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RESEARCH REGARDING CEREAL AUTHENTICATION BY USING EFFICIENT METHODS OF ANALYSIS. A REVIEW

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Abstract

Cereals are found in many foods. Testing their authenticity is necessary to comply with the rules of labeling and to avoid unfair competition. To protect consumers, European Union (EU) law requires labeling of ingredients that cause allergies or intolerances, especially for cereals containing gluten, such as wheat (including common wheat, durum wheat, spelled wheat), rye, barley, oats or their hybridized strains and products thereof (OJEU, 2011; OJEU, 2014). Thus, the identification of cereals in a product is of paramount importance, not only to prevent the risks related to food safety in sensitive or allergic persons but also to avoid economic fraud.

***Key words**: authenticity, Triticum aestivum, PCR methods, molecular characterisation, spectroscopy.*

INTRODUCTION

Cereals are present in many food products. Authenticity testing it is necessary in order to comply to labelling norms and avoid unfair competition.

EU regulations impose mentions on products labels of ingredients that could induce allergies or intolerance reactions, especially for gluten presence in such as wheat (including common wheat, durum wheat, spelled wheat), rye, barley, oats or their hybridized strains and products thereof (OJEU, 2011; OJEU, 2014).

Thus, the identification of cereals in a product is of paramount importance, not only to prevent the risks related to food safety in sensitive or allergic persons but also to avoid economic fraud (James and Schmidt, 2004).

The authenticity of cereals has been based in recent years on high performance techniques analyzing either protein content or DNA (Hernandez et al., 2005; Tavoletti et al., 2009; Bottero and Dalmasso, 2011).

The use of Real time PCR with TaqMan samples is a good alternative when specific and sensitive detection of the smallest DNA fragments is required, such as in processed foods (Bottero and Dalmasso, 2011; Hernandez et al., 2005; Tavoletti et al., 2009).

The increased number of researches generated the need of systematic revues (Simpkims and Harrison, 1995). However new results make necessary new revues.

MATERIALS AND METHODS

The present article reviews some of the main researches in cereal authenticity, focused on wheat. There are considered genetic studies, different analysis technics, including spectrometry or PCR. The selection considered also a diachronic approach for PCR studies section, in order to suggest the evolution of researches focuses in the field.

RESULTS AND DISCUSSIONS

Luo et al. (2015), Li et al. (2016) used the method of element analyser-stable isotope ratio mass spectrometry, in order to discriminate the geographical origin of wheat, d13C and d15N values. Studies on magnetic field effect on cell differentiation on different wheat genotypes showed that differences between wheat genotypes and level of magnetic fields were significant (Kahrizi et al., 2013). Brescia et al. (2002) established that the isotopic signature can be used to develop reliable fingerprints for

regional determination. IR spectrometry was used in order to develop simple, rapid technology to determine the origin of products (González-Martín et al., 2014; Zhao et al., 2013), as well as to determine wheat species (Ziegler et al., 2016). Fuzzy chromatographic mass spectrometry proved to be efficient in discriminate between whole wheat and refined wheat flours (Geng et al., 2016). Koenig et al. (2015) used HPLC technics to classify spelt cultivars, from 'typical spelt' to 'similar to common wheat'.

Mass spectrometry, combined with polyacrylamide gel electrophoresis and two-dimensional gel electrophoresis were used for a proteomic study to characterize serpin polymorphisms along 177 Australian and 19 foreign hexaploid as well as 6 tetraploid wheat varieties (Wu et al., 2012).

Konieczny et al. (2005), studied the extracellular matrix surface network transformation during plant regeneration for wheat anther culture. Microscopic observations revealed two distinct types of cells on the callus surface, arranged in multicellular clusters.

Determination of multi-element composition of wheat proved to be effective in developing a fingerprint of geographical origin (Zhao et al., 2013).

Popping (2002), Pauli et al. (2014), Zörb et al. (2009) used chemical methods to determine authenticity of wheat, or adulterations, including for *Triticum aestivum* L.

Voorhuijzen et al. (2011), developed a padlock probe ligation and detection method, a DNA-based multiplex detection tool to determine traceability and authenticity for crop plant materials, wheat included.

Mass spectrometry proves to be an effective method in contaminants and food adulteration detection (Gharechahi et al., 2016).

Escherichia coli expression, Western blotting and tandem mass spectrometry were used to identify and confirm authenticity of two novel x-type HMW-GS from wheat line CNU608, as possibly originated from one octapeptide deletion and two unequal cross-over events (Wang et al., 2016; Liang et al., 2015).

Genetic studies

Wang et al., 2000, contributed to the first comprehensive analysis made of restriction fragment length ploimorphism of the mitochondrial (mt) DNA of *Triticum aestivum* L. This led to clarification of the nature of mtDNA variability.

Maat, 2001, offers an overall analysis on genetic researches and stakeholders cooperation in wheat breeding in Netherlands, as a best practice experience.

Genetic analysis of Russian wheat, the history of prebreeding studies and the genetic diversity evaluation is reviewed by Mitrifanova (2012), Novoselskaya-Dragovich et al. (2015).

Results of some new approaches, like "systems biology", "genome informatics" or "computational genome science" are concentrated on pre-mRNA splicing, organisation of transposable elements, identification of protein-coding genes and RNA genes were developed into tools by Brendel et al. (2004). Gremme et al. (2005) proposed a software predictive tool for gene structure in higher organisms.

The analysis of several storage protein loci, allow differentiating Asian and European *Triticum spelta* L. (Kozub et al., 2014).

Microsatellite markers and in situ hybridization are valuable techniques in molecular analysis of triticale lines with different vrn gene systems (Leonova et al., 2005), as well in confirming the authenticity of inter-varietal chromosome substitution lines of *Triticum aestivum* L. (Pestsova et al., 2000).

Schmidt et al. (2004), Kara et al. (2018), used microsatellite SSR markers in molecular characterization of *Triticum aestivum* L. genotype. The results demonstrate the utility of microsatellite markers for detecting polymorphism to estimate genetic diversity.

Similar researches were conducted by Ahmad et al. (2018), on studying molecular diversity of *Triticum aestivum* L. genotypes resistance to rice weevi (*Sitophilus oryzae* L.). Results indicated that microsatellite markers are able to acces genetic diversity among wheat genotypes for weevil resistance.

Genetic similarity studies with SSR markers were conducted on 43 wheat varieties to reveal genetic relationships in wheat varieties by Zhang et al. (2002). Studies revealed that genetic similarities should be based on data from all genomes, rather than any one genome.

Assessing genetic modification impact on allergenicity of wheat species concluded that

the differences observed between GM wheats and their parents are within the range of cultivated wheats (Lupi et al., 2014)

Song et al. (2002), determined the abundance of nine different trinucleotide microsatellites in the wheat genome, the repeat length distributions of

each and the rates at which they could be developed into informative markers.

The stress tolerance traits in wheat revealed that 11 important quantitative trait loci clusters located on chromosomes 1 BL, 1D, 2A, 2B, 4A, 6B and 7B (Zhang et al., 2014). Disease resistance studies showed that a wheat - L. mollis double substitution line DM96 could induce high resistance to stripe rust and Fusarium head blight (Zhao et al., 2013).

Influence of regional origin, harvest year and genotypes are significant in the fingerprints of the wheat kernels (Liu et al., 2015).

Korzun et al. (1997) showed the role of microsatellites and their markers as a tool in determination of wheat authenticity.

The combining ability and authentication of F_1 hybrids in *Triticum aestivum* L. using SSR markers revealed that LU_26S as best general combiner for plant height (Ahmet et al., 2012).

New progress was reported on utilization of Golden Ball (GB) wheat cultivar and Langdon - GB lines for genetic and genomic studies in tetraploid wheat and for improvement of stem solidness in both durum and bread wheat (Xu et al., 2014).

Genetic studies on heat stress for *Triticum aestivum* L. observed significant differences among the 19 genotypes considered (Pankjj et al., 2019).

Low-molecular-weight glutenin subunits encoded by Glu-3 complex loci in hexaploidy wheat, were found to impact the flour quality, as a comprehensive study revealed. Molecular characteristics and functional properties were conducted (Zhen et al., 2014).

Researches on chinese wheat *Triticum aestivum* L. landrace Banjiemang identified two novel HMW-GS genes, designated as 1Bx14* and 1Bx15*, novel allelic variations of HMW-GS at Glu-B1 locus, which were probably exploitable as new resources for quality improvement of *Triticum aestivum* L. (Shao et al., 2015).

Authentication of *Triticum aestivum* L. lines with specific rust resistance using molecular markers were done for yielding cultivars PBW343, UP2338 and WH542, used to incorporate multiple rust resistance genes from winter wheat or agronomical inferior wheat lines (Datta et al., 2008).

By using quantitative trait locus (QTL) detection techniques, Cui et al. (2012), found that though co-located QTL were universal, every trait owned its unique QTL and even two closely related traits were not excluded.

Chromosome sequencing techniques were used to reveal the partitioning correlated with meiotic recombination for 1-gigabase chromosome 3B of hexaploidy bread wheat. Comparative analyses indicated high wheat-specific inter and intrachromosomal gene duplication activities, source of variability, for increased adaptability (Choulet et al., 2014).

Kabir et al. (2015) also used two wheat populations in mapping QTL's associated with root traits. Root morphological parameters were measured for both populations. In total, 54 QTLs for roots traits were detected.

Bagherikia et al. (2014), studied translocation of chromosome arm 1RS (*Secale cereale*) to *Triticum aestivum* L. improvement. 1AL.1RS offering higher biotic and abiotic stress tolerance. Results 1AL.1RS confirmed "Sholeh" wheat cultivar as the only cultivar (1.5%) that carries 1AL.1RS, as a successful translocation process result.

The full-length cDNA sequence (1158 bp) encoding a ribosomal L5 protein, designated as TaL5, was firstly isolated from common wheat (*Triticum aestivum* L.) using the rapid amplification of cDNA ends method (RACE). Stress studies indicated that TaL5 gene was dramatically induced by salt, drought and freezing. These implied that TaL5 gene could preserve function in several stress conditions in whaet plants (Kang et al., 2012).

Molecular cloning techniques were applied to isolate the starch-branching enzymes isoform SBEIII cDNA sequence (3,780 bp) from common wheat (*Triticum aestivum* L.) using RACE. The SBE activity of the protein expressed in *Escherichia coli* (BL21) was measured and verified. During the wheat grain filling period, TASBEIII was constitutively expressed (Kang et al., 2013).

Molecular characterization using real-time PCR method

Adulteration studies on wheat variety content in traditional Italian pasta addressed identification of *Triticum aestivum* L. presence, as adulteration agent to *Triticum durum.* The PCR of some sequences of *T. aestivum* has been optimised using two sets of primers designed on *puroindoline b* gene. The analyses showed that this method works well also on high-temperature dried pasta (Arlorio et al., 2003). Similar interest showed the studies of Terzi et al. (2003). They proposed qualitative and quantitative PCR-based methods to detect hexaploid wheat adulteration in pasta.

PCR techniques were designed for phylogenetic analysis of 59 external transcribed spacers (ETS) region of the 18S ribosomal RNA genes for some species, including *Triticeae*. It was demonstrated that the complete ETS sequences of the *Triticeae* yeld coherent phylogenetic information (Sallares and Brown, 2004).

Mafra et al. (2008), revue on main novelties on animal products food authentication based on PCR methods. They emphasized on the method effectiveness in species authentication or detection of allergens and GMOs.

PCR was revealed as most efficient method for a rapid and specific wheat virus diagnostic tool that also has the potential for investigating the epidemiology of viral diseases, like dwarf viruses or mosaic viruses (Deb and Anderson, 2008).

The method was extended in detecting also vector leafhopper (*Psammotettix alienus* Dahlb.)*,* by Zhang et al. (2010).

A combination of STS markers and multiplex PCR techniques for Glu-A3 alleles in *Triticum aestivum* L. The markers and multiplex-PCR systems were validated on 141 CIMMYT wheat varieties and advanced lines with different Glu-A3 alleles, confirming that they can be efficiently used in marker-assisted breeding (Wang et al., 2010)

Specific detection and quantification of *Aspegillus flavus* and *Asperigillus parasiticus* in wheat flour was studied using two qPCR assays. Both assays could detect spore concentrations equal or higher than 106 spores/g in flour samples without prior incubation. The assays proved to be valuable tools to improve diagnosis at an early stagein all critical control pointa of food chain (Patiño et al., 2011).

Real-time PCR was tested in quantification of wheat contamination in gluten-free for for celiac patients. Values obtained were compared with those from R5 ELISA. They were similar for majority of tests; however real-time PCR showed a better sensitivity of the DNA for some samples. The method was proposed to be used also as a non-immunological tool to confirm the presence of wheat (Mujico et al., 2011).

One hundred and eighty-two bread wheat cultivars were characterised for low molecular weight glutenins using SDS-PAGE and allele-specific PCR. Data found greater consistency between SDS-PAGE and PCR amplification patterns for some of the alleles and less consistency for others. More studies are needed in order to achieve unambiguous identifications (Ram et al., 2011)

Amar et al. (2012) studied predictive and early detection of mycotoxigenic *Fusarium culmorum* in wheat. They used multiplex PCR to detect toxigenic agent with no need of prior DNA extraction. They concluded the method is a suitable strategy for high throughput screening of mycotoxigenic *Fusarium.*

PCR assay was used in confirming the presence of HMW-GS in the 29 genotypes of wheat. Differences between Arabian Australian and American varieties were identified (Ghazi et al., 2012).

Kutateladze et al. (2013), have developed methods of reliable and fast detection of maize (*Zea mays* L.*)* wheat (*Triticum aestivum* L.) and soybean (*Glycine max* L.). They used novel multiplex PCR techniques. New soybean and maize specific PCR-primers were developed, as well as a species-specific triplex PCR targeting maize invertase gene, soybean lectin gene and wheat low-molecular-weight glutenin subunit.

Authentication of wheat, barley, rye and oats in food and feed was studied using four TaqMan real-time PCR assays. Three specific primers were used. The system showed high specificity and sensitivity in experimental flour binary mixture. Method was further applied for 270 food and pet food products, proving to be a effective tool in authentication of foods with different labelling schemes, in which the presence of the targeted cereals was either

declared, not declared or declared as possible traces (Pegels et al., 2015).

Studies on Fusarium head blight caused by *Fusarium graminearum* were performed in order to discriminate quantitative resistance in barley and wheat genotypes. A pathogen inoculation and a quantitative PCR based protocol were reported.

The method proved to be effective and could be applied for medium to high throughput barley and wheat breeding programmes (Kumar et al., 2015).

Carloni et al. (2017) developed new PCR-related techniques to detect common wheat adulteration of durum wheat for pasta production. They demonstrated the limits of the method based on gliadin gene. A new molecular method, based on DNA extraction from semolina and real-time PCR determination of *Triticum aestivum* L. in *Triticum* spp., was validated.

The variation of high-molecular-weight glutenin subunits in wheat was studied, based on a combination of two techniques, PCR amplification and digestion with endonucleases. Data allowed detection of allelic variations that were not clearly by one technique alone (Wang et al., 2018).

Silleti et al. (2019), studied untargetet DNA-based methods for authentication of ancient wheat species and other cereals, present in modern food products, particularly pasta, bread and cookies. They used DNA fingerprinting through tubulin-based species and tested a series of commercial food products. The assay has a sensitivity of 0.5-1% w/w in binary detect possible adulterations.

CONCLUSIONS

There are a large variety of analytical methods in determination and authentication of cereals. Infrared techniques, mass spectrometry, chromatography and chemical determinations are however less studied compared to genetic methods, PCR particularly. The last years the scientists are more focussed in refining old techniques, overcoming their limits by developing new assays or by using complex mixture of techniques.

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