



A New Technique for Genotyping Bread Wheat under Phosphorus Deprivation as microRNA-SSR Markers

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Abstract

Wheat is considered as a major staple food of our country. There are several genotypes of wheat. Some wheat genotypes are very much sensitive and some are very resistant to the nutrient stress caused by the deficiency of phosphorus. Identification of these two types of genotypes is possible through the use of microRNA-SSR based markers. The article will describe the potential of this modern tool for genotyping the bread-wheat under phosphorus deficiency condition.

Among various natural as well as human-controlled yield contributing factors in plant, mineral nutrients play a pivotal role. Amidst of various macro and micro nutrients, phosphorus (P) play crucial role in the growth and development of the wheat. P has many vital functions in plants in addition to the unseen roles it plays in photosynthesis, energy storage, and transfer, respiration, cell division and other plant processes. P is an important constituent of membrane lipids, nucleic acids and has role in energy transfer reactions. At plant level, P helps in development of roots more rapidly during seedling stage, improves winter hardiness, promotes early and uniform heading, hastens crop maturity and increases water use efficiency (WUE) besides being vital to seed formation and quality. The application of the nutrients in the forms of chemical fertilizers is necessary to obtain optimum yield. New cost effective and environment friendly strategies need to be developed to improve crop yield with minimum use of P fertilizers. This can be achieved by identifying nutrient efficient crops or crops tolerant to nutrient limited conditions, showing increase in both acquisition and uptake efficiency as P use efficiency of cereals has been reported to be 15-20%, respectively which is quite low. However, plant have developed several mechanisms for persistent adaptation under P deficiency which includes change in root morphology, exudation of organic acids and phosphatases and also involvement of symbiotic relation with arbuscular mycorrhizal fungi. These adaptive strategies in plants under low P are dependent on regulation of gene expression. The phosphate transporter1 (PHT1) gene family plays a major role in uptake of Pi from the soil, PHT 1:1 as a member of this family has crucial role in Pi uptake (Muchhal *et al.*, 1996, Shin *et al.*, 2004). Besides this, various transcription factors like- WRKY45, WRKY75, phosphate starvation response 1 (PHR1) and MYB62 are involved in regulation of the expression of PHT1:1 (Devaiah *et al.*, 2007, Dai *et al.*, 2012). Pi translocation from root to shoot is mainly regulated by PHO1 family and WRKY6 and WRKY42 are involved in down regulating this process (Chen *et al.*, 2009, Su *et al.*, 2015). The phosphorous starvation tolerance 1 (PSTOL1) which have relation with changes in root system architecture. Apart from this, phosphate homeostasis in plants are modulated by miR399, miR827 and ZAT6 which is a zinc finger transcription factor (Kant *et al.*, 2011). In

the majority of these factor were identified in Arabidopsis, rice and PSTOL1 was in sorghum (Hufnagel *et al.*, 2014). Alteration of these genes involved in P signalling, uptake, transport and metabolism is one of the most promising approach gaining momentum to develop nutrient efficient crop under limited P. But will be advantageous to develop nutrient stress tolerant plant through marker assisted breeding which is less time consuming compared to conventional breeding or transgenic technology. Various DNA based molecular markers are scrutinized for faster crop improvement. Markers have role in gene mapping, genetic diversity analysis, and evaluation of germplasm. In traditional molecular marker system it is tough to get a desirable marker which has well combination of abundant polymorphism, highly reproducible, good stability and high efficiency (Razna *et al.*, 2015). A new type of molecular marker was developed in by Fu et al. (2013) with high efficiency, sufficient polymorphism and they are also highly reproducible.

The miRNA and pre miRNA sequences are highly conserved throughout the genome which give the opportunity to develop novel molecular based on miRNAs (Fu *et al.*, 2013). Besides these pre-miRNAs have stem loop regions which are highly conserved between closely related species (Mendes *et al.*, 2009, Fu et al., 2013) providing good marker transferability. DNA based markers developed from miRNA genomic sequences was successfully used in biomedical research on the aspects of breast cancer (Luo *et al.*, 2011). SSR marker (microsatellite) are also the sensitive tool which are useful for evaluation of genetic structure, intraspecific variation and genetic diversity (Wang *et al.*, 2016). SSRs can be intragenic or intergenic (Toth *et al.*, 2000), therefore the variable length of repeat motifs of SSRs may have relation with different activity and functions of the segments of chromosomes in which they reside. miRNA based SSR markers are substantially got success in animal science but in plant a few reports are available. In Brassica and also in foxtail millets (*Setaria italica*) and related grass species, miRNA based markers was used for genotyping (Fu *et al.*, 2013; Yadav *et al.*, 2014). Another novel approach was taken in this aspect in rice (*Oryza sativa*) which identifies salt responsive miRNA- SSR markers and utilised for diversity analysis between the related genotypes (Mondal and Ganie, 2014), in lotus (*Nelumbo nucifera*) genome wide identification and characterization of miRNA- SSRs was done (Wang et al., 2016). Wheat (*Triticum aestivum*) is considered a major staple cereal as evident from its volume of production worldwide which was 762 million metric ton cultivated on 218 million hectares of land in 2017-18. Wheat generally uptake 0.5-0.6 pounds of P₂O₅ per bushel. When phosphorous deficiency is present, winter wheat is more susceptible to winterkill and vulnerable to disease pressure, among other plant issues. It may be difficult to improve both P uptake and P utilization efficiencies together due to a negative correlation. But it can be achieved by further studying the inter-relation and interaction between multi-dimensional approaches associated with PUE (Phosphorous use efficiency). Recent studies have also shown the involvement of miRNAs (micro-RNAs), long non-coding RNA (lnc-RNAs) having better adaptation to P limited conditions by modifying plant growth, phenology, architecture and production of secondary metabolites (Liu *et al.*, 2015; Fischer *et al.*, 2013; Zeng *et al.*, 2014; Paul *et al.*, 2015). Therefore, identification of the microRNA based SSR markers which will be useful for genetic diversity analysis for P stress tolerance among the tolerant and sensitive wheat genotypes. This characterization of low P responsive (trait specific) miRNA derived SSRs which may become a useful tool in future for marker assisted breeding program in wheat.

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