Chapter 14
Spelt (*Triticum spelta* L.) In Vitro Androgenesis Breeding for Special Food Quality Parameters

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Abstract Consuming gluten-containing foods derived from wheat, barley, rye and possibly oats can result in health problems for a significant proportion of people. However, in the case of several types of disorders related to consuming gluten-containing cereals, not only the gluten components are trigger compounds. According to medical experts the majority of people suffering from health problems because of gluten— except for celiac disease patients—instead of consuming gluten-free food have the option to choose food products containing healthier, low levels of fermentable oligosaccharides abbreviated FODMAP. In order to meet the health-related special needs of these particular consumer groups, cereal breeders aim to develop new germplasm, suitable for the food industry to produce healthier products. This chapter provides a summary of the latest developments in this booming research field, including: (i) describing the actual knowledge on cereal-related health problems, (ii) describing the current status of celiac-safe cereal breeding, (iii) enhancing the importance of developing healthier spelt-based cereal products through the advancement of an ongoing spelt breeding program and finally (iv) developing plant biotechnology improvements relative to special food quality parameters.

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14.1 Introduction

Islam et al. (2011), defined wheat gluten as a protein-lipid-carbohydrate complex formed through particular non-covalent and covalent correlations among flour constituents during dough making, as the constituents are hydrated and where the mixing process provides the mechanical energy input. Nevertheless, these days the use of technical terms such as *gluten-free* and *gluten* are more common (but inaccurate) among lay people. Gluten-free roughly refers to food products free from cereal prolamin proteins, i.e. wheat glutenin and gliadin, which are similar.

Consuming food with gluten content derived from wheat, barley, rye and possibly oats can result in health problems for a significant proportion of people. In this regard, considering oats as a *harmless cereal* is one of the oldest and open disputes among specialists. Some experts assert that a certain percentage of patients suffering from celiac disease are sensitive to epitopes located in the so-called avenins, the prolamin type storage proteins in oat (Comino et al. 2016; Pulido et al. 2009), while other publications explain its potential danger through contamination with other cereal.

However, in the case of several types of disorders related to consuming gluten-containing cereals, the gluten components are not the trigger compounds. We do not have enough specific knowledge about the reasons for different symptoms or diseases and the precise meaning of terms like *gluten*, *prolamins*, *gliadin* or *glutenin*, neither for average consumers nor for medical experts (Branchi et al. 2015).

Nowadays in most Western countries the general public has learned of the perceived dangers and adverse influence of cereals containing gluten, thanks to reports appearing in the lay press (Braly and Hoggan 2002; Ford 2008; Wangen 2009) recommending gluten-free diets. Many of these reports are not able to define the nature of the gluten *intolerance* an individual may have or enhance the significance of appropriate diagnosis. In this way they portray a severe public relations threat to the grain industry.

In Western countries the gluten-free food business has improved in the last 5 years. In 2013 the US retail trade in gluten-free products reached USD 10.5 billion, and up to USD 15 billion was predicted for 2016. It is forecast in Australia that in the following 5 years the gluten-free retail sales will exceed USD 100 million, where in 2014 already more than 20% of the newest baking products were launched in the market as being gluten free (Jargon 2014).

As information about mechanisms of certain disorders has been accumulating, it has been revealed that the causative components of cereal products are often the soluble proteins (albumins and globulins) or the soluble oligosaccharides instead of

gluten proteins. This fact enhances the importance of research dealing with an appropriate diet for different types of gluten-sensitive people, instead of just suggesting a gluten-free diet.

Marketing activities associated with *wheat allergy* or *gluten toxicity* is frequently concentrated on gluten-free products. However, to meet the needs of different consumer groups, the medium or long-term solution based on more and more information collected in the medical research field is not simply having gluten free and traditional products on the shelves of the stores. A gluten-free diet is incomparably more expensive but not healthier than consuming gluten-containing foods (Missbach et al. 2015). Except for individuals who suffer from celiac disease (about 1% of the Caucasian population), people with some kind of cereal-related health problems can consume foods with lower levels and/or modified gluten content and decreased amounts of certain soluble carbohydrate (FODMAPs) components (Halmos et al. 2015).

To meet the special health related needs of certain consumer groups, cereal breeding has the duty to develop new germplasm, suitable for the food industry to produce new, healthier products. Setting achievable goals, comprehending the numerous health-related disorders, their presence and trigger composites, is essential not only in the current basic research but also in profit-based cereal breeding as well. In this chapter we present a summary of the latest developments in this booming research field, describing the actual knowledge on cereal-related health problems, describing the current status of celiac-safe cereal breeding and finally—through an example— enhancing the importance of developing healthier spelt-based cereal products through the advancement of an ongoing spelt breeding program.

14.2 Cereal-Related Health Disorders

Health problems caused by gluten can be classified basically into three types: allergic, autoimmune and nonallergic - not autoimmune disorders (Fig. 14.1, Sapone et al. 2012). The first two of these, are quite well researched; although, to reveal mechanisms related to various symptoms of allergic reactions, further studies are required. The allergic reactions comprise food allergy (Mills et al. 2004), respiratory allergy (Amano et al. 1998), contact urticaria (Lahti 1986) and wheat-dependent exercise-induced anaphylaxis (Armentia et al. 1990). The innate and adaptive pathways leading to celiac disease (CD)-specific symptoms have been described and studied extensively (Plugis and Khosla 2015). The autoimmune disorders include gluten ataxia, CD and dermatitis herpetiformis (Anderson and Wieser 2006; Lauriere et al. 2006).

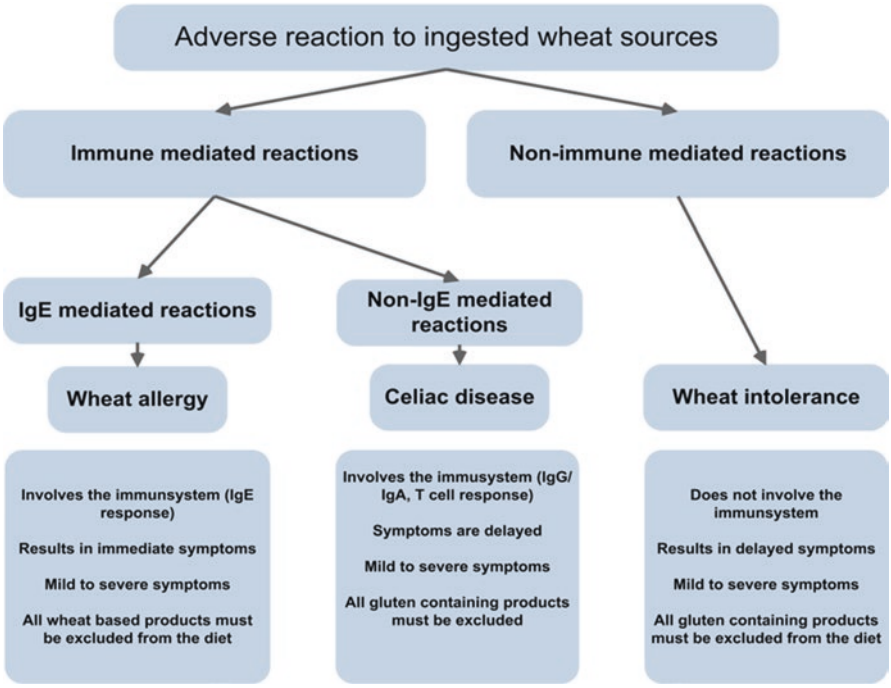

Fig. 14.1 The classification of wheat-related health disorders based on Sapone et al. (2012)

14.2.1 Celiac Disease

An abnormal response of autoantibodies causes the symptoms of celiac disease; for example, antibodies produced against glutamine- and proline-rich wheat gluten proteins, or their rye and barley homologues, or antibodies to tissue-transglutaminase (Green and Cellier 2007). There is a strong connection between celiac disease and one of the most essential genetic factors in developing detrimental symptoms, HLA-DQ alleles (Sollid et al. 2012). Heterodimers of these alleles, bound to the surface of antigen-presenting cells, function as surface-type receptor proteins. The presence of HLA-DQ compounds (DQ2.2, DQ2.5, DQ8), is a clear sign for the appearance of autoimmune symptoms, with modifying effects deriving from environmental and genetic factors (Anderson et al. 2000). The different peptides are recognized by different serotypes.

A set of criteria for the structure of an active CD epitope in a cereal protein can be described as follows: a) surrounded by amino acids with defined charge and hydrophobicity, b) the presence of a tissue transglutaminase 2 (tTG) enzyme-binding site and c) a size of nine amino acids fitting into the groove of the HLA-DQ heterodimers (Sollid et al. 2012). Patients suffering from celiac disease produce a range of autoimmune reactions to gliadins, LMW glutenins and some HMW glute-

nin subunits, just as to their analog polypeptides in the consumed cereal foods (Juhász et al. 2012).

14.2.2 Wheat Allergies

Due to specific IgE epitopes bound to mast cells, the development of wheat allergies is mediated more directly by the recognition of allergens (Catassi and Fasano 2008). Symptoms can be divided depending on the route of wheat allergen exposure into symptoms of classic food allergy: occupational asthma (bakers' asthma), wheat-dependent exercise-induced anaphylaxis (WDEIA) and urticaria (Sapone et al. 2012). Common food allergy signs may affect the respiratory or gastrointestinal tract or the skin. One of the most typical respiratory allergies is bakers' asthma, a serious symptom among adults who work with wheat flour.

Allergens presenting T-cells and B-cells have the same level of effects in a wheat allergy. Similar to other allergic reactions connected to food allergies, it is the result of cross-links between specific immunoglobulin E and short peptides that are rich in glutamine and proline and derive from the degradation of wheat grain proteins by endogenous proteases. This interaction stimulates mast cells and basophils to release chemical mediators, for instance, histamines resulting in different classes of inflammatory reactions. IgE-binding epitopes are the allergenic regions of proteins recognized by the binding sites of IgE molecules. These epitopes can be categorized into two groups: linear and conformational epitopes. Both types play a part in the development of allergic responses (Akagawa et al. 2007). In wheat, allergenic proteins are not just the prolamins (α, β, γ and ω gliadins, HMW and LMW glutenin subunits) but also proteins that are not elements of the gluten matrix {amylases, peroxidase, thioredoxin and serine proteinase inhibitors, the lipid transfer proteins (LTP)} (Tatham and Shewry 2008).

14.2.3 Non-Celiac Gluten Sensitivity

Non-celiac gluten sensitivity (NCGS) was first described about 40 years ago as mainly intestinal symptoms connected to consumption of gluten-containing cereals (Schuppan et al. 2015). There is significant proof that NCGS is induced by an innate immune response to wheat proteins (it is different from the adaptive, T cell-mediated response to gluten and non-gluten proteins in wheat allergy or to gluten in celiac disease) (Catassi et al. 2013; Sapone et al. 2012). A study by Junker et al. (2012) helped in the identification of the family of amylase-trypsin inhibitors (ATIs) as occasions of innate immunity in wheat. Nowadays NCGS has become a subject of growing interest for scientists. Research indicated a certain degree of awareness in connection with clinical relevance of gluten-related disorders among gastroenterologists (Zevallos et al. 2016).

14.2.4 Irritable Bowel Syndrome

A common gastrointestinal condition is irritable bowel syndrome (IBS) that has an impact on quality of life and can lead to significant financial costs. Generally, the efficacy of the therapeutic strategies to treat IBS, is disappointing, but has been improved significantly by the research of Gibson et al. (2007, 2015). Numerous abdominal symptoms may originate from factors which alter bowel digestion. They contribute to luminal digestion, particularly the osmotic load within the lumen, and the fermentative gas content may offer symptomatic benefit. FODMAPs (fermentable oligosaccharides, disaccharides, monosaccharides and polyols) is the collective term for candidate substrates that are highly fermentable causing an osmotic effect and are dietary, poorly absorbed and short-chain carbohydrates (Shepherd and Gibson 2006). Lactose and fructose are included in FODMAPs as there are patients in whom these are malabsorbed. Other FODMAP compounds are the polyols, for instance, sorbitol since they are poorly absorbed by humans, as well as galacto-oligosaccharides (such as raffinose) and fructo-oligosaccharides (fructans). The latter are always poorly absorbed since humans do not express suitable hydrolases (Shepherd et al. 2008). Fructans and fructose are included by major dietary FODMAPs. The most common food sources of fructans are cereals and onions; sources of fructose are fruits or honey (Biesiekierski et al. 2011; Muir et al. 2009).

A blinded placebo-controlled crossover study proved the efficiency of low FODMAP intake, confirming previous studies where about 75% of patients gain clinically-outstanding advantage (Halmos et al. 2014). As a first-line therapy, the low-FODMAP diet is progressively being applied by health specialists in patients with IBS (Halmos et al. 2014, 2015). Given the chronic nature and the high prevalence of IBS, many people may restrain the intake of FODMAPs over the short- or long-term (Catassi et al. 2017; Shepherd and Gibson 2006).

14.2.5 Prevalence of Different Disorders

In Western countries where the predisposing genes of celiac disease, *HLA DQ8* and *HLA DQ2* genes, can be found in approx. 30% of the population, celiac disease itself affect only 1% of these Western communities (Anderson and Wieser 2006).

Food allergy questionnaires were used with statistical analyses to establish relationships between individuals with clinical symptoms and immune responses (Vu 2014, Vu et al. 2014) in a study where a large number of blood samples was analyzed by the IgE RAST method against milk and wheat antigens from randomly-selected individuals (n = 1145) (Pasco et al. 2012). Outcomes indicated that the frequency of wheat allergy was 2.5% where both symptoms against wheat and a positive IgE immune response were observed. These results corroborate absolutely with similar wheat examinations where it was found that in European countries the prevalence of wheat IgE sensitization is 2.9% (Siles and Hsieh 2013; Zuidmeer

et al. 2008). In summary it is assumed that those individuals (12.8%; n = 125) who exhibit increased IgE levels without any symptoms might have a latent wheat sensitivity. They can be potential patients sooner or later as they have the chance of developing the symptoms. In the investigated population there is a large proportion (12.9%) who have symptoms connected to a wheat-consuming diet but did not show increased IgE. They may suffer from other wheat-related disorders (i.e. not IgE mediated) such as reaction to fructans (FODMAPs) for those with IBS, celiac disease or non-celiac gluten sensitivity (NCGS).

14.3 Developing Celiac-Safe Wheat

Identification of amino acid sequences of gluten proteins which are the inducing factors in sensitive individuals have shown that their distribution varies between and among the different groups of gluten proteins (Shewry and Tatham 2016). Detecting and determining the quantity of gluten proteins are crucial for two reasons: due to its direct impact on end-use quality and also for food safety reasons. There is a difference between seed composition among cereal genotypes which creates methodological problems in food allergen research and genotype selection for quality in breeding. The multi-species origin of prolamins and high sequence analogy, coupled with restrictions in the available methodologies, reviewed by Haraszi et al. (2011), make the precise identification of proteins that generate health disorders and their genotypic stability, variability and frequency, difficult to define. An accurate quantitative relationship is required between the final gluten/prolamin content and the prolamin peptide biomarkers in high-resolution methods; for instance, in mass spectrometry (MS) to connect the detection of peptide mass to their protein bases. Due to environmental and genotypic variability, these quantitative interactions, however, are difficult to detect.

To assist in protein selection, epitope mapping, peptide biomarker searches and medical studies, a database (ProPepper, https://propepper.net) was created which contains linear epitopes responsible for wheat-related food disorders, peptides obtained with single and multi-enzyme in silico digestion and the members of the prolamin superfamily proteins identified from Poaceae species (Juhász et al. 2015).

Information about the amount and composition of allergen and toxic epitopes existing in a single wheat sample can represent a substantial gap. The very soon to be completed grain proteome datasets can ensure the essential information to perform an estimation of allergen/toxicity calculation for a single cultivar. The combined use of allergen/toxic databases and genome sequence, cereal chemistry and prediction methodology results in a better understanding of the level of toxicity present from wheat flour in the end-products. Information is available about the distribution and number of epitopes for a single protein or a protein fraction based on the workflow presented in the review of Juhász et al. (2012). The most detrimental proteins and epitopes occurring in the highest frequency can be identified as

well. Researchers may obtain significant output from the analysis of large datasets such as the epitope toxicity value that can be well applied in food industry.

Two excellent reviews (Comino et al. 2013; Shewry and Tatham 2016) summarize recent research activities on the status of pseudo and alternative cereals and their derivatives, achieved by enzymatic or transgenic technology, breeding programs and natural selection, with the potential to develop products tolerated by celiac patients. Consequently, it is possible to use conventional breeding methods for selecting gluten protein fractions with lesser amounts of celiac epitopes. Molecular breeding methods can be applied to mutate celiac epitopes within individual proteins or to specifically downregulate celiac-toxic proteins. Gil-Humanes et al. (2008) provide well-known examples of this method, using RNA interference mediated gene silencing to downregulate the alpha and gamma gliadins in bread wheat (*Triticum aestivum* L.). A parallel method was applied by Altenbach et al. (2014a, b) to remove the omega-5 gliadins that are the most allergenic gluten proteins.

The combination of the above approaches may be used to develop celiac-safe wheat. However, due to the complex multigenic control of gluten protein composition, this remains a formidable challenge. Moreover, wheat must retain acceptable bread making or baking properties after any type of modification. It is not surprising that such celiac-safe wheat has not been developed, despite over a decade of research.

14.4 Gluten-Free Versus Low FODMAP Diet

According to a study of gluten-free foods (Market Research 2012), of individuals on a gluten-free diet, 65% consider it healthier, 36% do so for reasons other than sensitivity, 27% think it helps weight loss, 7% do so to lower inflammation and 4% to fight depression; only 5.7% claimed an certified medical diagnosis.

Due to the ubiquity of gluten in foods, a gluten-free diet (GFD) causes many social and economic repercussions (Comino et al. 2013). In the meantime, it cannot be considered as a healthy diet (Balakiereva and Zamyatin 2016), because gluten-free products usually are comprised of starches or refined flours containing a low amount of fiber. The fact is that consuming sufficient amounts of dietary fiber is associated with substantial health benefits, for instance, prevention of diabetes, colon cancer and cardiovascular disease. In consequence, GFD may cause different nutrient deficiencies in fiber which can lead to further subsequent health problems, as mentioned above. Numerous studies recommend using pseudo-cereal sources of fiber to maintain the required amount of fiber levels (Saturni et al. 2010). GFD can also induce a deficiency in folic acid and Vitamins B12, C and D, along with micro-elements, most importantly zinc, magnesium and calcium (Caruso et al. 2013; Hallert et al. 2002;). Besides, GFD comprises high amounts of hydrogenated fats and sugar, which can lead to an increased risk of obesity and hyperinsulinemia (Lamacchia et al. 2014). Hence, a GFD appears to be unbalanced and inadequate

with regard to both micro- and macronutrients. According to medical experts the majority of people, who may feel better consuming gluten-free foods—except for celiac disease patients—are automatically choosing a low FODMAP diet by selecting gluten-free or wheat-free products (Halmos et al. 2014, 2015).

Active research work and development in analyzing FODMAP-content, together with using food ingredients with low FODMAP content, were initiated to determine the crucial significance of FODMAP constituents related to health disorders. Establishment of a low FODMAP diet includes a much wider range of plant-origin products in contrast with the gluten-free phenomena, where the problematic food sources are a relatively small group of certain cereals. Studies by Biesiekierski et al. (2011) and Muir et al. (2007, 2009), along with computer and mobile phone applications, have helped to improve custom-designed low FODMAP diets: (http://www.med.monash.edu.au/cecs/gastro/fodmap/iphone-app.html).

Products made from traditional cereals (wheat, barley and particularly rye) are not recommended in FODMAP diets since they contain relatively high FODMAPs (mostly fructans). However, the genotypic variation within certain cereal species (Table 14.1) opens new perspectives for reducing/increasing kernel fructan

Table 14.1 Fuctan levels in different cereals

Crop	Species	Fructan concentration (% dry matter)			References
		Range	Average	No. of samples	
Rye	*Secale cereale L.*	3.6–5.0	4.2	25	Fretzdorf and Welge (2003a,b)
		4.5–6.4	5.5	25	Hansen et al. (2003)
		3.6–4.6	4.1	18	Andersson et al. (2009)
		4.6–6.6	5.6	13	Karppinen et al. (2003)
Wheat	*Triticum aestivum* L.	0.8–1.9	1.3	129	Andersson et al. (2013)
		0.9–1.8	1.4	25	Fretzdorff and Welge (2003b)
	Triticum durum L.	1.5–1.7	1.6	5	Fretzdorff and Welge (2003b)
	Triticum monococcum L.	1.6–2.3	1.9	8	Brandolini et al. (2011)
Spelt	*Triticum spelta* L.	0.9–1.3	1.1	5	Fretzdorff and Welge (2003b)
Triticale	*Triticosecale* Wittmack	1.6–2.9	2.3	16	Rakha et al. (2011)
		1.5–2.1	1.8	5	Fretzdorff and Welge (2003b)
Barley	*Hordeum vulgare* L.	Traces–1.0	0.4	92	Aman et al. (1985)
Oats	*Avena sativa* L.	Traces– 0.2	0.1	121	Aman (1987)
Maize	*Zea mays* L.	0			Fretzdorff and Welge (2003b)

Source: Based on Verspreet et al. (2015)

concentrations by breeding. In the case of wheat, it was shown that there is not a very strong genotype x environment (G x E) interaction, and that heritability is high, in a range of 0.64–0.94 (Huynh et al. 2008b).

Recent research studies to obtain information about gene regulation of fructan synthesis in cereals has primarily focused on barley and wheat. In the case of wheat, two major quantitative trait loci were identified so far on chromosomes 6D and 7A, and, in case of barley, several genes were mapped which encode fructan synthesizing enzymes (Huynh et al. 2008a, 2012; Wei et al. 2000).

A total of eight QTLs with two pairs of epistatic interactions were found for grain fructan concentration. Two QTLs on chromosomes 7A and 6D explained, respectively, 17 and 27% of the total phenotypic variation. Transgressive segregation was observed, and broad-sense heritability was estimated as 0.71 (Huynh et al. 2008b). Genes encoding the enzymes of fructan biosynthesis (*1-SST*, *1FFT*, *6-SFT*) form a functional cluster which was sequenced and mapped to the major QTL on chromosome 7A (Huynh et al. 2012).

Moreover, TaMYB13, a transcriptional activator, was identified, which could control the expression of major fructosyltransferases that are involved in fructan synthesis (Xue et al. 2011). In transgenic wheat lines the overproduction of this transcriptional activator has raised the fructan concentrations in the top internode and flag leaf, but the result on grain fructans was not examined (Kooiker et al. 2013). In transgenic triticale lines, kernel fructan concentrations were increased up to 20 times by overexpressing a fructan synthesizing enzyme from wheat or rye behind an aleurone-layer specific promoter (Diedhiou et al. 2012). In addition, it is possible to enhance fructan concentrations by producing mutant lines lacking GBSSI, granule-bound starch synthase I and/or SSIIa, starch synthase IIa (Shimbata et al. 2011; Yasui and Ashida 2011). Shimbata et al. (2011) showed that in sweet wheat cultivars, where functional GBSSI and SSIIa are lacking, kernel fructan concentration are up to 6.5 times higher than in corresponding wild-type wheat lines. Due to a mutation in the SSIIa gene, Clarke et al. (2008) generated a 42-fold growth in grain fructan concentration of barley. Downregulation of FEH activity in cereal grains by RNA interference is the final strategy for increasing cereal grain fructan content. Definitely, FEH activity is high during the second phase of grain maturation (Verspreet et al. 2013) and it is essential for fructan degradation (Wardlaw and Willenbrink 2000; Yang et al. 2004). It is assumed that a reduction of FEH translation will cause higher cereal grain fructan levels.

Under stress conditions (drought, waterlogging and lack of nutrients), fructan and other water-soluble carbohydrates (WSC) accumulate temporarily—with a peak around late booting and emergence of the inflorescence—in vegetative plant tissues such as stems or roots, and can be remobilized during grain filling where fructan is again synthesized in the mature grain (Gebbing 2003). Genetic variation was reported not only for fructan contents in stems but also for its remobilization efficiency (Ehdaie et al. 2006). In the grain, net synthesis of fructan is most rapid, 5–9 days after anthesis, then reaches its maximum concentration (15–30%) and diminishes thereafter (Costantini et al. 2008).

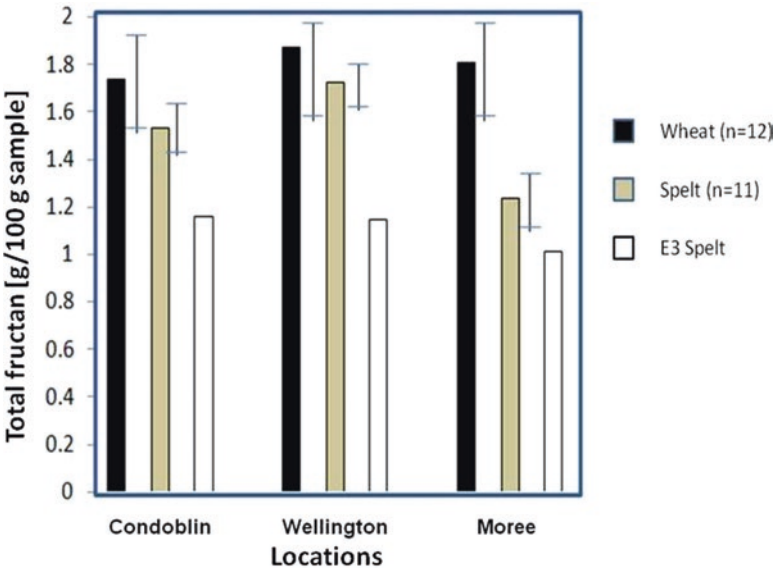

Fig. 14.2 Comparing the total fructan level found in the trade-marked cultivar, E3 spelt with those found in 12 wheat and 11 spelt samples grown at three locations in NSW, Australia. Error bars for wheat and spelt samples indicate the inter-variety variations. (Source: Békés 2015)

A significant environmental influence was observed in most fructan studies (Costantini et al. 2008); however, with no evidence of strong G x E interactions (Huynh et al. 2008a). No difference was observed between fertilization levels and management system (organic vs. conventional), only unfertilized wheat showed a slightly higher fructan concentration indicating stress due to a lack of nutrients (Langenkämper et al. 2006).

Spelt, compared to bread wheat, is comprised of considerably lower amounts of fructans and fructose. More importantly, the intervarietal differences among spelt genotypes is about five-fold larger than among bread wheat cultivars (Fig. 14.2), (Békés 2015). This provides the basis for screening spelt cultivars for low FODMAP content.

14.5 Spelt: A Nutritive Cereal

According to molecular evidence, spelt arose from hybridization between free-threshing hexaploid wheat and non-free threshing tetraploid wheat (2n = 4x = 28, genome = AABB). In the case of most hulled wild wheats and hexaploid varieties such as *Triticum aestivum* var. *vavilovii* (Tumanian) Sears, there is a recessive allele (q) at the Q locus that controls major domestication features in wheat (Faris 2014).

About 100 years ago, spelt was considered as the most important cereal in southern Germany, Austria, and Switzerland due to its hardiness and robustness concerning soil and climate, resistance to disease and nitrogen utilization efficiency. Bread wheat almost completely replaced spelt during the twentieth century because of higher yields, better baking performance and lower processing costs. However, spelt products have gained increasing popularity in the last 10 years. The major reasons are good digestibility, pleasant taste and aroma, and a better tolerance (in the case of wheat sensitivity) (Kling 1988). In addition, spelt is now being increasingly cultivated in water-protected areas and in organic farming as this cereal has fewer requirements with regard to use of herbicides or pesticides and fertilization compared to wheat (Kohajdova and Karovicova 2008).

Extensive systematic breeding and selection led to the creation of high-yielding and free-threshing cultivars of spelt, while retaining wide adaptability to different agro-climatic conditions. These new spelt cultivars have replaced most of the low-yielding traditional landraces and subspecies. However, in most parts of the world spelt has been superseded by bread wheat, leaving spelt to be grown as a marginal or niche crop with low inputs.

Recent interest in the use of spelt for ecologically-grown food has led to a revival in its cultivation after many years of marginal cultivation. As a matter of fact, in Europe spelt is used traditionally in the everyday diet, especially in Germany where numerous grain-based foods are produced using spelt (Cubadda and Marconi 2002). Several excellent works have been published in the last 10–15 years in connection with the evolution, physiology, genetics and the G x E effects on quality of spelt (Blatter et al. 2004; Förster et al. 2013, 2014).

The genetic diversity of major crops has suffered an overall reduction over time. Such genetic erosion can cause serious difficulties in the maintenance of the biodiversity of crop plants, as well as in crop improvement (Caballero et al. 2008). As a consequence of the reduced genetic variation, the stability of the crops in response to various stress factors has decreased in their local environments. In order to compensate for the loss of variation, breeders have turned to crops that were neglected in the recent past.

Spelt represents a useful gene reservoir for breeding programs, as it has the same ploidy level (2n = 6x = 42, AABBDD) as bread wheat. It has valuable nutritional quality due to its protein content and composition, which differ from those of wheat (Abdel-Aal and Huck 2002; Bonafaccia et al. 2002). The main difference is the variation in the amount and type of grain proteins, especially prolamins. Spelt dough is softer and stickier after kneading and is characterized by lower stability, less elasticity and higher extensibility than bread wheat dough (Schober et al. 2002).

In many modern breeding programs, spelt has intentionally been crossed with wheat cultivars for the improvement of yield, baking quality, and resistance to lodging and disease. It is essential to distinguish between spelt and bread wheat cultivars. The genetic diversity and chemical composition of spelt and modern wheat cultivars have been assessed and discussed from many perspectives. The gliadin protein patterns obtained with acidic polyacrylamide gel electrophoresis (A-PAGE)

were reported to be an excellent tool for qualitative cultivar identification (Ng et al. 1988). Schober et al. (2006) used size-exclusion high-performance liquid chromatography to classify spelt cultivars by their gluten proteins. Furthermore, a spelt-specific gamma-gliadin gene was discovered (Büren and Lüthy 2000) and then utilized in a simple polymerase chain reaction (PCR) method to detect the wheat adulteration of spelt flour and products (Büren et al. 2001). In addition, capillary zone electrophoresis can detect not only differences between the gliadin patterns of closely related spelt cultivars, but also the presence of wheat elements in the gliadin patterns of wheat/spelt crosses. In a study by Schober and Kuhn (2003), the presence of bread wheat was revealed in a number of spelt cultivars using capillary zone electrophoresis (CZE). The genetic diversity of spelt has been extensively examined on the basis of prolamin composition using reversed-phase HPLC (Wieser 2000), acid polyacrylamide gel electrophoresis (A-PAGE) (Caballero et al. 2004), one- and two-dimensional polyacrylamide gel electrophoresis (An et al. 2005) and capillary zone electrophoresis (Schober and Kuhn 2003). Other methods were also used to characterize genetic variation in spelt, by analyzing the rheological and viscoelastic properties of spelt gluten (Pruska-Kedzior et al. 2008; Schober et al. 2002) or its fiber content (Escarnot et al. 2010).

The molecular characterization of cultivars is also a useful way of evaluating genetic diversity during the breeding process. The genetic diversity of the Triticeae has been explored using a range of molecular markers (Manifesto et al. 2001) and with diversity arrays technology (DArT) markers (White et al. 2008). A high level of genetic diversity was revealed in spelt germplasm by microsatellite markers (Bertin et al. 2004). Gulyás et al. (2012) reviewed the milling, technological, compositional and bread making characteristics of European spelt cultivars and breeding lines, and estimated the phylogenetic relationships among spelt accessions using AFLP markers and tried to establish relationships between the presence of wheat genes and the technological and nutritional quality characteristics. The extent of genetic diversity in spelt germplasm, characterized by polymorphism information content (PIC) (Anderson et al. 1993), was far greater than found by Martos et al. (2005), for durum, or by Lelley (2000), for bread wheat. It was concluded that the diversity could be due in part to the extent of crossing between pure spelt cultivars and bread wheat. This suggests that spelt cultivars cannot be characterized without considering their origin. In view of the fact that it is mostly genotypes with bread wheat in their pedigree which achieve the quality required for bread making, spelt could be used as a source of lodging and disease resistance (Campbell 1997) and a source of genetic diversity for grain protein and mineral nutrients (Gomez-Becerra et al. 2010). Both the quality assessment and molecular analysis revealed a great diversity in spelt accessions that can be further utilized by breeders to incorporate them into specific breeding programs aiming to improve not only resistance but quality traits.

Spelt can be cultivated in harsh ecological conditions, without the use of pesticides because it shows a higher tolerance of environmental factors than bread wheat (Raman et al. 2008). Furthermore, spelt is a proper crop for organic farming as it can grow on low-fertility and poorly drained soils (Bonafacci et al. 2002). Spelt was not

among the targeted cereals in systematic breeding in the past; the objectives for improvement have only been identified recently (Neeson et al. 2008).

Spelt has been emerging as a worthy cereal for the past decade because of its repute as a healthy food not just for celiacs, but also for people suffering from wheat allergy (Elia et al. 2004; Galova and Knoblochova 2001). Spelt flour is also regarded to be more nutritious than bread wheat flour and to possess a unique flavor (Campbell 1997). Generally, spelt products, especially organic products, such as bread command a higher price in the marketplace.

In the last decade, the chemical, functional and nutritive properties of spelt compared to bread wheats have been described in numerous publications, recently reviewed by Escarnot et al. (2012). According to their results, on average, the spelt milling fractions and whole meal flour were higher in unsaturated fatty acids and lipids, but contained lower tocopherol content than in wheat samples. This suggests that there is no close relationship between high lipid content and high germ proportion in spelt. Although the proportion of bran and flour in milling fractionation is quite similar in wheat and spelt, there can be significant differences in mineral content. It was found that copper, zinc, phosphorus iron, magnesium and ash contents were higher in spelt samples, particularly in coarse bran and in aleurone-rich fine bran. Even spelt bran has the higher phosphorus content than wheat; the opposite trend was shown by phytic acid content, as it was 40% lower in spelt versus wheat fine bran. This suggest that spelt has either a lower phytic acid content or a higher endogenous phytase activity than wheat. Ruibal-Menieta et al. (2005) provide important suggestions about the true nutritional value of spelt compared to wheat. Furthermore, Gomez-Becerra et al. (2010) showed that the oleate/palmitate rate together with the Ca/Fe proportion provide a highly discriminating tool to face the growing issue of spelt-flour adulteration and to authenticate spelt from wheat flours. The results showed that spelt is a highly promising source of genetic diversity for mineral nutrients, particularly Zn and Fe, and for grain protein. Similar results have been obtained comparing wheat and spelt samples grown in Australia (Muir et al. 2014), (Table 14.2).

Spelt-based products have a slightly higher protein content and are more easily digestible than wheat. The variation in the amount and type of grain proteins, especially prolamins, is the main difference in the nutritive value of spelt flours (Galli et al. 2000; Shewry 2002).

Systematic crossbreeding of wheat and spelt was started at the beginning of the twentieth century to compensate, not for the detrimental properties of spelt in agriculture and processing, but to preserve its desirable properties. Not only pure spelt but also wheat/spelt crossbreeds have been cultivated and used in food processing since that time. The assignment of crossbreeds to sp. *spelt* is exclusively done based on morphological properties, for instance, shape of the ears and the fact that after threshing the husks are still attached to the seeds. Typical components of the seeds that assure the characteristics and quality of spelt are not accounted for at all and information on the rate of wheat crossing has not been obtainable to both consumers and producers of spelt products. To classify spelt cultivars, according to crossbreeding with bread wheat, is of special interest for persons tolerant towards spelt and sensitive to wheat (Catassi et al. 2013; Ludvigsson et al. 2013).

Table 14.2 Comparison of the levels of some important nutritive components in bread wheat and spelt samples, grown at the same location in NSW, Australia

		Nutritive components								
		Protein	Total fat	Mono unsaturated fatty acids	Poly unsaturated fatty acids	Total dietary fiber	Ca	Mg	Zn	Fe
Species	Parameter	%	%	g/100 g	g/100 g	g/100 g	mg/kg	mg/kg	mg/kg	mg/kg
Wheat (n = 12)	Mean	11.8	1.325	0.200	0.850	11.1	264.3	940.3	22.7	30.7
	Std. Dev.	1.1	0.096	0.082	0.058	5.0	287.9	338.2	7.9	9.4
	cv%	9.4	7.226	40.825	6.792	45.4	124.6	36.0	34.9	30.8
Spelt (n = 12)	Mean	13.8	1.843	0.457	1.086	10.6	287.9	1071.3	28.1	62.4
	Std. Dev.	1.9	0.310	0.053	0.318	4.1	124.6	364.8	8.5	28.9
	cv%	13.9	16.830	11.693	29.334	38.3	43.3	34.1	30.1	46.3

Source: Muir et al. (2014)

Several studies showed that reliable distinction between wheat and spelt can be provided by the differences in the protein patterns (Schober and Kuhn 2003; Wieser 2006); however, wheat and spelt seeds, due to their close botanical relationship, contain similar endosperm proteins.

Wheat proteins can be classified into the Osborne fractions of glutenins, GLUT; gliadins, GLIA and albumins/globulins ALGL. These can further be subgrouped into different types of gluten protein (α/β-,γ-, ω1,2-, ω5-, gliadins; low- and high-molecular-weight glutenin subunits, LMW-GS and HMW-GS (Wieser 2000). The main protein fractions of both cereals are the gliadins. Their patterns are determined by several components. For decades, the differentiation and identification of genotypes was based effectively on the variations of these patterns. SDS gel and acid gel electrophoresis, reversed-phase high-performance liquid chromatography (RP-HPLC) and gel isoelectric focusing have been applied, in particular, to the differentiation of wheat cultivars worldwide (Cornell and Hoveling 1998; Radic et al. 1997; Radic-Miehle et al. 1998; Wrigley and Bietz 1988).

Capillary zone electrophoresis (CE) of GLIA from 27 European spelt samples showed that differences were detected in the patterns of some wheat/spelt crossbreeds, even of closely-related cultivars and wheat elements (Schober and Kuhn 2003). Similarly, RPHPLC of GLIA allowed determination of the degree of wheat crossing in spelt and the differentiation of spelt and wheat cultivars (Wieser 2006). A total of 23 spelt cultivars were categorized into 5 groups ranging from pure spelts to spelts similar to wheat. Different degrees of wheat crossing and different GLIA patterns were detected in crossbreeds with identical parents (wheat and spelt). Therefore, no conclusion can be drawn from the pedigree on the real content of wheat elements. Until recently in spelt products the wheat contaminations could not be determined for authenticity and quality control of cereal grains and pure genotypes. It was demonstrated by Mayer et al. (2012) that a fast and simple observation of wheat in spelt flours can be accomplished by the combination of lab-on-a-chip

capillary gel electrophoresis and the polymerase chain reaction-restriction fragment length (PCR-RFLP) analysis.

The quantitative protein compositions of the Osborne fractions of whole meal flours from 62 spelt and 13 wheat cultivars were determined by Koenig et al. (2015) using reversed-phase high-performance liquid chromatography. The result shows that the chromatograms of the reduced gliadin fractions could be applied best for spelt classification and to distinguish spelt from wheat. Composition of the reduced spelt gliadins revealed one to three markers that cannot be found in wheat. Spelt cultivars were categorized into three groups based on these markers ranging from *typical spelt* to *similar to bread wheat*. Marker 1 was referred as ω1,2-gliadin and markers 2, 3a and 3b were identified as γ-gliadins by the determination of the relative molecular mass by mass spectrometry and the means of N-terminal sequence analysis. Glutenin-bound ω-gliadins may be used to quantitate and detect even small amounts of wheat in spelt products because this type of protein is absent in spelt but present in wheat.

There have been anecdotal clinical observations for a long time that a high percentage of non-celiac disease patients who suffer from wheat-related health problems can consume goods made from some spelt cultivars. The first research paper with robust statistical and experimental results also proved this important discovery (Armentia et al. 2012).

Since the endosperm of the kernels contain the major amount of FODMAP components, producing less bran in the flour or reducing the milling yield do not increase the FODMAP level. Baking and milling technology has an important effect on FODMAP levels in the products because during fermentation a certain amount of soluble carbohydrates are metabolized by yeast. Thus, FODMAP content in the end-products can be altered both by the length of fermentation and/or by the amount of yeast applied in the bread formulation. Sourdough technology has an even more substantial effect compared to yeasted dough products in reducing the FODMAP level (Fig. 14.3).

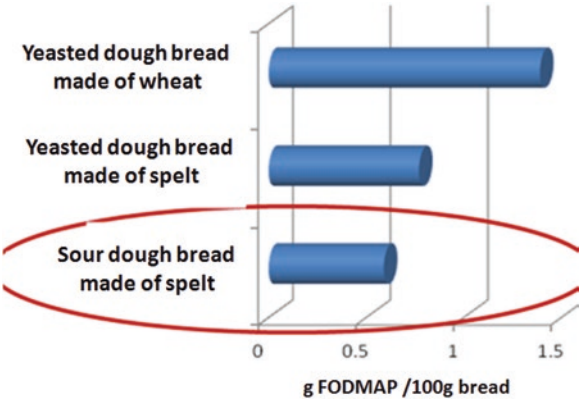

Fig. 14.3 FODMAP content of yeasted and sour dough breads made from wheat and spelt. (Source: based on the data of Muir et al. 2014)

14.6 Breeding less Allergenic Spelt with Low FODMAP Content

Australia grown spelt cultivar GWF was successfully selected with considerably lower FODMAP content. According to Muir et al. (2014), in the baked goods produced from this line, the FODMAP content—using an optimized technology and formulation—was significantly lower than the threshold defined for low FODMAP products.

Intriguingly, this same line was observed to have considerably less immune reactivity than any other spelt and wheat cultivars to wheat sensitive individuals (Vu 2014, Vu et al. 2014). It was also observed that the albumin-globulin protein composition of GWF spelt is different from other wheat-related and wheat species (Fig. 14.4). One of these discrepancies has been identified as a mutation in a CACTA retrotransposon region tightly connected to a gene coding a beta-expansin protein in many spelt and wheat germplasm (Fig. 14.5) (Breen et al. 2010).

A protein family closely connected to nonenzymatic proteins, the beta-expansins, located in plant cell walls, are known as pollen allergens. This alteration in the soluble protein composition was stated to be one of the reasons for the considerably lower immune reactivity of GWF spelt for wheat sensitive people (Vu 2014, Vu et al. 2014). The mutation in the expansin gene alone cannot account for the lower immune reactivity; however, it could be applied as an indicator for the uncommon

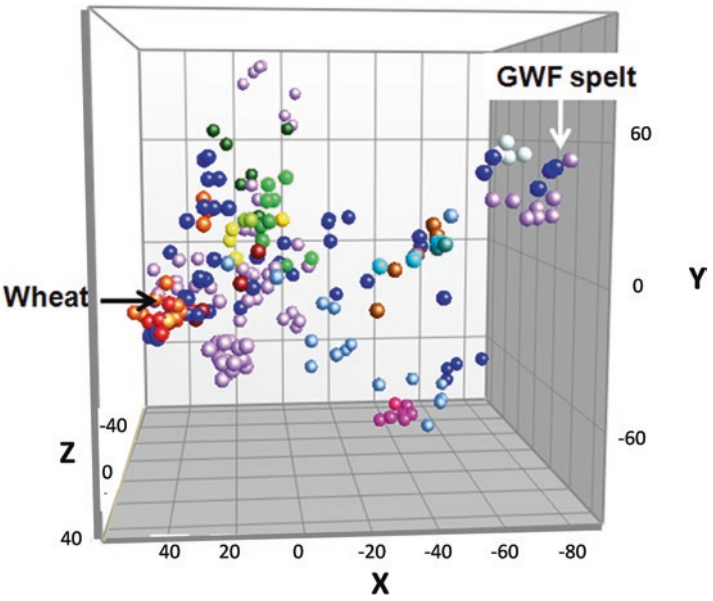

Fig. 14.4 Principle component analysis based comparison of soluble protein composition (albumins and globulins) of 127 wheats and wheat relatives. (Source: Vu 2014)

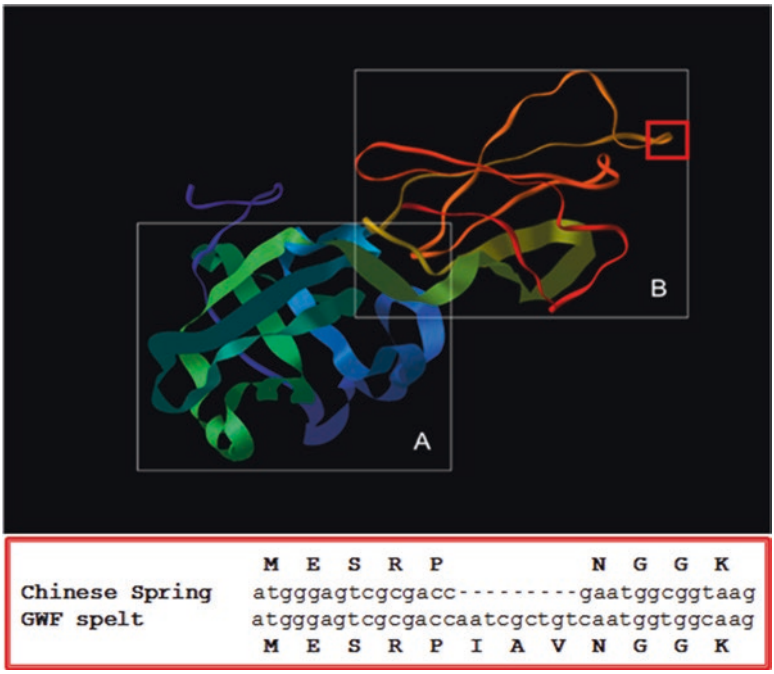

Fig. 14.5 Mutation in the expansin coding gene based on Breen et al. (2010)

soluble protein composition observed in GWF spelt. This modified protein configuration could be linked to the lower immune reactivity detected (Fig. 14.6).

Several bakeries in Australia have recognized the potential candidate source for producing cereal products with dual health benefits. A closed production line has been created, incorporating contract growing of GWF spelt with strict rules to avoid any contamination from other grains. A small milling capacity has been developed and special care has been devoted to clean the all milling equipment before GWF spelt processing. A PCR-based procedure has been established to reveal the above-mentioned mutation in the expansin gene (Suter and Békés 2012) to screen for any contamination in the flour or grain samples induced by any other cereals with the wild type expansin protein (Fig. 14.7). This procedure is used for quality guarantee purposes. A dedicated laboratory monitors the FODMAP content of flour and their goods (sourdough bread with unique formulation). Flour from this unique spelt source, characterized by lower immune reactivity and low FODMAP, is currently being supplied to Australian artisan bakeries. The name of this special flour is Hildegard spelt flour, after St Hildegard von Bingen, an eleventh century nun who first observed the health advantages of spelt.

In connection with this spelt line, there are prior experiences which provide some evidence to support the dual health benefits. The question is whether this observation is connected to the apparent lower allergenicity of spelt for those with wheat allergy. The answer needs a double-blind, placebo-controlled randomized clinical

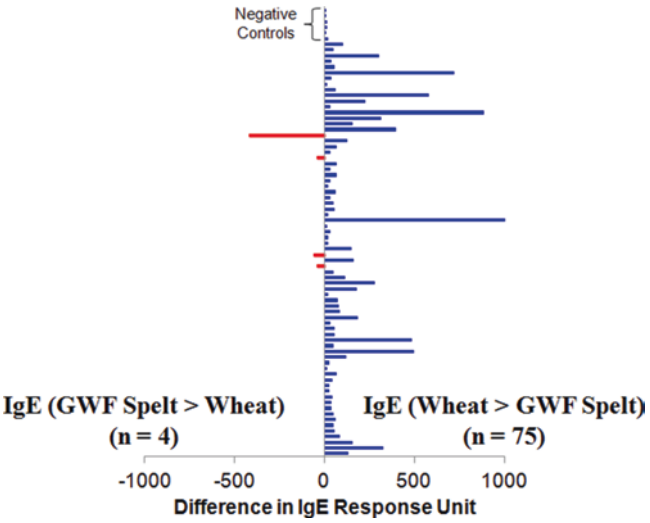

Fig. 14.6 The comparison of immuno-reactivity of wheat and GWF spelt, determined by RAST measurements. (Source: Based on the data from Vu 2014, Vu et al. 2014)

trial, compared to the allergic signs and IgE levels generated by consuming GWF spelt and wheat products, involving those who eat chiefly wheat and those who have replaced spelt for wheat in their diet.

A pre-breeding activity has been initiated in Hungary based on the positive experiences on evaluating GWF spelt to screen large spelt populations, selecting suitable processing quality and lines with low FODMAP content. The chosen lines are further examined by searching for genotypes containing the expansin-mutation, applying the ELISA based techniques (Bodinier et al. 2008) and PCR-based test of Suter and Békés (2012), respectively. As described in an initial survey carried out on 105 spelt lines (Pauk et al. unpublished results), the FODMAP content of spelt lines grown in the Carpathian Basin show large variation (0.7–1.8 g fructan in 100 g grain). More than 10% of the lines comprise lower or equal level of fructan than the Australian control, found to be helpful in low FODMAP diet (Fig. 14.8).

Selection for quality in this program is based on three independent attributes: low FODMAP content, satisfactory bread making properties and the presence of the expansin mutation to be able to monitor the purity of the samples on grain, flour, and even the end-product stage.

In the early stages of a breeding program, when the sample size is extremely limited for traditional dough testing methodologies, a set of small-scale testing methods is applied. The FQC2000 micro mill (MeteFEM Budapest, Hungary) is used to mill flour for the functional studies (Békés et al. 2000; Tömösközi et al. 2001). This equipment is capable of making good quality flour from only 2–5 g of grain. Mixing properties of the breeders' lines are monitored on a micro-doughLAB (Perten Instruments) mixer (Dang and Bason 2013; Haraszi et al. 2004). The Kieffer rig methodology and equipment (Stable MicroSystems) is applied for the determi-

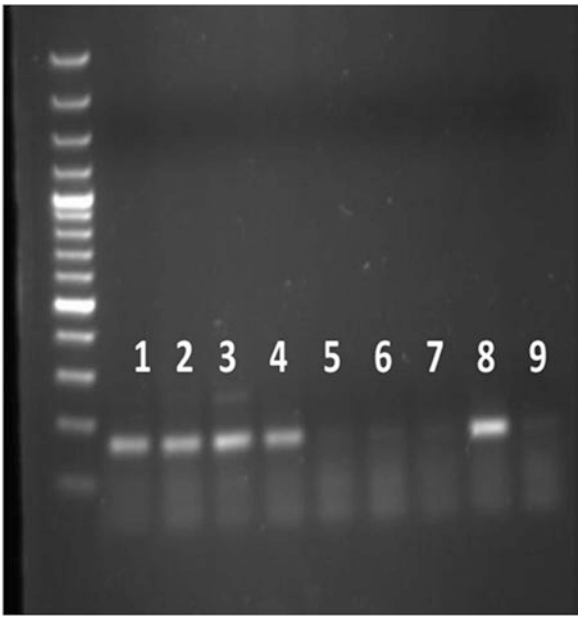

Fig. 14.7 Monitoring the presence of mutation in the expansin gene, using a molecular marker, developed by Suter and Békés (2012). 1 - wheat; 2 - kamut; 3 - emmer; 4 - normal spelt; 5,6,7 - GWF spelt - 9 negative control. (Source: Békés et al. unpublished results)

nation of extensional parameters (Rmax and extensibility) (Kieffer et al. 1998), while a prototype SediCom System micro Zeleny equipment (Lab-Intern Ltd., Budapest, Hungary) was used for determination of Zeleny values. The measurements are carried out according to the modified ICC Standard No. 116/1. (Tömösközi et al. 2010).

14.7 Spelt Breeding Via in Vitro Androgenesis Using Anther and Isolated Microspore Culture Methods

Classical breeding is a time-consuming process. It takes about 10–12 years to release a new cultivar, because the selection of segregated population and growing and testing of different generations requires much time. To accelerate the generation changing in plant breeding and to achieve the homogeneity of selected lines within one generation, an efficient plant biotechnology method—based on in vitro androgenesis—was developed in spelt (Lantos et al. 2016, 2018).

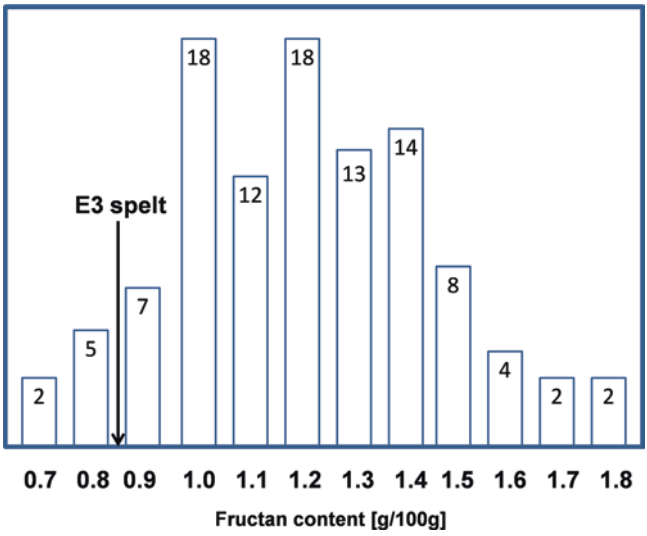

Fig. 14.8 Variation of fructan levels in 105 spelt cultivars grown in Hungary at the same location. (Source: Ács et al. 2017)

14.7.1 Induction of in Vitro Androgenesis in Anther Culture

In spelt, in vitro anther culture was first tested with Hungarian spelt cv. GK Fehér. Later the protocol was also implemented with three other spelt cultivars. The number of produced embrioids and embryo-like structures (ELS) was 62/100 anthers. These structures regenerated dominantly green plantlets in vitro. The green plantlets production was 89.0% among the regenerated plantlets while the number of albino plantlets was low, 3.8/100 anthers. More than 1000 in vitro green plantlets were produced from anther culture of different spelt cultivars. After ploidy-level analyses (measurement of stomata length and flow cytometric analyses), using the haploid plants, doubled haploid plants were produced via colchicine treatment (Pauk et al. 2003) and produced seeds after rediploidization. The colchicine induced and spontaneous DH spelt plants were propagated in the greenhouse. The in vitro doubled haploid plant production method was integrated into our spelt breeding program in the similar way as was done with bread wheat (Pauk et al. 2003).

In our study, the first anther culture-derived ELS were observed after 3 weeks of androgenesis induction. The microspore-derived ELS with 1–2 mm size (Fig. 14.9a) were transferred to a Petri dish containing regeneration medium. The ELS produced significantly more green plantlets than albinos (Fig. 14.9b). Green plantlets were rooted in individual culture tubes (Fig. 14.9c) and regenerated plantlets acclimatized and grown in the greenhouse (Fig. 14.9d).

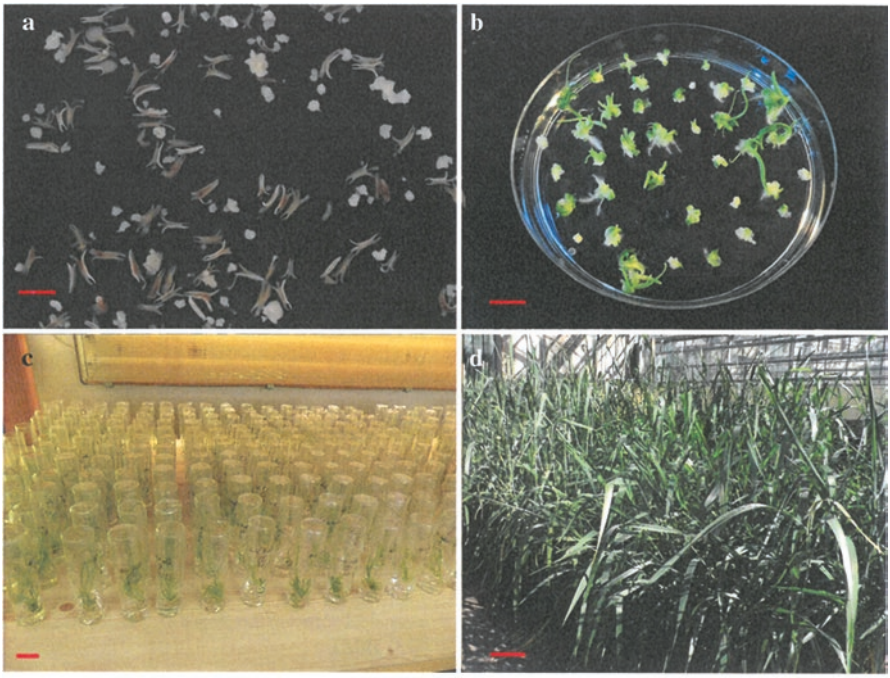

Fig. 14.9 Important steps of anther culture in spelt: (**a**) Microspore-derived ELS, (**b**) Green plantlets in regeneration, (**c**) Rooting of individual plantlets, (**d**) Acclimatized plantlets in greenhouse. Red bars 5 mm for **a**; 10 mm for **b** and **c**; 50 mm for **d**. (Source: Lantos et al. 2018)

14.7.2 Effect of Genotype and Cold Pre-Treatment in Anther Culture

ELS could be produced with or without cold pre-treatment of the donor tillers (0 and 12 day cold pre-treatment) in anther culture. However, cold pre-treatment significantly improved the production of ELS and the number of regenerated green and albino plantlets in anther culture of each tested genotype (Table 14.3). The number of albinos was limited in anther culture (3.48 albinos/100 anthers), these values had a range of 0.5–7.47 albino plantlets/100 anthers depending on the cultivar. However, the production of green plantlets achieved high values, on average 41.45 in vitro green plantlets were regenerated from 100 anthers. The number of produced in vitro green plantlets had a range of 20.93–83.07 green plantlets/100 anthers. The most green plantlets (83.07/100 anthers) was regenerated from anther culture-derived ELS of cv. Franckenkorn. Altogether, more than 1000 green plantlets were produced from the anther culture of the 4 cultivars.

In a two-way analysis of variance (Lantos et al. 2018), the effect of pre-treatment and genotype was tested in anther culture of four spelt cultivars (data are not shown here). Based on the ANOVA test, genotype and cold pre-treatment of donor tillers

Table 14.3 The effect of genotype and cold pre-treatment on the parameters of anther culture in spelt

	Embryo-like structures /100 anthers		Regenerated plantlets/100 anthers		Green plantlets/100 anthers		Albinos/100 anthers	
Genotype	0 day	12 days	0 day	12 days	0 day	12 days	0 day	12 days
Franckenkorn	9.17 b	134.80 a A	8.67 b	90.53 a A	5.50 b	83.07 a A	3.17 b	7.47 a A
GK Fehér	2.33 a	46.33 a B	1.33 b	43.33 a AB	1.00 b	38.33 a AB	0.33 b	5.00 a B
Mv Martongold	4.33 a	34.80 a B	3.00 a	21.87 a B	2.67 a	20.93 a B	0.33 a	0.93 a C
Oberkulmer Rotkern	3.60 a	27.07 a B	1.07 a	25.47 a B	0.67 a	23.47 a B	0.40 a	0.50 a C
Mean	4.86	60.75	3.52	45.3	2.46	41.45	1.06	3.48

Values followed by the same capital letters (A, B, C) are not significantly (P = 0.05) different for the genotypes. Values followed by the same letters (a, b) are not significantly (P = 0.05) different for different treatment within genotype
Source: Lantos et al. (2018)

significantly influenced the parameters of androgenesis (number of ELS, regenerated, green and albino plantlets). The effect of genotype × pre-treatment interaction was significant in the number of ELS, regenerated and green plantlets, too.

On the greenhouse grown plants, the fertile and partial fertile spikes were harvested. In the case of four tested spelt cvs. (Frankenkorn, Oberkulmer Rotkorn, GK Fehér, Mv Martongold) the values of seed setting were 11.8, 19.35, 44.44 and 21.47%, respectively. These data show that the anther culture method of spelt is ready for integration into the breeding process.

14.7.3 Induction of Androgenesis in Isolated Microspore Culture of Spelt

Because the anther culture technique needs significant manual work in isolation, to reduce this manual effort, we began to study the isolation microspore culture in spelt wheat as well. The microspore isolation was started, when in donor spikes, the stage of microspores was in uni- and binucleate stages (Fig. 14.10a, b). The ovary coculture was crucial in isolated microspore culture. Multicellular structures and ELS were not observed in isolated microspore culture without ovaries. The ovaries supported the development of multicellular structures and ELS (Fig. 14.10c, d). The transferred ELS produced both green and albino plantlets (Fig 14.10e) on the regeneration medium (Lantos et al. 2018).

In isolated microspore culture, cv. Franckenkorn produced the most ELS and regenerated plantlets, while the production of ELS was the lowest in case of cv. Oberkulmer Rotkorn. In isolated microspore culture, the growth regulators were not

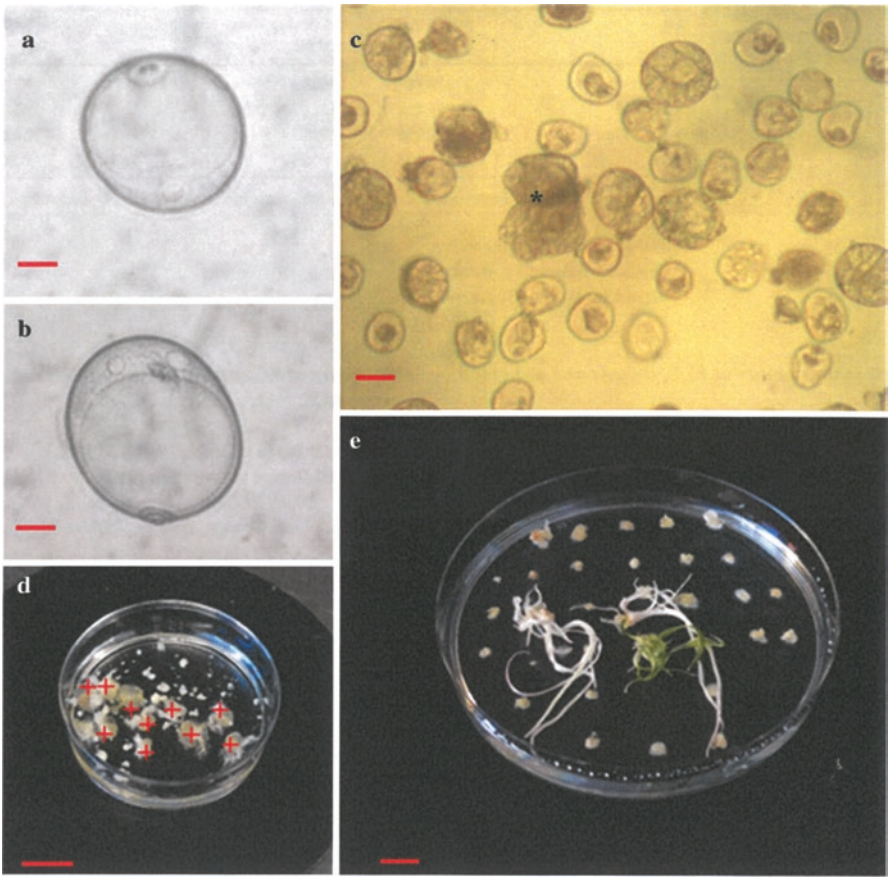

Fig. 14.10 Critical steps of isolated microspore culture: (**a**) Late uninucleate microspore, (**b**) Early binucleate microspore, (**c**) Multicellular structures (∗) and divided microspores, (**d**) Obtained ELS in ovary-supported (+) microspore culture, **e** Regenerated green and albino plantlets. Red bars 10 μm for (**a**) and (**b**); 50 μm for (**c**); 10 mm for (**d**) and (**e**). (Source: Lantos et al. 2018)

essential in induction of androgenesis, but growth regulators increased the efficiency of the method (ELS, green and albino plantlets). The ELS production was high in isolated microspore culture. In practical terms, it was a big problem that most of the regenerants were albino plantlets. Altogether, three isolated microspore culture-derived in vitro green plantlets were regenerated. Two from cv. Franckenkorn and one from GK Fehér, respectively.

Androgenesis was induced in isolated microspore culture of each cultivar. The cultivars influenced the number of produced ELS, in vitro regenerated plantlets and albinos, while the exogenous growth regulators had significant effect on the tested parameter.

14.8 Conclusions and Prospects

The abovementioned method of producing a cereal line which is beneficial to human health can be used as a model, demonstrating how to protect baked goods from contamination by any other cereal material from the field to the bakery and how to accomplish (protect, monitor, check) the quality of the final product. These kinds of issues will hopefully emerge soon because of selecting and producing germplasm with proven reduced allergenicity and altered gluten and/or non-gluten protein composition.

Developing cereals with largely reduced allergenicity and with low FODMAP content is a much more realistic approach, compared to the ultimate task, developing celiac-safe cereals. Most wheat sensitive individuals—except for celiac patients—can consume these kinds of products.

Further detailed basic and applied research is required for the development, manufacturing and commercial availability of such products. This must include developing cost-effective, reliable and high-throughput analytical tools to monitor the quantity and the presence/absence of certain key components in the source material, the intermediates and the final product through the whole production chain, from the farm to the supermarket. Nevertheless, developing the necessary infrastructure for the production and commercialization of a multipurpose, healthier cereal product basis is only one of numerous requirements needing attention in the complex matter of providing appropriate food for people suffering from cereal-based health problems. It is essential to alter the legislative environment in order to permit the production/commercialization of products with altered/low gluten content, giving instructions on analytical procedures, defining thresholds of certain key components, determining food safety control systems, labelling, policing and regulating the very often misleading media-based marketing. However, the ultimate purpose is a more effective collaboration and communication among plant science, the grain industry as well as medical researchers and practitioners, together with up-to-date, accurate and permanent information of customers.

An effective in vitro androgenesis method was developed in spelt to develop the homogeneity of newly-selected spelt lines and to accelerate the pace of generation changes in breeding (Lantos et al. 2016). In this activity we were able to apply successfully those small and micro scale dough testing methods which have been developed earlier for characterizing bread wheat samples. With Hungarian cv. GK Fehér the anther culture method was carried out; however, in three other registered spelt cultivars tested using the protocol, 62 ELS/100 anthers produced embryo-like structures (ELS), from which we were able to regenerate green plantlets. A total of 89% of green plantlets production was achieved among the regenerated plantlets whereas the number of albinos was limited (3.8/100 anthers). All in all, from an anther culture of different spelt genotypes over 1000 in vitro green plantlets were created. The doubled haploid plantlets were produced via colchicine treatment and obtaining seed after spontaneous diploidization, based on ploidy-level analyses

from the haploid plantlets (Pauk et al. 2003). In the greenhouse the colchicine treated and spontaneous DH plants were grown out. The in vitro haploid induction system was incorporated into the breeding program of spelt in the same way as was done with bread wheat (Pauk et al. 2003, 2004).

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Appendices

Appendix I: Research Institutes Relevant to Spelt

Institution	Specialization and research activities	Contact information and website
Cereal research non-profit ltd., Szeged, Hungary	Research and breeding	https://www.gabonakutato.hu/en/contact
Centre for Agricultural Research, agricultural institute, Martonvásár, Hungary	Breeding and research (FODMAP)	http://www.agrar.mta.hu/en

Appendix II: Spelt Genetic Resources

Cultivar	Important traits	Cultivation location
Center for Plant Diversity, Tápiószele, Hungary	Gene bank, genetic resources, germplasm collection	http://www.nodik.hu/english/?page_id=2348

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