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Short Communication

Interaction between AhMTP1s, Potential Candidate for Zinc Biofortication of Cereals, for their Functioning

ZAIGHAM SHAHZAD[1]†, FRANÇOISE GOSTI AND PIERRE BERTHOMIEU
Biochimie et Physiologie Moléculaire des Plantes, Unité Mixte de Recherche Montpellier, SupAgro/CNRS/INRA/Université Montpellier II, 2 Place Viala, 34060 Montpellier cedex 1, France
†*Department of Plant Breeding and Genetics, University of Agriculture, Faisalabad, Pakistan*
[1]Corresponding author's e-mail: zshahzaduaf@gmail.com

ABSTRACT

Zinc (Zn) biofortification is considered to be a valuable economical solution to ameliorate Zn malnutrition. *Metal Tolerance Protein 1* (*MTP1*) based on its role in Zn accumulation is considered to be a potential candidate to be expressed in cereals for their Zn biofortification. However, oligomer formation has been shown to be possible for MTP1s which could be a limiting step for successfully manipulating their expression in cereals. In the present study, interaction between different AhMTP1s when present in the same cell was explored. AhMTP1-A2 that displays the highest ability to complement Zn hypersensitivity of zrc1 cot1 yeast mutant was co-expressed with other lesser competent AhMTP1s. Zn assays in yeast indicated the occurrence of an interaction between different AhMTP1s and AhMTP1-A2 could have a positive effect on the functioning of other co-expressed AhMTP1. Our results provide additional argument to use AhMTP1 for Zn biofortification of cereals.

Key Words: Zinc; Biofortification; Cereals; *AhMTP1*; *zrc1 cot1*

INTRODUCTION

Zinc (Zn) is one of the most important essential micronutrient of human nutrition. It is the only metal represented in all six classes of enzymes (Coleman, 1998), and plays a structural role in regulatory proteins (Berg & Shi, 1996). Zn deficiency badly affects the health of both plants and animals (Bouis, 2003). Approximately, 25 to 30% of the population of developing countries is suffering from Zn deficiency that leads to growth retardation, weight loss, altered immune response, diarrhoea and many other health problems (Salgueiro *et al*., 2000; Hotz & Brown, 2004). Zinc biofortification of cereals to produce Zn enriched grains is considered to be a more cost-effective approach than ordinary Zn supplementation or fortification to ameliorate Zn malnutrition (Meenakshi *et al*., 2007). The major challenge in developing Zn biofortified crops is to increase the accumulation of Zn in edible parts, while limiting that of cadmium (Palmgren *et al*., 2008). Presently, lack of comprehensive genetic knowledge of accumulation of Zn in edible portions of cereals is a limiting step for their improvement through conventional breeding approaches. Over-expression of Zn transporters in cereals is considered to be the most reasonable solution to biofortify them with Zn. *Metal Tolerance Protein 1* (*MTP1*) involved in accumulation of Zn and not of cadmium is considered one of the potential candidates to be used for this purpose (Dräger *et al*., 2004; Desbrosses-Fonrouge *et al*., 2005). In addition, over-expression and presence of four additional copies of *MTP1* in *A. halleri*, a Zn hyperaccumulator, as compared to in *A. thaliana*, a Zn non-accumulator (Shahzad *et al*., 2010), suggests that this protein plays important role in Zn accumulation. However, there is an important point that must not be ignored before expressing a heterologous MTP1 in cereals. This relates to the possible formation of heterodimers of exogenous MTP1 with endogenous MTP1. Previously, it was shown that MTP1 of poplar forms oligomers when expressed in yeast as well as *in planta* (Blaudez *et al*., 2003). Thus, it was hypothesized that different AhMTP1s proteins when present in the same cell could interact with each other and this interaction could have functional consequences.

MATERIALS AND METHODS

Coding DNA sequences of *AhMTP1s* were amplified directly from *A. halleri* BAC clones using the proofreading *Pfu* DNA polymerase (Promega) and cloned in pYX212 (ura+) as described by Shahzad *et al*. (2010). AhMTP1-A2 was cloned in pFL38H (his+). Overnight *S. cerevisiae* cultures of *zrc1 cot1* mutant strain having an $O.D_{600nm}$ of 0.6 were double transformed with either empty pFL38H (his+) vector or containing AhMTP1-A2 and either empty pYX212 (ura+) or pYX212 (ura+) expressing AhMTP1-B, -C, -D, or AhPDF1.1 (Mirouze *et al*., 2006) using lithium acetate/ single-stranded carrier DNA/polyethylene glycol

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method (Gietz & Woods, 2002). Zinc assays were performed on selective modified Low Sulphate/Phosphate medium (Dräger *et al*., 2004), supplemented with various levels of $ZnSO_4$. At least four independent colonies were tested for each construct.

RESULTS AND DISCUSSION

Here, we tested if AhMTP1s interact with each other or not when present in the same cell and what could be the functional consequences if they interact? AhMTP1-A2, a most efficient AhMTP1 to complement Zn-hypersensitivity of the *zrc1 cot1* yeast mutant, was co-expressed in the yeast mutant along with AhMTP1-B, -C or -D that are lesser efficient than AhMTP1-A2 to complement Zn-hypersensitivity (Shahzad *et al*., 2010). At the same time as a control, AhMTP1-A2 was also co-expressed with another gene involved in Zn tolerance but not showing sequence similarity to MTP1, AhPDF1.1 (Mirouze *et al*., 2006). It was observed that yeast cells co-expressing AhMTP1-A2 and AhMTP1-B exhibited a phenotype that was intermediated between the phenotypes imparted by AhMTP1-A2 or -B when present alone (Fig. 1B). While, yeast cells co-expressing AhMTP1-A2 and either AhMTP1-C or -D exhibited a phenotype that was inferior than both of the AhMTP1s when present alone (Fig. 1C). However, cells co-expressing AhPDF1.1 and AhMTP1-A2 exhibited no change in the phenotype than the phenotype exhibited by AhMTP1-A2 alone. These findings indicated that different AhMTP1s interact with each other when present in the same cell probably through the formation of heterodimers. Formation of dimers or of oligomers has been suggested in previous studies on plant MTP1s (Bloss & Clemens, 2002; Blaudez *et al.*, 2003) and multimeric formation has been clearly demonstrated in animal cells (Palmiter & Findley, 1995). Both negative and positive types of effects were seen on the functioning due to the interaction between AhMTP1s. Co-expression of AhMTP1-A2 with AhMTP1-B showed a positive effect on the functioning of AhMTP1-B. Contrastingly, co-expression of AhMTP1-A2 with AhMTP1-C or -D showed a negative effect on the functioning of AhMTP1-C or D. This shows that co-expression of AhMTP1-A2 with other AhMTP1s could not always enhance the functioning. Thus, our work points to the requisite that when developing Zn biofortification approach for cereals using AhMTP1, compatibility between exogenous and endogenous MTP1 of cereals must be checked. If this interaction is not negative then AhMTP1 would likely produce Zn enriched seeds.

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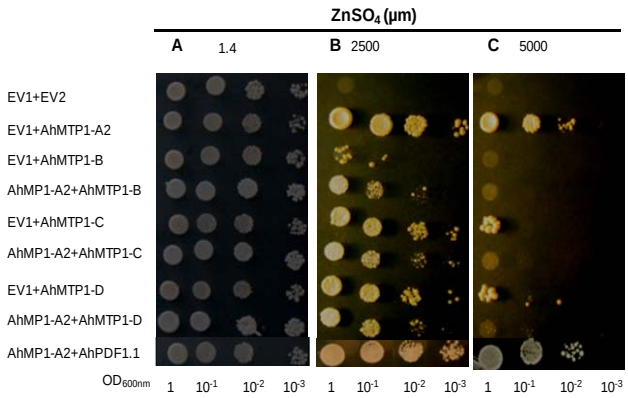
Fig. 1: Functional heterologous co-expression of AhMTP1-A2 and AhMTP1-B/-C/-D or AhPDF1.1

Saccharomyces cerevisiae zrc1 cot1 mutant strains was double transformed with either empty pFL38H (his+) vector or containing AhMTP1-A2 and either empty pYX212 (ura+) or pYX212 (ura+) expressing AhMTP1-B, -C, -D, or AhPDF1.1 (Mirouze *et al*., 2006). Serial dilutions were spotted on modified selective LSP medium supplemented with different concentrations of $ZnSO_4$ as indicated above the panels. Each spot was made with 10 µl of a yeast culture diluted at the OD_{600nm} mentioned below the drops. Pictures were taken after 2 days for control (1.4 µM $ZnSO_4$) and 4 days for other treatments



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