PATTERN OF EXPRESSION ON WEEDY RICE OF THREE MAJOR GENES ASSOCIATED WITH SEED DORMANCY IN CULTIVATED RICE

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ABSTRACT: Seed dormancy is associated with the invasiveness and persistence of weedy rice that is one of the main constraints of rice production. The aim of this study was to evaluate the expression of genes originally associated with seed dormancy in cultivated rice in different stages of seed development and germination in order to access the importance of these genes in weedy rice. Gene expression was evaluated during embryo formation, mature seeds and at five timepoints during seed germination by real-time PCR. The expression of the gene *OsMADS29* at all stages of seed development and germination was higher in the genotypes with lower seed dormancy. The expression of the gene *Sdr4* was directly correlated with seed dormancy during embryo formation and in mature seeds and was not associated with seed dormancy during germination in the weedy rice ecotypes. Despite its high importance in cultivated rice the gene *OsCYP707A5* was not associated with seed dormancy in weedy rice. The dormancy-related genes *OsMADS29* and *Sdr4* originally identified in cultivated rice are also important in weedy rice. However, the magnitude of the expression of these genes was different between weedy and cultivated rice, indicating the existence of other regulatory factors that result in a higher seed dormancy in weedy rice.

Keywords: *Oryza sativa*, weedy rice**,** cultivated rice, seed dormancy, seed germination.

INTRODUCTION

Seed dormancy is the temporary absence of germination of viable seeds even under favorable environmental conditions. The regulation of seed dormancy is determined by genetic factors with substantial environmental influences mostly associated with air humidity and temperature (CAI and MORISHIMA, 2000). In weedy rice (*Oryza sativa*), which is an important weed of rice worldwide, seed dormancy is one of the main traits responsible for its invasiveness and persistence (THURBER et al., 2013). Seed dormancy in weedy rice allows the seed to remain viable in the soil seed bank for up to 10 years, causing scaled germination, which complicates its control before crop establishment (NOLDIN et al., 2006). The lack of adequate control of weedy rice contributes to reinfestation of the area resulting in

increased soil seed bank. The wild Oryza species and weedy rice ecotypes are known to have more pronounced seed dormancy than cultivated rice (CAI and MORISHIMA, 2000).

Several studies demonstrated that seed dormancy in rice is controlled by many quantitative trait loci (QTLs) (LI et al., 2011; THURBER et al., 2013). However, few QTLs of dormancy have been mapped accurately. Recent studies in cultivated rice have associated the genes *OsCYP707A5* (LIU et al., 2011), *OsMADS29* (LI et al., 2011) and *Sdr4* (SUGIMOTO et al., 2010) with seed dormancy. These studies showed a correlation between the regulation of these genes and seed dormancy through physiological, morphological and molecular approaches. Based on the results found in previous studies, we hypothesise that these three genes identified in cultivated rice may also act in the regulation of seed dormancy in weedy rice considering that the origin of weedy rice is most related to the de-domestication process of cultivated rice. The aim of this study was to evaluate the relative expression of genes *OsCYP707A5*, *OsMADS29* and *Sdr4* during different stages of seed development from embryo formation until seed germination in order to access the importance of these genes for seed dormancy in weedy rice.

MATERIAL AND METHODS

The expression of genes *OsCYP707A5* (*Os02g07420*), *OsMADS29* (*Os02g07430*) and *Sdr4* (*Os07g39700*) was evaluated in rice genotypes which showed contrasting dormancy levels in the second year of the dormancy phenotyping (data not shown). The rice cultivars (IRGA 417 and Kaybonnet), *O. glaberrima,* and a weedy rice (WR) ecotype WR 508 have low seed dormancy, the dormancy of the ecotype WR 503 is intermediary and in the ecotypes WR 223, WR 511 the seed dormancy is high. The gene expression analysis was carried out in three experiments including different stages of seed development and germination. Each repetition consisted of 30 embryos collected from seeds present in the central part of the panicle of an individual plant. Each genotype had three replications.

In the first experiment, gene expression was evaluated during embryo formation. Fourteen days after anthesis, the seed embryo, which is the structure of 3-mm of the seed from the pedicel (SUGIMOTO et al., 2010) was collected and stored in liquid nitrogen. The second experiment was carried out in mature seeds. The seed was considered mature when the humidity was between 20 to 25%. Mature seeds were collected from a single panicle and maintained with the hull for six months at 8°C as described by LIU et al. (2011). The third experiment was performed at five different time-points after exposing the seeds for germination. Initially, mature seeds were subjected to treatment for breaking dormancy followed by germination as previously described. The analyses were carried out at 12, 24, 36, 48 and 60 hours after exposure to germination (HEG).

Total RNA was extracted using the TRIzol method (Invitrogen). RNA samples were pre-treated with RNase-free DNase I (Invitrogen). The first-strand cDNA was synthesized using SuperScript III reverse transcriptase (Invitrogen). Amplification analysis by RT-PCR was performed on the Applied Biosystems 7300 real-time PCR System. Diluted cDNA was amplified using the SYBR Green PCR kit (Invitrogen). The 28S rRNA gene was chosen as the endogenous control. The primers were designed using the program Primer3Plus. The statistical analysis was performed considering the values of the quadruplicate of the Ct values. This analysis was carried out by the formula $\Delta\Delta Ct = (Ct_{target} - Ct_{28S}) - (Ct_{calibrator} - Ct_{28S})$, and $\Delta\Delta Ct$ is the relative expression of the gene and $2^{-(\Delta\Delta Ct)}$ is the variability of the quantification. The results were presented as the means and standard deviations.

RESULTS AND DISCUSSION

The relative expression of *OsCYP707A5* was not correlate with dormancy in the evaluations during embryo formation (Figure 1A) and in mature seeds (Figure 1B), because the genotypes with distinct seed dormancy levels presented similar relative expression levels. In mature seeds, however, the genotypes *O. glaberrima* and WR 223, which had different dormancy levels, showed similar relative expression levels (Figure 1B).

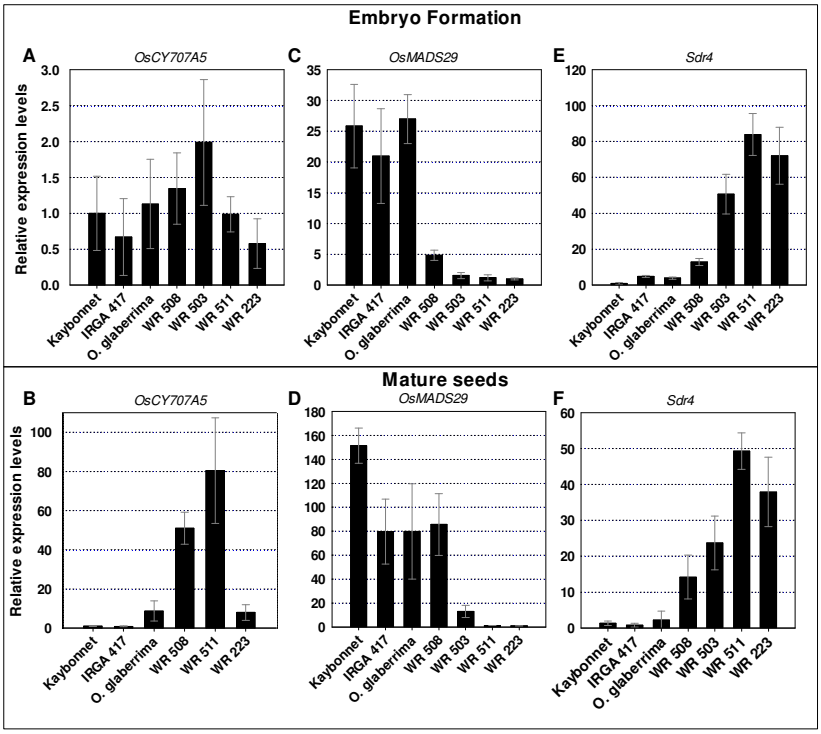

Figura 1. Relative expression of the *OsCY707A5* (A and B), *OsMADS29* (C and D), *Sdr4 (*E and F) genes in the embryo seeds of rice genotypes in the stages of embryo formation and in mature seeds. Bars represent the SD of three replicates with four replicates.

The gene *OsCYP707A5* was not amplified in the embryonic region during these stages of seed germination. In seed embryos of cultivated rice, the high expression of *OsCYP707A5* seeds assessed at six months after harvest correlated with higher germination rates (LIU et al., 2011). However, in the present study the expression of *OsCYP707A5* was not associated with seed dormancy in the weedy rice ecotypes and rice cultivars evaluated (Figures 1A and 1B).

The expression of the gene *OsMADS29* during embryo formation (Figure 1C), mature seeds (Figure 1D) and during seed germination 12 and 24 HEG (Figure 2A and B) was higher in the genotypes with lower seed dormancy. After 36 HEG, *OsMADS29* expression began to increase in relation to the earlier evaluations (Figure 2C, D and E) mainly for the weedy rice ecotypes that had lower germination speeds. The gene *OsMADS29* was identified in QTL qGR2 and was associated with the absence of seed dormancy in cultivated rice (LI et al., 2011). The present results indicated that *OsMADS29* gene expression is associated with seed dormancy in weedy rice similar to its effect in cultivated rice as described in previous studies.

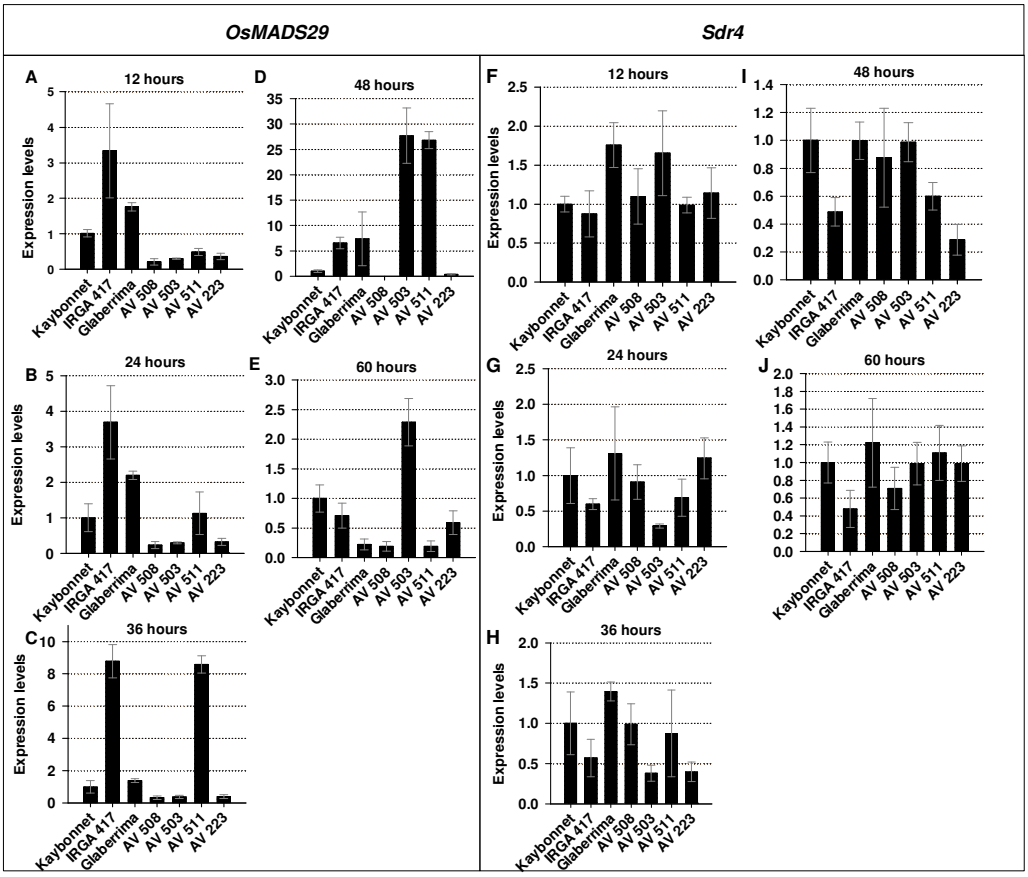

Figura 2. Relative expression levels of *OsMADS29* (A –E) and *Sdr4* (F–J), during seed germination at 12, 24, 36, 48 and 60 hours after exposure to germination (HEG), in the embryo seeds of rice genotypes. Bars represent the SD of three replications with four replicates.

The expression of the *Sdr4* gene was directly correlated with seed dormancy for the evaluations during embryo formation (Figure 1E) and in mature seeds (Figure 1F) because, in weedy rice ecotypes, the expression of this gene was higher compared to rice cultivars, which had lower seed dormancy rates. However, *Sdr4* expression during seed germination at 12, 24, 36, 48 and 60 HEG was not associated with the levels of seed dormancy in weedy rice ecotypes and cultivated rice (Figure 2). Although previous studies had characterized the effect of this gene during seed formation (SUGIMOTO et al., 2010), in the present study the evaluations were carried out also in the mature seed and the different stages of germination in order to standarise the analyses of the three stages for all three evaluated genes. The results presented above indicate a positive relationship between the expression of the *Sdr4* gene and dormancy.

CONCLUSIONS

Expression of the genes *OsCYP707A5*, *OsMADS29* and *Sdr4* analyzed together and during different physiological stages of seed development, maturation and germination showed the importance of these genes for seed dormancy regulation in weedy rice. During embryo formation and in mature seeds, the expression of the genes *OsCYP707A5*, *Sdr4* and *OsMADS29* show, respectively, no relationship, a positive and a negative relationship to seed dormancy in weedy rice.

The results found in this study are different for weedy rice in comparison with cultivated rice for the gene *OsCYP707A5* and are similar for the genes *OsMADS29* and *Sdr4*. These differences are due to the different selection of cultivated and weedy rice. Cultivated rice has been selected for low seed dormancy and in weedy rice the selection occurs for longer seed dormancy even with the continuous gene flow of alleles of low dormancy from cultivated rice.

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