GENETIC CHARACTERIZATION OF ANCIENT *TRITICUM* FROM BENEVENTO

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Abstract – Corn or wheat (*Triticum* L., 1753), also said tritico (usually indicates whether the plant is the kernels of the plant), it is a genus of the family Poaceae (or "grass"), the tribe Triticeae. It is an old crop corn, the area is located between the Mediterranean Sea, the Black Sea and the Caspian Sea. Considering the variability of its morphological characteristics, we used molecular analysis as a necessary tool for the recognition of the *Triticum aestivum*. We investigate the genetic variability of the protein (Rht-B1) gene nucleotide sequences in this species, focusing our attention on samples collected in the area of Benevento (Italy). Molecular analysis allowed us to identify a genetic difference between the most common *Triticum eastivum* and ancient Triticum aestivum from Benevento, called "Saragolletta del Sannio".

Keywords: Triticum, Rht-B1 protein, ancient wheat.

1. INTRODUCTION

Triticum aestivum, common or bread wheat, is an annual grass in the Poaceae (grass family) native to the Mediterranean region and southwest Asia, which is one of several species of cultivated wheat, now grown in temperate climates worldwide. Wheat one of the top two cereal crops grown in the world for human consumption, along with rice (Oryza sativa). Wheat is one of the most ancient of domesticated crops, with archaeological evidence of the cultivation of various species in the Fertile Crescent dating back to 9,600 B.C.

Genetically, wheat is an allohexaploid species ($2n = 6x = 42$), which has a complex original and evolutionary history, derived from three diploid donor species through two naturally interspecific hybridization events. The initial hybridization event was occurred between A genome donor (T. urartu, AA; $2n = 14$) and B geome donor (Aegilops speltoides, SS; $2n = 14$) to produce the allotetraploid (AABB, T. turgidum L) about 0.2 MYa ago, and then the AABB donor crossed with the D genome donor (A. Tauschii Coss) to form the allohexaploid wheat (AABBDD) about 9000 years ago. As a result, wheat possesses a large and complex genome with three homologous genomes (A, B and D) and the size more than 17 Gb, which makes it a huge challenge to conduct genomic study in wheat [1].

Wheat is high in carbohydrates, protein (although it lacks several essential amino acids), and vitamins B and E (if the grain is left whole) is used in countless breads and baked goods, and is an important source of calories for over 1 billion people in the world. Wheat can be refined into starch and wheatgerm oil, and wheat gluten (the proteins that make it sticky) is used in many products. Wheat is also used to make beer and as animal fodder

2. SARAGOLLA WHEAT

The Saragolla is an ancestor of the modern durum wheat.

Originally, it grows in the lands between Anatolia and Iranian upland and between Egypt and Mesopotamia. For this reason it is also called the "pharaoh wheat". It has long stalk, up to 180cm. The kernel is longer than any other wheat. From the Middle Ages onwards numerous historical documents praise the quality of grains called "Saragolla"; the known varieties were different: the Gypsy, the Bulgarian, the Sannio Saragolletta.

The abandon of the cultivation of this grains starts at the end of '700, when population growth shift the focus to import productive durum wheat from North Africa and the Middle East, relegating the cultivation of Saragolla in small property in the hilly area of the Adriatic coast of central Italy. Here the best seaes were stored for sowing. The cultivation without chemical aid, has given to this grain, survived thanks to its power, unique features.

Compared to the other types of wheat the Saragolla (resistant to plant diseases, lends itself very well to the organic cultivation) contains from 20 to 40% more protein, and higher percentages of amino acids, vitamins and minerals among which a high content of selenium and beta carotene (excellent antioxidants) and less gluten content (but is not free and is therefore not suitable for celiacs). Its characteristics and its great digestibility (due to the simple structure of gluten, never hybridised) make it so a delicious food and is ideal for those who are intolerant to the common wheat products. Better to consume "Wheat Saragolla", Italian wheat, because it is a cereal km 0 and is to be preferred to one that comes from the other side of the world; because a native cereal, like any other product, helps the local area and the local farmer who regains biodiversity and an ancient tradition (Fig.1)

Fig1. Saragolla wheat

3. MATERIALS AND METHODS

3.1. Molecular analysis

Genomic DNA of *Triticum* was extracted with a DNeasy Plant Mini Kit (QIAGEN, Hilden, Germany) according to the manufacturer's instructions. All PCR amplifications were performed using 100 ng of template DNA in a final volume of 50 μL containing 25 μL of Hot Start Taq Master mix (Qiagen, Hilden, Germany), 1μL each of primers, (BF-GGTAGGGAGGCGAGAGGCGAG and MR-1 CATCCCCATGGCCATCTCGAGCTA)and 18 μL sterile water DNAse/RNAse (Qiagen, Hilden, Germany) free to adjust to the final volume.

The heat of reaction profile used is shown below:
-1 ° step of 15 min at 95 ° C,
-7 Cycles, drop 1 ° C: 30 sec at 94 ° C, 30 sec at 65 °C and 1:20 min at 72 ° C
-30 Cycles: 15 sec at 94 ° C, 15 sec at 58 ° C, 50 sec at 72°C a final extension step of 2 min at 72°C.

PCR products were checked on agarose gel through the QiaXcel (Qiagen). Fragment separation on QiaXcel system was performed according to the manufacturer's recommendations.The PCR products, purified by the QIAquick PCR Purification Kit (Qiagen). Each amplified product was sequenced, using the same primers cited above, with the BigDye Terminator Cycle Sequencing Kit (Applied Biosystems, USA). The sequence reaction was performed with a thermal cycler (Applied Biosystems 2700, USA) and provides 25 divided into cycles: 10 sec at 96 °C for DNA denaturation, 5sec at 50°C for the primer hybridization and 4 min at 60 ° C for the extension of the fragment. The sequenced products were purified using DyeEX 2.0 spin kit (Qiagen) and analysed on the ABI Prism 3500 automated sequencer (Applied Biosystems, USA).

the ABI PRISM 3500 Genetic Analyzer automated sequencer (Applied Biosystems). It has eight capillaries is based on a completely automated capillary electrophoresis process. DNA sequence analyses were performed with BLAST software available through the National Center for Biotechnology Information (NCBI). A BLAST search has allowed us to compare the sequence of interest with an already known sequence databases, and identify between those ones that have similarities to the sequence of interest. It was later an alignment performed with the bioinformatics program Bioedit.

4. RESULTS

The identification of *Triticum* can be performed with the direct optical microscope analysis of the sample, with serological tests for detection of antigens or antibodies and biochemical tests.

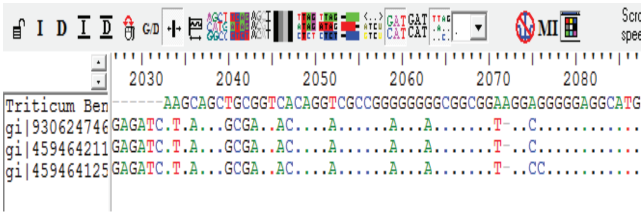
Today these techniques are no longer satisfactory, mainly for diagnostic level since techniques are laborious, little standardized and with very long execution times. In addition, in most cases, these techniques do not provide accurate results because they have a low specificity in the identification of the species [2].

The use of molecular techniques instead allows rapid and specific identification with a higher sensitivity such as PCR, Real-time PCR, PCR-Elisa, the RFLP (Restriction Fragment Polymorfism) etc. [3, 4, 5, 6]. We decided to identify Triticum of the Benevento area using PCR to amplify the region of the rth-b1 gene.

Rht-B1 and Rht-D1 encode DELLA proteins, which act to repress GA-responsive growth, and their mutant alleles Rht-B1b and Rht-D1b are thought to confer dwarfism by producing more active forms of these growth repressors.

The Rht-B1 and Rht-D1 genes were shown by Peng et al. (1999) to encode DELLA proteins, transcriptional regulators that act to repress GA signaling. Peng et al. (1999) found that Rht-B1b and Rht-D1b contain single nucleotide substitutions that introduce premature stop codons in the N-terminal coding region. [7].

Then, the obtained sequences were also compared with other *Triticum aestivum* sequences in GenBank using the BLAST (Basic Local Alignment Search Tool) of the NCBI (National Center for Biotechnology Information, USA) for the fragment identification. The alignment of the sequences was performed using the bioinformatics program bioiedit. The analysis of sequences showed that the genetic sequence of Triticum of Benevento is different, for some nucleotides, compared to the genetic sequences of other Triticum, Relating to (Rht-B1) gene. (Fig. 2 and Fig. 3).



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            2030      2040      2050      2060      2070      2080
Triticum Ben------AAGCAGCTGCGGTCACAGGTCGCCGGGGGGGGCGGCGGAAGGAGGGGGAGGCATG
gi|930624746GAGATC.T.A...GCGA..AC....A......A...A.......T-..C............
gi|459464211GAGATC.T.A...GCGA..AC....A......A...A.......T-..C............
gi|459464125GAGATC.T.A...GCGA..AC....A......A...A.......T-..CC...........
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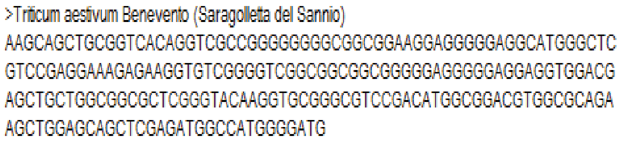
Fig. 2. Alignment (Bioedit software) of Benevento area (Saragolletta del Sannio) ,sequence with other sequences deposited in GenBank



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>Triticum aestivum Benevento (Saragolletta del Sannio)
AAGCAGCTGCGGTCACAGGTCGCCGGGGGGGGCGGCGGAAGGAGGGGGAGGCATGGGCTC
GTCCGAGGAAAGAGAAGGTGTCGGGGTCGGCGGCGGCGGGGGAGGGGGAGGAGGTGGACG
AGCTGCTGGCGGCGCTCGGGTACAAGGTGCGGGCGTCCGACATGGCGGACGTGGCGCAGA
AGCTGGAGCAGCTCGAGATGGCCATGGGGATG
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Fig. 3. Genetic sequence ancient *Triticum* or “Saragolletta del Sannio”.

5. DISCUSSION

Genetic analysis of *Triticum* sequences has clearly shown that the genetic sequences of ancient *Triticum* of Benevento area, also said Saragolletta del Sannio, is different in genetic sequence on the analyzed region, to *Triticum aestivum* samples in GeneBank. For this purpose, the sequences obtained were aligned with the sequences with GenBank accession numbers KT013263.1,KC614605.1,JX993616.1. So, the alignments performed with BioEdit, showed that the genetic sequence of *Triticum aestivum* ancient of Benevento is different, for some nucleotides, compared to other genetic sequences of *Triticum aestivum* deposited in GeneBank. Therefore the aim was to verify the genetic difference, setting the stage for subsequent studies

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