

A possible role of the aleurone expressed gene *HvMAN1* in the hydrolysis of the cell wall mannans of the starchy endosperm in germinating *Hordeum vulgare* L. seeds

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**Supplementary Table S1.** Major biochemical characteristics of the deduced protein MAN sequences of *Hordeum vulgare*, *Arabidopsis thaliana* and *Brachypodium distachyon*.

| <b>Protein name</b> | <b>Locus/Ref.</b>                           | <b>Protein size (aa)</b> | <b>Ip</b> | <b>Mw (Da)</b> | <b>SP (position)</b>                           |
|---------------------|---------------------------------------------|--------------------------|-----------|----------------|------------------------------------------------|
| <b>HvMAN1</b>       | <i>HORVU3Hr1g060060/</i><br><i>AK356724</i> | 427                      | 9.20      | 46077.81       | YES<br>(25-26 aa)                              |
| <b>HvMAN2</b>       | <i>HORVU3Hr1g071210/</i><br><i>AK356773</i> | 444                      | 5.85      | 49902.57       | NO                                             |
| <b>HvMAN3</b>       | <i>HORVU5Hr1g115310/</i><br><i>AK371032</i> | 480                      | 6.07      | 53214.95       | YES<br>(19-20 aa)                              |
| <b>HvMAN4</b>       | <i>HORVU0Hr1g017220/</i><br><i>AK373804</i> | 433                      | 6.72      | 47524.71       | YES<br>(30-31 aa)                              |
| <b>HvMAN5</b>       | <i>HORVU5Hr1g046370/</i><br><i>AK370093</i> | 420                      | 4.61      | 46964.79       | NO                                             |
| <b>HvMAN6</b>       | <i>HORVU7Hr1g045910/</i><br><i>AK369260</i> | 437                      | 8.09      | 49398.32       | YES<br>(22-23 aa)                              |
| <b>AtMAN1</b>       | <i>At1g02310</i>                            | 411                      | 5.68      | 46290.39       | Iglesias-<br>Fernández<br><i>et al.</i> , 2011 |
| <b>AtMAN2</b>       | <i>At2g20680</i>                            | 433                      | 6.47      | 49558.31       |                                                |
| <b>AtMAN3</b>       | <i>At3g10890</i>                            | 414                      | 8.41      | 46461.86       |                                                |
| <b>AtMAN4</b>       | <i>At3g10900</i>                            | 408                      | 6.67      | 45376.48       |                                                |
| <b>AtMAN5</b>       | <i>At4g28320</i>                            | 431                      | 8.38      | 49898.87       |                                                |
| <b>AtMAN6</b>       | <i>At5g01930</i>                            | 448                      | 5.95      | 50622.24       |                                                |
| <b>AtMAN7</b>       | <i>At5g66460</i>                            | 431                      | 9.33      | 48572.98       |                                                |
| <b>BdMAN1</b>       | <i>Bd2g45790</i>                            | 417                      | 8.60      | 45098.78       | González-<br>Calle <i>et al.</i> ,<br>2015     |
| <b>BdMAN2</b>       | <i>Bd2g49682</i>                            | 446                      | 6.24      | 49945.77       |                                                |
| <b>BdMAN3</b>       | <i>Bd1g32107</i>                            | 468                      | 5.67      | 51765.15       |                                                |
| <b>BdMAN4</b>       | <i>Bd3g57290</i>                            | 413                      | 6.84      | 45112.05       |                                                |
| <b>BdMAN5</b>       | <i>Bd4g44340</i>                            | 389                      | 4.44      | 43266.27       |                                                |
| <b>BdMAN6</b>       | <i>Bd1g42770</i>                            | 436                      | 8.75      | 49158.23       |                                                |

**Supplementary Table S2:** Characteristics and sequences of the primers used for quantitative PCR experiments

| Gene           | Primer Name                   | Sequences (5'-3')     | Amplicon size (bp) | Eff.(%) | Diss. T (°C) |
|----------------|-------------------------------|-----------------------|--------------------|---------|--------------|
| <b>HvMAN1</b>  | <i>S-HvMAN1-RT (RIF-53)</i>   | AGACCACCACGACAACAAAG  | 77                 | 80.4    | 72.4         |
|                | <i>AS-HvMAN1-RT (RIF-54)</i>  | AAGGCACGCATTCTTACTCC  |                    |         |              |
| <b>HvMAN2</b>  | <i>S-HvMAN2-RT (RIF-55)</i>   | ACATGGAGGAGTTCCACGAC  | 128                | 70.5    | 84.7         |
|                | <i>AS-HvMAN2-RT (RIF-56)</i>  | GATCCTCTTGGCCTCTTGTTT |                    |         |              |
| <b>HvMAN3</b>  | <i>S-HvMAN3-R (PCZ109)</i>    | TGCTCATCCTTACGTCCAAAG | 87                 | 86      | 74.1         |
|                | <i>AS-HvMAN3-RT (PCZ110)</i>  | ACACGGGACCAATCAAACAG  |                    |         |              |
| <b>HvMAN4</b>  | <i>S-HvMAN4-RT (PCZ111)</i>   | GGCTACGAAGTCGTGCTTG   | 92                 | 95.13   | 84.1         |
|                | <i>AS-HvMAN4-RT (PCZ112)</i>  | TAGCCTCAGGGCGTGATTC   |                    |         |              |
| <b>HvMAN5</b>  | <i>S-HvMAN5-RT (PCZ113)</i>   | CATGGACGATGGTTATGCAG  | 99                 | 100     | 74.8         |
|                | <i>AS-HvMAN5-RT (PCZ114)</i>  | GCAGCATGCTCTCAACATTC  |                    |         |              |
| <b>HvMAN6</b>  | <i>S-HvMAN6-RT (PCZ115)</i>   | AGAGACCCTGACTGGCAATG  | 72                 | 100     | 74.8         |
|                | <i>AS-HvMAN6-RT (PCZ116)</i>  | AACCAGGATTTCCCGGTATC  |                    |         |              |
| <b>HvGAPDH</b> | <i>S-HvGAPDH-RT (PCZ117)</i>  | GGGAAAGCTCAAGGGTATC   | 161                | 100     | 78.7         |
|                | <i>AS-HvGAPDH-RT (PCZ118)</i> | TGTAACCCCACTCGTTGTCA  |                    |         |              |

**Supplementary Table S3.** List of primers used for *HvMAN1* probe synthesis in the mRNA *in situ* hybridization analyses

| Gene                 | PRIMERS                                | SEQUENCES (5'-3')    | Probe size (bp) |
|----------------------|----------------------------------------|----------------------|-----------------|
| <b><i>HvMAN1</i></b> | <i>S-HvMAN1-IS</i><br>( <i>RIF57</i> ) | ACGCCAAGATCTACCAGTCG | 170             |
|                      | <i>S-HvMAN1-IS</i><br>( <i>RIF58</i> ) | CTTGAGCTTGCGGCTCTC   |                 |