Biofortification and Genomics Approaches to Improve Quality of Nutricereal Pearl Millet

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Keywords: Biofortification, genomic tools, malnutrition, MAS, nutricereal, pearl millet

Abstract

Pearl millet [*Pennisetum glaucum* (L.) R. Br.] is main staple food grown on more than 26 mha in the arid and semiarid tropical regions of Asia, Africa and Latin America. In India, it is the fourth most widely cultivated crop with an average production of 9.73 mt. It has high nutrition value and rightly termed as nutricereal as it is rich in protein, essential fatty acids, dietary fibre, vitamins, minerals such as calcium, iron, zinc, potassium and magnesium. It helps in rendering several health benefits but its direct consumption as food has significantly declined over the past three decades due to various reasons. In this context, it is important to raise the awareness on its nutritional value and reorient the efforts to generate demand through value-addition and quality improvement. Biofortification is a cost-effective process of nutrient fortification using modern breeding, improved agronomic practices, transgenic approaches and microbiological interventions. In addition, molecular tools and genomic studies are also promising approaches as they have massive prospective for improving efficiency and accuracy of conventional breeding. In India, through ICAR-All India Coordinated Research Project on Pearl millet, we are developing various biofortified hybrids rich in Fe and Zn and using MAS strategies and genomics tools, Fe-Zn QTL location has been identified and Improved HHB 67 has been released. Further, using consensus map, genomic positions of SSR markers related to grain iron and zinc content is being identified and research is in progress towards mapping QTLs for flour rancidity. Identification and tagging of genes underlying a trait has become possible due to advanced next-generation sequencing (NGS) and molecular profiling technologies. Recently, the whole genome sequencing of pearl millet has been carried out and the draft genome and resequencing data will help researchers better understand the trait variation while advancing genetic improvement of the crop. Thus, development and application of novel approaches such as biofortification, marker-trait associations, genomic selection tools, NGS and GBS need to be intensified to accelerate the genetic gain targets for improvement of pearl millet.

Introduction

Pearl millet [*Pennisetum glaucum* (L.) R. Br.] is grown on more than 26 million ha in the harsh climatic conditions in arid and semi-arid tropical regions of Africa, Latin America and Asia. India is the largest producer with an average production of 9.73 million ton where it is the fourth most widely grown crop after rice, wheat and maize. It is a highly nutritious cereal and established as a "nutricereal" due to the presence of good quality protein, vitamins, unsaturated fatty acids, carbohydrates, insoluble dietary fibre and minerals such as iron, zinc, magnesium, calcium, and potassium and year 2018 was declared as the "Year of Millets". It helps in rendering several health benefits like reduction in blood pressure, diabetes, thyroid, cardiovascular and celiac diseases. It has lower glycemic index and lacks gluten thus enhancing its health value and being accepted by many people (Dahlberg et al., 2004). But, still there is a need to raise basic awareness especially among youth about its nutritional value. Thus, efforts must be focused to generate its demand through value addition and quality improvement which may become possible by biofortification and genomic approaches. The major health problems among the poor in India are due to micronutrient deficiencies and to meet out the challenge of hidden hunger biofortification can prove to be an effective approach. It is cost-effective because it involves only a one-time investment in plant breeding. In addition, biotechnological tools and genomic studies using high throughput genomic tools are also promising approaches as they have huge potential to accelerate the genetic gains targets and improve breeding efficiency in the changing climatic scenario.

Biofortification as a potential tool

Micronutrient deficiencies lead to poor health and considerable efforts are being done in this direction to improve the health of poor people by breeding staple food crops enriched with essential micronutrients using strategies like biofortification at global and national fronts. Biofortification is the approach to enhance the content and bioavailability of essential vitamins/minerals in crops using modern breeding, transgenic approaches, improved agronomic practices and microbiological interventions. It helps in changing genetic architecture, increasing micronutrient uptake and properly distributing them in edible tissues to safe levels, reduction in antinutrients in food staples ultimately leading to crop improvement (Bouis et al*.,* 2011). It is an upcoming, cost-effective, sustainable and effective strategy to deliver essential micronutrients to a larger population that has limited access to diverse diets. Further, efficiency of biofortification approach mainly depends on the farmer's and consumer's acceptance and future policy interventions.

Approaches and strategies of biofortification

Plant breeding, biotechnology, genetic engineering, transgenic approaches, improved agronomic practices are some of the sustainable approaches of biofortification to achieve the desired targets (Stein *et al*. 2008). Although, presently very few

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commercial plants with better nutrition derived from these methods are available, yet these approaches have cost-effectiveness and might have a vital impact in future. Plant breeding and genetic engineering can effectively change the genotype of a target crop by developing germplasm lines with genes favoring the most efficient buildup of bioavailable minerals. This could be achieved by undertaking crossing programme of the best performing crop plants and selecting only the plants with desirable traits. While, genetic engineering involves introduction of genes accessed from any source directly into the crop plants. The main advantage of these approaches is that investment is essentially required for research and development activities and mineral rich plants give higher yields, more vigorous and tolerant to biotic stresses (Nestel et al., 2006). There are two main delivery strategies for biofortification- the push and pull strategies where push strategy is supply oriented while pull strategy is demand oriented and a combined approach is proposed for pearl millet in India, with a marked emphasis on the push strategy.

Biofortification in pearl millet

Biofortification research in pearl millet has shown large variability for Fe (31-125 ppm) and Zn (35-82 ppm) content with good prospects of developing cultivars with higher contents of these micronutrients. Biofortification Priority Index (BPI) indicates pearl millet is a major target crop for iron and zinc biofortification. Pearl millet has relatively higher content of Fe & Zn but commercially available cultivars have lower Fe and Zn content. Thus, pearl millet biofortification research focuses towards development of high-yielding and high-Fe/Zn hybrids in India. The gene banks of pearl millet contain varieties with high levels of iron and zinc which may be used to produce new pearl millet varieties with elevated iron levels bred with the high-yielding varieties.

Several promising donors have been identified and breeding material is being generated combining high nutrient content and yield. Biofortification of pearl millet is underway in India and ICAR is supporting a Consortia Research Platform (CRP) in several crops including pearl millet to target development of biofortified crops with enhanced nutrients since 2014. One variety and six hybrids were identified as biofortified/iron rich pearl millet. A first high-iron pearl millet variety 'Dhanashakti' released in Maharashtra state in 2013, was subsequently released and notified by Central Variety Releasing Committee in April 2014, for cultivation in all pearl millet growing states of India. This variety has also been included in the Nutri-Farm Pilot Project, Govt. of India for addressing issue of Fe deficiency in India. Thus, along with yield improvement, focus on the nutritional improvement was also taken care in pearl millet and in order to develop biofortified varieties/hybrids with enhanced Fe and Zn, a landmark decision of including minimum Fe (42 ppm) & Zn (32 ppm) in promotion criteria was taken by ICAR-AICRP on Pearl millet which is the first of its kind in the world.

Genomic interventions for pearl millet improvement

Conventional approaches are good but time consuming and need support of genomics, genetic transformation and several modern biotechnological tools to accelerate millet development programs. Molecular tools and genomic studies are gaining lot of momentum these days as they are playing major role in crop improvement programs. The advanced next-generation sequencing (NGS) and molecular profiling tools are more efficient in detecting DNA sequence variations over several loci and identification and tagging of genes underlying a trait. Enormous development in area of genomics during past years has rendered various novel tools for precise and faster breeding programs. Recently, the whole genome sequencing of pearl millet has been carried out and the draft genome and resequencing data will help researchers better understand the trait variation while advancing genetic improvement of the crop. Genetic maps, NGS, GBS, GWAS, transgenic approaches, molecular tools, genomic studies, synteny studies, expression profiling, fine QTL mapping, candidate gene identification and genetic engineering technology, transcriptomics, proteomics and metabolomics are some of the useful platforms which can be used for the advancement of nutrient rich pearl millet (Langridge and Fleury, 2011).

Use of molecular markers like RFLP, AFLP, SSR and SNP markers and applications of MAS may be anticipated ultimately giving prospective benefits of rapid release of useful varieties. Pearl millet is the first crop where MAS strategies and tools have been applied to develop downy mildew-resistant variety "Improved HHB 67" (Hash et al., 2006). Fe-Zn QTL location has been worked out and research is being focused towards mapping QTLs for flour rancidity. Omics approaches can be widely used to analyze the genes responsible for stress adaptation and identification of various QTLs governing the adaptive response under stressful conditions and predict the molecular mechanism of stress response/tolerance, disease resistance and nutritional quality by studying gene, protein or metabolite profile and their phenotypic effects. NGS, GBS approaches along with genome-wide expression profiling studies can resolve the issues put forward by large genomes particularly those like pearl millet. In addition, genome editing using Cas9/sg RNA system is very reliable, functional and proficient in two model system and thus gives lot of prospective for gene manipulations.

Conclusion

Biofortification is very useful for addressing the issue of malnutrition and will be more accessible in long-term because it eliminates hurdle and is independent of any infrastructure or procurement. Also, normal taste and texture of grains remains as such as plants absorb minerals in organic forms and which is obviously bioavailable. Biofortification journey is quite long and are being discussed in several forums but yet more efforts are

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required to take it into mainstream including focus on its bioavalablity. In conclusion, biofortification strategies and multidisciplinary research is required to move forward and raise the importance of nutricereal pearl millet in a more effective way so that the products can be made reliable and available to the consumers.

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