

Hypoimmunogenic Gluten: A Way Out for Wheat Gluten Intolerance

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SUMMARY

Wheat gluten is majorly divided into two fractions: glutenins and gliadins. Wheat with hypoimmunogenic gluten denotes no or reduced immunogenic epitopes such as α -gliadins, γ -gliadins, ω -gliadins, and some LMW glutenins. These epitopes creates health issues like celiac disease, dermatitis herpetiformis and gluten ataxia among gluten intolerant individuals. As wheat is a major staple crop, following a gluten-free diet to combat this is nearly impossible. The purpose of breeding for hypoimmunogeneity in wheat is to make the wheat products safe for gluten-intolerant people to consume. Since no cultivated or wild sources are available, gluten-safe wheat is developed using mutation breeding and molecular breeding techniques to knock-out, knock-down, or edit the gene.

INTRODUCTION

Wheat (*Triticum aestivum* L.) is one of the leading cereals in terms of production and utilization. Wheat, unlike other cereals, has the ability to produce a variety of processed forms from its flour due to its key protein, gluten. It is the major target while considering the quality breeding aspects in the crop. So far concern was to improve gluten content and composition. The first reports of gluten intolerance came way back in 1980s (Cooper *et al.* 1981). After the ground breaking work published in 2010 by Sapone and colleagues describing the clinical and diagnostic features of gluten sensitivity, a serious discussion and research into gluten free diets began. To avoid the problems associated with gluten consumption, wheat with hypoimmunogenic gluten could be developed.

Wheat Gluten and Classification:

The end-use quality of wheat grain is determined by its protein, starch and lipids constituents of whom gluten proteins play a pivotal role (Sonia goel *et al.*, 2015). Wheat gluten was unearthed in 1754 by Jacopo Giacomo Beccari, a chemistry professor at the University of Bologna, who reported the preparation of a water-insoluble fraction from wheat flour called "glutinis". Gluten is the primary protein in wheat that imparts the weak and extensible property. When the dough is washed with water, most of the soluble and particle debris is removed, leaving a mass of protein called gluten that has the ability to remain cohesive when stretched. Albumin, globulin, gliadin, and glutenin are four types of wheat seed proteins based on their solubility qualities (Osborne 1907). While albumin and globulin are minor proteins in the wheat endosperm that aren't thought to have a significant impact on end-use quality, gliadin and glutenin, the two major components of gluten, are the most important determinants of wheat flour quality for bread, biscuits, chapati, noodles, and other products (Shewry *et al.*, 1990). Gluten contains 75 percent of the protein (Anjum *et al.*, 2007) and is made up of a complex mixture of monomers called gliadins (prolamins) and polymeric glutenins (or glutelins) (Wall *et al.*, 1979). Polymeric glutenin is responsible for the strength or elasticity of wheat flour dough, but monomeric gliadin is responsible for its viscosity, according to fractionation and reconstitution investigations with wheat flour (MacRitchie 1985). The major monomeric low molecular-weight (LMW) and polymeric high molecular-weight (HMW) glutenin subunits (GS) are unequally distributed among the glutenin protein subunits (Payne *et al.*, (1981). Although both fractions have an impact on flour extensibility and strength, HMW-GS appears to be more important for dough strength and LMW GS for dough extensibility. The gliadins consist of monomeric proteins, which are separated into α/β , γ , and ω groups by polyacrylamide electrophoresis at low pH.

Gluten Intolerance:

Wheat gluten can generate health problems in gluten intolerant individuals. Along with abdominal pain, bloating, and diarrhoea this ailment often causes headaches, fatigue, rashes, and joint pain (Servick. 2018). According to the estimates, this condition affects more than 1% of the world's population (Raju *et al.*

2020). The prevalence of gluten intolerance in northern India has lately been revealed, despite the fact that it is generally thought of as a western disease. This is because wheat is a major staple in the northern states. Based on an Indian Council of Medical Research survey, gluten intolerance affects 8 out of every 1,000 persons in Haryana, 4 out of every 1,000 in Assam, and only 0.1 out of every 1,000 people in Tamil Nadu (Anonymous, 2019). Therefore, addressing the gluten intolerance is as important as the gluten quality improvement in wheat. The term "gluten intolerance" refers to conditions such as non-celiac gluten sensitivity (NCGS), wheat allergy (WA), and indeed the autoimmune Celiac Disease (CD) (Balakireva et al. 2016). The CD is the major disease associated with the ingestion of the gluten proteins of wheat and related prolamins. It is associated with certain alleles of Human Leukocyte Antigen (HLA) genes. Two much rarer forms of intolerance which are related to CD have been described: these are dermatitis herpetiformis (DH) and gluten ataxia (GA). CD is a T cell-mediated autoimmune disease in which the ingestion of prolamins results in the inflammation and flattening of the villi in the intestinal tract there by malabsorption of nutrients (Shewry and Tatham. 2015). The solutions for gluten intolerant people are either to follow gluten-free diet (excluding wheat, barley, rye) or to consume wheat products containing only hypoimmunogenic gluten proteins.

Hypoimmunogenic Wheat:

A gluten-free diet can be more expensive and less healthy. On the other hand, since gluten is a key element for end-use quality, it is absolutely impossible to remove the entire gluten from wheat. Hence, development of wheat varieties with hypoimmunogenic gluten holds immense importance. Certain short protein amino acid sequences called epitopes have the ability to trigger a more severe and immediate immune response (Palatnik-de-Sousa *et al.* 2018). Wheat varieties without these immunogenic epitopes are known as hypoimmunogenic. Among the gluten fractions, gene families encoding α -gliadins on chromosomes 6 trigger CD strongly, followed by γ -gliadins, ω -gliadins, and LMW glutenins on chromosomes 1. The immunogenic epitopes identify and activates the HLA-DQ2.5 and/or HLA-DQ8 protein receptors on T cell surface of intolerant individuals (Sollid *et al.* 2012). In order to attain hypoimmunogeneity in wheat, the genes that encode these epitopes can be deleted or edited.

Genetic Improvement for Gluten-Safe Wheat:

There is no cultivated wheat or wild relative has been identified that contains only CD safe gluten epitopes so far (Van den Broeck *et al.*,2010b). Therefore, several approaches like basic screening for natural coeliac safe variants, mutagenesis, transgenic techniques and genome editing can be followed to derive lines free from CD epitopes. *In vivo* studies of the developed hypoimmunogenic wheat lines are also required to ensure that no CD reaction occurs.

Mutation Breeding

Various mutagens can induce desirable mutations in the gliadin genes, reducing wheat immunogenicity. So far, γ irradiation and Ethyl methane sulfonate (EMS) have been predominantly used for this purpose which creates large deletions in the chromosome 1 and 6 of wheat. Hybridization followed by selection can be used to combine selected mutations from different plants (Tanner *et al.* 2016).

RNAi and CRISPR Techniques

Recently, RNA interference and CRISPR-Cas9-mediated genome editing have become popular and are highly effective tools for precisely modifying the genes that code for epitopes. Jouanin *et al.* (2018) transformed immature embryos of the bread wheat variety Fielder with constructs of Cas9 and multiplex guide RNA constructs to reduce immunogenicity without affecting dough rheology.

CONCLUSION

Working with wheat gluten is a challenging task. It is important to retain the baking or end-use quality while developing gluten-safe wheat varieties. Molecular breeding techniques pave a new direction in wheat improvement to efficiently reduce the immunogenic gliadins in wheat. This area of research is underexplored due to the poor detection of gluten intolerance among the Indian population.

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