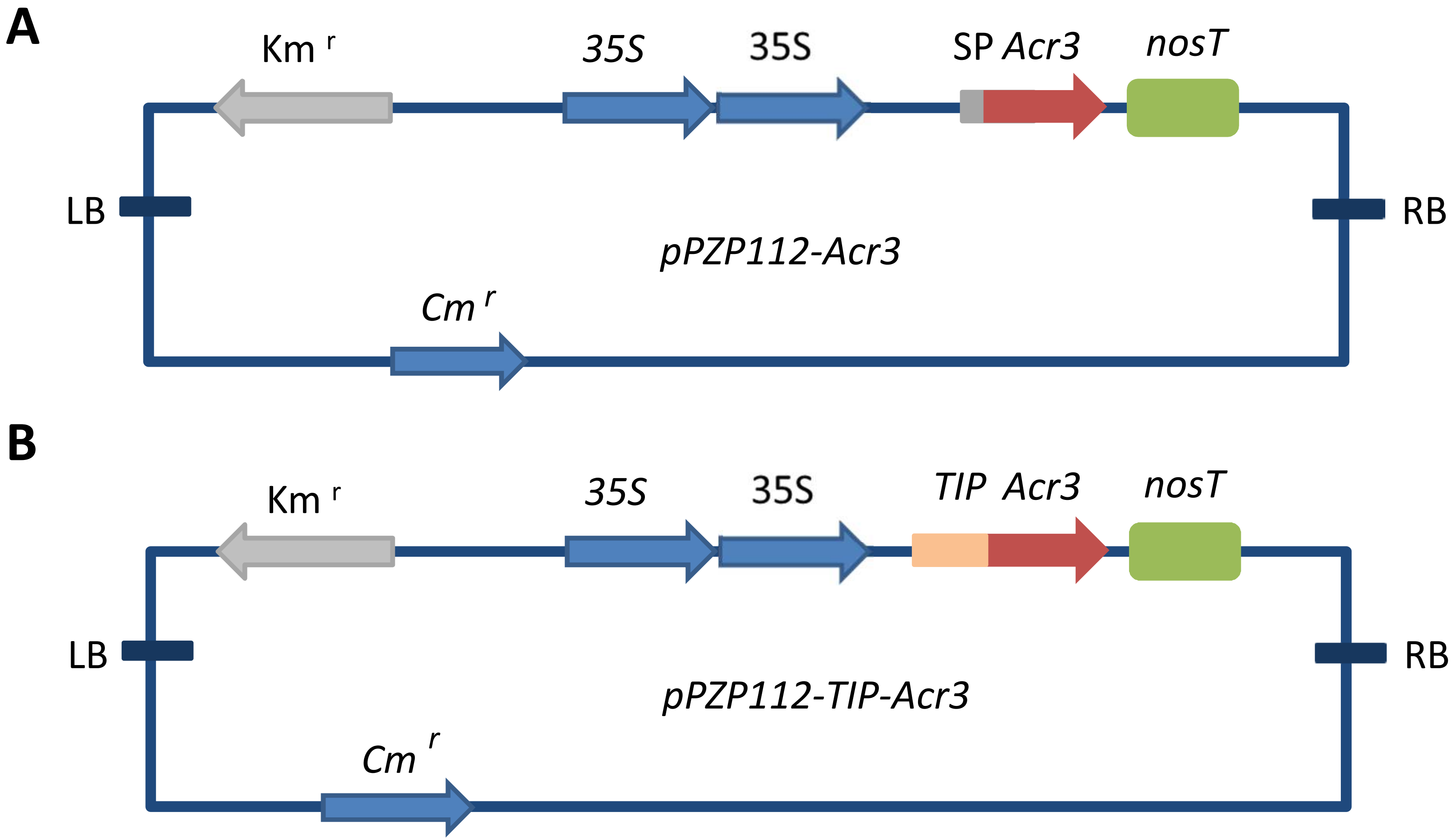
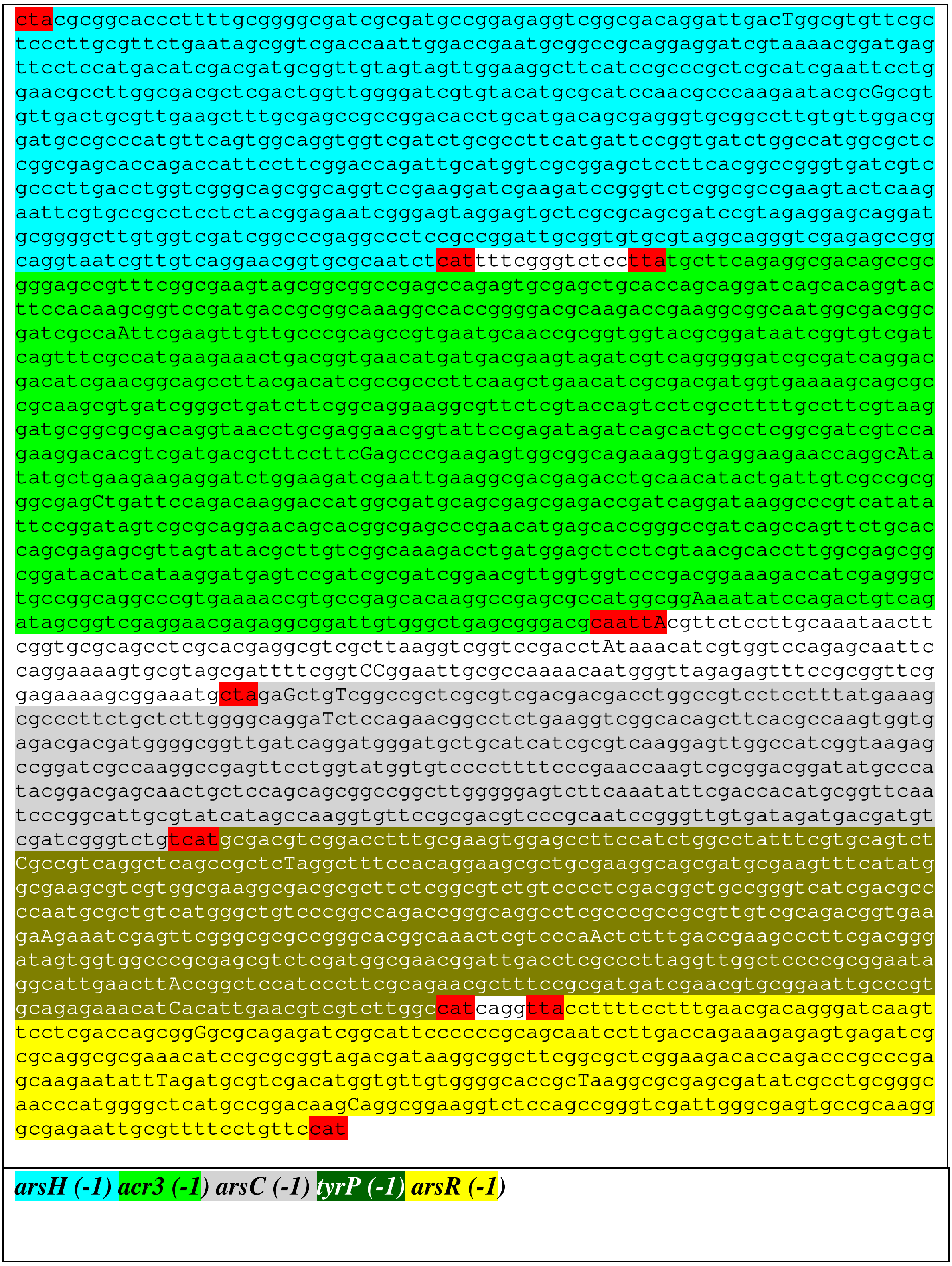
Pérez-Palacios et al., Electronic Supplementary Information Figure S1



**Electronic Supplementary Information S1**: Plasmids used for infection of *N. tabacum* roots. A) Acr3 targeted to plasma membrane (the signal peptide SP was maintained in the *acr3* cloned gene); B) TIP-Acr3 fusion protein for targeting to the tonoplast of the central vacuole (the signal peptide SP was removed from the sequence of the gene).



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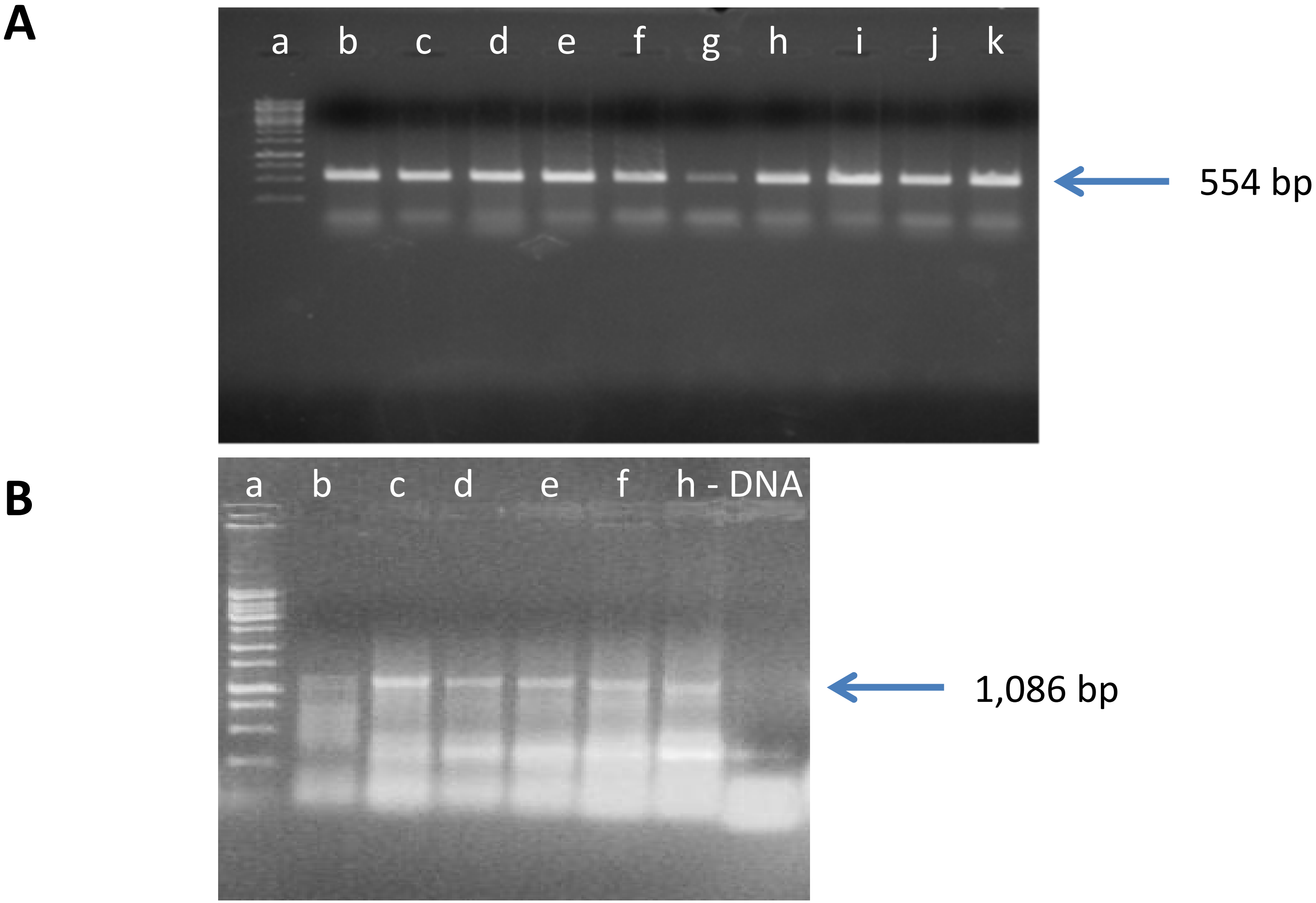
*arsH (-1) acr3 (-1) arsC (-1) tyrP (-1) arsR (-1)*

**Electronic Supplementary Information Figure S2.** Sequence of the 3.3 Kb PCR product corresponding to the complete arsenic resistance operon from *E. medicae* MA11. The operon is composed of 5 genes (*arsH, acr3, arsC, tyrP* and *arsR*) codified in the minus strand. Codons in red correspond to translation initiation and stop codons. Genes *tyrP* and *arsC* share nucleotides in the stop and start codons, respectively.

**Electronic Supplementary Information S3.** Transformation frequency of *N. tabacum* with the different constructs *pPZPY112-Acr3* and *pPZPY112-TIP-Acr3* and with control *A. rhizogenes Arqua109* carrying the empty vector.

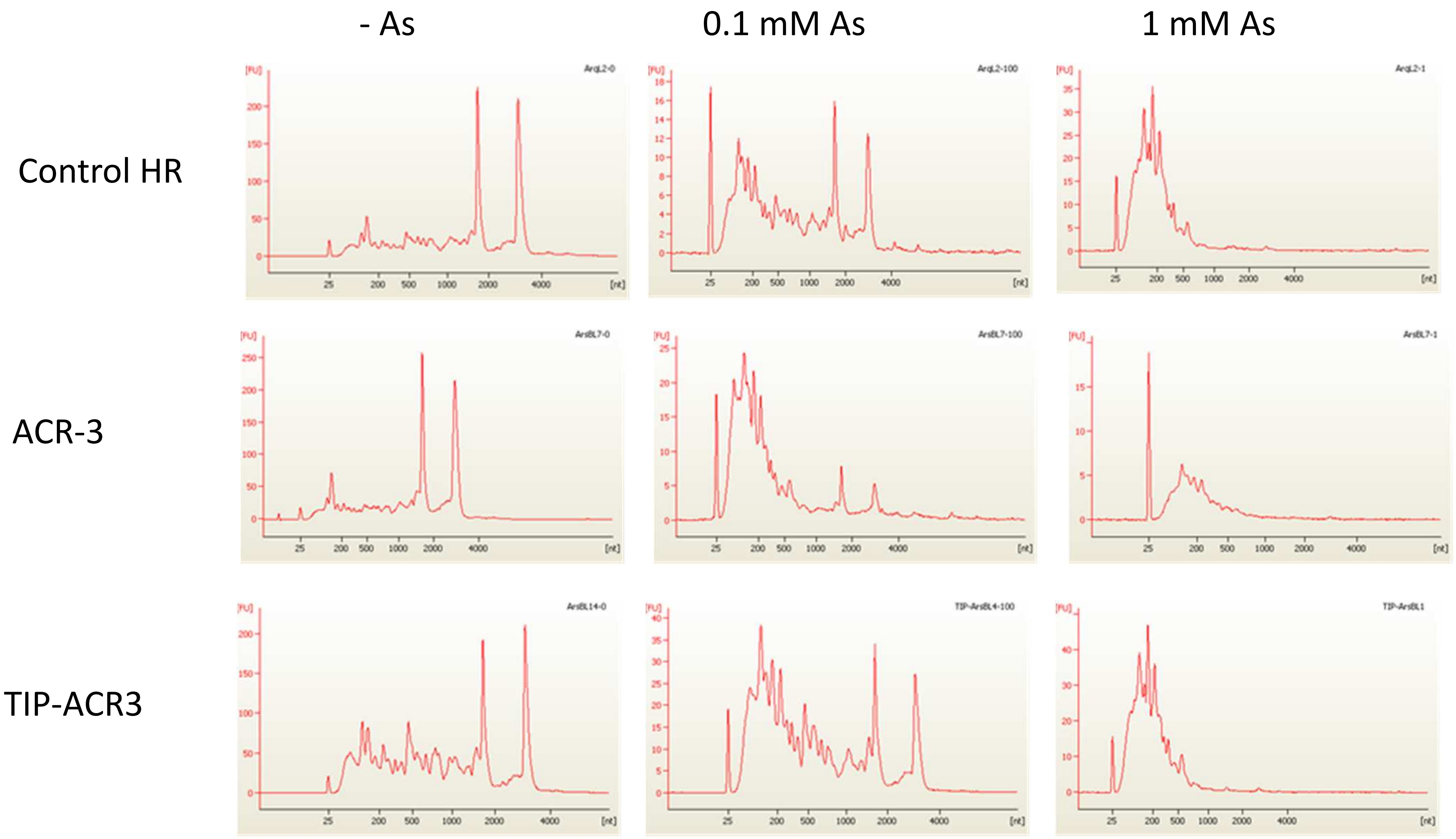
| **Lines** | **Transformation frequency (%)** | | |
|---|---|---|---|
| | **10 days** | **12 days** | **15 days** |
| **HR** | 36.30 | 42.65 | 47.60 |
| **ACR3** | 21.42 | 31.50 | 38.44 |
| **TIP-ACR3** | 20.95 | 30.90 | 41.50 |



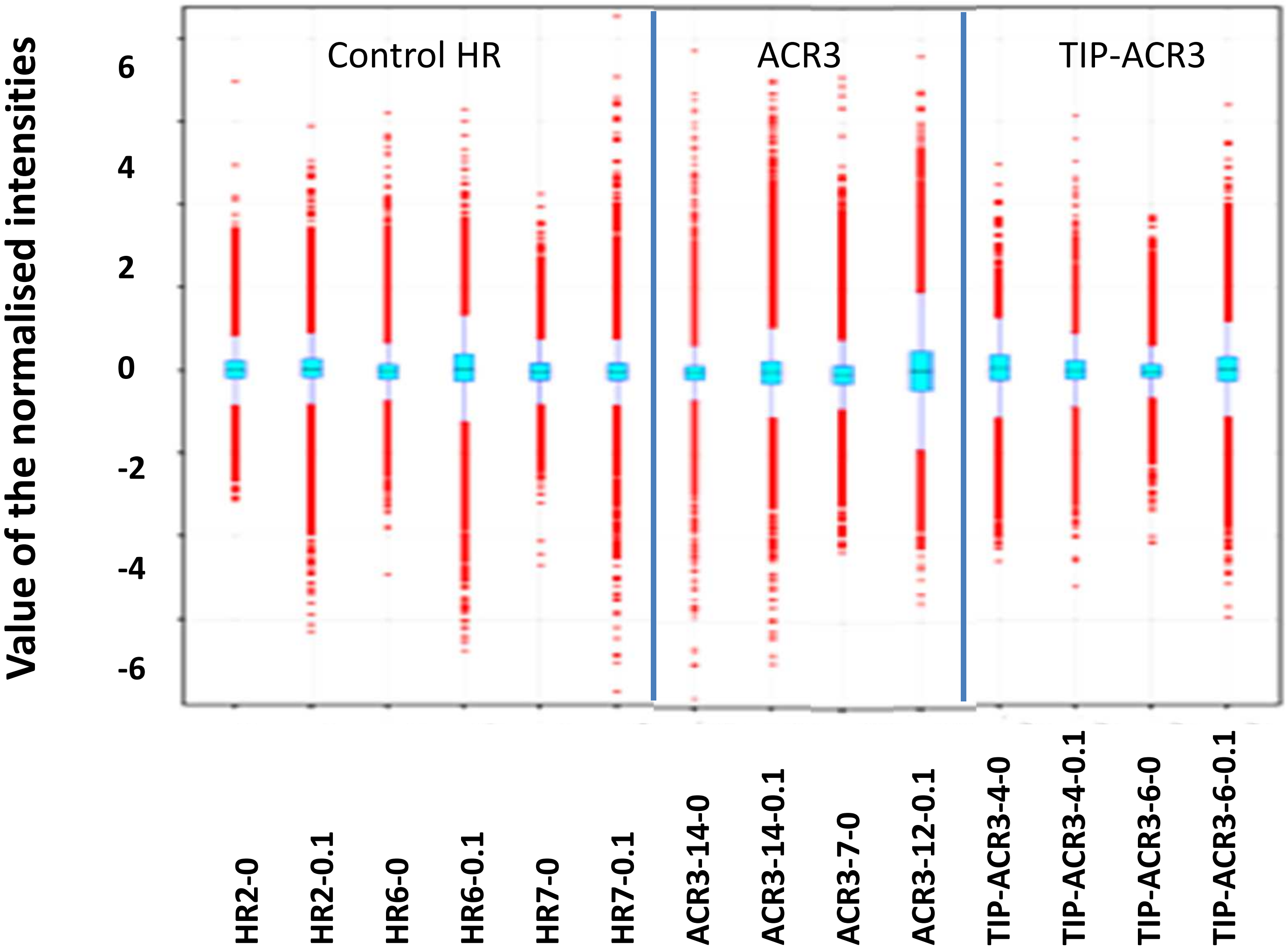
**Electronic Supplementary Information S4.** A. PCR product of the gene *rolC* that supports the presence of the transgene in hairy roots; (a) is the molecular weight marker; b-k) PCR products of the gene *rolC* of different transgenic lines. B. Amplification of the 1,086 bp PCR product of the gene *acr3; (*a) molecular weight marker; b) control HR; c-h) PCR products of the gene *acr3* in different transgenic lines.

**Electronic Supplementary Information S5.** Values of the activities of ROS-scavenging enzymes as function of: type of the root HR, ACR3 and TIP-ACR3), chemical species of As ($As^{III}$ or $As^{V}$), concentration of As (0.1 or 1 mM) and exposure time (6 h or 1 week). Enzyme activities are expressed in U $mg^{-1}$ protein. Content of malondialdehyde (MDA) is given in μg $g^{-1}$ protein.

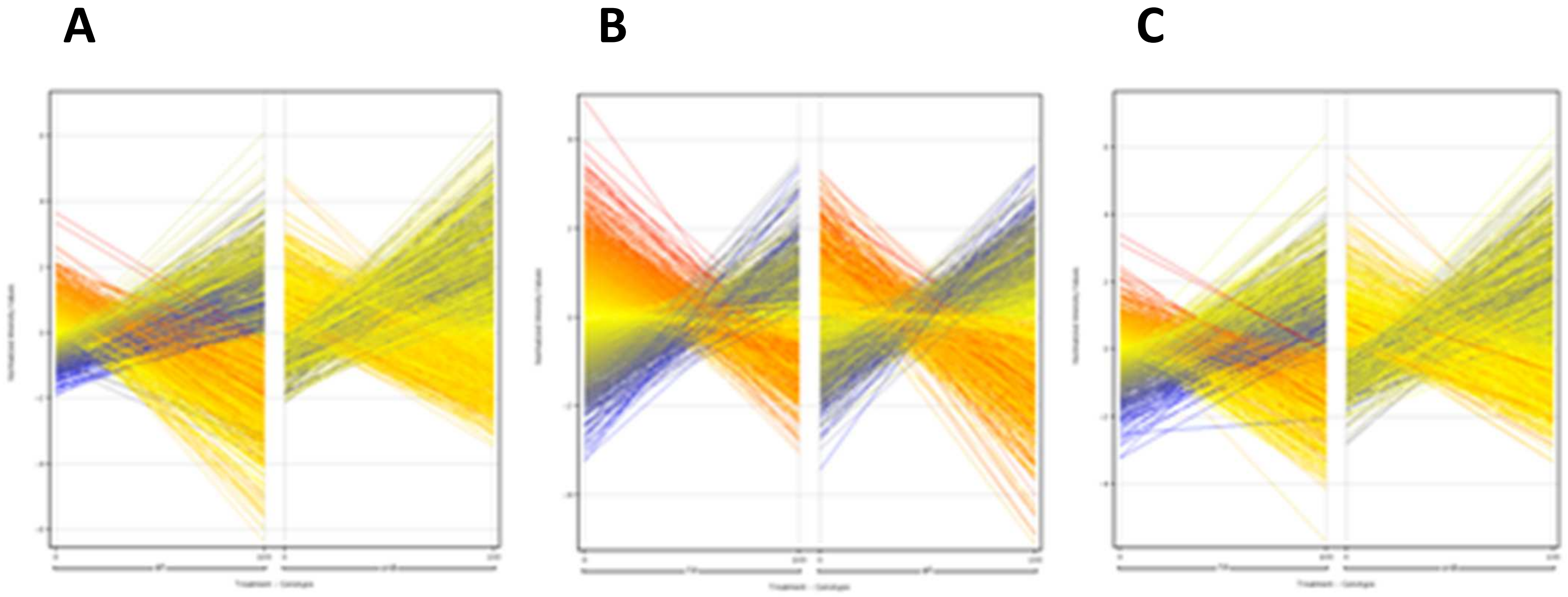
| **Enzyme** | **As concentration (mM)** | | Average enzyme value | | |
|---|---|---|---|---|---|
| | Exposure time | Chemical species | **Lines** | | |
| | | | **HR** | **ACR3** | **TIP-ACR3** |
| **Peroxidase (PX)** | | Without As | 31.79 ± 5.06 | 35.75 ± 5.26 | 35.18 ± 4.14 |
| | 6 hours | As(V)-0.1 | 32.30 ± 8.06 | 24.70 ± 0.61 | 70.55 ± 6.04 |
| | | As(III)-0.1 | 59.76 ± 5.12 | 53.15 ± 1.47 | 69.11 ± 4.20 |
| | | As (V)-1 | 3.18 ± 0.07 | 6.56 ± 0.24 | 74.24 ± 4.34 |
| | | As (III)-1 | 53.74 ± 3.23 | 3.96 ± 0.62 | 64.71 ± 3.87 |
| | 1 week | As(V)-0.1 | 28.27 ± 3.75 | 23.18 ± 1.86 | 14.87 ± 2.37 |
| | | As(III)-0.1 | 28.76 ± 5.65 | 36.32 ± 9.05 | 34.48 ± 10.65 |
| | | As (V)-1 | 23.17 ± 7.75 | 40.05 ± 5.02 | 24.15 ± 1.55 |
| | | As (III)-1 | 59.89 ± 2.10 | 73.24 ± 2.59 | 33.75 ± 6.54 |
| **Superoxide dismutase (SOD)** | | Without As | 191 ± 23 | 106 ± 28 | 327 ± 62 |
| | 6 hours | As(V)-0.1 | 388 ± 106 | 343 ± 87 | 658 ± 7 |
| | | As(III)-0.1 | 468 ± 133 | 184 ± 33 | 442 ± 14 |
| | | As (V)-1 | 465 ± 51 | 292 ± 98 | 640 ± 5 |
| | | As (III)-1 | 405 ± 41 | 401 ± 29 | 309 ± 50 |
| | 1 week | As(V)-0.1 | 402 ± 148 | 307 ± 33 | 304 ± 12 |
| | | As(III)-0.1 | 189 ± 14 | 386 ± 115 | 243 ± 10 |
| | | As (V)-1 | 596 ± 72 | 353 ± 64 | 168 ± 24 |
| | | As (III)-1 | 377 ± 53 | 277 ± 60 | 308 ± 8 |
| **Ascorbate peroxidase (APX)** | | Without As | 0.30 ± 0.06 | 0.36 ± 0.05 | 0.34 ± 0.06 |
| | 6 hours | As(V)-0.1 | 1.94 ± 0.21 | 1.21 ±0.17 | 0.52 ± 0.09 |
| | | As(III)-0.1 | 4.68 ± 1467 | 3904 ±1482 | 1.05 ± 0.51 |
| | | As (V)-1 | 2.32 ± 613 | 1747 ±564 | 1.61 ± 0.15 |
| | | As (III)-1 | 6.97 ± 3921 | 2006 ±68 | 1.33 ± 0.11 |
| | 1 week | As(V)-0.1 | 0.40 ± 146 | 333 ±43 | 0.67 ± 0.06 |
| | | As(III)-0.1 | 0.65 ± 117 | 741 ±260 | 0.48 ± 0.16 |
| | | As (V)-1 | 0.45 ± 96 | 397 ±85 | 0.26 ± 0.09 |
| | | As (III)-1 | 1.68 ± 637 | 1.64 ± 0.71 | 1.50 ± 0.69 |
| **Lipid peroxidation (MDA)** | | Whitout As | 55 ± 8 | 45 ± 6 | 30 ± 4 |
| | 6 hours | As(V)-0.1 | 84 ± 17 | 85 ± 69 | 102 ± 19 |
| | | As(III)-0.1 | 85 ± 16 | 191 ± 83 | 307 ± 67 |
| | | As (V)-1 | 312 ± 46 | 251 ± 137 | 126 ± 2 |
| | | As (III)-1 | 55 ± 13 | 157 ± 8 | 178 ± 32 |
| | 1 week | As(V)-0.1 | 1489 ± 1082 | 1885 ± 744 | 560 ± 117 |
| | | As(III)-0.1 | 676 ± 237 | 586 ± 272 | 527 ± 189 |
| | | As (V)-1 | 3201 ± 270 | 523 ± 268 | 4965 ± 1061 |
| | | As (III)-1 | 849 ± 203 | 1154 ± 700 | 783 ± 162 |



**Electronic Supplementary Information S6.** Evaluation of the qualilty of total RNA spectrophotometrically. The presence of two peaks corresponding to ribosomal RNA together with the absence of degradation peaks and the ratios A260/A280 and A230/A260 were the parameters used for assesing the quality. All samples corresponding to roots incubated in the presence of 1mM As showed no quality enough and hence were discarded.



**Electronic Supplementary Information S7.** “Boxplot” of the raw data obtained from microarray hybridizations 1, 2 and 3 corresponding to control HR, ACR3, and TIP-ACR3 exposed to 0.1 mM of $As^{III}$ vs. non-exposed roots. In the Figure, hairy roots are grouped in pairs, the first of each pair is the root in the absence of As and the second one is the same root exposed to As. Middle line divides the box into two parts, in which half the genes are greater than or equal to this value and half are less. The interquartile range box represents the middle 50% of the data, whereas the whiskers extend to either side of the box and represent the extreme quartiles: the bottom 25% and the top 25% of the data values, excluding outliers.



**HR *vs.* ACR3**

**HR *vs.* TIP-ACR3**

**TIP-ACR3 *vs.* ACR3**

**Electronic Supplementary Information S8.** Graphic representation of gene expression levels in contrasting hairy roots for (A) Hybridization 4; control HR *vs*. ACR3, (B) Hybridization 5; HR *vs*. TIP-ACR3, and (C) Hybridization 6; TIP-ACR3 *vs*. ACR3. Genes are coloured according to their expression level in the first group of each panel for high (red), medium (yellow), and low (blue) expression.