The Compromised Recognition of Turnip Crinkle Virus1 Subfamily of Microrchidia ATPases Regulates Disease Resistance in Barley to Biotrophic and Necrotrophic Pathogens[1][C][W][OPEN]

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MORC1 and MORC2, two of the seven members of the Arabidopsis (*Arabidopsis thaliana*) Compromised Recognition of Turnip Crinkle Virus1 subfamily of microrchidia Gyrase, Heat Shock Protein90, Histidine Kinase, MutL (GHKL) ATPases, were previously shown to be required in multiple layers of plant immunity. Here, we show that the barley (*Hordeum vulgare*) MORCs also are involved in disease resistance. Genome-wide analyses identified five MORCs that are 37% to 48% identical on the protein level to AtMORC1. Unexpectedly, and in clear contrast to Arabidopsis, RNA interference-mediated knockdown of *MORC* in barley resulted in enhanced basal resistance and effector-triggered, powdery mildew resistance locus A12-mediated resistance against the biotrophic powdery mildew fungus (*Blumeria graminis* f. sp. *hordei*), while *MORC* overexpression decreased resistance. Moreover, barley knockdown mutants also showed higher resistance to *Fusarium graminearum*. Barley MORCs, like their Arabidopsis homologs, contain the highly conserved GHKL ATPase and S5 domains, which identify them as members of the MORC superfamily. Like AtMORC1, barley MORC1 (HvMORC1) binds DNA and has Mn^{2+}-dependent endonuclease activities, suggesting that the contrasting function of MORC1 homologs in barley versus Arabidopsis is not due to differences in their enzyme activities. In contrast to AtMORCs, which are involved in silencing of transposons that are largely restricted to pericentromeric regions, barley MORC mutants did not show a loss-of-transposon silencing regardless of their genomic location. Reciprocal overexpression of MORC1 homologs in barley and Arabidopsis showed that AtMORC1 and HvMORC1 could not restore each other's function. Together, these results suggest that MORC proteins function as modulators of immunity, which can act negatively (barley) or positively (Arabidopsis) dependent on the species.

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G.L. and S.v.E. performed the experiments and drafted the initial manuscript; J.I. generated transgenic barley; A.K. and S.P. performed the MORC mutant analysis; M.M. and H.W. performed the ATPase activity; K.E. performed the microscopy; M.C. and R.S. performed the transposon analysis; H.-G.M. and Y.B. performed the coimmunoprecipitation. H.-G.K., D.F.K., and K.-H.K. conceived the project, analyzed the data, and wrote the paper.

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The evolution of a complex defense system has been the consequence of plants being constantly exposed to pathogenic microbes and pests. One of the first lines of active defense is based on a perception of pathogen-associated molecular patterns (PAMPs) by pattern recognition receptors located in the plant cell membrane. The defense response to PAMP recognition is called PAMP-triggered immunity (PTI). While PTI is sufficient to stop colonization by many microbes, some microorganisms overcome this immune response by releasing effectors (formerly called virulence factors). In a coevolutionary process, some plants have evolved resistance (R) proteins for direct or indirect recognition of microbial effectors (avirulence [Avr] factors) leading to effector-triggered immunity (ETI). ETI is frequently characterized by a rapid and locally restricted programmed cell death response (also known as hypersensitive reaction [HR]), which helps to limit pathogen proliferation and disease symptoms. On the contrary, the absence of an Avr-R protein interaction results in virulence of the pathogen. In addition, ETI is counteracted by some microbes by the release of additional

virulence factors that block or overcome effector recognition and ensure pathogenicity. The mutual evolution of host and microbe leading to elicitation or suppression of ETI is summarized by the "zigzag" model proposed by Jones and Dangl (2006). PTI and ETI are associated with activation of various defense responses both at infection sites and in distal tissue, including production and accumulation of reactive oxygen species, salicylic acid, and pathogenesis-related proteins. Systemic activation of such responses, triggered in the uninfected tissue, leads to long-lasting, broad-based resistance to subsequent pathogen infections, termed systemic acquired resistance.

A genetic screen in Arabidopsis (*Arabidopsis thaliana*) searching for mutants with compromised resistance mediated by the R protein HR to Turnip Crinkle Virus (HRT) against Turnip Crinkle Virus (TCV) led to the discovery of the Compromised Recognition of TCV1 (CRT1) subfamily of the microrchidia (MORC) subclade of the GHKL (for Gyrase, Heat Shock Protein90, Histidine Kinase, MutL) ATPase superfamily (Watson et al., 1998; Iyer et al., 2008; Kang et al., 2008). Genome analysis of Arabidopsis revealed that MORC1 (formerly named CRT1 in Kang et al., 2008, 2010, 2012) has two close (>70% sequence similarity on amino acid [aa] level) and four distant (<50% aa similarity) homologs. A double knockout mutant, *morc1-2 morc2-1*, lacking *MORC1* and its closest homolog *MORC2* also displayed compromised ETI to avirulent *Pseudomonas syringae*, suppressed basal resistance, systemic acquired resistance, and/or PTI to TCV and virulent *P. syringae* and compromised nonhost resistance to *Phytophthora infestans* (Kang et al., 2012). Arabidopsis MORC1 physically interacts with at least eleven R proteins belonging to three different structural classes (Martin et al., 2003), including HRT, the R protein involved in recognition of TCV. This interaction is a dynamic process, as MORC1 bound inactive R proteins, while little or no interaction was observed when the R proteins were activated (Kang et al., 2010). Taken together, these results argued that MORC1 protein family members in Arabidopsis are key components in multiple layers of resistance against a variety of pathogens. Recently, it was shown that a small fraction of AtMORC1 translocates to the plant nucleus after ETI and PTI activation (Kang et al., 2012). Because Arabidopsis MORC1 possesses DNA/RNA-binding capacity and endonuclease activity in vitro, these findings suggest a potential role in DNA recombination and repair (Kang et al., 2012). In addition, three recent independent studies identified Arabidopsis MORC1 and its homolog MORC6 (also named Defective in Meristem Silencing11) as novel factors involved in gene silencing and/or chromatin superstructure remodeling in response to epigenetic signals (Lorković et al., 2012; Moissiard et al., 2012; Brabbs et al., 2013).

Given that the CRT1 subfamily of MORC ATPases is involved in multiple layers of disease resistance against various pathogens, these genes may have relevance for agronomic applications. To assess whether MORCs are involved in crop plant resistance and thus could be exploited in breeding strategies, *MORC1* homologous genes were identified in the model cereal crop barley (*Hordeum vulgare*). We show here that all five barley MORCs, discovered in the not yet fully annotated barley genome, are involved in resistance to agronomically important diseases. Unexpectedly, however, and in clear contrast to Arabidopsis, barley plants silenced for *MORC* genes were more resistant, while overexpression compromised resistance to infections by both biotrophic and necrotrophic fungal pathogens. Moreover, reciprocal overexpression in Arabidopsis and barley showed that AtMORC1 and HvMORC1 homologs are not functionally interchangeable.

RESULTS

Isolation of *MORC1* Homologous Genes from Barley

To identify *MORC1* homologs in the barley genome, a genome-wide analysis based on fragmentary barley DNA sequence data from National Center for Biotechnology Information, Leibniz-Institut für Pflanzengenetik und Kulturpflanzenforschung Gatersleben, and The Institute of Genome Research Plant Transcript Assemblies databases was conducted (Childs et al., 2007; Matsumoto et al., 2011; Mayer et al., 2012). Five genes have been identified that are 47% (HvMORC1, HG316119), 48% (HvMORC2, HG316120), 38% (HvMORC6a, HG316122), 37% (HvMORC6b, AK372785), and 35% (HvMORC7, HG316121) identical on the aa level to Arabidopsis AtMORC1 (At4g36290; Fig. 1; Supplemental Fig. S1). HvMORC1 and HvMORC2 are closely related to each other (90.2% aa similarity). The open reading frame of HvMORC1 encodes 605 aa, and the deduced protein of 67.7 kD is very similar to MORC1 of rice (*Oryza sativa*; AAK70637; 77.3% identity, 86.1% similarity). HvMORC6a and HvMORC6b show highest similarity to AtMORC6 (At1g19100; 44% and 42% aa identity, respectively), while they exhibit less similarity to HvMORC1 (35% and 36% aa identity, respectively). HvMORC7 shows highest similarity to AtMORC7 (At4g24970; 49% identity, 60% aa similarity), while it shows lower similarity to HvMORC1 (34% identity, 46% similarity).

Barley MORC proteins, like Arabidopsis MORC1 (Kang et al., 2008), are related to mouse MORC proteins, which contain a GHKL ATPase domain and a S5 domain, the hallmarks of this superfamily (Watson et al., 1998; Iyer et al., 2008). The CRT1 subfamily members in addition contain a coiled-coil (CC) domain at their C termini (Fig. 1B; Supplemental Fig. S1). The CC region, which forms a putative basic leucine zipper (bZIP) domain, is predicted to be involved in protein-protein interactions. Sequence comparison reveals that the N-terminal region and the C-terminal part of barley MORCs are highly variable in their aa composition (Supplemental Fig. S1). The evolutionary history of MORCs from several monocot and dicot plant species was inferred and a phylogenetic tree was constructed, in which the MORCs divide into three clades (Fig. 1C).

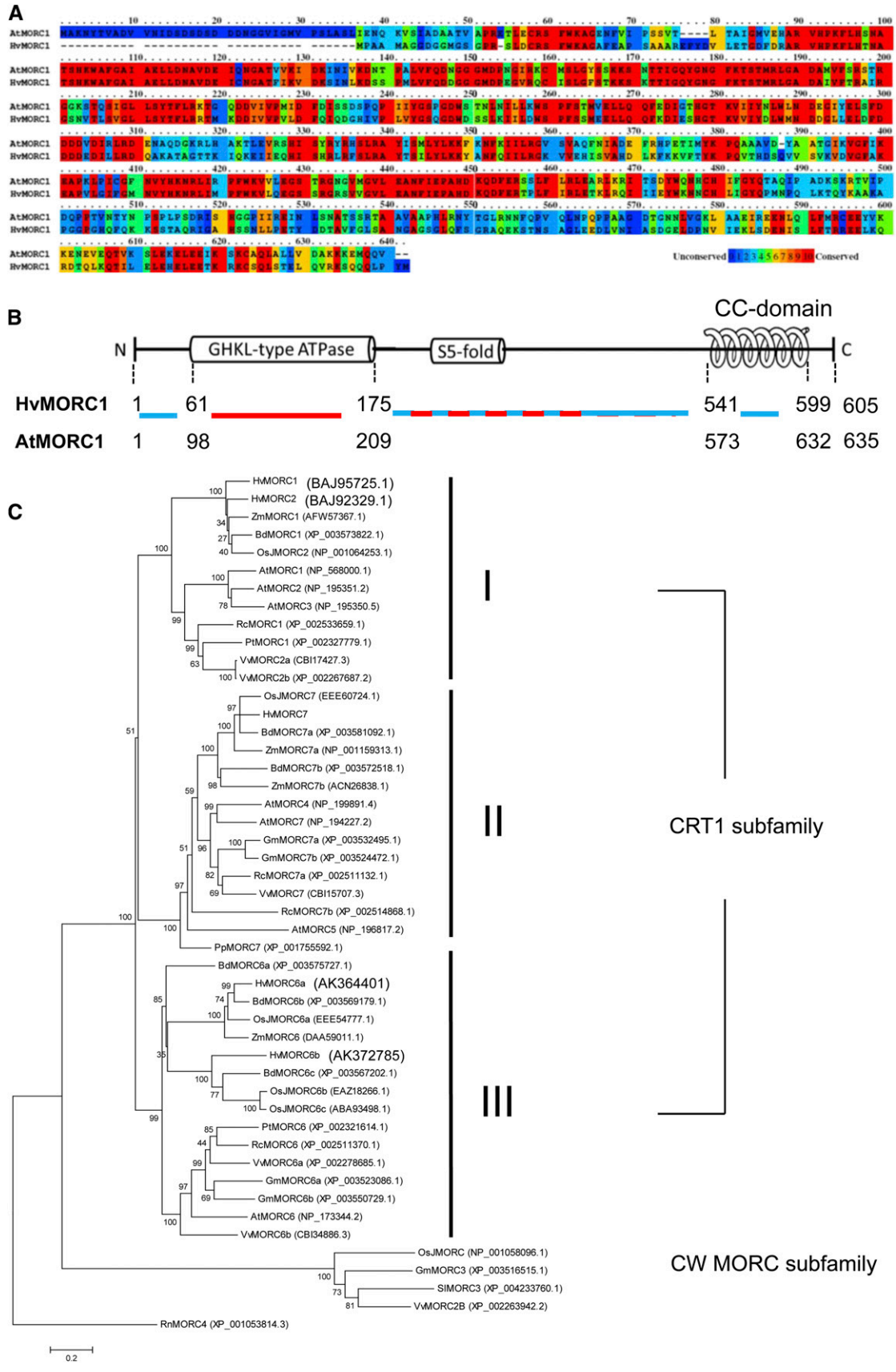

Figure 1. Sequence, domain structure, and phylogenetic relationship of the CRT1 subfamily of MORC ATPases in plants. A, Sequence alignment and aa conservation profile for barley HvMORC1 versus Arabidopsis AtMORC1 generated by PRALINE (Simossis et al., 2005). B, Domain structure of HvMORC1 versus AtMORC1. Numbers denote aa positions. Conserved regions

Altered Expression of Barley MORCs Affects Basal Resistance to Powdery Mildew Fungus

Arabidopsis MORC1 and its closest homolog AtMORC2 were previously shown to be required for various types of disease resistance including basal resistance and ETI against various pathogens (Kang et al., 2008, 2010, 2012). To assess whether barley homologs also are required for resistance, we generated transgenic barley 'Golden Promise' with altered expression of MORCs. Quantitative reverse transcription (RT)-PCR confirmed RNA interference (RNAi)-mediated knockdown in representative, independent *HvMORC2*-silenced lines (KD-*hvmorc2*) L11, L40, and L55 in which the *MORC2* transcription levels were reduced approximately 50% compared with control plants transformed with an empty vector (Fig. 2A). KD-*hvmorc2* plants infected with powdery mildew fungus (*Blumeria graminis* f. sp. *hordei*) race A6 (*Bgh*A6) displayed reduced numbers of fungal colonies compared with transgenic control plants (L11: 42%; L40: 53%; L55: 30%; Fig. 2B). Moreover, plants constitutively overexpressing *HvMORC1* under control of the *Cauliflower Mosaic Virus* 35S promoter were compromised for resistance. Compared with the transgenic empty vector control, the number of powdery mildew fungus colonies increased in the overexpressor lines by 145% in L5, 63% in L8, and 45% in L13 (Supplemental Fig. S2A). Quantitative RT-PCR showed that all three lines overexpressed *HvMORC1* approximately 10-fold (Supplemental Fig. S2B). Because barley 'Golden Promise' lacks an *R* gene matching the corresponding *Avr* gene(s) of *Bgh*A6, we concluded that in barley, like in Arabidopsis, MORCs are involved in basal resistance. However, very unexpectedly, altering expression of barley and Arabidopsis MORC homologs resulted in opposite effects on plant immunity.

Silencing of Barley *MORC* Genes Increases Resistance to Necrotrophic *Fusarium graminearum*

To address the question whether barley MORCs act in multiple plant organs and in resistance to pathogenic microbes with different life styles, we assessed the involvement of HvMORC2 in resistance to the root rot-causing necrotrophic fungus *F. graminearum* (*Fg*) in barley roots. To this end, roots of 3-d-old transgenic barley seedlings were inoculated with *Fg* macroconidia and later evaluated for infections (Fig. 3). Control plants, like *MORC1* overexpressors, showed symptoms of heavy root rot infections, while KD-*hvmorc2* plants retained a healthy appearance. Shoot and root lengths were significantly greater in KD-*hvmorc2* lines L11 and L40 compared with either control or *HvMORC1* overexpressing lines L27 and L30 (Fig. 3A). Consistent with this, quantification of fungal DNA in KD-*hvmorc2* roots by quantitative RT-PCR analysis revealed up to 60% reduced fungal colonization compared with control plants and *HvMORC1* overexpressors (Fig. 3B).

Altered Expression of MORCs Affects ETI in Barley

We assessed whether barley MORCs are involved in ETI. To address the contrasting effect of barley and Arabidopsis MORCs, we also included AtMORC1 in the analysis. To this end, we assessed whether MORCs alter the resistance of barley 'Sultan5,' which contains the *MLA12* gene, against *Bgh*A6 (containing *AvrMLA12*). To circumvent the problem of long generation times for stably transformed barley plants, experiments were conducted in which barley leaf epidermal cells were transiently transformed using a biolistic method (Schweizer et al., 1999). Interactions of *Bgh*A6 with transformed epidermal cells, assessed by GUS staining, were counted under the microscope and classified for the parameters (1) nonpenetrated papillae (PAP), (2) epidermal HR, (3) mesophyll cell clusters with HR (HRcc), (4) haustoria (HAU), and (5) elongated secondary hyphae (ESH). Note that MLA12, in contrast to other R proteins, such as Powdery Mildew Resistance Locus G or the recessive resistance allele *powdery mildew resistance locus O*, allows relatively frequent penetration and formation of initial primary HAU in epidermal cells upon attack by avirulent *Bgh*A6 (Hückelhoven et al., 1999). Formation of haustorial initials is then followed by either an epidermis HR or mesophyll HR (HRcc) beneath living epidermal cells that have been attacked. This eventually leads to MLA12-mediated arrest of fungal growth, as evidenced by a lack of ESH formation. By contrast, basal resistance is characterized by a high percentage of effective PAP. Based on these earlier parameters, we were able to assess whether MORCs modify ETI, basal, or both types of resistance. cv Sultan5 cells transiently overexpressing *HvMORC1* were strongly compromised for MLA12-mediated resistance, as revealed by a reduced number of infection sites with HR (9% versus 26% empty vector control) and HRcc (15% versus 47% empty vector control), while HAU and mycelium formation (ESH) increased compared with the empty vector control (Fig. 4A; Supplemental Fig. S3). Moreover, overexpression of *HvMORC1* also reduced incidences of PAP. As formation of effective papillae is known to be unaffected by MLA12 (Hückelhoven

Figure 1. (*Continued.*)
are marked in red, and nonconserved regions are marked in blue. C, Molecular phylogenetic analysis identified three clades for the plant CRT1 subfamily. The analysis involved 49 protein sequences. Following taxa were included in the analysis: barley (Hv), *Brachypodium distachyon* (Bd), rice 'Japonica' (OsJ), Arabidopsis (At), *Glycine max* (Gm), *Populus trichocarpa* (Pt), *Ricinus communis* (Rc), *Vitis vinifera* (Vv), maize (Zm), and *Physcomitrella patens* (Pp). The closest nonviridiplantae homolog (RnMORC4-1 from *Rattus norvegicus*) was used as outgroup. Molecular phylogenetic analysis was inferred by using the neighbor-joining method using MEGA5. CW, Cysteine Tryptophan domain.

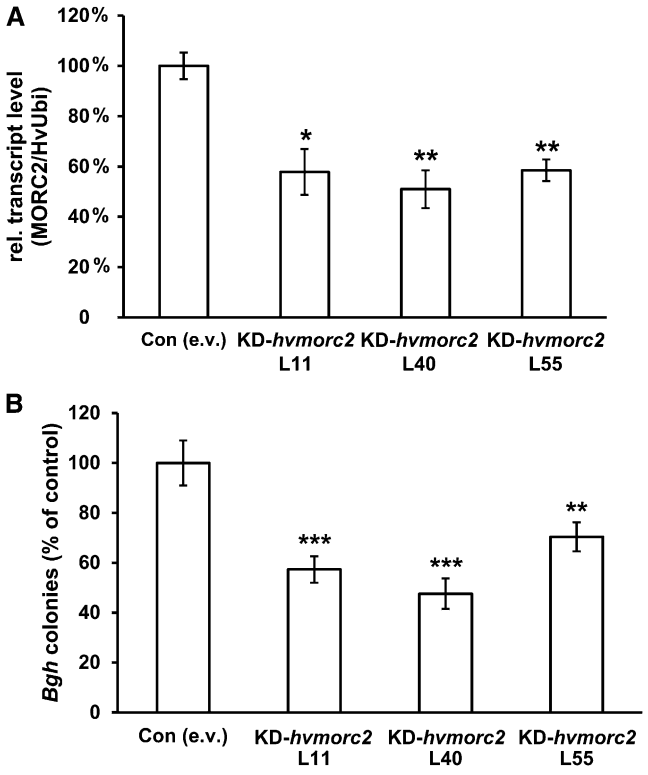

Figure 2. Altered expression of barley *MORC* genes affects basal resistance against powdery mildew fungus. Silencing of a barley MORC results in enhanced resistance. A, Average transcript levels of *HvMORC2* in lines L11, L40, and L55 (cv Golden Promise silenced for *HvMORC2*) as quantified by quantitative RT-PCR with normalization to barley *ubiquitin* and comparison to the empty vector control (Con [e.v.]). Values are calculated from 10 T1 plants per line. B, Detached second leaves of 12-d-old L11, L40, and L55 seedlings or Con (e.v.) were inoculated with three to five conidia per mm^{-2} of *Bgh*A6. Powdery mildew colonies were counted 5 d post inoculation (dpi). The number of powdery mildew colonies on Con (e.v.) is displayed as 100%. Presented are the means ± SE of 60 plants from three independent biological repetitions. Significant differences are marked: $*P < 0.05$, $**P < 0.01$, $***P < 0.001$ (Student's *t* test).

et al., 1999), the result confirms that basal resistance also is affected by HvMORC1. Occasionally, epidermal cells transformed with 35S::HvMORC1 had small *Bgh*A6 colonies with conidiophores, which suggests that the MORC1-mediated break of resistance in cv Sultan5 is very efficient (Fig. 4, B and C). By contrast, overexpression of Arabidopsis MORC1 in cv Sultan5 had no significant effect on *Bgh*A6 development.

These analyses were extended to additional barley MORCs in MLA12-mediated resistance using the transient transformation technique. Overexpression of *MORC* genes in cv Sultan5, including those from clade III (*HvMORC6a*) and clade II (*HvMORC7*), rendered plants more susceptible to *Bgh*A6 (Fig. 5), while RNAi-mediated knockdown led to enhanced resistance (Supplemental Table S1). While the effects of clade I member (*HvMORC1* and *HvMORC2*) overexpression compromised resistance equally strong and statistically indistinguishable from each other, altering expression of clade II (HvMORC7) and clade III (HvMORC6a) members had less, but still statistically significant, effect on resistance. These data argue that barley MORCs are involved in plant immunity.

Expression of Barley *MORC1* Does Not Restore ETI to *P. syringae* and Basal Resistance to *Botrytis cinerea* in the Arabidopsis *morc1-2 morc2-1* Mutant

To begin to assess whether the contrasting function of barley versus Arabidopsis homologs is due to the proteins themselves versus the cellular environments in which they function, we tested whether barley MORCs could restore resistance in the Arabidopsis *morc1-2 morc2-1* mutant. To this end, the *morc1-2 morc2-1* mutant was transformed with *HvMORC1* or *AtMORC1*, which were under control of the estradiol-inducible promoter (Zuo et al., 2000), sprayed with 50 μM β-estradiol, and then

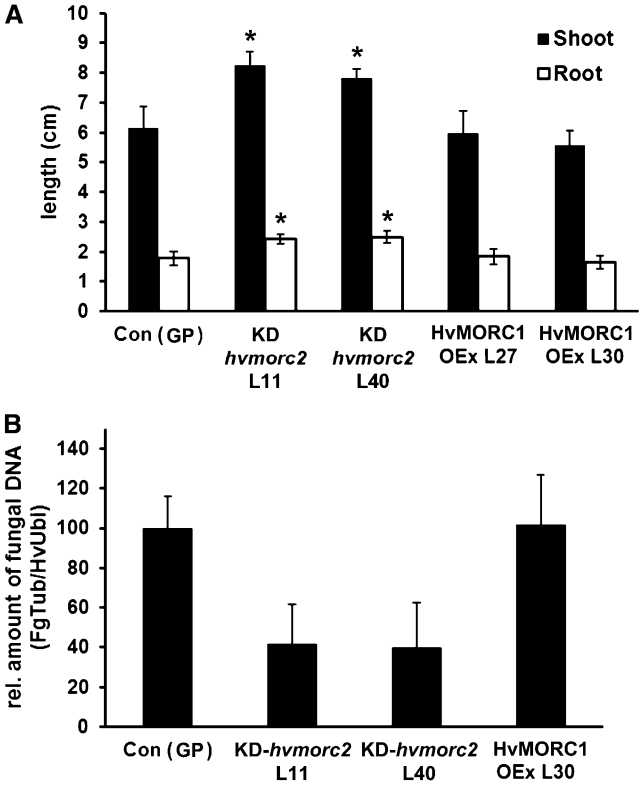

Figure 3. Altered expression of *HvMORC1* and *HvMORC2* affects basal resistance against root rot disease caused by *Fg*. A, Shoot and root lengths of infected seedlings. B, Quantification of *Fg* in the roots by quantitative RT-PCR based on the ratio of fungal tubulin (*Fg*Tub) to plant ubiquitin (*Hv*Ubi). Shown are phenotypic effects of fungal infections of 10-d-old cv Golden Promise (GP) seedlings (empty vector control), the knockdown lines L11 and L40 (silenced for *HvMORC2*), and *HvMORC1* overexpressor (OEx) lines L27 and L30. For inoculation, roots of 2-d-old seedlings were dipped into a solution of 50,000 *Fg* macrospores. Presented are mean ± SE of 30 seedlings from three biological repetitions. Significant changes are marked: $*P < 0.05$ (Student's *t* test).

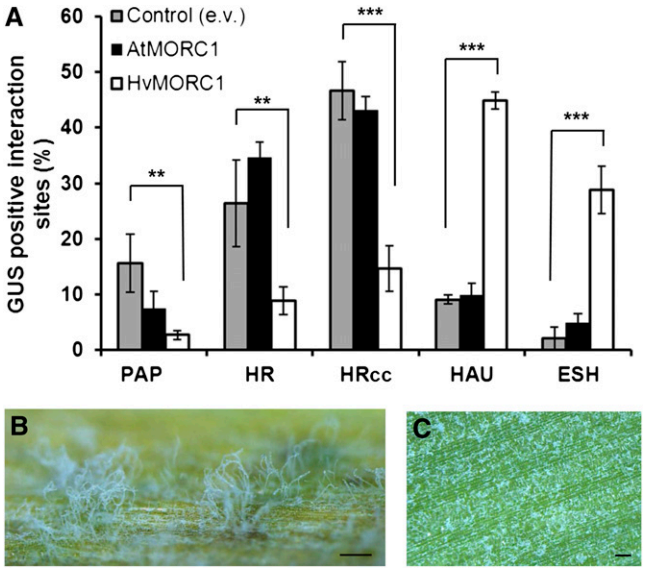

Figure 4. Overexpression of *HvMORC1* in barley 'Sultan5' breaks MLA12-mediated ETI against *Bgh*A6, while *AtMORC1* slightly but insignificantly enhances ETI. Detached first leaves of 7-d-old plants were shot with gold particles carrying either p35S::*HvMORC1*, p35S::*AtMORC1*, or p35S-BM (empty vector control [e.v.]) together with a GUS reporter construct (p35S::*GUS*) and, 24 h later, inoculated with avirulent *Bgh*A6. A, At 72 h post inoculation, leaves were costained with 5-bromo-4-chloro-3-indolyl-β-glucuronic acid to identify transformed cells and 3,3-diaminobenzidine to visualize H_2O_2 production indicative of the HR. Interaction of the fungus with GUS-stained epidermal cells was counted and classified for parameters PAP, HR, HRcc, HAU, and ESH. B and C, cv Sultan5 leaves that were transiently transformed with *HvMORC1* (B) or the empty vector control (C) were harvested at 6 d post inoculation (dpi) and microscopically analyzed for small powdery mildew fungus colonies normalized to GUS-stained epidermal cells. Bars represent mean values ± SDs of three independent experiments. Significant changes are marked (**$P < 0.001$, ***$P < 0.0001$; Student's *t* test). Bars = 1 mm.

inoculated either with the hemibiotrophic bacterium *P. syringae* pv *tomato* (*Pst*) bearing the avirulence gene *Avirulence against Pst2* (*AvrRpt2*; *Pst AvrRpt2*) or the necrotrophic fungus *B. cinerea*. Consistent with previous reports (Kang et al., 2010), overexpression of *AtMORC1* restored resistance to *Pst AvrRpt2* in the resistance-compromised *morc1-2 morc2-1* mutant (Fig. 6A). In clear contrast, *HvMORC1*-overexpressing plants remained as susceptible to *Pst AvrRpt2* as *morc1-2 morc2-1*. Similarly, while *AtMORC1* overexpression enhanced the resistance against *B. cinerea*, HvMORC1 rendered *morc1-2 morc2-1* plants even more susceptible to the fungus (Fig. 6B; Supplemental Fig. S4). Western analysis showed that *AtMORC1* and *HvMORC1* were expressed at similar levels, thereby excluding the possibility that the failure of HvMORC1 to restore resistance was due to its reduced expression and/or altered stability in Arabidopsis (Supplemental Fig. S5).

HvMORC1 Possesses ATPase and Endonuclease Activities

Arabidopsis MORC1 and MORC6 were shown to exhibit ATPase and endonuclease activity (Kang et al., 2008, 2010, 2012; Lorković et al., 2012). While the GHKL-type ATPase domain in MORCs is highly conserved and similar to the well-characterized prototypic GHKL family member MutL, their domain(s) associated with endonuclease activity is not equally conserved compared to that of MutL. We next addressed the question of whether the contrasting function of barley versus Arabidopsis MORC homologs in plant immunity could be explained by differences in either of these enzymatic activities. To assess this possibility, HvMORC1 was produced in *Escherichia coli* and purified to homogeneity for enzyme analyses. Consistent with our expectation, HvMORC1 exhibited ATPase activity comparable to AtMORC1 and AtMORC6 (Fig. 7A), which was approximately 10-fold lower than that obtained with a commercially available ATPase under similar assay conditions (data not shown). Recombinant HvMORC1 also exhibited endonuclease activity, converting supercoiled plasmid DNA into relaxed DNA (Fig. 7B). Like AtMORC1, endonuclease activity of HvMORC1 was dependent on bivalent metal ions with preference for Mn^{2+} and Co^{2+}, and to a lesser degree for Mg^{2+}. Taken together, these results suggest that the contrasting function of barley versus Arabidopsis MORC1 is not due to differences in enzyme activities, although subtle differences cannot be ruled out at this time.

Barley MORC1 Binds DNA and Accumulates in the Nucleus after Induction of PTI Triggered by the PAMP Flagellin

To assess if the species specificity was due to differences in DNA binding capacity, HvMORC1's ability

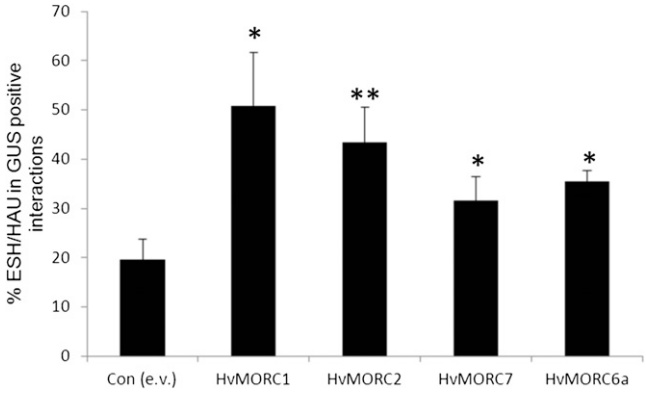

Figure 5. Overexpression of barley MORCs from phylogenetic clade I (*HvMORC1* and *HvMORC2*), clade II (*HvMORC7*), and clade III (*HvMORC6a*) in barley 'Sultan5' breaks MLA12-mediated ETI against *Bgh*A6. Detached first leaves of 7-d-old plants were transformed with the p35S::*HvMORC* construct or p35S-BM (empty vector control [e.v.]) together with a GUS reporter construct (p35S::*GUS*) and, 24 h later, inoculated with avirulent *Bgh*A6. At 24 h post inoculation, leaves were stained with 5-bromo-4-chloro-3-indolyl-β-glucuronic acid to identify transformed cells. Interactions of the fungus with GUS-stained epidermal cells were counted and classified for the parameter ESH. The mean ± SD values are presented ($n = 4$). Statistical difference from control is indicated: *$P < 0.05$, **$P < 0.01$ (Student's *t* test).

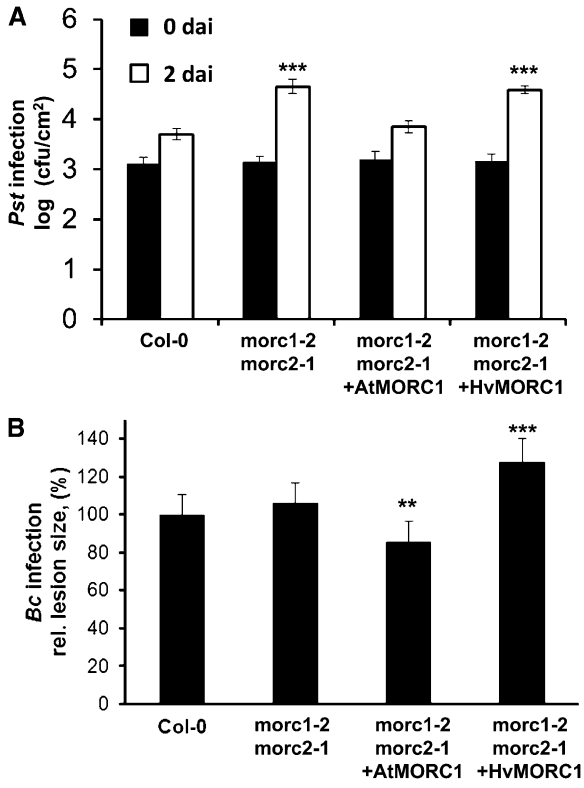

Figure 6. Barley MORC1 does not restore resistance in the Arabidopsis *morc1-2 morc2-1* mutant. A, *R* gene-mediated resistance against *Pst* AvrRpt2 in *morc1-2 morc2-1* is restored by ectopic expression of *AtMORC1* but not by *HvMORC1*. Four-week-old leaves were infiltrated with *Pst* AvrRpt2 at 10^5 colony-forming units ml^{-1}, and their growth was determined at 0 and 2 dpi. B, Basal resistance in the *morc1-2 morc2-1* mutant against the necrotrophic fungus *B. cinerea* is enhanced by *AtMORC1* and reduced by *HvMORC1* overexpression. Ten microliters spore suspension (50,000 conidia mL^{-1}) were placed in the leaf center. Disease symptoms were evaluated 3 dpi. Data are mean ± SD ($n = 4$). Significant differences from the wild type (ecotype Columbia) are indicated: $^{**}P < 0.01$, $^{***}P < 0.001$ (Student's *t* test).

to bind DNA was assessed by measuring the intrinsic fluorescence of the protein in the presence and absence of DNA. As previously demonstrated for AtMORC1 (Kang et al., 2012), the fluorescence intensity of HvMORC1 was quenched in the presence of DNA (Fig. 8), suggesting that barley MORC1 interacts with DNA and that DNA binding of HvMORC1, like AtMORC1, involves surface-exposed aromatic aas. To exclude the possibility that the contrasting function of HvMORC1 versus AtMORC1 is due to variations in the subcellular localization, the presence of HvMORC1 in the nucleus was examined. Transmission electron microscopy via indirect immunogold labeling of Arabidopsis *morc1-2 morc2-1* leaves, which were overexpressing *Myc-HvMORC1* under control of estradiol-inducible promoter, revealed immune reactivity with αMyc in cytosolic microbodies/endomembrane-like vesicles 24 h after estradiol spraying (Supplemental Fig. S6, A–C). This is consistent with earlier confocal microscopy analyses using GFP-tagged AtMORC1 (Kang et al., 2010) and indirect immunogold labeling of Myc-tagged AtMORC1 (Kang et al., 2012). Induction of PTI by infiltration of flg22, a peptide corresponding to the elicitor-active epitope of the bacterial PAMP flagellin, increased the amount of gold labeling in the nucleus within 10 min (Fig. 9; Supplemental Fig. S6, D–H), just as previously observed with AtMORC1 (Kang et al., 2012). Together with the endonuclease and DNA binding activities shown above, this result suggests

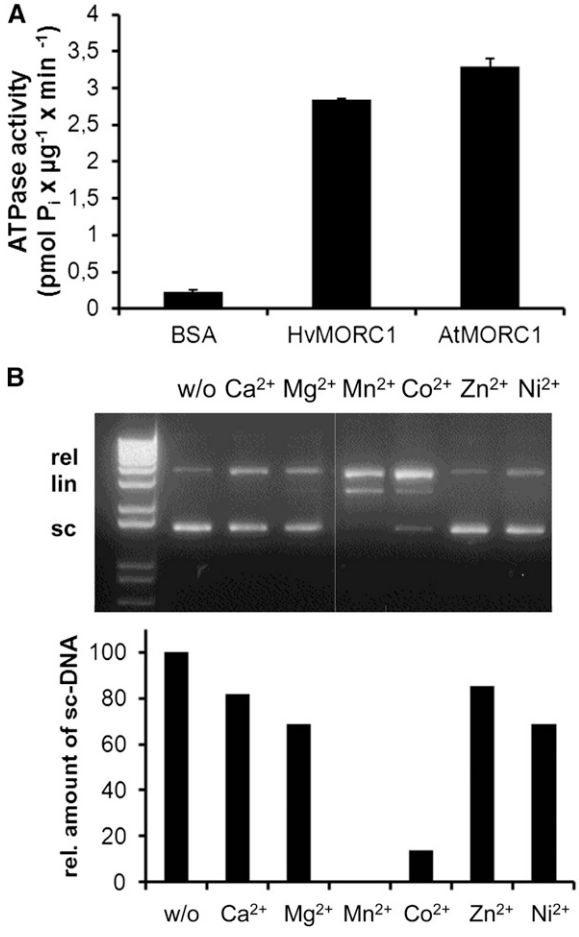

Figure 7. Barley MORC1 displays ATPase and endonuclease activity and binds to DNA. A, ATPase activity of purified His-HvMORC1 and His-AtMORC1. The data present the mean of two independent measurements, with the error bars representing SD. One additional set of experiment was performed with similar results. Bovine serum albumin was used as a negative control. B, Agarose gel electrophoresis showing endonuclease activity of HvMORC1. Recombinant HvMORC1 (400 nM) was incubated with 100 ng of pUC19 supercoiled plasmid DNA for 1 h at 37°C in presence of 2 mM of the indicated bivalent cations. Reaction mixtures were electrophoretically separated. Endonucleolytic cleavage results in accumulation of relaxed and linearized DNA. DNA was assessed using ImageJ software. The experiment was repeated two times using different protein preparations with similar results. sc, Supercoiled; rel, relaxed; lin, linearized DNA.

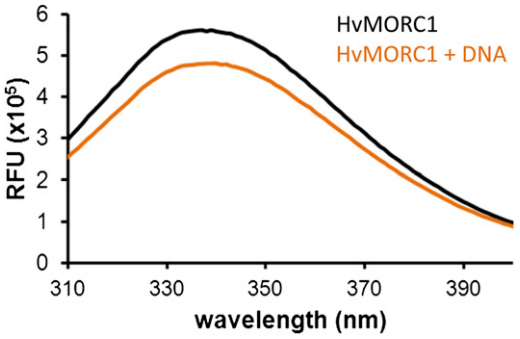

Figure 8. Barley MORC1 displays DNA-binding activity. Intrinsic fluorescence (Trp fluorescence) in relative fluorescence unit (RFU) of recombinant HvMORC1 (10 μg mL^{-1}) after excitation at 280 nm was monitored in presence or absence of 100 ng of λ-DNA. [See online article for color version of this figure.]

that HvMORC1 likely functions in the nucleus just as AtMORC1.

Barley Plants Silenced for MORC Expression Show Little or No Derepression of Transposons

Moissiard et al. (2012) reported that the Arabidopsis mutants *atmorc1* and *atmorc6* show decondensation of pericentromeric heterochromatin and transcriptional derepression of transposons that are largely residing in pericentromeric regions. To assess whether barley MORC also is involved in transposon silencing, we selected barley transposons for comparative expression analysis in wild-type barley 'Golden Promise' and the mutant *KD-hvmorc1*. Three categories of transposons were tested (Supplemental Table S2): barley transposons selected by sequence similarity to the Arabidopsis transposons that were shown to be derepressed in *atmorc1* and *atmorc6* (e.g. ROMANIAT5), barley transposons that are known to be located at loci residing in the pericentromeric region (e.g. Cereba), and other barley transposons. In clear contrast to Arabidopsis, the barley *KD-hvmorc1* mutant did not show increased transposon expression but rather exhibited slightly reduced transcription (Fig. 10). This suggests that HvMORC1, in contrast to AtMORC1 and ATMORC6, is not involved in transposon silencing.

Barley MORC1 Poorly Interacts with MLA12

Previous studies showed that AtMORC1 physically interacts with a wide variety of R proteins from Arabidopsis and other plant species in a transient coexpression assay in *Nicotiana benthamiana* (Kang et al., 2008, 2010). Using the same assay, the interaction of HvMORC1 with two R proteins, barley MLA12 and Arabidopsis HRT, was assessed. To perform coimmunoprecipitation (co-IP), HvMORC1 and either R protein were tagged with Myc and Human Influenza Hemagglutinin (HA), respectively. The Leucine-Rich Repeat (LRR) domain in both HRT and MLA12 proteins were removed for these co-IP experiments because of frequent involvement of LRR domains in nonspecific protein-protein interaction (Kang et al., 2008; Bai et al., 2012). As anticipated, AtMORC1 interacted with MLA12 as well as with HRT. By contrast, HvMORC1 showed little or no interaction with either R protein (Fig. 11). Although a small amount of HRT-HvMORC1 complex was visible, this was very low compared with the HRT-AtMORC1. This result suggests that HvMORC1 likely has very different protein interaction properties from those of AtMORC1, which provides a good explanation for the contrasting function of barley and Arabidopsis MORC1 homologs in plant immunity.

DISCUSSION

The family of MORC proteins represents a subset of the ubiquitous GHKL superfamily of ATPases. The GHKL superfamily is named for the prototypical members DNA Gyrase B, Heat Shock Protein90, His Kinase, and MutL. The common motif is a highly conserved and well-characterized ATP-binding domain that has a so-called Bergerat fold. Sequence alignment and comparison suggested that the MORC proteins in plants subdivide further into two groups: the zing finger (ZF)-MORCs and the CC-MORCs (CRT1 subfamily), both subfamilies being distantly related to mammalian MORCs (Fig. 1C; Supplemental Fig. S7). ZF-MORCs possess a CW-type ZF motif (Pfam accession number PF07496) in the C-terminal part of the proteins. Exclusively found in vertebrates, vertebrate-infecting parasites, and higher plants, the CW-ZF folding motif is predicted to promote protein-protein and/or protein-DNA interactions in eukaryotic processes.

MORCs of the CRT1 subfamily are predicted to have a CC domain in the C-terminal part of the proteins that putatively forms a bZIP folding motif (Pfam accession number PF00170). This bipartite domain confers, on the one hand, sequence-specific DNA binding and possesses, on the other hand, a Leu zipper required for protein dimerization (Ellenberger, 1994).

All plant species for which genome data are available to date contain CRT1 subfamily members, suggesting that they have an evolutionary conserved function. However, in contrast to recent reports (Kang et al., 2008, 2010, 2012), which demonstrate that AtMORC1 and AtMORC2 are required for full resistance in Arabidopsis, we discovered that barley MORC members appear to negatively affect basal resistance and ETI to various microbial pathogens in barley.

Five MORCs, named HvMORC1, HvMORC2, HvMORC6a, HvMORC6b, and HvMORC7, were identified in this study. Altering expression of these members, either by RNAi-mediated gene silencing or overexpression, changed the resistance of barley to powdery mildew caused by biotrophic powdery mildew fungus or root rot caused by necrotrophic *Fg* (note that

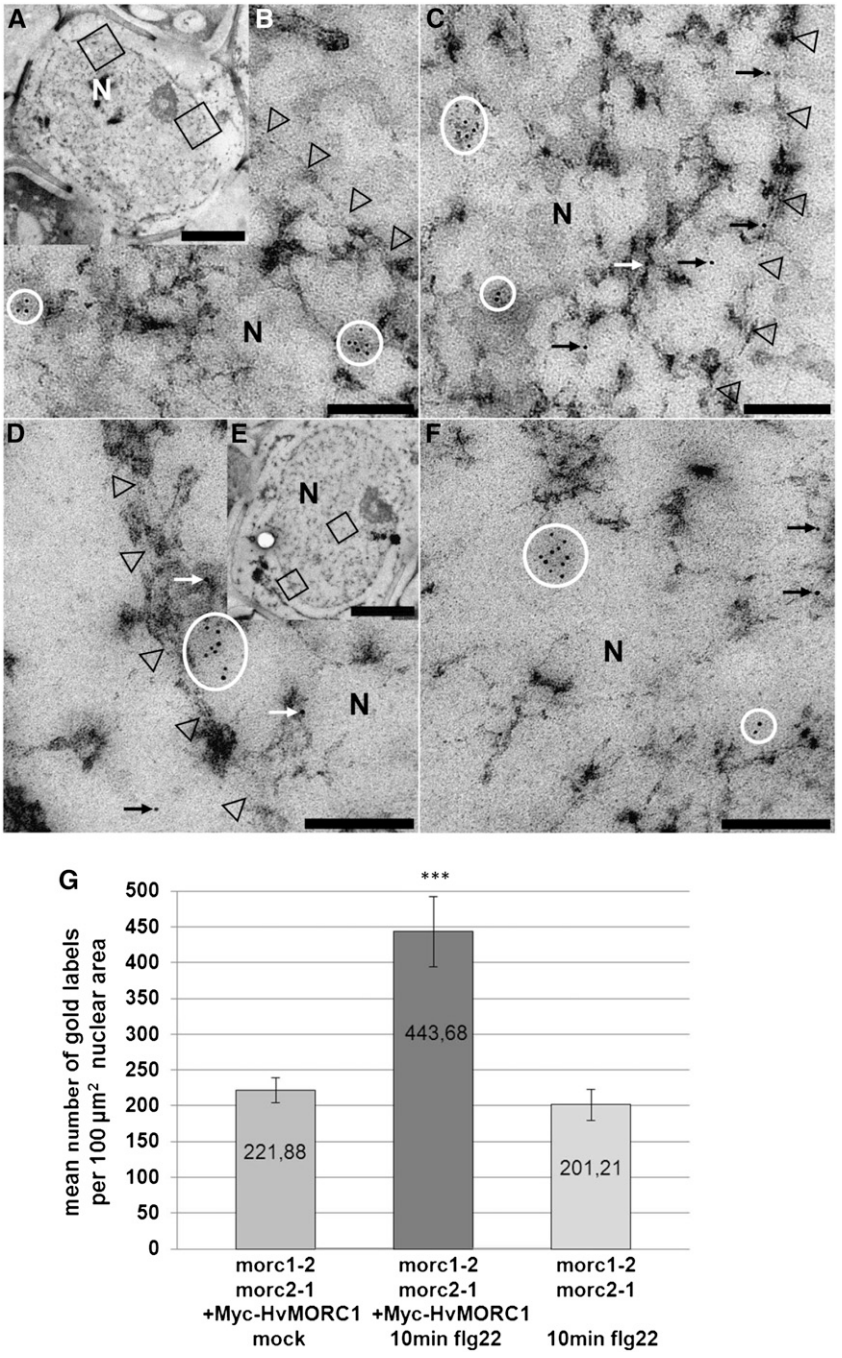

Figure 9. Transmission electron microscopy detects HvMORC1 in the nucleus of transgenic Arabidopsis. Indirect immunogold labeling was performed on leaf tissue of 6-week-old Arabidopsis mutant *morc1-2 morc2-1* overexpressing Myc-HvMORC1 under control of the estradiol-inducible promoter 24 h after spraying the plants with estradiol. αMyc mouse antibodies were used to detect Myc-tagged HvMORC1, followed by αmouse antibodies conjugated to 5-nm gold particles. Single gold labels are denoted with arrows, and circles mark clusters of more than one gold label. A and E, Nuclei (N) in phloem parenchyma cells 10 min after PTI induction by leaf infiltration of 5 μM flg22 in 10 mM $MgCl_2$. Details of the nuclear regions contained in the boxed areas are shown at a higher magnification in B and C for A and in D and F for E. Arrowheads mark the nuclear membranes. Gold labels were often observed on the gray filamentous structures in the nucleus, which most likely represent euchromatin fibers, and they often occurred in large clusters (see also Supplemental Fig. S6H). Bars = 2 μm (A and E) and 200 nm (B, C, D, and F). G, Mean number of gold labels per 100 μm^2 nuclear area in leaf cells of mutant *morc1-2 morc2-1* overexpressing Myc-HvMORC1 after estradiol spraying and infiltration with 10 mM MgCl2 or 5 mM flg22. *morc1-2 morc2-1*, which lacks the Myc-HvMORC1 transgene, was treated with estradiol and 5 μM flg22 in 10 mM $MgCl_2$ and served as a negative control. The mean ± SE values are presented ($n \geq 19$). Asterisks indicate statistically significant difference (***$P < 0.0005$, Student's *t* test) to the Myc-HvMORC mock control.

HvMORC6b was not analyzed in the biological assays due to its very high similarity to HvMORC6a). Given these results, we strongly suspect that most or all CRT1 subfamily members participate in plant immunity to microbial pathogens with different life styles in Arabidopsis, barley, and other plant species.

Barley 'Golden Promise' does not contain a known *R* gene that interacts with a corresponding *Avr* gene in *Bgh*A6 used throughout this study. Thus, altered growth and development of *Bgh*A6 on MORC-suppressed barley plants can be attributed to altered basal resistance. By contrast, barley 'Sultan5' contains a functional *MLA12* gene that matches the *AvrMLA12* gene of *Bgh*A6. Thus, using these interacting partners, we were able to provide clear evidence that barley MORCs modulate both basal resistance and ETI.

This study takes advantage of rapidly increasing understanding of MORCs' role(s) in plant immunity, which initially was discovered in Arabidopsis. Transforming a resistance-compromised Arabidopsis *morc1-2 morc2-1* mutant with *AtMORC1* restored ETI to avirulent *P. syringae*, corroborating earlier finding that

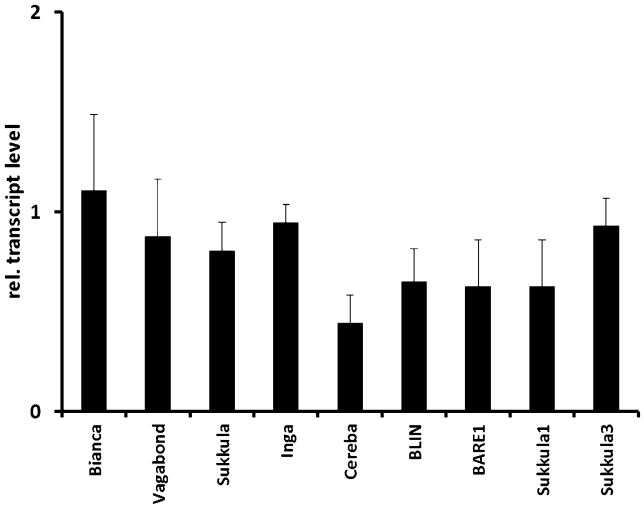

Figure 10. Knockdown of HvMORC1 does not derepress expression of transposons in barley. Relative fold increase of transcripts for 10 transposons in 11-d-old leaves of KD-*hvmorc1* over the wild type (cv Golden Promise) assayed by real-time quantitative PCR and normalized to ubiquitin (see Supplemental Table S2 for more information on transposon sequences and genomic location). The mean ± SE values are based on three biological experiments ($n = 5$).

AtMORC1 is required for full resistance to *Pst* carrying AvrRpt2 (Kang et al., 2010). Unexpected from our previous work, but consistent with the above finding in barley, overexpression of *HvMORC1* did not restore ETI to avirulent *Pst* AvrRpt2 in Arabidopsis (Fig. 6A). These data together demonstrate that MORC proteins from barley and Arabidopsis are not functionally interchangeable.

Like AtMORC1, barley MORC1 contains a functional and highly conserved GHKL ATPase domain and a S5 domain (Fig. 1), characterizing it as a member of the MORC superfamily, many of whom are involved in DNA metabolism, chromatin superstructure remodeling, and signal transduction (Iyer et al., 2008). Consistent with this, recombinant HvMORC1, like Arabidopsis MORC1, exhibited ATPase, DNA binding, and endonuclease activities in vitro (Figs. 7 and 8). Recent work (Kang et al., 2012) and data presented here (Fig. 9) show that PAMP (flg22) treatment or infection with avirulent *Pst* increases levels of nuclear-localized Arabidopsis and barley MORC1, supporting a possible role in DNA binding/modification. Moreover, the Arabidopsis *morc1-2 morc2-1* mutant exhibited altered tolerance to DNA-damaging agents, suggesting that nuclear-localized MORC1 may be involved in DNA repair/recombination and possibly genome stability. In addition, recent independent studies published by the groups of Steve Jacobsen, Marjori Matzke, and more recently Louise Jones implicate Arabidopsis MORC1 and MORC6 in gene silencing and/or modification of heterochromatin, perhaps via RNA-directed DNA methylation (Lorković et al., 2012; Moissiard et al., 2012; Brabbs et al., 2013). Together, these and our results argue that MORCs have important nuclear functions during the immune response in plants, and perhaps also in animals. Interestingly, we found that all the tested barley MORCs are involved in plant immunity, whereas only MORC1 and MORC6 were found to be involved in transcriptional gene silencing in Arabidopsis (Brabbs et al., 2013). Although the immunity and gene silencing studies were done in different species, we suspect that most, if not all, family members within a species are involved in immunity, while only a subset may participate in gene silencing, because a knock-out mutation of *AtMORC6*, like silencing of *HvMORC6*, also compromises resistance (Y. Bordiya, H.W. Choi, D.F. Klessig, and H.-G. Kang, unpublished data). Furthermore, our analysis revealed that barley mutant *KD-hvmorc1* was not compromised in transposon silencing regardless of their chromosomal locations (Fig. 10; Supplemental

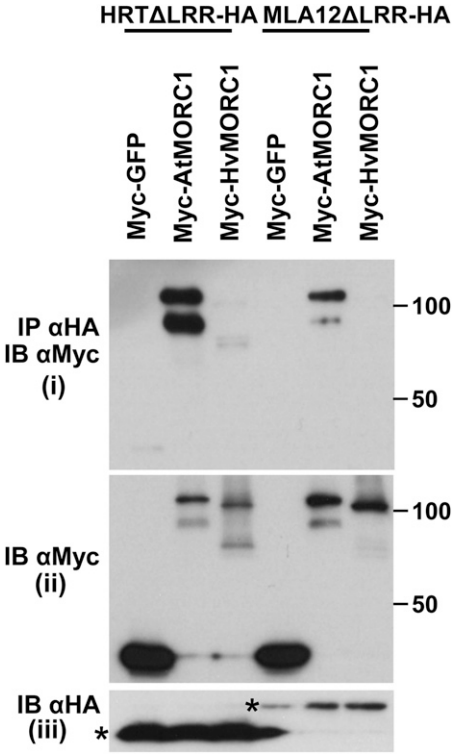

Figure 11. Barley MORC1 displays significantly weaker interaction with the two R proteins AtHRT and HvMLA12 than Arabidopsis MORC1. Myc-tagged HvMORC1, AtMORC1, or GFP were transiently overexpressed in *N. benthamiana* leaves together with HA-tagged AtHRT or HvMLA12. Asterisks indicate the expected sizes of HA-tagged proteins. Note that the LRR domain in these R proteins was removed to enhance the interaction specificity. Myc-GFP served as a negative control. Soluble leaf extracts were separated by 10% SDS-PAGE, followed by immuno-blot (IB) analysis with the indicated antibodies or co-IP with αHA antibody linked to agarose, followed by IB. Size markers are shown on the right in kilodaltons. For each, at least three independent experiments were performed with similar results. Input (ii and iii) and IP (i) proteins were analyzed using IB with αHA (i and iii) and αMyc (ii).

Table S2). Further analysis is required to elucidate if this difference can explain the contrasting immune phenotypes of barley and Arabidopsis. These results also raise the possibility that immunity is not tightly associated with gene silencing in the MORC family.

The finding that altering expression of MORCs in barley had just the opposite effects on resistance compared with altering their expression in Arabidopsis indicates MORCs can negatively (barley) or positively (Arabidopsis) modulate plant immunity. This species-specific effect could be due to the proteins themselves and/or the cellular environment in which they function. The inability of HvMORC1 to replace AtMORC1 in Arabidopsis and AtMORC1 to replace HvMORC1 in barley might suggest that the proteins themselves are responsible. However, an alternative explanation is that MORCs cannot function in the cellular environment of a distant plant species. This is particularly relevant because Arabidopsis is a dicot and barley is a monocot. The contrasting poor interaction of HvMORC1 with the R proteins MLA12 from barley and HRT from Arabidopsis compared with that of AtMORC1 (Fig. 11) again suggests that the proteins themselves are responsible for the species-specific effect. However, because transient coexpression and co-IP assays were done in *N. benthamiana*, a dicot, one cannot rigorously rule out the possibility that *N. benthamiana* contains one or more cellular factors that facilitated interactions of AtMORC1, but not HvMORC1, with the R proteins. In this regard, it should be noted that AtMORC1 appears to interact indirectly with R proteins because we have not been able to detect this interaction in a yeast (*Saccharomyces cerevisiae*) two-hybrid assay (data not shown). In summary, together the data presented here suggest that the proteins themselves are responsible for the species-specific effect, although this proposition still requires rigorous proof.

Based on the finding that silencing of MORCs enhances ETI and basal resistance in barley to a biotrophic (*Blumeria* spp.) and basal resistance to a necrotrophic pathogen (*Fusarium* spp.), it is anticipated that these genes may have high potential for agronomical applications. Phylogenetic analysis reveals that maize (*Zea mays*), rice, and wheat (*Triticum aestivum*) each contain single highly conserved orthologs of barley and Arabidopsis MORC1, suggesting that disease resistance in these crops could also be enhanced by altering these MORC1 homologs.

MATERIALS AND METHODS

Plant Material and Inoculation

Seeds of barley (*Hordeum vulgare*) 'Golden Promise' and 'Sultan5' (containing the *MLA12* gene for powdery mildew fungus [*Blumeria graminis* f. sp. *hordei*] resistance) were germinated for 2 d on filter paper. Seedlings were transferred to soil (Frühstorfer Erde Typ T) and maintained under 16-h/8-h photoperiod with 60% relative humidity and 22°C light (200 μmol photons m^{-2} s^{-1}) 18°C dark cycles. After complete emergence (10–12 d), the second leaves were detached, laid on 0.8% (w/v) water agar, and inoculated with *Bgh*A6 (containing *AvrMLA12*) at a density of 2 to 5 conidia mm^{-2}.

For *Fusarium graminearum* inoculation, surface-sterilized 3-d-old barley seedlings were dip inoculated with *Fg* 1003 suspension (Jansen et al., 2005) at a density of 50,000 ml^{-1} macroconidia in 0.1% (v/v) Tween 20 for 2 h by gentle shaking at room temperature. Subsequently, seedlings were transferred to 6-cm-diameter pots filled with a substrate of sand and Oil-Dri (3:1; Damolin) and grown as described above but at 125 μmol photons m^{-2} s^{-1}. Plants were harvested at 10 d after inoculation (dai), root and shoot lengths were measured, and disease symptoms assessed using ImageJ (National Institutes of Health, http://imagej.nih.gov/ij/).

For *Pseudomonas syringae* pv *tomato* inoculation, leaves of 4-week-old Arabidopsis (*Arabidopsis thaliana*) plants grown under short-day conditions (8-h light) were infiltrated with 10^5 colony-forming units mL^{-1} of *Pst* AvrRpt2. Bacterial growth was determined 72 h post inoculation as described (Kang et al., 2008).

For *Botrytis cinerea* inoculation, leaves of 4-week-old Arabidopsis plants, grown under short-day conditions, were detached and placed on 0.5% (w/v) agar containing 50 μg mL^{-1} β-estradiol with the petiole embedded in the medium. Inoculation was performed by placing 10 μL spore suspension of *B. cinerea* strain B05.10 (gift of M. Hahn) adjusted to 50,000 mL^{-1} conidia in 12 g L^{-1} potato (*Solanum tuberosum*) dextrose broth in the leaf center. Disease symptoms were evaluated 3 dai by determining lesion size using ImageJ software.

Isolation of Barley MORC Genes

Full-length sequences of HvMORC1 (accession no. HG316119), HvMORC2 (HG316120), and HvMORC7 (HG316121) were obtained from complementary DNA of barley 'Golden Promise;' the HvMORC6a (HG316122) and HvMORC6b (AK372785) sequences were obtained from cv Ingrid using indicated oligonucleotides (Supplemental Table S3). PCR amplicons were ligated into pGEMt-easy (Promega) and verified by sequencing.

Plant Transformation

HvMORC1 was cut from pGEMt-easy and cloned into *Sma*I and *Hin*dIII sites of plasmid p35S-Nos (for nopaline synthase terminator; DNA Cloning Service) and *HvMORC2* and *HvMORC7* using *Eco*RI. *HvMORC6a* was cloned into *Spe*I *and Eco*RI sites of plasmid pUbi-Nos (DNA Cloning Service; Supplemental Fig. S8A). For transient knockdown experiments, plasmid pAB-35S-RNAiZeBaTA (Supplemental Fig. S8B) was produced by replacing the GUS fragment of intermediate plasmid pAB-35S-RNAi, which harbors two *Sfi*I-flanked opposing *Cauliflower mosaic virus* 35S promoters and terminators (derived from p7i-Ubi-RNAi, DNA Cloning Service), with *Xcm*I-flanked negative selection marker gene *control of cell death toxin* from ZeBaTA plasmids (Chen et al., 2009) to facilitate thymine/adenine cloning of PCR amplicons between the inverted promoters. PCR amplified fragments of *HvMORC2* (primers 996-*HvMORC2* and 997-*HvMORC2*; size, 384 bp), *HvMORC6a* (566-*HvMORC6a*/565-*HvMORC6a*, 266 bp), and *HvMORC6b* (561-*HvMORC6b*/562-*HvMORC6b*, 161 bp) were cloned into *Xcm*I sites of p35S-AB-RNAiZeBaTA. *AtMORC1* from pET28-*CRT1* (Kang et al., 2008) was cloned into *Bam*HI/*Hin*dIII sites of p35S-Nos-BM.

For stable barley transformation, the *HvMORC2* fragment in plasmid pAB-35S-RNAiZeBaTA was cloned together with flanking terminators into the *Sac*I/*Spe*I sites of p7i-Ubi-RNAi plasmid (DNA Cloning Service), replacing the GUS fragment. Expression cassettes from plasmids p35S::*HvMORC1*, p35S::*HvMORC2*, p35S::*AtMORC1*, and p7i-Ubi::*HvMORC2*-RNAi were cloned into *Sfi*I sites of binary plasmid pLH6000 (AY234328, DNA Cloning Service), which was also used to produce the transgenic control plants (Supplemental Fig. S8C). Plasmids were electroporated (Gene Pluser, Biometra) into *Agrobacterium tumefaciens* strain AGL1 (Lazo et al., 1991) and used to transform spring barley 'Golden Promise' as described (Schultheiss et al., 2005; Imani et al., 2011). Stable transformants of the *morc1-2 morc2-1* mutant (Kang et al., 2010) were produced using floral dip (Clough and Bent, 1998).

Transient barley transformation was performed as described (Schweizer et al., 1999, 2000). For each experiment, 24 detached 7-d-old first leaves of barley 'Sultan5' were shot using a particle inflow gun with DNA-coated, 1.1-μm tungsten particles. One microgram per shot of the following plasmid constructs was used for overexpression of p35S::*HvMORC1*, p35S::*HvMORC2*, p35S::*HvMORC6a*, and p35S::*HvMORC7*. To visualize successfully transformed epidermal cells, leaves were cotransformed with 1 μg pUbi::GUS. As control, the empty vector p35S-BM was used. Twenty-four hours after transformation, leaves were inoculated with *Bgh*A6 at a density of approximately 200 conidia mm^{-2}, and 72 h later, leaves were loaded with 3,3-diaminobenzidine (1 mg mL^{-1})

via the transpiration stream for 4 h and subsequently stained for GUS. Finally leaves were destained with 0.15% trichloroacetic acid in ethanol/chloroform (4:1) and evaluated by counting the parameters PAP, HR, HRcc, HAU, and ESH at single-cell level using fluorescence microscopy. A minimum of 100 sites was evaluated for each construct.

To visualize transformed epidermal cells upon transient gene silencing, leaves were cotransformed with 0.5 μg pGY1::*GFP* (containing a GFP reporter gene; Schweizer et al., 2000) and p35S::*Mlo* (containing the *HvMlo* gene that enhances penetration rates of powdery mildew fungi). Interaction sites were evaluated 48 h after *Bgh*A6 inoculation at single-cell level using fluorescence microscopy. A minimum of 100 sites was evaluated for each experiment.

Phylogenetic Analysis

The evolutionary history was inferred using the neighbor-joining method (Saitou and Nei, 1987). The bootstrap consensus tree inferred from 1,000 replicates is taken to represent the evolutionary history of the taxa analyzed. Branches corresponding to partitions reproduced in less than 50% bootstrap replicates are collapsed. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test is shown next to the branches (Felsenstein, 1985). The tree is drawn to scale, with branch lengths in the same units as those of the evolutionary distances used to infer the phylogenetic tree. The evolutionary distances were computed using the Jones, Taylor, Thornton matrix-based method (Jones et al., 1992) and are in the units of the number of aa substitutions per site. The rate variation among sites was modeled with a γ distribution (shape parameter of 5). The analysis involved 49 aa sequences. All positions with less than 0% site coverage were eliminated. That is, fewer than 100% alignment gaps, missing data, and ambiguous bases were allowed at any position. There were a total of 533 positions in the final dataset. Evolutionary analyses were conducted using the MEGA5 program (Tamura et al., 2011).

DNA Isolation and Quantitative PCR Analysis

DNA/RNA extraction and quantitative RT-PCR was performed as described (Doyle and Doyle, 1987; Jacobs et al., 2011). Primer pairs used for the respective genes are *HvUbiquitin*-F/*HvUbiquitin*-R and *FgTubulin*-F/*FgTubulin*-R for colonization studies. For expression analysis, *HvMORC1*-F/*HvMORC1*-R, *HvMORC2*-F/*HvMORC2*-R, and *HvUbiquitin*-F/*HvUbiquitin*-R were used (Supplemental Table S3). Primer pairs for transposable elements are listed in Supplemental Table S3.

Generation of Recombinant HvMORC1

Full-length *HvMORC1* complementary DNA was cloned into pET28a. Recombinant expression was carried out in *Escherichia coli* strain BL21 (DE3) cotransformed with pLysS to suppress expression in the uninduced state. Cells were grown at 37°C to an optical density at 600 nm of 0.6 to 0.8. Heterologous expression was induced by adding isopropylthio-β-galactoside to a final concentration of 1 mM for 3 to 4 h. Cells were harvested by centrifugation. For cell disruption, cell mass was resuspended in cell disruption buffer (25 mM Tris/acetate, pH 7.5, 10% [v/v] glycerine, and 200 mM NaCl) supplemented with 5 mM imidazole, 100 μg mL^{-1} DNaseI, and 1 mg mL^{-1} lysozyme (20 mL cell disruption buffer g^{-1} cell wet weight) and sonicated. After centrifugation, the supernatant was purified by immobilized metal ion affinity chromatography employing the N-terminal His_6-tag by use of a prepacked HisTrap column (5-mL bed volume; GE Healthcare). The loaded column was washed with cell disruption buffer containing 10 mM imidazole, and bound protein was eluted with cell disruption buffer containing 200 mM imidazole. Fractions containing the target protein were diluted 1:5 in 25 mM Tris/acetate, pH 7.5, and 10% (v/v) glycerine and loaded onto a QXL ion exchange column (1 mL-bed volume; GE Healthcare). Elution was done with increasing concentrations of NaCl (up to 500 mM) in 25 mM Tris/acetate, pH 7.5, and 10% (v/v) glycerine.

Endonuclease Assay

Reaction mixtures were set up in a total volume of 10 μL with use of 25 mM Tris/acetate, pH 7.5, 10% (v/v) glycerine, and 200 mM NaCl as buffer background and supplemented with $MgCl_2$, $MnCl_2$, or $CoCl_2$, respectively, to a final concentration of 2 mM. pUC19 (M77789) plasmid DNA served as substrate in a total amount of 100 ng per reaction. The reaction was carried out with 200 nM HvMORC1 purified protein at 37°C for 1 h. The cleavage was stopped by addition of DNA loading dye, and the reaction mixture was subsequently electrophoretically separated in a 1% (w/v) agarose/TRIS-Borat-EDTA gel. Quantification of the DNA bands was done by ImageJ.

ATPase Assay

ATPase assay was performed using a colorimetric kit (Innova Bioscience) in 50 mM Tris/HCl, pH 7.5, 2.5 mM $MgCl_2$, 10 mM NaCl, and 10 mM KCl containing 0.5 mM ATP. Reaction mixtures were incubated for 150 min at 37°C. ATPase activity was calculated on basis of a standard curve obtained with serial dilutions of free inorganic phosphate.

Microscopy

Plant culture, flg22 treatment, fixation, ultrathin sectioning, immunocytochemistry, and transmission electron microscopy were performed as previously described (Kang et al., 2012). All plants were sprayed with 50 μM β-estradiol 24 h prior to mock or flg22 treatment.

Supplemental Data

The following materials are available in the online version of this article.

Supplemental Figure S1. Sequence alignment of HvMORC1, HvMORC2, HvMORC6a, HvMORC6b, and HvMORC7.

Supplemental Figure S2. Altered expression of barley *MORCs* affects basal resistance against *Blumeria graminis*.

Supplemental Figure S3. Microscopic images of specific interactions of powdery mildew fungus *Blumeria graminis* with transiently transformed barley leaf epidermal cells.

Supplemental Figure S4. Basal resistance in the Arabidopsis *morc1-2 morc2-1* mutant against the necrotrophic fungus *Botrytis cinerea* is enhanced by *AtMORC1* and reduced by *HvMORC1* overexpression.

Supplemental Figure S5. Stability of HvMORC1 and AtMORC1 proteins in the Arabidopsis *morc1-2 morc2-1* mutant.

Supplemental Figure S6. Transmission electron microscopy detects HvMORC1 in the nucleus of transgenic Arabidopsis.

Supplemental Figure S7. Sequence alignment of HvMORC1, OsJMORC, and RnMORC4.

Supplemental Figure S8. Plasmids for transient expression, transient silencing, and stable transformation.

Supplemental Table S1. RNAi-mediated silencing of barley MORC homologs affects penetration efficiency of *Bgh* into barley leaf epidermis cells.

Supplemental Table S2. Barley transposable elements used for RT-qPCR.

Supplemental Table S3. Oligonucleotide primers used in this study.

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