

FUNCTIONAL ANALYSIS OF THE 5' REGULATORY REGION OF THE MAIZE *PHOSPHORUS-STARVATION TOLERANCE 1 (ZMPSTOL8.02)* GENE

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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. Recently, 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 with root length, root surface area, root:shoot ratio and P content and is highly expressed in the root of L22, the donor line of the favorable QTL alleles. In the present study, we aimed to characterize the *ZmPstol8.02* promoter region. The upstream region (-1 to -2039 bp) of *ZmPstol8.02* from L3 and L22 lines were fully sequenced and analyzed using SIGNALSCAN program provided by NEW PLACE database and Software GENOMATIX in order to identify their cis-regulatory elements (CREs). Using the NEW PLACE, we found 450 and 444 CREs in the promoter of L3 and L22, respectively. Five CREs were found in a larger number in L3. One of them is an ABRE-related sequence, a key component of Mem1 (Mesophyll expression module 1). Another key component of Mem 1, CACTFTPPCA1, was the most frequent element in both lines, and the other three elements are RY repeats. Some CREs related to abscisic acid (ABA) that regulates many aspects of plant growth and development, including inhibition of root elongation were found in a higher number in L3. GENOMATIX analyses showed the presence of cis elements related to salicylic acid, ABA and auxin phytohormones and also to P, water and salt stresses. In order to validate the promoter region and better comprehend its regulation we cloned ~2 Kb of L3 and L22 promoter region in pTF102 vector with *Bar* as a selective marker and *Gus* as a reporter gene. 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* and *Gus* genes were amplified by PCR, confirming the integration of the cassettes in the transformed plants. All events presented from one to three copies of *BAR* gene. Both promoters presented *GUS* expression in the root and shoot and some events had similar intensity as CaMV 35S promoter. The promoters identified and characterized in this study have the potential to be applied in crop biotechnology for constitutive expression of transgenes.

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