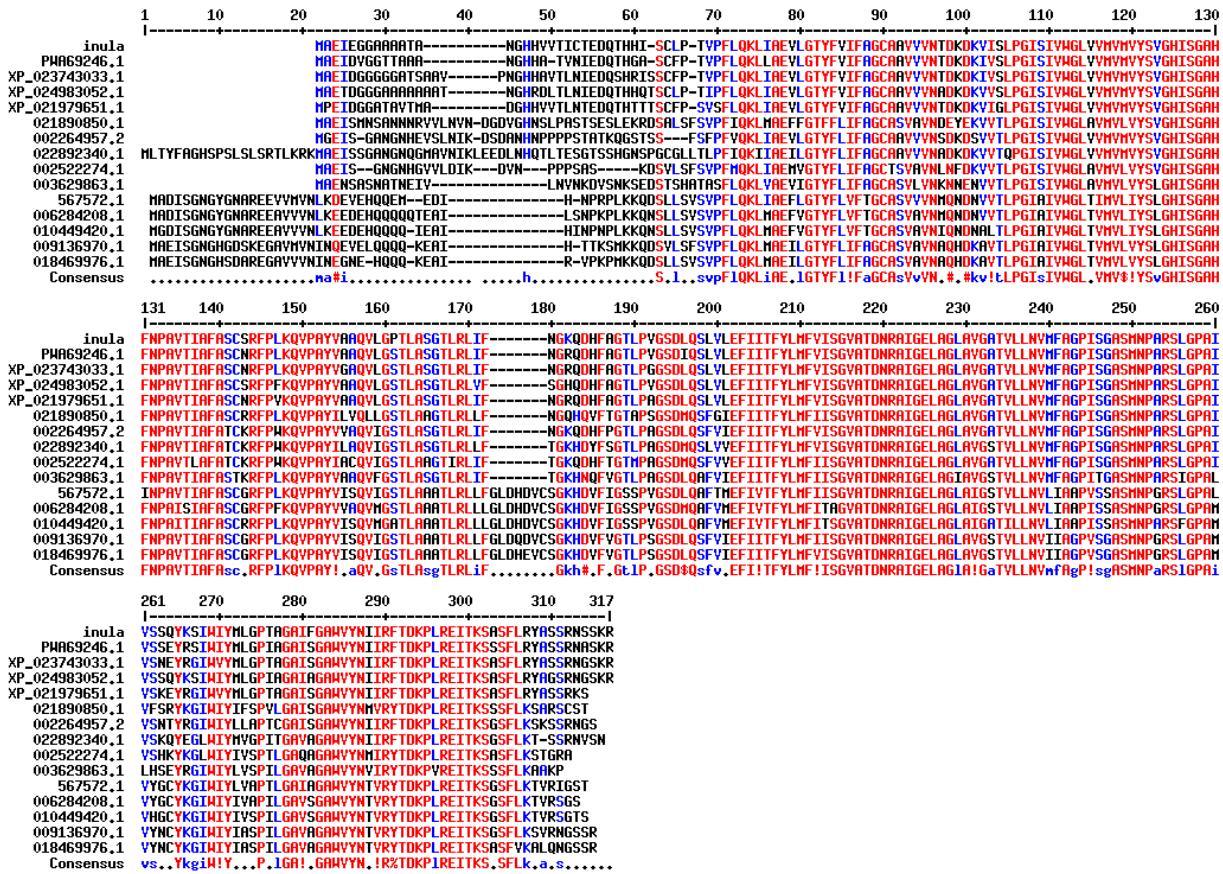
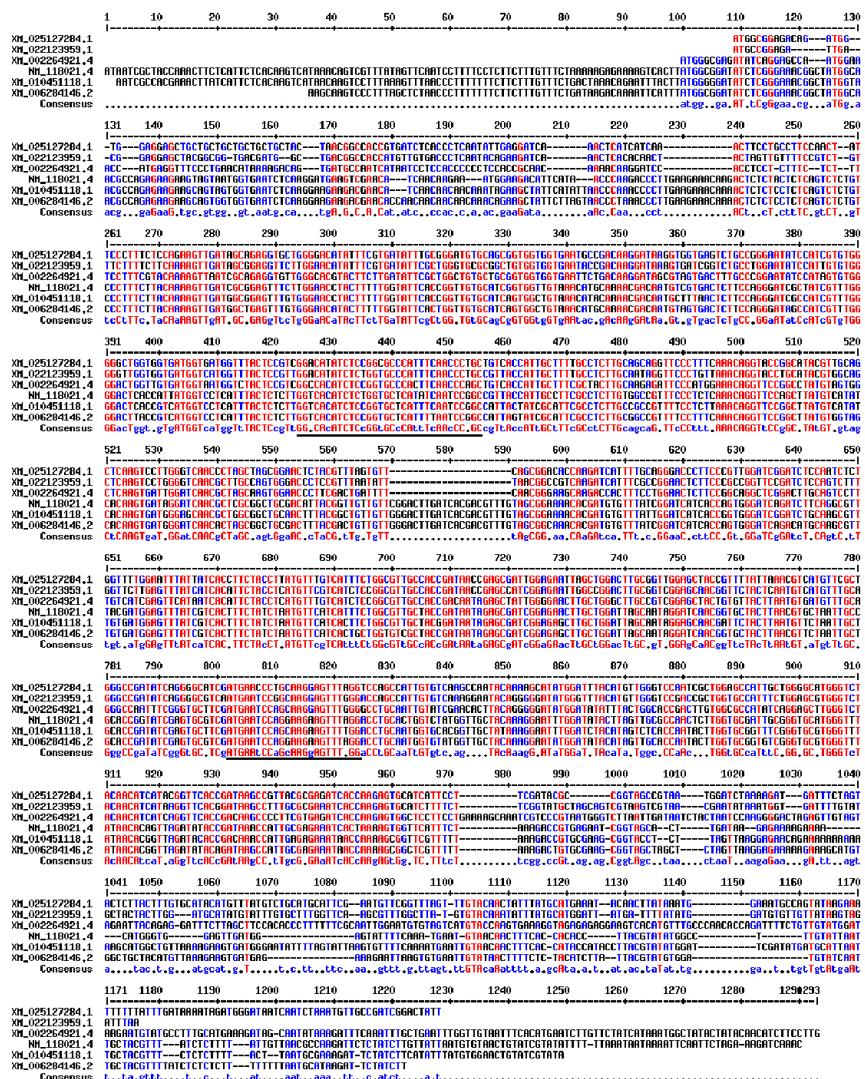


Supplementary Material

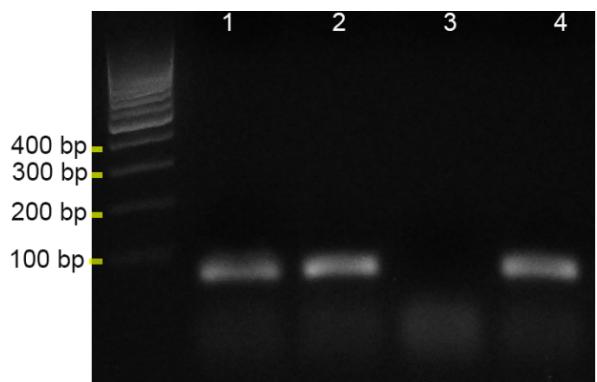


Supplemental figure s1: Alignment of the predicted aminoacid sequence of DvNip1 with members of *Arabidopsis* and other Nip⁺. Putative *Dittrichia viscosa* DvNip1 amino acid sequence were aligned using MultiAlign. The identical residues are shaded in red. >inula nip [Dittrichia viscosa]; >PWA69246.1 NOD26-like intrinsic protein 1,2 [Artemisia annua]; >XP_023743033.1 aquaporin NIP1-2-like [Lactuca sativa]; >XP_024983052.1 aquaporin NIP1-1-like [Cynara cardunculus var. scolymus]; >XP_021979651.1 aquaporin NIP1-1-like [Helianthus annuus]; >567572.1 NOD26-like major intrinsic protein 1 [Arabidopsis thaliana]; >006284208.1 aquaporin NIP1-1 [Capsella rubella]; >010449420.1 PREDICTED: aquaporin NIP1-1 isoform X1 [Camelina sativa]; >009136970.1 PREDICTED: aquaporin NIP1-2 [Brassica rapa]; >018469976.1 PREDICTED: aquaporin NIP1-2 [Raphanus sativus]; >021890850.1 aquaporin NIP1-2 [Carica papaya]; >002522274.1 PREDICTED: aquaporin NIP1-2 isoform X1 [Ricinus communis]; >002264957.2 PREDICTED: aquaporin NIP1-1 [Vitis vinifera]; >003629863.1 nodulin-26 isoform X1 [Medicago truncatula]; >022892340.1 aquaporin NIP1-1-like isoform X1 [Olea europaea var. sylvestris].

Supplementary Material



Supplemental figure s2: Nucleotide sequence alignment of Nip1.1 gene. XM_025127284.1 PREDICTED: *Cynara cardunculus* var. *scolymus*, >XM_022123959.1 PREDICTED: *Helianthus annuus* aquaporin NIP1-1-like , >XM_002264921.4 PREDICTED: *Vitis vinifera* aquaporin NIP1-1, >NM_118021.4 *Arabidopsis thaliana* NOD26-like major intrinsic protein 1 (NLM1), >XM_006284146.2 PREDICTED: *Capsella rubella* aquaporin NIP1-1 were aligned with multiAlign (ref. Multiple sequence alignment with hierarchical clustering" F. CORPET, 1988, Nucl. Acids Res., 16 (22), 10881-10890. The position of degenerate primers was indicated.



Supplemental Figure s3. Conventional PCR products amplified with each pair of primers revealed a single product of the expected size. Obtained amplimers with AtEF1-A (1), AtAct2 (2), AtAct8 (3) and NtEF1-A (4) primer pairs, visualized on 2.0% agarose gel.

Descriptions Graphic Summary Alignments Taxonomy

Sequences producing significant alignments

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GenPept Graphics Distance tree of results Multiple alignment MSA Viewer

	Description	Scientific Name	Max Score	Total Score	Query Cover	E value	Per. Ident	Acc. Len	Accession
<input checked="" type="checkbox"/>	elongation factor 1-alpha [Helianthus annuus]	Helianthus annuus	64.7	64.7	100%	1e-13	100.00%	449	XP_021981523.1
<input checked="" type="checkbox"/>	elongation factor 1-alpha-like [Cynara cardunculus var. scolymus]	Cynara cardunculus ...	64.7	64.7	100%	1e-13	100.00%	449	XP_024976594.1
<input checked="" type="checkbox"/>	elongation factor 1-alpha [Artemisia annua]	Artemisia annua	64.7	64.7	100%	1e-13	100.00%	449	PWA49076.1
<input checked="" type="checkbox"/>	elongation factor 1-alpha [Helianthus annuus]	Helianthus annuus	64.7	64.7	100%	1e-13	100.00%	449	XP_021973407.1
<input checked="" type="checkbox"/>	elongation factor 1-alpha-like isoform X1 [Cynara cardunculus var. scolymus]	Cynara cardunculus ...	64.7	64.7	100%	1e-13	100.00%	449	XP_024970426.1
<input checked="" type="checkbox"/>	elongation factor 1-alpha isoform X1 [Lactuca sativa]	Lactuca sativa	64.7	64.7	100%	1e-13	100.00%	449	XP_023747111.1
<input checked="" type="checkbox"/>	elongation factor 1-alpha-like [Lactuca sativa]	Lactuca sativa	64.7	64.7	100%	1e-13	100.00%	447	XP_042755374.1
<input checked="" type="checkbox"/>	hypothetical protein CTI12_AA312610 [Artemisia annua]	Artemisia annua	64.7	64.7	100%	1e-13	100.00%	447	PWA65970.1
<input checked="" type="checkbox"/>	elongation factor 1-alpha [Cynara cardunculus var. scolymus]	Cynara cardunculus ...	64.7	64.7	100%	1e-13	100.00%	447	XP_024983118.1
<input checked="" type="checkbox"/>	hypothetical protein CTI12_AA312610 [Artemisia annua]	Artemisia annua	64.7	64.7	100%	1e-13	100.00%	447	PWA65971.1
<input checked="" type="checkbox"/>	elongation factor 1-alpha 1 [Cynara cardunculus var. scolymus]	Cynara cardunculus ...	64.7	64.7	100%	1e-13	100.00%	447	XP_024985721.1
<input checked="" type="checkbox"/>	elongation factor 1-alpha-like [Cynara cardunculus var. scolymus]	Cynara cardunculus ...	64.7	64.7	100%	1e-13	100.00%	447	XP_024968429.1
<input checked="" type="checkbox"/>	hypothetical protein CTI12_AA400390 [Artemisia annua]	Artemisia annua	64.7	64.7	100%	1e-13	100.00%	447	PWA58403.1
<input checked="" type="checkbox"/>	elongation factor 1-alpha [Artemisia annua]	Artemisia annua	64.7	64.7	100%	1e-13	100.00%	447	PWA48367.1
<input checked="" type="checkbox"/>	elongation factor 1-alpha [Helianthus annuus]	Helianthus annuus	64.7	64.7	100%	1e-13	100.00%	447	XP_022006019.1
<input checked="" type="checkbox"/>	hypothetical protein Ccrd_021776 [Cynara cardunculus var. scolymus]	Cynara cardunculus ...	64.7	64.7	100%	1e-13	100.00%	437	KVI00009.1
<input checked="" type="checkbox"/>	hypothetical protein Ccrd_012208 [Cynara cardunculus var. scolymus]	Cynara cardunculus ...	64.7	64.7	100%	1e-13	100.00%	389	KVI09405.1
<input checked="" type="checkbox"/>	elongation factor 1-alpha 1 isoform X2 [Lactuca sativa]	Lactuca sativa	64.7	64.7	100%	1e-13	100.00%	380	XP_042754064.1

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elongation factor 1-alpha [Helianthus annuus]

Sequence ID: [XP_021981523.1](#) Length: 449 Number of Matches: 1

[See 2 more title\(s\)](#) ▾ [See all Identical Proteins\(IPG\)](#)

Range 1: 226 to 244 GenPept Graphics ▾ Next Match ▲ Previous Match

Score 64.7 bits(145)	Expect 1e-13	Identities 19/19(100%)	Positives 19/19(100%)	Gaps 0/19(0%)
Query 1 PKRPSDKPLRLPLQDVYKI 19				
Sbjct 226 PKRPSDKPLRLPLQDVYKI 244				

Supplemental Figure s4. BLAST-P analysis results performed with the aminoacid sequence deduced from amplification product obtained with AtEF1-A primer pairs.

Sample	AtAct2			AtEF1-A			NtEF1-A					
	Ct value	Experiment mean	SD	CV	Ct Value	Experiment mean	SD	CV	Ct Value	Experiment mean	SD	CV
Shoot CTRL1	30,34	30,4866667	0,57422411	0,05053975	23,79	23,09	0,63624793	0,02676146	35,6	34,76	0,73430239	0,02419668
Shoot CTRL2	30				23,19				34,44			
Shoot CTRL3	31,12				22,29				34,24			
Shoot AsIII(6h)1	32,55	30,6366667	1,68953643		23,89	23,2333333	0,63610796		34,33	34,53	0,71140706	
Shoot AsIII(6h)2	30,01				23,19				35,32			
Shoot AsIII(6h)3	29,35				22,62				33,94			
Shoot AsIII(48h)1	26,16	27,6	1,29799846		22,62	23,2133333	0,61614392		33,74	33,9133333	0,52204725	
Shoot AsIII(48h)2	27,96				23,17				33,5			
Shoot AsIII(48h)3	28,68				23,85				34,5			
Shoot AsV(6h)1	30,54	29,3066667	1,06884673		22,79	23,6666667	0,94044316		34,89	34,8833333	1,38001208	
Shoot AsV(6h)2	28,65				23,55				36,26			
Shoot AsV(6h)3	28,73				24,66				33,5			
Shoot CdII(6h)1	30,1	29,5933333	0,83464563		23,03	22,9166667	0,36350149		33,72	34,1533333	0,93831409	
Shoot CdII(6h)2	28,63				22,51				33,51			
Shoot CdII(6h)3	30,05				23,21				35,23			
Root CTRL1	33,61	32,2733333	1,23313962	0,04484553	25,535	25,6616667	0,15892871	0,03929649	34,18	35,2266667	1,09258104	0,03391399
Root CTRL2	32,03				25,61				35,14			
Root CTRL3	31,18				25,84				36,36			
Root AsIII(6h)1	30,27	29,61	0,77948701		25,8	25,4666667	0,28884829		35,23	34,9266667	0,65957057	
Root AsIII(6h)2	29,81				25,31				35,38			
Root AsIII(6h)3	28,75				25,29				34,17			
Root AsIII(48h)1	30,21	30,3533333	1,4503218		26,57	25,8866667	0,78449559		35,21	34,3066667	0,8292366	
Root AsIII(48h)2	31,87				26,06				33,58			
Root AsIII(48h)3	28,98				25,03				34,13			
Root AsV(6h)1	31,78	31,73	0,836122		23,1	23,9033333	0,74096784		35,85	33,9033333	1,7350024	
Root AsV(6h)2	32,54				24,05				32,52			
Root AsV(6h)3	30,87				24,56				33,34			
Root CdII(6h)1	32,56	32,2633333	0,34151623		24,32	24,02	0,26457513		34,42	33,6566667	1,24500335	
Root CdII(6h)2	31,89				23,92				32,22			
Root CdII(6h)3	32,34				23,82				34,33			

Table S1. Validation of reference genes in *D. viscosa*. Raw Ct values of the three amplification products given by the primer pairs used to identify the reference genes for *Dittrichia* in all experimental condition and samples (shoots and roots). Means \pm SD of single independent experiments and Coefficient of Variation (CV) are reported.