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"MOLECULAR BREEDING FOR LOW PHYTATE MAIZE (*ZEA MAYS L.*)"

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ABSTRACT

Maize is the third important major food crop. Phytic Acid (PA) in maize kernel is an anti-nutritional factor; it chelates mineral cations in human gut and causes mineral deficiency in humans. Therefore, development of low-PA maize is required. Breeding for low phytate maize genotypes is an effective strategy for decreasing the content of kernel PA and thereby increasing the bioavailability of nutritive minerals in human diet and animal feed. In maize, three low phytic acid (lpa) mutants have been isolated viz., lpa1, lpa2 and lpa3. These mutant lines are important genetic resources to develop low phytic acid maize. Scientists have developed markers for lpa mutants which can be introgressed to develop low phytate maize using marker assisted backcross breeding. Here we discuss phytic acid and its effects in maize kernels, low phytic acid mutants in maize, molecular markers for lpa mutants and marker assisted backcross breeding to develop low phytate nutritive maize. This knowledge will help to understand the effects of phytic acid, lpa mutants and their use in development of low phytate maize.

Keywords : Maize, low phytate, lpa mutants, marker assisted backcross breeding.

INTRODUCTION :

Maize (*Zea mays L.*) is an important human food, livestock feed and an industrial raw mater. A major concern in maize is its phytate (phytic acid/ PA) content, which may go up to 4.0 mg/g of seed. PA also known as myo-inositol hexakisphosphate or InsP6. PA is a major storage form of phosphate in plant seeds (Raboy, 2007) accounting for up to 80% of total seed phosphorus and contributing as much as 1.5% to the seed dry weight. The negatively charged phosphate in PA strongly binds to metallic cations of Ca, Fe, K, Mg, Mn and Zn making them insoluble and thus unavailable as nutritional factors

(Bohn et al. 2008). When released during food or feed processing or in the gut, PA binds minerals and makes them unavailable and hence PA is an anti-nutritional factor (Raboy, 2007), which causes malnutrition in human (Zhou and Erdman, 1995). PA also reduces the phosphorous availability required for growth in monogastric animals, which digest PA poorly. Moreover, undigested PA eliminated by the monogastric animals into the environment leads to an increase in phosphorous level in the environment and contributes to water pollution through eutrophication (Cromwell and Coffey 1991).

Supplementation of feed with inorganic phosphate or with industrially produced phytase enzyme, which breaks down PA and releases phosphorous for animal use, can address the phosphorous requirement for animal growth and reduce phosphorous pollution. However, phosphate and phytase supplementation increase the animal feeding costs. Therefore, breeding for low phytate maize genotypes is an effective strategy for decreasing the content of kernel phytic and thereby

I) increasing the bioavailability of nutritive minerals in human diet,

ii) increasing the availability of phosphorous in animal feed,

iii) decreasing the environmental pollution by phosphorous released from undigested and unutilized phytic acid derived from animal feed and

iv) reducing the amount of phytase used to supplement animal feed for breaking down seed derived phytate and release phosphorous for animal growth (Ertl et al. 1998).

Phytic acid mutants

Several low PA mutants have been developed successfully in soybean, barley, maize and rice by disrupting PA biosynthesis pathway through mutagenesis breeding (Larson et al., 2000; Raboy et al., 2000; Wilcox et al., 2000) and these mutant lines were used in genetic breeding as donors for the development of low PA lines (Raboy et al., 2001). So far, in maize, three low phytic acid (lpa) mutants have been isolated viz., lpa1, lpa2 and lpa3. These mutant lines are important genetic resources to develop low phytic acid maize crops.

The lpa1mutation is caused by a mutation in a gene that encodes transmembrane transporter protein (ZmMRP4), which is hypothesized to load phytic acid into protein storage vacuoles of maize seed. The lpa2 mutation is caused by a mutation in inosotol phosphate kinase gene (ZmIpk4), which along with other kinases leads to phytic acid synthesis. The lpa2-1 mutation is caused by genomic sequence rearrangement in the ZmIpk. The lpa2-2 mutation caused by a single nucleotide change (i.e. C to T at nucleotide position 158) generates a stop codon in the N-terminal region of the ZmIpk open reading frame (Shi et al. 2003). The lpa3 mutation is caused by a mutation in a gene that encodes myoIns kinase, which catalyzes the production of Ins (3) P1 in maize seed. Compared with wild type kernels, the lpa1, lpa2-1, lpa3 mutations achieved 66%, 50% and 50% reduction in phytic acid content respectively (Raboy et al. 2000; Shi et al. 2005). The lpa2-2 mutation achieved a 30% reduction in phytic acid content and a three-fold increase in inorganic phosphate (Shi et al. 2003). The mutant lines are temperate maize lines that are not adapted to local tropical and subtropical conditions.

Therefore, there is a need to have the lpa locus introgressed into locally adapted agronomically superior lines to improve their nutritional benefit.

Marker assisted backcross breeding for low phytate maize

Marker assisted backcross breeding (MABB) provides a great opportunity for transfer of desirable trait of interest into the genetic background of a recipient genotype by recurrent backcrossing and also to recover the recurrent parent genome as rapidly and completely as possible. Therefore, MABB that involves introgression of lpa2-2 recessive allele for low phytate trait from the donor lpa2-2

mutant into a locally well-adapted agronomically superior line using a series of backcrosses and selection of lines possessing lpa2-2 trait from each backcross progenies, with the help of markers, is an effective strategy for developing low phytate maize. The selection of lines possessing lpa2-2 trait from each backcross progenies is a challenging task because it requires destructive sampling to measure the amount of phytic acid in maize grain. Also, the selection takes time and therefore the selection has to be deferred until when adequate seed can be produced to allow destructive sampling. Therefore, the development of a co-dominant molecular marker will enable quicker selection and make maize breeding for LPA efficient and fast, and it will enable the earlier release of lpa2-2 varieties.

Recently researchers have successfully developed and applied the lpa1-1 SNP marker for foreground selection in a backcross breeding programme and reported that this SNP marker can enhance efficiency of maize breeding for LPA and it may enable the earlier release of lpa1-1 varieties (Naidoo et al. 2012, 2013). But profiling of plants using SNP marker includes a PCR step and a post PCR step (i.e. high resolution melt analysis). Sureshkumar et al. (2014) constructed the genetic map of chromosome 1 of maize and found that a SSR marker umc2230 present in the short arm is in proximity (0.4 cM downstream) of the lpa2-2 allele. Further they validated that SSR marker umc2230 cosegregates with phytate trait conferred by lpa2-2 alleles in a mendelian fashion in both selfed and backcross progenies, and concluded that umc2230 can be dependably used in a MABB for the efficient selection and transfer of low phytate trait for the development of low phytate nutritive maize lines. Tamilkumar et al. (2014) made use of umc2230 for efficient, fast and cost effective selection of plants possessing lpa2-2 allele in MABB and successfully developed four lines with both low PA trait similar to that of donor parent and agronomical traits similar to that of the recurrent parent. Breeding for maize genotypes with low kernel phytic acid content is an effective strategy for increasing the bioavailability of minerals in human diet and availability of phosphorous in animal feed, decreasing the environmental phosphorous pollution and reducing the cost of phytase supplementation in animal feeds.

Conclusion

The MABB that involves introgression of lpa2-2 recessive allele (which confers low phytate trait) from the donor lpa2-2 mutant into a well adapted agronomically superior line with the help of a series of backcrosses and selection of lines possessing lpa2-2 allele in each backcross population with the help of markers is an effective strategy for developing low phytate maize. A recent advance in genetic engineering has led to development of transgenic lines with low phytic acid by expressing exogenous phytase genes in maize (Chen et al. 2008). Phytic acid is part of plant metabolism and plays a major role in plants response to different abiotic stresses, besides possessing positive effects on imparting resistance against pathogens and insect-pests (Graham et al. 2001; Welch and Graham, 2004). It is also required for higher seedling vigour and reduced aflatoxin development in grain (Morris, 1995). Besides, phytate has been found to protect seeds against oxidative stress during the seed's life span (Doria et al. 2009). Thus, development of agronomically suitable high yielding genotypes with sufficiently low phytic acid is a challenge for the researchers.

References

1. Bohn, L., Meyer, A. S. and Rasmussen, S. K. 2008. Phytate: impact on environment and human nutrition. A challenge for molecular breeding. *J. Zhejiang Univ. Sci.* 9: 165 – 191.
2. Chen, R., Xue, G., Chen, P., Yao, B., Yang, W., Ma, Q., Fan, Y., Zhao, Z., Tarczynski, M. C. and Shi, J. 2008. Transgenic maize plants expressing a fungal phytase gene. *Transgenic Res.* 17: 633-643.

3. Cromwell, G. L. and Coffey, R. D. 1991. Phosphorus: a key essential nutrient, yet a possible major pollutant. Its central role in animal nutrition. In: Lyons, T. P. (ed.), Biotechnology in the feed industry. *Alltech Tech Publishers, Nicholasville, KY.* pp. 133-145.
4. Doria, L., Galleschi, L., Calucci, L., Pinzino, C., Pilu, R., Cassani, E. and Nielsen, E. 2009. Phytic acid prevents oxidative stress in seeds: evidence from a maize (Zea mays L.) low phytic acid mutant. *J. Exp. Bot.* 60: 967-978.
5. Ertl, D. S., Young, K. A. and Raboy, V. 1998. Plant genetic approaches to phosphorus management in agricultural production. *J. Environ. Qual.* 27: 299 – 304.
6. Graham R. D., Welch R. M. and Bouis H. E. 2001. Addressing micronutrient malnutrition through enhancing the nutritional quality of staple foods: principles, perspectives and knowledge gaps. *Adv. Agron.* 70: 77-142.
7. Larson, S. R., Rutger, J. N., Young, K. A. and Raboy, V. 2000. Isolation and genetic mapping of a non-lethal rice (L.) mutation. Crop Sci. 40: 1397-1405.
8. Morris, H. 1995. Phytate: a good or a bad food component? *Nutrition Res.* 15: 754-773.
9. Naidoo, R., Watson, G., Derera, J., Tongoona, P., Laing, M. 2012. Marker-assisted selection for low phytic acid (lpa1-1) with single nucleotide polymorphism marker and amplified fragment length polymorphisms for background selection in a maize backcross breeding programme. *Mol Breed.* 30: 1207-1217.
10. Naidoo, R., Watson, G., Tongoona, P., Derera, J. and Laing, M. 2013. Development of a single nucleotide polymorphism (SNP) marker for detection of the low phytic acid (lpa1-1) gene used during maize breeding. *Afr J Biotech*. 12: 892-900.
11. Raboy, V., Gerbasi, P. F., Young, K. A., Stoneberg, S. D., Pickett, S. G., Bauman, A. T., Murthy, P. P., Sheridan, W. F. and Ertl, D. S. 2000. Origin and seed phenotype of maize low phytic acid 1-1 and low phytic acid 2-1. *Plant Physiol.* 124: 355-368.
12. Raboy, V., Young, K. A., Dorsch, J. A. and Cook, A. 2001. Genetics and breeding of seed phosphorus and phytic acid. *J Plant Physiol.* 158: 489-497.
13. Raboy, V. 2007. The ABCs of low-phytate crops. *Nat Biotech.* 25: 874-875.
14. Shi, J., Wang, H., Wu, Y., Hazebroek, J., Meeley, R. B. and Ertl, D. S. 2003. The maize low-phytic acid mutant lpa2 is caused by mutation in an inositol phosphate kinase gene. *Plant Physiol.* 131: 507-515.
15. Shi, J., Wang, H., Hazebroek, J., Ertl, D. S. and Harp, T. 2005. The maize low-phytic acid 3 encodes a myo-inositol kinase that plays a role in phytic acid biosynthesis in developing seeds. *Plant J.* 42: 708-719.
16. Sureshkumar, S., Tamilkumar, P., Thangavelu, A. U., Senthil, N., Nagarajan, P., Vellaikumar, S., Ganesan, K. N., Balagopal, R. and Ravindran, M. 2014. Marker-assisted introgression of lpa-2 locus responsible for low-phytic acid trait into an elite tropical maize inbred. *Plant Breed*. 133: 566-578.
17. Tamilkumar, P., Senthil, N., Sureshkumar, S., Thangavelu, A. U., Nagarajan, P., Vellaikumar, S., Ganesan, K. N., Natarajan, N., Balagopal, R., Nepolean, T. and Raveendran, M. 2014. Introgression of low phytic acid locus (lpa2-2) into an elite maize (Zea mays L.) inbred through marker assisted backcross breeding. *Aus. J. Crop Sci.* 8: 1224-1231.
18. Welch, R. M. and Graham, R. D. 2004. Breeding for micronutrients in staple food crops from a human nutrition perspective. *J. Exp. Bot*. 55: 353-364.
19. Wilcox, J. R., Premachandra, G. S., Young, K. A. and Raboy, V. 2000. Isolation of high seed inorganic P, low-phytate soybean mutants. *Crop Sci.* 40: 1601-1605.
20. Zhou, J. R. and Erdman, J. W. Jr. 1995. Phytic acid in health and disease. *Crit. Rev. Food Sci. Nutr.* 35: 495-508.