LAST FINDINGS IN MOLECULAR BASED PLANTS AUTHENTICITY – MINI-REVUE –

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Abstract
Products adulteration became one of the most damaging practices in modern economy. Food sector faces many threats, on every category, from diaries to meat products or panification. Along the time there were developed and applied many methods to determine authenticity of raw materials, recipes or nutritional factors. Genetic based methods proved to be most accurate, reliable and efficient. The present study continues to revue findings in vegetables authenticity determination. Bibliometrics data from 1980 are discussed. Results are also presented, focused on latest findings, in articles published in 2019 and 2020.

Key words: PCR (polymerase chain reaction), QTL (quantitative trait locus), grain authenticity, Triticum aestivum, adulteration

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INTRODUCTION

The food adulteration, plants included - wheat especially, became one of the most wide spread behaviour of these days (Ichim, 2019). The quest for lower costs and higher incomes generate a continuous effort to identify adulteration techniques and define unperceived limits (Primrose, 2019).

In response to these efforts, various researches are conducted to develop new methods of authentication, and the corresponding responsible bodies develop guidance and regulations (FDA - RSSC, 2019).

The DNA-based methods and PCR technics proved to be the most efficient and accurate analytical tools to provide quality and reliable food / plants authenticity information.

The complexity of the research determined various essays to systematize the results, on general level (Simpkins & Harrison, 1993; Mafra et al., 2008; Böhme et al., 2019). Other reviews covered the wheat authentication researches, in a specific area (Mitrofanova, 2012) or on global scale (Li et al., 2016; Dumitru et al., 2020). Some other significant crops are also subject of systematization process (Fan et al., 2020; He et al., 2020).

MATERIALS AND METHODS

The documentation reviewed over 300 articles related to products authentication and preventing adulteration, for a period of 25 years (1995 – 2020). We used different searching engines, Elsevier, Science Direct, Springer Link, Taylor & Francis, Willey, as well as independent sources. A new filter process concentrates the results for plant authentication, mainly to cereal and particularly on wheat studies. We retained 109 articles considered as best responding to these criteria.

The results were included in a bibliometric study, made by using the technical software facilities of SPSS program, in which we

identified authors, institutions involved, main countries sponsoring these studies and the time progression of these studies. Finally, we decided to detail the review focusing the latest findings, published in 2019 and 2020.

RESULTS AND DISCUSSION

Bibliometric results

Authors distribution

There are 495 authors involved in the studied articles, which indicates a wide scientifical interest. Out of these, 29 authors signed more than 3 presences, 8 of them co-authoring more than 4 articles. All these top authors are located in China (Fig.1).

The reviewed articles have 156 main or correspondent authors and other 444 co-authors (Figure 2a). The calculated mean authors' team size was 5.46 (Figure 2b).

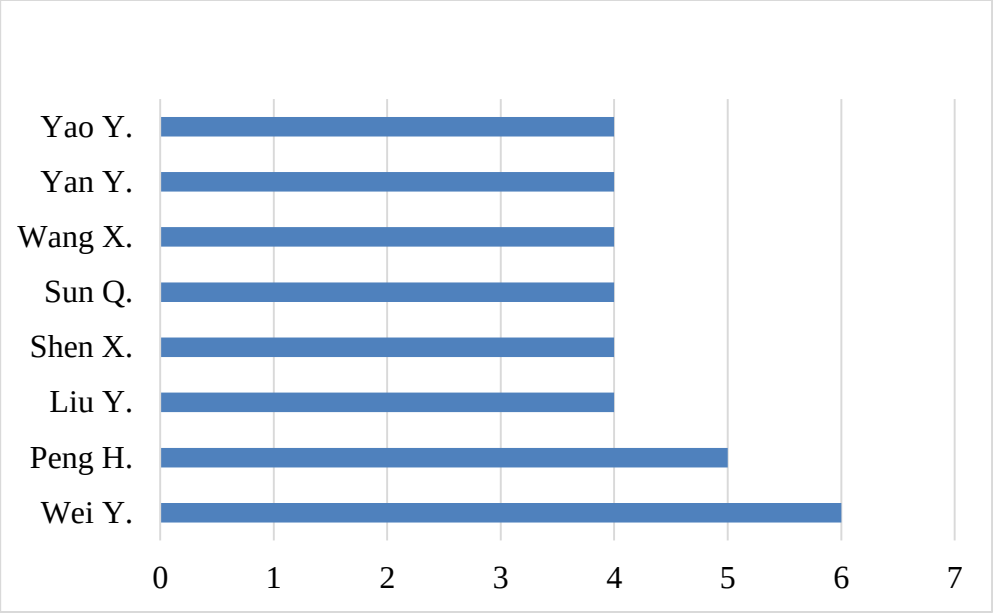
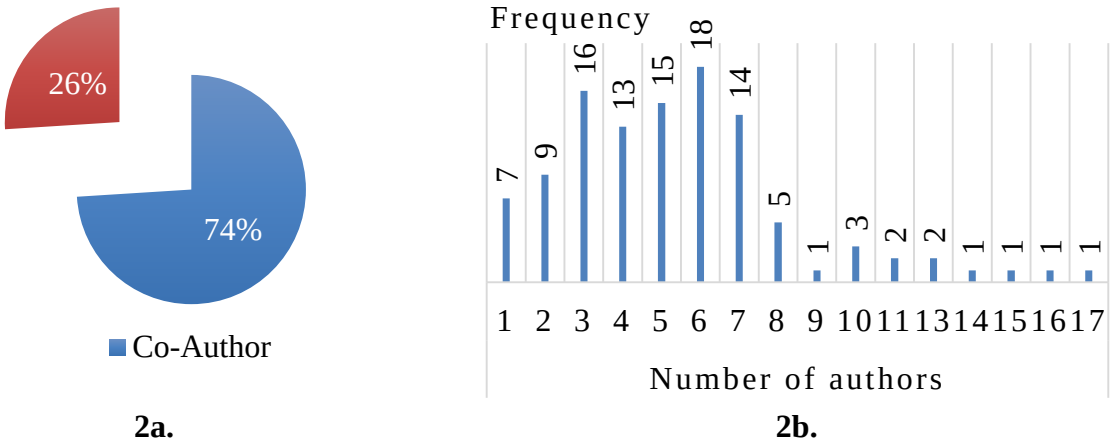


Figure 1. Top 8 of most prolific authors



2a. **2b.**

Figure 2. Authoring structure of studied publications: **2a.** Distribution main authors (first and corresponding) *versus* co-authors; **2b.** Authors' team sizes

Table 1. Top 10 institutions hosting wheat authenticity researches

#	Institute	Frequency
1	Institute of Cytology and Genetics, Novosibirsk	3
2	Center for Agricultural Resources Research, Institute of Genetics and Developmental Biology, Chinese Academy of Sciences, Hebei	2
3	College of Biological Sciences, China Agricultural University, Beijing	2
4	College of Life Science, Capital Normal University, Beijing	2
5	Hubei Collaborative Innovation Center for Grain Industry, Jingzhou	2
6	International Maize and Wheat Improvement Center (CIMMYT), Mexico	2
7	Plant Breeding Institute, University of Sydney	2
8	Proteomics Unit, Maimonides Institute for Biomedical Research (IMIBIC), E-14004 Córdoba	2
9	State Key Laboratory for Agrobiotechnology/Key Laboratory of Crop Heterosis and Utilization, Ministry of Education/Beijing Key Laboratory of Crop Genetic Improvement/College of Agronomy and Biotechnology, China Agricultural University, Beijing	2
10	Triticeae Research Institute, Sichuan Agricultural University, Wenjiang, Chengdu	2

The authors' affiliation indicates 203 institutions that hosted researches on wheat authenticity and adulteration prevention in the studied period. 10 of them are mentioned in 2 or more articles (Table 1).

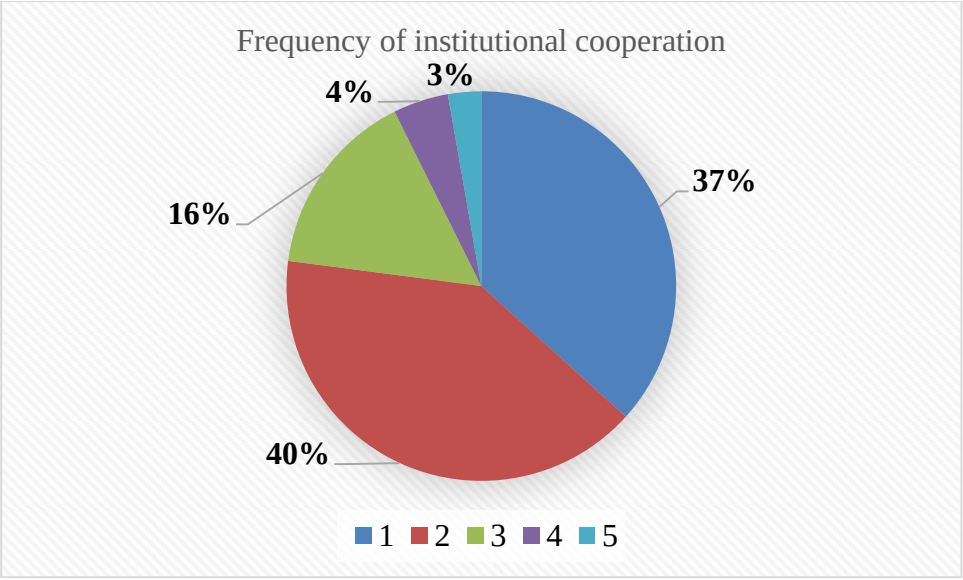


Figure 3. Articles *vs.* institutional cooperation structure (1, 2, 3, 4 or 5 institution/s that cooperated per article)

The institutional cooperative structure of the articles indicates a mean of 1.96 institutions (Figure 3).

The *geographical structure* of research institutions involved indicates a number of 33 countries, with a total of 192 presences. Out of these, 20 countries have at least 2 presences and the first six most present countries represent 20 % of the total (120 presences). China is significantly leading the top-ten countries (51 presences) in the studied period, as may be observed in Table 2.

The level of *international cooperation* reveals that 78.9 % of the published articles are results of national research efforts. There are 22 articles resulted from 2 countries consortiums and 1 from 3 countries consortium. The mean is 1.22 (Figure 4).

Table 2. Top ten most present countries in research activity related to wheat authentication subjects, in the studied period

Rank	*Country*	*Number of articles published*
1	China	51
2	Italy	19
3	India	14
4	USA	13
5	Spain	12
6	Australia	11
7	Germany	8
8	UK	8
9	Iran	6
10	Russia	6

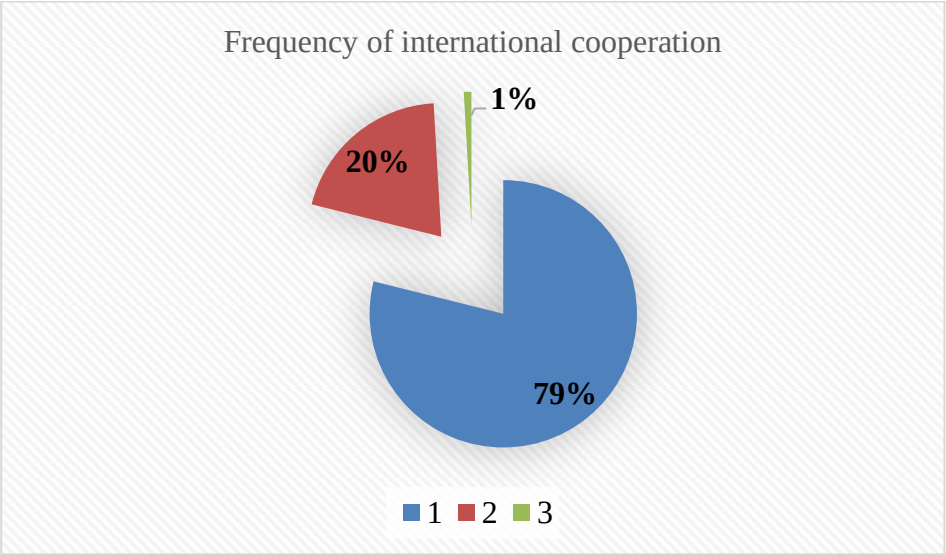


Figure 4. Articles international cooperation structure distribution (1, 2 or 3 countries involved in corresponding research team)

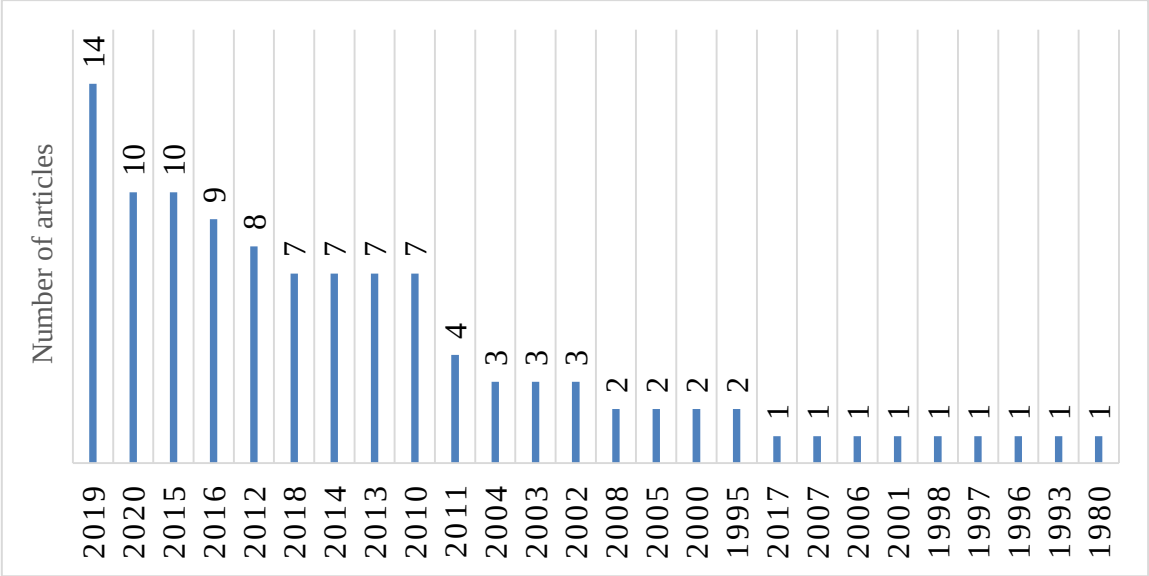


Figure 5. Articles distribution in time

Time distribution

The 109 selected articles were published between 1980 to 2020. From 2016 till mid 2020 were published almost 43 of these (39.45 %). The increasing trend was consistent all along this period, starting with 1 article in 1980 to 14 articles in 2019 and 10 articles in the first semester of 2020 (Figure 5).

The present article updates the field review contributions, focused on the latest articles, published in 2019 and 2020.

The globalization of the food chain market generates high level of resources therefore a high interest towards widespread adulteration actions (Ichim, 2019). Usually involve replacement of one valuable ingredient with a cheaper substitute, followed by misdescription of food: non-declaration or false declaration of processes, over-declaration of a quantitative ingredient or false claims (on geographical origin or production method) (Primrose, 2019). A major quest in preventing adulterations is to set an effective methodological frame of operations. FDA Foods Program Regulatory Science Steering Committee of the Food and Drug Administration developed a comprehensive validation of analytical methods, for rice four-way MAGIC population (FDA - RSSC, 2019). 97 % of the genome was covered, with a total of 5934 bins with an average length of 65 kb constructed. DNA based real-time PCR proves to be a valuable way to quantify virulent agents infecting different plants (Yepuri et al., 2020). The method demonstrates more versatility than protein methods and PCR can be used to screen the presence of several Living Modified Organisms (LMO) traits (Viljoen et al., 2019). Pankajj et al., 2019 determined that relatively stable genotypes may be evaluated at various climatic conditions, for heat tolerance in order to select the ideal plant type for specific agro-regions. Genetic methods provide useful insights in understanding the molecular mechanism of tolerance to salinity stress and develop tolerant genotypes of wheat (Mahajan et al., 2020). Qian et al., 2020 analyzed phylogenetic relationship of Methyl-CpG-binding domain (MBD) gene families from Arabidopsis, rice, wheat, and maize. All 14 MBD proteins in maize were categorized into four subclasses. They concluded that results offer a theoretical basis for future experimental research of epigenetic regulation in plants. Bitskinashvili et al., 2019 studied PCR-based detection methods to detect genetically modified (GM) maize. They found that the effectiveness of detections depends on combinations of parameters such as temperature and exposure duration, DNA extraction method, DNA marker and size or location of amplicons.

Genetical cluster analysis conducted on aromatic Indonezian rice cultivars. PCA and 2D scatter diagram indicate four major groups (Sholehah et al., 2019). Data concluded for a possibility of genetic proximity based of the physicochemical properties of Indonesian aromatic rice brought a useful information for the parental selection. He et al., 2020, developed an accurate quantification of *Colletotrichum camelliae* pathogen growth in tea. Mapping quantitative trait locus (QTL) in chromosome 4AL in *Triticum aestivum* was proved to maximize the grain yield for breeders (Guan et al., 2020; Tao et al., 2019). The physical position related to PCR-based markers was systematically characterized by Zhao et al., 2019. The BLAST analysis confirmed positions in accordance with those of nullisomic/aneuploidy/linkage analysis for 96.2 % markers. Results facilitated the integration of physical and genetic information of molecular markers. Wang et al., 2020 revealed the influence of nitrogen content fluctuation in alteration of thousands of genes expression which profoundly affect the root growth. They demonstrated that many genes are involved in N-response modulation through Alternative Splicing mechanism. Lin et al., 2020, identified and validate two stable grain filling rate QTL in wheat, that influence several traits related to yield. The results facilitate molecular-marker selection of wheat, with significant contribution to yield improvement. Similar study was conducted by Guan et al., 2019, to provide a basis for map-based cloning of the major QTL in thousand-grain weight in wheat. Zhou et al., 2020, identified 5399 lncRNAs transcripts in wheat, using bio-informational analyses. The data enable further investigation into the functions and roles of key candidate lncRNAs participation in different developmental processes of the plants. The PCR method proved to be more sensitive than DNA-based methods to detect possible gluten contamination in processes food. Ahmed et al., 2019 studied the detection limits for determinations DNA markers within mitochondrial DNA were of three primers, for wheat, barley and rye. All the three designed primers could detect the presence of 0.2 pg wheat, barley, and rye DNA, 10−5 (10 ppm) of wheat, barley, and rye DNA diluted with soya DNA, or 10−5 (10 ppm) of wheat flour mixed within corn flour. Similar higher specificity of q-PCR determination was demonstrated for pasta (Silleti et al., 2019). The assay had a sensitivity of 0.5–1 % (w/w) in binary mixtures of durum wheat in einkorn or emmer flour. Hu et al., 2019 used allelic-specific polymerase chain reaction (AS-PCR) method to clone the complete encodes of ten special high-molecular-weight glutenin subunits from *Aegilops longissima L*. They found that at least one genome with superior potential application values for improvement the quality of wheat bread.

CONCLUSION

PCR – based methods proved their superior sensitivity compared to other DNA-based methods. They are effective in preventing adulteration of processed food, as well in helping the yield improvement. The time distribution indicates an increasing trend of studies performed in this domain, their number increased in the last years, as resulted from the bibliometric analyze of the indicated timeframe. Although a widespread interest, recent years showed the Chinese leading role in researches on general plant issues, as well as on wheat area. The continue integration of the food market suggest that the scientifical effort will diversify along the entire food chain links, and for a large variety of products.

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