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Functional characterization of maize *Phosphorus-Starvation Tolerance 1* (*ZmPstol8.02*) promoter

(submitted by Sylvia Morais de Sousa <sylvia.sousa@embrapa.br>)

Full Author List: Negri, Barbara F¹; Ferreira, Nataly F²; Palhares, Patrícia LS²; Lana, Ubiraci GP^{2,3}; Alves, Meire C³; Guimarães, Claudia T^{1,3}; Carneiro, Andrea A³; de Sousa, Sylvia M^{1,2,3}

¹ Universidade Federal de São João del-Rei – UFSJ, São João del-Rei, MG, Brazil, 36307-352

² Centro Universitário de Sete Lagoas - UNIFEMM, Sete Lagoas, MG, Brazil, 35701-242

³ Embrapa Maize and Sorghum, Sete Lagoas, MG, Brazil, 35701-970

Phosphorus (P) is an essential macronutrient required for a range of key biochemical processes associated with plant growth and function; however, most soils throughout the world are deficient in readily available forms of P, and poor availability of P limit cereal production. Modifications in root morphology are important strategies to maximize soil exploitation under P starvation in plants. Our group performed a multiple interval QTL mapping in a maize recombinant inbred line population derived from a bi-parental cross of lines L3 and L22, P-efficient and inefficient, respectively, under low-P condition. The QTL mapping revealed candidate genes as maize homologs to *Phosphorus-Starvation Tolerance 1* (*Pstol1*) that is a gene responsible to enhance root surface, P acquisition and grain yield in rice under P deficiency. One of the candidates is the *ZmPstol8.02* that co-localizes root length, root surface area, root:shoot ratio and P content and was highly expressed in roots of L22, the donor line of the favorable QTL alleles. In the present study, we aimed to characterize *ZmPstol8.02* promoter region. The upstream region (-1 to -2039 bp) was analyzed using SIGNALSCAN program provided by NEW PLACE database in order to identify their cis-regulatory elements (CREs). Using this approach, we found 450 and 444 CREs in the promoter of L3 and L22, respectively. Five CREs were found in a larger number in L3. All elements that were found in a higher number in L3 are related to abscisic acid (ABA) that regulates many aspects of plant growth and development, including inhibition of root elongation. In order to validate the promoter region and better comprehend its regulation; we cloned around 2 Kb of L3 and L22 promoter region in pTF102 using *Bar* gene as a selective marker and *Gus* as a reporter. Maize HiII plants were genetically transformed via *Agrobacterium tumefaciens* EHA101 strain and regenerated from selected callus in shooting and rooting medium. Fragments of the *Bar* gene (~400 bp) and *Gus* gene (~700 bp) were amplified by PCR, confirming integration of the cassettes in the transformed plants. All events presented from one to three copies of *Bar* gene. Both promoters presented *Gus* expression levels in roots and shoots with a similar intensity as CaMV 35S promoter.

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Functional studies of divergent prolamins

(submitted by Zhiyong Zhang <zhiyong@waksman.rutgers.edu>)

Full Author List: zhang, zhiyong¹; zhang, wei¹; Messing, Joachim¹

¹ Waksman Institute of Microbiology, Rutgers University, 190 Frelinghuysen Road, Piscataway, NJ, US 08854-8020

Prolamins, the major storage proteins present in most cereal grains, are encoded by a large gene family that arose from a tandem duplication of an α -globulin gene. Although carbohydrates are the major calorie source in cereal grains, representing about 70% of seed weight, the proteins with only about 10% play an important part in the nutritional quality of cereals. Cereal proteins also determine important physical properties of crop seeds. For instance, the gamma prolamins in maize, also called gamma zeins, contribute to kernel hardness, which is a critical property for storage and transport of corn. On the other hand, the wheat prolamins, also known as glutens, play key roles in the rheological properties of dough, which are essential for bread and noodle products. These divergent physical properties of prolamins are reflected in the evolution of the prolamin gene family. Interestingly, despite the divergence of prolamins into different classes and chromosomal locations their regulation of gene expression appears to be quite conserved based on gene transfer from one species to another. To further characterize these physical properties, we use different evolutionary distances to express the combination of different prolamins in maize. An interesting intermediate evolutionary distance between wheat and maize is teff (*Eragrostis tef*), a cereal consumed mainly in Ethiopia. It is used to make a kind of flatbread called injera. While teff has in this respect similar properties as wheat, its prolamins are more closely related to the maize prolamins. For instance, it has alpha zeins instead of gliadins. However, in contrast to maize it has a higher expression and amplification of globulin genes. To test the role of globulin gene expression in endosperm, we constructed chimeric teff globulin genes with a strong alpha zein promoter for expression in maize.

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