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**Drd. Eng. ALECU (SCUTELNICU) ANCA**

**ASSESSMENT OF GENETIC VARIABILITY OF**  
***CASTANEA SATIVA* BIODIVERSITY AND ITS**  
**PRESERVATION**

**(SUMMARY OF DOCTORAL THESIS)**

**Scientific Coordinator**

**Prof. Univ.PhD. Mihai BOTU**

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# **„Assessment of genetic variability of *Castanea sativa* biodiversity and its preservation"**

**(Summary)**

**Key words:** comestible chestnut, biodiversity, preservation

## **Paper scope:**

**Identification, assessment and transition to preservation of valuable biotypes of comestible chestnut from the semi-spontaneous flora located in the Northern area of Oltenia.**

## **Objectives of the paper:**

- Analysis of the geomorphological, orographic and climatic characteristics of the Northern area of Oltenia, comprising Valcea and Gorj counties;
- Identification of biotic and abiotic factors involved in the degradation of the situation of comestible chestnut in the Northern area of Oltenia;
- Selection and assessment of chestnut biotypes based on certain morphological and agro-productive characteristics;
- Identification of existing relationships between processes of growth and fruition and ecological conditions for comestible chestnut biotypes;
- Assessment of manifestation of main physiological elements at the chestnut biotypes studied in Northern area of Oltenia;
- Determination of certain correlation and regression between physiological indicators at the comestible chestnut biotypes selected in Northern area of Oltenia.

## **Biological material**

The biological material studied consists of comestible chestnut biotypes from Northern area of Oltenia. The biological material used in the present work paper is derived from adjacent areas of following locations: Dăești, Călimănești, Bistrita and Horezu (Valcea County) and Polovragi (Gorj). There were studied a total of 75 biotypes of comestible chestnut, with ages comprised between 15 and 350 years from semi-cultivated flora.

### **Research methods:**

The present doctoral paper includes a series of approaches and methods specific to the objectives pursued and relate to:

- Analysis of the geographical and ecological areas where researches were performed;
- Identification of geographical position of comestible chestnut selections using a GPS type device;
- Assessment of growth and fruiting processed at chestnut selections studied based on biometrics data;
- Analysis of phenology specific to stages of vegetation and fruiting;
- Quantitative and qualitative of fruits production registered at the selections of comestible chestnut selections;
- Utilization of certain methods of biostatic analysis, adequate to this type of study (arithmetic mean, variance, standard deviation, variation coefficient, correlations and regression analysis).

### **Results of own research:**

Comestible chestnut (*Castanea sativa* Mill.) is a species in visible regress during the last three decades, both in Romania and in Europe, especially due to the attack of diseases such as cancer of bark (caused by *Cryphonectria parasitica*). Since this very dangerous and fatal disease for the very old specimens was identified in all the areas in Romania where chestnut is found, the chestnut biodiversity is seriously affected.

In the present paper, a series of valuable biotypes of chestnut located in a perimeter comprised between 45°12'14" (Dăești locality) and 45°11'30" (Polovragi locality), Northern latitude and 24°23'07"(Dăești) and 23°47'30"(Polovragi) East latitude were analysed and assessed.

The identified genotypes are natural hybrids on their own roots, located at altitudes comprises between 261 m (Dăești) and 624 m (Polovragi).

#### **a) Geographical, orographic and ecological framework within which research was conducted**

The Northern area of Oltenia comprises Sub-Carpathian hills separated by narrow meadows, crossed by numerous rivers. Sub-Carpathian area of Oltenia presents various types of soil, arranged in

mosaic, thin, with a high contents of clay (sometimes more than 60%), with low drainage, low fertility and a pH between 5.0 and 8.0.

The areas where comestible chestnut grow are still disposed on deep soils, with a PH comprised between 5.5 and 6.5.

In Northern Oltenia, the climate is temperate continental with Mediterranean influences. The average annual temperature at the locations of study (during 2012-2014) was 12.0<sup>0</sup>C, at the meteorological station in Rm. Valcea and 10.2<sup>0</sup>C at the meteorological station in Polovragi. The average rainfall amount of during the period of study was 725.2 mm, respectively 921.4 mm. The absolute minimum temperature recorded in the period 2012 - 2015 was -15.8<sup>0</sup>C at the meteorological station in Rm. Valcea (01.01.2015) and -24.0<sup>0</sup>C at the meteorological station in Polovragi (24.01.2015) and the maximum reached 40.4<sup>0</sup>C (06.08. 2012), respectively 36.6<sup>0</sup>C (22.08.2015).

Flora and fauna are very rich in this area, being specific to deciduous forests, with an amplitude altitude of 200 to 1700 m. Chestnut is located at altitudes between 200 and 600 m.

#### **b. Identification and localization of genetic resources of comestible chestnut**

In Northern Oltenia, the comestible chestnut may be found in semi-spontaneous and cultivated state, usually around the monasteries (In Oltenia north chestnut is able semi-spontaneous and cultivated, usually around the monasteries (Tismana, Polovragi, Horezu, Bistrița, Frăsinei, Turnu, etc.), therefore it is considered that this species had been introduced in the area by the monks that came from Mount Athos.

Among chestnut biotypes that exist around localities of settlements around Dăești, Călimănești, Bistrița and Horezu (Vâlcea County) and Polovragi (Gorj County), 75 genotypes were identified and selected. The geographical coordinates of each genotype were recorded using a GPS type device.

#### **c. Selection and assessment of comestible chestnut biotypes, based on morphological and agro-productive characteristics**

##### **Growth characteristics of comestible chestnut biotypes**

Comestible chestnut selections from Northern area of Oltenia presented differentiations in the growth process, according to the age of trees and their location.

At chestnut selections from Dăești area (Vâlcea County), trunk circumference ranged between 2.00 m at biotype D 1013 and 3.60 m at biotype D 1008. Values of sectional surface of the trunk

(SST) are comprised between 3185 cm<sup>2</sup> and 10 318 cm<sup>2</sup>. Diameter of tree crown oscillated between 7.50 m at selection D 1005 and 16,0 m at D 1011, 92.3% out of the genotyped having crown diameter higher than de 10 m. Tree height is comprised between 11 m (D 1013, D 1009, D 1008, D 1006, D 1005) and 15 m (D 1001, D 1003). The growth type is semi-spread at 7 selections (53.85%), 5 selections have spread port (38. 46%) and a single specimen has erect port (D 1006).

According to the summed up points for the four elements of growth, in Dăești area were obtained values comprised between 522.9 points (D 1010) and 1106.1 (D 1008). All selected biotypes from this area are classified in the category of trees with high vigour.

At chestnut selections from Călimănești area (Vâlcea County), the trunk circumferences varies among 1.20 m and 2.20 m and sectional surface of the trunk (SST) oscillates between 1911 cm<sup>2</sup> (selections C 1052 and C 1053) and 3503 cm<sup>2</sup> (selections C 1055 and C 1056). Tree crown diameter is comprised between 4.90 m (selection C 1053) and 13.1 m (C 1056). The height of selected plants from Călimănești area ranged between 5 m (genotypes C 1052 and C 1053) and 14 m (C 1056). Crown diameter recorded growths comprised between 56.5 m<sup>3</sup> (C 1053) and 1414.7 m<sup>3</sup> (C 1056).

For the chestnut biotypes selected from Călimănești area, the points obtained for the four elements of growth were comprised between 203.3 points (C 1053) and 433.6 (C 1056). Two of the biotypes have average vigour (C 1052 and C1053), while the rest of selections present high growth vigour.

At chestnut selections from Bistrița area (Vâlcea County), the trunk circumference varies among 1.65 m (selection B 1103) and 6.30 m (B 1101). Values of sectional surface of the trunk (SST) are comprised between 2168 cm<sup>2</sup> (B 1103) and 25581 cm<sup>2</sup> (B 1101 and B 1107). Biotypes crown diameter is comprised between 5.60 m (B1103) and 15.5 m B 1107. Height of genotypes is comprised between 8 m (B 1103) and 16 m (B 1106). Crown diameters recorded increases comprised between 157.3 m<sup>3</sup> (B1103) and 2505.8 m<sup>3</sup> (B1106).

Biotypes force identified have a high vigour. The type of growth is in a share of 62% semi-spread, biotype B 1104 has spread habitus, and selections B 1103 and B 1104 B have erect growth. High vigour is given by summing over 652.6 points for biotype B 1106 and 2702.3 points for biotype B 1107.

At chestnut selections from Horezu area (Vâlcea County), trunk circumference recorded values comprised between 2.60 m (H 1210 and H 1202) and 5.30 m (H 1205). Sectional surface of the trunk (SST) in case of biotype H 1205 reached a value of 22365 cm<sup>2</sup>. The lowest value of SST was recorded at H 1204 (only 4212 cm<sup>2</sup>).

Crown diameter had values comprised between 11.7 (H 1202) and 15.0 m (H 1205). All 8 selections have the height more than 10 m, in case of biotype H 1201 the height of the tree reaching 19 m. The crown volume recorded growths comprised between 1293.7m<sup>3</sup> (H1207) and 2435.2 m<sup>3</sup> (H1205).

The tree vigour of chestnut selections from Horezu area is high, and their port is semi-spread.

At chestnut selections from Polovragi area (Gorj County) the trunk circumference varies from 1.20 m (PO 1325) up to 7.60 m (PO 1311).

Sectional surface of the trunk (SST) is comprised between 2866 cm<sup>2</sup> (PO 1323) up to 30859 cm<sup>2</sup> (PO 1311). The crown diameter varies highly, reaching values comprised between 4.71 m (PO 1323) up to 20.50 m (PO 1324). Trees height is comprised between 12 m (PO 1301; PO 1302; PO 1327; PO 1325; PO 1337) and 28 m (PO 1331). The crown volume has recorded increases comprised between 300.5 m<sup>3</sup> (PO1309) and 5288.7 m<sup>3</sup> (PO1326).

The approximate age of selections is comprised between 60 years (PO 1339) and more than 300 years (PO 1310 and PO 1311). The two selections are unique by their age in the region. The type of growth is in proportion of 52% semi-spread and 42.50% spread.

For the biotypes in Polovragi area, according to the points summed up for the four elements of growth, there were obtained values comprised between 318.9 points (PO 1323) and 2026.9 points (PO 1311). The vigour of selected chestnut trees from Polovragi area is average (at genotypes PO 1338 and PO 1339) and high (at all the others).

The plants vigour is influenced by the genotype, by the environment conditions, and by the trees age, which generally is very high.

Biostatistics determinations (regressions and correlations) relating to the existing relations between the sectional surface of the trunk (SST) and the crown volume at the chestnut selections reflected the following:

- Dăești area: regression equation  $y = 0.066x + 996,5$  and correlation coefficient  $r = 0.275$ ;
- Bistrița area: regression equation  $y = 0.014x + 1306$  and correlation coefficient  $r = 0.141$ ;
- Horezu area: regression equation  $y = 0.053x + 1177$  and correlation coefficient  $r = 0.872$ ;
- Polovragi area: regression equation  $y = 0.112x + 1015$  and correlation coefficient  $r = 0.412$ .

Biostatistics determinations (regressions and correlations) relating to the existing relations between the sectional surface of the trunk (SST) and the approximate age of chestnut biotypes underlined positive correlations:

- Dăești area: regression equation  $y = 0.004x + 55.55$  and correlation coefficient  $r = 0.484$ ;
- Bistrița area: regression equation  $y = 0.01x + 58.77$  and correlation coefficient  $r = 0.901$ ;

- Horezu area: regression equation  $y = 0.09x + 132.3$  and correlation coefficient  $r = 0.787$ .

### **Phenology characteristics of comestible chestnut selections**

Knowledge of phenology for vegetative and fruiting organs has a huge relevance for the culture of tree species. Development of vegetative buds is an important vegetation phase of the chestnut. Although development is occurring later than at other tree species, there is a risk that it may be followed by periods of negative temperatures, which may adversely affect the plants.

First genotypes of comestible chestnut that begin the vegetation in Dăești area (Vâlcea County) are: D 1006 (development occurs earliest on 14<sup>th</sup> of April), D 1003 (19<sup>th</sup> of April), D 1004 (20<sup>th</sup> of April), D 1010 (21<sup>st</sup> of April) and the last genotypes for which this phenophase occurs are: D 1013 (23<sup>rd</sup> of April); D 1002 (24<sup>th</sup> of April) and D 1009 (25<sup>th</sup> of April). The length of the vegetative period is comprised between 183 days (D 1004 and D 1009) and 200 days (D 1003).

In the case of the chestnut selections from Călimănești area (Vâlcea County), development of vegetative buds was triggered at the earliest on 18<sup>th</sup> of April and ended the latest on 13<sup>th</sup> of May. The vegetation period sums up to 191 up to 195 days.

At the 8 genotypes selected from Bistrita area (Vâlcea County), development occurred at the earliest between 23<sup>rd</sup> of April and 11<sup>th</sup> of May and at the latest between 27<sup>th</sup> of April and 12<sup>th</sup> of May (B 1102). The vegetation period at genotypes studied sums up to 169 days (selection B 1101) and up to 214 days (B 1105).

At Horezu (Vâlcea County), development at chestnut selections began earlier in the first part of April (13<sup>th</sup> to 19<sup>th</sup> of April), leaf fall prolonged until the first decade of November, resulting thus a vegetative period from 192 days at biotype H 1201 and 205 at biotype days H 1207.

At Polovragi (Gorj County), development was triggered towards the end of April, only selections PO 1302 and PO 1308 began the vegetation period on the 19<sup>th</sup> of April. The whole vegetation period ranged from 193 days (PO 1306) to 219 days (PO 1336).

The vegetation period of chestnut biotypes of the 5 areas was comprised between 196 days and 216 days, within each zone the vegetation period being differentiated between biotypes. Differences between vegetation periods varies from 4 days (Călimănești) to 40 days (Bistrita), being the result of interaction between genotypes and ecological conditions of the area.

In the conditions of Northern Oltenia, fruits of comestible chestnut biotypes have reached maturation from 1<sup>st</sup> of September and ended on the 1<sup>st</sup> of November. The earliest biotypes of comestible chestnut selected proved to be: D 1002, H 1202, H 1204 and H 1206. Late maturation of fruits may be met at biotypes: PO 1304, PO 1307, PO 1312, PO 1325. The remaining selections of chestnut present a medium or middle maturation.

The maturation age of the fruits is very different from one genotype to another, but oscillated according to the area. The variable climatic conditions that are characteristic to Sub-Carpathian area of Oltenia differentiate very much the maturation period of fruits from one year to another.

### **Productive characteristics of comestible chestnut selections**

According to the average fruit production, the chestnut biotypes can be classified into the following groups:

- Very good productions (more than 50 kg/tree). Here are included the biotypes: D 1001, D 1002, D 1003, D 1004, D 1005, D 1006, D 1007, D 1008, D 1009, D 1010, D 1011, D 1012, D 1013, B 1101, B 1102, B 1103, B 1104, B 1105, B 1106, B 1107, B 1108, H 1202, H1203, H 1204, H 1205 H 1206, H 1207, PO 1309, PO 1315, and PO 1320
- Good production (30-50 kg/tree) - biotypes H 1201 and H 1207;
- Average production (20-30 kg tree) – most biotypes of chestnut from Polovragi area;
- Low production (below 20 kg/ tree) - biotypes C 1052, C 1053, PO 1308, PO 1313, PO 1316, PO 1322, PO 1323, PO 1328 and PO 1331.

Fruit production varies from one year to another, and are influenced by the age and the vigour of the plants.

### **Morphological characteristics of the fruits of comestible chestnut biotypes selected**

The fruits size at comestible chestnut biotypes studied in the Northern area of Oltenia were classified according to FAO - CIHEAM (2002) into the following categories:

1. Very big (<60 chestnuts/kg);
2. Big are (61 - 80 chestnuts /kg);
3. Average (81 - 100 chestnuts /kg);
4. Small (101 - 120 chestnuts /kg).
5. Very small (> 121 chestnuts /kg)

Taking into account this classification, the biotypes of comestible chestnut identified and selected in the Northern area of Oltenia are classified as follows:

- With very big fruits: selection H 1205;
- With big fruits: D 1007, D 1010, D 1011, B 1102, B 1103, H 1203 and H 1204;
- With average fruits: D 1001, D 1005, D 1008, D 1009, B 1101, B 1104 H 1201, H 1202, H 1206 and H 1208;
- With small fruits: D 1004, D 1013, B 1106, B 1108, H 1207, PO 1309, PO 1312, PO 1318, PO 1320, PO 1330 and PO 1337;



- With very small fruits: D 1003 and D 1012, to which are added 22 biotypes from Polovragi area.

According to this classification, there is a high variability regarding the weight and size of fruits from chestnut genotypes of Northern Oltenia.

As for the size of fruits, it is found that the variability between the biotypes of comestible chestnut from Dăești (8.4%), Bistrița (8.2%) and Horezu (10.2%) is very low, while for the biotypes from Polovragi (20.2), it is very high. If we relate at all locations in the area, biotypes show a high variability in the size of fruit (21.7%).

The fruits of comestible chestnut with the roundness index exceeding 1.0 round have globular shape (D 1001, D1003, D1010, D 1011, D 1012, C 1054, B 1101, B 1102, B 1105, H 1202 and H 1206) and other types from Polovragi. Most biotypes have an ellipsoidal transversal shape and transversally broad ellipsoidal. We find though biotypes with fruits in the ovoid shape (H 1203, H 1204, H 1207, PO 1325, PO 1336). A single biotype (D 1013) presents fruits with a large ovoid shape.

The weight of comestible chestnut fruits from genotypes selected in Northern area of Oltenia oscillates from 3.9 g (PO 1338) up to 15.5 g (H 1205).

In case of fruit weight, the variability is higher within populations of each locality (17.3 to 28.4%), but the most obvious variability is found across the entire areas ( $s^2 = 34.5\%$ ). This high variability of fruit weight biotypes signals that biotypes come from different populations.

The chestnut fruits from Dăești area have tegument colour reddish-brown to brown - dark, only biotype D 1009 has brown colour fruits. Selections fruits from Călimănești area are brown reddish. At biotypes from Bistrita area, we remark the dark brown to brown blackish, and biotypes of the Horezu are classified as reddish (H 1205), reddish brown (H 1201, H 1206, H 1207 and H 1208) to brown dark (H 1202, H 1203 and H 1204). Chestnut tegument colour from Polovragi area varies from reddish to dark brown.

#### **d. Behaviour of comestible chestnut selections to natural infections with pathogens**

##### ***Cryphonectria parasitica* and *Phytophthora cambivora***

Biotypes of comestible chestnut from the spontaneous semi flora of Northern area of Oltenia are strongly attacked by *Cryphonectria parasitica* (causing cancer of chestnut bark) and *Phytophthora cambivora* (ink disease).

*Cryphonectria parasitica* is responsible for the cancer of chestnut bark, the disease being particularly dangerous because it causes damages on mature or old specimens, causing their drying.

Fighting against this disease can be done by using strains hypo-virulent inoculations. If action is not taken to protect valuable genetic resources in affected areas, they shall disappear.

Out of the 75 genotypes studied, a number of 28 genotypes (37.3%) are affected by *Cryphonectria parasitica*, and 13 genotypes (17.3%) are affected by *Phytophthora cambivora*.

#### **g. Manifestation way of the main physiological elements**

Determinations were performed on some selections of comestible chestnut relating to the intensity of photosynthesis, photosynthesis rate, transpiration rate and stomatal conductivity of CO<sub>2</sub>.

**Photosynthesis intensity** is very different between in all 3 intervals of the day taken into study. It should be noted that the intensity of photosynthesis does not follow a linear path but it modifies itself.

There are highlighted cases where the intensity of photosynthesis has higher values in the morning and decreases throughout the day (at selections H 1208 and B 1101), or the photosynthesis intensity has the highest value in the last hour interval (selection D 1107).

**Photosynthesis rate** at chestnut biotypes was very different and oscillated from one hour to another, a significant differentiation being recorded between the biotypes from Dăești comparatively with other localities.

**Transpiration rate** manifested a visible increase of 0.07 mmol/m<sup>2</sup>/s to 1.0 mmol/m<sup>2</sup>/s as response to temperature increase from 27.4 to 31.5°C. A higher diversity of transpiration rate was observed when temperatures are higher.

**Stomatal conductivity of CO<sub>2</sub>** proved to be different, but within reduced limits, at all biotypes of comestible chestnut. This depends on the time of day when observations were performed, on light and temperature. The value of stomatal conductivity proved to be very reduced, independently of the time interval when observations were performed, being under the level of 0.1 mol/m<sup>2</sup>/s.

#### **f. Preservation of valuable genetic resources of comestible chestnut in Northern area of Oltenia**

Maintaining biodiversity is essential to preserve the ecosystem functions on long-term.

The results obtained within this thesis suggest adequate options for managing current resources of comestible chestnut, in order to preserve biodiversity. In the absence of a quick action, there exists the possibility to lose biodiversity by genetic erosion.

Of the 75 biotypes analysed, 10 were selected: D 1006, D 1010, D 1011, B 1101, B 1102, B 1105, H 1205, PO 1317 and PO 1305. These are valuable and can be useful for programs on *ex situ* preservation and genetic improvement of varieties and rootstocks.

From the biotypes selected, branches were harvested and multiplied vegetatively, in order to introduce them in the national collection of the UCV - SCDP Vâlcea.