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1 **Running head: OsMADS26 negatively regulates stress resistance**

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OsMADS26 negatively regulates resistance to pathogens and drought tolerance in rice.

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One sentence summary: OsMADS26 acts as a repressor of resistance against pathogenic microorganisms and water deficit and its down-regulation results in improved biotic and abiotic stress tolerance of rice.

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49 ## Abstract

50 Functional analyses of MADS-box transcription factors in plants have unraveled their role in
51 major developmental programs (e.g; flowering and floral organ identity), as well as in stress-
52 related developmental processes such as abscission, fruit ripening and senescence. Over-
53 expression of the *OsMADS26* gene in rice (*Oryza sativa*) has revealed a possible function
54 related to stress response (Lee et al., 2008b). Here we show that *OsMADS26* down-regulated
55 plants exhibit enhanced resistance against two major rice pathogens, *Magnaporthe oryzae* and
56 *Xanthomonas oryzae*. Despite this enhanced resistance to biotic stresses, *OsMADS26* down-
57 regulated plants also displayed enhanced tolerance to water deficit. These phenotypes were
58 observed both in controlled and field conditions. Interestingly, alteration of *OsMADS26*
59 expression has no strong impact on plant development. Gene expression profiling revealed
60 that a majority of genes miss-regulated in over-expresser and down-regulated *OsMADS26*
61 lines compared to control plants are associated to biotic or abiotic stress response. Altogether,
62 our data indicate that *OsMADS26* acts as an upstream regulator of stress-associated genes and
63 thereby as a hub to modulate the response to various stresses in the rice plant.

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Introduction

MADS box transcription factors belong to a multigenic family and have been identified in yeasts, plants, insects, nematodes and lower vertebrates and mammals where they control different aspects of development and cell differentiation (Shore and Sharrocks, 1995). For example, the yeast MINICHROMOSOME MAINTENANCE 1 (MCM1) MADS-box transcription factor is involved in diverse regulatory mechanisms underlying cell viability, cell-cycle control, mating, minichromosome maintenance, recombination but also osmotolerance (Messenguy and Dubois, 2003). The MADS-BOX PROTEIN REQUIRED FOR INFECTIOUS GROWTH 1/RESISTANCE TO LEPTOSPHAERIA MACULANS 1 MADS-box transcription factor is required for pathogenicity of the causal fungal agent of the rice blast disease, *Magnaporthe oryzae* (Mehrabi et al., 2008). In plants, analyses of MADS box transcription factors have mainly revealed a function in flower development, flowering induction or fruit development (Theissen et al., 2000; Arora et al., 2007; Smaczniak et al., 2012). Expression of other MADS genes in pollen, endosperm, guard cells, roots and trichomes suggests a function in the differentiation of these organs and tissues (Alvarez-Buylla et al., 2000; Parenicova et al., 2003; Puig et al., 2013). Some plant MADS-box transcription factors are involved in the control of stress-related developmental programs such as abscission, fruit ripening and senescence. For example, in *Arabidopsis thaliana*, over-expression of *AGAMOUS-LIKE 15* (*AGL15*) was found to delay flowering, senescence, fruit ripening and floral organ abscission suggesting that this MADS-box transcription factor is a negative regulator of these processes (Fernandez et al., 2000; Fang and Fernandez, 2002). Similarly FOREVER YOUNG FLOWER (FYF) represses floral organ senescence and abscission in *Arabidopsis* (Chen et al., 2011). SHATTERPROOF1 (SHP1) and SHP2 are involved in the cell specification of the dehiscence zone in *Arabidopsis* fruits where they promote the lignification of cells adjacent to this zone (Liljegren et al., 2000). In *Solanum lycopersicum*, the MADS domain protein JOINTLESS is necessary to specify pedicel abscission zones MADS-RIN and TOMATO AGAMOUS-LIKE 1 (TAGL1) controls fruit ripening (Mao et al., 2000; Vrebalov et al., 2002, Itkin et al., 2009, Vrebalov et al., 2002). Nevertheless no MADS box gene has been yet identified in plants to have a function related to biotic or abiotic stress-response regulation.

The *Oryza sativa* genome contains 75 genes encoding MADS-box transcription factors but the function of only few of them has been determined. Most of the studied genes are involved in the control of development, including tillering, flower development and flowering time (Arora et al., 2007; Guo et al., 2013). Some of them are involved in development by controlling stress-related processes such as *OsMADS3* that is involved in reactive oxygen species homeostasis during anther development and *OsMADS29* that controls cell degeneration during seed development (Hu et al., 2011; Yang et al., 2012). A possible specific involvement of rice MADS genes in stress response has been reported only for *OsMADS26*, the rice ortholog of *AGL12* (Lee et al., 2008b; Lee et al., 2011). In *Arabidopsis AGL12* regulates cell proliferation in the root apical meristem as well as flowering transition, and was suggested to control root secondary cell-wall synthesis (Tapia-Lopez et al., 2008; Montes et al., 2014). When over-expressed in *Catharanthus roseus* cell suspension, *AGL12* promotes cell aggregation and stimulates expression of genes involved in the biosynthesis of terpene indole alkaloids (Montiel et al., 2007). In rice, *OsMADS26* over-expression causes a severe stress phenotype that generally leads to plant death. Expression of *OsMADS26* under the control of a dexamethasone-inducible promoter provokes the differential regulation of genes involved in jasmonic acid biosynthesis and reactive oxygen species production (Lee et al., 2008b).

In order to precise the involvement of *OsMADS26* in stress response in rice, we succeeded in generating viable plants over-expressing *OsMADS26* and plants where *OsMADS26* expression was down-regulated through RNA interference. Our data showed that *OsMADS26* down-regulated plants have no dramatic alteration of their development and were more resistant to *Magnaporthe oryzae* and *Xanthomonas oryzae* pv *oryzae*, the main fungal and bacterial pathogens of rice. On the other hand, *OsMADS26* over-expression increased moderately their susceptibility to these pathogens. Enhancement of recovery capacity after a severe water stress was also observed in *OsMADS26* down-regulated plants. These phenotypes were further confirmed in the field with *OsMADS26* overexpression increasing *M. oryzae* susceptibility and *OsMADS26* down regulation promoting resistance against water deficit. A transcriptome analysis revealed that genes differentially regulated between control and over- or down-regulated *OsMADS26* plants were enriched with already known biotic and abiotic stress-related genes. Altogether, these results indicate that *OsMADS26* is a major negative regulator of both biotic and abiotic stress responses in rice.

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130 ## Results

131 ### *OsMADS26 is preferentially expressed in peripheral tissues and regulated by biotic and*
132 ### *abiotic stresses*

133 Accumulation of *OsMADS26* transcripts in roots, leaves and panicles has been
134 previously reported (Shinozuka et al., 1999; Pelucchi et al., 2002; Arora et al., 2007) and was
135 found to increase with organ aging (Lee et al., 2008b). To further precise the expression
136 pattern of *OsMADS26* we carried out RT-qPCR and *in situ* hybridization assays in the organs
137 of 7 day-old rice seedlings. *OsMADS26* was found to be expressed in all the investigated
138 organs (i.e. leaf blade, stem bases, seminal and crown roots (Figure 1 A), in a consistent
139 manner with regards to the available expression data (see www.genevestigator.com with
140 Os.4174.1.S1_at). In seminal roots, the expression of *OsMADS26* in the 0.5 cm segment
141 above the root tip was two-fold higher than in the root tip itself (the 0.5 cm apical part of the
142 seminal root) (Figure 1 A). *In situ* hybridization specified RT-qPCR data showing that
143 *OsMADS26* transcripts accumulate in the differentiated epidermis, exodermis, sclerenchyma
144 and cortical aerenchyma layers but neither in the meristematic zone of the root nor in the root
145 cap (Figure 2, A to H). *OsMADS26* mRNA was not detected in the stele tissues (Figure 2, A
146 and E). In leaves, *OsMADS26* was expressed in the epidermal cells, bulliform cells, phloem,
147 and xylem associated parenchyma cells (Figure 2, I to L).
148 To determine whether *OsMADS26* expression is influenced by osmotic stress, rice
149 seedlings were grown on culture media supplemented with 100 mM mannitol. Under these
150 conditions, the seedling growth is reduced but not abolished (data not shown). Mannitol
151 treatment induced the expression level of *OsMADS26* both in shoot and in root tissues (Figure
152 1 B and C).
153 As available microarray data indicate that *OsMADS26* is slightly down-regulated late
154 after infection (48 hpi) by the FR13 virulent isolate of the blast fungus *M. oryzae* (Ribot et al.,
155 2008); GEO accession GSE7256), we further investigated its expression time course
156 following inoculation with virulent and avirulent isolates (FR13 and CL3.6.7, respectively;
157 (Delteil et al., 2012)) of *M. oryzae* (Figure 3). We confirmed that *OsMADS26* transcription is
158 slightly repressed late after inoculation (72 hpi) with the virulent isolate FR13 but not the
159 avirulent isolate CL3.6.7. More strikingly, *OsMADS26* was strongly repressed in an early

phase of infection by both isolates (4 and 8 hpi), before the fungus has penetrated into the leaf (Figure 3).

OsMADS26 mis-regulation does not strongly affect plant development

To precise the function of *OsMADS26*, we investigated the effect of its over-expression and of its RNAi-mediated down-regulation in rice plants. For over-expression, the *OsMADS26* cDNA was placed under the control of the maize ubiquitin 1 promoter that allows high level, constitutive expression in rice (Cornejo et al., 1993). We selected two independent, homozygous single T-DNA copy events, OX1 and OX2, accumulating *OsMADS26* transcripts at a 30- and 20-fold higher level than the control, respectively (Figure 4 A). *OsMADS26* over-expression remained stable in further generations (Figure S1 A). For constitutive RNAi-mediated down-regulation (DR) of *OsMADS26*, two constructs specifically targeting either its 5'UTR (DR5) or the 3'UTR (DR3) regions were prepared. Two independent, homozygous, single T-DNA copy events were randomly selected for each construct (DR5-1 and DR5-2; DR3-1 and DR3-2). A wild-type line regenerated from untransformed callus used for the transformation experiment was kept as control (WT). In addition, one line transformed with the empty over-expression T-DNA (OX0) and one line obtained by transformation with the empty RNAi T-DNA (DR0) were used as additional controls. Plantlets of these three control lines accumulated *OsMADS26* transcripts at a similar level (Figure 4 A and B). In all the RNAi lines, *OsMADS26* expression was reduced strongly and stably over the subsequent generations (Figure 4 B, Figure S1 B) and did not respondanymore to an osmotic stress (Figure S1 C).

In order to further establish the influence of *OsMADS26* on rice development, the phenology of the transformed lines was investigated. First, the height of 7 day-old seedlingsgrown *in vitro* was scored. All control lines (WT, OX0 and DR0) exhibited similar development while the height of the OX1, OX2, DR5 and DR3 lines was significantly reduced (Table I). DR5 and DR3 plantlets were the most affected. However, two months following transfer in pots in the greenhouse (76 days after germination), the average heights OX1, OX2, DR5 and DR3 lines were similar to those of control lines, except the DR5-1 line which still exhibited a reduced size (Table I). At the same time all the down-regulated lines displayed a reduction in tiller number (Table I; Figure 4 C). This was particularly significant

for the DR5-2 line which displayed a 45% reduction in number of tillers compared to its control (DR0) (Table I). The dry weights (DW) of the aerial part of the DR plants, especially the two DR5 lines, were lower than those of the control and OX plants (Table I). The two DR3 lines also exhibited significant delay of 3-4 days in flowering (Table I). No significant difference for these two traits was observed among the rest of the lines. Total weight and 1000-seed weight of the main panicle were comparable in all the lines studied (Table I). In summary, while the over-expressing and down-regulated *OsMADS26* lines exhibited a retarded growth at early stages of development following germination further transfer and growth in the greenhouse allowed them to recover and exhibit a performance generally similar or close to that of control plants. The weak impact of constitutive *OsMADS26* over-expression or down-regulation on plant development was confirmed in the field where we observed only a reduced height for the OX2 line and a higher biomass and yield for the DR3-1 line in comparison with their relative controls (Figure S2).

OsMADS26 is required for resistance against blast fungus and bacterial blight

As *OsMADS26* was found to be a stress-related gene in rice (Lee et al., 2008b; Lee et al., 2011), we further evaluated the response of the *OsMADS26* transgenic lines to pathogen infection.

First, plantlets of the different *OsMADS26* lines were inoculated with the moderately virulent fungal isolate GUY11 of *Magnaporthe oryzae* (Delteil et al., 2012). This isolate triggers lesions in the leaf blade of cv. Nipponbare consisting of an average of 50% greyish lesions surrounded by brown margins that are characteristic of successful invasion of the fungus (disease). The other are small and dark spots characteristic of unsuccessful invasion events (see WT, OX0 and DR0 plants in Figure 5 A). Differences in the degree and development of disease symptoms caused by *M. oryzae* between transformed and untransformed plants were clearly visible at 7 days post inoculation (dpi) (Figure 5 A). The two over-expressing lines (OX1 and OX2) presented more disease symptoms compared with the controls (WT and OX0). In contrast, all the down-regulated lines, displayed many small and dark spots characteristic of resistance and very few disease symptoms. These observations were further confirmed by calculating the percentage of susceptible lesion versus the total number of observed lesion on each infected leaf (Figure 5 B). Thus, this suggested that

OsMADS26 negatively regulates blast resistance. In addition, the susceptibility to *M. oryzae* of OX0, OX2 and DR3-1 lines was challenged in a nethouse in Vietnam on 10 weeks old plants inoculated with the VT15 Vietnamese isolate virulent on Nipponbare (Figure S3). In this experiment the number of susceptible lesions was significantly higher in OX2 line and slightly lower in DR3-1 line than in the control (OX0), confirming the opposite phenotypes observed for over-expressing and down-regulated *OsMADS26* lines. The expression of a set of selected major defence-related genes *PEROXIDASE 22.3* (*POX22.3*) (Vergne et al., 2007), chitinase (CHI7) (Kaku et al, 2006), *PATHOGENESIS-RELATED PROTEINS 5 (PR5), NONEXPRESSOR OF PATHOGENESIS-RELATED* (*NPR1*) *HOMOLOGUE 1* (*NH1*), Flagellin-receptor (*OsFLS2*), *OsWRKY28* and *PROBENAZOLE-INDUCIBLE 1* (*PBZ1*) (Delteil et al., 2012) was examined in OX2 lines 2 days following inoculation with *M. oryzae* GY11 isolate or mock treatment (Figure 6). This showed that in mock-treated and inoculated plants, the expression of most of these genes (*POX223*, *CHI7*, *PR5*, *NH1*, *FLS2* and *WRKY28*) was significantly reduced in the OX2 line in comparison with OX0, before and/or after infection. This results suggests that *OsMADS26* acts as a negative regulator of defense-gene expression.

Secondly, in order to evaluate whether constitutive deregulation of *OsMADS26* affects the susceptibility to a bacterial pathogen, we challenged the over-expressing and down-regulated *OsMADS26* lines with *Xanthomonas oryzae* pv. *oryzae*. Similar data were obtained for resistance to bacterial blight *X. oryzae* pv. *oryzae* as with *M. oryzae.* In this case the length of the necrotic and yellowing zone extending from the wounded extremity of the infected leaves was measured 14 days after inoculation. The symptoms had a significantly higher severity for OX1 and OX2 lines, compared to the control lines (Figure S4 A and B). Conversely, the symptoms developed by down-regulated lines (DR5-1, DR5-2, DR3-1 and DR3-2) were limited to a short necrosis just below the inoculation zone (Figure S4 A and B), suggesting that these lines were strongly resistant to *X. oryzae* pv. *oryzae* and supporting a negative role of OsMADS26 on blight resistance.

Finally, we tested whether the response to the Rice Yellow Mottle Virus (RYMV, Kouassi et al., 2005) could be affected by *OsMADS26* over-expression or down-regulation. We did not observe any difference in the development of symptoms or in virus accumulation

between the over-expressing lines, the down-regulated lines and their respective controls (Figure S5), suggesting that mis-regulation of *OsMADS26* expression had no impact on the resistance against RYMV.

OsMADS26 inhibition favours plant tolerance against drought stress

Because mannitol stress induces the expression of *OsMADS26* (Figure 1 B and C) we investigated the tolerance of over-expressing and down-regulated lines to the drought stress. Following the drought stress, plants were re-watered for a period of two weeks to allow recovery. While plants of all the control and *OsMADS26* over-expressing lines were mostly wilted and died, *OsMADS26* down-regulated plants fully recovered from the water stress (Figure 7 A).

All the lines exhibited at the beginning of the experiment a similar Relative Water Content (RWC, nearly 95%) that decreased to around 85% following 11 days of water deficit (Figure 7 B). However, 15 days after water deprivation, the leaf RWC of all the control and *OsMADS26* over-expressing lines dropped to a 47 to 62 % range while the two *OsMADS26* down-regulated lines maintained a significantly higher RWC falling within a 81 to 84% range. This suggests that the inhibition of *OsMADS26* expression enhances the capacity of the rice plant to maintain its water content under water deficit.

The expression of two drought-responsive genes was analyzed: *RESPONSIVE TO ABA21 (RAB21)*, a rice dehydrin and *SALT-STRESS-INDUCED PROTEIN (SALT)* (Claes et al., 1990; Oh et al., 2005). Their expression levels were similar in all lines before or 5 days following the water stress. Following 11 days of water stress however, their expression was significantly higher in the two *OsMADS26* down-regulated lines compared to control and *OsMADS26* over-expression lines (Figure 7 C and D). This suggests that *OsMADS26* may play a negative role in the regulation of some drought stress-responsive genes in response to water deficit.

In addition we challenged in the field the capacity of OX0, OX2, DR0 and DR3-1 lines to tolerate water deficit. The DR3-1 line presented a much better tolerance to water deficit conditions associated with a slower decrease of chlorophyll a content and a better capacity to maintain yield under drought than the other lines (Figure 8). Other measurements (leaf rolling, chlorophyll content, biomass) confirmed that DR3-1 plants had an increased

capacity to sustain drought stress (Figure S6). This confirmed that a constitutive down regulation of *OsMADS26* increases the capacity of the plant to tolerate water deficit.

Transcriptome profiling of OsMADS26 over-expressing and down regulated lines

Preliminary evidence of altered expression of stress related genes in *OsMADS26* over-expressing and down regulated lines led us to further identify the pathways potentially regulated by *OsMADS26*, through transcriptome profiling. Transcriptome profiles were established from two independent biological replicates per line. Genes significantly and reproducibly induced or repressed (fold change > 2 and p-value, $P \leq 0.05$) across lines and replicates compared to their values in the appropriate controls were selected for further analysis (see material and methods for more information). We finally selected genes at least one time inversely regulated in OX compared to DR lines or reproducibly over-expressed or repressed in OX or control lines. In order to compare our results to other available data, we converted the rice probes into MSU transcriptional units (Table S1). This represented a total of 400 non-redundant genes. A total of 71 non-redundant genes presented an inverted regulation profile in OX and DR lines (Figure 9, Table S1). Overall, 212 genes were down-regulated in DR lines and/or up-regulated in OX lines. These genes should belong to pathways induced by OsMADS26. On the contrary, 200 genes were up-regulated in DR lines and/or down-regulated in OX lines. These genes should belong to pathways inhibited by OsMADS26.

We then looked for overlaps between a set of >6800 probes that were known to be transcriptionally regulated upon pathogen infection (Vergne et al., 2008) and the 400 genes that were significantly mis-regulated in DR and/or OX lines (Table S1). We found that 53% of the 200 genes up regulated in DR and/or down-regulated in OX lines are known to be transcriptionally regulated during pathogen challenge whereas only 30% were expected by chance in a random selection of 2000 genes ($P < 0.001$ as evaluated with a Chi square test; Vergne et al, 2008). In contrast there was no such enrichment in the 212 genes up-regulated in DR lines and/or down-regulated in OX lines. Thus OsMADS26 seems to down-regulate the transcription of a large number of genes known to be involved in disease resistance. Similarly, a large proportion (41%) of genes mis-regulated in *OsMADS26* lines was found in previous published drought dataset (Minh-Thu et al., 2013). The extent of this overlap is proportinal to

312 the one observed with genes found to be deregulated in DEX-inducible *OsMADS26* lines
313 (39%) (Lee et al., 2008b). Our analysis thus resulted in a list of putative OsMADS26 target
314 genes that may be involved in the regulation of biotic or abiotic stress resistance.

315 ## Discussion

316 ### *Alteration of OsMADS26 expression does not deeply affect Nipponbare plant development*

317 The *OsMADS26* over-expressing lines presented a delayed development at the
318 seedling stage but their development in the greenhouse and field was almost similar to the
319 development of control plants, aside a slight reduction in tiller number (Table I). This
320 contrasts with the previous study of Lee and co-workers (2008b) who reported that over-
321 expression of *OsMADS26* driven by the same constitutive promoter triggered several
322 dramatically abnormal developmental phenotypes, including anthocyanin accumulation or
323 lethality. A tentative explanation might lie in the use of different genetic backgrounds
324 (Nipponbare vs. Dongjin) for expressing *OsMADS26*. To our knowledge, there is at least one
325 report where over-expression in different rice genetic background resulted in the opposite
326 effects (Tao et al., 2009). Alternatively, it is possible that our transformation procedure
327 (Sallaud et al., 2003) that differs from that used by Lee and colleagues, has counter selected
328 plants presenting a severe reduction of their development or lethality due to very high levels
329 of expression. Although we cannot explain the strong phenotypic differences between our
330 over-expressing lines and the lines analyzed by Lee et al (2008b), these differences may
331 explain at least in part why we found little overlap between our and their micro-array
332 experiments (16 genes in total, see below). Similarly, except for a delay in development
333 observed at early stages, the overall development of the down regulated lines was not strongly
334 modified (Table I).

335 ### **OsMADS26** *is a negative regulator of both biotic and abiotic stresses*

336 Our data showed that *OsMADS26* down-regulated lines displayed decreased
337 susceptibility to two major pathogens of rice (Figures 5, S3 and S4) as well as an increased
338 water deficit tolerance and a better recovery capacity following a drought stress (Figures 7, 8
339 and S2). The observation of consistent phenotypes in the *OsMADS26* down-regulated lines

obtained with two independent constructs targeting 5' or 3' UTR, reduces the risk of misinterpretation related to trans-interference with transcripts of other genes. As the observed phenotypes are similar between the different down-regulated lines we can assume that they are the consequence of a specific degradation of *OsMADS26* mRNAs.

Up to 60% and 40% average disease symptom reductions were observed in down-regulated lines inoculated with *X. oryzae* pv *oryzae* and *M. oryzae* respectively (Figures 5 and S4). This corresponds to a high level of disease reduction when compared to the range attained in transgenic lines obtained through mis-regulation of a set of defense-associated genes (Delteil et al., 2010). Consistently, an increased susceptibility of *OsMADS26* OX lines to *M. oryzae* was also observed in the nethouse experiments whereas the tested *OsMADS26* down-regulated lines presented a reduction of susceptible lesions in comparison with the DR0 control (Figure S3). This shows that the negative regulation of *OsMADS26* on the resistance mechanisms to *M. oryzae* can be observed at different developmental stages, with different virulent isolates and independently of the growth conditions. It is interesting to stress that there is a coincidence between the tissue localization of *OsMADS26* transcripts and the cell barriers that pathogens have to cross in the plant (Figure 2). For instance, *OsMADS26* is expressed in the epidermis, a barrier that *M. oryzae* has to cross to perform its life cycle. Transcripts of *OsMADS26* also accumulated in cells around the vessels where *X. oryzae* pv *oryzae* develops. To our knowledge this is the first report of the involvement of a *MADS* gene in disease resistance in plants. The resistance of rice against RYMV was not affected by *OsMADS26* down-regulation. Resistance against bacteria and fungi on the one hand and virus on the other hand involves different mechanisms, such as RNA silencing for the latter and pathways producing antimicrobial molecules for the former. Thus *OsMADS26* negatively participates in resistance to a wide range of rice pathogens but not to RYMV.

Besides this strong effect on biotic stress resistance, the *OsMADS26* down-regulated lines showed an increased ability to maintain their RWC under soil water deficit and to recover from a severe drought stress as well as a better capacity to maintain yield in drought condition in the field (Figure 7, 8, S6) The preferential localization of *OsMADS26* transcripts (Figure 2) in peripheral tissues such as epidermis and bulliform cells in leaves and exodermis in roots supports a role for this transcription factor in the response mechanism to

environmental clues. To our knowledge, *OsNAC6* and *OsNAC10* are the only transcription factors for which the deregulation had a joint benefit on both biotic and abiotic stresses tolerances (Nakashima et al., 2007; Sun et al., 2012). *OsNAC6* over-expressing rice plants showed an improved tolerance to dehydration and high-salt stresses as well as increased tolerance to blast disease. However, constitutive overexpressers also exhibit growth retardation and low reproductive yields, in contrast to *OsMADS26* down-regulated lines that presented only discrete developmental changes.

OsMADS26 alters the transcription of a wide range of biotic and abiotic stresses-related genes

We showed that the expression of a set of defense genes is lower in OX *OsMADS26* lines than in the control before and after inoculation with a virulent isolate of *M. oryzae* (Figure 6). This was confirmed by micro-array analysis (Table S1) where several other genes coding for Pathogenesis-Related proteins were down regulated in OX *OsMADS26* lines. Similarly the expression of a set of drought resistance related genes is higher in *OsMADS26* DR lines after the application of a water deficit (Figure 7). This suggests a direct or indirect involvement of *OsMADS26* as a repressor of stress responsive genes.

By using transcriptome analysis, we investigated whether the modified response to biotic and abiotic stresses was associated to a more global differential expression of stress-related genes before application of the stress itself. Using the Archipelago database referencing genes in rice involved in disease resistance (Vergne et al., 2008) or the drought responsive genes dataset (Minh-Thu et al., 2013), we could establish that a large proportion of the genes differentially regulated in down-regulated and over-expressing lines are known to be regulated by biotic (53%) or abiotic (41%) stresses. This was similar (49% and 39% respectively) to what was found by Lee and colleagues (2008b) following DEX-induced over expression of *OsMADS26*. Thus these transcriptome analyses demonstrate that *OsMADS26* participates in the transcriptional regulation of defense-related genes. The low overlap with the data set obtained by Lee and colleagues 2008b probably reflects the fact that we determined the genes regulated at steady-state levels after constitutive over-expression or down-regulation of *OsMADS26* expression whereas Lee and colleagues 2008b identified the genes deregulated upon a sudden increase of *OsMADS26* transcription triggered by the

dexamethasone induction treatment. Based on their transcriptome analysis, Lee and colleagues (2008b) stressed that OsMADS26 may be involved in the regulation of genes involved in jasmonate and ethylene stress hormone biosynthesis. Here we found that *OsLOX8* (*Os08g39840*) is consistently up-regulated in DR lines and down-regulated both in OX *OsMADS26* lines and dexamethasone-induced *OsMADS26* lines (Lee et al., 2008b). This gene was reported to be regulated during the early stage of *M. oryzae* infection (Peng et al., 1994; Agrawal et al., 2004), by wounding (Marla and Sing, 2012) and during the senescence process (Kong et al., 2006). Two genes involved in ethylene biosynthesis *OsACO3* (*Os09g27750*) and *OsARD1* (*Os10g28350*) are down regulated in OX *OsMADS26* lines. *OsACO3* and *OsARD1* are strongly up regulated by ethylene and contribute to maintain elevated ethylene rate in stressed plants (Rzewusky and Sauter, 2009). Similarly the ethylene responsive *ERF063* transcription factor (*Os09g11480*) (Ma et al., 2013) was found to be down regulated in OX *OsMADS26* lines suggesting that these lines are impaired for ethylene biosynthesis and response.

Other stress related transcription factors were found to be differentially regulated in OX and/or DR *OsMADS26* lines. *OsNAC103* (*Os07g48450*) known to be up regulated by water deficit treatment, salt stress and jasmonate (Murruzaman et al., 2012; Fang et al., 2008) was found to be up and down regulated in DR and OX lines, respectively. *OsNAC045* (*Os11g03370*) down regulated in OX lines is up regulated in response to salt or cold stress (Fang et al., 2008). *OsWRKY24* (*Os01g61080*) represses ABA and GA signaling in aleurone cells (Xie et al., 2005; Zhang et al., 2009) and is induced by chilling stress (Yun et al., 2010). It is up regulated in DR lines and down regulated in OX lines. *OsWRKY53* (*Os05g39720*), down regulated in OX lines is induced by elicitors, jasmonate, *M. oryzae* infection and during the *Xa21*-mediated resistance to *Xanthomonas oryzae* pv. *oryzae*. Its overexpression enhances rice resistance to *M. oryzae* (Chujo et al., 2007; 2014). Interestingly, we identified that *RH1* (*Os05g30500*) is up regulated in OX line. RH1 is an NRR homologue that can interact with and inhibit NH1/OsNPR1 that is a master regulator of defence genes and systemic acquired resistance (Chern et al., 2012). The Wall-Associated kinase *WAK25* (*Os03g12470*) was down regulated in OX plants. This is consistent with the published function of this gene as a positive regulator of *Xanthomonas* resistance (Seo et al., 2011). Finally, the *OsRMC* (*Os04g56430*) Receptor-like kinase known to be highly induced by salt treatment (Serra et al.,

2013) was up-regulated in DR plants and down-regulated in OX plants. Whether OX or DR *OsMADS26* plants are more resistant to salt stress remains to be established.

Taken together this shows that OsMADS26 contributes to the regulation of several stress-related transcriptional and regulatory pathways and that its over-expression or down regulation impact on the expression of a wide range of biotic and abiotic defense related genes and which is consistent with the observed phenotypes of DR and OX lines.

OsMADS26 a hub for stress resistance regulation in plants?

Our data indicate that OsMADS26 probably mainly acts as a negative regulator of stress response. This has also been reported for *OsMADS22* and *OsMADS55* which act as negative regulators of the brassinosteroid response (Lee et al., 2008a). Whereas the down-regulation of *OsMADS26* transcription upon rice blast infection (Figure 3), irrespective of the virulence of the isolate, can constitute a basal defense response, its up-regulation during osmotic stress (Figure 1) is more difficult to interpret. We propose that this up-regulation of *OsMADS26* could be part of a negative feed-back loop that would dampen abiotic stress response.

Nevertheless, it cannot be excluded that *OsMADS26* might have both activating and inhibiting activity on stress response genes depending on post-translational modifications or interaction with other regulatory proteins. Indeed, MADS box proteins are combinatorial transcription factors and their regulatory specificity is affected by the interaction with other DNA binding or accessory factors (Messenguy and Dubois, 2003). In this context OsMADS26 could be a hub that integrates different signals and contributes to a short term activation of defense mechanisms and becomes afterwards partly responsible for their cancellation. In this respect, it will be interesting to identify the proteins that can interact *in vivo* with OsMADS26.

Conclusion:

Our data show that *OsMADS26* is a negative regulator of different stresses of major agronomical importance in rice. It also represents the description of a new range of functions

for *MADS* genes in plants and opens the door towards the achievement of drought tolerant and disease resistant plants. To reach this goal, it will be very interesting to identify in rice tilling population plants with *OsMADS26* null alleles and to test their resistance against stresses. These alleles could be introduced in future breeding programs.

Materials and methods

Plant material and growth conditions

Dehulled and surface sterilized seeds of *Oryza sativa*, cv. Nipponbare were incubated in sterile distilled water in a growth chamber (16 h of light per day, 500 µE m^{-2} s^{-1}, 28°C/25°C day/night) for 2 days at 25°C. Imbibed seeds were transferred in square Petri dishes (245 mm x 245 mm, CORNING, 7 seeds per dish) containing 250 ml of half strength Murashige and Skoog (DUCHEFA) standard medium (MS/2) solidified with 8 g L^{-1} of agarose type II (SIGMA). These dishes were transferred and placed vertically in a growth chamber at 28°C under 16h light. Roots and shoots of 7 day-old seedlings were collected and used for *in situ* hybridization and RNA isolation for RT-qPCR or transcriptome analyses. Salt and osmotic stresses were applied by supplementing the culture medium with 150 mM NaCl (DUCHEFA) or 100 mM mannitol (DUCHEFA), respectively.

Plants were grown in 3L pots filled with EGO 140 soil substrate (TREF, www.Trefgroup.com) in a containment greenhouse (16-h-light/8-h-dark cycles, at 28°C to 30°C). For plant phenotyping, the plants belonging to the different lines were randomly distributed in the greenhouse. Twenty days after germination (DAG), plant height and tiller number were measured once a week until the early flowering stage. The latter stage was defined as the date when the first spike emerges from the flag leaf sheath on a plant. The flowering date corresponds to the date when spikes are observed on 50% of the tillers of a plant. After harvesting, the dry weight of the aerial part of the plant part was determined following drying the plant tissues at 70°C for 96 h. Panicles of each plant were also individually weighted following a drying treatment at 37°c for 3 days. The 1000 seed-weight was evaluated using seeds borne by the master tiller panicle. This experiment was repeated twice using three plants per line.

Specific culture conditions used for evaluation of pathogen and drought tolerance are detailed in the corresponding sections.

Plasmid construction for plant transformation

The isolation of *OsMADS26* (*Os08g02070*) cDNA from *O. sativa* cv Nipponbare was achieved by RT-PCR. Total RNA was extracted from 100mg of leaf tissue of 7 day-old seedlings grounded in liquid nitrogen using 1ml of TRIzol (INVITROGEN) following the recommendation of the supplier. A PCR amplification was performed with a couple of specific primers designed in the 5' and 3' UTR of *OsMADS26* (Figure S7). The amplified cDNA was cloned using the pGEM-T easy cloning kit of Promega. From the cDNA further PCR reactions were done using specific primers to amplify a 215 bp fragment located in the 5' UTR of *OsMADS26*, named GST1 and a 321 bp fragment comprising the end of the last exon and the major part of the 3' UTR region, named GST2 (Figure S4). PCR cycling conditions were: 94 °C for 4 min (1 cycle) and 94 °C for 1 min, an annealing step at various temperatures depending on the *T*m of the primers used (typically *T*m -5 °C), for 1.5 min, and 72 °C for 1 min (35 cycles) with a 5 min final extension step at 72 °C. PCR was performed in a final volume of 25 µL with 0.25 u of *Taq* polymerase in MgCl2-free buffer (PROMEGA), 2 mM MgCl2, 200 nM each dNTP, appropriate oligonucleotides (1µM) and cDNA (2 µL) or pGEMT-PC8 plasmid (10 ng). The BP tailed *OsMADS26* amplified cDNA was cloned with the BP recombinase (INVITROGEN) in a modified pCAMBIA 1300 binary vector for over-expression named PC5300.OE where the *Ccdb* gene surrounded by the BP recombination sites were cloned between the constitutive promoter of ubiquitin gene from maize and the terminator of the nopaline syntase gene from *Agrobacterium tumefaciens (*J.C. Breitler, CIRAD, unpublished). After cloning, the presence of the *OsMADS26* cDNA in frame was ascertained by sequencing. The plasmid named PC5300.OE-PC8 was transferred into *A. tumefaciens* strain EHA105. For RNA interference, the BP tailed amplified GST1 or GST2 were cloned by BP recombination in the pDON207 entry plasmid (INVITROGEN) and transferred with the LR recombinase (INVITROGEN) in the siRNA binary plasmid pANDA (Miki and Shimamoto, 2004). The insertion of the GSTs in pANDA was controlled by sequencing. The resulting plasmids, named pANDA-DR5 and pANDA-DR3, were mobilized into *A. tumefaciens* strain EHA105 for plant transformation.

Transgenic plants were obtained by co-culture of seed embryo-derived callus with *A. tumefaciens* strain EHA105 carrying the adequate binary plasmids following the procedure detailed in (Sallaud et al., 2003). Single locus and homozygous T2 lines were selected on the basis of the segregation of the antibiotic resistance gene carried by the T-DNA and Southern blot analysis.

The expression of *OsMADS26* in selected transgenic lines was analyzed by RT-qPCR using specific primers (Table SI).

Real-time quantitative reverse transcriptase polymerase chain reaction (RT-qPCR) analysis

Total RNA were extracted from 100 mg of grounded leaf tissues with 1ml of TRIzol (INVITROGEN) following the recommendation of the supplier. Two µg of RNA were treated by RQ1 DNAse (PROMEGA) to remove residual gDNA. The first strand cDNA synthesis was performed in 20 µl of final volume using the kit Superscripts III (INVITROGEN) following the manufacturer's instructions.

For RT-qPCR analysis, specific forward (F) and reverse (R) primers were designed to amplify a fragment of 200-400 bp in the 3' untranslated region (3'-UTR) of each studied gene using the Vector NTI (version 10.1) software with default parameters. Primer sequences are given in Table SII. RT-qPCR was performed with a LighCycler 480 (ROCHE) using the SYBR green master mix (ROCHE). The reaction was carried out in 96-well optical reaction plates (ROCHE). The reaction mix contained 7.5 µL SYBR Green QPCR Master Mix (ROCHE), 250 nM of each primer (F and R), and 3µL of 10 fold diluted cDNA template. All reactions were heated to 95°C for 5min, followed by 45 cycles of 95°C for 10s and 60°C for 30s. Melt curve analysis and gel electrophoresis of the PCR products were used to confirm the absence of non-specific amplification products. The primer efficiencies observed for the couples of primers used was ranged between 1.86 and 2.05. Transcripts from the *EXP* (Expressed Protein, *Os06g11070*) or actin (*Os03g50890*) genes were also detected and used as an endogenous control to normalize expression of the other genes. *EXP* or actin was chosen as reference genes because their expression appeared to be the most stable in different tissues and physiological conditions (Caldana et al., 2007). We verified that in all our experiments, the Ct (threshold cycle) value of the EXP and Actin genes remained stable irrespective of the treatment applied to the plants and ranges between 26 and 28.. Relative expression level was

546 calculated by subtracting the C_t values for *EXP* or Actin from those of the target gene (to give
547 ΔC_t), then $\Delta\Delta C_t$ and calculating $2^{-\Delta\Delta Ct}$ (Giulietti et al., 2001). Reactions were performed on
548 technical triplicates from duplicated biological experiments.

549 ***In situ hybridization***

550 For *OsMADS26* probe preparation, we used the same primers designed for
551 *OsMADS26* RT-qPCR amplification (Table S1). A 18S ribosome coding sequence was used
552 as positive hybridization control and PCR amplified from cDNA using the primer couple:
553 Rib-Up (5'-CCGACCCTGATCTTCTGTGAAGGG-3') and Rib-Down (5'-
554 CAAGTCAGACGAACGATTTGCACG-3'). Primers containing the above specific
555 sequences but extended at their 5' ends with the T7 RNA polymerase promoter sequence (5'-
556 GCGAAATTAATACGACTCACTATAGGGAGA-3') were also designed and were named
557 *OsMADS26*-T7-Up, *OsMADS26*-T7-Down, RibT7-Up and RibT7-Down. Finally, one primer
558 corresponding to the T7 end was also designed and named E-T7 (5'-
559 GCGAAATTAATACGACTCAC-3'). To generate sense and antisense probes, specific
560 cDNAs were amplified by PCR with one primer Up and one primer T7-Down or with one
561 primer Down and one primer T7-Up respectively. These cDNAs were used to generate sense
562 or antisense digoxigenin-labeled RNA probes by *in vitro* transcription using the T7 primer
563 (T7 MAXIScript Kit; AMBION). Plant samples were fixed in 4% (v/v) paraformaldehyde
564 in phosphate buffer (0.2 M, pH 7.5), inclusion, section preparation and hybridization were
565 done as previously described (Jabnoune et al., 2009). Sections were observed with a DM6000
566 (LEICA) microscope under white light. Photographs were taken with a Retiga 2000R camera
567 (QIMAGING), and images were processed through Volocity 4.0.1 (IMPROVISION). *In situ*
568 hybridization experiments have been conducted on the Plate-Forme d'Histocytologie et
569 d'Imagerie Cellulaire Végétale (http://phiv.cirad.fr/) using microscopes of the Montpellier Rio
570 Imaging platform (www.mri.cnrs.fr).

571 ***Microarray hybridization and analysis***

572 For microarray hybridization experiments, total RNA was extracted from 100 mg of
573 frozen leaves and roots after removal of the remaining seeds from 7-day-old seedlings using a
574 RNeasy Plant Mini Kit (QUIAGEN) according to manufacturer's instructions. Residual

genomic DNA was removed with the RNAse-Free DNase Set (QUIAGEN) during RNA purification. Two independent biological experiments were used for each studied plant line.

Microarray hybridization and data processing were carried out with Affymetrix custom service (AFFYMETRIX) by following the standard protocol for Affymetrix DNA chip as previously described (Coudert et al., 2011). The complete transcriptome data are accessible through GEO Series accession number GSE52640 (http://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE52640). Expression values were normalized with the robust Multi-Array average method (Irizarry et al., 2003). Differential analysis and extraction of mas5 FLAG calls were done with linear models and empirical Bayes and TREAT methods within affy and limma R packages (www.r-project.org, Gautier et al., 2004; Smyth, 2004; Smyth et al., 2005; McCarthy and Smyth, 2009). Raw P-values were adjusted with the Benjamini-Hochberg (BH) method to control the false discovery rate (Benjamini and Hochberg, 1995). Empirical Bayes method with the Benjamini-Hochberg correction was kept for further analysis as it allowed to confirm the respective down- and up-regulations of *OsMADS26* in the two replicates in the down- and over- expressing lines. Orygenes DataBase (http://orygenesdb.cirad.fr/; Droc et al., 2006) was used to retrieve gene annotation corresponding to selected Affymetrix probes. Microarray control probesets and probesets without annotation were discarded for further analysis. Only probesets with "Present" Detection Call were kept for subsequent analysis. The 2 biological repetitions for each type of down- or over- expressing transgenic lines were compared to the corresponding controls. A gene was considered significantly regulated if it present a fold change ≥ 2 and a BH corrected *p*-value P ≤ 0.05 in at least two out of the four different contrasts. Genes showing inconsistent regulations such as i) inverse regulation in two biological repeats of the same type of down- or over- expressing line or ii) similar regulation in the two different types of down- and over- expressing line were discarded. A set of up-regulated genes from DNA chip analysis were confirmed by RT-qPCR analysis as previously described using specific primers (Table SI).

Disease resistance assays

The GUY11 (CIRAD collection, Montpellier, France) or VT15 (LMI RICE collection, Hanoi, Vietnam) isolates of *Magnaporthe oryzae* were used for inoculation. GUY11 and

VT15 isolates are compatible with *O. sativa* cv Nipponbare and generate moderate susceptibility symptoms. For gene expression studies (Figure 3), we used the fully virulent FR13 isolate and the avirulent isolate CL3.6.7 (Delteil et al., 2012). In laboratory, inoculations were performed on 4-5 leaf stage plantlets as described in (Berruyer et al., 2003), *O. sativa* japonica cv Maratelli was used as a susceptible control in the experiments in addition to the studied transgenic lines. The data presented are representative of data obtained from three independent replicated experiments. For gene expression studies (Figure 3), we used the fully virulent FR13 isolate and the avirulent isolate CL3.6.7 (Delteil et al., 2012). Leaves were collected before and after inoculation in liquid nitrogen and used for RNA extraction and RT-qPCR analysis to measure the expression level of different defence genes using specific primers (Table SII).

For nethouse experiments in Vietnam plants were grown in pots (28 l) filled with organic soil (10 kg by pots) and supplemented with nitrogen (2g by pots) 3 and 9 weeks after planting. After germination in water plants were planted (5 plants by pots, 1 pot by line) following a randomized design where OE, DR and control lines were interspersed with Maratelli and Sariceltick susceptible lines. Plants were grown in a nethouse, in natural conditions and irrigated permanently to saturation. After 6 weeks of growth plants were sprayed twice a week during 6 weeks using a fresh *M. Oryzae* VT15 isolate spore solution (50 0000 spore by ml, 1% w:v gelatin). Symptoms were observed 15 weeks after sowing. Leaves were collected and scanned and the number of susceptible lesions was numbered according to Berruyer et al., 2003.

Resistance assays against *X. oryzae* pv. *oryzae* were carried out on 8 week-old rice plants. The *Xoo* strain PXO99A (Salzberg et al., 2008) was inoculated using the leaf-clipping method as previously described (Kauffman, 1973). The data presented are representative of two independent experiments. Before inoculation and after symptom development, infected leaves were collected in liquid nitrogen and used for RNA extraction and RT-qPCR analysis to measure the expression level of different defense genes using specific primers (Table SII).

For resistance assay against Rice Yellow Mottled Virus (RYMV), ten plants per line were inoculated by finger rubbing the leaves in presence of Carborundum (600 mesh) with purified RYMV particles at a concentration of 100 µg mL^{-1} as previously described (Quilis et al., 2008). Virus accumulation in tissues was measured by ELISA analysis using an antibody

against the RYMV coat protein (N'Guessan, 2000). Presented data are representative of two independent replicated experiments.

Resistance assay to water deficit

Plants were germinated directly in soil and grown in the greenhouse. Each pot was filled with EGO 140 soil substrate (TREF, www.Trefgroup.com), planted with 5 seedlings and watered with the same volume of water. After one month, plants were subjected to 18 days of withholding water followed by 15 days of re-watering. Drought tolerance was evaluated by determining the percentage of plants that survived or continued to grow after the period of recovery. This experiment was performed using 20 plants per line and repeated three times.

During the water stress period, the relative water content (RWC) of plants was monitored using a 7 cm-long segment of the last expanded leaf in a random set of five plants per line according to (Barr and Weatherley, 1962). The other leaves were also harvested, frozen in liquid nitrogen and stored at -80°C for RNA extraction and RT-qPCR analysis of stress related genes expression using two plants per line exhibiting closest RWC. RT-qPCR analysis was conducted as described earlier with specific primers of genes identified as drought and high salinity stress markers in rice: *RAB21*, a rice dehydrin (AK109096) and *SALT-STRESS-INDUCED PROTEIN* (*SALT*, AF001395) genes (Claes et al., 1990; Oh et al., 2005). The primer sequences used are given in Table SI.

Upland field experiments were carried out under confined rain-out shelter field facility, at the International Center for Tropical Agriculture (CIAT, Palmira, Colombia). This field trial was laid out in a randomly complete block design with three replicates. Drought stress was imposed from panicle initiation (56 days after direct seeding) and continued around 3 weeks (or) until severe leaf rolling & wilting appeared in non-transgenic control. Then the plants were rewatered til physiological maturity. The intensity of drought was monitored through volumetric soil water. Leaf rolling (LR) scores were recorded on a 1-9 IRRI scale standardized for rice. The following agronomic traits were scored according to the criteria established in the Standard Evaluation System for Rice (SES) (IRRI, 2002): plant height (cm), single plant dry biomass (g) and single plant yield were recorded. The degree of relative chlorophyll content in the fully expanded flag leaf was determined using a SPAD-502

chlorophyll meter (Minolta Co., Tokyo, Japan) under stress at different stages of crop development. Chlorophyll-a fluorescence parameters were also measured using a fluorpen FP100 chlorophyll fluorometer. Fv/Fm represented the maximal photochemical efficiency. Leaves were kept in the dark for 20 min before measurement. Fv/Fm was calculated with the following formula: Fv/Fm=(Fm–Fo)/Fm, where Fo is initial fluorescence, Fm is maximum fluorescence, and Fv is variable fluorescence (any reference to the technique?).

Acknowledgements:

Affymetrix microarrays were processed in the Microarray Core Facility of the Institute of Research of Biotherapy, CHRU-INSERM-UM1 Montpellier, France, http://irb.chu-montpellier.fr/ by Véronique Pantesco.

677 ## Figure legends:

678 **Figure 1**. *OsMADS26* is expressed in shoots and roots and is induced by osmotic stress.

679 A, expression of *OsMADS26* in different organs of 7-day-old rice seedlings cultivated in
680 standard condition (MS/2). L: leaf, S: stem base, CR: crown root, SR-A: seminal root without
681 apex, SR+A: seminal root apex. B-C, expression patterns of *OsMADS26* in root (B) and shoot
682 (C) in standard condition (c) or under osmotic stress (OS: MS/2 + 100 mM Mannitol). Mean
683 and standard error were calculated from two independent experiments consisting of three
684 technical replicates each. A Student t-test was used to compare the relative expression level
685 observed in standard andstress conditions; *: significant difference with $p<0.05$.

686 **Figure 2.** *OsMADS26* is expressed in differentiated peripheral tissues.

687 *In situ* hybridizations were revealed with the VectorBlue Kit III. Antisense (A, E, I) and sense
688 (B, F, J) *OsMADS26* probe hybridizations on a longitudinal section of the root tip (A, B),
689 transverse section in the seminal root (E, F) and transverse section in the third leaf (I, J) of 7-
690 day-old rice seedling. Hybridization with antisense (C, G, K) and sense (D, H, L) 18S
691 ribonucleic RNA probe were used as a positive and a negative control, respectively. ep,
692 epidermis; ex, exodermis sc, sclerenchyma; ae, aerenchyma; st: stele; ph, phloem; xy, xylem;
693 abe, abaxial epidermis; ade, adaxial epidermis; bc, bulliform cells; fib, fiber; bds, bundle
694 sheath. Scale bars = 70 µm.

695 **Figure 3**. *OsMADS26* expression is regulated by *Magnaporthe oryzae* infection.

696 Three-week-old rice seedlings of Nipponbare were challenged with two isolates of *M.oryzae*
697 virulent FR13 and avirulent CL3.6.7 or mock treated. The expression of each gene was
698 normalized using the actin gene as control. The mean and SD were calculated from three
699 independent experiments. A Student T-test (*: $P<0.05$; **: $P<0.01$) was done to establish
700 whether the relative expression level in inoculated condition was different from mock treated.

701 **Figure 4**. Over-expression and down-regulation of *OsMADS26* do not interfere with overall
702 plant development.

703 A, *OsMADS26* relative expression levels in 3-weeks-old T2 overexpressing (OX1, OX2, dark
704 bars) and controls (WT, OX0, white bars) plants cultivated in greenhouse. B, *OsMADS26*
705 expression levels in RNA down-regulated (DR5-1, DR5-2, DR3-1, DR3-2, grey bars) and
706 control (WT, DR0, white bars) plants cultivated in greenhouse. Mean and standard error were
707 obtained from two individual plants of each line. C, Control and transgenic *OsMADS26* T2
708 plants cultivated in greenhouse observed at flowering stage. A Student t-test was done to
709 establish whether the relative expression level in transgenic line was different from
710 corresponding null segregant line; *: significant difference with $p<0.05$; **: significant
711 difference with $p<0.01$; ***: significant difference with $p<0.001$.

712 **Figure 5.** *OsMADS26* negatively regulates resistance against *Magnaporthe oryzae.*

713 Plants overexpressing (OX1, OX2, black bars), down-regulated (DR5-1, DR5-2, DR3-1,
714 DR3-2, grey bars) *OsMADS26* lines and corresponding control lines transformed with empty
715 vectors or untransformed line (OX0, DR0 WT, white bars) and Maratelli, a highly susceptible
716 cultivar, were tested. A, symptom severity in leaves of transgenic and control plants
717 inoculated with the GUY11 strain of *M. oryzae.* Photographs were taken 7 days post
718 inoculation. B, percentage of susceptible versus total lesions observed in *Mo*-infected leaves 7
719 days after inoculation. Mean and standard error were from ten inoculated plants for each line.
720 Results shown are from one of two independent experiments that produced similar results. A
721 Student t-test was done to establish whether one given transgenic line was different from its
722 corresponding null segregant line; *: significant difference with $p<0.05$; **: significant
723 difference with $p<0.01$.

724 **Figure 6.** Expression of defense genes is down regulated in *OsMADS26* over-expressing
725 before and after infection by *Magnaporthae oryzae*.

726 Three-week-old rice seedling of *OsMADS26* over-expressing (OX2) line and control line
727 (OX0) were challenged with the moderately virulent isolates of *M. oryzae* GY11 (black bars)
728 or mock treated (grey bars). The RNA were extracted at post-inoculation. The expression of
729 each gene was normalized using the actin gene as control. The *POX223*, *PBZ1*, *CHI7* and
730 *PR5* genes are coding for Pathogenesis-related proteins used as classical markers of defense.
731 The *NH1*, *OsFLS2* and *WRKY28* genes are coding for regulator proteins of defense in rice.

732 The mean and SD were calculated from three independent experiments. A Student T-test (*:
733 P<0.01) was done to establish whether the relative expression level in the OX2 lines was
734 different with the line used as control.

735 **Figure 7.** *OsMADS26* negatively regulates water stress tolerance

736 Six independent lines: over-expressing (OX2) or down-regulated (DR5-2, DR3-1)
737 *OsMADS26* and corresponding control lines transformed with empty vectors (OX0, DR0) or
738 wild type (WT) were used for this experiment. A, Drought stress was applied on twenty days
739 old plants growing in greenhouse in pots, by stopping watering during 18 days followed by 15
740 days of rewatering. The pictures were taken 15 days after rewatering. B, Relative water
741 content (RWC) of plants was measured on the last expanded leaf before and at 5 days, 11
742 days and 15 days after watering stopping. Mean value and standard error were calculated from
743 five individual plants for each line. C and D, RT-qPCR expression analysis of drought- and
744 salt-responsive rice genes *RAB21* (C) and *SALT* (D) in control and transgenic plants before
745 and during drought stress. RNA were extracted from leaves of two plants of each line that had
746 closest relative water content (RWC). We did not measure gene expression 15 days after the
747 water deficit period since the control and *MADS26* overexpressing plants were already highly
748 damaged. Mean and standard error were from two individual plants for each line. A Student t-
749 test was done to establish whether the RWC or the gene expression level in transgenic lines
750 was different from corresponding control line; *: significant difference with p<0.05; ** :
751 significant difference with p<0.01; *** : significant difference with p<0.001.

752 **Figure 8.** *OsMADS26* down-regulation confers tolerance to water deficit under field
753 conditions.

754 Plants were grown in the field in CIAT (Colombia) and a drought stress was applied (see
755 Methods). The shape of the plant 17 DAS (DAS= days after stress) is shown (A) and the
756 chlorophyll fluorescence (B) was measured at the indicated times after stress in three
757 independent blocks on three plants. Yield was measured at the end of the experiment (C). The
758 mean and SD are shown and a T-test (n=9;***: P<0.001) was used to evaluate statistical
759 difference between the over-expressing OX2 and down-regulated DR3-1 transgenic lines with
760 their respective controls OX0 and DR0.

761 **Figure 9.** Genome wide gene expression regulations in OsMADS26 over-expressing or down
762 regulated lines.

763 Number of genes significantly differentially expressed in the microarray experiment. 71 (32 +
764 39) genes presented an inverted regulation profile in OE and DR lines. Green and red colors
765 depict respectively genes induced or repressed by OsMADS26 expression.

766 ## Tables

Table I: Plant phenotype of control and transgenic *OsMADS26* lines after 7-day of *in vitro* culture (MS/2), 72 days after germination in greenhouse and from flowering to harvest.

Line name	HTG_7 (cm)	HTG_76 (cm)	TIL_76	BEG (DAG)	FD (DAG)	DW (g)	PW (g)	P1000 (g)
WT	6.06 ± 1.51	97.53 ±0.59	12.33 ± 0.33	80.33 ± 0.33	81.67 ± 0.88	9.74 ± 2.34	15.18 ± 2.45	21.80 ± 0.71
OX0	6.34 ± 1.33	97.47 ± 2.06	10.67 ± 0.33	82.33 ± 1.20	84.00 ± 1.00	8.73 ± 0.87	9.52 ± 0.95	20.34 ± 0.62
DR0	6.72 ± 1.27	95.23 ± 1.36	11.33 ± 1.33	81.67 ± 0.67	83.67 ± 0.67	7.96 ± 3.80	8.88 ± 4.28	17.89 ± 3.93
OX1	3.84 ± 0.67**	100.60 ± 2.17	10.33 ± 1.45	80.00 ± 1.53	81.67 ± 1.86	8.00 ± 1.42	8.78 ± 1.50	21.39 ± 0.30
OX2	2.41 ± 0.92***	93.40 ± 2.84	12.33 ± 0.88	83.67 ± 0.88	86.00 ± 1.00	8.21 ± 1.12	8.93 ± 1.34	20.38 ± 0.72
DR5-1	1.68 ± 0.68***	87.90 ± 2.51*	7.80 ± 2.08	83.00 ± 1.15	85.67 ± 0.67	3.86 ± 1.07	4.21 ± 1.14	16.32 ± 0.48
DR5-2	1.61 ± 0.29***	95.37 ± 1.84	6.67 ± 0.67*	82.33 ± 0.67	85.00 ± 0.00	4.93 ± 0.40	5.48 ± 0.39	19.79 ± 1.15
DR3-1	1.61 ± 0.31***	90.53 ± 1.79	9.67 ± 1.33	85.00 ± 0.00**	87.00 ± 0.58**	6.62 ± 1.37	7.33 ± 1.65	21.42 ± 0.73
DR3-2	0.84 ± 0.18***	97.20 ± 1.73	9.00 ± 1.00	84.67 ± 0.33**	86.33 ± 0.33*	7.76 ± 0.73	8.41 ± 0.67	20.01 ± 0.68

BEG: flowering beginning; DAG: day after germination; DW plant dry weight after seed harvesting; FD: flowering date; HTG_7: Plant height measured at 7 DAG; HTG_76: Plant height measured at 76 DAG; PW: panicle weight; TIL_76: number of tillers counted at 76 DAG; W1000: weight of 1000 seeds; Reported values are the mean value and standard error obtained for three individual plants. Results shown are from one of two independent biological repetitions that produced similar results.

HTG_7: Height of 7-d-old plants cultivated *in vitro* condition (MS/2). Reported values are the mean and standard error for 14 individual plants of each line.

780 A Student t-test was done to establish whether the parameter measured in transgenic lines was different from corresponding control line; *: significant difference with $p<0.05$;
781 **: significant differentce with $p<0.01$; ***: significant difference with $p<0.001$.
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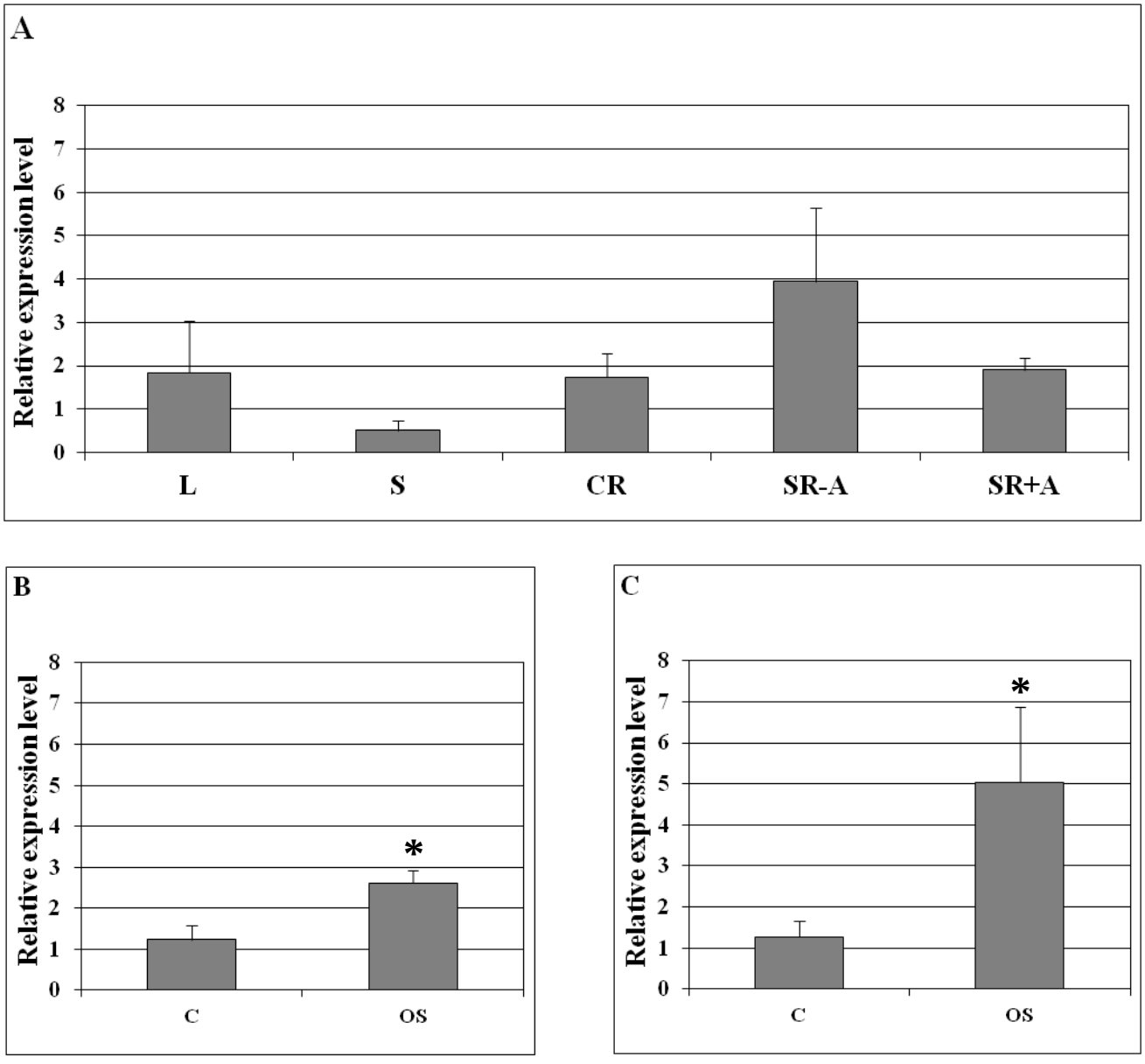

Figure 1. ***OsMADS26*** **is expressed in shoots and roots and is induced by osmotic stress.**
A, expression of *OsMADS26* in different organs of 7-day-old rice seedlings cultivated in standard condition (MS/2). L: leaf, S: stem base, CR: crown root, SR-A: seminal root without apex, SR+A: seminal root apex. B-C, expression patterns of *OsMADS26* in root (B) and shoot (C) in standard condition (c) or under osmotic stress (OS: MS/2 + 100 mM Mannitol). Mean and standard error were calculated from two independent experiments consisting of three technical replicates each. A Student t-test was used to compare the relative expression level observed in standard andstress conditions; *: significant difference with $p<0.05$.

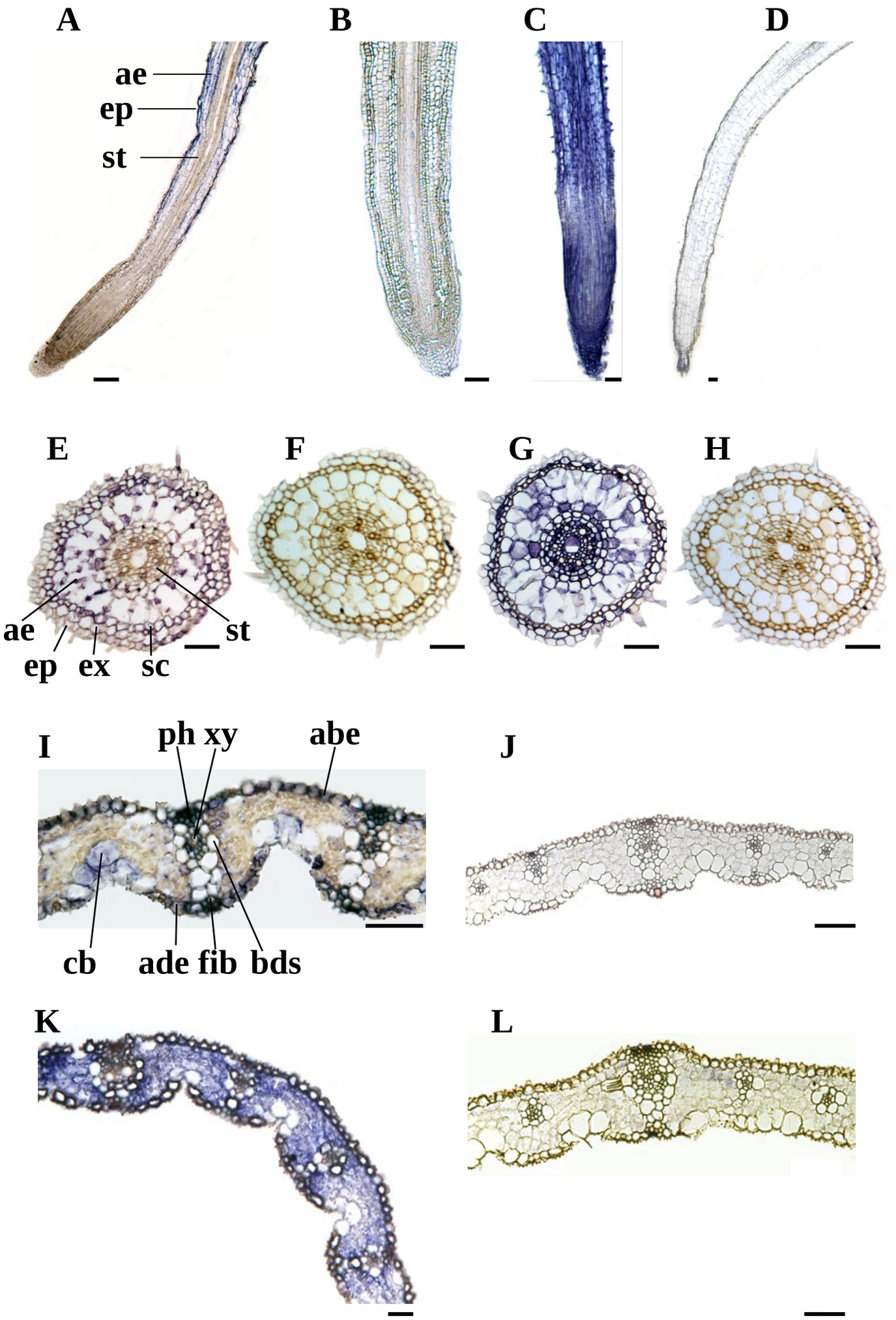

Figure 2. *OsMADS26* is expressed in differentiated peripheral tissues.
In situ hybridizations were revealed with the VectorBlue Kit III. Antisense (A, E, I) and sense (B, F, J) *OsMADS26* probe hybridizations on a longitudinal section of the root tip (A, B), transverse section in the seminal root (E, F) and transverse section in the third leaf (I, J) of 7-day-old rice seedling. Hybridization with antisense (C, G, K) and sense (D, H, L) 18S ribonucleic RNA probe were used as a positive and a negative control, respectively. ep, epidermis; ex, exodermis sc, sclerenchyma; ae, aerenchyma; st. stele; ph, phloem; xy, xylem; abe, abaxial epidermis; ade, adaxial epidermis; bc, bulliform cells; fib, fiber; bds, bundle sheath. Scale bars = 70 µm.

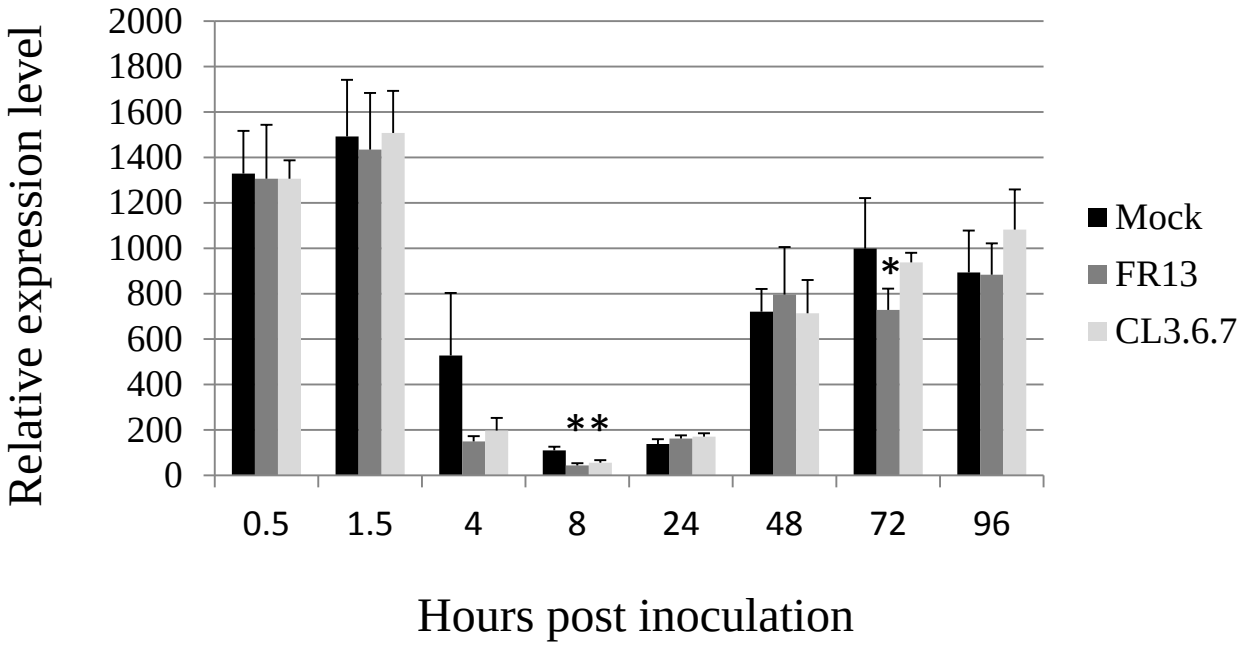

Figure 3. *OsMADS26* expression is regulated by *Magnaporthe oryzae* infection.
Three-week-old rice seedlings of Nipponbare were challenged with two isolates of *M.oryzae* virulent FR13 and avirulent CL3.6.7 or mock treated. The expression of each gene was normalized using the actin gene as control. The mean and SD were calculated from three independent experiments. A Student T-test (*: P<0.05; **: P<0.01) was done to establish whether the relative expression level in inoculated condition was different from mock treated.

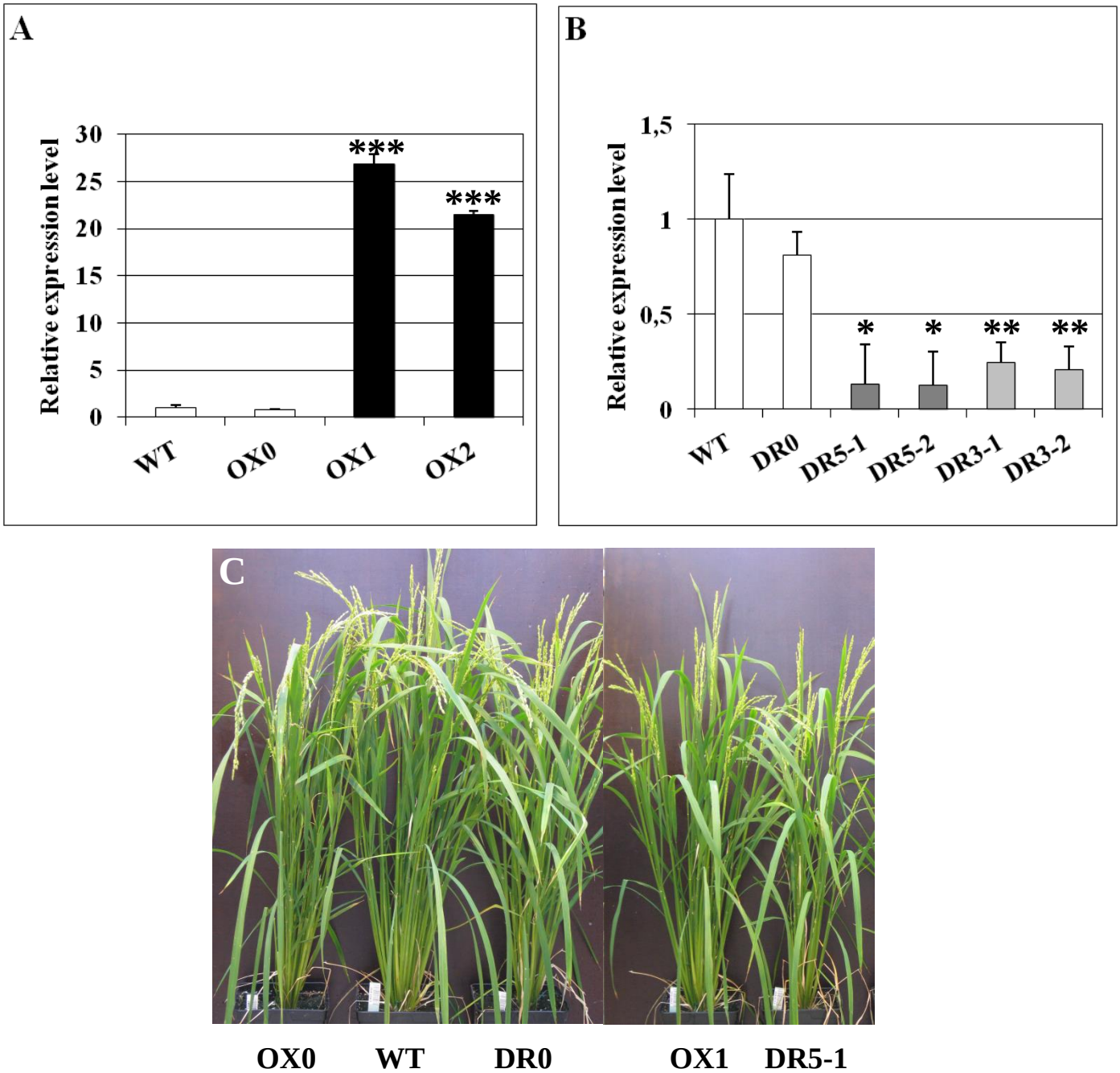

Figure 4. Over-expression and down-regulation of *OsMADS26* do not interfere with overall plant development.

A, *OsMADS26* relative expression levels in 3-weeks-old T2 overexpressing (OX1, OX2, dark bars) and controls (WT, OX0, white bars) plants cultivated in greenhouse. B, *OsMADS26* expression levels in RNA down-regulated (DR5-1, DR5-2, DR3-1, DR3-2, grey bars) and control (WT, DR0, white bars) plants cultivated in greenhouse. Mean and standard error were obtained from two individual plants of each line. C, Control and transgenic *OsMADS26* T2 plants cultivated in greenhouse observed at flowering stage. A Student t-test was done to establish whether the relative expression level in transgenic line was different from corresponding null segregant line; *: significant difference with $p<0.05$; **: significant difference with $p<0.01$; ***: significant difference with $p<0.001$.

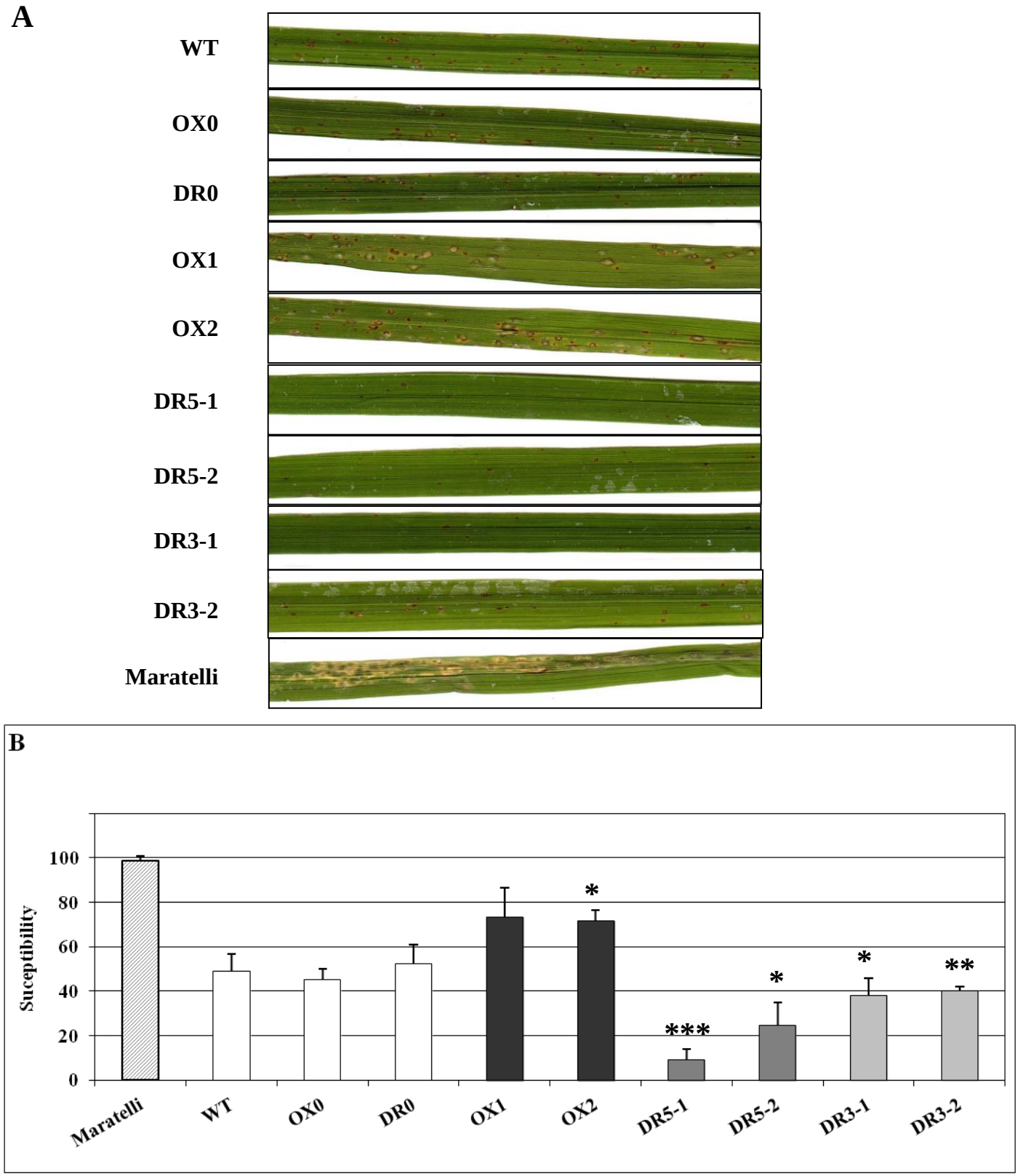

Figure 5. *OsMADS26* negatively regulates resistance against *Magnaporthe oryzae*.
Plants overexpressing (OX1, OX2, black bars), down-regulated (DR5-1, DR5-2, DR3-1, DR3-2, grey bars) *OsMADS26* lines and corresponding control lines transformed with empty vectors or untransformed line (OX0, DR0 WT, white bars) and Maratelli, a highly susceptible cultivar, were tested. A, symptom severity in leaves of transgenic and control plants inoculated with the GUY11 strain of *M. oryzae*. Photographs were taken 7 days post inoculation. B, percentage of susceptible versus total lesions observed in *Mo*-infected leaves 7 days after inoculation. Mean and standard error were from ten inoculated plants for each line. Results shown are from one of two independent experiments that produced similar results. A Student t-test was done to establish whether one given transgenic line was different from its corresponding null segregant line; *: significant difference with $p<0.05$; **: significant difference with $p<0.01$.

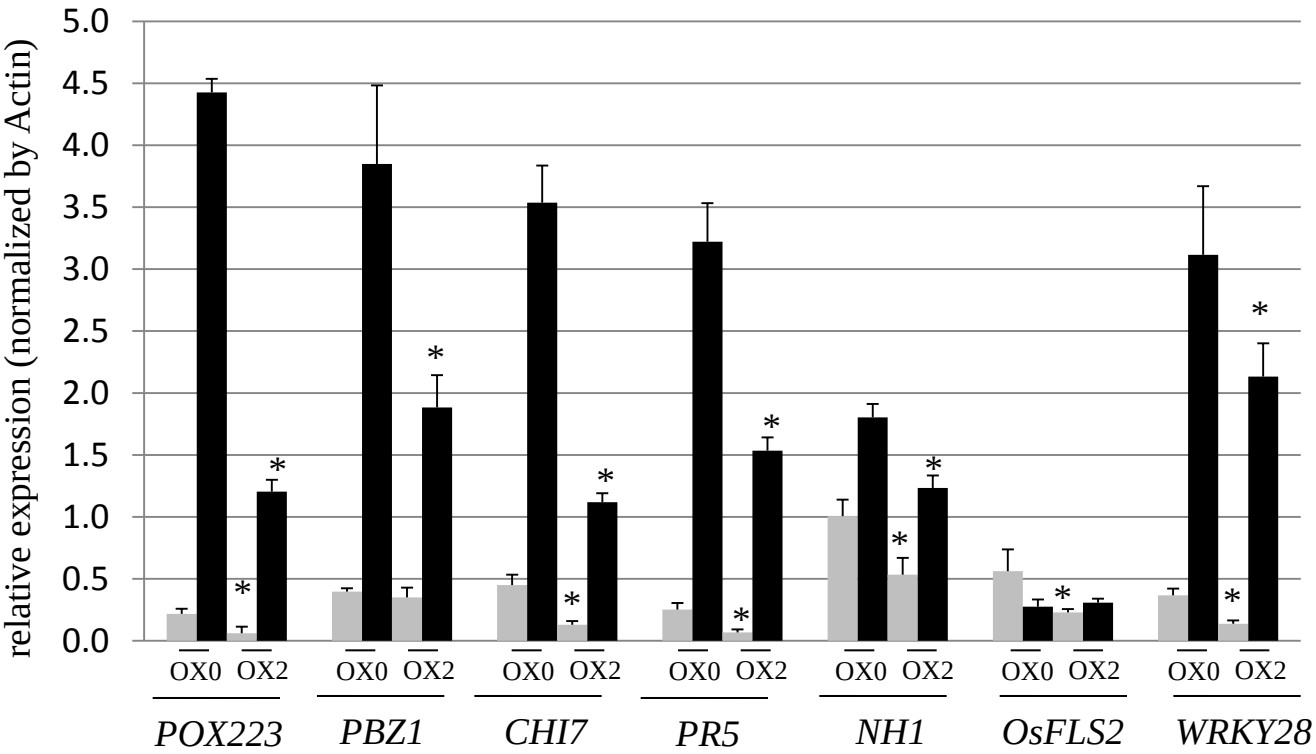

Figure 6. Expression of defense genes is down regulated in *OsMADS26* over-expressing before and after infection by *Magnaporthae oryzae*.

Three-week-old rice seedling of *OsMADS26* over-expressing (OX2) lines and a control line (OX0) were challenged with the moderately virulent isolates of *M. oryzae* GY11 (black bars) or mock treated (grey bars). The RNA were extracted at 48h post-inoculation. The expression of each gene was normalized using the actin gene as control. The *POX223*, *PBZ1*, *CHI7* and *PR5* genes are coding for Pathogenesis-related proteins used as classical markers of defense. The *NH1*, *OsFLS2* and *WRKY28* genes are coding for regulator proteins of defense in rice. The mean and SD were calculated from three independent experiments. A Student T-test (*: P<0.01) was done to establish whether the relative expression level in the OX2 lines was different with the Ox0 line used as control.

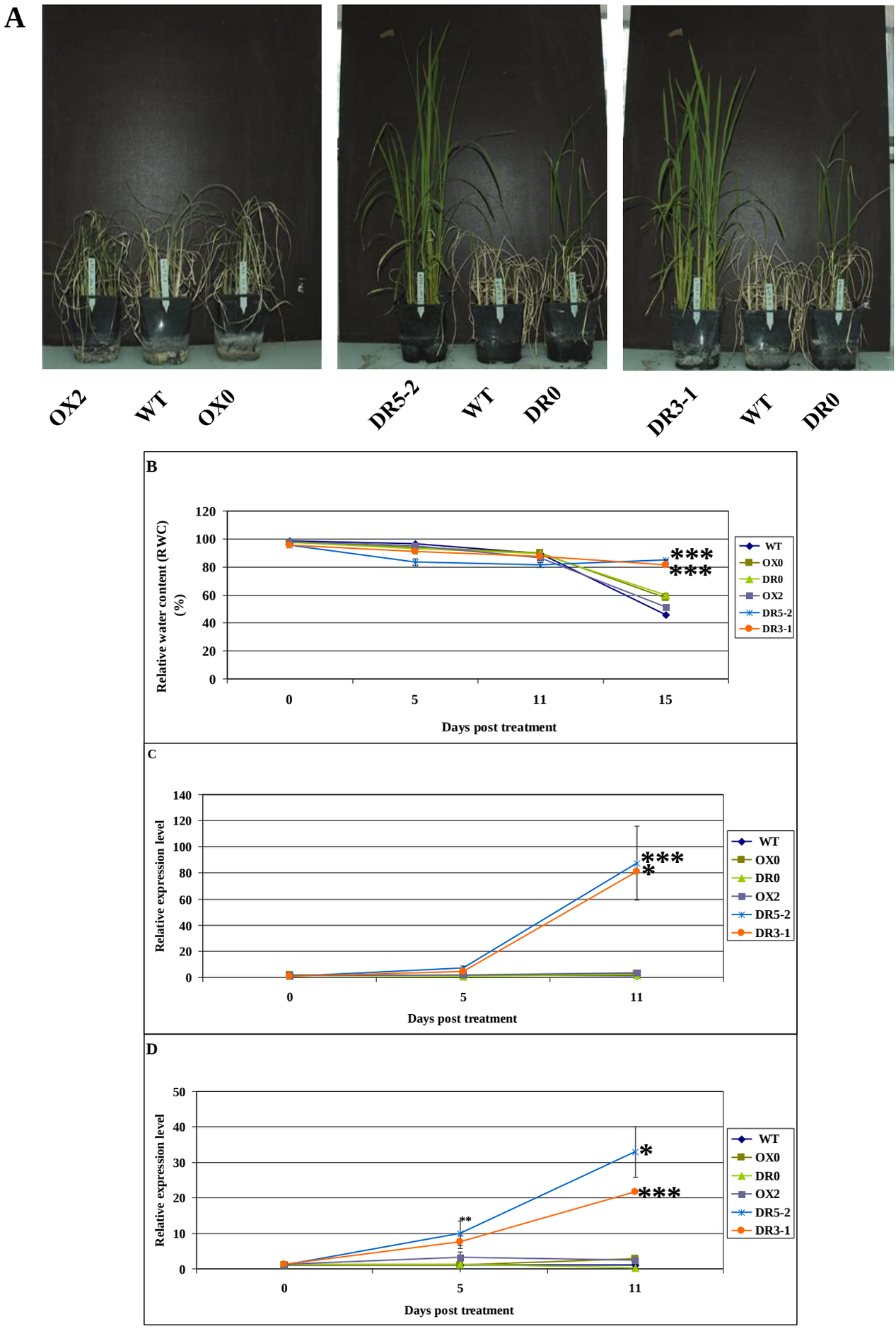

Figure 7. *OsMADS26* negatively regulates water stress tolerance

Six independent lines: over-expressing (OX2) or down-regulated (DR5-2, DR3-1) *OsMADS26* and corresponding control lines transformed with empty vectors (OX0, DR0) or wild type (WT) were used for this experiment. A, Drought stress was applied on twenty days old plants growing in greenhouse in pots, by stopping watering during 18 days followed by 15 days of rewatering. The pictures were taken 15 days after rewatering. B, Relative water content (RWC) of plants was measured on the last expanded leaf before and at 5 days, 11 days and 15 days after watering stopping. Mean value and standard error were calculated from five individual plants for each line. C and D, RT-qPCR expression analysis of drought- and salt-responsive rice genes *RAB21* (C) and *SALT* (D) in control and transgenic plants before and during drought stress. RNA were extracted from leaves of two plants of each line that had closest relative water content (RWC). We did not measure gene expression 15 days after the water deficit period since the control and *MADS26* overexpressing plants were already highly damaged. Mean and standard error were from two individual plants for each line. A Student t-test was done to establish whether the RWC or the gene expression level in transgenic lines was different from corresponding control line; *: significant difference with $p<0.05$; ** : significant difference with $p<0.01$; *** : significant difference with $p<0.001$.

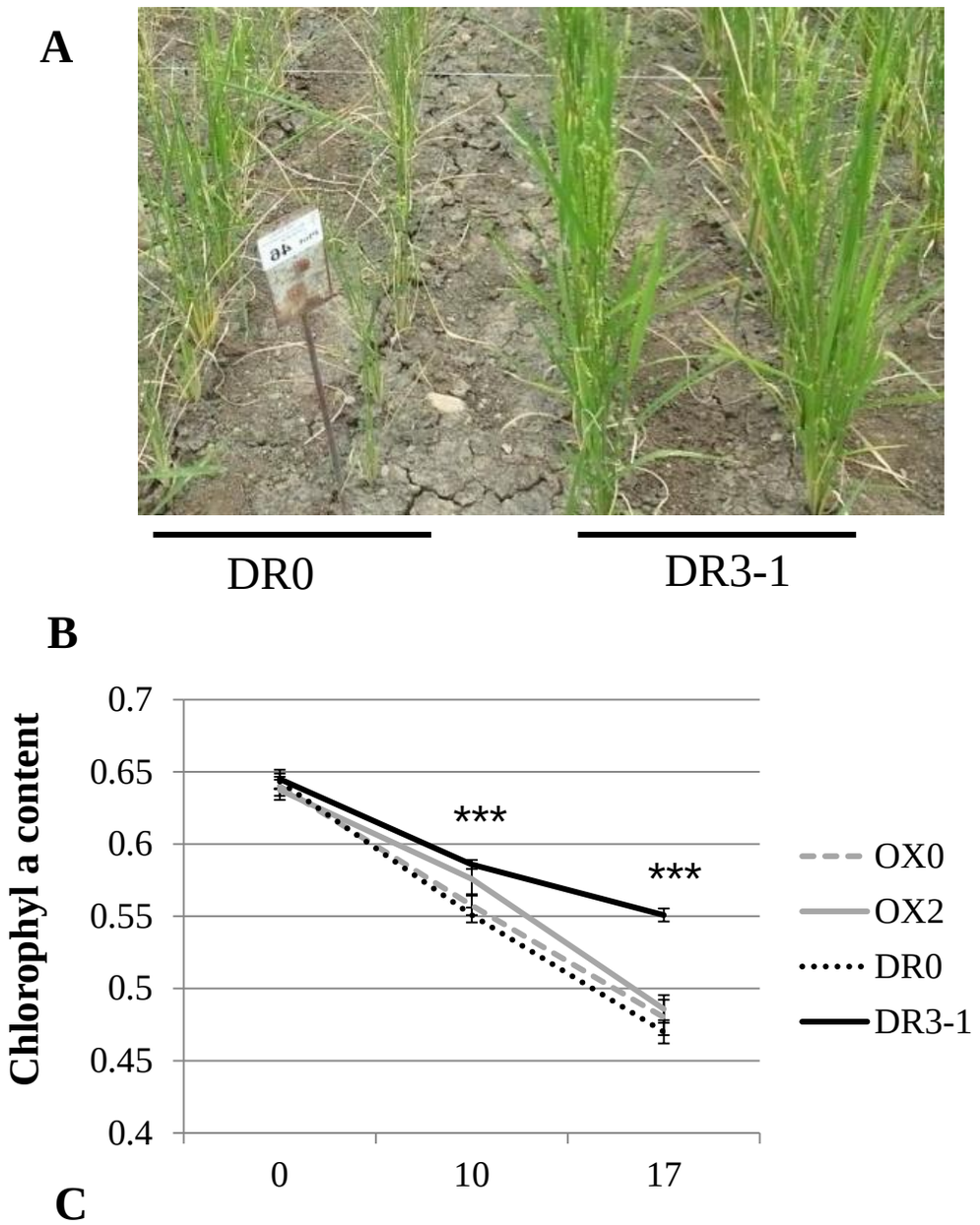

Figure 8. *OsMADS26* down-regulation confers tolerance to water deficit under field conditions.
Plants were grown in the field in CIAT (Colombia) and a drought stress was applied (see Methods). The shape of the plant 17 DAS (DAS= days after stress) is shown (A) and the chlorophyll fluorescence (B) was measured at the indicated times after stress in three independent blocks on three plants. Yield was measured at the end of the experiment (C). The mean and SD are shown and a T-test (n=9;***: P<0.001) was used to evaluate statistical difference between the over-expressing OX2 and down-regulated DR3-1 transgenic lines with their respective controls OX0 and DR0.

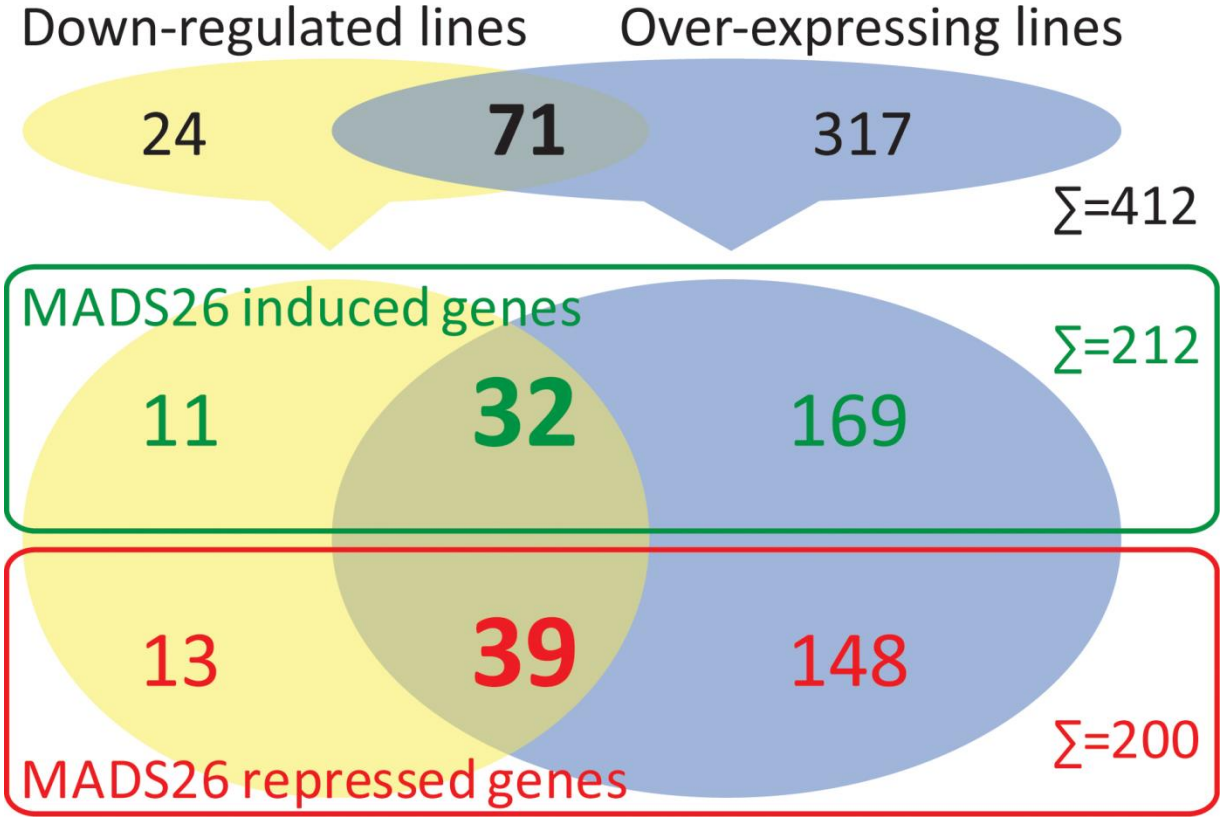

Figure 9. Genome wide gene expression regulations in OsMADS26 over-expressing or down regulated lines.

Number of genes significantly differentially expressed in the microarray experiment. 71 (32 + 39) genes presented an inverted regulation profile in OE and DR lines. Green and red colors depict respectively genes induced or repressed by OsMADS26 expression.

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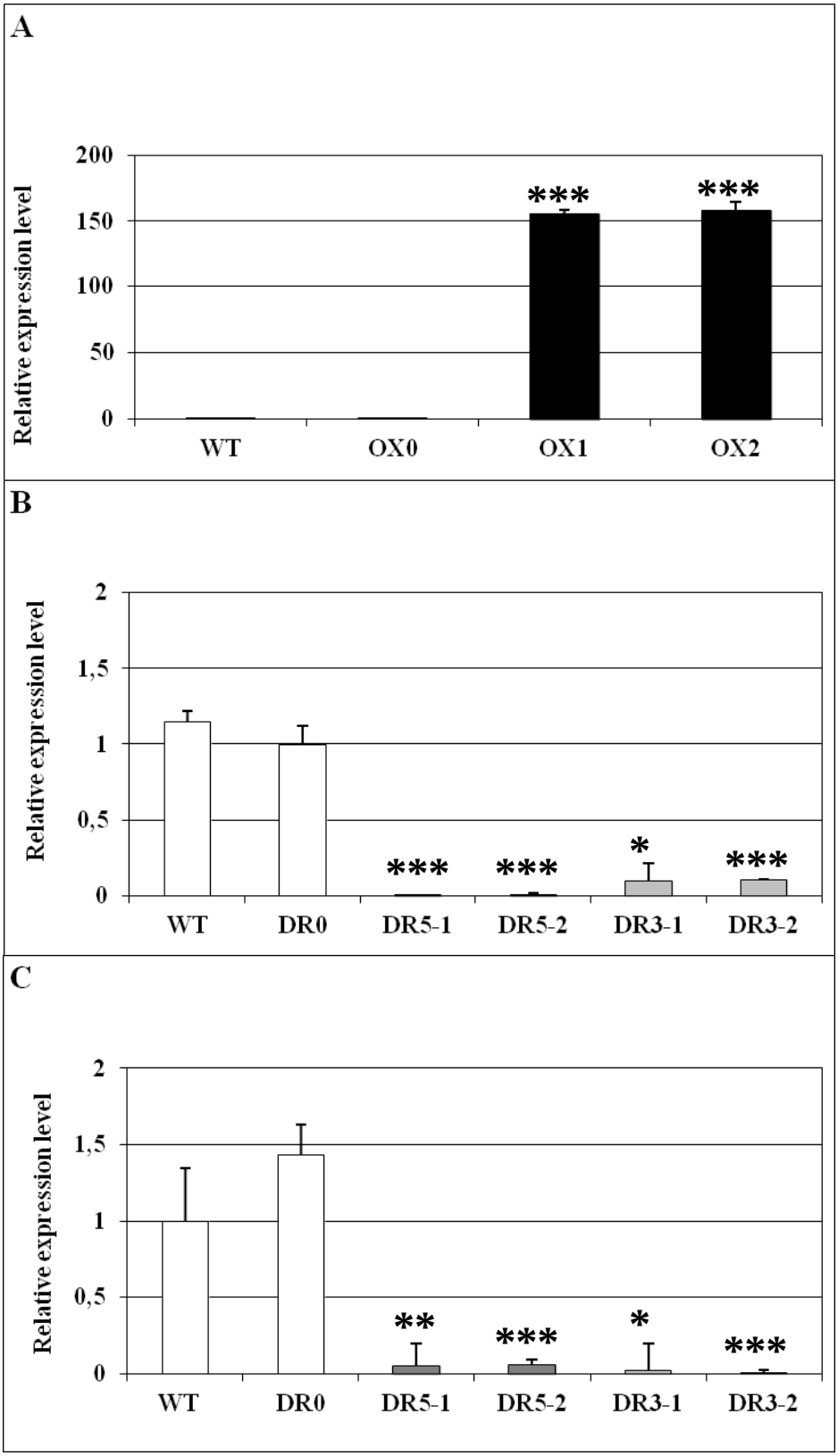

Figure S1: ***OsMADS26* over- or down-expression is stable across generations**

A, *OsMADS26* expression in overexpressing (OX1, OX2, dark bars) and corresponding control (OX0, WT, white bars) T4 plants. B, *OsMADS26* expression in interfered (DR5-1, DR5-2, DR3-1, DR3-2, grey bars) and corresponding control (PDP, WT, white bars) T4 plants. Mean value and standard error were obtained from two independent experiments. C, *OsMADS26* expression levels in RNA interfered (grey bars) and control (white bars) of 7-day-old T2 seedlings cultivated on MS/2 medium added with 125 mM of Mannitol. Mean and standard error were obtained from 14 individual plants of each line. A Student t-test was done to establish whether the RWC or the gene expression level in transgenic lines was different from corresponding control line; *: significant difference with $p<0.05$; ** : significant difference with $p<0.01$; *** : significant difference with $p<0.001$.

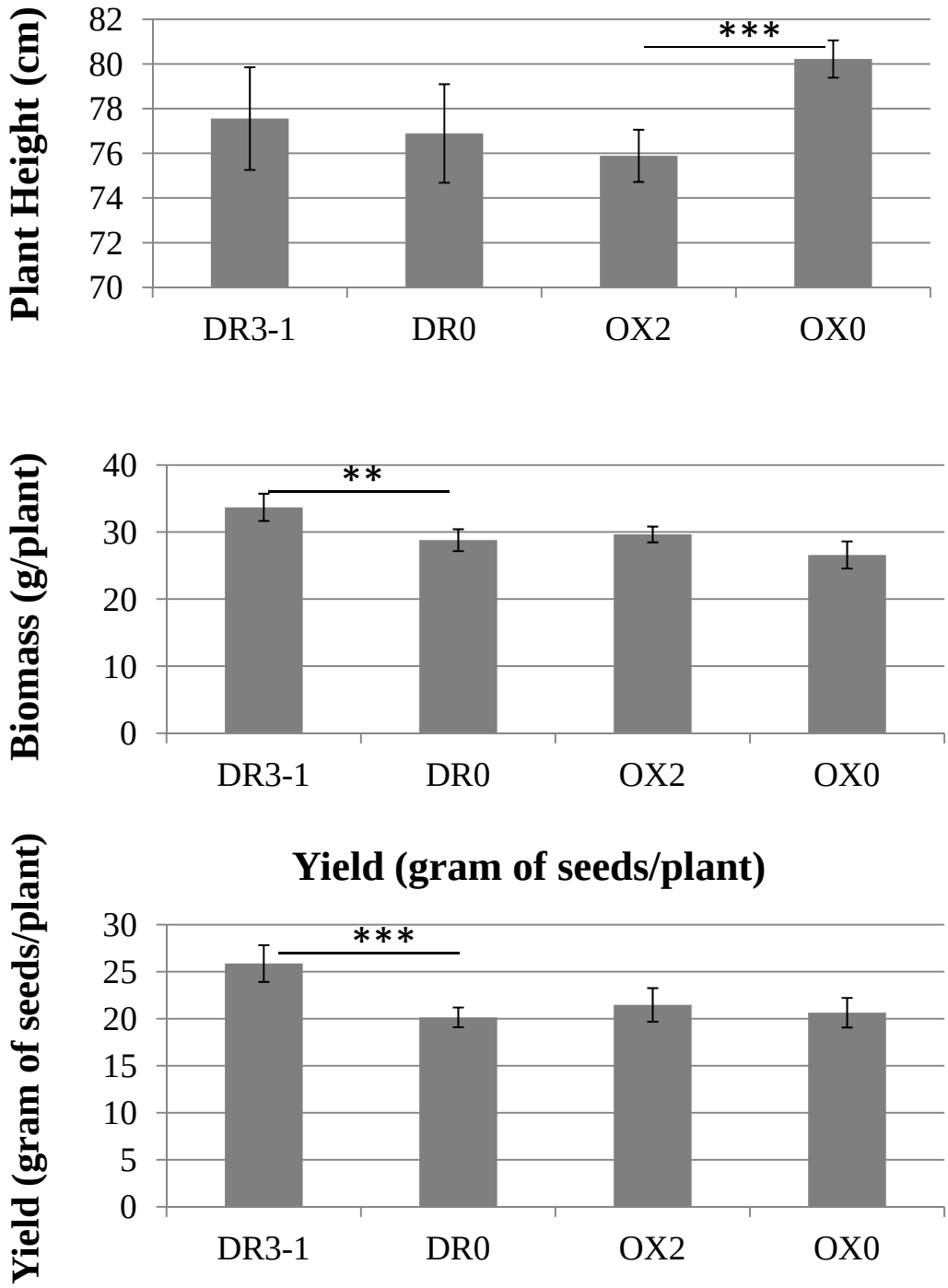

Figure S2: *OsMADS26* over-expressing and down-regulated lines growth under normal watering condition in the field.

Plants were grown under normal water condition in the field in CIAT (Colombia). The height, biomass and yield were measured at the end of the experiment. The mean and SD are shown and a T-test (n=9;**: P<0.01; ***: P<0.001) was used to evaluate statistical difference between the over-expressing OX2 and down-regulated DR3-1 transgenic lines with their respective controls OX0 and DR0.

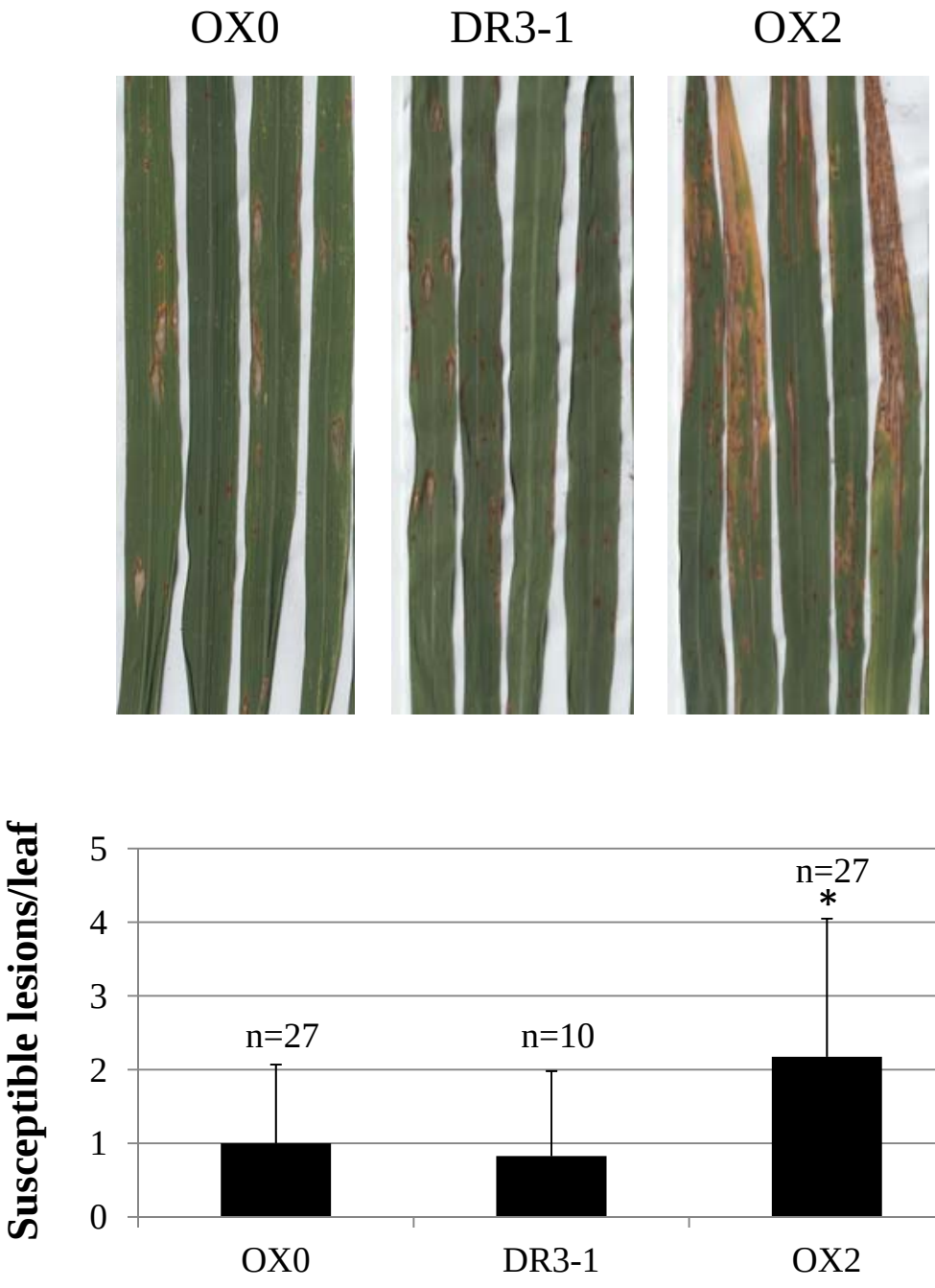

Figure S3: **Rice blast resistance evaluation of over-expressing or down-regulated *OsMADS26* lines under semi-controlled field conditions.**

Plants were grown in nethouses in LMI-RICE (Hanoi, Vietnam) and inoculated each week for four weeks with spores of the virulent *M. oryzae* isolate VT15. Symptoms were measured every week after epidemics started and one time point is provided. The greyish lesions were counted as a measure of susceptibility. The mean and SD are shown and a T-test (*: $P<0.05$) was used to evaluate statistical difference between the *OsMADS26* over-expressing OX2 and down-regulated DR3-1 transgenic lines with their respective controls OX0 and DR0.

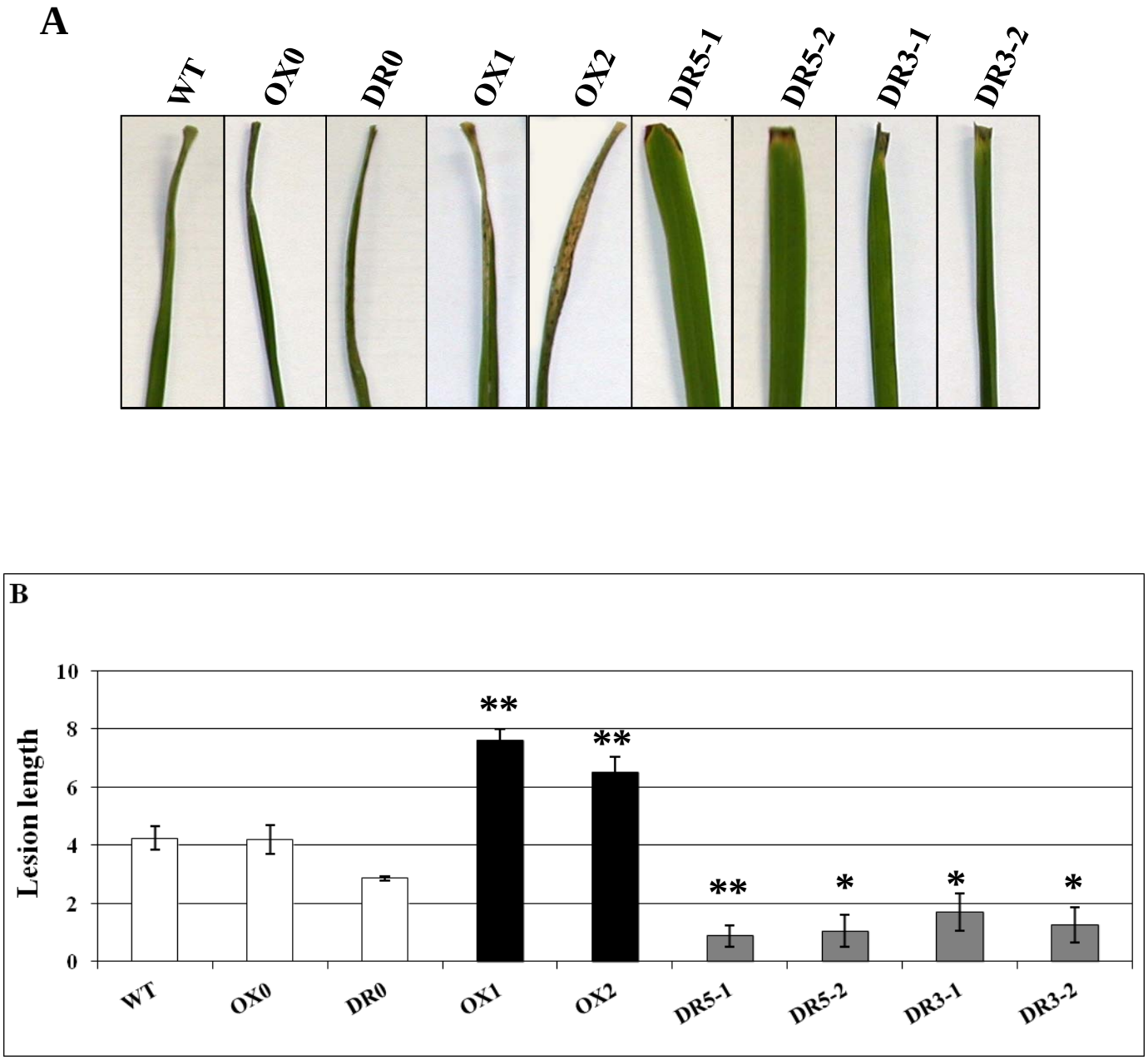

Figure S4: OsMADS26 negatively regulates resistance against *Xanthomonas oryzae* pv. *oryzae* (*Xoo*).

Plants over-expressing (OX1, OX2) (black bars) or down-regulated (DR5-1, DR5-2, DR3-1, DR3-2) (grey bars) *OsMADS26* and corresponding control lines transformed with empty vectors (OX0, DR0) or untransformed line (WT) (white bars) were tested. A: Symptom severity in leaves of transgenic and control plants inoculated with the PXO99A strain of *Xoo.* Photographs were taken at 14 days post inoculation (dpi). B: Length of lesion produced in *Xoo*-infected leaves at 14 dpi. Mean and standard error were obtained from nine inoculated plants for each line. Results shown are from one of two independent experiments that produced similar results.

A Student t-test was done to establish whether one given mutant line was different from its corresponding control line; *: significant difference with $p<0.05$; **: significant difference with $p<0.01$.

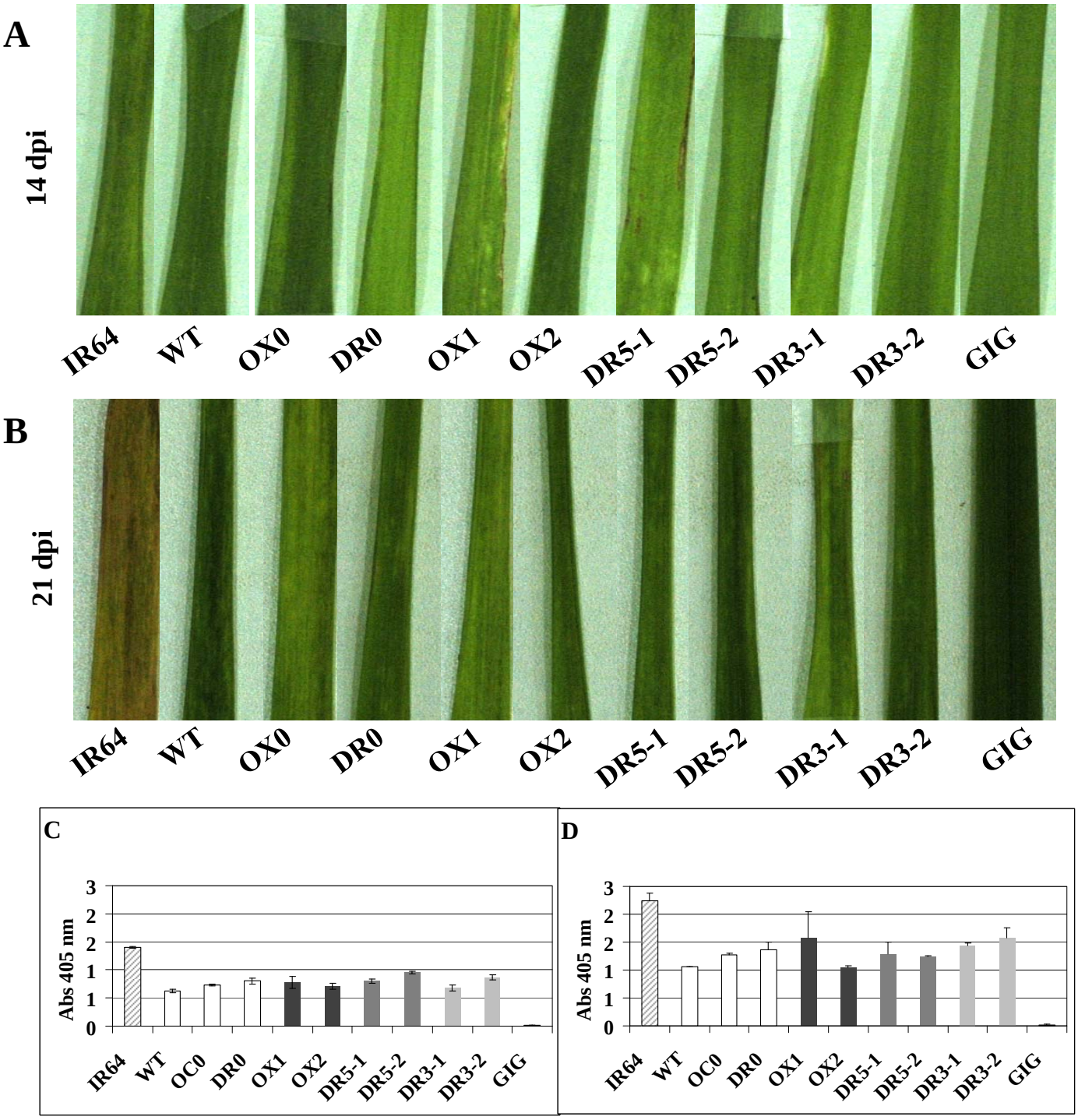

Figure S5: *OsMADS26* expression level does not affect resistance against Rice Yellow Mottle Virus (RYMV).

Nine independent lines of over-expressing (OX1, OX2, black bars), down-regulated (DR5-1, DR5-2, DR3-1, DR3-2, grey bars) *OsMADS26* lines and corresponding control lines transformed with empty vectors or untransformed line (OX0, DR0 WT, white bars), IR64 (susceptible control, dashed bar) and Gigante (resistant control) cultivars were tested. A,B, Symptom severity in leaves of transgenic and control plants inoculated with RYMV at 14, and 21 days postinoculation (dpi). C,D, ELISA virus accumulation quantification in leaves of transgenic and control plants inoculated with RYMV at 14 and 21 (dpi). WT and control transformed with empty vectors (white bars), over-expressing lines (black bars), down-regulated lines (grey bars) and reference cultivars (dashed bars) Gigante (GIG), and IR64. Leaves from ten plants for each line were pooled and the virus content determined by enzyme-linked immunosorbent assay using an antibody generated against the coat protein as described (N'Guessan et al. 2000). Mean and standard error were obtained from ten inoculated plants for each line. Results shown are representative of data obtained from two independent experiments.

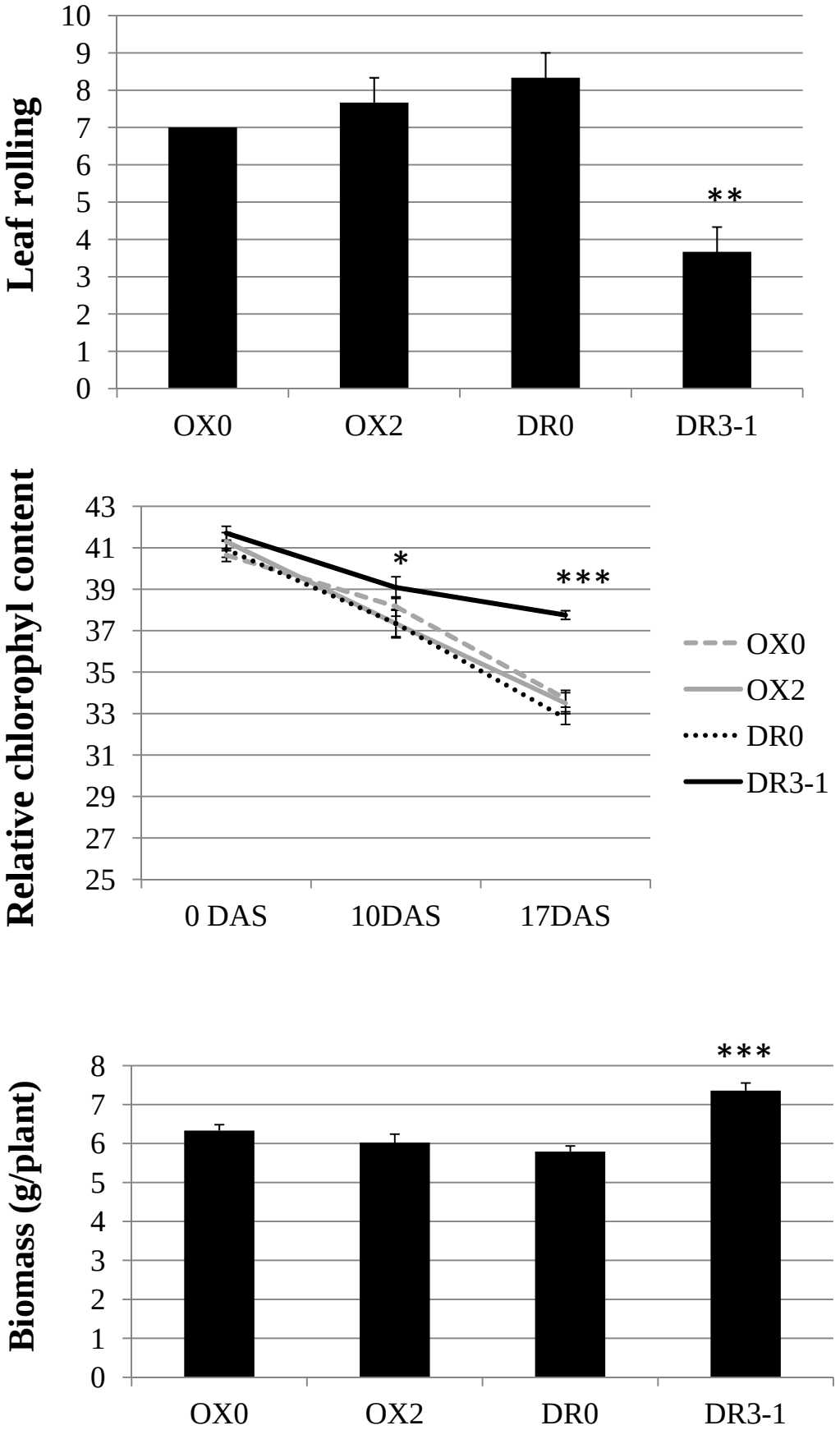

Figure S6: *OsMADS26* down regulation enhances water deficit tolerance in the field.
Plants were grown in the field in CIAT (Colombia) and a drought stress was applied (see Methods). The leaf rolling score (0-9 scale from the less to the more) of the plant 17 DAS (DAS= days after stress) is given (A) and SPAD value (B) was measured at the indicated times after stress in three independent blocks on three plants. The total biomass was measured at the end of the experiment (C). The mean and SD are shown and a T-test (n=9;*: P<0.05; **:P<0.01; ***: P<0.001) was used to evaluate statistical difference between the over-expressor OX2 and interfered DR3-1 transgenic lines with their respective controls OX0 and DR0.

GST1

gtaagcaagagatagggataaggg**GAAGAGGAGGAAGAAGGAGG**aggtgtagggaga
aaccggagcaacctcgaagctagtccaaactagtgggaggttgtctttccggcaagccggagcccggagc
tatcgatcatcaagctttctaccccgaccgacgaggaagaagacgactgatcaattgatcaaaccgatctct
ccatagctaggtag**acaggaggagaggaggaagaagaggg**ggagaggagacttatcttgatcg***ATG***gcg
cgaggcaaggtgcagctccgtcgcatcgagaacccggttcACCGTCAGGTCACCTTCTGCAA
gcgccgtgccggcctgctgaagaaggccagggagctctccatcctctgcgaggccgacatcggcatcatcat
cttctccgcccacggcaagctctacgacctcgccaccaccggaaccatggaggagctgatcgagaggtacaa
gagtgctagtggcgaacaggccaacgcctgcggcgaccagagaatggacccaaaacaggaggcaatggt
gctcaaacaagaaatcaatctactgcagaagggcctgaggtacatctatgggaacagggcaaatgaacaca
tgactgttgaagagctgaatgccctagagaggtacttagagatatggatgtacAACATTCGCTCCGC
AAAGATGCagataatgatccaagagatccaagcactaaagagcaaggaaggcatgttgaaagctgcta

GST2

*acgaaattctccaagaaaaga**tagtagaacagaatggtctg**atcgacgtagg**catgatggtagcagatcaac***
agaatgggcattttagtacagtcccactgttagaagagatcactaacccactgactatactgagtggctattcta
cttgtaggggctcggagatgggctattccttcTAA**cactaataatggcctgggggatacttgtgttcattacta**
gtgtgtaatatggttaataatgcttgtgttgctgtttgctttgctattctgatgtaccttatttagacaagttcccg
caggaagtgtcttttagtattgtatttgtcttgggctgtggtgctttgtttttccCTAAAGAACTCTT
GAGGAGCtctgttgttgaaccatttcaagtaattgagactattgtttcc

Primers used for *OsMADS26* cDNA amplification

Forward: 5'-gaagaggaggaagaaggagg -3'
Reverse: 5'-gctcctcaagagttctttag -3'

Primers used for GST1 amplification and cloning

I^st^ Amplification

Forward: 5'-aagcaagagatagggataag -3'
Reverse: 5'-cgatcaagataagtctcctc -3'

2^nd^ Amplification (with *att*B sequence)

Forward: 5'-**ggggacaagtttgtacaaaaaagcaggct**gaagaggaggaagaaggagg-3'
Reverse: 5'-**ggggaccactttgtacaagaaagctgggt**ccctcttcttcctcctcc -3'

Primers used for GST2 amplification and cloning

I^st^ Amplification

Forward: 5'-tagtagaacagaatggtctg -3'
Reverse: 5'-gttgaaccatttcaagtaat -3'

2^nd^ Amplification (with *att*B sequence)

Forward: 5'-**ggggacaagtttgtacaaaaaagcaggct**catgatggtagcagatcaac -3'
Reverse: 5'-**ggggaccactttgtacaagaaagctgggt**gctcctcaagagttctttag -3'

Figure S4: Sequence of *OsMADS26* cDNA, GST1 and GST2 position in 5' and 3'-UTR and primer sequences used for PCR amplification.

In bold: GST sequences cloned in pANDA vector and used for RNA interference induction; underlined: nested primers used for amplification of GST1 and GST2; Underlined capitals: primers used for the amplification of the cDNA sequence cloned in PC5300.OE vector for *OsMADS26* overexpression; Capitals: primers used for the analysis of *OsMADS26* expression by RT-qPRC in transgenic plants. In italic: Open reading frame (ORF), in italic, capital and bold: start and stop codons. In grey: BP recombination sequence (gateway cloning technology of INVITROGEN).

Table SII: Primers used for RT-qPCR gene expression studies

Name	Gene	Function	Forward	Reverse	Reference
Actin	Os03g50890	Actin	GCGTGGACAAAGTTTTCAACCG	TCTGGTACCCTCATCAGGCATC	-
CHI7	Os06g51050	chitinase	CAATGCACACGAGATTGTGA	CCGCATTGTGTTAACGTCCA	Kaku et al, 2006
PR5	Os08g04580	CsAtPR5, putative, expressed	TTGGCTTCTGTCTGCTTGAA	AGCTGCATCAACCATGCTAA	-
EXP	Os06g11070	Expressed protein	TCCATCTGCTCCCGTTGTTGTG	AAAGAGTTCGCCACCAACCGTC	(Caldana et al., 2007)
NH1	Os01g09800	Regulatory protein NPR1, putative, expressed	CCTGATGGTTGCCTTCTGTC	ATTCAAGCACTTGTATTACACCTC	(Chern et al., 2005)
OsFLS2					
OsMADS26	Os08g02070	Transcription factor activity	GCTCGGAGATGGGCTATTCCTTC	GACACTTCCTGCGGGAACTTGTC	(Shinozuka et al., 1999)
PBZ1	Os12g36880	Probenazole induced protein PBZ1/PR10	CCGGGCACCATCTACACC	CCTCGATCATCTTGAGCATGC	(Midoh and Iwata, 1996; Swarbrick et al., 2008)
POX223	Os07g48020	Peroxidase 2 precursor, putative, expressed	ACGACGCCCAACGCCTTC	CTTCCAGCAACGAACGCATCC	(Vergne et al., 2007)
Rab21	AK109096	Rice dehydrin	TGTGTGATCGGTGTTTCGAT	CCACACGCGCACTTACATAC	(Claes et al., 1990; Quilis et al., 2008)
Salt	AF001395	Salt-stress-induced protein	CCCCATTGTCTGTGTACGTG	GGGATTAGTTGCCCATGGAT	(Oh et al., 2005; Quilis et al., 2008)
WRKY28		Os06g44010	CGCCGATGAACTTTGCTC	CCACCTTGGCACGTGTAGA	Delteil et al, 2012