STORAGE PROTEINS IN WHEAT (*Triticum aestivum* L.) AND THE ECOLOGICAL IMPACTS AFFECTING THEIR QUALITY AND QUANTITY, WITH A FOCUS ON NITROGEN SUPPLY

Csaba HORVÁTH

Szent István University, Crop Production Institute, Gödöllő
e-mail: hoce.csaba@gmail.com

Abstract
Wheat (*Triticum aestivum* L.) is the most widely used basic staple for mankind. Wheat is also one of the most important cereals in Hungary with a high economic value. The aim of wheat production is twofold; to provide quantity and quality. Milling and baking quality of wheat is mainly determined by the genetic basis, however it can be influenced by management techniques.

Storage proteins account for more than half of the total protein in mature cereal grains and have important impacts on their nutritional quality for humans and livestock and on their functional properties in food processing. Gluten proteins - gliadins and glutenins of wheat determine the quality of the grain for breadmaking and their amount and composition can be influenced by agronomic impacts leading to changes in dough properties and that of baking quality.

The present review is dealing with the impact of various agronomic and environmental factors on the performance of storage proteins and within them gluten proteins.

Keywords: review, wheat, proteins, nitrogen suply

Introduction

Wheat is one of the most important cereals used for bread making, a key source of food all over the world. The total global wheat output exceeded 670 million tonnes in 2012, according to FAOSTAT data (FAOSTAT 2012). Bread made from wheat is a staple foodstuff because it fills the stomach, it is easy to digest and it is not expensive (Pollhamerné 1973).

The success of wheat relies partly on its adaptability and high yield potential but it is also important that the dough made from its flour is suitable for making a wide variety of bakery products. Wheat contains essential amino acids, minerals and vitamins, along with useful secondary metabolites and dietary fibres (whole grain products are particularly rich in the latter two). It should also be noted however, that meals containing wheat products may be responsible for a variety of undesired side effects, such as intolerance (wheat sensitivity) or allergic symptoms (respiratory allergy and food allergy).

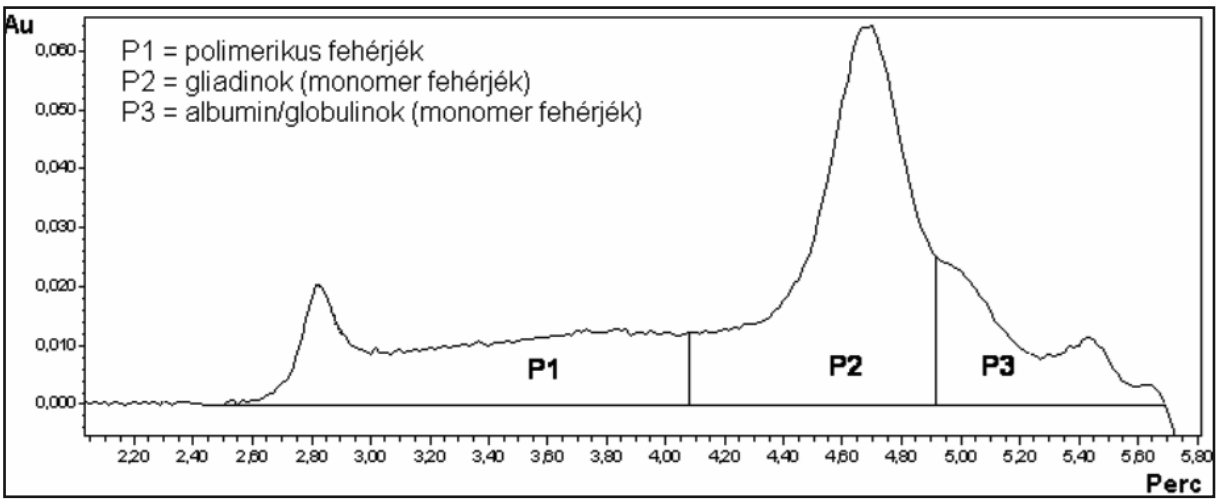
The topics of ongoing and prospective research projects include sustainable wheat production and quality using reduced amounts of chemicals, breeding of new wheat strains for end-users with special requirements, including the bio-fuel industry and public catering (Shewry 2009).

Plant breeders have, for quite some time now, been working on developing wheat varieties and strains meeting a wide range of different requirements on the part of users. The quality attributes of common – or bread – wheat are highly important for different users (Bedő et al. 1998). Different types of wheat of different protein contents are required for the production of biscuits, different types of bread or pasta. The possibilities of using a given wheat variety are determined primarily by two of its attributes: grain hardness and protein content: higher protein contents make it possible to make more valuable, higher quality products (Békés 2014).

Wheat's storage proteins

It has been known since 1728 when Jacopo Beccari carried out his studies that it is possible to produce more or less clean gluten – a sticky

Figure 1. Polymeric (glutenin-P1) and monomeric (gliadin-P2 and albumin, globulin-P3) proteins in the flour of the "Ukrainka" wheat variety (24. DPA) separated on the basis of its SE-HPLC chromatogram (Abonyi Tibor, 2010. p. 72.) (P1 = polymeric proteins, P2 = gliadins (monomeric proteins), P3 = albumin/globulins (monomeric proteins) **Minutes**)



matter of a peculiar consistency – by rinsing dough produced from wheat flour in water. This matter has a complex structure that is created by the interaction between elastic glutenin and plastic gliadin proteins. The gluten proteins are the most important storage proteins of a wheat grain, to be found exclusively in the starchy endosperm, the part of the grain that is ground into flour. During the ripening process the proteins combine with each other forming large polymers and when the flour is mixed with water while being worked into dough they build a continuous network of proteins. This network of protein molecules gives flexibility and viscosity to the dough, making it possible for instance to produce raised bakery products (Tosi et al. 2011). It is the storage proteins of wheat, called prolamins, making up what is commonly known as gluten, that make it possible to create such a wide variety of products from wheat flour. The relative proportions of the different types of proteins making up a given wheat flour's protein content however, are extremely important. Accordingly, the baking quality of wheat is determined by the quantity of the total protein content and the relative proportions of the different types of proteins to be found in it.

Thomas Burr Osborne (1859-1929) categorised grain proteins according to water-solubility: Albumins: water-soluble, globulins: salt-soluble, prolamins (gliadins): alcohol-soluble, glutelins (glutenins): alkali-soluble. The different types of wheat proteins can be separated according to size. In general, wheat flours contain 45% glutenin, 45% gliadin and 10% soluble proteins.

Wheat proteins are conventionally assigned to two different groups: the density and extensibility of the dough is determined by the monomeric gliadins, while its flexibility and strength is determined by polymeric gluteins. Within these groups the various proteins are categorised further according to their electrophoretic mobility: gliadins are sub-divided into type α-, γ- and ω proteins, depending on their mobility during electrophoresis, in a low pH environment.

Under chemical reduction glutenin decomposes into its subunits. The glutenin components are categorised into high molecular weight (HMW) and low molecular weight (LMW) groups based on the SDS-PAGE separation technique (Shewry et al., 2009).

Similarly to other wheat storage proteins its prolamins are also polymorphic, encoded by multigene families that are present in the form of homeologous alleles on three genomes (A, B and D). A high degree of variation of the gluten protein alleles is observed across different genotypes as well. Different wheat varieties can be separated from one another

Figure 2. Fractioning of gliadin proteins according to charge (Békés 2014)

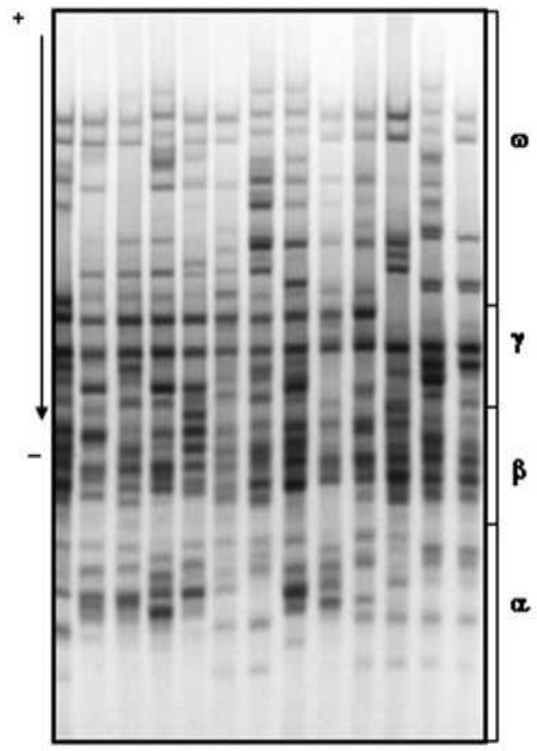
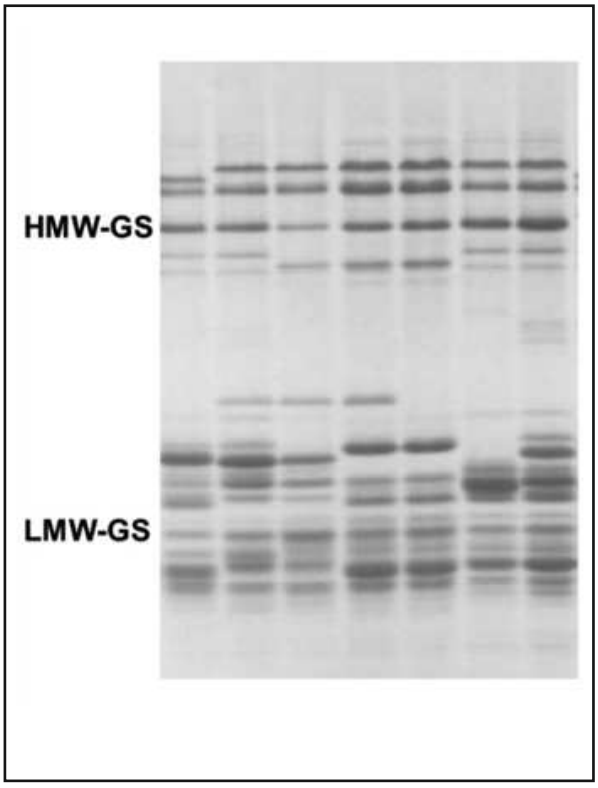


Figure 3. Separation of glutenin components by size (Békés 2014)



on the basis of the identification of the storage proteins, e.g. by the electrophoresis of gliadins (Bushuk et Zillman 1978).

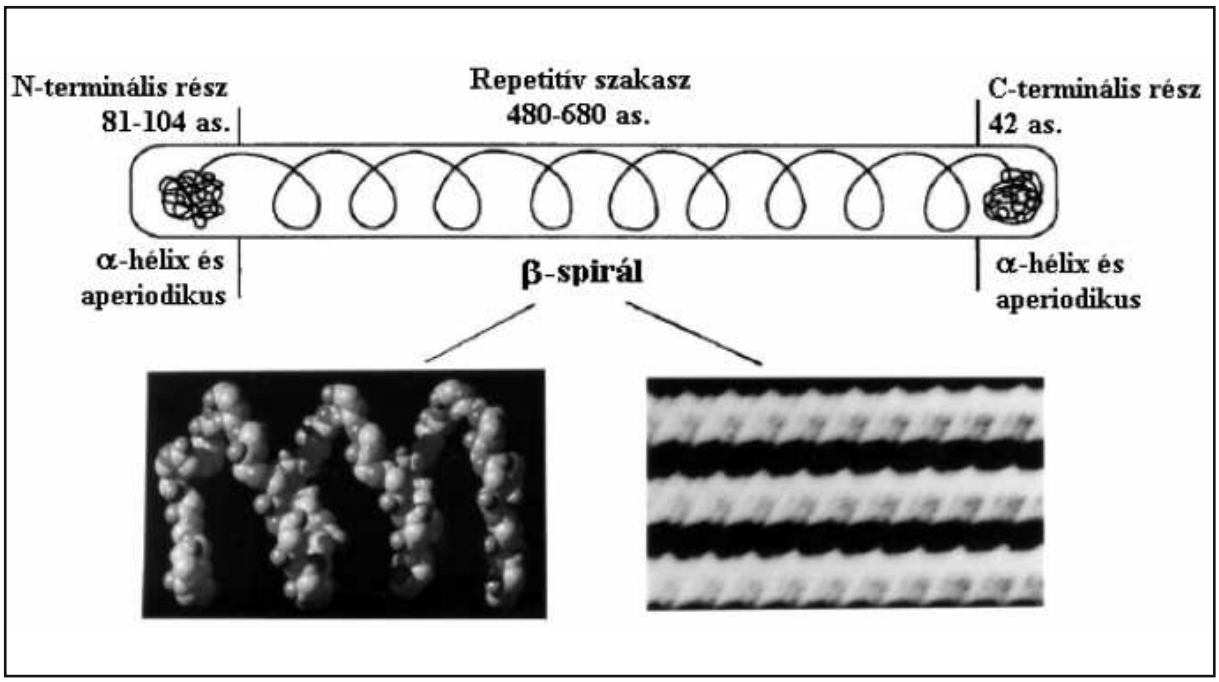
The division into gliadins and gluteins - though it has proven to be a rather durable technique over time – sheds no light onto the molecular and evolutionary aspects of proteins. In this regard only three groups can be distinguished (Shewry et al., 1986): HMW proteins (comprising glutenins' HMW components), prolamins that are rich in sulphur (comprising α-, β- and γ-gliadins and glutenins' LMV components) and low-sulphur prolamins (comprising ω -gliadins and this type of proteins of the glutenin fraction – these are referred to as group D within the LMW category) (Masci et al. 1993, 1999).

Most ω-gliadins are encoded by the genes (called *Gli-A1*, *Gli-B1* and *Gli-D1*) on the *Gli-1 loci* on chromosomes A, B and D however, some other loci are also located on the same chromosome arm.

The structures of the proteins encoded by the genes *Gli-A1*, *Gli-D1* and *Gli-B1* are clearly different from each other. Although both groups of the proteins are created by repetitions of – for the most part – short peptide patterns, the patterns themselves are different from each other: in the case of the proteins encoded by *Gli-A1* and the *Gli-D1* genes a repetition of the PQQPFPQQ sequence, in the case of those encoded by the *Gli-B1* genes a repetition of the PFQ_{2-4} sequence is observed (P=proline, Q=glutamine, F=phenylalanine). These differences in pattern appear, of course, in the amino-acid compositions of the resulting proteins as well: the ω-gliadins encoded by the genes *Gli-A1* and *Gli-D1* contain about 40 n/n% Glutamine and 30 n/n% Proline while the proteins encoded by the *Gli-B1* genes contain approx. 50 n/n% Glutamine and n/n% Proline. Moreover, these two different types of ω-gliadins are easily separated by electrophoresis at a low pH value. The *Gli-A1* and the *Gli-D1* proteins move much slower: these are called ω-1/2 gliadins, while the faster moving *Gli-B1* proteins are called ω-5 gliadins. The different ω-gliadins are distinguished from each other according to

their N-terminal amino acid sequences, such as the SRLLSPQ sequence in ω-5 gliadins, the ARQLNPSNKELQ or KELQSPQQS sequence in ω-1 gliadins and the ARELNPSNK sequence in ω-2 gliadins (A=alanine , E=glutamic acid , L=leucine, S=serine , R=arginine, N=asparagine, K=lysine) (Shewry et al., 2009). The protein subunits making up gluten protein are bound together through disulphide bridges in the polymer. distribution of the special gluten proteins in the endosperm. The large molecule weight glutenin subunits (HMW - glutenin) and the γ gliadins are to be found primarily in the innermost layers of the endosperm, while the low molecule weight subunits of glutenin (LMW - glutenin), the ω and the α gliadins, are more typically to be found in the subaleuron layer. Immune localisation made it possible to demonstrate that the segregation of

Figure 4. The assumed structure of the HMW gluten subunit, according to spectroscopic and hydrodynamic examinations (Shewry et al., 2000)



The distribution and accumulation of proteins

Proteins are concentrated predominantly in the subaleuron layer of the ripe grain (Bradbury et al., 1956; Normand et al., 1965; Kent, 1966; Kent and Evers, 1969; Tosi et al., 2009, 2011; He et al., 2013). Accordingly, the endosperm is not a homogeneous tissue and its key components (proteins, starch and cell-wall polysaccharides) show a qualitative and a quantitative gradient as well. The gradient observed in the protein content and its composition is clearly evident and highly important because it plays a major role in the determination – by the gluten proteins – of the value of wheat for the milling industry. By means of Western blot analysis, using antibodies, it is possible to display the the gluten proteins occurs between and within the protein particles in the course of protein accumulation and this is preserved in the ripe wheat grain as well. Accordingly, a qualitative and a quantitative gradient develops among the gluten proteins of the endosperm during the development and maturing of the wheat grain. This may be caused perhaps by the origin of the subaleuronic cells which – unlike the other endosperm cells – develop from redifferentiated aleuronic cells but it may also possibly be a consequence of special controlling signals created by the matured tissue on the special domain of the gluten protein's gene promoter (Tosi et al. 2011).

The application of nitrogen fertilisers however, affects the expression patterns within the grain:

Figure 5. LMW glutenin subunits (Békés 2014)

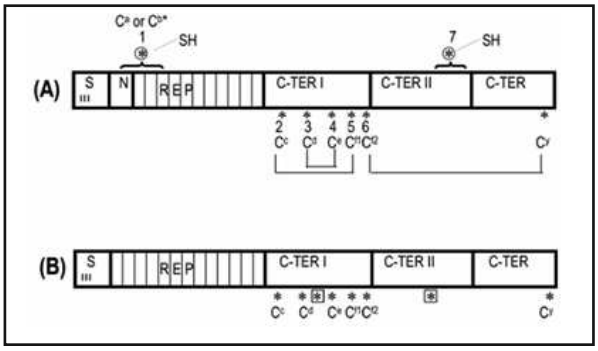
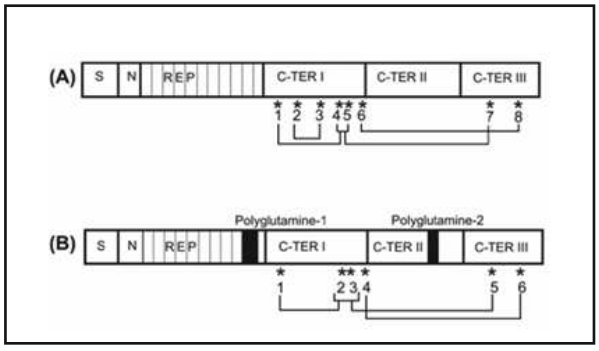


Figure 6. Gliadins (Békés 2014)



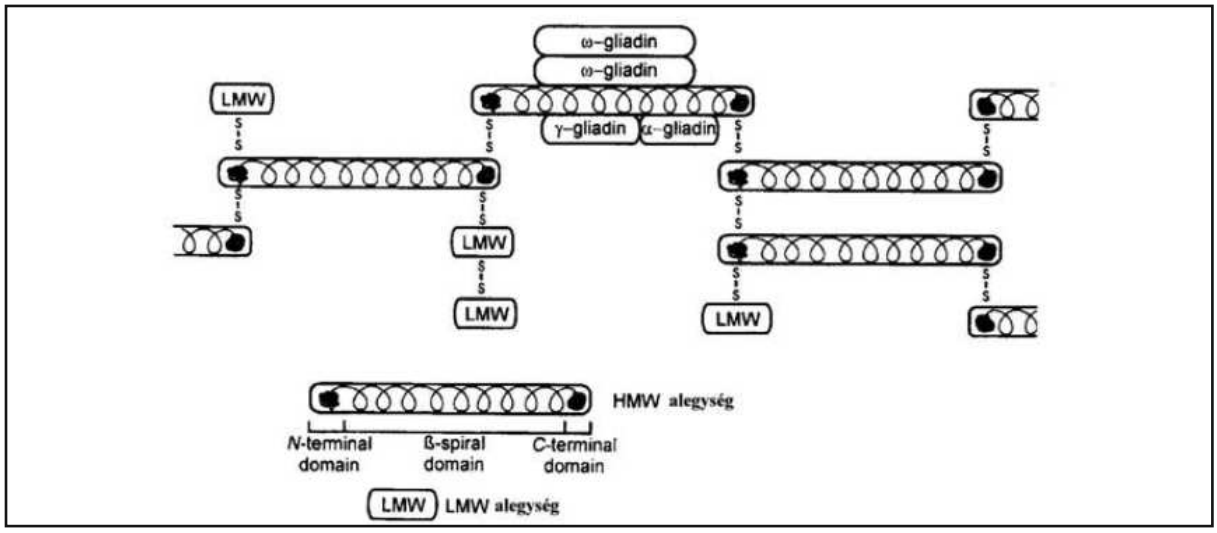
the ω gliadin genes got expressed mainly in the core of the endosperm rather than in the subaleuronic layer in the case of low N levels (100kgN-1), while in the case of high (350kgN-1) nitrogen levels they got expressed in the subaleuronic cells. (Wan et al., 2013b).

NIR spectroscopy may be an effective tool for monitoring the physiological processes of plants in terms of both qualitative and quantitative aspects also in regard to protein accumulation, the interaction between gliadins and glutenins and the development of the gluten network, while the spectrum also contains some additional hidden information that can be used for determining the degree of development of a particular wheat grain (Salgó and Gergely 2012).

The impacts of fertilisers/manure on plant development, yields and quality

Excellent raw materials is the first prerequisite for good bakery products. Of plant nutrients nitrogen has the most dramatic impact on both the quality and the amount of the yield. The application of a nitrogen fertiliser boosts protein content, the amount of wet gluten, vitrenousness and the thousand grain weight, indeed even the starch content of the grain (Pollhamerné 1973). The genetically determined quality of the various wheat varieties may only be attained by applying appropriate agronomical techniques. In the array of up-to-date agricultural techniques roper application of fertilisers is the means that can have the most profound impact on the quality and quantity of wheat yields. A number of authors have come to the conclusion that the improvement of the genetic stock contributes 30-50 %, while agricultural techniques contribute 50-70 % to increasing yields (Jolánkai 1985). Findings of long term experiments that have been continued in Hungary since 1967 at nine sites of different agro-ecological conditions, involving

Figure 7. A model illustrating the role of proteins making up gluten protein (Wieser et al. 2006)



long term N and P fertiliser experiments as well, show that increasing the amount of the P and the N fertiliser has a remarkable impact on the quantity, composition and quality of the yield, in comparison to the average of the data collected and evaluated during the first 20 years of the experiment (Ragasits et al., 2000). These impacts depend on the agro-ecological features of the area concerned. Even the application of a smaller dose of fertiliser triggers an increase in yield, while a higher dose generates a quality improvement as well.

In the case of winter wheat a harmonised supply of nutrients (NPK) is a dominant technique in crop production even in areas with favourable nutrient and water supply. Experiments carried out in 2001 showed an increase from the 3193 kg/ha average yield, without fertilisation, of the various wheat varieties involved in the trial, to 4 tonnes after the application of optimised quantities of fertilisers. Moreover, the application of fertilisers also affected, besides the quantity of the yield, the quality and the stability of the quality of the produce. The effects of the application of fertilisers are influenced by agro-ecological, biological and agro-technical elements (e.g. genotype, water supply, the preceding crop, the crop protection technology) (Pepó P. 2002, 2005, 2006, 2007). Nitrogen top dressing, even in doses as low as 40 kg/ha, but much more so in doses up to 80-120 kg/ha, triggers yield increases even under very dry conditions. Experiments have shown that increasing doses of nitrogen top dressing can enable outstanding quality improvements even under unfavourable ecological conditions (Szentpétery 2004). It is also confirmed by the results of long term fertiliser application experiments set up back in 1967, that the decrease in yields caused by aridification can be alleviated by the application of the right doses of fertilisers. (Láng et al 2007.)

As a general rule, yields, as well as grain protein contents, are also increased significantly by irrigation besides the application of fertilisers. These details are highlighted by the experiments carried out by Pushman and Bingham (1976): they applied fertilisers in amounts of 90 kg/ha, delivered to the experiment plots in the form of granules, as a result of which the yield increased by 12. 4 % and 6.1 %, while the grain protein content grew by 13.0 % and 33.7 % , without and with irrigation, respectively. The application of another dose of N fertiliser – liquid urea, in the form of top dressing during the anthesis phase – resulted in a 12.4 % and a 8.8 % increase in the amount of proteins with and without irrigation, respectively, along with a positive impact on the total amount of the yield as well. The significant difference in the yields and the protein contents of the produce of the different varieties resulted, in the case of each nitrogen treatment, in an inverse relationship between yield quantity and protein content. The amount of protein produced (N mass/unit of area) was similar in the case of every variety but the flour yield decreased as a consequence of the urea treatment. The differences between the varieties in terms of flour yield appeared to be stable and they were in no correlation with the thousand grain weight or with the bulk density. The bulk density may be a useful aid in the calculation of the flour yield on the basis of a sample taken from one variety but it would be probably misleading when it comes to comparing different varieties (Pushman and Bingham 1975). The test loaf volume increased as a consequence of the application of the granulated fertiliser. Urea top dressing had no such effect, despite the fact that it lead to increased flour protein content and decreased α-amylase activity showing that the application of nitrogen fertiliser after flowering is somewhat belated if the aim is to improve the product's value for the baking industry (Pushman and Bingham 1976).

Berecz and Ragasits (1990) examined the impacts of N fertiliser applied in the form of ammonium nitrate, in various doses (80-200 kg/ha), during different Feeks phases of flowering, in different distributions among the examined Feeks phases, on the dry matter accumulation in the winter wheat variety named Martonvásári-4 (*Triticim aestivum, L.*). In the

initial growth phase neither the amount of nitrogen fertiliser applied, nor the timing of its application had any impact on dry matter accumulation. The plant's N accumulation positively correlated with the amount of N, with a 349.5 mg maximum amount per 10 plants when 200 kg/ha nitrogen was applied. Distributed application of the N fertiliser had no material impact on N accumulation. The grains' raw protein content increased along with the increase in the applied amount of nitrogen, but it was not affected by distributed allocation. The highest raw protein content was observed after the distributed application of 160 kg/he nitrogen fertiliser. The lysine and threonine content decreased as the raw protein content increased.

According to experiments performed by Győri (2006) the protein content determining the quality of wheat is radically increased by the application of a harmonised combination of nitrogen, phosphorous and potassium (NPK). This is also confirmed by the results of experiments carried out in 2001 and 2003, in the course of which the lower than 10 % protein content of the wheat produced on the control plot without fertilisers had been increased to 12 % or higher by the application of 60 kg/ha N+PK fertiliser. The wet gluten content of the produce on the control plots where no fertiliser was applied was below 25 %, while the application of 60 kg/ha N+PK resulted in a higher than 30 % wet gluten content in each of the experimental years. The application of the combined fertiliser in an 60 kg/ha dose improved the bakery value of the output in the case of each of the varieties involved in the experiments, however, the application of a higher doses does not result in further improvements in the case of every variety. In the course of their experiments Tanács et al. (2006) found that the application of higher doses of fertilisers usually lead to higher wet gluten contents. Reliable wet gluten content increase was produced by the application of 40+40 kg/ha N, 40 kg/ha P, 40 kg/ha K. The application of fertilisers in combination with fungicide treatments further increased the wet gluten contents. The baking value numbers were significantly different across varieties in both the annual evaluations and in the three-year averages in the wake of the treatments.

In experiments carried out by Szentpétery et al. (2005) increasing doses of nitrogen top dressing and their distributed application lead to quality improvements even in unfavourable ecological circumstances. In a series of experiments with the following doses: 40, 80, 120, 40+80 and 80 + 40 kg/ha the 120 kg/ha dose reduced the yield but in terms of total protein and gluten content the higher nutrient doses produced higher quality yields. Applying the amount of nitrogen used for top dressing in several rounds had a very positive impact on the crop. From the aspect of baking quality the growing amounts of fertiliser, particularly when applied in two rounds (one applied at a later stage) resulted in an increase in value. The best combination of treatments was the one (80+40 kg/ha) where a relatively large dose provided the wheat with the nutrient boost required for the first phase of its growth and the during the flowering phase another application of top dressing definitely helped the variant in coming closer to the maximum possible baking quality enabled by its genetic potential and the conditions of the given season. According to Fuertes-Mendizábal et al. (2010) the N-content of the wheat grain depends on the variant, the environmental impacts and the distribution of the applications of fertilisers. In comparing the response to increasing doses of nitrogen and to their application in different quantities at different times Soissons found that not only the increased amounts but also the distribution of their application over time had a positive impact on the quality of the wheat. Indeed, the distribution of the applications of a small total amount of nitrogen fertiliser over the growing season had an added positive impact in making the quality of the wheat more evenly balanced. The composition of the metabolic proteins remained unchanged regardless of the grains' nitrogen content, yet the amount

of storage proteins increased along with the growth of the grains' nitrogen content. The flexibility, extensibility and strength of the dough improved dramatically, which was related to a slight increase in the glutenin fraction whereby it exceeded a certain threshold, indicating that a higher degree of glutenin polymerisation explains the improvement in quality. Both the raising of the doses of the N fertiliser and the distribution of the applications of the given amount over time, led to an increase in the amount of HMW-GS, enabling to the forming of an increased number of disulphide bridges, leading, in turn, to a higher degree of polymerisation which may ultimately drive the improvement in quality.

Raising the doses of the N fertiliser (from 0 to 288 kg/ha) resulted in an increase in the proportion of gliadin proteins and the extensibility of the dough (Godfrey et al., 2010). The flour ground from wheat produced on a field where 192 kg/ha N and 0 kg/ha S fertiliser had been applied was similar in quality to the flour made from wheat grown in a field where a total of 192 kg/ha N and 53 kg/ha S fertiliser had applied. The share of ω-gliadins however, increased and the strength of the dough was similar to that of the doughs made from wheat grown with smaller doses of N fertilisers. The N content of wheat grown on fields after the delivery of 35 t/ha farmyard manure equalled to that of wheat grown on fields where 144 kg/ha N fertiliser had been applied, indicating that most of the nitrogen present was not available for the wheat plants. The protein composition of the flour ground from wheat grown on this plot and the parameters of the dough produced from that flour were similar to those of flour and dough made from wheat of similar N content grown on a field enriched by conventional farmyard manure. Similar differences were observed in terms of the grains' N content, protein composition and functional attributes between wheat produced by organic farming and wheat grown by conventional techniques.

The protein content of grains is of relevance not only for the purposes of the baking industry but also from the aspect of crop production. Ayers et al. (1976) examined the relationships between grain size, total, fractional and individual protein compositions and the viability of the seedlings in winter wheat (*Triticum aestivum* L.) in response to the application of urea top dressing. They found that there was a close interrelationship between the viability of the seedling and the total protein content of the grain and of the endosperm. The viability of the seedling was greatly affected by the relative proportions of the salt solution-soluble and the salt solution insoluble fractions in the endosperm. The majority of the individual proteins separated by SDS gel electrophoresis - both the endosperm's proteins that are soluble in a thin salt solution and those that are not – positively correlated with the seedlings' viability. In the growth experiments while the weight and protein content of the grains of high protein contents decreased more rapidly than did those of the low protein grains, no such difference could be observed in the relative growth rates or the leaf surface area ratios. The ratio of the insoluble to the soluble proteins did not change along with changing grain sizes, but this ratio increased after the application of urea. The N content of the gliadin fraction grew in response to the application of N fertiliser, and upon the combined application of urea top dressing and herbicides in sub-toxic amounts the N content of the gluten fraction decreased.

Monitoring the biosynthesis of gluten forming polypeptides shows that small amounts of gliadin and glutenin monomers can be identified in the early stages of grain development but the bulk of these proteins is synthesised during later stages of development. Most experts think that the synthesis and accumulation of glutenin polymers begins later than that of monomers and this is confirmed by the conclusions drawn from experiments. It has been found that during the initial phase of protein synthesis the monomers are in a "free" state and it is not until later stages that polymeric glutenin can be identified. The HMW glutenin subunits are synthesised

concurrently and the quantity is dominated by polypeptides encoded by chromosomes B and D. Although there may be significant differences between specific varieties in terms of total protein quantity, gliadin, glutenin and the individual gluten-forming polypeptides, the curve of the accumulation of the various protein components – in terms of protein mass/grain – is of a similar sigmoid shape (Abonyi et al. 2007). The experiment conducted by Liu et al. (2007) showed that the application of a N fertiliser ((0, 120, 240 and 360 kg/ha urea) resulted in a significant increase in the albumin and globulin content during the early stage of the grain-filling period but this impact gradually dampened later on. The different doses of N had no perceptible impacts on the albumin and globulin content by the ripening of the grains. The application of N fertiliser resulted in increased levels of both gliadin and glutenin. The latter increased by a somewhat higher percentage therefore the glutenin to gliadin ratio increased to some extent. The N treatment also raised the flour's wet gluten content whereby it extended the farinographic dough forming time, increasing the its stability and longevity.

Two-dimensional gel electrophoresis has shown that the application of fertilisers after flowering causes changes to the proteome that has a profound impact on the quality of the flour and on immunogenicity (Altenbach et al. 2011). The supply of mineral nutrients has a great impact during grain development on the protein content and composition of the flour ground from the grains which, in turn, affects the quality and the immunogenic potential of this economically highly valuable commodity. As a result of the complexity of the wheat flour proteome it is difficult to precisely identify the impacts of mineral supply on the composition of the protein. The use of tandem mass spectroscopy (MS/MS) has improved the identification of flour proteins and the comprehensive proteome maps of flour ground from an American wheat (Butte 86) are now available. All of these make it possible to document the changes taking place in the individual proteins of flour that are caused by the application of fertilisers. The Butte 86 wheat variety was treated with fertiliser after flowering, while the same variety was also grown on a control plot. Quantitative 2D gel electrophoresis was applied in order to determine the protein compositions of the resulting flours. The treatment was found to have caused significant changes to the proportions of 54 individual proteins. The ratios of most of the omega-gliadins, the HMW-GSs and the serpins (serine protease inhibitors) as well as those of some alpha-gliadins increased as a result of the treatment. By contrast, the ratios of alpha-amylase/protease inhibitors, farinins, purinins and puroindolines decreased. The ratios of a number of low molecule weight glutenin subunits (LMW-GS), globulins and enzymes also increased. The HMW-GS to LMW-GS ratio increased from 0.61 to 0.95, while the gliadin to glutenin ratio grew from 1.02 to 1.30 in response to the application of fertilisers. Since the protein content of the flour doubled (from 7 % to 14 %) in response to the application of fertiliser after flowering, the absolute quantities of most types of proteins increased. The data indicate that the flour proteins may change in response to the application of fertilisers after flowering in line with the amounts of sulphur containing amino acids (Cys and Met).

The dosages of N fertilisers have a significant impact not only on protein content but – through the protein content – on the alcohol yield as well. Using two varieties Kindred et al. (2008) found that an average of 10 kg of protein increase per tonne resulted in a 5.7 litre decrease in the alcohol output. The genotype had only a minor impact on the protein content. The two varieties responded to the application N in the same way, i.e. the interaction between the application of fertiliser and the wheat variety had no impact on either attribute being examined. Accordingly, in the case of the different grain protein levels the scientists found similar differences between the starch contents and consequently in the alcohol yield as well. An examination based on size

exclusion chromatography revealed that upon each gram increase in the total grain protein the amount of gliadins grew by 0.56 g, i.e. gliadins made up the most important storage protein as regards mass itself. All of these findings indicate that plant improvement with the aim of reducing the grain's gliadin content reduces the total amount of grain protein and increases the alcohol yield. It has been proven statistically that the economically optimum amount of nitrogen is close to the amount that produces the maximum amount of alcohol.

The impacts of fertilisers on gene expression controlling

Gene expression in wheat is affected heavily by the application of farmyard manure or fertilisers (Lu et al. 2005). The greatest impact on yield and quality is produced by nitrogen, therefore the adequate and accurate planning of the application of N fertilisers is crucial for agriculture as well as for environmental protection. Nonetheless, we still know fairly little about the impacts of different doses and forms of nitrogen on gene expression in the case of field-grown cereals. Samples originating from the Rothamsted Broadbalk winter wheat experiment and from three other experimental fields were examined using the EST (expressed sequence tag) based wheat microarray technique, finding that the various genes responded to nitrogen delivered in the form of farmyard manure or fertiliser with surprisingly different expression levels. A number of genes showing different gene expression levels are known to participate in N metabolism and in the synthesis of storage proteins. Others play hitherto unknown roles which may be a suitable subject for future research. Typical gene expression may be used for distinguishing between organic and conventionally grown wheats.

The controlling of one of the new ω gliadin gene families is largely influenced by the N supply in the course of grain development (WAN et al. 2013a). A total of 6 different wheat varieties were grown at Rothamsted with different (100, 200 and 350 kg/ha) N treatments in 2009 and 2010. The gene expression of the developing wheat grains were described using the Affymetrix wheat GeneChip® on the 21st day after flowering. Of the 105 transcripts 4 – whose transcription was significantly overridden by N – were identified as γ-3 hordein. The identification of the expressed sequences prove that their amino acid sequences were different from those of the earlier described (typical) γ-gliadins, representing a new family of γ gliadins. The transcripts were examined during the ripening of the wheat grain using the technique called Real time reverse transcriptase PCR method on the 14th, 21st, 28th and 35th day after flowering and they found this transcription to be the most abundant on the 21st day which is when it responded most markedly to N treatment. As many as four new γ-globulin genes were isolated from the wheat variety Hereward and from the close relative species *Aegilops tuschii* and *Triticum monococcum,* with the PCR technique, while three such genes were identified from the wheat (Chinese Spring variety) genome sequence database. The amino acid sequence associated with the seven genes so identified revealed that they displayed a mere 44.4-46.0 % correspondence to the typical γ-gliadins but there was a 61.8-68.3 % match with the γ-3 hordein sequences of the wild barley species *Hordeum chilense*. The new γ-gliadin gene was localised in the first chromosome group (1, 1B, 1D)

Other factors' impacts on the quality and quantity of the yield

According to Van Lili et al. (1995) the baking industry is supplied with flour of rather widely varying quality, as a consequence of environmental impacts affecting both yield quantities and technological quality, having a negative impact on the market value of wheat. The baking quality of flour may be affected by a variety of genetic and environmental factors. Yields and protein quantities (concentrations) are affected by environmental impacts while dough quality is determined predominantly by wheat's inherited qualities.

According to Dupont and Altenbach (2003) when the grains are filling up environmental factors have a major impact on both yield and flour quality. Environmental variables (temperature, water and nutrient supply) affect the duration and ratios of the growth and development of wheat, the accumulation of protein and the deposition of starch in unique ways, through various mechanisms. The environmental impacts are added to gene expression's internal chronological pattern in the course of the development of wheat. Comparisons of genetic and proteomic research to experiments carried out under controlled environmental conditions may reveal the complex pattern of gene expression during the development and maturing of the grain, identifying the key controlling processes that are affected by environmental impacts and demonstrating the molecular foundations of the impacts of environmental factors on flour quality and composition.

According to Borojevic and Williams (1982) choosing the best variety for the given environmental conditions is a crucial prerequisite for maximising the yield. This is said because it is the genotype that most dominantly determines the number of grain per ear ratio, the thousand grain weight, resistance to diseases and endurance, just like the yield level. Experiments conducted over a decade showed how the interaction between genotype and environment affect the parameters that determine the plant's nutrient absorption and source capacity and their impact on the yield. In relation to leaf area index (LAI) and leaf area durability (LAD) the "year effect" is more important than the genotype or the year or the interaction between the two. Environmental factors had a greater impact on source capacity than on the factors relating to the utilisation of nutrients. The direct impacts of other variables differed across varieties: some had positive, others had negative direct impacts on yield. Of the climatic impacts only higher numbers of sunny hours in May and June showed a positive correlation with yield, for instance during the period of the formation of the reproductive cells, fertilisation and grain filling.

The impacts of the different years on the quality of the produce manifested in different ways in the case of wet gluten content and valiographic value. Experiments showed that while in the case of the examined varieties the wet gluten content could be kept at a high level – subject to the year effect – in the premium quality category, the valiographic value number was much more exposed to the year effect, ranging in a very wide interval of 39 (C1) to 80 (A2) even in the case of suitable application of fertilisers or manure. Experiments proved that in years of drought the process of grain fill-up was disrupted, the required gluten composition could not develop and no favourable gluten skeleton could grow (Pepó P. 2004).

The application of N fertilisers combined with farmyard manure increases gluten content and the farinographic value, and even the Zeleny number grows substantially. When N fertilisers are applied in low doses no general improvements are observed despite the favourable impacts of farmyard manure. These results were found in an experiment carried out on Ramann brown forest soil of average K supply, low phosphorous level and medium N content (Kismanyoky and Ragasits 2003). The experiment conducted at Keszthely examined how the application of farmyard manure and N fertiliser affects wheat yields and quality, applying 0-200 kg/ha N fertiliser and 100 kg/ha phosphorous (P2O5) and 100 kg/ha potassium (K2O), farmyard manure, straw manure and green manure, along with a control plot without manure or fertiliser. The application of N fertiliser had a profound impact on yield (the control 1.98 t/ha yield was tripled by the application of 200 kg/ha N fertiliser). The treatments had a marked impact on the quality of wheat as well. Grain weight, protein content, gliadin composition and content are affected in different ways by the temperature and the nitrogen supply. Higher temperatures and more ample nitrogen supplies raise the ratios of proteins and gliadins in the flour, while at the same time higher temperature has an adverse

impact on the quality of proteins or gliadins, while these are favourably influenced by ample nitrogen supply. Both factors increase the ratios of ω gliadins within the total gliadin content, while the ratio of α- and β-gliadins increases as the temperature rises or the supply of nitrogen decreases. The proportion of γ-gliadins is reduced by higher temperatures and it is increased by higher nitrogen supplies. Unlike the total amounts of proteins or gliadins accumulated in the grain, the different impacts of temperature and nitrogen supply on the ratio of gliadin within the proteins contained in the flour and on the relative proportions of the different gliadins can be explained. The two approaches are found to supplement one another at the level of flour and grain, the first can be used for describing the raw material, while the other can be used in understanding and modelling the variants of grain composition. (Daniel and Triboi 2000)

Gliadin content and composition at harvest time play a key role in determining the attributes and utility of wheat flour. An increase in temperature after flowering results in increased daily gliadin accumulation and in reduced periods of gliadin accumulation during the day. This impact is more marked in the case of the α- and the β-gliadins, than in that of the ω-gliadins. The application of nitrogen fertilisers increases the rate and duration of accumulation within a day. The N level before flowering affects the impact of N supply at flowering. The N supply has a relatively greater impact on the accumulation of ω-gliadin than on that of α-, β- and γ-gliadins. The final composition of gliadins is affected by the ratio of accumulation and the duration of synthesis, which, in turn, is determined by the timing of the beginning and the end of the period of synthesis. The dynamic allocation across the various gliadin fractions – which may be described from the aspect of thermal treatment – is suitable for modelling the gliadin content and composition of wheat (Daniel és Triboï 2001).

The impacts of the application of nitrogen fertilisers, the temperature after flowering, and dry spells are reflected by winter wheat's (*Triticum aestivum* L.) dry matter accumulation kinetics, the total N quantity and the relative proportions of the protein components (albumins, globulins, amphiphile compounds, gliadins and glutenins). Temperature or water supply effects applied during the post-anthesis period have no significant impact on the kinetic of the accumulation of the protein fractions, while the application of nitrogen fertilisers have a profound impact on the duration of the accumulation of storage proteins and their relative proportions. The accumulation of albumin-globulin proteins takes place during the early phase of grain development. The rate of the accumulation of this fraction significantly decreases after flowering (at about 250°Cd), when the intensive accumulation of storage proteins (albumins and globulins) begins. Simple allometric relationships exist under different environmental conditions between the total N per grain and the quantity of each protein fraction. The process of N distribution is not affected by post-flowering temperature or drought, or by the timing of the application of N fertiliser or its dose. Changes in the composition of protein fractions are affected primarily by the total N accumulated during the grain-filling phase (Triboï et al. (2003).

The impacts of mineral nutrients and temperature on the accumulation and composition of wheat grain proteins and on its baking quality were studied under controlled circumstances (Dupont et al., 2006). At controlled daytime and overnight temperature of 24°C and 17°C respectively (24/17°C), the continuous supply of nitrogen, phosphorous and potassium fertiliser (N:P:K=20:20:20) in a drip irrigation system after flowering increased the rate of protein accumulation, doubled the ratio of flour protein and slightly increased the grain weight. By contrast, post-flowering NPK treatment had nearly no impact at all on the rate or duration of protein accumulation or on the ratio of flour proteins when the nutrients were supplied in parallel with a high temperature treatment (37/28°C). The 37/28°C treatment shortened

the period of grain fill-up, along with the period of dry matter accumulation and reduced the grain weight by 50 %. In the case of the 37/28°C temperature regime the rate and duration of grain protein accumulation and the quantity of total protein per grain was similar – with or without NPK supply – to the above characteristics of the wheat yield produced by wheat raised under a 24/17 ºC temperature regime but without NPK treatment after flowering. The transcription and the protein profile studies confirmed that the 37/28 ºC treatment shortens the development period without disruption of the coordinated synthesis of gliadins and glutens, although some specific impacts of the supply of NPK fertiliser and the temperature on the relative amounts of certain gliadins and gluteins could be observed. The transcription levels of the ω-gliadins, the α-gliadins and some high molecule weight glutenin subunits (HMW-GS) dropped during the 24/17 ºC temperature treatment without the application of NPK after the flowering phase, while the transcription level of the low molecule glutenin subunits (LMW-GS) and the γ-gliadins changed only modestly. The two-dimensional gel electrophoresis examination showed that the relative quantities of a number of ω-gliadins, α-gliadins and HMW-GS were lower without than with NPK treatment after flowering, whereas the relative quantities of the majority of the LMW-GSs were lower after NPK treatment. The impact of the temperature on the relative quantities was usually less dramatic than that of the application of NPK. The relative quantities of some α-gliadins and the HMW-GS were higher at 37/28 ºC with and without NPK treatment after flowering than at 24/17 ºC without NPK, while at 37/28 ºC the relative quantities of the majority of the LMW-GSs decreased. The test loaf volume was in correlation with the ratio of flour protein, regardless of temperature treatment, but the highest kneading tolerance indices were measured in the case of the flours ground from wheat grown at 24/17 ºC with the application of NPK treatment as well.

Transgenic strains to improve wheat quality

Some experiment findings show that it is possible to create transgenic wheat strains whose grains make higher baking quality flour. The transgenic wheat strain identified as B73-6-1 developed and described by British scientists includes the original HMW-glutenin gene (*1Dx5*) in 10-15 extra copies, increasing the amount of the protein encoded in the gene to about four times the original quantity. The technological and rheological attributes of this transgenic wheat strain were studied at Martonvásár between 2000 and 2002, comparing them to the non-transgenic control. The results show that the transgenic and the associated functional attributes are reliably passed down through a number of generations. No differences could be identified between the yields of the transgenic and the original genotypes, but in terms of grain hardness and grain size there were genotype related differences. The transgenic strain grew harder but smaller grains. The transgenic B73-6-1 plants tended to have higher protein contents than the control (L88-6) but the difference was not statistically significant. By contrast, the amount of the *1Dx5* HMW glutenin subunit and the *Dx/Dy*, the HMW/LMW and the glutenin/gliadin ratios increased by a significant 400 %. At the same time, the wet gluten ratio and the SDS sedimentation index decreased. The attributes of the flour changed owing to the changes in the protein matrix structure, caused by the altered x:y ratio of the HMW-GS. The attributes that characterising the stability and strength of the dough showed that the dough made with the flour ground from B73-6-1 was stronger but it was less extensible. The flour produced from this transgenic strain may be suitable for improving lower quality flours (Rakszegi et al. 2005). A bread cereal (Cadenza) (*Triticum aestivum*) was transformed by the albumin gene encoding the 35 kDa AmA1 grain protein with a high level of essential amino acids, contained in a cockscomb species (*Amaranthus hypochondriacus*). The

Southern-blot analysis of the T1 line proved the integration of the alien gene while the Rt.-PCR and Western-blot analyses of the samples confirmed the transcription and translation of the same transgenes. The impact of the extra albumin protein on the attributes of the flour – ground from grains propagated from the T2 line – were identified by examining the total protein content, the essential amino acid content and the polymer/monomer and HMW/LMW ratios. The findings showed that not only the essential amino acid content may be increased but some parameters relating to the quality of the flour can also be improved by the expression of the AmA1 protein. (Tamás et al. 2009.)

Environmental aspects

Studies performed by Ragasits et al. (1996) have proved that different forms of nitrogen (ammonium nitrate, AN, urea (Formurin/FO) and paraffin coated urea (Paramid/PA) have an impact on nitrogen leaching and nitrogen absorption but they have no effect on yield or baking quality in the case of winter wheat, therefore their use may be justified more by environmental than by direct economic considerations. One of the most favourable attributes of slow-release N fertilisers is that they provide a steady nitrogen supply, while reducing the risk of N leaching. The application of FO and PA in the late autumn resulted in 38 % and 15 % lower N-minimum percent levels in the top 90 cm soil layer, respectively, than did the application of AN. The N-minimum level was not excessively high in this layer in the control plots. The differences between the nitrogen supply levels were reflected by wheat's nitrogen uptake as well. After AN or PA treatment the rate of nitrogen uptake was similar in both the stem growth and the flowering phases but when FO was applied, the N uptake slowly decreased during the life of the wheat plants. Nonetheless, the yields were not influenced by the form of N supply. The baking quality was not affected more favourably by the slow-release N fertilisers than by the application of AN. An amount of 160 kg/ha of N was required for reaching the maximum wet gluten content, Zeleny number and valorigraphic value. Neither FO, nor PA treatments resulted in significant differences in the above parameters in comparison to the AN treatment.

The application of N fertilisers is a costly technique for the farmer and at the same time the leaching of N, the consumption of fossil fuels during the production and delivery of the fertilisers as well as the N_2O emission from the soil during denitrification may also entail environmental impacts. The development of N-efficient cultures will provide farmers with economic benefits and helps reduce the environmental loads entailed by the application of excessive amounts of N fertilisers. It is concluded that (i) increased root length density (RLD), (ii) the stalk's high nitrogen absorbing capacity perhaps in combination with a high maximum N uptake ratio, (iii) the low N concentration of the leaf blade, (iv) more efficient N remobilisation capability from stalk to grain after flowering but reduced efficiency of N remobilisation from leaf blade to grain, both preferably together with delayed senescence and (v) the grain's lower N concentration may be particularly crucial in the efforts at attaining high nitrogen use efficiency (NUE) in the case of wheat varieties grown for livestock feed, and (vi) in the case of bread wheat varieties the high NUE may be accompanied by the highly efficient uptake and assimilation of N, with high efficiency in N remobilisation after flowering and/or special grain protein compositions (Foulkes et al., 2009).

Health implications

The processing industry and food allergy research are avidly interested in wheat ω-gliadins. The ω-5 sub-group is particularly important, owing to its role in the development wheat-dependent exercise-induced anaphylaxis (WDEIA) (Morita et al., 2003; Matsuo et al., 2005). This allergic response occurs when wheat was digested by the sensitive individual before physical exertion and the symptoms may be extremely acute – in some cases fatal (Palosuo et al., 2001; Morita et al., 2003). Similarly to the other gluten

proteins these also show genetic polymorphism. (Metakovsky, 1991; Denery-Papini et al.,2007). Accurate identification of the number of the wheat ω-gliadin proteins and genes is still to be worked on. Sabelli and Shewry (1991) applied the method of Southern blot analysis and found that bread wheat contains up to 15-18 ω-gliadin genes. A number of scientists have identified various ω-gliadin N-terminal by sequencing or electrophoresis or reverse phase HPLC (Kasarda et al., 1983; Masci et al., 1993, 1999; Dupont et al., 2000). Dupont et al. (2011) identified 7 ω-gliadins by 2-D gel electrophoresis and tandem mass spectrometry but they did not specify whether those were monomeric or polymeric. Wan et al. (2013b) identified two patterns of ω-gliadins in six wheat varieties, including the monomeric "gliadin" proteins and the subunits contained in the polymeric "glutenin" fraction. They concluded that the two groups of the six wheat varieties they studied contained at least seven and five ω-gliadin proteins, respectively. The polymeric type of the ω-5 gliadin (ω-5b) was found in three varieties (Hereward, Istabraq, Malacca), the polymeric form of the ω-2 gliadin (ω-2b) was identified in each of the six varieties. This indicates that the polymeric forms of the ω gliadins are widely present in modern wheat varieties and shows that continued studying of their roles in glutenin's polymeric structure and in the baking quality of the dough will be fully justifiable.

Moss et al., (1981); Wieser and Seilmeier (1998); Godfrey et al. (2010); Altenbach et al. (2011) proved that and the application of higher quantities of N fertilisers leads to an increase in the ratio of ω-gliadins. Raising the N dose within a plot drove the proportion of ω-5 gliadins also upwards. The increase in the proportion of ω-5 gliadins as a result of the application of more ample doses of N fertilisers is probably related to the fact that the N content of these proteins is higher than those of ω-1/2 gliadins.

The role of ω-gliadins in determining the baking value has not yet been clarified. The adding of purified protein to the flour may have either a positive (Khatar et al., 2002a, b) or a negative (Uthayakumaran et al., 2001; Fido et al., 1997) impact on baking quality, though these studies were limited to the monomeric fractions. The marked growth of the polymeric ω-5b gliadins may lead to an increase in the overall glutenin polymer content but it is not likely that they would result in improved quality because the polymeric ω-5 gliadins (that is, their free cysteine groups) may form disulphide bridges between chains therefore they may act as chain terminators reducing the lengths of the polymers (Gianibelli et al., 2002).

The increased accumulation of ω-gliadins and other gluten proteins in the subaleurone layer of the starch-containing endosperm is enabled by the influx of a large quantity of amino acids into these cells as a consequence of the ample N supply. The impact of the level of N supply on the spatial distribution and composition of the ω-gliadins in the wheat grain's endosperm shows that the composition of ω-gliadins in wheat and in foodstuffs can be controlled by plant improvement, agronomical solutions or industrial methods in order to optimise functional.

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