url)
finding could influence the public's current perception that transgenic crops are "unnatural."

Materials and Methods

Plant Material. In total, 304 plant samples were included in this study (Dataset S3): 291 samples of cultivated I. batatas [L.] Lam. (haxlopphid) originating from South and Central America, Africa, Asia, and Oceania; nine samples belonging to wild, uncultivated I. batatas [L.] Lam. (tetraploid) from South and Central America; and four samples belonging to one of the related species [I. tabacana (tetraploid), I. triloba (diploid), and I. triloba (diploid)]. The plant materials were obtained from the germplasm collections of the International Potato Center (Lima, Peru) and the National Genetic Resources Program (US Department of Agriculture).

DNA Extraction. DNA extraction from leaf tissues of 303 samples was performed using the Cetyl trimethylammonium bromide (CTAB) method (54). DNA quality and quantity were measured using a Nanodrop spectrophotometer (Thermo Fisher Scientific Inc.) and agarose gel electrophoresis.

Screening for IBT-DNAs in Ipomoea spp. Detection of Ibt-DNA1 and Ibt-DNA2 genes in Ipomoea samples was carried out by conventional PCR or high-resolution DNA melting (55), using the primers listed in Dataset S4. Part of the Ipomoea-specific malate dehydrogenase gene was amplified on each DNA sample as a positive PCR control. PCR reactions were accomplished in 25-μL volumes containing 1× PCR buffer (Invitrogen); 0.4 mM each dGTP, dATP, dTTP, and dCTP; 0.3 μM forward and reverse primer; 1 U Taq DNA polymerase (Invitrogen); and 100 ng genomic DNA. The PCR conditions were 94°C for 5 min, followed by 35 cycles of 95°C for 30 s, 50°C–60°C for 30 s, and 72°C for 2 min, and then a final extension at 72°C for 10 min. PCR products were separated on 1% agarose gels for visual detection of presence or absence.

Genome Walking on "Huachano." The joining and extension of partial sequences identified through small RNA sequencing and PCR were carried out using the Genome Walker Universal Kit (Clontech) according to the manufacturer's instructions and some minor modifications to the PCR conditions as follows: First-PCR: 94°C for 2 min, followed by seven cycles of 94°C for 25 s and 72°C for 3 min, 32 cycles of 94°C for 25 s and 67°C for 3 min, and a final cycle of 67°C for 7 min. Nested-PCR: 94°C for 1 min, followed by five cycles of 94°C for 25 s and 72°C for 3 min, 20 cycles of 94°C for 25 s and 67°C for 3 min, and a final cycle of 67°C for 7 min. PCR products were recovered using the Wizard SV gel extraction kit (Promega) according to the manufacturer's recommendation. The eluted DNA was ligated into plasmid vector pCR 2.1 (Invitrogen), according to the manufacturer's instructions, and cloned in Escherichia coli strain DH5α. PCR products were sequenced by Macrogen, using the Sanger method.

BAC Library of Sweet Potato Variety "Xu781." A BAC library of 15,360 bacterial colonies was constructed from total genomic DNA partially restricted with HindIII from the sweet potato variety "Xu781," following standard procedures. PCR screening was done using the primers for amplification of the IaaM and C-prot gene (Dataset S4). After identification of a positive BAC clone, its insert was sequenced at the Beijing Genomics Institute, using the Sanger method in a shotgun approach.

Sequence Analysis, Blast Search, Annotation, and Accession Number. Sequences were predicted using the program FGENESH2.6 (56) and annotated on the public database NCBI. Alignment and phylogenetic analysis were performed using the software MEGA 5 (57). All sequences were deposited in the GenBank database under the following accession numbers: KM052616 (Ibt-DNA1 "Huachano"), KM052617 (Ibt-DNA2 "Huachano"), KM113766 (Ibt-DNA1 BAC clone "Xu781"), KM658948 (ORF13 CIP_402065), KM658949 (ORF13 CIP_440031), KM658950 (ORF13 CIP_440166), KM658951 (ORF13 PI_561255), KM658952 (ORF13 CIP_403552), KM658953 (ORF13 CIP107655.9), KM658954 (IaaM CIP_440132), KM658955 (IaaM CIP_440166), KM658956 (IaaM CIP_440274), KM658957 (IaaM CIP_440398), KM658958 (IaaM CIP_400450), KM658959 (IaaM CIP_441724), KM658960 (IaaM CIP_440116), KM68961 (IaaM CIP_404106), and KM68962 (IaaM CIP_400031).

Southern Blot Hybridization. A total of 30 μg genomic DNA was digested with Spel, separated on a 0.8% agarose gel under 25 eV for 18 h, and transferred to a Hybond-N+ nylon membrane (Amersham Pharmacia Biotech) with transfer buffer (20x SSC).

Primers (Dataset S4) were designed to amplify three different DNA probes (probe 1, C-prot; probe 2, the F-box gene sequence; and probe 3, ORF17n). Probe labeling was performed using the PCR DIG Probe Synthesis Kit (Roche). Prehybridization and hybridization steps were carried out using the buffer DIG Easy Hyb (Roche), according to the manufacturer's instructions. After hybridization, membranes were washed twice (5 min) at low stringency (2× SSC, 0.1% SDS) at room temperature and two additional times (15 min) at high stringency (0.1x SSC, 0.1% SDS) at 65°C. The images were captured by chemiluminescence on a phosphorimager (FujiFilm Life Science).

RNA Extraction, cDNA Synthesis, and qRT-PCR. RNA from leaf, stem, root, shoot apex, and storage root tissues of sweet potato plants (landrace "Huachano") was extracted using the RNeasy Plant Mini Kit (Qiagen GmbH). The RNA concentration and purity were measured using the Nanodrop spectrophotometer (Thermo Fisher Scientific Inc.). To remove contaminating DNA, the sample was treated with Dnase I (Fermentas). First-strand cDNA synthesis was generated using SuperScript II Reverse Transcriptase (Invitrogen). All qRT-PCRs were performed in triplicate by using the SensiMix SYBR No Rox Kit (Biolene) in the Rotor-Gene 3,000 (Qiagen), using Rotor Discs (72, Qiagen); results were generated by the Rotor-Gene 6.2 software. PCRs were performed under the following conditions: 10 min at 95°C and 45 cycles of 25 s at 95°C, 60 s at 58°C, and 20 s at 72°C. Data were analyzed using the REST 2008 software (Qiagen). Normalization of the expression level of the target genes was done using two reference genes: phospholipase D1 (PLD) and ribosomal protein L (RPL) (see Dataset S4 for all used primer pairs). Expression levels are shown in comparison with the expression level of the stably expressed housekeeping gene Cytochrome c oxidase (Cox). As negative controls, "no reverse transcriptase" and "no template" samples (I. trifida a- cession CIP 460112) were tested and confirmed to be negative.

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