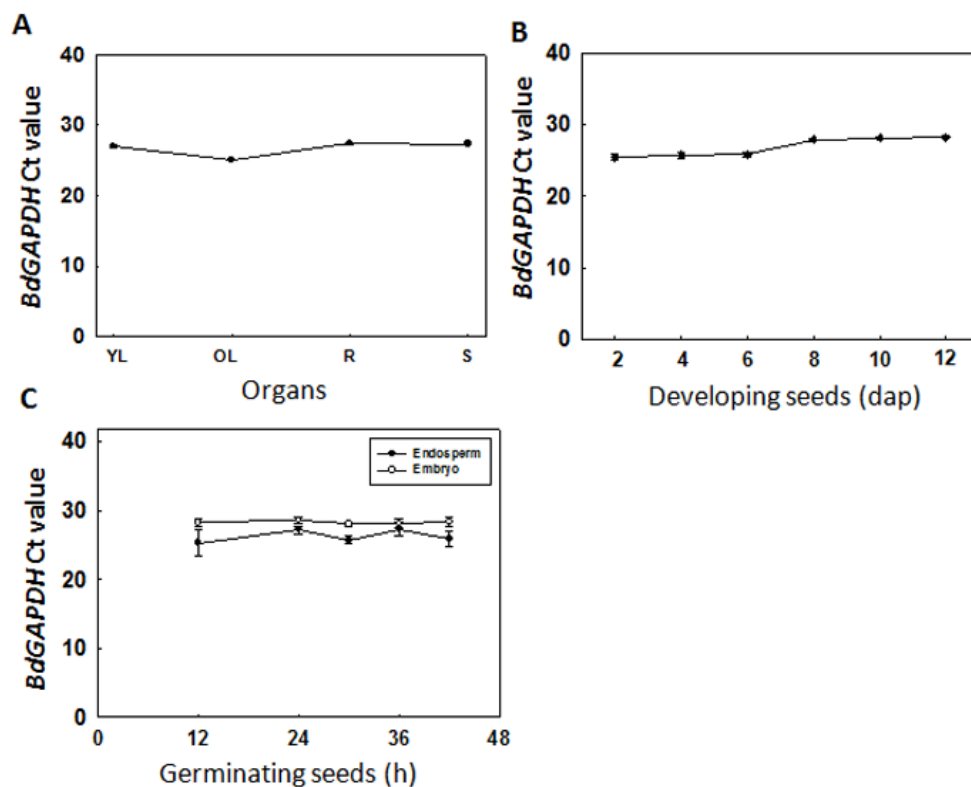


**Mannans and endo- β -mannanases (MAN) in *Brachypodium distachyon*:
Expression profiling and possible role of the *BdMAN* genes during
coleorhiza-limited seed germination**

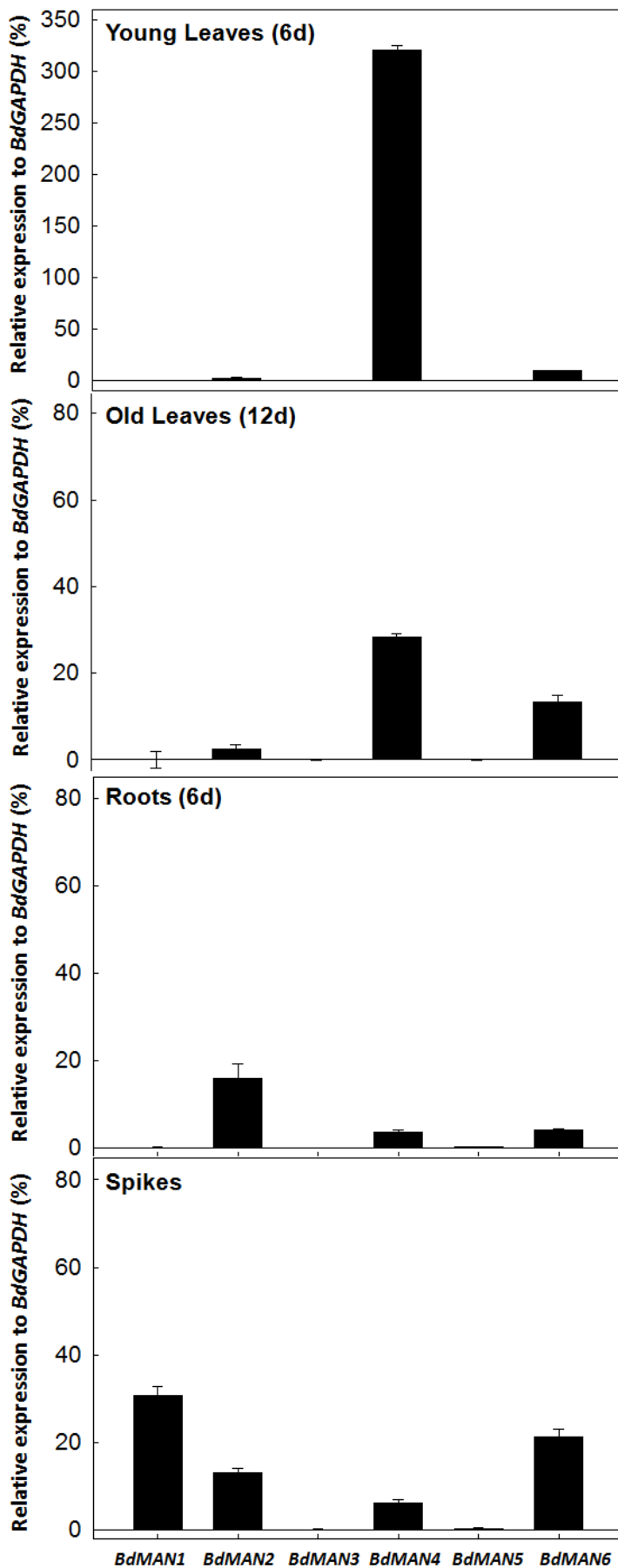
Virginia González-Calle, Cristina Barrero-Sicilia¹, Pilar Carbonero, Raquel Iglesias-Fernández*

Centro de Biotecnología y Genómica de Plantas (UPM-INIA), and E.T.S.I. Agrónomos, Campus de Montegancedo, Universidad Politécnica de Madrid, Pozuelo de Alarcón, 28223-Madrid, Spain

Supplementary data:



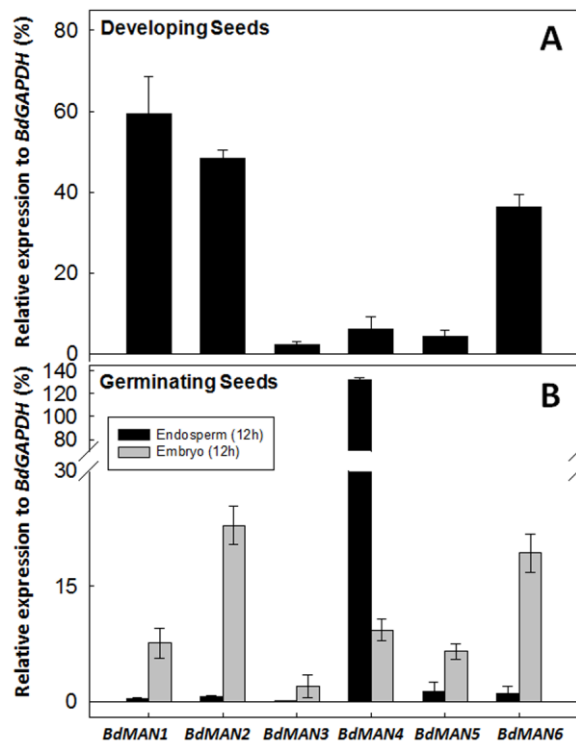
Supplementary Fig. S1. Transcription levels of the housekeeping (*BdGAPDH* gene), presented as Ct mean values, in different organs (OL: Old Leaves; R: Roots; S: Spikes; YL: Young Leaves; **A**), in developing seeds (dap: days after pollination; **B**) and during seed germination (from 12 to 42 h; **C**) of *B. distachyon*.



Supplementary Fig.

S2.

Transcripts analysis by RTqPCR of the *BdMAN1-6* genes in different organs. Young leaves (6 d); Old leaves (12 d); Roots (6 d) and Spikes. Data are means \pm standard error (SE) of three technical replicates of three biological samples.



Supplementary Fig. S3. Expression analysis by RT-qPCR of *BdMAN1-6* genes in developing seeds (mix of different stages; **A**) and germinating seeds (12 h of imbibition; **B**). Embryos and de-embryonated seeds (endosperm) were separately analysed of *Brachypodium distachyon*. Data are means \pm standard error (SE) of three technical replicates of three biological samples.

Table S1. Major biochemical characteristics of *Brachypodium distachyon* and *Oryza sativa* endo- β -mannanase proteins. SL: Sub-cellular localization

Protein name	Locus	Protein size (Aa)	pI	Mw(Da)	Signal peptide position	SL prediction
BdMAN1	Bd2g45790	417	8.60	51765.15	Cleavage site 21-22	Secretory pathway (P=0.78)
BdMAN2	Bd2g49682	446	6.24	49945.77	No	Secretory pathway (P=0.92)
BdMAN3	Bd3g57290	413	6.84	45112.05	Cleavage site 30-31	Secretory pathway (P=0.88)
BdMAN4	Bd1g32107	468	5.67	51765.15	Cleavage site 20-21	Secretory pathway (P=0.85)
BdMAN5	Bd4g44340	389	4.44	43266.27	No	Other (P=0.91)
BdMAN6	Bd1g42770	436	8.75	49158.23	No	
OsMAN1	Os01g0663300.1	432	9.27	46874.91	Cleavage site 30-31	Secretory pathway (P= 0.96)
OsMAN2	Os01g0746700.1	445	5.79	50062.80	No	Secretory pathway (P= 0.90)
OsMAN3	Os03g0828300.1	468	6.02	51216.68	Cleavage site 23-24	Secretory pathway (P= 0.97)
OsMAN4	Os03g0828500.1	461	5.38	50739.43	No	-----
OsMAN5	Os05g0319100.1	491	5.60	53465.08	No	Secretory pathway (P=0.70)
OsMAN6	Os06g0311600.1	440	8.12	49455.08	No	Secretory pathway (P=0.96)
OsMAN7	Os11g0118200.1	379	4.95	42984.55	No	-----
OsMAN8	Os12g0117300.1	372	4.85	41839.18	No	Secretory pathway (P=0.96)

Table S2. Oligonucleotide sequences, amplicon length and PCR efficiency of primers used for RT-qPCR analyses.

Gene	Primer sense	Primer antisense	Amplicon size (bp)	T _m (°C)	Slope	E*
<i>BdGAPDH</i>	CTCCCGCTATTTGTTTTGTC	TGAAGATGTTGGAGCTGACG	76	71.5	-3.07	2.11
<i>BdMAN1</i>	TATTCATCCGGGAGAGAAGG	ATTGGCATCTCCTGATCCAC	104	71.2	-3.36	1.98
<i>BdMAN2</i>	GGTGTACGACATCGCCTACG	GAGAAGCCGTCGTGGTAGTC	108	83.3	-4.22	1.73
<i>BdMAN3</i>	TGTATGACGCGATCTACGC	ATCACCTGCCAGAACATTCC	70	77.2	-3.64	1.88
<i>BdMAN4</i>	TAGGTTCAGTTTCAGGTTG	ACACTTGCACTGCACAT	120	70.3	-3.09	2.10
<i>BdMAN5</i>	CCAAGATCCAATCGTGAAATG	CTCACAGAAGCATAAGACCCAAC	86	65.9	-3.16	2.07
<i>BdMAN6</i>	GCTCATCAAAGTTGCCTGTG	TTACCGGCGCAACTAGAAAG	106	71.7	-3.40	1.96

*E=efficiency=10^(-1/slope)

Table S3. Primers used for the synthesis of the *in situ* mRNA hybridization probes

Primer name	Sequence 5'-3'
<i>BdMAN2-insitu-s</i>	CTCTCTCAAGCCGAAGCACC
<i>BdMAN2-insitu-as</i>	CGTAGGCGATGTCGTACACC
<i>BdMAN4-insitu-s</i>	CTGCCGACTCGTTTCGTCTC
<i>BdMAN4-insitu-as</i>	GACCAATCATCCTGACCGAC
<i>BdMAN6-insitu-s</i>	GCAAATAAAGCCCAAACACTAC
<i>BdMAN6-insitu-as</i>	GTCCGCCAAGTTTATACAAC

s, sense; *as*, antisense