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BIOCHEMICAL COMPOSITION OF THE HORSE CHESTNUT SEED (*AESCULUS HIPPOCASTANUM* L.)

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Abstract - This study presents research on the chemical composition of 15 horse chestnut seed genotypes (*Aesculus hippocastanum* L.) conducted on three different sites. The seeds were picked from selected trees in Bačka Palanka, Novi Sad and Sremski Karlovci at the end of September 2010. The statistical parameters point out the variables of the examined features of the seed. On the basis of the moisture, starch, fat and protein contents in the horse chestnut seeds, the genotypes, the reproductive material which would be used for the intensive production of the high grade seedlings for the greening of the different area categories in urban areas, are distinguished.

Key words: *Aesculus hippocastanum* L., seed, biochemical composition, urban cenosis

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INTRODUCTION

The Horse Chestnut (*Aesculus hippocastanum* L.) is an Arcto-Tertiary endemic species inhabiting the south part of the Balkan Peninsula. In the 17th century it was introduced to the whole of Europe and since then it has been intensively grown for the needs of horticulture and landscape architecture (Vukićević, 1997). Because of its exceptional adaptability to the prevailing ecological conditions in Serbia, it is often found in urban communities and in different categories of green areas. Its appeal for further studies is due to its decorative features, high adaptability in urban cenosis conditions, healing characteristics and as a significant source of seed material.

The seed of the horse chestnut contains a series of biologically active substances in different quantities which affect the time duration of seed preservation, its germination, and therefore the production of good quality seedlings. Regarding the high level of water and its irretrievable loss from ripening to sowing, the horse chestnut seed is considered to be a recalcitrant seed. Starch, as organic matter, has the highest incidence in the seed while fats, which play a significant role in the pharmaceutical industry, come second. Proteins, as organic matter, have the lowest incidence in the horse chestnut seed, which, besides its structure, plays a role in the prevention of excessive drying up of the seeds. A two-year research was conducted on the selected trees of secondary origin on the green areas in Bačka Palanka, Novi Sad and Sremski Karlovci. The selection included 50 individual horse chestnut trees. By narrower selection, the variability of the biochemical composition of 15 collected genotype seeds was researched along with the influence of the site of growth on the quantity of the examined substances. Our aim was to determine optimal conditions for seed preservation during winter for subsequent germination. Regarding plant production, the basic benefits of ornamental trees and undergrowth as a source of

seeds for natural and artificial population renewal are considerable (Stilinović, 1985).

MATERIALS AND METHODS

The selection of five horse chestnut trees, taken from green areas in the three towns: Bačka Palanka, Novi Sad and Sremski Karlovci, was based on the criteria for separation of the seed in horticulture (Stilinović and Tucović, 1977) as well as on their phenotypic characteristics, functional degree and aesthetic criteria on the selected green areas. The seed was collected at the stage of technical (complete) ripeness. Most of the selected trees are from tree rows, while four trees on the Novi Sad site were from distinct green areas. The collected seed material was stored and preserved in the controlled environment of a cold storage plant at a temperature of 8°C and relative moisture of 90%. The seed was kept in sand. Preservation of substrate moisture and fungicide treatment (Benomil) at a concentration of 0.03% were done regularly. Bearing in mind that the horse chestnut seed is a recalcitrant seed, in other words, that it belongs to the group of seeds that are intolerant to drying up, during preservation conditions which enabled the seed to preserve their biological, technological and thereby market value, were provided.

Testing the moisture, fat, starch and protein contents was done at the end of the seed preservation period, i.e., six months after picking. The moisture content was examined by the standard method for drying seeds in a drying room at constant temperature (103±2°C), for 17±1 h. The fat content was determined using the Soxlet method; the starch content was established by the Ewers method, while the protein content was determined from the nitrogen content by the standard method used for the determination of elements AOAC 972.43.2000, using the apparatus ELEMENTAR VARIO EL III.

The biochemical composition of horse chestnut seeds shown in the tables was analyzed using appropriate statistical methods. Data processing was performed with Statistica 9.0.

RESULTS AND DISCUSSION

The horse chestnut (*Aesculus hippocastanum* L.) showed a great variability in the biochemical composition of the seed. The content of moisture in the seed was the most significant factor for the successful conservation of recalcitrant seeds, of which the horse chestnut seed is one. To preserve this type of seeds for germination, conditions of conservation have to be provided which will not significantly affect the reduction of moisture content in the seed (Bonner et al., 1994). It has also been observed that the moisture content in the seed significantly decreases with the rise of preservation time, so that the moisture content in the seed was the lowest at the end of the preservation period, but enough to let the seed germinate normally.

The content of the basic components of the horse chestnut seed is shown in Table 1.

The content of water in the tested seed in 2008 was 36.91% on average. The BP_2 genotype had the highest moisture content in the seed (48.83%) that was statistically significantly higher in relation to the other tested genotypes of the horse chestnut. The lowest moisture content in that year of research was observed in the IV_1 genotype (22.82%). In 2009, the average moisture content in the tested seeds was lower by 3.13% compared to the previous year when it was 33.78%. The genotype BP_7 had the highest moisture content in the seeds (39.87%). This was statistically highly significant with regard to the other horse chestnut genotypes, except for the SK_4 (39.83%) where there were no statistically significant differences in the moisture content in the seeds. The sites where the seeds were collected influenced the moisture content in them. Thus, the highest average moisture percentage in both research years was registered in the genotypes taken from the Bačka Palanka site. In 2008 it was 43.00% and in 2009 it was 34.22%. This can be credited to the prevailing conditions on the sites which are, to a certain degree, under the influence of the unfavorable factors of urban areas. Using the LSD test, the existence of statistically very significant differ-

Table 1. Moisture content, starch, fat and protein in seeds collected from horse chestnut trees at three sites

Locality	Genotyp	2008. year				2009. year			
		moisture	starch	fat	proteins	moisture	starch	fat	proteins
Bačka Palanka	BP2	48,83	29,76	5,89	5,97	37,89	29,78	6,73	6,10
	BP4	42,09	40,37	5,02	6,10	39,02	38,31	3,78	6,08
	BP7	42,56	39,57	4,23	5,99	39,87	37,52	6,79	6,00
	BP12	40,06	39,04	6,13	5,92	24,13	37,48	4,10	6,02
	BP26	41,44	38,14	5,37	6,02	30,19	38,05	5,19	5,86
	Mean	43,00	37,38	5,33	6,00	34,22	36,23	5,32	6,01
Novi Sad	IV1	22,82	31,15	7,75	5,83	20,07	30,53	6,61	5,80
	IV2	22,91	30,25	7,80	5,82	30,93	31,20	5,85	5,72
	BDS1	35,87	30,92	8,14	5,93	38,36	30,18	7,26	6,05
	BDS2	35,15	30,51	7,71	6,03	33,04	31,05	7,09	5,91
	VS8	38,79	37,64	7,94	5,98	38,73	37,42	5,15	6,10
	Mean	31,11	32,09	7,87	5,92	32,23	32,08	6,39	5,92
Sremski Karlovci	SK3	42,78	29,87	6,18	6,00	36,16	30,93	7,24	5,81
	SK4	35,52	38,10	5,68	5,98	39,83	38,72	4,53	5,78
	SK5	41,33	38,75	5,31	5,90	32,95	37,69	4,16	6,10
	SK7	31,61	30,85	6,88	6,00	30,12	31,24	4,88	5,91
	SK9	31,91	30,71	5,89	5,95	35,42	31,35	5,35	6,00
	Mean	36,63	33,66	5,99	5,97	34,90	33,99	5,23	5,92
	General mean	36,91	34,38	6,39	5,96	33,78	34,10	5,65	5,95

ences between the Bačka Palanka-Novi Sad and Bačka Palanka-Sremski Karlovci sites was observed in 2008. The differences between Novi Sad-Sremski Karlovci were statistically significant. In 2009, statistically significant differences between Sremski Karlovci-Novi Sad sites were observed.

In comparison to the research results of the moisture content in the seeds of the horse chestnut, which, depending on the author ranges from 15.00% to 50.00% (Suzka et al., 1966; Bonner, 1978; Tompset et al., 1998), the moisture content in the seeds of the tested genotypes was compatible with the range as shown in the above-mentioned literature.

A number of authors (Tucović, 1983; Lemajić et al. 1985; Stilinović, 1985; Stanković, 1995), report that starch, as a reserve nutriment, is predominant in the horse chestnut seed. Starch is an energy source and is needed for complex physiological and biochemical processes during seed preservation; in other words, during stratification and germination. The starch content analysis in both research years showed that it is present in the horse chestnut seed

in significant quantities. In 2008, the average starch content in the seed was 34.38%, while in 2009 it was slightly lower (34.10%). The highest starch content in the seed in the first research year was found in the genotype BP_4 (40.37%), and this was highly statistically significant compared to most of the tested genotypes. There were no statistically significant differences in the starch content in the seeds from BP_7 (39.57%), BP_{12} (39.04%), BP_{26} (38.14%), SK_4 (38.10%) and SK_5 (38.75%). In the second year of research, the genotype SK_4 had the highest starch content (38.72%), while the lowest starch content was registered with the genotype BP_2 (29.78%). There were no statistically significant differences in the starch content in the seeds from BP_4 (38.31%), BP_7 (37.52%), BP_{12} (37.48%), BP_{26} (38.05%), VS_8 (37.42%) and SK_5 (37.49%). Regarding site levels, the starch content in the seed in 2008 was as follows: Bačka Palanka 37.38%; Sremski Karlovci 33.66%; Novi Sad 32.09%. In 2009, the average starch content by site was: Bačka Palanka 36.23%; Sremski Karlovci 34.00%; Novi Sad 32.08%. The results of the variant analysis for starch content in 2008 show highly significant differences between the sites Bačka Palanka – Novi Sad and Bačka Palanka –Sremski Karlovci, while in 2009, highly significant differences were registered among the trees on the sites Bačka Palanka – Novi Sad.

The starch content analysis in both research years shows that it is present in the horse chestnut seed in significant quantities. The highest starch content was registered in the seed collected at the Bačka Palanka site, so, in this case, it can be concluded that the modified site conditions that we find in the Novi Sad could influence starch accumulation. That starch content influences germination is shown in the germination results of the tested seed in the field and laboratory conditions. The horse chestnut genotypes BP_4, BP_7, SK_4, which have a high starch content in the seed in both research years, distinguish themselves by high germination percentages in the laboratory and in field conditions (over 70.00%).

The starch content in the tested horse chestnut seeds had a range of 29.76% to 40.37%, and this is in accordance with the literature data. Tucović (1983) reported that the horse chestnut seed contains 34% of starch, Lemajić et al. (1985) that there is 40-60% of starch, and Stanković (1995) states that there is 30-40% of starch in the horse chestnut seed.

Apart from starch, in the horse chestnut seed there are also fats. Fats in seeds of this kind are used as raw material in pharmaceutical and chemical industries. Bearing in mind that the horse chestnut seed is mostly carbohydrates, i.e. starch, it can be said that fats have a less important role in the process of providing the seed the necessary energy for germination.

The average fat content in the tested seed, in 2008 was 6.39%, while in 2009 it was 5.65%. It was registered that the genotype from the Novi Sad site had the highest fat content in both research years. The genotype BDS_1 had a statistically significant higher fat content (8.14%) compared to the other genotypes in 2008. The lowest fat content in the seed in the same research year was that of the genotype BP_7 (4.23%). In 2009, the BDS_1 tree also had the highest fat content in the seed (7.26%), while the lowest was registered in the BP_4 tree (3.78%). Regarding site level, the fat content in the horse chestnut seeds in 2008, was: Novi Sad 7.87%, Sremski Karlovci 6.00% and Bačka Palanka 5.33%. In the second research year, the average fat content in the seeds was: 6.39% (Novi Sad), 5.32% (Bačka Palanka) and 5.23% (Sremski Karlovci). Using the LSD test, statistically significant differences among all sites in both research years were established.

The examined seeds had a similar average fat content compared to previous researches. Tucović (1983) states that the horse chestnut seed has 4-6% oil or fat, Lemajić et al. (1985), 6.10-7.73%, Stanković (1995) 5.5%. In relation to Stilinović's data (1985), which states that the horse chestnut seed has 2% fat on average, the examined seed had a significantly higher average fat content.

In recalcitrant seed research, the relation between the protein content and its resistance to water loss was established. It is assumed that the proteins

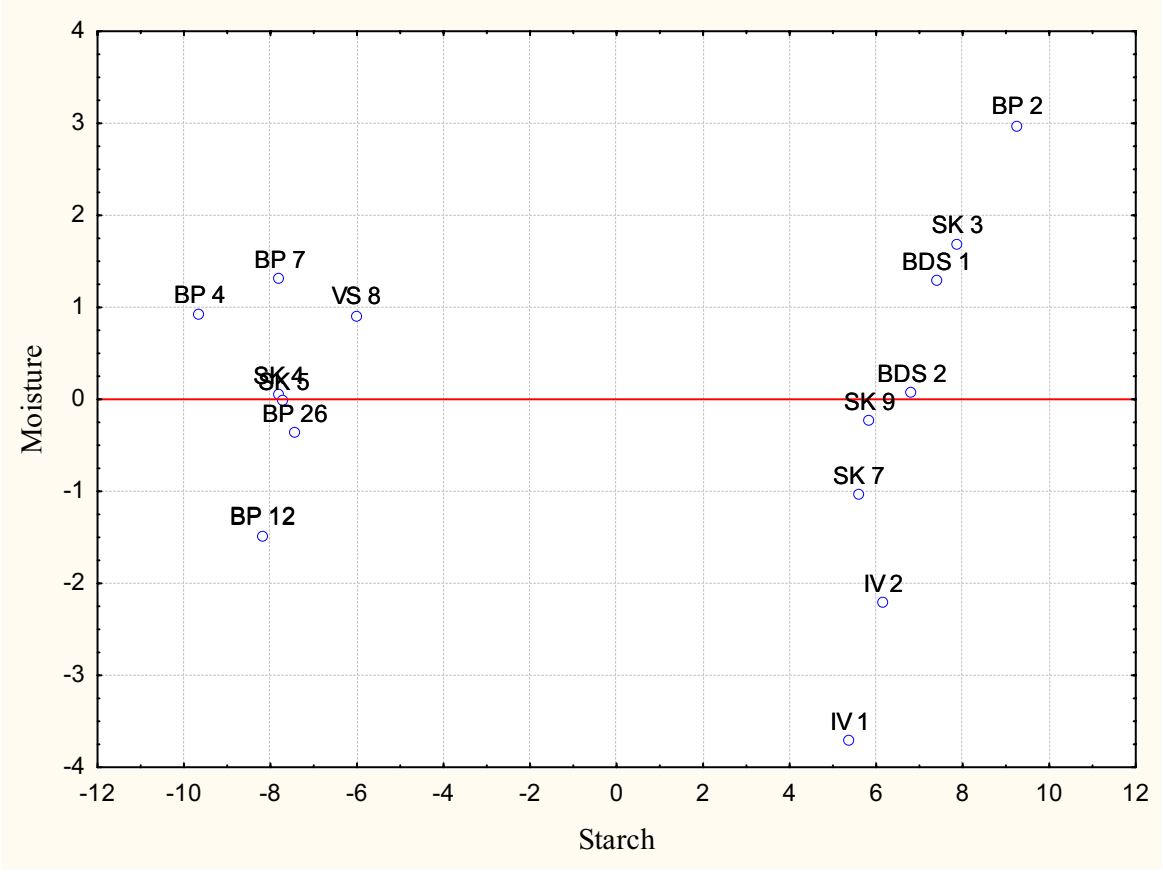

Fig. 1. Discriminant analysis Scatter plot based on investigated genotypes and parameters

in the horse chestnut seed prevent the cell damage which arises due to moisture loss in the seed (Finch-Savage *et al.*, 1994), and only seeds that contain enough moisture quantity can successfully germinate. The average protein content in the horse chestnut seed, in both research years, was 5.95%. In 2008, the genotype BP_4, with the highest protein content (6.10%) was statistically significantly higher compared to the other genotypes, except for BP_7 (6.00%), BP_{26} (6.02%), BDS_2 (6.03%), SK_3 (6.00%) and SK_7 (6.00%), where there were no statistically significant differences in the protein content in the seed. In the second research year, the genotypes with the highest protein content in the seed were BP_2, VS_8 and SK_5, all with 6.10% protein. The lowest protein content in the seed in 2008 was measured in IV_2 (5.82%). The same genotype had the lowest protein content (5.72%) in 2009. Regarding the site level, the average protein content in the seeds in 2008 was: Bačka Palanka 6.00%; Sremski Karlovci 5.97%; Novi Sad 5.92%. In 2009, the average protein content in was: Bačka Palanka 6.01%; Sremski Karlovci 5.95%; Novi Sad 5.92%. Based on the statistical analysis in both research years there were no statistically significant differences among the tested sites regarding protein content.

The average values of the protein content in the horse chestnut seed were lower in relation to the research conducted by Tucović (1983), who states that the average protein content in the seed is 15%, as well Stilinović (1985), who indicates that the average protein content in the horse chestnut seed is 7%. Regarding Stanković's research (1995), which states

Table 2. Discriminant analysis on the basis of the selected parameters. Marked loadings are >0.7000 and significant for the axis.

Features	Root 1	Root 2	Root 3	Root 4
Moisture	0.39071	1.146521*	0.040190	-0.184849
Starch	-1.13170*	-0.055072	0.160711	0.004003
Fat	0.12106	0.172396	1.136278*	0.136788
Proteins	-0.23839	0.256049	-0.066257	0.969658*
Eigenval	65.11529	3.113659	0.810791	0.091727
Cum.Prop	0.94191	0.986945	0.998673	1.000000

that the average protein content in the seed is 6%, it can be said that the protein content in the examined seed is of a similar value.

The examined trees whose seeds had the highest protein content were the BP_4, BP_7 and BP_{26} trees. It was registered that the high moisture content in the seed confirms the relation between the protein and moisture contents. Also, the mentioned horse chestnut genotypes had the high percentage of germination in both laboratory and field conditions.

By applying discriminant analysis it is possible to confirm the above results. The most important parameters to effect a separation of the selected horse chestnut trees are moisture content and starch (Table 2). In the first there are genotypes with significantly higher moisture content and starch in the seed (BP_2, BP_4, BP_7, BDS_1, VS_8, SK_3). In the other there are individual trees with significantly lower contents of the investigated seed components (IV_1, IV_2 and SK_7).

CONCLUSIONS

Due to its extraordinary adaptability to the ecological conditions in Serbia, the horse chestnut (*Aesculus hippocastanum* L.) is often found in an urban cenosis at different green area categories. In order to obtain seedlings of the best possible quality, there is a need to study the variability of its secondary origin populations and selected tree reproductive functionality.

The analysis and estimation of the all trees confirmed that most of the genotypes had adapted to the existing ecological conditions, which further justified the collection and preservation of the seeds. The biochemical analyses of the seeds, i.e., the determination of moisture, starch, fat and protein contents, confirmed the existence of the differences among individual secondary populations on the mentioned sites. Examination of the seed's biochemical composition is also an important parameter of its germination potential. The high water and starch contents in the seeds of the isolated genotypes enabled as high a percentage of germination in laboratory as under field conditions. It was also noticed that the fat content does not have a decisive influence on seed germination, but, although present in small quantities, it can serve as a source of energy and as a starting base for the synthesis of numerous substances necessary for the growth and development of the germ. Also, the horse chestnut's fat has broad uses in pharmaceutical industry. Although the fat content in the seed is relatively low, it can be easily and quickly collected in great quantities. The biological value of protein is in its role of preventing cell damage during dehydration.

Based on all examined parameters, the following genotypes stand out:

from Bačka Palanka site: BP_2, BP_4 and BP_7
from Novi Sad site: BDS_1 and VS_8
from Sremski Karlovci site: SK_4

These findings can serve as a basis for the controlled production of horse chestnut seedlings (*Aesculus hippocastanum* L.) for the needs of horticulture and landscape architecture.

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