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SHORT COMUNICATION

Biofortification of barley grains by cell-type-specific expression of a vacuolar metal transporter

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Abstact

Transition metals are universally present in our environment occurring either naturally or resulting from anthropogenic activities. Many of the transition metals (e.g. Fe, Zn, Cu, Mn, Ni, Co) are essential for all forms of life, playing important roles as cofactors of numerous enzymes, in stabilising transcription factors and in regulatory and structural proteins. However, when present at high concentrations these metals become toxic alongside with other metals (e.g. Cd, Pb) that have no known biological function. The only source of metals for humans is through diet and ultimately the source of mineral elements is therefore plant tissues. Consequently, the presence of minerals and the maintenance of their homeostasis within the edible tissues of plants are of great importance for human nutrition.

One of the essential elements for both plants and humans is Zn. Zinc is a trace element, essential in sustaining all biological organisms and is present in all enzyme classes – oxidoreductases, transferases, hydrolases, lyases, isomerases and ligases (Broadley *et al*., 2007). Over 3200 putative zinc-binding proteins have been estimated within the human proteome (Andreini *et al*., 2006) *i.e*. more than 10% percent of proteins encoded by the genome. The WHO places Zn and Fe among the micronutrients - alongside Vitamin A – for which deficit is most acute in the human diet and for which enhancement is necessary. To a large degree, these deficiencies worldwide arise from the low content of Fe and Zn in polished grains and from the tendency of other components in predominantly vegetable diets to chelate the already-low concentrations of these essential metals. Recently, biofortification has been proposed as a complementary solution to address human mineral malnutrition. Biofortification has been defined as the process of increasing the bioavailable concentrations of essential elements in the edible portions of crop plants through agronomic intervention or genetic selection.

I order to address biofortification of grains with mineral elements, the transporters involved in the deposition of these elements in grains and their metal specificity has to be identified. A recent barley grain microarray, consisting of cDNA clones of relevance for heavy metal transport and deposition, suggested that Metal Tolerance Proteins (MTPs) might be involved in the deposition of Zn in the endosperm during grain filling (Tauris et al., 2009). MTPs are plant members of the large Cation Diffusion Facilitator (CDF) family of transporters. CDFs are ubiquitous to all form of life (Archaea, Bacteria and Eukaryotes), more than 400 members being reported so far. CDFs are all localized either at the plasma membrane, vacuolar membrane (in plants), vesicles or at the endocompartments and are involved in maintaining the cellular metal homeostasis by catalyzing the efflux of transition metal cations from the cytoplasm to the outside of the cell or into subcellular compartments.

Functional aspects of CDFs have been little investigated within plants and not at all in cereals. In barley, likewise in rice, HvMTP1 appears to be the major representative of the Zn-group of CDF transporters and it is expressed ubiquitously in root, shoot and grain tissues

(Podar, unpublished). Cellular localization of HvMTP1 is apparent at the vacuolar membrane both when is expressed heterologously in yeast or transiently in protoplasts from barley mesophyll. Ionic selectivity of HvMTP1 was assessed by heterologous expression in a number of yeast (*Saccharomyces cerevisiae)* mutants deficient in different metal transporters. Results showed that HvMTP1, in contrast to AtMTP1 and OsMTP1 which are highly selective for Zn, can complement the phenotype of mutant yeast deficient in both Zn and Co transporters (Podar unpublished).

In order too investigate the potential of HvMTP1 in biofortifying barley grains with Zn two constructs were created for targeted over-expression of barley vacuolar heavy metal transporter (HvMTP1) in the endosperm, under D-Hordein promoter and in the aleurone cells, under Ltp2 promoter. Barley immature embryos (1-1.5 mm in diameter) were transformed for each of the constructs *via Agrobacterium tumefaciens.* As a control for the transformation efficiency, GFP constructs have been used. After transformation, the embryos were transferred to a succession of media with the final aim of regenerating transformed plants. Numerous lines of transformed plants were obtained and further screened for the presence of T-DNA insertion and for segregation. A summary of stages and steps performed can be seen in the table 1.

Table 1. Summary of stages and steps performed for barley transformation, regeneration, selection of homozygous plants and screening for gene expression and mineral analysis.

Event	Steps	Construct	Number of samples	Designated name	Month done
Transformation	Inoculation Co-cultivation with *Agrobacterium* Calli formation	D-Hordein	400 immature embryos + 50 for GFP (as control)	T0 generation (parental material)	1 -2
		Ltp2	400 immature embryos + 50 for GFP (as control)		
Re-generation	Shoot formation	D-Hordein	109 lines (not including sisters)	T1 generation (transformed re-generated plants)	3
		Ltp2	81 lines (not including sisters)		
	Root formation	D-Hordein	75 lines (not including sisters)		4
		Ltp2	53 lines (not including sisters)		
	Plant selection	All rooted lines were tested both by PCR and by leaf test for the insertion of T-DNA (for being transformed). Based on the results, the number of lines further grown for seed formation was reduced to:			5-6
		D-Hordein	45 lines (not including sisters) grown for seeds formation		
		Ltp2	36 lines (not including sisters) grown for seeds formation		
Screening for segregation	Seed collection	D-Hordein	39 lines (not including sisters)	T2 generation heterozygous (seeds of primary re-generated transformed plants)	7-8
		Ltp2	28 lines (not including sisters)		
	1st screening for segregation	All lines from which the seed were collected were screened for segregation on hygromycin (leaf test)			9-12
		D-Hordein	12 lines (6 plants per line)		
		Ltp2	8 lines (6 plants per line)		
		GFP	4 lines (6 plants per line)		
	2nd screening of segregation	All T2 plants selected after 1st screening of segregation were left to set seeds. These seeds represented T3.		T3 generation homozygous	13-14
		Seeds of all these plants were germinated and a 2nd screening of segregation (by leaf test) was performed in order to select for homozygous plants.			15-19
Analysis of barley		Selected homozygous plants (1-3 plants/line – 12 lines for D-Hordein, 6 lines for Ltp2) were grown in soil to produce seeds (T4).		T4 generation homozygous	20-24
		Samples of seeds were collected at 20 days after pollination (DAP) for extraction RNA and cDNA preparation.			25-
		To be done further:			
		First analysis of level of expression of HvMTP1.			
		Mineral analysis of seeds and plants.		T5 generation homozygous	

RNA was extracted from grains collected from homozygous selected plants from several lines at 20 days after pollination (20DAP). The level of expression of HvMTP1 within filling grains is to be investigated. Furthermore, selected homozygous lines with different level of expression of HvMTP1 will be grown on substrate with different concentrations of Zn. The barley kernels will be then analysed for mineral, especially Zn, content.

References

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