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Biofortification in microelements by modulating the accumulation of phytic acid in durum wheat grain

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INTRODUCTION

Phytic acid (PA) in seeds is considered an anti-nutritional compound, as it limits the bioavailability of phosphorus and microelements in the kernel. Therefore, the production of low phytic acid (*lpa*) mutants is a successful strategy to obtain biofortified crops and to improve the nutritional quality of derived foods[1]. The research here presented is focused on the development and characterization of durum wheat genotypes biofortified in essential minerals obtained by silencing two key genes through a Targeting Induced Local Lesions IN Genomes (TILLING) strategy[2]:

- ✓ *Inositol pentakisphosphate 2-kinase 1* (*IPK1*), involved in the last step of the PA biosynthetic pathway[3];
- ✓ *Multidrug-resistance associated protein 3* (*MRP3*), a transporter involved in the accumulation of PA into the vacuole[4].

MATERIALS AND METHODS

MOLECULAR ANALYSIS :

- ✓Selection of single null mutants in the homeoalleles of *IPK1* and *MRP3* genes using the TILLING platform available at the University of Davis.
- ✓Piramyding of null mutations for target genes.
- ✓Marker-assisted selection (MAS) based on PCR and HRM-genotyping[5].

BIOCHEMICAL ANALYSIS:

- ✓Phytic acid content (K-PHYT kit Megazyme).
- ✓Concentration of nutrients by ICP-MS.
- ✓Determination of the ferrous deposits within the kernel using the Perls' Prussian blue method[6].

RESULTS

The selection of the mutant lines was carried out through a MAS assay based on the HRM-genotyping (**Fig.1**).

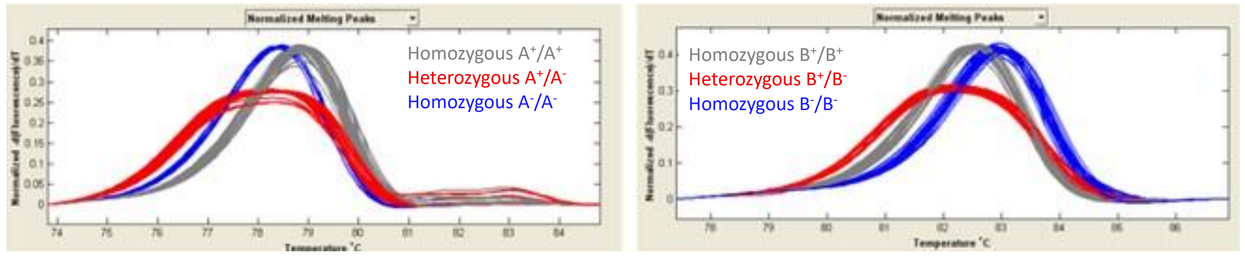
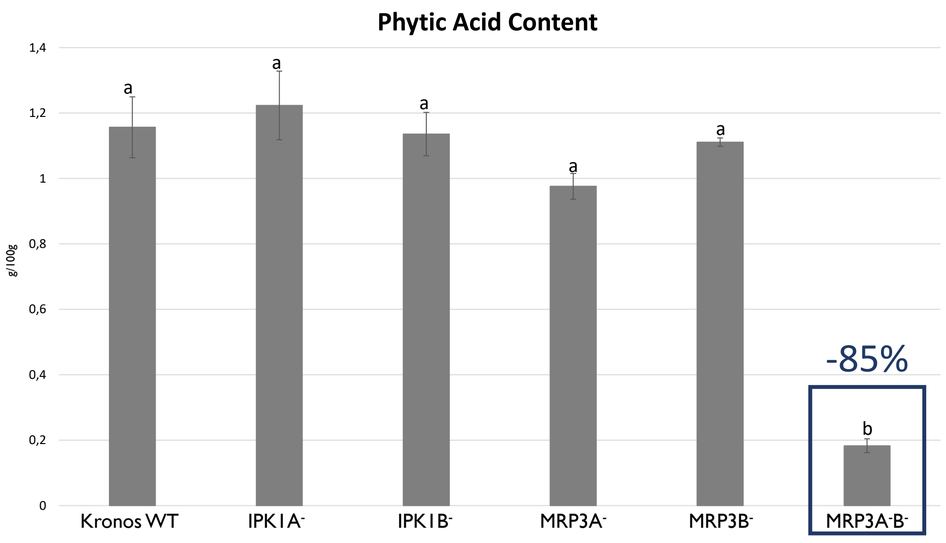


Fig. 1 HRM-genotyping of F_2 progeny from the cross MRP3A$^-$ x MRP3B$^-$. On the left HRM analysis of the MRP3A homeoallele; on the right HRM analysis of the MRP3B homeoallele.

HRM analysis allowed to identify four homozygous double null mutants MRP3A$^-$B$^-$. Although an elevate number of F_2 plants were analysed, no double null IPK1 mutants were identified.

The selected mutant genotypes were characterized for the content of PA (**Fig.2**).



The PA content is drastically reduced in the double null mutant MRP3A$^-$B$^-$ compared to the control wild type, while no differences were observed in the single mutants.

Fig.2 Analysis of the phytic acid content in mature seeds. The reported data represent the mean values of three biological replicates, the error bars indicate the standard error. One-way ANOVA,Tukey HSD test, p< 0.01.

Ferrous deposits are strongly increased in the scutellum, aleuronic layer and endosperm of the MRP3A$^-$B$^-$ mutant. The single null mutants also revealed visible differences compare to the control wild-type (**Fig.3**).

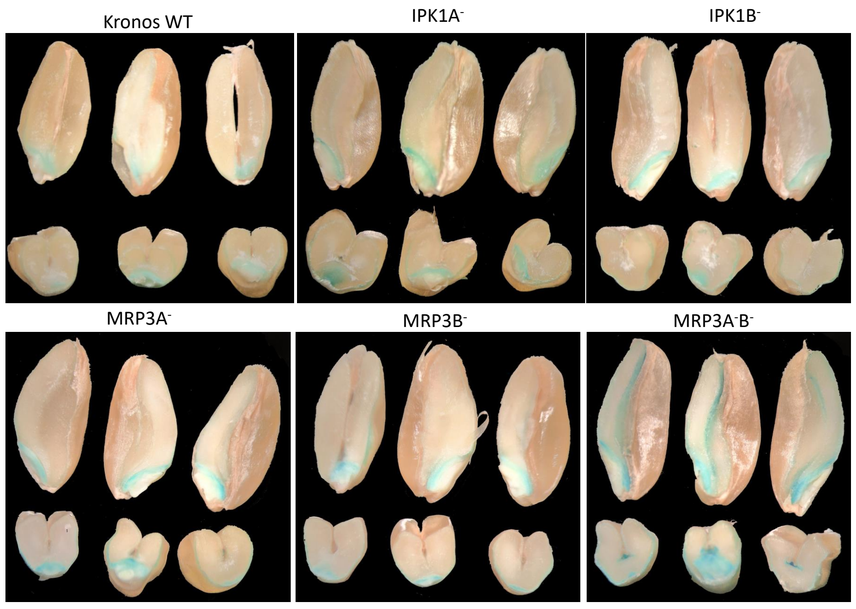


Fig. 3 Colorimetric test to determine the localization of iron deposits in the kernel was performed using the Perls' Prussian blue method.

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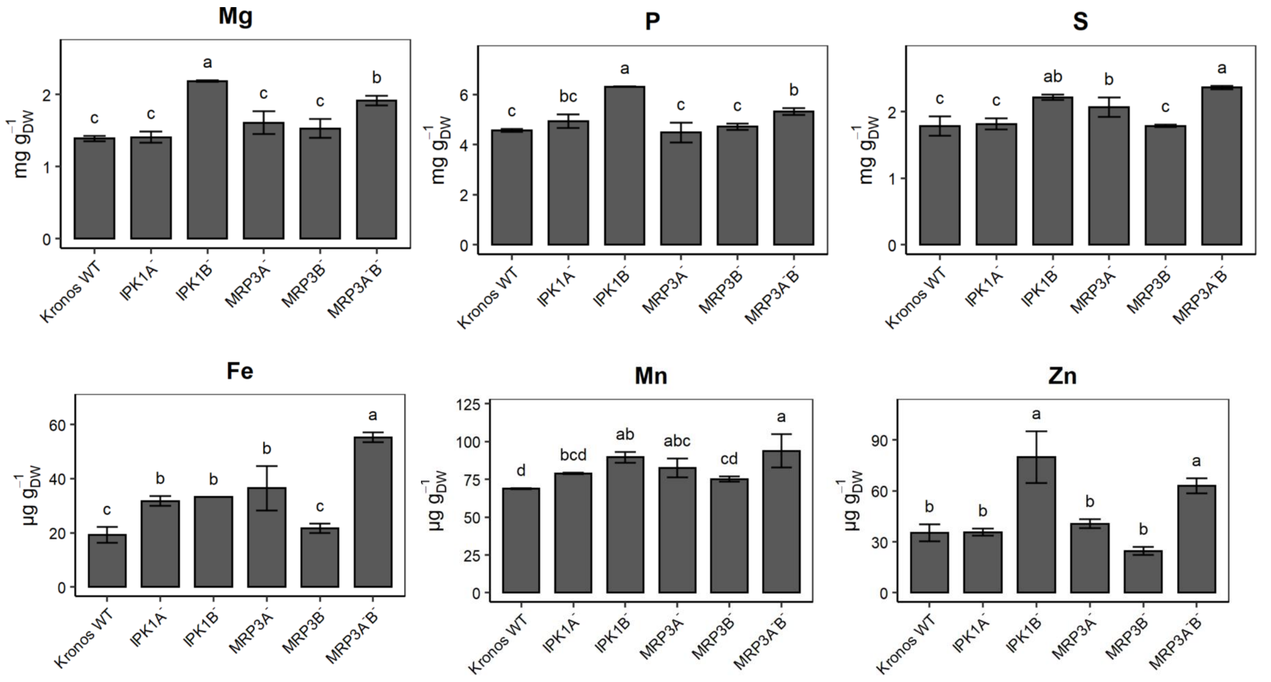

Fig. 4 Concentration of nutrients in *lpa* mutants and control. Mean values of three biological replicates ± St. Dev. One-way ANOVA, LSD test, p <0.05.

The ICP-MS analysis revealed a significant accumulation of macro- and micro-nutrients in the homozygous double null mutant MRP3A$^-$B$^-$ compared to the control wild-type. The IPK1B$^-$ mutant also showed a greater accumulation of Mg, P, S, Mn, Zn (**Fig.4**).

The complete MRP3 mutants compared to the control were evaluated for the morphology of the root apparatus (**Fig. 5**) and agronomic performance (**Tab.2**).

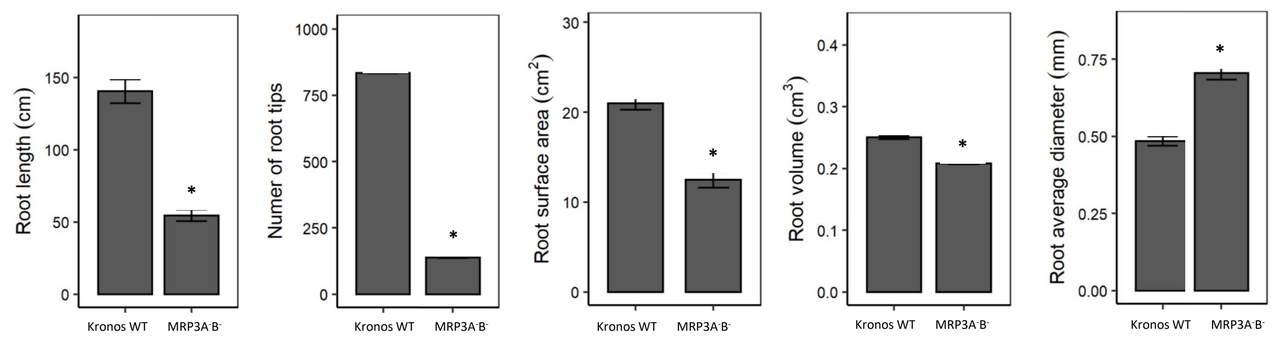

Fig. 5 Morphological analysis of the roots. Mean values of three replicates ± St. Dev. T-stu, p <0.05

Tab. 2 Agronomic traits analysis. Mean values of three replicates ± St. Dev. T-stu, p <0.01

Lines	Spike weight (g)	Spike lenght (cm)	Spikelets/ spike	Kernels/spike	Kernel weight/ spike (g)	Single kernel weight (mg)
Control	1.64±0.37	6.81± 0.61	19.20± 1.91	42.63± 9.06	1.12± 0.26	26.28± 3.89
MRP3A$^-$B$^-$	1.54±0.66	6.74± 0.91	15.80± 1.56 **	27.93± 10.80 **	1.01± 0.49	35.29± 6.67 **

Agronomic analysis did not reveal strong pleiotropic effects, despite the morphology and development of the root were significantly altered in the complete null MRP3A$^-$B$^-$ mutant lines compared to the control. Although the number of spikelets and seeds per spike are reduced, the negative agronomic performances are balanced by the increased grain weight.

CONCLUSIONS

- ✓ The silencing of the *MRP3* genes represents a successful strategy to obtain biofortified durum wheat lines able to accumulate a higher quantity of essential microelements (Fe, Mn, Zn).
- ✓ Preliminary analyses suggest that the complete silencing of the *IPK1* gene may have a negative impact on pollen fertility or seed formation since homozygous double null mutants were not identified.