DNA ANALYSIS OF ROMANIAN WHEAT CULTIVARS FROM THE VEGETAL GENETIC RESOURCES BANK SUCEAVA

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Abstract

Development of improved technology, new high-yielding varieties, mechanization, enhanced use of chemical and fertilizers led to increased wheat yields, but this development adversely affected agroecology and labor demands in agriculture. Wheat (*Triticum aestivum* L.) is the most important crop plants, with a significant dietary importance, all due to the high carbohydrate and protein content of the grain and the ratio between them, which satisfies the requirements of human nutrition. Also, it is one of the ancient crops of agronomic importance, having been taken into cultivation by man about 10-12 thousand years B.C. In Romania, wheat cultivation dates back more than 2500 years. On the shores of the Black Sea, on the sites of ancient cities, coins have been found with the image of the goddess Demeter inscribed on one side and wheat ears on the other. The objectives of the research paper are the DNA-based genetic analysis of wheat (*Triticum aestivum* L.) and einkorn (*Triticum monococcum*) cultivars belonging to different subspecies or varieties. This study used 50 wheat genotypes, containing local populations, varieties and breeding material provided by the Vegetal Genetic Resources Bank "Mihai Cristea" Suceava.

Key words: (wheat, DNA, genotypes)

Bread wheat (*Triticum aestivum* L.) has a long history, cultivated 10-12 thousand years B.C, being considered one of the ancient crops of agronomic importance. Wheat, in Romania, was first cultivated approximately 2500 years ago, as shown by archeological discoveries on ancient sites across the South parts of Romania. Worldwide, wheat provides 20% of the food needed by the human population. Increasing the productivity of this crop is a global task of great importance. The demand for this plant is growing faster than its productivity, but it must be produced without harming the environment. Plant breeding, in combination with advanced production systems with increased efficiency, can be applied to meet population demands by 2050 (Crespo-Herrera L. A. *et al*, 2017).

Cultivated wheat varieties and their close wild relatives belong to the genus *Triticum* L., a member of the tribe *Triticeae*, which contains around 300 species (Clayton W. D., Renvoize S. A., 1986). The genus *Triticum* consists of six species: *Triticum monococcum* L. (AA genome); *Triticum urartu* Tumanian ex Gandilyan (AA genome); *Triticum turgidum* L. (AABB genome); *Triticum timopheevii* (Zhuk.) Zhuk. (AAGG genome); *Triticum aestivum* L. (AABBDD genome); and *Triticum zhukovskyi* Menabde & Ericz. (AAAAGG genome). All *Triticum* species are native to the „Fertile Crescent" of the Near East. Common wheat is composed of three genomes (2n = 6x = 42; AA, BB and DD) from three diploid ancestors. The donors of genomes A and D are relatives of *Triticum urartu* Tumanian ex Gandilyan and *Aegilops tauschii* Coss., respectively. The origin of the B genome is not completely known, but some research points to *Aegilops speltoides* Tausch as a relative of the donor. Hexaploid wheat (AABBDD) originates from the cross of *T. turgidum* L. (AABB) and *A. tauschii* (DD) (Matsuoka Y, 2011).

Wheat seeds have a high content of carbohydrates (62-75% of the mass of the grain), of which starch has a weight of over 90%, the rest being dextrins and other simple carbohydrates. Carbohydrates are stored, mainly, in the central level of the caryopsis. Proteins measure a percentage of 10-16% of the weight of the wheat grain, and its qualitative characteristics are given by the amount and composition of the proteins (Roman G. V. *et al*, 2012)

The main objectives pursued in wheat breeding have been similar over many decades. The priority was to increase the production potential of this species in order to satisfy the food requirements of the population that is constantly

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growing (Borlaug N. E., 2007). The second most important goal is disease resistance, and the third is considered to be tolerance to abiotic stress factors, especially drought and high temperatures (Reynolds M. P., Borlaug N. E., 2006).

Mutagenesis (physical or chemical) has been used to increase the genetic variability of many cultivated plants, including wheat. The polyploid nature of wheat exhibits a buffering effect, where mutations in a genome can be masked by certain genes, making them difficult to detect (Parry M. A. J. et al, 2009).

From 1960 until 2017, 256 wheat varieties were obtained by this method and are registered in the FAO database (https://mvd.iaea.org). Among the many induced mutations of agronomic importance, resistance to various herbicides and increased amylose content are just few examples (Pozniak C. J., Hucl P. J., 2004; Slade A. J. *et al*, 2012). Genetic transformation and the cultivation of transgenic wheat are still highly debated topics in our society, not being unanimously accepted in all states, for social or religious reasons. However, good results were obtained, genotypes with resistance to wheat powdery mildew (*Erysiphe graminis*) and some physiological races of *Fusarium* spp. Also, resistance to drought, low temperatures and tolerance to soil salinity have been increased and some traits of the ripening process have been improved (Venske E. et al, 2019).

Among the most recent and promising innovations in biotechnology and plant breeding is gene editing. This technique can identify with high precision genome segments to be edited, by removing a fragment and inserting the one with genes of interest, or by replacing nucleotides. Despite the great complexity of the wheat genome, given its size and polyploidy, several attempts at genetic editing have been successful. A specific protocol was established for this species using the CRISPR/CAS9 system (Zhang Y. et al, 2016).

In this article we analysed the DNA-based genetic content of wheat (*Triticum aestivum* L.) and einkorn (*Triticum monococcum*) cultivars belonging to different subspecies or varieties. In total we used 50 wheat genotypes, containing local populations, varieties and breeding material provided by the Vegetal Genetic Resources Bank "Mihai Cristea" Suceava.

MATERIAL AND METHOD

The research material consisted of 50 wheat genotypes (varieties and local populations), from a total of 352 genotypes provided by the Vegetal Genetic Resources Bank "Mihai Cristea" Suceava. The researched seed material was divided into bags with 50 seeds for each wheat genotype, labeled with the genus, species, variety and germination percentage.

From each genotype, around 3 – 6 seeds (depending on the germination percentage provided by the Genebank) were sown in alveolar pallets with 54 holes (*figure 1*). Each cultivar was sown in 3 holes, thus, there were 18 wheat genotypes in each palette. The sowing activity was carried out in the greenhouse of the plant breeding department of JLU Giessen, and the alveolar pallets were kept in greenhouse conditions until the moment when the plants needed to be subjected to vernalization.

Figure 1 **Alveolar palette and the material for sowing**

Cultivars marked with the ID number *300* are represented by local populations, and those marked with the number *500* are represented by varieties. For the development of the study, 10 local populations of *Triticum monococcum*, 20 varieties and 20 local populations of *Triticum aestivum* L. were chosen.

Each sample was represented by one or two young wheat leaves that I inserted into Eppendorf tubes, along with two glass beads that help to break up the plant tissue.

The grinding of the plant tissue in the tubes was done using the QIAGEN TissueLyser II machine (*figure 2*).

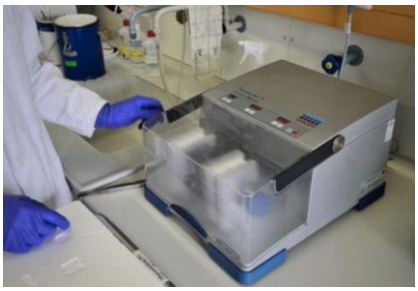

Figure 2 **QIAGEN TissueLyser II machine**

Before placing in the special machine stand, the Eppendorf tubes were left in liquid nitrogen for freezing (*figure 3*). After this step, the tubes were inserted with the help of tweezers into the supports of the machine, they were clamped between 2 special plates on one side and the other, and then fixed on the grinding machine. The machine was set to work with a vibration frequency of 30 Hz for 30 seconds for each cycle.

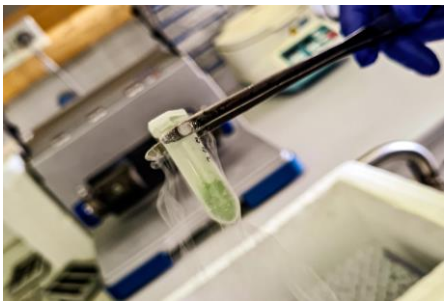

Figure 3 **Sample frozen in liquid nitrogen**

DNA extraction and purification was done with the QIAGEN BioSprint 96 automated DNA extraction robot (*figure 4*). 7 plates with 96 holes each were prepared. For each individual plate, the number of holes to be filled with buffer solution must correspond to the number of samples to be processed.

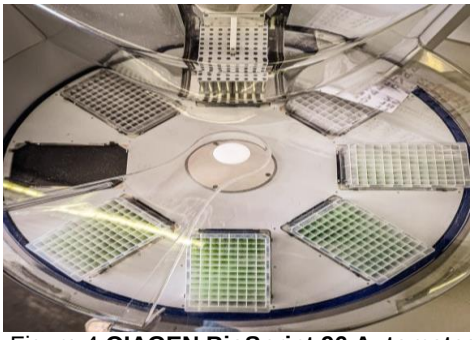

Figure 4 **QIAGEN BioSprint 96 Automated DNA Extraction Robot**

The working principle and procedure for the BioSprint 96 DNA extraction kit uses MagAttract technology (magnetic particles) to purify DNA from plants. The working material with which the extraction begins can be fresh, frozen or lyophilized and must be mechanically ground beforehand to obtain a very fine powder. The solution responsible for cell lysis is added to the obtained powder, mixed, and then sedimented through a short centrifugation step. The lysed material is transferred to an S-Block for DNA extraction with BioSprint 96. The DNA fragments, in the presence of a chaotropic salt, attach to the surface of the MagAttract magnetic particles. The DNA attached to the magnetic particles is effectively washed with ethanol-based buffer solutions, and then quickly rinsed with distilled water, a process that has the purpose of increasing its purity. DNA obtained and of high quality is eluted in water or in a solution with a low salt content. The amount of DNA obtained following the extraction process with QIAGEN BioSprint 96 depends on the type of sample taken for analysis, the method of sampling and the method of tissue destruction.

In the first step, transfer 200 µl of purified plant lysate to each hole of an S-Block, add 200 µl of isopropanol (99% purity) to each sample in the S-Block, then add 20 µl of MagAttract Suspension G (suspension of magnetic powder) of each sample in the plate. Before to adding the magnetic suspension to the S-Block, vortexing is required for 3 minutes for the first use, and 1 minute for subsequent uses.

In the next step of the DNA extraction protocol, turn on the BioSprint 96 automated DNA extraction robot, open the protective cover and select the working protocol for DNA extraction from plant tissue, then press the Start key to start the protocol run. The screen will show a message asking to load the S-Block boards.

After the previously described step, it is checked that the protective cover is closed correctly to prevent contamination of the samples and the Start button is pressed to start the actual processing. At the end of the extraction protocol, the plates will be removed according to the instructions on the device display. The first plate to be extracted from the machine contains the samples with purified DNA. After removing all plates, press the Stop key.

The DNA concentration in the samples was determined with the Qubit 2.0 Fluorometer (*figure 5*).

Figure 5 **The Qubit 2.0 Fluorometer**

To obtain favorable results, it is recommended to store the dye and other solutions at room temperature.

For fluorometer calibration, two tubes are used, and for analysis, one tube for each sample. Prepare working solution by diluting Qubit reagent 1:200 in Qubit buffer. For each individual sample, prepare 200 µl of working solution. The 200 µl of solution from each sample to be analyzed consisted of 198 µl of Qubit buffer solution and 2 µl of DNA solution. For determinations, use only clear, thin-walled PCR tubes with a volume of 0.5 ml. Before analysis, the tubes will be vortexed for 2-3 seconds and then kept for 2 minutes at room temperature. The next step involves inserting each tube into the fluorometer and reading the measurements, and through the computer feature, the concentration of the sample can be determined.

RESULTS AND DISCUSSIONS

The DNA content of the analyzed samples was quantified using the QUBIT 2.0 fluorometer. The 50 wheat cultivars that are the object of the present study, recorded the following results regarding the DNA concentration (*table 1*).

Table 1

The results obtained after the DNA quantification

Genus	Species	Subspecies	Code	Type of cultivated variety	DNA concentration (ng/µl)
Triticum	*monococcum*		SVGB-9458	300	241
Triticum	*monococcum*		SVGB-9462	300	248
Triticum	*monococcum*		SVGB-9464	300	243
Triticum	*monococcum*		SVGB-9466	300	355
Triticum	*monococcum*		SVGB-9473	300	396
Triticum	*monococcum*		SVGB-9485	300	254
Triticum	*monococcum*		SVGB-9487	300	443
Triticum	*monococcum*		SVGB-9511	300	286
Triticum	*monococcum*		SVGB-9515	300	263
Triticum	*monococcum*		SVGB-9521	300	121
Triticum	*aestivum*		SVGB-10196	300	291
Triticum	*aestivum*		SVGB-11381	300	291
Triticum	*aestivum*		SVGB-11413	300	225
Triticum	*aestivum*		SVGB-12187	300	149
Triticum	*aestivum*		SVGB-12217	300	259
Triticum	*aestivum*		SVGB-12570	300	328
Triticum	*aestivum*		SVGB-12579	300	260
Triticum	*aestivum*		SVGB-12605	300	257
Triticum	*aestivum*		SVGB-12614	300	218
Triticum	*aestivum*		SVGB-12649	300	255
Triticum	*aestivum*		SVGB-12653	300	389
Triticum	*aestivum*		SVGB-12675	300	235
Triticum	*aestivum*		SVGB-12693	300	341
Triticum	*aestivum*		SVGB-12694	300	253
Triticum	*aestivum*		SVGB-12816	300	327
Triticum	*aestivum*		SVGB-15122	300	290
Triticum	*aestivum*		SVGB-15204	300	193
Triticum	*aestivum*		SVGB-4239	300	182
Triticum	*aestivum*		SVGB-5262	300	334
Triticum	*aestivum*		SVGB-5642	300	170
Triticum	*aestivum*		SVGB-10168	500	210
Triticum	*aestivum*		SVGB-11382	500	257
Triticum	*aestivum*	*subsp. aestivum*	SVGB-11401	500	232
Triticum	*aestivum*		SVGB-11434	500	266
Triticum	*aestivum*		SVGB-12571	500	382
Triticum	*aestivum*	*subsp. aestivum*	SVGB-12586	500	261
Triticum	*aestivum*		SVGB-12594	500	308
Triticum	*aestivum*		SVGB-12615	500	433
Triticum	*aestivum*		SVGB-12658	500	400
Triticum	*aestivum*	*subsp. aestivum*	SVGB-12672	500	368
Triticum	*aestivum*		SVGB-12778	500	435
Triticum	*aestivum*	*subsp. aestivum*	SVGB-12808	500	350
Triticum	*aestivum*	*subsp. aestivum*	SVGB-12823	500	358
Triticum	*aestivum*	*subsp. aestivum*	SVGB-12844	500	443
Triticum	*aestivum*		SVGB-15203	500	278
Triticum	*aestivum*		SVGB-4144	500	277
Triticum	*aestivum*	*subsp. aestivum*	SVGB-5056	500	273
Triticum	*aestivum*		SVGB-5258	500	317
Triticum	*aestivum*		SVGB-5950	500	360
Triticum	*aestivum*	*subsp. aestivum*	SVGB-8117	500	127

The International Maize and Wheat Improvement Center's (CIMMYT) germplasm bank is one of the largest providers of wheat and maize germplasm in the world, distributing around 20,000 wheat seed samples to around 100 countries per year. An initiative of an organization (Seeds of Discovery), which aims to facilitate the efficient use of genetic diversity in wheat and other plants of agronomic importance, has characterized 80,000 genotypes from two of the largest wheat germplasm banks in the world: the first CIMMYT, which holds 140,812 genotypes and the second International Center for Agricultural Research in Dry Areas (ICARDA), with 43,924 wheat genotypes. Genotypic information obtained from the material of these Gene banks has been used for a wide range of applications in wheat breeding (Sansaloni C. et al, 2020).

An extensive study carried out for the genetic analysis of wheat had as research material

79,191 wheat genotypes, of which 50,053 from CIMMYT and 29,138 from the ICARDA Genebank. The material included local populations, elite breeding lines, cultivars, synthetic varieties and wheat wild relatives. Five seeds of each genotype were randomly selected and grown for 2 weeks in CIMMYT greenhouses in Mexico. Young leaves were harvested from a single plant for each genotype, then frozen at -80°C and lyophilized for 24 h. To track and organize such a large number of samples, it was used CIMMYT's DNA Sample Database, a purpose-built platform for tracking samples from the seed stage to DNA. Genomic DNA was extracted in 96-well plates from lyophilized leaves using a modified method with cetyltrimethyl-ammonium bromide. DNA purity and concentration were determined by electrophoresis on 1% agarose gel.

Similar with the results from the CIMMYT experiments, our values of the content of DNA extracted from each sample, were quantified with the QUBIT 2.0 fluorometer and ranged between 121 – 443 ng/µl. The lowest value, as well as the highest, was recorded in a cultivated variety of *Triticum monococcum*, local population (121 ng/µl and 443 ng/µl). Similarly, high values were observed for a cultivated variety of *Triticum aestivum subsp. aestivum.*

Wheat genotypes from the local population category, recorded relatively uniform values, ranging between 149 ng/µl and 389 ng/µl.

The genotypes of *Triticum aestivum* and *Triticum aestivum subsp. aestivum* recorded higher values than those presented previously, except for one (SVGB-8117 - 127 ng/µl). Four varieties of common wheat obtained DNA concentration values above 400 ng/µl (SVGB-12658 – 400 ng/µl; SVGB-12615 – 433 ng/µl; SVGB-12778 – 435 ng/µl; SVGB-12844 – 443 ng/µl) (*figure 6*).

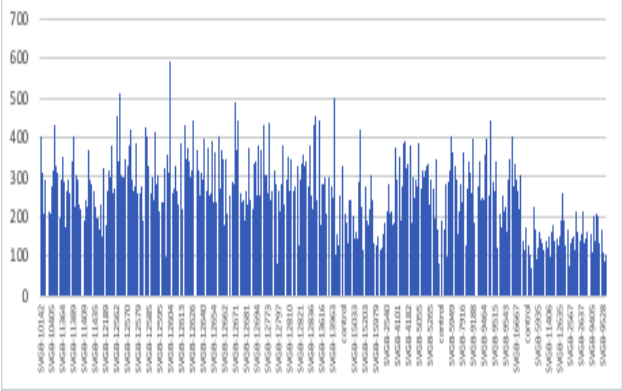

Figure 6. **Variation in DNA concentrations**

CONCLUSIONS

Wheat breeding has an important role in both human and animal nutrition, with an increased demand for new varieties or hybrids with superior yields, nutritional quality and high tolerance to biotic and abiotic stress factors.

Genomic analysis of wheat is essential for understanding the genetic mechanisms underlying evolution and allopolyploidy, as well as the biology of agronomically important traits influencing yields. Even thought, wheat has a complex genome and a high degree of polyploidy, researchers have devised innovative strategies for detailed structural and functional analysis of the wheat genome (Faris J. D. et al, 2002).

In our studies, DNA concentrations observed for 50 different wheat cultivars, ranged between 121 – 443 ng/µl. DNA extraction is of particular importance in genetics research, being the first step in the development of molecular genetics studies. It is necessary to extract high purity DNA from various samples with concentration higher than 50 ng/µl for advanced genomic studies such as sequencing, genotyping and genetic mapping.

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