ScienSage

Journal of Advanced Scientific Research

Available online through http://www.sciensage.info

ISSN
0976-9595
Review Article

GLUTEN FREE WHEAT (*TRITICUM AESTIVUM L.*): A GENETIC WAY TO CURB COELIAC DISEASE

Mahek Sharan*[1], Meghana DP[2]

[1]*Department of Biotechnology, Institute of Applied Medicine and Research, Ghaziabad, India*

[2]*Department of Genetics and Plant Breeding, Punjab Agricultural University, Punjab, India*

**Corresponding author: Mahek01091998@gmail.com*

ABSTRACT

There are three main cereal crops, rice, maize and wheat, in which, latter constitutes the major portion in the category. According to 2017 reports, it is known to be harvested around 772 million tonnes. Wheat is present in a diverse range and gets its elasticity, viscosity and properties of binding by the storage protein, Gluten, which is a secretory protein synthesised on rough endoplasmic reticulum. Gluten is roughly 78-85% of total wheat protein and made of gliadin and glutenins. There is a part of population with genes which develop autoimmune response against gluten that majorly affects the small intestine by damaging its lining and have to suffer from problems like pain in abdomen, joints, anaemia, osteoporosis and various gastro intestinal problems, developmental problems and problems like cramping, itching and lactose tolerance and excess weight loss. The patients who suffer from this disease undergo either biopsy of small intestine or serological assay like Rapid or ELISA for diagnosis. There are currently no treatments available for coeliac disease as the only way to curb this is strict gluten-free diet. There have been advances made in the field of agriculture, which resulted in production of the low gliadin content by use of gene editing technology and RNAi but the customer acceptance is the major issue faced. The most recent technological advancements in the field of biotechnology, like CRISPR-Cas9 is capable of producing the genetically modified wheat with lowest gliadin composition to curb the celiac disease impact in population.

Keywords: Wheat, Gluten, Coeliac disease, ELISA, Rapid, RNAi, CRISPR-Cas9.

1. INTRODUCTION

The cereal crops are basically comprised of three crops, wheat, rice and maize. In which, wheat hold the position of one of the primary cereals in the world. Wheat is a cereal crop where the leaves from shoot apical meristem emerges and transforms to flowering by reproduction. Wheat is majorly consumed as a food and used in different food processing industries for manufacturing of bread and other bakery products, raw wheat is used as flour, livestock feed and if germinated and dried then used in the alcoholic beverage industry as malt. Wheat is majorly composed of carbohydrates (73gm/100gm) along with moderate amount of protein (12gm/100gm) present in the grain. According to 2018 reports of Food and Agriculture Organisation on International Wheat Production Statistics, the countries that acquired the top position for the production of Wheat, were China followed by India with 99.7 million metric tonne. The study on transcriptomics and proteomics of wheat showed that in a developing wheat grain, there are over 30,000 genes expressed and 1125 individual components involved in grain protein expression. Gluten protein which consists of glutenin and gliadins are responsible for the stretchibility and viscoelasticity of the dough of wheat flour and the gliadins are present in four forms encoded by the genes. Coeliac disease is a genetic disorder which causes an autoimmune response when interacted with gluten protein results in inflammatory T cells specific response leading to various gastrointestinal and extrgastro-intestinal symptoms and deterioration of small intestine lining. There is no treatment present currently for the coeliac disease and lately a proper diagnosis set has been created but gluten free wheat production by the genome editing technology can make it possible.

2. WHEAT

Wheat is basically a grass which is cultivated on a large scale for its seeds that constitutes of the worldwide staple food [1-3] (Fig.1). The cereal crops are basically

comprised of three crops, wheat, rice and maize. In which, wheat hold the position of one of the primary cereals in the world. The reason behind this is mainly the widest cultivation range of wheat from plains to elevated regions in tropics and sub-tropics along with its role played in different cultures and religions [4].

Fig. 1: *Triticum aestivum*: A staple crop

There are different species of wheat which collectively form the Triticum genus (Table1). The most common wheat grown is *Triticum aestivum*. The global demand of wheat is increasing day by day due to the ideal features showed by it for industrial food processing like viscoelasticity and gluten proteins adhesive properties [5, 6].

Table 1: Kingdom classification of wheat

Kingdom	Plantae
Clade	Angiosperms
Clade	Commelinids
Class	Monocots
Order	Poales
Family	Poaceae
Subfamily	Pooideae
Supertribe	Triticodae
Tribe	Triticeae
Genus	Triticum

3. PHYSIOLOGY

Wheat is a cereal crop where the leaves from shoot apical meristem emerges and transforms to flowering by reproduction. The flag leaf is the last leaf produced by it as it has higher rate of photosynthesis and denser in chlorophyll, to supply energy in form of carbohydrates to developing ear [7, 8]. Wheat is very unusual when compared to other plants as they have more stomata on their adaxial side than abaxial side of leaf, which may be the consequence of domestication [9]. Wheat is generally preferred to be grown in winter as it produces 15 leaves per shoot giving 35 tillers per plant whereas in spring it is only 9.

The roots of wheat are deep and can extend down up to 2m [10] and it stores its energy in stem by accumulating in form of fructans [11], making it tolerant to disease and drought [12]. The wheats are of different variety depending on the production on awn, where the awn production results in more photosynthesis in leaves [13] and more frequently observed in drought prone areas [14].

4. HISTORY OF WHEAT

Around 10,000 years ago, wheat was a part of the Neolithic Revolution, which was the major transition from hunting to cultivation. Among the cultivated forms, the earliest of the cultivated wheat forms were Diploid (AA genome), known as einkorn and Tetraploid (AABB genome), known as emmer. When the genetic relationships of the cultivated wheats were studied, it was found that the origin of wheat is basically from Turkey's south-eastern part [15-17]. Around 9000 years ago, hexaploid bread wheat started to be cultivated in the near east [18]. The wheat variety evolved by natural selection and clearly from the non-scientific way of plant breeding, as the traits selected of wheat over other wild varieties were dependent on two characteristics. The first one is seed loss by the spike shattering at maturity which was a primary trait for dispersal of seeds and the mutations in brittle rachis (Br) is responsible for non-shattering [19]. Second one is the change to naked forms, which arose from the dominant mutant Q locus that modified the recessive mutations at Tg locus effects, making it easier to thresh unlike from earlier hulled forms where the grains were tightly attached to the glumes [20-22]. The evidence of evolution and selection could easily be seen by observing the early domesticated forms of emmer, einkorn and spelt as they both are in hulled forms while modern forms like tetraploid and hexaploid are in the free threshing form.

In 1880s, John Benner Lawes while performing his long term famous Broadbalk experiment at Rothamsted, decided to allow part of it to return to the natural form [23]. In 1882, he left one part of the crop unharvested and observed the growth for years. He recorded the weed dominancy in 1883. From this, it was quite clear that hexaploids of *A* genome are related to wild *A*

genomes and cultivated eikorn, while hexaploids of *D* genome is derived from the cultivated ancestor *Triticum tauschii*. The B genome of tetraploid and hexaploid on the other hand, is derived from *S* genome that occur in Sitopsis section of Aegilops with the closest extant species of *Aegilops Speltoides,* which is closest to *G* genome of *T. timopheevi* [18].

5. NUTRITIONAL INDEX OF WHEAT

Wheat is majorly consumed as a food and used in different food processing industries for manufacturing of bread and other bakery products, raw wheat is used as flour, livestock feed and if germinated and dried then used in the alcoholic beverage industry as malt. Wheat is majorly composed of carbohydrates along with moderate amount of protein present in the grain. Table 2, denotes the nutritional index per 100 grams of wheat grain.

Table 2: Nutritional index of wheat

Calories	**340**
Water	11%
Protein	13.2 grams
Carbs	72 grams
Sugar	0.4 grams
Fiber	10.7 grams
Fat	2.5 gram
Vitamins	39.31 milligrams
Minerals	81.75 milligrams

6. PRODUCTION OF WHEAT IN INDIA OVER THE YEARS

In 2018, Food and Agriculture Organization, developed a report on International Wheat Production Statistics, deriving from the FAOSTAT database unlike from International Grain Council which derive from Grain Market report.

The report showed the comparison of countries wheat production in million metric tonnes, along with the data for it over last 10 years.

The countries that acquired the Top 5 position for the production of wheat were China with the highest production of 131.4 million metric tonne in 2018, followed by India with 99.7 million metric tonne. The third country was Russia with 72.1 million metric tonne followed by United States with 51.3 million metric tonne and the fifth country was France with 35.8 million metric tonne wheat production in 2018 (Fig.2).

According to the reports, India showed Gradual increase in the wheat production from the year 2014, but there was a sudden drop observed in 2015, which may be there due to the external or physical factors affecting the wheat production (Fig. 3). India is one of the top wheat producing country and the consumption rate of wheat is also very high in India due to the connection in cultural and religious matters.

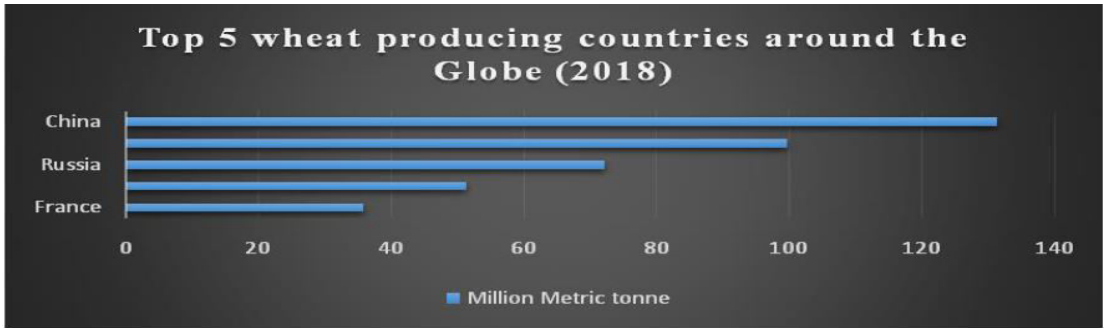

Fig. 2: Top 5 countries producing wheat

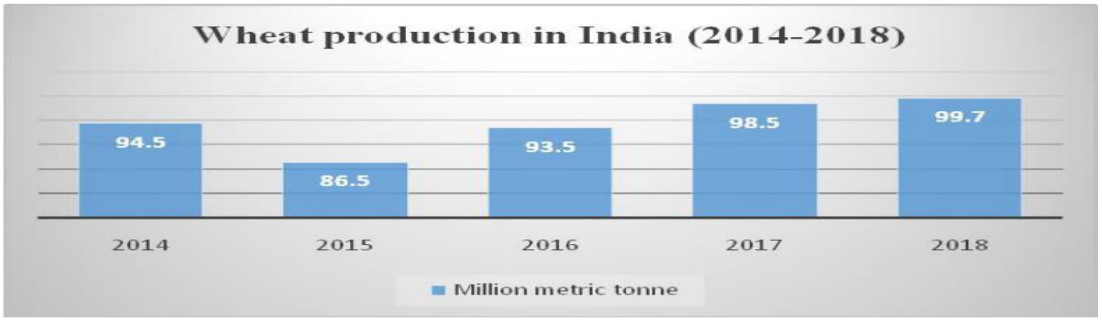

Fig. 3: Wheat production in India (2014-2018)

7. GLUTEN PROTEIN

The study on transcriptomics of wheat showed that in a developing wheat grain, there are over 30,000 genes expressed [24] and the proteomic study showed 1125 individual components involved in grain protein expression [25]. The maximum components involved have either little or no effect on grain utilization due to small amount expression, where in terms of impact or amount, one is dominant. The dominant one is the prolamin storage protein, which was analysed precisely by different techniques like 2D gel analysis which showed that this protein accounted for about 80% in European wheat [26]. In 1728, one of the earliest protein, Gluten was described by Beccari [27]. Traditionally, the wheat dough is gently washed under dilute salt solution or water which leaves the 80% protein consisting cohesive mass and protein matrix traps the starch granules. There are some unusual properties of gluten which is the basis of the different procedures applied to obtain the pure state of it. The first property was defined by TB Osborne that gluten is soluble in alcohol and water mixtures but insoluble in dilute salt or pure water solution and so named as prolamins [28]. The second property of gluten was defined as the association of individual gluten protein by non-covalent and covalent forces, resulting in formation of cohesive mass.

Gluten protein is made up of by combining two proteins- gliadins and glutenins. Gluten proteins are defined as secretory proteins that synthesise on rough endoplasmic reticulum and transported into the lumen of the endoplasmic reticulum after co-translation. Once it enters the lumen, the seed storage protein can follow any of the two routes, one is defined as Golgi-independent route where the formation of protein deposits occur inside ER lumen that ultimately fuse with vacuole originated protein bodies, other route is Golgi-dependent route which involved the deposition of vacuole originated protein bodies [29]. In a study conducted by Galili and colleagues, it was observed by using epitope tags and specific antibodies attached to individual proteins that wheat gluten proteins can follow both routes in developing grain [30-33]. This storage protein deposits fuse as continuous matrix which follow up in endosperm drying and getting depleting during maturation and results in network formation when interact with water.

8. MOLECULAR BASIS OF WHEAT PROTEINS

Wheat is majorly comprised of starch, which is around 60-75% and has only 9-18% protein, which makes it ideal for the food processing industry, especially bread making. Wheat proteins are basically divided into two types, based on the function: Gluten and Non-Gluten proteins (Albumins and Globulins). When proteins are considered, then around 20% of the total grain protein comprise of non-gluten proteins, which play important role in structural and metabolic functions of crop. On the other hand, Gluten proteins comprise of 80% of the total grain protein and are majorly responsible for the wheat quality (rheological properties of dough). Gluten protein is also known as Prolamins, as it contains Proline and Glutamine in very high amount [34, 35], including gliadians, which is of four types, α, β, ω, and γ-gliadins along with glutenins, which is of two types- HMW-GS (High Molecular Weight- Glutenin Subunits), LMW-GS (Low Molecular Weight- Glutenin Subunit). Gene on Gpc-B1 loci on chromosome 6B, which coded for about 70% of protein content variation [36, 37].

Gliadins form intramolecular disulphide bonds and are monomeric in nature, whereas, glutenins are polymeric protein complexes which are linked by disulphide bonds both intramolecular and intermolecular to glutenins and gliadins (Fig. 4). The important property inherited by both glutenins and gliadins is formation of viscoelastic network which traps the released CO_2, during fermentation leading to the typical wheat bread characteristic [38].

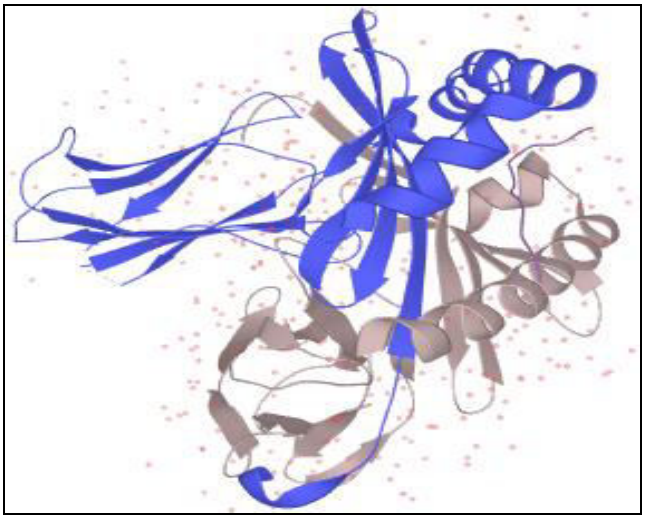

Fig. 4: Gliadin 3D structure showing alpha/beta chains retrieved from UniProt

The major role is played by gliadins, as it is solely responsible for the extensibility and viscosity of the dough. On the other hand, glutenins (especially HMW-GS) provide strength and elasticity to the dough by forming long polymers [39].

Multiple genes present at complex loci on chromosome 1 and 6 code for gluten proteins. The genes that have been sequenced under genomics are for α-gliadins, which is coded by Gli-2 genes on chromosome 6 short arm [40], ω-, and γ-gliadins are linked genetically and coded by Gli-1 genes on chromosome 1 short arm, genes at Glu-3 loci code for LMW-GS that is genetically linked to Gli-1, genes at Glu-1 loci code for HMW-GS on chromosome 1 long arm. The final product quality is totally dependent on these proteins balance [41].

9. COELIAC DISEASE

An autoimmune disorder that was defined in 1887 develops in individuals who are genetically allergic to gluten is known as Coeliac disease. This chronic disease mainly affects the small intestine of the human body, causing inflammation of the bowel that lead to low or malabsorption of nutrients and the patient develops many intestinal and extra-intestinal symptoms including abdominal and joint pain, belching, dairrhoea, fat in stool, heartburn, indigestion, heart burn, vomiting, bone loss, fatigue, malnutrition, lactose intolerance, skin rashes, osteoporosis etc. Around 1% of the Western Europe population is estimated to suffer from this disease [42]. There is a broad presentation of its clinical spectrum by the large range of age for onset of the disease and the eventual increase in the mortality and morbidity.

10. EPIDEMOLOGY

The occurence of the coeliac disease varies from different regions of the world and it affects individuals 1 in 100 [43]. Due to the lack of the awareness and knowledge, it has been observed that about 85% of the people remain undiagnosed [44]. The people who come under the clinically diagnosed list are only 0.05-0.27%. The individuals who complain about the gastrointestinal symptoms are not necessarily suffering from coeliac disease.

In the earlier days, coeliac disease was considered as a rare condition [45]. Different countries have different epidemiology of the disease, in USA the patients suffering from disease were 1, in Australia it is 1 in 70.The countries where the people are not even diagnosed properly for this are China, Japan and Africa, which is due to the low genetic risk factors [46]. This disease is more common in women than men [47]. This disease if not timely diagnosed then mortality cases have also been reported.

11. GATROINTESTINAL AND EXTRA-INTESTINAL MANIFESTATIONS

Coeliac disease is considered to be underdiagnosed and characterised with normal gastrointestinal symptoms but it has been observed that extra-intestinal manifestations can also help to diagnose the autoimmune disorders like this. The more detailed studies and increase awareness is important for the proper diagnosis of this disease to improve the treatment [48].

12. PATHOPHYSIOLOGY OF COELIAC DISEASE

Coeliac disease represents a basic immune-based disease model that gets affected by environmental risk factors and strong genetic factors. Its relationship with the cereal crop, wheat was established in late 1940s by Dicke [49] with series of studies and experiments that resulted in the observation that this response is generated only when the gluten binds to the T cells peptides in some individuals that have different genetic makeup, where the specialised antigen-presenting cells expresses the human leucocyte antigens (HLAs): DQ8 or DQ2. HLA-DQ is basically a part of MHC class II antigen-presenting receptor system which helps to differentiate between self and foreign cells. HLA-DQA1 and HLA-DQB1 genes on chromosome 6 short arm encode for the two subunits for HLA-DQ. Specific CD4+ T cells recognise the presented peptides which results in the inflammatory cytokine release that eventually leads to the intestinal epithelium flattening. An intestinal epithelium enzyme known as Transglutaminase has also been demonstrated to play a major role in the coeliac disease by deamidating the glutamine residues to transform into glutamate, leading to generation of toxic peptides [1].

Majority of patients of this disease consist of HLA-DQ2 two gene haplotype (DQ2.5 haplotype) consisting of two alleles, DQB1*0201 and DQA1*0501 encoding subunits, DQ α^5 and DQ β^2. In majority of the patients this DQ2.5 is encoded by inherited chromosome 6. The case of the HLA-DQ2 antigen presence causing the disease was observed to be 95% [50] and intestinal T cells lines are been further studied by different researchers to identify the peptide sequences which by either synthetic peptides or gluten produced peptide

fractions, are recognised by T cells. This was followed by the two epitopes definition which were overlapping and immunodominant and corresponded to the residues 57-68 (α-9) and 62-75 (α-2) of a type of α-form of gliadin, A. gliadin [51-54]. In γ-gliadins, related epitopes were defined that corresponded to residues at 60-79, 102-113, 115-123, 228-236 [51, 55, 56] along with the determination of the spacing between proline and glutamine [56], leading to the pathway of peptide activation and its interaction with novel T cell specific stimulatory gluten protein peptide.

As compared to DQ2, there has been less studies done on Coeliac disease caused by DQ8 which is the reason of only 10% of patients with HLA-DQ2 and 6% with HLA-DQ2 [50] which raises rhe possibilities of identifying other set of epitopes with immunodominant property [57-59]. The detailed study of both the mechanisms is important as they may differ on molecular level [60]. LPP or lipoma-preferred partner gene is a locus which is involved in the extracellular matrix to cell surface adhesion and even a single minor variant in this can increase the possibility of individual suffering from this disease to 30% [61].

Two levels of immune response, adaptive and innate develops when the peptides of gliadin leaks the membrane. α -gliadin produces a protease resistant peptide which result in interleukin-15 release when stimulates the lymphocytes. These events lead to innate response against gliadin by signalling that triggers the release of chemicals from the inflammatory cells [58]. This signal is strongest when is directed to 33 amino acid, α2 -gliadin fragment when the patient has DQ-2 isoform.

13. ENVIRONMENTAL FACTORS

The genetic factors are involved but still only 1% population is affected, which clearly suggest the involvement of other factors apart from it. Other factors are like, breastfeeding and infant feeding practices as the delay of gluten intake by a child approx. 12 month lowers the risk of the coeliac disease [62]. Season of birth, elective caesarean, rotavirus in children are also considered as risk factors for disease development [63, 64].

14. TREATMENTS AVAILABLE

There are symptoms which could be diagnosed for the coeliac disease by various methods available and modifying technically with recent trends (Table 3). Currently, there is no treatment available for the coeliac disease other than gluten free diet as the intestinal damage and disappearance of symptoms takes around months [71]. This treatment has various drawbacks like the non-feasibility of the gluten-free substitutes products due to high cost [72], no clear specification of the names of the foods that are gluten free [73], potential exposure to gluten during festivities or travelling [74], societal pressure especially in the adolescence [75], trace amount of gluten in medicines [76].

The patient just after getting diagnosed with this disease should be immediately tested for any deficiency of micronutrient [77], bone density measurement and should be given the pneumococcal vaccine due to association of pneumonia and coeliac disease [78]. Even after giving up gluten, patients are advised to monitor their symptoms.

Table 3: Diagnostic tests available for coeliac disease

DIAGNOSTIC TESTS	INFORMATION	REFERENCES
Serological blood test	1. IgA type antibodies known as anti-endomysial presence is measured. 2. IgG antibody reaction with transglutaminase is also measured	[65, 66]
Pathology	March Classification 1. Stage 0: normal mucosa 2. Stage 1: increased number of intra-epithelial lymphocytes, usually exceeding 20 per 100 enterocytes 3. Stage 2: proliferation of the crypts of lieberkuhn 4. Stage 3: partial or complete villous atrophy and crypt hypertrophy 5. stage 4: Hypoplasia of the small intestine architecture	[67, 68]
Endoscopy	Biopsy of jejunum or duodenum and upper endoscopy	[69]
Capsule endoscopy	The mucosal changes identification with precision along with T cell lymphoma diagnosis refractory and ulcerative jejunum	[70]

15. GLUTEN FREE WHEAT

The approach to produce wheat with lack of toxic celiac peptide has been considered from a very long time and the molecular detailed study of the mechanism of the coeliac disease are on the way to be more refined to achieve the treatment possibilities.

By classic plant breeding method, there is a possibility to identify the natural form of gliadin and no celiac toxic epitopes [79]. The search of wheat varieties which are naturally devoid of coeliac epitopes encoded by *A, B, D* genomes of wheat in gliadin sequences was analysed by van Den Broeck using monoclonal antibodies. The varieties with deletion of α-gliadins in the D genome showed less T cell stimulatory response [80]. The reference mapping of immuno-stimulatory proteins provided a targeting traits tool forselection of low protein variety [81]. There was a drawback in this technique as there are other epitopes that could also lead to coeliac disease like ω-gliadins, which were not all deactivated at the same time [82]. The involvement of the findings that gliadin genes are inherited in blocks, this high complexity of selection makes conventional method difficult.

The epitopes that are related to coeliac disease is expressed in gliadins and silencing of this particular protein encoded gene can give rise to the non-gluten wheat [83, 84]. RNA interference technology is a technology in Biotechnology where the antisense RNA is being injected to cure the disease by forming double stranded unstable RNA. The silencing of γ-gliadins complex genes was carried out by Gil Humanes using D-Hordein promoter [85]. Further analysis showed the increase in other gliadin fractions which suggested a compensatory effect by crop, resulting in increase of total gluten content and this compensation mechanism with other gliadin fractions could eventually lead back to toxicity [86]. Later, 3 gliadin families were silenced from the most conserved regions of ω and α-gliadins with D-Hordein promoter and γ-gliadin promoter [87]. Significant decrease in the gluten content, around 98.1% was observed in line E82 [86] and this time there was no change in total protein content as increase in non-gluten protein occurred [88]. There was a decrease in gliadins and LMW-GS in transgenic line which was compensated by albumins and globulins [89, 90] and also triticins, which codes for lysine residue [88].

16. FUTURE ASPECTS

Whenever there is a release of Genetically Modified crop with a promising result, the only things that can make this all pointless is the rejection by the consumers by the fear of genetically modified food. The acceptance rate has however increased in the past years.

Different approaches besides RNA interference are being considered for the gluten free wheat like Site specific Nuclease (SSN) that can cut or modify any DNA sequence which eventually leads to target mutation that could be performed on the multiple genes coding for gliadins it forms. CRISPR-Cas9 is the new and the future of the genome editing technologies where the specific genes could be edited on the same point to produce breaks which can later be repaired by homologous recombination or non-homologous end joining. The possibility of rejection by consumer would be less in this due to no involvement of introduction of foreign gene but the editing or deletion of the natural gene present in the crop. When the CRISPR-Cas9 was used for the modification, high mutation frequency was observed in the α-gliadin gene family with immunoreactivity reduced to 80%. There should be further studies conducted in the CRISPR-Cas9 and gluten free wheat production as the base editors should be more studied to mutate CD epitopes, as the specificity and the efficiency could be improved for the process, if not well defined could result in the change of amino acid sequencing and could lead to different expression of characters sometime which should be closely monitored to detect the potential risk.

17. CONCLUSION

Wheat is a staple crop which is produced over 770 million metric tonne every year by most of the developed and developing countries and serve as an important crop as related to culture and religion of many countries especially of Europe. Wheat is basically made of starch and proteins with traces of vitamins and mineral but the most important are carbohydrates and proteins which also are the reason behind the different use of wheat as a raw product for various food processing industries, beverage industry and livestock feed. Wheat has the characteristic of being viscoelastic due to protein called gluten which is of 2 types gliadin and glutenins where the gliadins of all 4 types are encoded by a multigene family and majorly responsible for the gluten specific epitope production. India is among the top 5 wheat producers and every year there

is a significant increase in the harvest and also leading to the increase in the coeliac disease patients. Coeliac disease is a chronic genetic autoimmune disorder caused by the glutens which causes stimulatory response by T cell specific reaction leading to inflammation and damage of the small intestinal lining. The symptoms include the gastrointestinal along with extra intestinal manifestations like anaemia, osteoporosis, abdominal pain, oral cavity damage, diarrhoea, slow growth, vomiting, lactose intolerance and so on. This disease has always been under the category of underdiagnosed which eventually results in the worsening of the case but now by awareness there are different tests present for its detection, blood test, endoscopy, biopsy and so on. This disease occurs by the genetic cause in human developing T cells innate and adaptive immunity in the HLA-DQ variant of DQ2 or DQ8 and also by the transglutaminase enzyme accompanied by environmental risk factors.

There was a conventional method developed for the selection of the gluten free wheat was not successful enough due to the linkage of all the forms of gliadin multigene family and their effect on the production of the gluten protein. The RNA interference is the genome editing technology applied for the silencing of the gene encoding for the gliadin protein which in turn, first increased the total protein content by compensation factor then later its multigene family was targeted and silenced, leading to the compensating factor application only on non-gluten proteins- globulin and albumin. The non-acceptance of the genetically modified crop by the consumers is still a problem and the gluten free diet is the only treatment available but not feasible for the low income group people and different problems faced by following the diet. CRISPR-Cas9 is the new age technology and promising approach for the gluten free wheat production as no other molecule or gene is introduced in this which will make it come under the category of non-genetically modified crop and its specific editing and mutation of the DNA or gene can lead to non-expression of the gliadin proteins but for that many promoters, base editors with more efficiency and specificity have to be further discovered.

18. REFERENCES

1. Shewry PR. *Journal of Experimental Botany*, 2009; **60**(6):1537-1553.
2. James D, Mauseth. *Botany*, 2014:223.
3. Belderock BR, Hans M, Dingena DA. *Springer*, 2003; **3**.
4. Feldman M. *Lavoisier Publishing*, 2001:3-56.
5. Shewry PR, Hey SJ. *Food and Energy Security*, 2015; **4**(3):178-202.
6. Day L, Augustin MA, Batey IL, Wrigley CW. *Trends in Food Science & Technology*, 2006; **17**(2):82-90.
7. Pajević S, Krstić B Stanković Z, Plesničar M, Denčić S. *Cereal Research Communications*, 1999; **27**(1):155-162.
8. Araus J L, Tapia L, Azcon-Bieto J, Caballero A. *Biological Control of Photosynthesis*, 1986:199-207.
9. Milla R, De Diego-Vico, N, Martín-Robles N. *Journal of Experimental Botany*, 2013; **64(11):**3137-3146.
10. Das N R. Wheat Crop Management, 2008.
11. Hogan ME, Hendrix JE.*Plant Physiology*, 1986; **80**(4):1048-1050.
12. Zhang J, Chen W, Dell B, Vergauwen R, Zhang X, Mayer JE, Van Den Ende W. *Frontiers in Plant Science*, 2005; **6**:624.
13. Duwayri, Mahmud. *Field Crops Research,* 1984; **8**:307-313.
14. Kahiluoto H, Kaseva J, Balek J, Olesen Jørgen E, Ruiz-Ramos M, Gobin A, et al. *Proceedings of the National Academy of Sciences,* 2009; **116**(1):123-128.
15. Heun M, Scha¨fer-Pregl R, Klawan D, Castagna R, Accerbi M, Borghi B, Salamini F. *Science,* 1997; **278**:1312-1314.
16. Nesbitt M. *Trends in Plant Science*, 1998; **3**:1360-1385.
17. Dubcovsky J, Dvorak J. *Science,* 2007; **316**:1862-1866.
18. Feldman M. *Lavoisier Publishing*, 2001; 3-56.
19. Nalam VJ, Vales MI, Watson CJW, Kianian SF, Riera-Lizarazu O. *Theoretical and Applied Genetics,* 2006; **112**:373-381.
20. Jantasuriyarat C, Vales MI, Watson CJW, Riera-Lizarazu O. *Theoretical and Applied Genetics*, 2014; **108**:261-273.
21. Dyke GV. *Hoos Press,* 1993.
22. van Heel DA, West J. *Gut* (Review), 2006; **55**(7):1037-1046.
23. Singh S, Sethi GS. *Cereal Research Communications*, 1995; **23**(1):103-108.
24. Wan Y, Poole RL, Huttly AK, Toscano-Underwood C, Feeney K, Welham S et al. *BMC Genomics,* 2008; **9**:121.

25. Skylas DJ, Mackintosh JA, Cordwell SJ, Basseal DJ, Walsh BJ, Harry J, et al. *Journal of Cereal Science,* 2001; **32**:169-188.
26. Seilmeier W, Belitz H-D, Wieser H.*Zeitschrift Lebensmittel-Untersuchung Und -Forschung*, 2001; **192**:124-129.
27. Bailey CH.*Cereal Chemistry*, 1941; **18**:555-561.
28. Osborne TB.*The vegetable proteins. Longmans Green & Co,* 1924.
29. Kumamaru T, Ogawa M, Satoh H, Okita TW. *Plant Cell Monographs*, 2007; **8**:141-158.
30. Galili G. *The Netherlands:Kluwer*, 1997; 221-256.
31. Galili G, Altschuler Y, Levanony H, Giorini-Silfen S, Shimoni Y, Shani N, Karchi H. *Journal of Plant Physiology*, 1995; **145**:626-631.
32. Levanany H, Rubin R, Altschuler Y, Galili G.*Journal of Cell Biology*, 1992; **119**:1117-1128.
33. Tosi P, Parker M, Gritsch C, Carzaniga R, Martin B, Shewry PR. *Journal of Experimental Botany*, 2009; **60**:619-627.
34. Shewry PR, Napier JA, Tatham AS. *Plant Cell*, 1995; **7**:945-956.
35. Shewry PR, Halford NG. *Journal of Experimental Botany*, 2002; **53**:947-958.
36. Chee PW, Elias EM, Anderson JA, Kianian SF. *Crop Science*, 2001; **41**:295-301.
37. Dolores M, García-Molina, María JG, Sánchez-León S, Barro F. *Nutrients.*, 2019; **11**(3):487.
38. Distelfeld A, Uauy C, Fahima T, Dubcovsky J. *New Phytologist*, 2006; **169**:753-763.
39. Shewry PR, Halford NG, Tatham AS, Popineau Y, Lafiandra D, Belton PS. *Advance Food Nutrients*, **2003**; 45:219-302.
40. Payne PI. *Plant Physiology*, 1987; **38**:141-153.
41. Wrigley CW, Békés F, Bushuk W. *AACC International*, 2006; 3-32.
42. Feighery C, Coeliac disease, *British Medical Journal*, 1999; **29**:236-239.
43. Fasano A, Catassi C. *The New England Journal of Medicine (Review),* 2012; **367**(25):2419-2426.
44. Guandalini S, Assiri A. *JAMA Pediatrics,* 2014; **168** (3):272-278.
45. Barker JM, Liu E. *Adv Pediatr*, 2008; **55**:349-365.
46. Buchanan N. *Williams & Wilkins*, 1987; 164.
47. Hischenhuber C, Crevel R, Jarry B, Mäki M, Moneret-Vautrin DA, Romano A, et al. *Alimentary Pharmacology & Therapeutics*, 2006; **23**(5):559-575.
48. Leffler DA, Green PHP, Fasano A. *Nature Reviews:Gastroenterology & Hepatology,* 2015; 131.
49. Losowsky MS, *Digestive Diseases*, 2008; **26**:112-120.
50. Karell K, Louka AS, Moodie SJ, Ascher H, Clot F, Greco L, et al. *Human Immunology,* 2013; **64**:469-477.
51. Arentz-Hansen H, Korner R, Molberg, Quarsten H, Vader W, Kooy YMC, et al. *Journal of Experimental Medicine,* 2001; **191**:603-612.
52. Arentz-Hansen H, McAdam SN, Molberg, Fleckenstein B, Lundin KEA, Jorgensen TJD, et al. *Gasteroenterology,* 2002; **123**:803-809.
53. Anderson RP, Degano P, Godkin AJ, Jewell DP, Hill AVS. *Nature Medicine,* 2002; **6**:337-342.
54. Ellis HJ, Pollock EL, Engel W, Fraser JS, Rosen-Bronson S, Wieser H, Ciclitira PJ. *Gut*, 2003; **52**:212-217.
55. Sjostrom H, Lunkin KEA, Molberg O, Korner R, McAdam SN, Anthonsen D, et al. *Journal of Immunology,* 1998; **48**:111-115.
56. Vader LW, Kooy YMC, van Veelen P, de Ru A, Harris D, Benckhuijsen W, et al. Gaste*roenterology,* 2002; **122**:1729-1737.
57. Van de Wal Y, Kooy YMC, Van Veelen PA, Pena SA, Mearin LM, Molberg O, et al. *Proceedings of the National Academy of Sciences,* 1998; **95**:10050-10054.
58. Van de Wal Y, Kooy YMC, van Veelen P, Vader W, August SA, Drijfhout JW, et al. *European Journal of Immunology*, 1999; **29**:3133-3139.
59. Mazzarella G, Maglio M, Paparo F, Nardone G, Stefanile R, Greco L, et al. *Gut*, 2003; **52**(1):57-62.
60. Henderson KN, Tye-Din JA, Reid HH, Chen Z, Borg NA, Beissbarth T, et al. *Immunity*, 2007; **27**:(1). 1-12.
61. Dubois PC, Trynka G, Franke L, Hunt KA, et al. *Nature Genetics*, 2010; **42**(4):295-302.
62. Lionetti E, Castellaneta S, Francavilla R, Pulvirenti A, et al. *N Engl J Med*, 2014; **371**(14):1295-303.
63. Tanpowpong P, Obuch JC, Jiang H, McCarty CE, et al. *Journal of Pediatrics*, 2013; **162**:501-04.
64. Mårild K, Stephansson O, Montgomery S, Murray JA, Ludvigsson JF. *Gastroenterology*, 2012; **142**:39-45.
65. National Institute for Health and Clinical Excellence, Clinical guideline.86:Recognition and assessment of coeliac disease, 2015
66. van der Windt DA, Jellema P, Mulder CJ, Kneepkens CM, van der Horst HE. *JAMA*, 2010; **303**(17):1738-1746.
67. Marsh MN. *Gastroenterology,* 1992; **102** (1):330-354.

68. Corazza GR, Villanacci V. *Journal of Clinical Pathology*, **58(6):**573-574.
69. American Gastroenterological Association medical position statement:Celiac Sprue, *Gastroenterology*, 2001; **120**(6):1522-1525.
70. *Redondo -Cerezo E, Sánchez-Capilla AD, De La Torre-Rubio P, De Teresa J. World Journal of Gastroenterol,* 2014; **20**(42):15664-15673.
71. Murray JA, Watson T, Clearman B, Mitros F. *Journal of Clinical Nutrients*, 2004; **79**:669-673.
72. Lee AR, Ng DL, Zivin J, Green PH. *Journal of Human Nutritional Diet*, 2007; **20**:423-430.
73. England CY, Nicholls AM. *Journal of Human Nutrient Diet*, 2004; **17**:547-559.
74. Barratt SM, Leeds JS, Sanders DS. *Journal of Gastrointestinal Liver Disease,* 2011; **20**:241-245.
75. Ludvigsson JF, Agreus L, Ciacci C, Crowe SE, et al. *Gut*, 2016; **65:**1242-1251.
76. Mangione RA, Patel PN, Shin E, Fiebert J. *Journal Pharmacology Association*, 2011; **51**:734-737.
77. Rubio-Tapia A, Hill ID, Kelly CP, Calderwood AH, Murray JA. *Journal of Gastroenterol*, 2013; **108**:656-676.
78. Zingone F, Abdul Sultan A, Crooks CJ, Tata LJ, Ciacci C, West J. *Aliment Pharmacology Therapy*, 2016; **44**:57-67.
79. Spaenij-Dekking L, Kooy-Winkelaar Y, van Veelen P, Wouter Drijfhout J, et al. *Gastroenterology,* 2005;**129**:797-806.
80. Van den Broeck HC, van Herpen TWJM, Schuit C, Salentijn EMJ, et al. *BMC Plant Biology,* 2009; **9**:41-52.
81. Juhász A, Belova T, Florides CG, Maulis C, et al. *Sci. Adv.*, 2018; **4**(8):eaar8602.
82. Tye-Din JA, Stewart JA, Dromey JA, Beissbarth T, et al. *Science Translation Medicine*, 2010; **2**(41):41-51.
83. Altenbach SB, Allen PV. *GM Crops*, 2011; **2**:66-73.
84. Wen S, Wen N, Pang J, Langen G. *Proc. Natl. Acad. Sci.*, 2012; **109**(50):20543-20548.
85. Pistón F, León E, Lazzeri PA, Barro F. *Euphytica*, 2008; **162**:371-379.
86. Gil-Humanes J, Pistón F, Tollefsen S, Sollid LM, Barro, F. *Proc. Natl. Acad. Sci,* 2010; **107**:17023-17028.
87. Pistón F, Marín S, Hernando A, Barro F. *Mol. Breeding*, 2009; **23**:655-667.
88. Gil-Humanes J, Pistón F, Shewry PR, Tosi P, Barro F. *Journal of Experimental Botany*, 2011; **62**:4203-4213.
89. García-Molina MD, Muccilli V, Saletti R, Foti S, Masci S, Barro F. *Journal of Proteomics,* 2017; **165**:102-112.
90. Gil-Humanes, J, Pistón F, Altamirano-Fortoul R, Real A. *PLoS ONE*, 2014; **9**(3).