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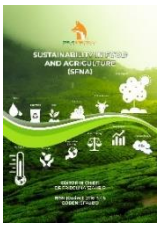

REVIEW ARTICLE

REVIEW ON PROSPECT OF GENETICALLY MODIFIED DIET IN OVERCOMING CELIAC DISEASE

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ABSTRACT

The advancements on biotechnological techniques and improvement in genetic makeup of plant cell structure gave rise to the genetic modification of plants in the 1980s. Genetically Modified plants have been a key to combat various diseases, Celiac Disease (CD) being one of them. Gluten, the structural protein of wheat, is identified as the prime cause of the autoimmune CD. Gluten-Free Diet (GFD) has been the only appropriate solution so far. In this review, we aim to shed some light on the numerous health implications caused by CD along with the merits and demerits of gluten-free diet. The inclination towards GFD is increasing even among non-celiac individuals. But GFD is considered inferior to products containing gluten though its claim to lessening gluten-related disorders and nutritional imbalance is pretty strong. The strict long term restriction to gluten is challenging to achieve, hence leading patients to seek for alternatives, possibly some manipulations in a genetic level that would create downregulation of the genes causing CD, while maintaining the nutrition, quality, and taste of the food. Approaches such as RNAi, EMS, Gene editing, Peptidase treatment, Gamma irradiation, and Deletion lines have shown convincing results in silencing the immunogenic epitope of gluten. This paper focuses on discussing various approaches on solving the issue of CD along with the prospects and challenges on obtaining a genetically modified diet against the disease. It highlights various biotechnological advances in obtaining such genetically modified diet as a promising guide to overcome Celiac Disease.

KEYWORDS

Celiac Disease, Gluten, Gluten-free diet, GMO.

1. INTRODUCTION

Gastrointestinal problem is a worldwide health issue affecting the digestive system that displays wide range of symptoms like bloating, burn, constipation, diarrhea, nausea, pain, and vomitus (Avramidou et al., 2018). One of such chronic gastrointestinal disease is Celiac Disease (CD). Celiac Disease, also called coeliac disease, is an auto-immune condition caused due to dietary gluten in genetically susceptible individuals characterized by intolerance to wheat, barley, rye, and triticale occurring in 1 out of 100 individuals in the western region (Dubé et al., 2005). Historically it used to be known as coeliac sprue, a gluten sensitive enteropathy that affects the small bowel in genetically disposed individuals from the ingestion of gluten (Ludvigsson et al., 2013). It is now recognized as a global disease affecting 1% of the total world population (Caio et al., 2019). CD or gluten related disorders were assumed to be affecting to people of European origin only, but recent data of past decades have shown that it is a disease found worldwide and they were simply undiagnosed in developing countries due to the lack of knowledge on the separate clinical representations of CD (Cataldo and Montalto, 2007). When people with celiac disease consume gluten, it disrupts the intercellular tight junction system of the intestine and damages the small finger-like projections lining the small intestine called villi that prevents nutrient absorption (Sharma et al., 2020). This is due to the triggering of the specific toxic peptides within the gliadin fraction of gluten protein which initiates an auto-immune response damaging the small intestine. CD also causes skin allergy due to presence of insoluble gliadins in gluten, which shows the symptoms of itching, swelling, skin rash, and anaphylaxis (Biesiekierski, 2017). Celiac disease, that affects all age group, can lead to additional health issues if left untreated (Ludvigsson et al., 2013). CD has been found to complicate cardiovascular disorders; most patients possess the constant threat of developing myocardial infection, angina pectoris, and stroke (Reynolds, 2019). As it is a hereditary disease, individuals with celiac patients as first-degree relatives have 10% higher chances of developing CD (Ludvigsson et al., 2013). A particular Human Leukocyte Antigen (HLA) is said to be related to this disease (Rai et al., 2018).

1.1 HLA Gene

Among the various HLA-DQ genes present such as HLA-DQ1, HLA-DQ2, HLA-DQ7, HLA-DQ8, and HLA-DQ9, the main two genes involved in the pathogenesis of CD are HLA-DQ2 and HLA-DQ8 (Cecilio and Bonatto,

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2015). The HLA-DQ2 itself has many different versions like HLA-DQ2.2 and HLA-DQ2.5.

Table 1: Risk of prevalence of CD depending on HLA-DQ gene (Al-Hussaini et al., 2018)

Each copy of HLA-DQ2.5 and HLA-DQ8	Very high risk
One copy of HLA-DQ2.5	High
Two copies of HLA-DQ8	High
One copy of HLA-DQ2.2	High-Low
One copy of HLA-DQ8	Low

The chances of prevalence of CD depends on the type and dosage of HLA-DQ genes such as the major role of HLA gene is to bind with foreign antigens which then interacts with T- lymphocytes cells and activates them. This results in an immune response system against those foreign antigens (Kårhus et al., 2018). When gluten containing food-items are consumed by the CD patients, this auto-immune response gets triggered

Table 2: Types of Celiac Disease (CD) (Ludvigsson et al., 2013)

S.N.	Type of CD	Signs and Symptoms
1.	Asymptomatic CD	Not accompanied by symptoms
2.	Typical/Classical CD	Malabsorption (such as diarrhea or malnutrition) or malabsorption syndrome (such as weight loss, steatorrhea, hypoalbuminemia)
3.	Atypical CD	Gastro-intestinal symptoms including irritable bowel syndrome and liver dysfunction; extra intestinal manifestations such as thyroid dysfunction (hypo/hyper); neurologic findings including depression and gluten ataxia; reproductive disease including abnormalities in menarche and menopause and disease including Dermatitis Herpetiformis (DH)
4.	Non-classical CD	Without signs and symptoms of malabsorption
5.	Subclinical CD	Extra-intestinal symptoms; clinical or laboratory signs (iron deficiency anemia, abnormalities in liver function tests, enamel defects, incidental endoscopic features, osteoporosis, etc.) but no symptoms
6.	Symptomatic CD	Gastro-intestinal and/or extra-intestinal symptoms
7.	Overt CD	Gastro-intestinal (dyspepsia, diarrhea and bloating) or extra-intestinal (neuro-logical symptoms and fatigue)
8.	Refractory CD	Persistent or recurrent mal-absorptive symptoms and signs with villous atrophy (VA)
9.	Potential CD	No signs or symptoms but are at risk of developing CD

which causes various forms of Celiac disease (Salentijn et al., 2012).

Research hasn't found a definite cause of CD. However, gluten is considered a major cause that has been provoking a range of clinical disorders collectively termed as Gluten related disorders (KAMER et al., 1953). Gluten is a water-insoluble protein mass that is the reason behind the viscoelastic and extensible properties of dough resulting in a variety of food products like bread, pasta, noodles, cakes, and pastries (Sharma et al., 2020). The main source of gluten is wheat whereas it is also found in some primitive varieties of wheat such as Spelt and Kamut and few other plants such as secalin in rye, hordein in barley, and avenins in oats (Biesiekierski, 2017). Gluten is divided into two proteins- gliadins and glutenins. The Gliadins found are α-gliadins, β -gliadins, ω -gliadins whereas glutenins could be either high molecular weight (HMW) or low molecular weight (LMW) glutenins (Spaenij-Dekking et al., 2005). The baking property of bread wheat is influenced by the HMW subunit of glutenin (Dunwell, 2002). Human beings cannot effectively digest these proteins and are found to possess a differential immune target that makes them immunotoxin by nature (Spaenij-Dekking et al., 2005). This difficult digestion is due to the high content of amino acids, proline, and glutamine which many proteases can't cleave (Ludvigsson et al., 2013).

1.2 Role of T cell in causing Celiac Disease

T cells or T lymphocyte cells are the integral part of adaptive immune system that are involved in controlling the immune responses by either destroying the infected host cells straight away or initiating other immune systems (Heath, 1998). An enzyme playing a key role in determining the severity of CD is tissue transglutaminase (tTG) that deamidates gliadin into glutamate which triggers a more powerful immune response (Di Sabatino et al., 2012). The HLA-DQ2 or HLA-DQ8 genes bind with these deamidated gliadin and are presented to the CD4+ T cells (a type of helper T cells) by antigen presenting cells (Salentijn et al., 2012). The CD4+ T cells identifies these deamidated gliadin as pathogens hence signaling autoimmune responses such as diarrhea, malabsorption and other disorders categorized under Celiac disease (Hisamatsu et al., 2016). Other gluten-related disorders besides CD are- Gluten ataxia, Dermatitis Herpetiformis, Gluten allergy, and Non celiac gluten sensitivity (Biesiekierski, 2017).

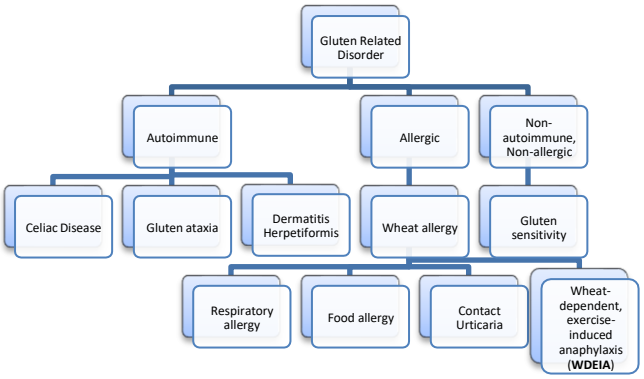

Figure 1: Classification of gluten related disorders (Sapone et al., 2012)

1.3 Diagnosis of celiac disease

To diagnose celiac disease, some few methods have been used by health care professionals. The most common method is serology test where the blood sample of the suspected patient is tested for the anti-tissue transglutaminase antibodies present in celiac disease (Parzanese et al., 2017). Histology, also called the 'gold standard' for CD diagnosis, is performed if the blood tests suggest positive for CD (Caio et al., 2019). For the duodenal biopsy, a small tissue from small intestine is taken and analyzed for any lesion in the villi which are categorized into various divisions as per Marsh classification (Schuppan and Zimmer, 2013). In case of dubious results, the doctors may ask for genetic analysis where they test for the presence of HLA DQ2 and HLA DQ8 genes in patients (Parzanese et al., 2017).

1.4 Available Treatment Options

The only available treatment for CD is a lifelong strict Gluten-Free Diet (GFD). Some of the non-dietary treatments include Gluten detoxification, TG2 inhibitors, HLA blockers, Biological therapies, Cortisone that work by improving gluten tolerance (Lähdeaho et al., 2012). In the case of a severely damaged small intestine, steroids are given. Drugs like Azathioprine and Budesonide can be taken as medication.

2. Gluten-free Diet: Dietary Management

The main treatment of Celiac disease-GFD are the foods containing less than 20 ppm of gluten according to the Codex Alimentarius Standard for gluten-free foods and the EU regulation 41/2009 (Knorr et al., 2016). Patients with CD should be educated to avoid cereals and food products derived from wheat, barley, or rye, and rather should be encouraged to use

gluten-free foods such as maize, oats (Currie et al., 2014). Patients are suggested to use separate cooking utensils and cooking surface should be well maintained. Attention should be given on proper food labeling making it feasible for CD patients to check the packaged food items (Fry et al., 1969). There have been pieces of evidence of improvement in celiac patients by following GFD. While they may need to avoid certain food items but many healthy gluten-free foods are also available in the market. However, food labeled 'gluten-free' tends to be more expensive than the same food having gluten. These food items should be easily attainable in terms of price and accessibility (Reynolds, 2019). You may find naturally gluten-free food such as potato, rice, soy, amaranth, quinoa, buckwheat, or bean flour less expensive than wheat flour (Hausch et al., 2002).

3. Nutritional Implications of Gluten-free Diet

Gluten-free diet is getting popular not only among the patients of CD but also in the general population. It is perceived as an effective approach to improve general health. The rapid rise in GFD is mainly from perpetuation in popular media, celebrity endorsements and health-focused television shows. It is shown that fiber intake is lower in individuals following the GFD plan. Since the small intestine is affected by gluten, micronutrients and minerals including calcium, phosphorus, copper, selenium, and zinc are not absorbed and hence their levels turn low in patients with untreated CD (Theethira et al., 2014). Gluten-free cereal products contain an inferior amount of thiamin, riboflavin, niacin, folate, and iron compared with the enriched gluten containing products (Niewinski, 2008).

Fiber can be obtained from gluten-free whole grain such as amaranth, millet, oats, flaxseed, etc. whereas the deficiency of nutrients can be treated by taking long or short term supplements with gluten-free vitamins, minerals, and proteins (Fasano and Catassi, 2001). In place of gluten, dairy products like milk powder, milk protein isolate, albumin are used as replacement strategies to help in refining the standard of gluten-free products (Collar, 2019). However, surveys have shown that a strictly gluten-free diet isn't much preferred by CD patients (Aziz et al., 2011). This is because of the poor taste in diet and an overall decrease in quality of life. (Samasca et al., 2014). In comparison to food containing gluten, GFD has a higher Glycemic Index (GI) and lower dietary fiber (Aziz et al., 2011). About 40% of CD patients were displeased with GFD according to a study (Vaquero et al., 2019).

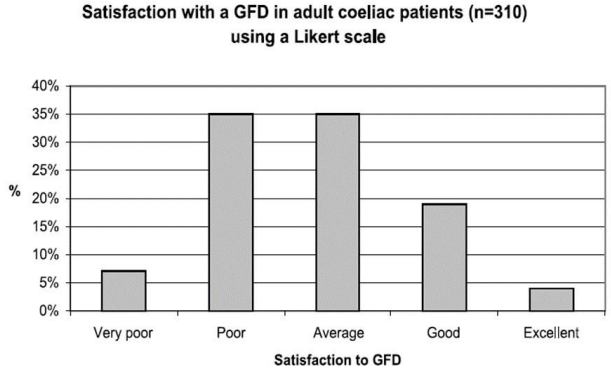

Figure 2: Satisfaction with a GFD in adult coeliac patients using a Likert scale

Hence, there is a need for genetically modified diets so that CD patients can consume products like wheat, barley, etc. without having to face gastrointestinal complications. Wheat (a hexaploid) and other gluten containing crops consist of numerous gliadin genes occurring at various loci which makes it difficult to minimize gluten ratios through conventional breeding (Aurélie Jouanin et al., 2019). The broad analysis of the human genome involved in pathogenesis of CD has led to the detection of various genetic variants and altered gene expression (Aaron, 2011). The sector of nutritional genomics must be explored rapidly for the production of transgenic crops without compromising its nutritive value (Bouis, 2007).

4. Methods of Genetic Modifications

4.1 RNA Interference

RNA interference or RNAi is a biological response that uses the post-transitional gene slicing process to manipulate the genes related to the protein (Chander et al., 2018). Since primordial time, this technique has been used as a defensive response by plants, fungi and invertebrates against invasion from unfamiliar harmful genes (Al-Khayri et al., 2016). The RNAi mechanism involves introduction of small non-coding double stranded RNA sequences that interfere with the gene activity by switching off the sequential gene expression (Pandita, 2018). This technology is based on gene modification through the cleavage of viral double-stranded RNA (dsRNA) (Aurélie Jouanin et al., 2018). This approach can be used to alter the gluten genome in treating Celiac disease. One of the effective use of this method was to create downregulation of α-gliadins that ultimately reduced the possibilities of the disease, along with maintaining the quality of dough. (Chander et al., 2018). There have been two significant pieces of research with the help of the RNAi technique: one by who were able to downregulate gliadins from wheat, and another by who terminated 20 various storage proteins by silencing α-gliadins (Aurélie Jouanin et al., 2018; Gil-Humanes et al., 2010; Becker et al., 2012).

4.2 Peptidase treatment

One of the effective methods of getting a gluten-modified diet is by degrading the protein through peptidase treatment. This treatment is conducted by the use of prolyl endopeptidase from fungus *Aspergillus niger*, also known as ANPEP (Knorr et al., 2016). The degradation in gluten content is recorded with the help of ELISA using the R5 antibody which amounted to the decrease in gluten level of below 20mg/kg (Walter et al., 2014). This method is fairly appreciated since it also adds the nutritive value to the diet through higher soluble fiber content (Aurélie Jouanin et al., 2018).

4.3 EMS (Ethyl Methane Sulfonate)

EMS is an organic ethylating compound that is used in plant breeding to conduct point mutation (Kamili et al., 2016). EMS has a molecular weight of 124.2, whereas its boiling point is 213.5°C and density is 1.1452 at 22°C relative to water at 4°C (Sega, 1984). This is a type of mutation breeding through transitioning of G/C nucleotides into A/T in DNA (Rai et al., 2018). Along with producing required breeding line, EMS is used in recognizing the function of the amino acids in protein and the role of corresponding genes in creating non-lethal alleles (Gillmor and Lukowitz, 2020). TILLING (Targeting Local Lesions IN Genomes) is used to recognize the presence of EMS mutations. This mutation results in disrupt bindings within epitopes that leads to low gliadin content (Aurélie Jouanin et al., 2018). But this method is considered quite challenging due to the complex nature and composition of gluten protein with over 50 multiple expressed genes (Shewry and Tatham, 2016).

4.4 Gene editing by CRISPR technology-Cas9

CRISPR (Clustered Regularly Interspaced Short Palindromic Repeats) - associated protein 9 is one of the recently developed gene editing technology that allows addition, alteration or removal in targeted location of the gene sequence (Ma et al., 2016). This technique has the potential to simultaneously and precisely modify multiple gliadin-encoded epitopes, and/or delete some of the genes, while maintaining the food-technological quality of the gliadin proteins (Aurelie Jouanin et al., 2020). As per this technology, the genomes undergo highly site-specific alterations without any foreign DNA addition (Rai et al., 2018). It targets the coding sequence of the *33-mer* in α-gliadin genes. Certain gliadin genes were mutated by adding sg Alpha-1 and sg Alpha-2. This generated low-gliadin mutant lines which were stable and heritable (Ribeiro et al., 2018). Genome editing by CRISPR/Cas9 method is highly effective due to its process of removal of transgene integration and small DNA insertions to the mutants (Liang et al., 2017). But this is a rather demanding outlook due to multiple genes and expressed proteins (Rai et al., 2018).

4.5 Deletion lines

This approach follows the elimination of 6D-gliadin locus from gluten which decreased the chances of occurrence of CD, but this had a side effect of poor mixing behavior of dough. This deletion is termed as 'Chinese Spring' deletion (Aurélie Jouanin et al., 2018). Similarly, while deleting the -gliadin and gliadin, it was possible to successfully remove the T-cell stimulatory isotopes without a reduction in the dough properties (Rai et al., 2018). The elastic properties of dough can be enhanced by replacing the deleted gliadin lines with non- immunogenic gliadin (Vaquero et al., 2019).

4.6 Gamma irradiation

Gamma irradiation is one of the earliest used technology causing physical mutations in the sector of plant breeding (Beyaz and Yildiz, 2017). Reactive oxygen species (ROS) are produced by this approach that breaks the DNA single/double strand. Such breakage is then repaired with the help of a non-homologous end joining (NHEJ) mechanism. This damage-repair process may result in the mutation of the T cell epitope. It could also lead to the removal of one or more gliadin genes (Aurélie Jouanin et al., 2018). Gamma irradiation was also able to create progressive reduction in the High Molecular Weight (HMW): Low Molecular Weight (LMW) Glutenin Subunits ratio (HMW: LMW-GS) (Köksel et al., 1998). Hence, the change in the DNA pattern of gliadin could be a good prospect in achieving gluten-free diet, but the deterioration in dough mixing property caused due to exposure to gamma irradiation may lead to inferior quality diet (Köksel et al., 1998) .

5. Conclusion

The quest for obtaining a potential solution to celiac disease has given rise to several studies, researches, and experiments intending to achieve a genetically modified diet. Genetic techniques such as CRISPR technology/CAS9, Gamma irradiation, etc. present exciting opportunities via altering the gluten content and composition in crops. The main objective of these strategies is to change the genetic characteristics of the gluten so that people with CD can consume gluten without having to sacrifice food items made up of crops like wheat. Few kinds of research have been successful at this attempt but failed to maintain the dough quality. Broader study on this subject matter is a necessity as purely gluten-free diet are expensive and projects low quality in comparison to products containing gluten, and aren't easily accessible (El Khoury et al., 2018). While GM crops may appear as a promising solution in combating CD, the occurrence of possible allergy from GM crops must be sincerely checked before their commercialization (Goodman et al., 2005). Further researches should be carried out investigating if the reintroduction of gluten in patients with CD is harmful or not to check whether patients develop gluten tolerance over time. Also, various surveys have shown that the CD patients are less inclined towards a strictly Gluten-free diet (Samasca et al., 2014). GFD can be applied as a temporary dietary solution until such genetically modified crops have been successfully commercialized. Till then, Gluten- free diet should be incorporated into the diet of CD patients and they should be counseled accordingly about the disease and the micronutrient and macronutrient inadequacy that comes with GFD.

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