Evidence for the Role of Phytosiderophores in Zinc Efficiency of Wheat

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INTRODUCTION

Zinc deficiency has been recognized as a global nutritional constraint with its prevalence in more than 30 and 50% of the cultivated soils of the world and India, respectively. It is the most widespread micro-nutrient deficiency in cereals especially in wheat. The so-called "Zn deficiency" is in terms of the availability of Zn in readily usable forms for plant uptake and not in terms of the total amount of Zn in soils. Nevertheless, graminaceous species possess a mechanism that involves rhizosphere modification through the secretion of phytosiderophores (phytometalophore, PS) by roots which is induced mainly under Fe-deficiency and determines Fe-deficiency tolerance. Although there have been attempts to identify genes and gene products responsible for PS synthesis, their regulation has so far only been related to Fe deficiency (Takahashi et al. 2001). Their role under Zn deficiency, though suggested, is far from clear. Therefore, experiments were conducted to ascertain if the expression of Zn efficiency in wheat species is related to phytosiderophore release, biosynthesis and / or transport of Zn-PS complex from the rhizosphere into the plant.

METHODS

Triticum aestivum L (bread wheat, PBW-343, HD- 2329) and *Triticum durum* L. (durum wheat, HI-8498, PDW-233) procured from the Division of Genetics and Plant Breeding, IARI, New Delhi, were used as experimental material. Plants were raised in nutrient solution with (1mM $ZnSO_4$) and without Zn under controlled climatic conditions (light/dark regimes of 16/8 h at 18/22^oC) in the Phytotron facility of the institute. Phytosiderophore release from roots (for 6h from 8am-2pm) between 11-14 days of transfer to the respective nutrient solution and the phytosiderophore content of roots before and after release periods were measured following methods as described by Singh et al. (2002) and the Fe mobilization test of Takagi (1976).

A mixed culture experiment was conducted where bread and durum wheat types were precultured in Zn-deficient and sufficient solutions for seven days and then transferred to 500 ml pots containing micronutrient free solution for 6h, 2 h after the onset of light, in mixed and individual culture (8 replicates/treatment). During the 6 h, each pot was supplied with 10 ml of a Zn-loaded resin suspension in dialysis bags of 15 cm length. Plants were transferred back to the respective Zn-deficient or control solutions after 6 h. This procedure was repeated everyday for 10 days consecutively. Afterwards, root and shoot tissues of each genotype and treatment were collected for mass and tissue-Zn measurements. The phytosiderophore release was also monitored.

For gene expression analysis of Nicotianamine amino transferase (NAAT), total Ribonucleic Acid (RNA) was isolated from roots of 11 days-old Zn-sufficient and Zn-deficient plants of all wheat genotypes. The expressed sequence tag (EST) (Accession No. BE 490536) of wheat genome, showing high homology to gene sequence of barley NAAT A (gene:1600 bp long), procured from Dr. Onil Anderson, Wheat Research Lab, Agricultural

Research Services, USDA, California, USA, was labelled (^{32}P) and used as a specific probe for the northern analysis of NAAT.

RESULTS AND DISCUSSION

In Zn-deficient nutrient solution, durum wheat showed a more rapid development of visible Zn-deficiency symptoms than bread wheat types. Bread wheat types were Zn-efficient and produced more biomass and durum wheat types under Zn-deficiency. Under Zn deficiency, Zn-inefficient durum wheat lines HI-8498 and PDW-233 released significantly lower amounts of phytosiderophores than the Zn-efficient bread wheat PBW-343 and HD-2329. The phytosiderophore release in bread and durum wheat types was related to efficiency of Zn uptake across genotypes. The phytosiderophore release in durum types under Zn deficiency was limited by phytosiderophore availability in the roots rather than by release of phytosiderophores. The mixed culture experiment showed that rhizospheric availability of phytosiderophores is a factor limiting the acquisition of Zn by Zn-inefficient wheat. When grown in mixed culture with bread types, Zn-inefficient durum grew better and acquired more Zn compared to growth in monoculture (Fig. 1). Furthermore, durum types showed a low transcript level of NAAT which correlated well with the production and release of phytosiderophores in Zn-deficient plants (Fig. 2).

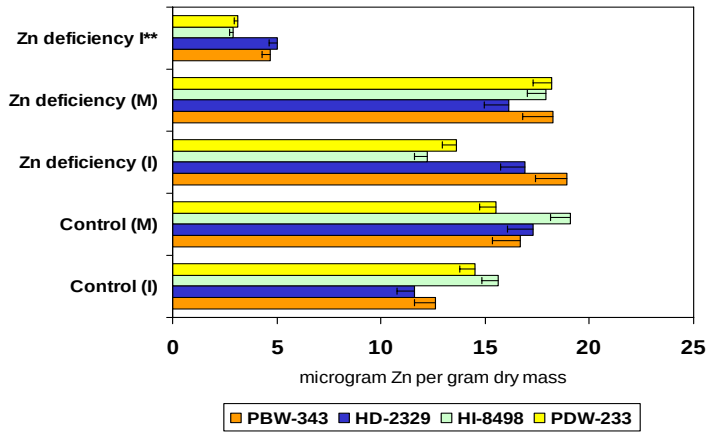

Fig. 1. Zinc concentration of durum wheat increased and were at par with those of bread wheat when grown in mixed culture.

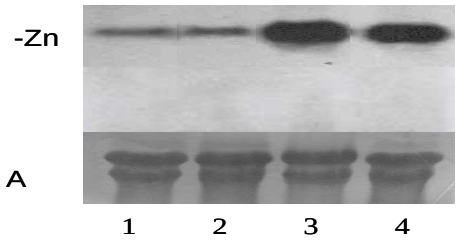

Fig. 2. Northern analysis of root mRNA of *Triticum aestivum* and *T. durum* wheat raised under Zn deficiency (Zn-) and nutrient sufficiency (untitled), hybridised with a specific cDNA probe wheat EST, BE490536 showing sequence similarity with Barley NAAT A; PDW-233 (1), HI-8498 (2) and bread wheat - HD-2329 (3), PBW-343 (4) genotypes at 11 days after transfer to control, Zn- and/or Fe-deficient nutrient solution. The symbol "A" refers to loading controls.

CONCLUSIONS

It is suggested that over-expression of genes regulating the synthesis of phytosiderophores could help enhancing Zn-deficiency tolerance of wheat and consequently grain production on calcareous or alkaline soils with low Zn availabilities because the acquisition of Zn mediated by phytosiderophores resembles that of Fe at the physiological level.

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