EFFECT OF BISPYRIBAC-SODIUM AND COLD STRESS ON GENE EXPRESSION OF RICE SEEDLINGS

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Abstract: Abiotic stresses are the main factors that restrict some areas to practice agriculture worldwide. Rice, a staple food for more than half of the world's population, has limited tolerance to cold stress. In subtropical areas, early planting exposes rice seedlings to cold stress which impairs their development, turning them more vulnerable to other stresses such as herbicide injury. The objective of this study was to evaluate the effect of cold stress combined with bispyribac-sodium application on molecular patterns of rice seedlings. A growth chamber experiment was conducted twice in 2012 at University of Arkansas, Fayetteville, AR, US. The treatments were composed by: factor A - rice cultivars: IRGA 424 (cold tolerant) and EPAGRI 109 (cold susceptible); factor B - bispyribac-sodium application and a untreated check; and factor C - cold for two days before herbicide spray (short term acclimation), cold for two days immediately after spray (non-acclimated plants); cold since emergence (long term acclimation) and a warm check. Quantitative gene expression at 48 hours after herbicide application (HAT) of OsRAN2 and OsFAD8 - cold; OsGSTL2 and CYP72A21 - herbicide related markers were recorded. OsRAN2 and OsGSTL2 showed good response to cold, herbicide and cultivars, characterizing a cross-talk and indicating that these genes were not only involved in herbicide metabolism but also in cold tolerance. OsFAD8, was a good indicator of cold acclimation observed in cold-susceptible short-term acclimated plants, however, it was not responsive to bispyribac-sodium. CYP72A21 also presented cross-talk, being stimulated not only by herbicide as expected, but also by cold. These results show that cold stress affects the herbicide metabolism on rice plants.

Keywords: cross-talk, herbicide metabolism, reactive oxygen species.

INTRODUCTION

Rice production in Southern Brazil is marked by high technology and higher yields. Among the practices responsible for this achievement, earlier planting time stands out. Currently, rice planting time in RS has being shifted to early spring. This practice allows the timing of the reproductive stage with the maximum solar radiation period, improving the grain yield grain due to a better efficiency on nitrogen assimilation during microsporogenesis and panicle initiation (YOSHIDA, 1981). On the other hand, this practice may expose rice seedlings to cold, one of the main abiotic stress which impairs the overall plant metabolism (THEOCHARIS et al. 2012), turning them more vulnerable to other stress factors.

Herbicide selectivity in crops is a function of absorption, translocation, and metabolism. These processes are highly correlated with environmental conditions (OLIVEIRA JR., 2011) and might be negatively affected by cold stress. This fact has been observed in the South and Southwest regions of Rio Grande do Sul when farmers applied bispyribac-sodium on early-planted rice. Therefore, the interaction of cold and herbicide stress markers might be a good start to improve cold tolerance in rice cultivars, promoting knowledge about herbicide physiology under that stress. So, the objective of this study was evaluate the effect of cold stress combined with bispyribac-sodium stress on molecular patterns of rice seedlings.

MATERIAL AND METHODS

A growth chamber experiment was conducted twice in 2012 at the Altheimer Laboratory – Crop, Soil and Environmental Sciences Department of the University of Arkansas, Fayetteville, AR, US. The experimental units consisted of 2 L plastic pots filled with a ratio of 2:1 sieved Captina silt-loam soil and potting mix. The experiment was arranged in a completely randomized scheme with four replications. The treatments were composed by: factor A - rice cultivars: IRGA 424 and EPAGRI 109, tolerant and susceptible to cold, respectively; factor B - bispyribac-sodium application (50 g ai ha^{-1}) and untreated check; and factor C - cold stress occurrence: two days before the herbicide spray (short term acclimation); immediately after spray (non-acclimated plants), cold since emergence (long term acclimation) and a warm grown plants check. The temperature were set for 16/22 C and 25/30 C (night/day) simulating the early (cold) and late (warm) planting time, respectively.

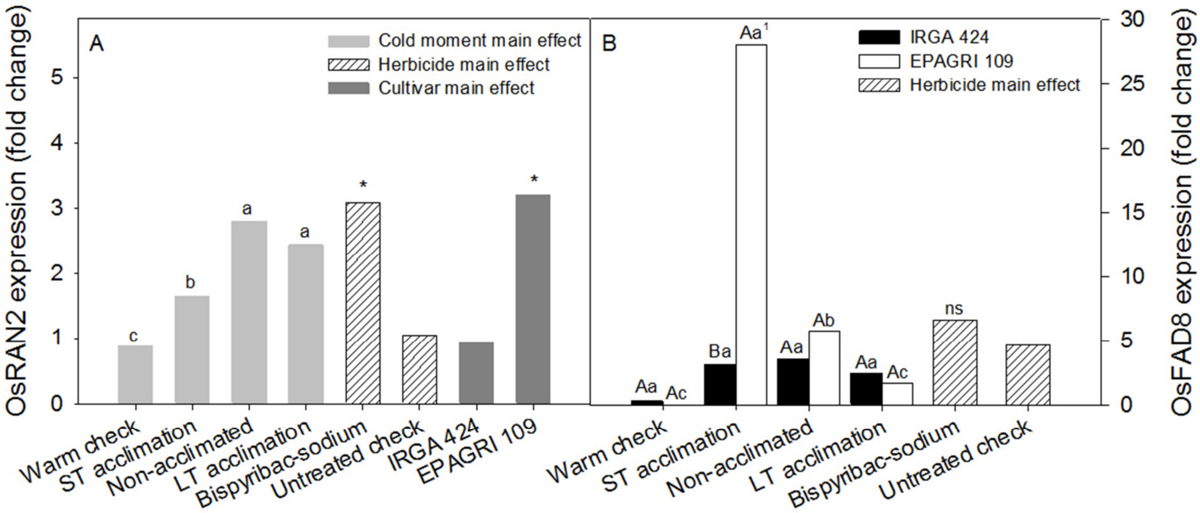
Differential gene expression at 48 hours after herbicide application (HAT) was recorded. The markers selected were: OsFAD8 - a lipid desaturase which improves membrane fluidity under cold stress (NAIR et al. 2009); OsRAN2 - a small GTP protein that acts on mitosis under cold conditions (CHEN et al. 2011). CYP72A21 - a P450 monoxygenase which acts on herbicide molecule oxidation, producing metabolites which further conjugate with glicose and/or reduced glutathione by Glutathione-S-transferase, such as OsGSTL2 (VAN EERD et al. 2003). UBQ5 was selected as a housekeeping gene. The gene expression, given in fold change, was calculated based on the check (warm grown plants without herbicide).

Data were initially tested for normality and homogeneity of variance and submmited to analysis of variance. A test for the interaction among cultivars (factor A), herbicide application (factor B) and cold occurrence (factor C) was carried out. In case of significant differences among cold occurrence treatments, the Tukey's test ($p \leq 0.05$) was performed.

RESULTS AND DISCUSSION

Cold stress promoted slight increase on OsRAN2 gene expression in all cold treatments, indicating that gene is responsive to low temperatures, in order to maintain the cellular division and consequently growth even in cold stress conditions (Figure 1A), similar to the results obtained by Chen et al. (2011). Moreover, the overexpression of OsRAN2 when the plants were exposed to bispyribac-sodium might be correlated to ALS herbicide mode of action through growth inhibition, which might be a signal to small GTP's such as OsRAN2 expression. Cold susceptible cultivar EPAGRI 109 presented greater expression, indicating the greater stress compared to the cold tolerant IRGA 424.

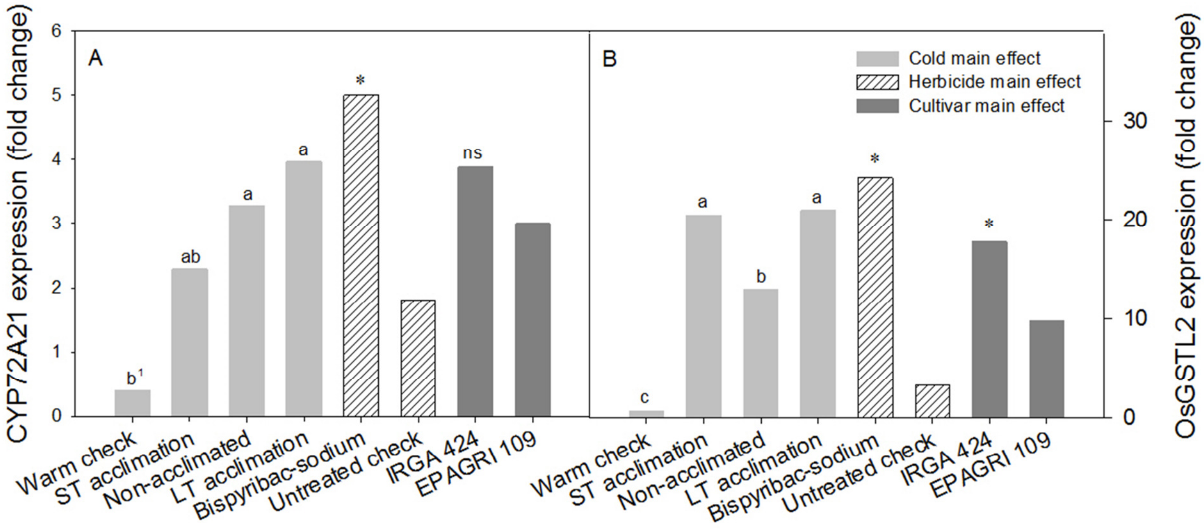
For OsFAD8 analysis, a significant interaction between cultivars and cold treatments was observed (Figure 1B). Likely by cold tolerance, IRGA 424 showed low response to OsFAD8 expression. On the other hand, for EPAGRI 109 was observed that short-term acclimation expressed 28 fold greater than warm grown plants, meaning that gene is an effective response concerning cold acclimation of susceptible cultivar. Therefore, non-acclimated plants had not enough time to proper acclimation due the similar response to check. In the same sense, the lower OsFAD8 expression by long-term acclimation plants might be related to earlier response of this gene to cold. In addition to that, OsFAD8 did not presented significant response to bispyribac-sodium application.



[1] Means not followed by same lowercase (cold treatments) differ by Tukey's test ($p \leq 0.05$); Means not followed by same uppercase letters (cultivars) differ by F test ($p \leq 0.05$). * two means are significantly different according to F test ($p \leq 0.05$); [ns] not significant by F test ($p \geq 0.05$).

Figure1. OsRAN2 (A) and OsFAD8 (B) expression (fold change) in relation to warm grown plants.

$P_{450's}$ are membrane-bound enzymes that are responsible to oxidative reactions in plant tissues, including pesticide detoxification (VAN EERD et al. 2003). Plants long-term acclimated and non-acclimated plants showed greater CYP72A21 expression, indicating that the cold impairs the $P_{450's}$ activity, increasing their expression under the stress conditions (Figure 2B). That fact can be related with the membrane rigidification under cold stress (MURATA & LOS, 1997) which impairs the activity of membrane-bounded enzymes such as $P_{450's}$. Bispyribac-sodium stimulated CYP72A21 expression, indicating that this gene has participation on herbicide metabolism. This result support the previous study by Hirose et al. (2007) which stated that CYP72A21 was induced by several kind of herbicides, including ALS inhibitors herbicides such as chlorotoluron.



[1] Means not followed by same lowercase differ among cold treatments by Tukey's test ($p \leq 0.05$); * two means are significantly different according to F test ($p \leq 0.05$); [ns] not significant by F test ($p \geq 0.05$).

Figure2. CYP72A21 (A) and OsGSTL2 (B) expression (fold change) in relation to warm grown plants.

For OsGSTL2 expression, no significant interaction among treatment factors was observed (Figure 2A). Plants previously exposed to cold either short as long-term showed greater OsGSTL2 expression. This response might be related to their role in detoxification reactions, acting as glutathione-peroxidases on Reactive Oxygen Species – ROS neutralization (Jain et al. 2010). Moreover, GST's are known to be involved in phase II of pesticide metabolism in plants, acting on conjugation of metabolite derived from phase I, modified by $P_{450's}$, with reduced glutathione. That fact explains the higher expression level of this gene when bispyribac-sodium was applied. In addition, OsGSTL2 presented differential expression pattern between cultivars, agreeing with the cold tolerance observed for IRGA 424, which showed greater expression compared to EPAGRI 109. Those results presents a cross-talk between cold and herbicide, meaning that the plants are capable to cope not only with the cold but also with herbicide stress.

CONCLUSIONS

OsRAN2 and OsGSTL2 shows good response to cold, herbicide and cultivars, characterizing a cross-talk, acting on herbicide and cold tolerance.

OsFAD8, is a good indicator of cold acclimation for cold-susceptible plants, however is not responsive to bispyribac-sodium.

CYP72A21 also presented cross-talk being stimulated not only by herbicide as expected, but also by cold stress.

Those results show the complex extent of interaction between cold and herbicide, indicating that cold might affect herbicide selectivity in rice.

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