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SUMMARY

PhD thesis

„MORPHO – CYTOLOGICAL AND MOLECULAR STUDIES REGARDING THE EVOLUTION OF *ALLIUM SATIVUM* L. LANDRACES''

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Introduction

In a continuous moving an developing world, sometimes under no control, preservation and conservation of plant genetic resources is one of the most important task humans must fulfill. Plants and animals are disappearing everyday even before they are discovered. Along with them the hope for a better world is disappearing as well. The conservation of plant genetic resources is now becoming more important than ever, regardless of developmental level of involved countries but with different approaches due to technical support and possibilities.

The research community along with political and administrative authorities realized the need for collection, evaluation and conservation of plant germplasm, of local landraces and wild relatives in order to establish a gene poll for research and breeding programs. (**Zender şi colab., 2006**).

Garlic cultivation (*Allium sativum* L.) is so old as the human race and so wide as the civilization itself. References for this plant can be found in the Bible and Coran, reflecting the importance as a food and as a horticulture plant (**Tapsell**, **2006)**

Alliaceae family comprises one of the most important plant groups, considering the economic advantages, like garlic (*Allium sativum* L.), onion (*Allium cepa*L.) **(Brewster, 1994)**.

The knowledge of garlic utilization in nutrition and medicine have been used from studies of old archeological sample since antiquity in Middle East. The evidence about garlic utilization come from pictograms, sculpture in stones and dry plant collections (**Parejo, 2002)**
Garlic is rich in antioxidants that prevent formation of free radicals -particles that damage celular membrane, DNA and have a major contribution to aging and disease development **(Rivlin, 2006,2001)**

Easy to cultivate, with no special requirements, garlic can generate yield of 20-22 tones/ha. In 2009, according to FAO, the global production for garlic was 18 million tones, from which 16 mil. were obtained by the first 10 world producers. From these China takes the first place with over 12 mil tones (78% from world production) followed by India (645000 tones) and South Korea (325000 tones). European Union is the 4th large producer of garlic (300052 tones); the list continues with: Egypt (258608 tone), continues with Russia (254000 tones), USA (222000 tones), Argentina (140000 tones) and Ukraine (125000 tones).

With the rapid development of the global economy and continued increase in world population, natural environments face serious deterioration and change, which has led to the extinction or severe endangerment of many plant species including important crops and their wild relatives At the same time, humans depend more and more on the wild relatives to enhance crop productivity in breeding by transferring genes from the wild gene pool. Effective conservation and exploitation of these wild relatives and their genetic diversity become essential factors in guaranteeing global food security and sustainable crop production.

Plant genetic resources are one of the most valuable natural resources (Ramanatha and Hodgkin 2002), providing the genetic diversity necessary for both farmers and breeders to obtain new cultivars either with high yield, or better quality, or more adapted to abiotic stress, or more resistant to pest and pathogens. Therefore, conservation and use of plant genetic resources plays an important role in agriculture, food security, and forestry.

Genetic diversity is a fundamental component of biodiversity as it is the basis of species diversity and ecosystem diversity. Genetic diversity can be defined as the range of genes within a species and can be studied at the individual, population and species level (Nei 1987; Lowe et al. 2004). Genetic diversity can be characterized by the set of possible alleles (different variants of the same gene) and their frequencies, by entire genes, or by even units larger than genes such as structures on chromosomes. Populations of all organisms in their habitat contain an abundant variation in morphology, physiology, and behavior. Much of this abundant variation may be

reflected in the genetic diversity of organism, which often interact with habitat variation and thus produces the phenotypic variation of organism (Lowe 2004).

In such context our major objective was to identify new sources for germplasm that can be used widely in research having in mind the actual social and economical conditions that impose the need to employ alternative ways for a rational utilization of genetic resources. In this sense we focused on the phenotypic and genetic analysis of several garlic land races from West part of Romania having as a target selection of new garlic genotypes with a high potential for economical utilization

The impact and relevance of research in the European, National and Regional Strategies

Our research fits to the European strategy for genetic resources conservation.

1.Ex situ conservation of genetic resources Art. 9 from Rio de Janeiro Convention concerning the biological diversity, 1992 (L 58/94) http://www.cbd.int/default.shtml
2.Bnera Convention concerning the wild species and habitats, 1979 (L13/93) http://www.coe.int/t/dg4/cultureheritage/nature/Bern/default_en.asp
3. Washington Convention CITES, 1973 (69/94)http://www.cites.org/

ITGRA Specifications:
- "genetic resources *are conserved and available to present and future generations, with no restrictions*" as part of the "*world legacy*."
- the principle regarding property of countries on their own genetic resources (FAO Resolution 3/91)
- the principle can be found later in the Convention regarding biological diversity (1992)
- in 1996 - officially there is a recognition for the first time of the importance to accumulate knowledge about species and their wild relatives that are used in agriculture and other areas. These species represent a valuable source for breeding and future research.

What do we need?
According to ITCPGR/96/3
- long lasting e*x situ* collections
- rehabilitation of affected resources *ex situ*
- widespread of *ex situ* conservation
- concentration of *ex situ* conservation
- monitoring the possible threats on the agriculture biodiversity
- improving education
- promoting public dissemination regarding the importance to conserve and preserve genetic resources with importance in agriculture

THE OBJECTIVE OF THESIS

The research we proposed targets the analysis of variability and heredity of different characters involved directly in garlic productivity based on collected landraces from Western Part of Romania.

Selection of at least one valuable landrace that can be further used in classic breeding programs, pharmaceutical industry or in alternative medicine. The climate changes and large temperature fluctuations are factors of yield loss worldwide. In this sense there is a permanent need to identify new ways to increase yield production and to make advantage at maximum of the available genetic resources. To accomplish our goals we set the following objectives:

1. Characterization of the landraces collected in order to identify potential valuable lines for breeding.
2. Genetic characterization of vegetative reproduction of morphological traits
3. Evaluation of cell cycle in the landraces by making use of FlowCytometry
4. Genetic relationships among investigated population based on molecular DNA markers
5. Evaluation of metabolic products in garlic landraces.

BIOLOGICAL MATERIAL

Biological material consisted from 16 garlic landraces (*Allium sativum* L.) collected from Timis, Arad and Hunedoara County (table 1). Garlic landraces have been collected from original sites and all data regarding life cycle, yield, quality and other features have been registered in data sheets for each population.

Table 1. General characteristics of *Allium sativum* landraces at *in situ* collection time

No.	Catalog No.	Collection site/Address	Year of collection	Observations
1	1252	Mărăuş, nr. 18, jud. AR	2003	**autumn garlic**
2	1269	Şeitin, jud. AR	2003	**autumn garlic**
3	1484	Căpâlnaş, jud. AR	2004	**autumn garlic**
4	1763	Sebiş nr. 5, jud AR	2004	spring garlic
5	1765	Sălăjeni nr. 21, jud. AR	2004	spring garlic
6	1768	Sebiş, nr. 18, jud AR	2004	spring garlic
7	1770	Sebiş, nr 94, jud AR	2004	spring garlic
8	1231	Cenad,nr.1259, jud TM	2002	spring garlic
9	1235	Chizătău, nr. 80, jud TM	2002	spring garlic
10	1279	Căpăt, nr. 107, jud. TM	2003	spring garlic
11	1480	Valcani, nr. 556, jud TM	2004	spring garlic
12	753	Curechiu, nr. 95, jud TM	2001	spring garlic
13	750	Băcâia, nr. 75, jud.HD	2001	spring garlic
14	754	Poiana, nr 6, jud. HD	2001	spring garlic
15	755	Poienița, NR 46, jud. HD	2001	spring garlic
16	772	Oprişeşti, nr. 4, jud.HD	2001	spring garlic

WORKING METHODS

- ***Ex situ* field experiments**

Generally, garlic (*Allium sativum* L.) is characterized by a vegetative reproduction through adventives bulbs. In classic breeding methods there is still a practice to use garlic seeds.

Vegetative reproduction *ex situ* was established as follows:

The garlic culture has been initiated on a sunny field, fertilized with compost and the sowing was done manually in autumn (Timisoara location,) and in Cenad location at a distance of 25 cm between rows and 15 cm inside the row at a 3cm depth. During vegetation the garlic culture has been removed from weeds and irrigated at need. At the first signs of plant raising, 1rst of November field data collection started and have been registered continuously.

Yield has been collected at full maturity - total yellowing, at the beginning of June for the autumn landraces-beginning of July for the spring of landraces. We did not allowed full dry of garlic landraces leaves before yielding since in this way we try to prevent unwilling of bulbs and loss of yield during preservation.

Yielding has been performed on dry weather, the collected bulbs have been left on ground to avoid mechanical damage and left to dry for 2 days in sun and after drying the leaves have been removed and a 1.5-2 cm tail was kept above the bulb and the root was shortened to 1 cm. The yield was stored at 1 – 3 ºC and 75% humidity.

Quantitative measurements were made for plant height, bulb diameter, bulb height, no of cloves and their weight for all landraces. The data were analyzed using multiple variance test analysis. For statistical coverage we made use of Stastistica7/Windows and the following parameters:

- average ± standard deviation
- correlation coefficient
- difference signification- Duncan Test
- variance analysis – ANOVA/MANOVA

DNA extraction

DNA have been extracted from 50 mg fresh leaves using 2xCTAB method (Saghai-Maroof, 1984, with few modifications). DNA was cleaned up DNA Clean Kit de ZymoResearch (BioZyme). For each PCR reaction 50 ng/µl was used in the mixture.

PCR-RAPD Reaction

For RAPD reaction 10 oligomers OP-A (Operon Techologies, Aalmeda) were used (Table 1)DNA amplification was carried out in 20µl in BioRad DNA Engine Termocycler Amplification conditions were as follows: 3 min denaturation at 94°C, followed by 36 cycles 1 min at 94°C, 1 min at 36°C, extension at 72°C for 2 min and a final extension 72°C for 7 min. PCR products were separated on agarose gel and visualized after etidium bromide staining. For reproducibility the reactions were carried form 2 independent samples.

Table 2 RAPD oligomers sequence (Biosearch Technologies, INC)

Reference No.	5'-3'
1	P2-5'd(GGT-GGC-CAA-G)3'
2	P5-5'd(CAC-TGG-CCC-A)3'
3	P7- 5'd(TGG-TCG-GGT-G)3'
4	P8- 5'd(CTA-AGC-GCA)3'
5	P9- 5'd(TTG-CTG-GGC-G)3'
6	P11- 5'd(CCG-CTG-GAG-C)3'
7	P12 -5'd(CGG-AGA-GCG-A)3'
8	P13- 5'd(CGA-CCA-GAG-C)3'
9	P15- 5'd(GCT-CCC-CCA-C)3'
10	P16 -5'd(TTG-CTG-GGC-G)3'

In case of polymorphism analysis we counted only those amplification bands that were clear and present in both repetitions

For a correct quantification the amplified products were scored with 1 (for present) and with 0 (for absent). According to electrophoresis profiles a cluster could be constructed that indicated the similarity between garlic landraces. In this sense we made used of the available indices:

- Genetic similarity coefficient
- Genetic distance (DG) calculated as follows as a Jaccard coefficient:

$$\mathbf{J(i,j)=C_{ij}/(n_i+n_j)-C_{ij}}$$

Using the - UPGMA (Unweighted Pairwise Group Method with Arithmetic Mean) similarity cluster has been assembled.

- **FlowCytometry**

Nuclei have been extracted form 0,5 cm² leaves according to manufactures specifications (Fig. 1).Samples were processed in the FCM and data were recorded and analyzed using manufacturer software.

Fig. 1 FlowCytometrul used in our research (The Partec CyFlow SL flow Cytometer)

- **Biochemical methods for extraction of active metabolite compounds form garlic.**

The biochemical procedure was standard.Water content was determined by air drying, fat content by Soxhlet method, glucose were calculated by difference, ash content by brut incineration fiber by incineration and digestion with acid and base and proteins by Kjedahl method. **(AOAC, 1990)**. Calorimetric value was calculated based on Atwater factor **(FAO, 2006a).**

The samples have been investigated by Mass-Spectrometry using the following conditions:

Temperature of ionization source 200°C;
Ionization tension 70eV in EI module
Mass scanning interval 25 – 400M/z;
Solvent passing through time: 2min.

Results and Discussion

- **Characterization of collected material regarding the important traits that can be used in breeding programs**

The data were analysed for bulb weight, cloves weight/bulb and no. of cloves/bulb. The data are presented form general point of view regarding the collected material to differences among the garlic genotypes concerning the important traits that can be exploited for breeding program.

In table 3 and graphics (Fig. 2; 3; 4) one can follow the general average for important traits (bulb weight, cloves weight/bulb and no. of cloves/bulb) established for *ex situ* cultivation period (2005 – 2008), and separatelly for sping and autumn garlic. According to bulb weight data, in Timisoara location, we registred the highest values in 2008. The 2008 year was very good as for climate conditions with proper rainfalls in autumn at rooting period and in march when the green part of garlic plants are growing. Similar conditions we observed in 2006 when the values for same traits were very close to those of 2008 cultivation year. In Cenad with soft soil the maximum values were registered during 2005-2006 due to proper humidity. The no. of cloves/ bulb varies very little in both garlic types, beeing the trait with the greater stability.

Table.3. The overall average values for landraces cultivated ex situ during 2005-2008

Years of the cultivation	Spring garlic				Autumn garlic			
	Timişoara		Cenad		Timişoara		Cenad	
	bulb weight (g)	Nr. of cloves in bulb	bulb weight (g)	Nr. of cloves in bulb	bulb weight (g)	Nr. of cloves in bulb	bulb weight (g)	Nr. of cloves in bulb
2005	9,16	8,89	10,35	9,63	13,32	7,53	14,37	8,17
2006	10,15	9,81	10,06	9,81	14,32	8,47	14,27	8,60
2007	9,26	9,60	8,99	9,44	14,08	8,83	13,60	8,87
2008	10,44	9,44	9,31	9,47	15,07	8,40	14,12	8,53
MEANS								
2005 - 2008	9,75	9,43	9,67	9,58	14,44	8,31	14,08	8,54

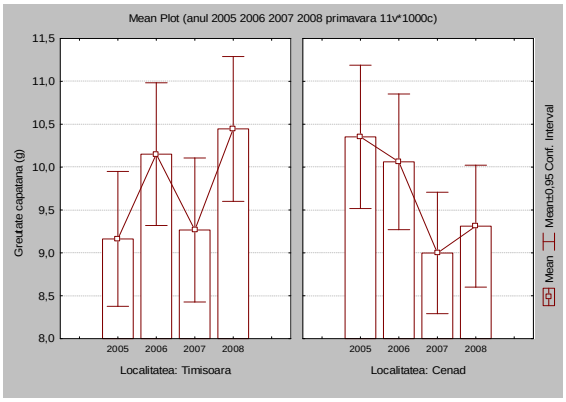

Fig 2. The differences among landraces through bulb weight (g) during overall cultivation period in Timisoara and Cenad *ex situ* locations

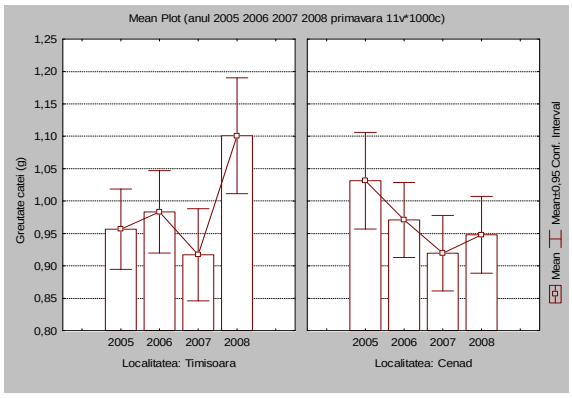

Fig 3. The differences among landraces through cloves weight (g) during overall cultivation period in Timisoara and Cenad *ex situ* locations

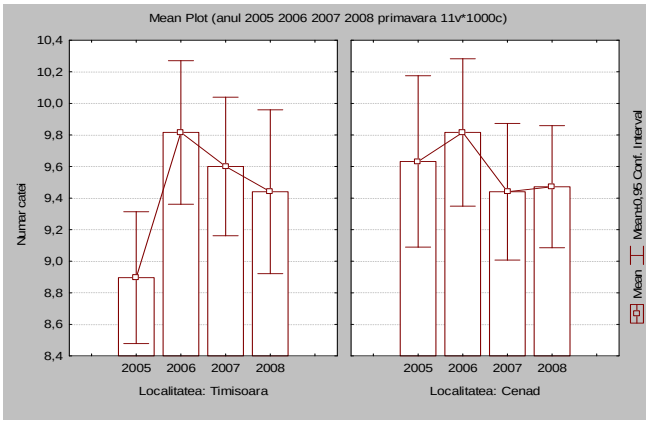

Fig. 4 The differences among landraces through cloves no/ bulb during overall cultivation period in Timisoara and Cenad *ex situ* locations

În fig. 5 – 6 we poltted the differences among spring and autumn garlic as a level of expression of productivity traits.

Fig.5 Landraces of spring garlic Sebis (original)

Fig. 6 Landraces of autumn garlic Maraus (original)

- **The characterization of spring garlic genotypes regarding the traits involved in productivity**

The highest no of cloves /bulb and the fact that Seitin landrace dose not form floral stalk confirms our observations that this population tends to behave like a autumn galic type.

Table 4 The differences among autumn garlic landraces by average values of expressed quantitative traits during the overall *ex situ* cultivation period and locations

Nr. crt.	Landraces	bulb weight (g)	clove weight (g)	no of cloves/bulb
		Mean±Ab.st	Mean±Ab.st	Mean±Ab.st
1	Şeitin	15,75±3,07	1,75±0,24	8,92±2,12
2	Căpâlnaş	15,38±2,94	1,72±0,29	8,69±1,45
3	Mărăuş	11,67±2,60	1,49±0,34	7,66±1,62
MEANS		14,27±3,41	1,65±0,31	8,43±1,83

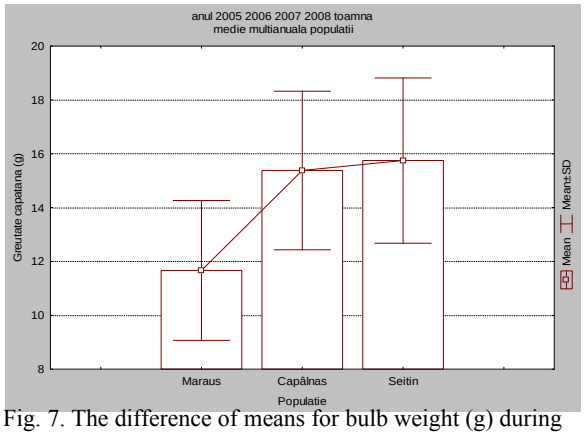

Fig. 7. The difference of means for bulb weight (g) during the overall *ex situ* cultivation for autumn landraces

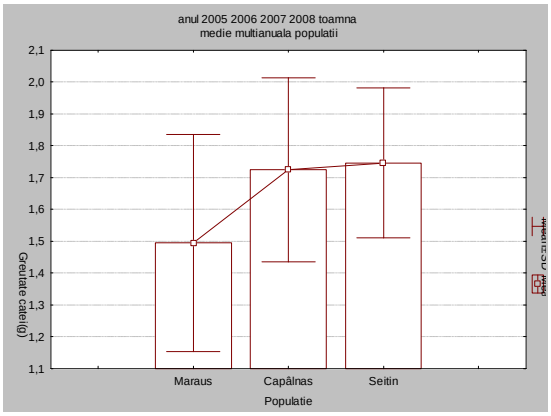

Fig. 8. The difference of means for cloves weight (g) during the overall *ex situ* cultivation for autumn landraces

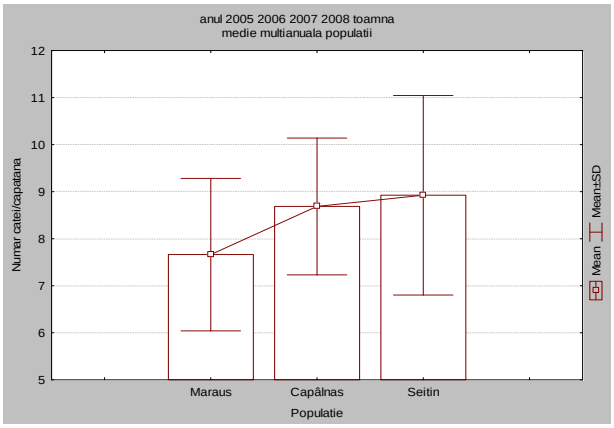

Fig.9. The difference of means for cloves no./bulb during the overall *ex situ* cultivation for autumn landraces

The data obtained above show a large variation among several characters that were taking into account at the begining of aour evaluation of the initial collected material and their usage for breeding programs.

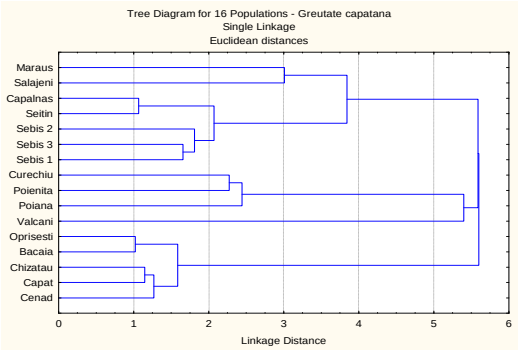

Fig. 10. Dendogram obtained from the analysis of the landraces of *Allium sativum* L.

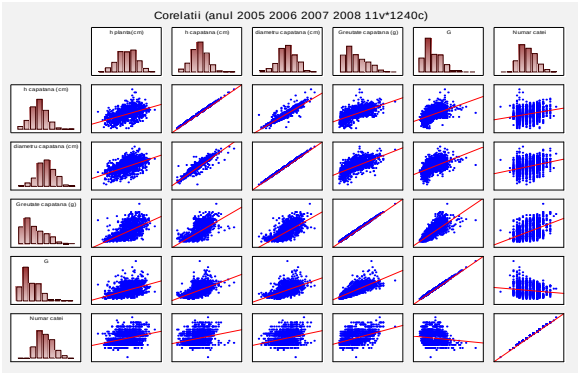

Fig. 11. The correlation coefficent between plant traits for 2005 – 2008 *ex situ* cultivation period

We could observe a positive correlation between analyzed traits with higher signification. (Fig. 11.). The only negative correlation we could obtain between the calves weight and no of cloves/bulb (r= - 0,1338; p= 0,000).

- **The filogenetic relationships among garlic landraces based on molecular DNA markers**

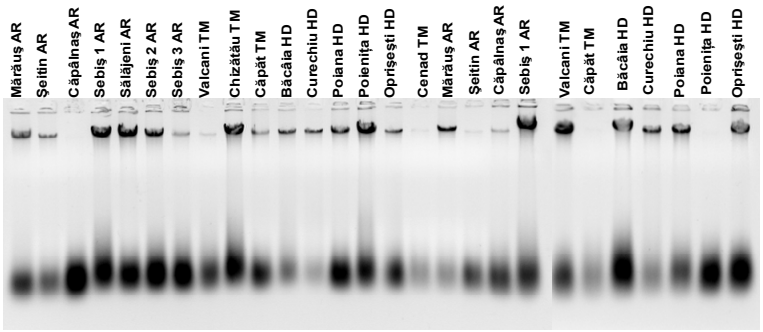

Fig. 12. DNA quality in garlic landraces by agarose gel (1%) electrophoresis. (1-16 single probes from each garlic population; 17-27 duplication of samples)

The figures below show the RAPD PCR amplification using different random oligomers in all 16 garlic populations (The Cenad garlic was used as control). The amplification products were separated on agarose gel 1,2% and visualized after etidium bromide staining.

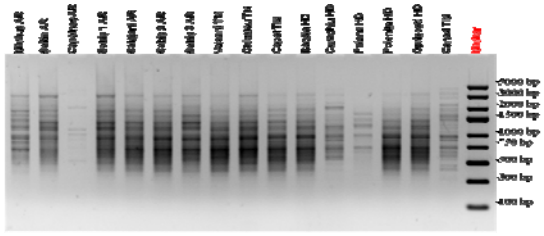

Fig. 13. RAPD profile in garlic landraces using oligomer P5-5'd(CAC-TGG-CCC-A)3'

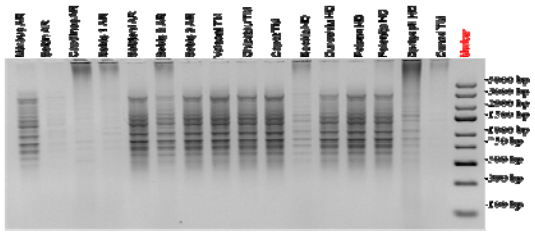

Fig. 14. RAPD profile in garlic landraces using oligomer P9- 5'd(TTG-CTG-GGC-G)3'

In case of RAPD (Fig.13;14), amplification the reproducibility of reaction is very important in order to validate the data. In this sense we repeated the DNA extraction and RAP reaction from 2 independent samples and compared the bands for each reaction. In order to analyze the molecular polymorphism in garlic landraces we considered only that amplification band that was present in both repetitions. The polymorphism (P, %) was calculated according to presence and absence of bands compared to the total amplified bands in for each primer used. To appreciate the P of a population we considered that a single individual band is present in 94% of individuals from the group then the band is polymorphic. If a amplification band is present only in 6% of individuals that form the analyzed group the band is absent so it dose not appear in all other 94% of individuals so its polymorphic. If a band appears to 95% of the individuals then is a monomorphic. For a better quantification the band were scored 1 for present and 0 for absent. Using the electrophoresis profiles we could build a cluster that reflects the similarity degree between garlic populations. The genetic distances were calculated using the Jaccard coefficient and UPGMA for dendogram construction.

In garlic landraces the polymorphism is low, 16.39% that can be explained by genetic stability of garlic and the close geographical origin of the collected landraces. The no of amplified bands varied form 2 (oligomer 2) to 10 (oligomer 5). The bands intensity can be classified as low, medium and intense. We can conclude that the garlic landraces are genetically stable and are closely related to each other.

The calculated genetic distances are below 0.5 which indicated a very close relation between the analyzed population and a high degree of similarity. The UPGMA clustering (Fig. 15) is grouping the garlic landraces into geographical associations and confirms the Cenad garlic as the oldest in the group. Few population are independently classified which indicates a separate evolution.

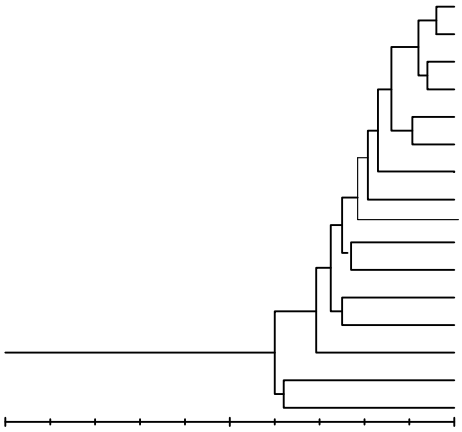

Fig. 15. Clustering of garlic landraces based on UPGMA analysis.

We can see a differentiation inside the geographical group. In all garlic landraces we see grouping bade on site of origin which indicated isolation based on distance model. Overall the genetic variability among analyzed populations is low which indicated a conserved stable genome and behavior. Since we

don’t know how this population have appeared we cant conclude about their genetic base in our limited conditions for molecular characterization namely the RAPD technique. Other more sensitive markers will be able to discriminate better this landraces.

FlowCytometry Results and Discussions

FlowCytometry is one of the methods commonly used in determination of DNA content and analysis of cell distribution during cell cycle progression. During mitosis four phases can be distinguished: G1,G2,S (DNA synthesis) and M (mitosis). In case of G2 and M that contain the same amount of DNA, the differentiation is not possible based only on DNA.

The FlowCytometry technique used in garlic landraces followed the manufacturer indications in order to calculate the percentage of cell undergoing different cell cycle phases. The cell cycle is very important in plant growth and father development. Miss regulation of this process leads to abnormalities and poor development and as a consequence a poor yield. Only a few histograms are presented below that are relevant of our study.

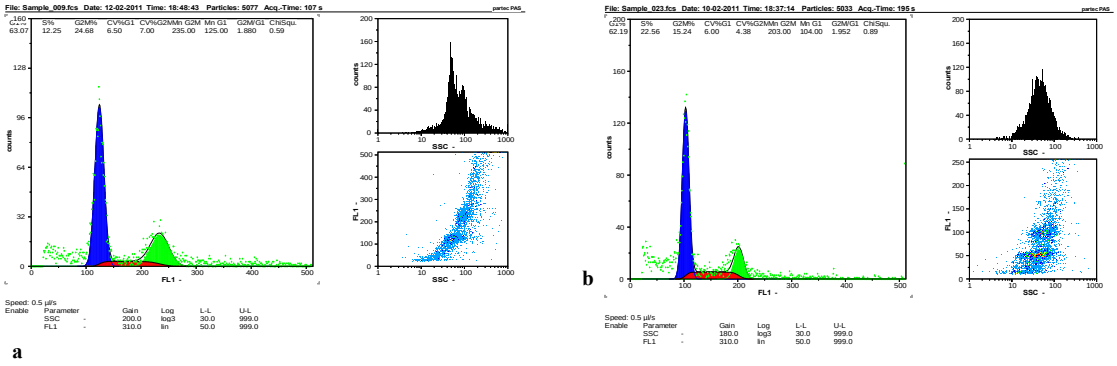

Fig. 16. The cytogram of cell cycle distribution in common cultivated garlic (**a.** Cenad; **b**.Sebiş)

The FlowCytometry analysis revealed a normal cell cycle for all garlic landraces regardless of *ex situ* conditions. This indicated again a stable genome in garlic landraces and that the *ex situ* conditions have no effect on plant growth. The cytological data obtained from each garlic landrace indicate a normal mitosis with no chromosomal aberration.

- **The *Allium sativum* L. metabolites analysis by biochemical methods**

The garlic quality is a genetic characteristic regulated by a series of genes and by the environmental and technological cultivation conditions. Is a very complex trait that is hard to control and to breed. The chemical composition of garlic comprises water in 65 – 87% and dry matter for 13 – 35 %, from which allin is the most important component.

Table.5. Water, ash, sugar, lipids, protids, fibres, and allin content in garlic landraces

Proba	Water %	Ash %	Sugar %	Lipids %	Protids % (N x 6,25)	Fibers %	Energetic value (kcal)	Aliin (mg /g)
Cenad TM 1231	65,0	1,3	27,3	1,2	6,1	1,1	2	6,12
Chizătău TM 1235	87,0	0,6	12.1	0,1	0,5	0,6	4	7,49
Căpăt TM 1279	76,4	0,4	12.6	0,1	2,0	0,5	5	7,03
Valcani TM 1480	88,0	0,7	19,8	0,1	1,3	0,7	3	7,55
Mărăuş AR 1252	94,0	0,7	15,8	0,1	2,0	0,8	6	3,9
Şeitin AR 1269	81,0	0,7	18,7	1,1	3,8	0,9	7	3,1
Căpâlnaş AR 1484	74,0	0,9	18,3	1,4	2,9	0,4	4	4,2
Sebiş AR 1763	88,0	0,9	19,8	0,3	1,3	0,5	2	4,39
Sălăjeni AR 1765	65,0	1,1	27,3	1,2	6,1	0,9	13	2,8
Sebiş AR 1768	78,0	1,3	16,0	0,6	8,3	0,8	10	3,2
Sebiş AR 1770	79,0	0,9	22,8	0,7	8,0	0,5	8	4,0
Curechiu HD 753	76,4	0,9	19,8	1,0	6,4	0,3	6	5,1
Poiana HD 754	84,0	0,6	19,8	0,8	6,2	0,9	13	4,9
Poienița HD 755	90,0	0,5	16,0	0,3	3,0	0,3	3	5,2
Oprişeşti 772 HD	65,0	1,1	27,3	1,4	6,1	1,0	2	4,6
Băcâia HD 750	76,4	0,5	20.6	1,8	2,0	0,8	3	5,7

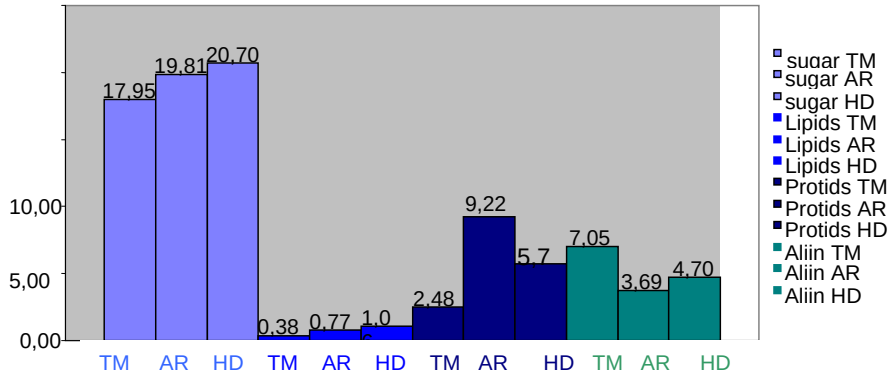

Fig. 17. Water, ash, sugar, lipids, protids, fibres, and allin content in garlic landraces

Our results showed a high aliin content in Timis landraces (7,05 ± 0,29 mg/g) (Table.5) compared with Hunedoara landraces (5,14 ± 0,16 mg/g) and Garlic populations from Arad (3,67 ± 0,21 mg/g). In common cultivated garlic the aliin content varies between 2,8 - 7,7 mg/g, for fresh weight, or 8 – 25%, representing 63 – 84% form dry matter. Taking into account that for 100 g fresh garlic the sugar content varies between 20 -25 %, we obtained values close to the low sugar limit in garlic as follows: 20,7 % for Hunedoara landraces, for Arad (19,81%) and for Timiş (17,95 %).

The lipid content was high in Arad populations with an average of 0,77 ± 0,17 % followed by Hunedoara 1,06 ± 0,23 %, that correspond to the standard limits imposed by vegetable legislation. The lipid content in Timis locations were below the limits 0,6 %, with values of 0,38 ± 0,24% (Table.5). The protein content was generally between 6% and 7% from fresh weight.

The cultivation conditions, specially the fertilization with nitrogen have a great influence on the protein content sometimes above the limits imposed by genetic background. This trait is a polygenic trait with a very complex regulation.

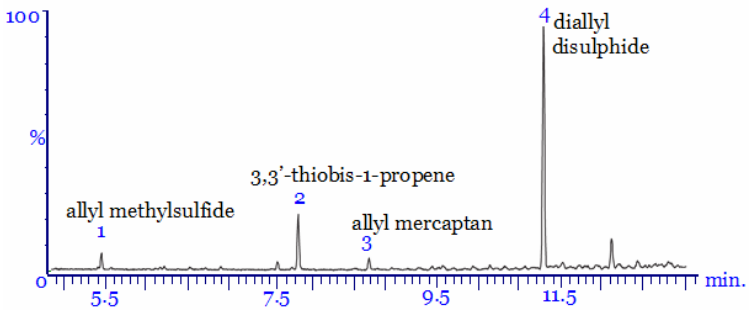

Fig. 18. GC – MS chromatogram obtained form garlic crude extract

CONCLUSSIONS AND RECOMMENDATIONS

1. Our results confirms necessity to analyze and select of new germplasm for improving garlic yield;
2. The garlic landraces were evaluated for four years in ex situ cultivation and are classified according to the yield in both ex situ location.
3. The garlic landraces can be used for different purposes in breeding programs depending on each area needs.
4. The selection for cultivation can be based on the large variation among collected garlic landraces.
5. The biogenetic analysis based on DNA markers indicated very close relationships between garlic landraces and a high degree of similarity.
6. The cell cycle in garlic populations analyzed by FlowCytometry is normal although in some population the variability was higher then 2%, variations that can be the effect of nuclei isolation technique.

7. The no of cells analyzed by FlowCytometry varied between 5000-10000 being enough for cell cycle analysis. No abnormal evolution was observed in all 16 garlic landraces indicating that these populations express a high capacity for adaptation to ex situ cultivation conditions.
8. The allin content present in garlic bulbs is in accordance with standard limits.;
9. Timis landraces are a good source for alliin and can be exploited for pharmaceutical industry.

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