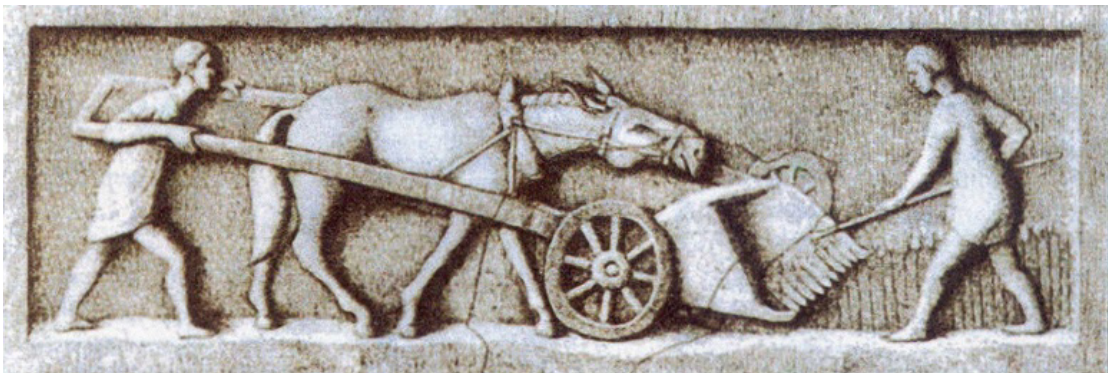
Volume 4, Number 1 (2017)

Columella

Journal of Agricultural and Environmental Sciences



Szent István University Press
Gödöllő

COLUMELLA
Journal of Agricultural and Environmental Sciences

This peer reviewed journal of the Faculty of Agricultural and Environmental Sciences of the Szent István University, Gödöllő, Hungary publishes papers in English language.

Technical assistance is provided by the respective Scientific Committees of the Hungarian Academy of Sciences.

The journal is published in yearly volumes of two issues annually.

Editor-in-chief:
Katalin POSTA

Edited by:
Zoltán KENDE

Responsible publisher:
Mihály LAJOS

Editorial correspondence:
Faculty of Agricultural and Environmental Sciences of the Szent István University

Páter Károly utca 1.
H-2100 Gödöllő, Hungary
Phone: (+36 28) 522067
Fax: (+36 28) 410-804
web: www.columella.mkk.szie.hu
E-mail: columella@mkk.szie.hu

HU ISSN 2064-7816 (print)
HU ISSN 2064-9479 (online)
DOI: 10.18380/

Printed in Hungary, Gödöllő
Printed by Szent István Egyetemi Kiadó Nonprofit Kft. (Szent István University Press)
HU-2100 Gödöllő, Páter Károly utca 1.

Columella

Journal of Agricultural and Environmental Sciences

Volume 4, Number 1 (2017)

HU ISSN 2064-7816 (print)
HU ISSN 2064-9479 (online)
DOI: 10.18380/

Editor-in-chief
Katalin POSTA

Guest editor
Márton JOLÁNKAI

Edited by
Zoltán KENDE

The edition of this volume was supported by the
Kutató Kari Kiválósági Támogatás - Research Centre of Excellence - 11476-3/2016/FEKUT

© 2017 Szent István University
Faculty of Agricultural and Environmental Sciences, Gödöllő,
HUNGARY

Authors of current volume of Columella

Zsolt ANCSIN
Eren BARIS
Janka BEDŐ
Andrea BÓCSAI
Gábor CSILLÉRY
Szilárd CZÓBEL
András DEMETER
Ogboro Samson EDOSA
Márta ERDÉLYI
Dominika FALVAI
Csaba FERNYE
Csilla FICSOR
Erzsébet KISS
Zsófia KOVÁCS
Miklós MÉZES
Péter PEPÓ
Antal SZŐKE
Petra TRENYIK
Anikó VERES

Reviewers of current volume of Columella

Ilona BANCZEROWSKI
Ernő FÜHRER
Borbála HOFFMANN
Márton JOLÁNKAI
Tamás KISMÁNYOKY
Desimir KNEŽEVIĆ
Mária KOVÁCS-WEBER
Lászlóné HAJÓS
Csaba MÁTYÁS
László MIKLÓS
András SZABÓ
Éva SZAKÁCS
Milan SKALICKÝ

Scientific board of Columella

Manuel ACOSTA (CZ)
Adalbert BALOG (RO)
Zoltán BEDŐ (H)
János BERÉNYI (RS)
Sándor CSÁNYI (H)
Dénes DUDITS (H)
Werner EUGSTER (CH)
György FÜLEKY (H)
Lajos HELYES (H)
László HESZKY (H)
István HOLLÓ (H)
László HORNOK (H)
Márton JOLÁNKAI (H)
Tamás KISMÁNYOKY (H)
Vlado KOVAČEVIĆ (HR)
Jan KUCHTÍK (CZ)
Magdalena LACKO-BARTOŠOVÁ (SK)
Éva LEHOCZKY (H)
Miklós MÉZES (H)
Erika MICHÉLI (H)
László MIKLÓS (SK)
Péter István NAGY (H)
János NAGY (H)
Zoltán NAGY (H)
Miklos NEMÉNYI (H)
Alberto PARDOSSI (IT)
János PAUK (H)
Károly PENKSZA (H)
Péter PEPÓ (H)
Katalin POSTA (H)
Péter PÓTI (H)
Erich M. PÖTSCH (A)
Rezső SCHMIDT (H)
Peter SURAI (UK)
János TŐZSÉR (H)
Béla URBÁNYI (H)
Ottó VEISZ (H)
György VÁRALLYAY (H)

© 2017 Szent István University
Faculty of Agricultural and Environmental Sciences, Gödöllő,
HUNGARY

Table of Contents

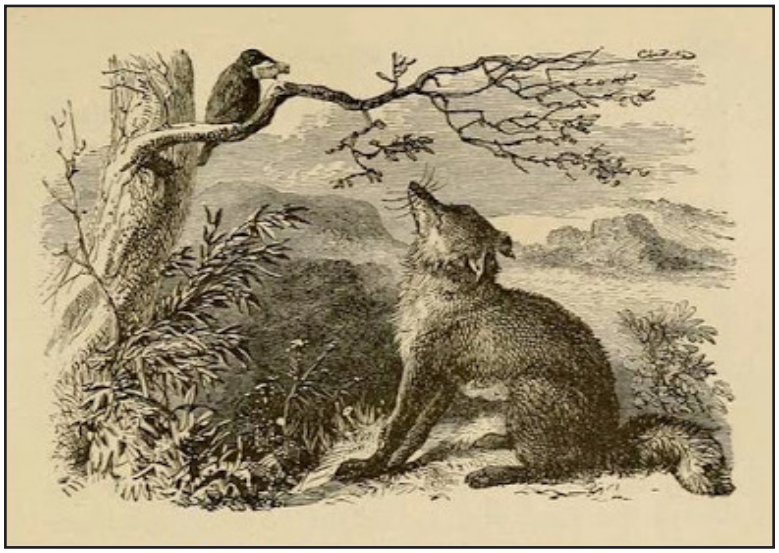
Preface
Superstition *versus* gullibility

Emerging from the cradle of humankind we have to face an evergreen problem of our mental boundaries – namely how to define truth, and how to make distinction between good and bad.

Science is the way and it also represents the means to learn more about the relation of the world and ourselves. What is science? Branch of knowledge involving systematized observation of, and experiment with phenomena. Simply we seek, we learn. That may be enough to define the truth, however will not enable us to determine the differences between the good and the bad. The whole story can be read in the Bible written in a sort of a philosophic manner in the Book of Genesis when it tells us that God placed human being in the **Garden of Eden,** in the middle of which stood "the tree of knowledge of good and evil" (2:17). The Garden of Eden was lost, and so the knowledge of the distinction between good and bad will never be delivered to us, and all members of the human race have to solve this problem themselves. How? Just in a primitive but practical way; by getting acquainted with the facts.

Our intellectual activities are influenced by two serious factors. One of them is superstition. The definition of superstition is as follows: a belief or practice resulting from ignorance, fear of the unknown, trust in magic or chance, or a false conception of causation. In other assessment superstition is any belief or practise that is irrational i.e., a misunderstanding of science or causality, a positive belief in fate or magic, or furthermore – accepting theories, ideas or advices as if they were proven principles.

The latter is the case when we face the other influential factor – gullibility. Gullibility is a failure



The story of the crow and the fox in the fables of La Fontaine is an etalon of gullibility.

of social intelligence in which a person is easily tricked or manipulated into an ill-advised course of action. It is closely related to credulity, which is the tendency to believe unlikely propositions that are unsupported by evidence. Classes of people especially vulnerable to exploitation due to gullibility include children, the elderly and the developmentally disabled and in general all people with no or insufficient educational background.

Apart from the credulity problems of ourselves that may emerge from lack of information escorted by our personal goodwill and open minded behaviour, there is a special field of gullibility when intellectual and educational positions may fail or even can be ruined.

One of the most profound philosophic pamphlets ever written about gullibility is the book of Jonathan Swift edited in the 18th Century. Even the name of his hero "Gulliver" was chosen to label the story. From among the specific characters of the book, Gulliver himself was given a role to be the narrator of the social adventures described. "Although Lemuel Gulliver's vivid and detailed style of narration makes it clear that he is intelligent and well educated, his perceptions are naïve and gullible. He has virtually no emotional life, or at least no awareness of it and his comments are strictly factual. Indeed, sometimes his obsession with the facts of navigation, for example, becomes unbearable for us. Gulliver never thinks that the absurdities he encounters are funny and never makes the satiric connections between the lands he visits and his own home. Gulliver's naïveté makes the satire possible, as we pick up on things that Gulliver does not notice."

At this point we return to the initial question again; what is the way and what are the means of approaching truth? I believe it is science in general, and the dissemination of scientific results in a controlled, broad and open access way to the public. Our journal, Columella provides a forum for scientific publications in the field of agricultural and environmental sciences. The journal of one of the most ambitious agricultural faculties of Hungary has a mission to disseminate novel research results in favour of improving our world.

The editors would say thanks to the authors of the present issue, and also welcome the readers who may read, use and broadcast the scientific information compiled. Also we do hope that they may become future authors as well.

Márton Jolánkai

References

Greenspan, S. (2009): Annals of gullibility: why we get duped and how to avoid it. Praeger Publishers, ISBN 978-0-313-36216-3

La Fontaine, J. (1922): La Fontaine összes meséi. Dante Kiadó Budapest

SparkNotes Editors. (2003): SparkNote on Gulliver's Travels. Retrieved July 3, 2017, from http://www.sparknotes.com/lit/gulliver/

Swift, J. (1726): Gulliver's Travels. Benjamin Motte, London.

Some chemical and physical characteristics of farmed pheasant hens (*Phasianus cholchicus*) breast meat

Csaba FERNYE – Márta ERDÉLYI – Zsolt ANCSIN – Andrea BÓCSAI – Miklós MÉZES

Szent István University, Faculty of Agricultural and Environmental Sciences, Department of Nutrition, H-2103 Gödöllő, Páter Károly u. 1., Hungary; E-mail: fernye.csaba@gmail.com

Abstract: Ring necked pheasant is the most significant game bird in Hungary. Around 300.000 pheasant harvested (hunted) annually and generally these birds are consumed by the hunters. As there are limited data on the quality of pheasant meat, in the present study we aimed to analyze some physical and chemical properties of it. At 20 weeks of age 63 pheasant hens were exterminated by cervical dislocation and meat, liver, spleen and heart samples were taken. The live weight of the birds was 1045±92g (870 to 1300g). The average weight of the liver, spleen and heart was 14.12±2.58, 0.47±0.13 and 4.30±0.49, respectively. The average drip loss was 5.90±2.38% (0.68±0.28g). As was expected the average protein content (26.2±0.7%) of the pheasant breasts was markedly higher than in broiler or turkey. The average fat content (0.4±0.2%) was similar to that in turkey. The unique chemical and physical properties of the pheasant meat make it suitable to fit in the human nutrition.

Keywords: pheasant, tenderness, drip loss, colour, chemical composition

Introduction

Ring necked pheasant is the most significant game bird in Hungary with the estimated population of 630.435 individuals (Csányi *et al.* 2016). Around 300.000 pheasants are harvested (hunted) annually, and most of them are consumed by the hunters. It is a widespread opinion that game meat has high protein, but low fat content (Aidoo and Haworth 1995; Crawford 1968). It also has to be highlighted that chemical composition of meat is not a constant attribute, but is known to be affected by season (Smankó *et al.* 2007), gender (Piaskowska *et al.* 2015; Purchas *et al.* 2010), age (Dannenberger *et al.* 2013) and type of muscle (Razmaité *et al.* 2015).

However, there is only limited information available on the chemical composition of pheasant meat. According to the results of Straková *et al.* (2011) 93.72 % protein and 2.95 % fat were present in the breast meat on dry matter bases, while the thigh had 78.18 % protein and 16.37% fat content. The same parameters on wet matter bases are between 20.73% protein and 0.13% fat for the breast, while 25.66% protein and 3,9% fat for the thigh, respectively (Severin *et al.* 2006; Hofbauer *et al.* 2010; Franco and Lorenzo 2013). These parameters can vary between farmed and wild pheasants, as well. According to Saeki and Kumagai (1990) and Tucak *et. al.* (2004, 2008) the meat of the farmed pheasant contains less protein, but more fat, compared to the wild birds. On the other hand, Hofbauer *et al.* (2010) did not confirm these differences between the wild and farmed pheasants. Even less data are available about the physical characteristics of pheasant meat, such as color, pH, water holding capacity and tenderness. Similarly to the meat of slaughtered animals (Fletcher, 1999; Bendall, 1988; Mach *et al.* 2008) pheasant meat has a slightly acidic pH (5.66-6.03) (Hofbauer *et al.* 2010). The drip loss ranges from 1 to 3% and an average of 30 N/cm^2 shear force characterizes the tenderness (Hofbauer *et al.* 2010).

The purpose of the present study was to provide further data on the chemical and physical attributes of farmed pheasant hen breast meat.

Materials and methods

Housing and feeding

A total of 63 pheasant hens at 16 weeks of age were kept in an aviary with 2 m^2/bird stocking density for 4 weeks. Feed and water were provided *ad libitum*. The feed was supplied in mashed form, and its guaranteed chemical composition (Vitafort Zrt. Dabas) is presented in Table 1.

DOI: 10.18380/SZIE.COLUM.2017.4.1.7

Table 1. Chemical composition of the diet

Metabolisable energy (MJ/kg)	10.69
Crude protein (g/100g dry matter)	19.34
Crude fat (g/100g dry matter)	2.90
Crude fiber (g/100g dry matter)	4.10
Crude ash (g/100g dry matter)	7.20
Lysine (g/100g dry matter)	0.95
Methionine (g/100g dry matter)	0.45
Ca (g/100g dry matter)	1.02
P (g/100g dry matter)	0.70
Na (g/100g dry matter)	0.15

Sampling

At 20 weeks of age individual live weight of the pheasants was measured. Then the birds were exterminated by cervical dislocation and exsanguination and *post mortem* meat, liver, spleen and heart samples were taken, and their weight was determined. Besides absolute organ weight, relative weight of the organs was also calculated, to normalize the variability due to different body weight. The physical and chemical characteristics of the meat were determined in the breast (*m. pectoralis major*) of the birds. For chemical analyses and tenderness measurements the samples were stored at -20°C until analysis, while the other measurements were made on fresh or chilled (4°C) samples.

Physical characteristics

The pH, colour and water holding capacity were tested on meat samples from 43 pheasants. After the slaughter both pectoral muscles (left and right) were removed from the carcass. The left muscles were tested for pH and colour, while the right muscles were used to determine the water holding capacity.

Measurements of the pH were carried out with WTW pH 330 (Weilheim, Germany) sensor simultaneously with colour measurements.

Water holding capacity (WHC) is normally described with drip loss analysis. For this purpose the small pectoral muscle (*m. pectoralis minor*) was removed from the right breast and modified Honikel (1998) method was used to determine weight loss. Thus the samples were pierced and hanged in the fridge at 4 °C and left there for 96 hours. Weight of the samples was measured at hanging (0) and 96 hours later and drip loss was calculated accordingly (Lesiak *et al*. 1995).

Objective colour analysis was done with Minolta CR-300 chromameter on the fresh cut surface of the muscles at 0 and 36 hour *post mortem*. This device works with reflectance spectrometry and the evaluation was done with the use of CIELAB (Commission Internationale de l'Eclairage, Paris, France, 1976) coordinates of lightness (L*), redness (a*) and yellowness (b*). For tenderness analysis the left pectoral muscle of 20 separate birds were taken (the same bird were used in chemical analysis). After weighing, the muscles were fried in an electric contact griller until their core temperature reached 72°C. Tenderness was tested on the grilled meats after a short cool-down period (until the samples reached room temperature). The samples were cut into prismatic slices with a cross section of 1cm^2. The slices were tested with a TA.XT. plus Texture Analyser (Stable Micro Systems, Godalming, United Kingdom) attached with a Warner-Bratzler device. The shear blade (1.016 mm thick) had vee-shaped 60° angle. The blade was moving with 2 mm/sec speed. Three cuts were made on each muscle prism and the mean value of the cuts was recorded.

Chemical composition

Due to the number of different tests the samples for chemical analysis (and tenderness) had to be taken from 20 separate pheasant hens. After slaughtering the right pectoral muscle was removed, packed in plastic bags, and stored at -20^{o}C for further processing. Before analysis, the samples were thawed to room temperature and all visible adipose and connective tissue was cut away, then the meat was ground and homogenized. Dry matter content was measured by drying at 105°C up to constant weight (MSZ ISO 1442 - 2000). The crude protein content was determined with Kjeldahl method (MSZ 5874/8-78). Soxhlet extraction with hexane as solvent (MSZ ISO 1443-2002) was used for the total lipid content analysis, while ash content of the meat was measured with incineration at 550 °C to constant weight (MSZ ISO 936).

DOI: 10.18380/SZIE.COLUM.2017.4.1.7

Statistical analysis

Correlation coefficient for pH and drip loss was generated using the Pearson's Correlation Coefficient in GraphPad InStat 3.05 software (GraphPad Software, San Diego).

Results and discussion

Physical characteristics

The average live weight of the birds was 1045±92g (ranged from 870 g to 1300 g). In previous reports of Tucak *et al.* (2008) (970±157 g) and Hofbauer *et al.* (2010) (912±142 g) slightly lower weights were outlined, but accurate comparison is not possible, because the age and diet of the birds was not defined in those articles. However, Kuzniacka and Adamski (2010) found 978±10.5 g average live weight at 24 weeks of age using a finisher diet from the 17th week of growing period with higher energy, but somewhat lower protein content than in our investigation. The difference was only 67 g lower than in our investigation. Altogether, it can be said that growth performance of our pheasant hens can be considered as typical for the species.

The weight of the breast muscles was comparable to the earlier findings of Hofbauer et al. (2010), however, both the absolute (177.72±24.33 g) and the relative breast weight (17.70±2.22%) were markedly lower than those in the report of Tucak *et al.* (2008). The average weight of the liver spleen and heart were 14.12±2.58 g, 0.47±0.13 g and 4.30±0.49 g, respectively. Interestingly, in earlier studies Szabó *et al.* (2006; 2010) found different weights for liver and heart (18.6±2.29 g, 2.1±0.3 g respectively). However, these results have been found in adult males, and the age of the birds is not known. Other authors presented results about the weight of visceral organs together, such as "edible viscera" (Hofbauer *et al.* 2010) and "liver and heart" (Tucak *et al.* 2008) accurate comparison between these parameters can not be done. The only report available on female pheasants with separate organ weight was published by Straková *et al.* (2005), and they found 15.89 g for liver and 5.58 g for heart. Relative organ weights calculated from the data given in the cited article are 1.91% and 0.67% for liver and heart, respectively. In our experiment relative weight of liver was found to be 1.35% and 0.41% for the heart. Referred to the fact that the measurements of Straková *et al.* (2005) were done at 13 weeks of age, our results are considered to be below the literature data.

Considering quality traits of the breast meat, slight increase was found in the pH during the 36 hours of chilling period (Table 2.).

Table 2. Colour and pH values of the meat samples 0 and 36 hour post mortem (n=43)

	L* (Lightness)	a* (Redness)	b* (Yellowness)	pH
0h	49.27±4.25	5.84±2.17	6.55±1.90	5.52±0.14
36h	48.53±3.82	5.61±1.78	7.59±2.74	5.67±0.19

However, even after culling the pH was as low as 5.52 and remained below 6.0 after 36 hours of storage. This data is important, because it is known that when the pH is lower than 6.0 the muscle protein denaturation may increase. The pH values found in our investigation were similar to those of Kokoszynski *et al.* (2012) for 16-week-old hens (5.87±2.60) or by Hofbauer *et al.* (2010) for pheasant (5.55±0.16). This pH range, between 5.6 to 6.0 is considered to be normal for raw poultry meat (Fletcher, 1999). The average weight of the small pectoral muscles was 11.87±2.78 g and the average drip loss was 5.90±2.38% (0.68±0.28 g), which is notably higher as was found by Hofbauer *et al.* (2010) in pheasant (2.19±1.37%). Corresponding values were found in pale broiler breast fillets (Woelfel *et al.* 2002) and turkey breast stored at high temperature (30^0C) (Lesiak *et al.* 1996). According to the results of Hofmann (2004) the lower pH in the muscle has correlation with higher drip loss during storage. There was a moderate negative correlation ($r = -0.4421$) between the pH and drip loss of the meat samples ($p < 0.01$). However, other factors may also contributed resulting higher drip loss such as stress during the handling and slaughtering. Game meats are typified with a deeper red colour than that of the meat of other livestock animals. Our results (Table 2) partially confirmed this concept as - even with the dominant yellowness (b*) -

the redness (a*) values were markedly higher than in broiler meat (Allen *et al.* 1997; Fletcher 1999). Turkey meat however poses similar redness; while its yellowness is considerably lower (Fraqueza *et al.* 2006). However, the average redness (a* value) was slightly higher than in other pheasant hens (Hofbauer *et al.* 2010). Considerably higher red (a* 16.5 and 18.1) values were revealed in different genetic groups of pheasants at 16 weeks-of-age by Kokosznynski *et al.* (2012), while lightness (L*) and yellowness (b*) values were similar.

According to the shear force results (2.62±0.93kg) of present study, the pheasant meat was found to be slightly less tender than the broiler chicken (1.98-2.10 kg) (Castellini *et al.* 2002) or the turkey meat (1.69 kg) (Ngoka *et al.* 1982). However, our result was still below as compared to previous literature data for the tenderness of pheasant meat as 2.9-3.2 kg value as was reported Hofbauer *et al.* (2010). These characteristics could be hypothetically attributed to the higher proportion of connective tissue, and increased heat stable cross-links between collagen fibrils and subsequent tensile strength in different species and ages (Owens *et al.* 2004).

Chemical composition of meat

As was expected, the average protein content (26.2±0.7%) (Table 3.) of the pheasant breasts was markedly higher than in broiler chicken (Castellini *et al.* 2002) or turkey breasts (Ngoka *et al.* 1982). The average protein value in our study was even higher than in the earlier studies (25.38±0.68 and 25.03±1.08 respectively) of Tucak *et al.* (2008) and Hofbauer *et al.* (2010). Hofbauer *et al.* (2010) mentions that higher protein content can be associated with higher dry matter (lower moisture content) and lower fat content. In our case, the dry matter content (27.2±0.5%) of the samples was even lower than the values found by other authors. The average fat content (0.4±0.2%) was similar to that in turkey (Ngoka *et al.* 1982) and it was in accordance with the earlier findings of Hofbauer *et al.* (2010) and Straková *et al.* (2011) in pheasant. According to the latter literature, the reported chemical composition of pheasant meat showed 5.16% higher protein and 55.3% lower fat content compared to broiler chicken breast meat. There is a notable difference in the crude ash content, as well. Our results (1.9±0.4%) were almost two times higher than in other pheasants (Tucak *et al.* 2008) or turkey (Ngoka et al. 1982) and three times higher than in broiler chicken (Castellini *et al.* 2002). However, ash content of pheasant meat is normally similar or even lower than that of the broiler meat (Straková *et al.* 2011; Hofbauer *et al.* 2010).

Table 3. Chemical composition and tenderness properties of pheasant meat (n=20)

	Mean	SD
Shear force (kg)	2.62	0.93
Dry matter (g/100g)	27.2	0.5
Crude protein (g/100g)	26.2	0.7
Crude fat (g/100g)	0.4	0.2
Crude ash (g/100g)	1.9	0.4

Conclusions

Quality of game meat is normally different than that of other livestock meat. Only a few data are reported in the literature on sensory value and chemical composition of pheasant meat. Furthermore, intrinsic (age, sex, genotype) and environmental factors (feeding, housing, culling) are quite different in the various experiments, therefore no consistent meat quality data are available and our results might help to determine the normal range for certain sensory properties and macronutrient content of the pheasant meat.

Our results mostly agree with the previous findings of Hofbauer *et al.* (2010), except a major difference in the shear force and ash content among the analysed parameters. However, high level of discrepancies were found when our data were compared with other literature sources (Franco and Lorenzo 2013; Straková *et al.* 2005). These variances might be at least partially due to the different age of birds, and the different growing, feeding and slaughtering conditions.

Considering the sensory parameters pheasant meat shows major difference from that of the intensive poultry species. Drip loss was much

DOI: 10.18380/SZIE.COLUM.2017.4.1.7

higher and as juiciness of the meat is determined by its water holding capacity, juiciness is poor for pheasant meat. This finding agrees with the results of tenderness analysis, with higher shear force. Finally, our colour results have confirmed that game meat is more red than the meat of other domesticated avian species.

It was proven in our experiment, that pheasant breast meat has low fat and high protein content. However, crude ash data are quite contradictory. To clarify the reason of the variance among the different literature data further research is to be done. Altogether, pheasant meat is different in its sensory attributes and chemical composition from the meat of commercial poultry species, but it is a good candidate to fit in the human nutrition as it has good protein, low fat content and preferable red colour. To increase its consumption further analysis of quality traits and the influencing factors is needed.

Acknowledgement

The present study was supported by the Research Centre of Excellence 9878-3/2016 FEKUT grant.

References

Aidoo, K. E., Haworth, R. P. (1995). Nutritional and chemical composition of farmed venison. Journal of Human Nutrition and Dietetics. **8**: 441-446.doi: http://dx.doi.org/10.1111/j.1365-277X.1995.tb00339.x

Allen C. D., Russel S. M., Fletcher D. L. (1997). The relationship of broiler breast meat color and pH to shelf-life and odor development. Poultry Science. **76**: 1042-1046. doi: http://dx.doi.org/10.1093/ps/76.7.1042

Bendall J. R. (1988). A review of the relationship of pH with physical aspects of pork quality. Meat Science. **24**: 85-126. doi: http://dx.doi.org/10.1016/0309-1740(88)90052-6

Castellini C.,Mugnai C., Dal Bosco A. (2002). Effect of organic production system on broiler carcass and meat quality. Meat Science. **60**: 219-225. doi: http://dx.doi.org/10.1016/S0309-1740(01)00124-3

CIE (Commission Internationale de l'Eclairage). 1976. Recommendations on uniform color spaces-color difference equations, Psychometric Color Terms. Supplement No. 2 to CIE Publications No. 15 (E-1.3.1.) 1978, 1971/ (TC-1-3). Commission Internationale de l'Eclairage, Paris, France.

Crawford, M. A. (1968). Fatty-acid ratios in free-living and domestic animals. Possible implications for atheroma. The Lancet,29**1 (7556)** 1329-1333. doi: https://doi.org/10.1016/s0140-6736(68)92034-5

Csányi, S., Kovács, I., Csókás, A., Putz, K. and Schally. G. (ed.) (2016). Vadgazdálkodási Adattár - 2015/2016. vadászati év. Országos Vadgazdálkodási Adattár, Gödöllő.

Dannenberger D., Nuernberg G., Nuernberg K., Hagemann E. (2013): The effects of gender, age and region on macro- and micronutrient contents and fatty acid profiles in the muscles of roe deer and wild boar in Mecklenburg-Western Pomerania (in Germany). Meat Science. **94:**39-46. doi: https://doi.org/10.1016/j.meatsci.2012.12.010

Fletcher D. L. (1999). Broiler breast meat color variation, pH, and texture. Poultry Science **78:** 1323-1327. doi: https://doi.org/10.1093/ps/78.9.1323

Franco D., Lorenzo M. (2013): Meat quality and nutritional composition of pheasant (Phasianus colchicus) reared in an extensive system. British Poultry Science **54**: 594-602. doi: https://doi.org/10.1080/00071668.2013.828195

Fraqeza M. J., Cardoso A. S., Ferriera M. C. and Barreto A. S. (2006). Incidence of pectoralis major turkey muscle with light and dark color in a Portuguese slaughterhouse. Poultry Science 85: 1992-2000. doi: https://doi.org/10.1093/ps/85.11.1992

Hofbauer D., Smulders F. J. M., Vodnansky M., Paulsen P., El-Ghareeb W. R. (2010): A note on meat quality traits of pheasants (Phasianus colchicus) European Journal of Wildlife Research **56**: 809-813. doi: https://doi.org/10.1007/s10344-010-0396-7

Hofmann K. (2004): What is quality? Definition, measurement and evaluation of meat quality. Meat Focus International. **3**:73-82.

Honikel K. O. (1998): Reference methods for the assessment of physical characteristics of meat. Meat Science **49**: 4. 447–457. doi: https://doi.org/10.1016/s0309-1740(98)00034-5

Kuzniacka J., Adamski M. (2010): Growth rate of body weight and measurements in pheasants reared up to the 24th week of life. Archiv für Tierzucht **53**: 360-367.

Kokoszynski D. Bernaczki Z., Duszynski L. (2012): Body conformation, carcass composition and physicochemical and sensory properties of pheasants of different origin. Czech Journal of Animal Science **57**:115-124.

Lesiak M. T., Olsoqb D. G., Lesiak C. A. and Ahd D. U. (1996) Effects of post mortem temperature and time on the water-holding capacity of hot-boned turkey breast and thigh muscle. Meat Science. **43**: 51-60. doi: https://doi.org/10.1016/0309-1740(95)00062-3

Mach N., Bach A., Velarde A. and Devant M. (2008). Association between animal, transportation, slaughterhause practice and meat pH in beef. Meat Science. 78: 232-238. doi: http://dx.doi.org/10.1016/j.meatsci.2007.06.021

MSZ 5874/8-78. Hungarian National Standard. Analytical methods for meat products – Protein analysis [In Hungarian].

MSZ ISO 1442:2000: Hungarian National Standard. Analytical methods for meat products – Analysis of moisture content [In Hungarian].

MSZ ISO 1443:2002: Hungarian National Standard. Analytical methods for meat products – Analysis of total fat content [In Hungarian].

MSZ ISO 936. Hungarian National Standard. Analytical methods for meat products – Analysis of crude ash content [In Hungarian].

Ngoka D. A., Froning G. W., Lowry S. R. and Babji A. S. (1982). Effect of sex, age, preslaughter factors and holding conditions on the quality characteristics and chemical composition of turkey breast muscles. Poultry Science. 68: 1996-2003. doi: https://doi.org/10.3382/ps.0611996

Owens C.M., Cavitt L.C., Meullenet JF.C. (2004): Tenderness evaluation in poultry meat. Proceedings of the 57th American Meat Science Association Reciprocal Meat Conference. Lexington, KY, USA. pp. 115-121.

Piaskowska N., Daszkiewicz T., Kubiak D., Janiszewski P. (2015):The effect of gender on meat (*Longissimus Lumborum* muscle) quality characteristic in fallow deer (*Dama dama L.)* Italian Journal of Animal Science **14**: 389-393. doi: https://doi.org/10.4081/ijas.2015.3845

Purchas R.W., Triumf E.C, Egelandsdal B. (2010): Quality characteristics and composition of the longissinus muscle in the short loin from male and female farmer red deer in New Zealand. Meat Science. **86**: 505-510. doi: https://doi.org/10.1016/j.meatsci.2010.05.043

Razmaité V., Siukscius A., Pileckas V., Svirmickas G.J. (2015): Effect of different roe deer muscles on fatty acid composition in intramuscular fat. Annals of Animal Science **15**: 775-784. doi: https://doi.org/10.1515/aoas-2015-0012

Saeki K., Kumagai H. (1990): Nutritional composition of tissues of wild and bred pheasants. Journal of Food Hygiene Society of Japan **31**: 522-526. doi: https://doi.org/10.3358/shokueishi.31.522

Severin K., Mašek T., Horvatek D., Konjević D., Janicki Z., Cvrtila Ž., Kozačinski L., Hadžiosmanovič L. and Barić-Rafaj R. (2006). The effects of castration on the growth parameters, carcass yield and meat chemical composition of intensively reared common pheasant (Phasianus colchicus colchicus L.). Italian Journal of Animal Science. **5**: 213-219. doi: http://dx.doi.org/10.4081/ijas.2007.213

Smanko T., Góreczka J., Korzeniowska M., Maliski A., Eeremenko E. (2007): Comparison of chosen quality parameters of meat from wild boar and domestic pigs. Polish Journal of Food and Nutrition Science **57**:523-528.

 DOI: 10.18380/SZIE.COLUM.2017.4.1.7

Straková E., Vitula F., Suchy P., Vecerek V. (2005): Growth intensity and carcass characteristics of fattened pheasant poults. Krmiva **2**:73-82.

Straková E., Suchy P., Karásková K., Jámbor M., Navrátil P (2011): Comparison of nutritional values of pheasant and broiler chicken meats. Acta Veterinaria Brno **80**:373-377. doi: https://doi.org/10.2754/avb201180040373

Szabó A., Fébel. H., Mézes M., Balogh K., Horn P. and Romvári R. (2006). Body size related adaptations of the avian myocardial phospholipid fatty acyl chain composition. Comparative Biochemistry and Physiology, part B **144**: 496-502. doi:10.1016/j.cbpb.2006.05.001

Szabó A., Mézes M., Romvári R. and Fébel H. (2010). Allometric scaling of fatty acyl chains in fowl liver, lung and kidney, but not in brain phospholipids. Comparative Biochemistry and Physiology, part B **155**: 301-308. doi:10.1016/j.cbpb.2009.11.018

Tucak Z, Skrivanko M, Posavcevic S, Periskic M, Boskovic I, Jumic V (2008): The influence of keeping pheasants in captivity vs. nature on the biological value of meat and its use in human nutrition. Colloquium on Antropology **32**: 959-962

Tucak Z., Skrivanko M., Krznaric M., Posavcevic S. and Boskovic I. (2004). Indicators of biological value of the pheasant meat originated from natural and controlled breeding. Acta agriculturae slovenica**, Suppl 1** 87–91.

Woelfel R. L., Owens C. M., Hirschler E. M., Martinez-Dawson R., and Sams A. R. (2002). The characterization and incidence of pale, soft, and exudative broiler meat in a commercial processing plant. Poultry Science 81:579–584. doi: https://doi.org/10.1093/ps/81.4.579

Ecological indicator based comparative study of tree of heaven (*Ailanthus altissima*) stands' herb layer

András DEMETER – Dominika FALVAI – Petra TRENYIK – Szilárd CZÓBEL

Department of Nature Conservation and Landscape Ecology, Faculty of Agricultural and Environmental Sciences, Szent István University, H-2100 Páter Károly u. 1. Gödöllő, Hungary. Email: demetex@gmail.com

Abstract: Tree of heaven (*Ailanthus altissima*) is one of the most dangerous and spread woody-stemmed invasive plant species in Hungary. By its nature it transforms its environment and reduces the biodiversity of the area. These processes can be observed studying species composition of habitats heavily infected by tree of heaven. In our research we studied the herb layered species composition of plant communities that were tree of heaven dominated in canopy in Fót, Gyermely, Makád and Tököl. After determining the species associated Borhidi's relative ecological indicators and Raunkiaer's life-forms have been applied and analysed for the distribution of the categories. Accordingly most of the species occurring in the herb layer of studied areas were herbaceous. In these stands disturbance tolerant species, generalists and weed species are common, but more, aggressive competitors also appear. These species are indicators of degraded and disturbed habitats however there was also a protected area (Fóti-Somlyó). Summing the indicators of relative temperature figures it can be stated that most of the species indicated the relative temperature demand of submontane broad-leaved forests. According to wetness requirement of the species, studied tree of heaven stands were semihumid. Looking at the relative nitrogen figures, species show a wide variety, but most of them indicate a moderately nutrient-rich, or richer habitat. On herb layer halflight plant species prevailed which indicates that these tree of heaven infected stands were less closed, allowing more light to come down to the herb layer. In continentality suboceanic and intermediate types dominated. The majority of the species is halophob, that does not tolerate the salty environment.

Keywords: tree of heaven, stands, herb layer, relative ecological values, life-form

Introduction

By development of transport, trade, industry and agriculture man dragged or immigrated more and more animal and plant species into areas far from these species habitats. These species often become successful invaders and by its strong spreading and environmental conversion activities threaten the ecosystem of the habitats. In our days invasive species connected conservational and economical losses are so huge, that to fight them became necessary (Csiszár 2012).

Mihók *et al.* (2014) during their research – in which they collected the currently most important questions of the Hungarian conservation – invasive species also came to the focus moreover tree of heaven has been highlighted. In 1998 in a workshop in Jósvafő this species was ranked among the 36 most dangerous species in Hungary (MÁT 2007). Tree of heaven is also on the list of Europe's 100 most dangerous invasive species compiled by DAISIE (Delivering Alien Invasive Species Inventories for Europe) (2006) and has the area of temperate and Mediterranean climate of the 5 continents (Kowarik & Böcker 1984).

Today tree of heaven can be found in a large part of Hungary, mostly on warmer hilly and plain areas (Udvardy & Zagyvai 2012), and it is present in a total of 12 different A-NER habitat types as one of the 3 most spread invasive plant species (Demeter *et al.* 2016). In Hungary it is receiving increasing attention, several publication has published about its spread, damage and potential usability. These have been summarized by Csiszár (2007) earlier and by Demeter and Czóbel (2016) last time. Most intensively Udvardy went in for ecological effects of the species. His results prove that tree of heaven transforms its environment and in these infected stands proportion of specialists is decreasing and proportion of competitors is increasing. It happens first because of allelopathic compounds releasing from its roots, and later by increasing shading than by the nitrogen enrichment effect of the large amount of falling leaves. In these stands nitrophilic, disturbance tolerant, shadow tolerant plant species appear with time (Udvardy 1997; 1998a; 1998b; 2004; Udvardy

& Facsar 1995; Udvardy & Zagyvai 2012). In this article we study the herb layer of areas heavily infected by tree of heaven and we typify it based on Borhidi's (1995) ecological indicators of plant species found there.

Material and methods

For our research we looked for areas heavily infected by tree of heaven with different characteristics and a minimum of 70 % tree of heaven coverage in the canopy. Besides this we wanted the study areas not to be close to the roads in order to avoid the effect of this kind of disturbance on our results. Based on recommendation from the professionals of Pilisi Parkerdő a total of 21 quadrats have been marked out in four areas: Foti-Somlyó, Gyermely, Makád and Tököl in which investigations were conducted. The quadrats of Fót had southern and western exposure with 0-40° slope and altitudes of 179 to 199 m. Tree of heaven trees had an average height of 15 m, 3,5-14,2 cm average and 6-43 cm maximum stem diameters at breast height, seed and sprout origin estimated. The quadrats of Gyermely had southern and eastern exposure with 5-40° slope and altitudes of 183 m. Tree of heaven trees had an average height of 17-20 m, 15,4-24,9 cm average and 25-33,5 cm maximum stem diameters at breast height, seed origin estimated. The quadrats of Makád had eastern exposure with 0-2° slope and altitudes of 96 m. Tree of heaven trees had an average height of 16-18,5 m, 10,5-12,8 cm average and 14-22 cm maximum stem diameters at breast height, seed origin estimated. The quadrats of Tököl were flat and had altitude of 100 m. Tree of heaven trees had an average height of 7-9,5 m, 3-4,5 cm average and 4,8-8 cm maximum stem diameters at breast height, seed origin estimated. After choosing the study areas we marked the corners of the typically square shaped, 10 square meter sized quadrats visibly with colourful marker, than we recorded the GPS coordinates of their center. For this we used Garmin eTrex 20x. Thereafter we estimated % of coverage total and for each representing species for each layer in the quadrats. Exposure, gradient, typical height of the trees and estimated origin of tree of heaven stands have also been recorded for each quadrat. It was important to wait until tree of heaven is completely leafy so data recording has been carried out on 07.06.2016 in Fót, 06.07.2016 in Gyermely and on 20.07.2016 in Makád and Tököl. The data were organized and arranged in Microsoft Excel program. From Borhidi' ecological indicators SBT (Social Behaviour Type), TB (relative temperature figures), WB (relative moisture figures), NB (nitrogen figures), LB (light figures), KB (continentality figures), SB (salt figures) and Raunkier's life-form type have been assigned to the species occurred in our quadrats. After these – managing the different indicators separately – by summarizing data we could typify the vegetation of studied tree of heaven stands. Figures were prepared by SigmaPlot 8.0 program.

Results and discussion

SBT (Social behaviour type)

Plant species found in the studied areas could be classified to 10 groups (Figure 1.) From these, disturbance tolerant species (DT) and generalists (G) were represented with the highest number of species, but proportion of native weed species (W) in Fót and alien competitors (AC) in species poor Tököl was also significant.

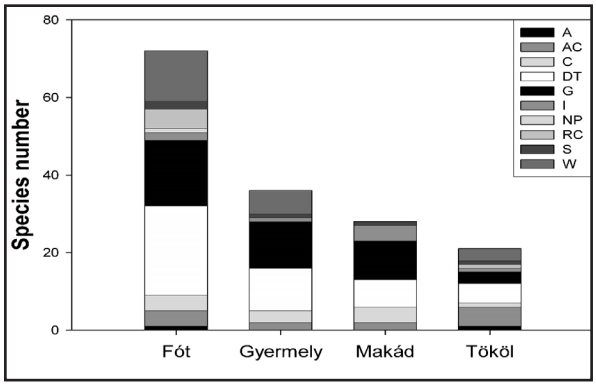


Figure 1. Distribution of Borhidi's social behaviour types of plant species occur in the studied sample areas Categories: A (Adventitious weeds); AC (Alien competitors); C (Natural competitors); DT (Disturbance tolerant plants of natural habitats); G (Generalists); I (Introduced crops running wild); NP (Natural pioneers); RC (Ruderal competitors of the natural flora); S (Specialists); W (Native weed species)

DOI: 10.18380/SZIE.COLUM.2017.4.1.15

TB (relative temperature figures)

The species represent 5 categories of relative temperature figures: from montane needle-leaved forest or taiga (TB_4) to submediterranean woodland and grassland (TB_8) (Figure 2.). Most of the species occurring on the studied areas indicated relative temperature demand of submontane broad-leaved forests (TB_6), the montane mesophilous broad-leaved forests (TB_5) furthermore thermophilous forest or woodlands (TB_7), thus they have middle or bigger temperature demand. These are followed by species of submediterranean woodlands and grasslands (TB_8). Relative temperature demand of montane needle-leaved forests or taigas (TB_4) is represented only by one species in Makád.

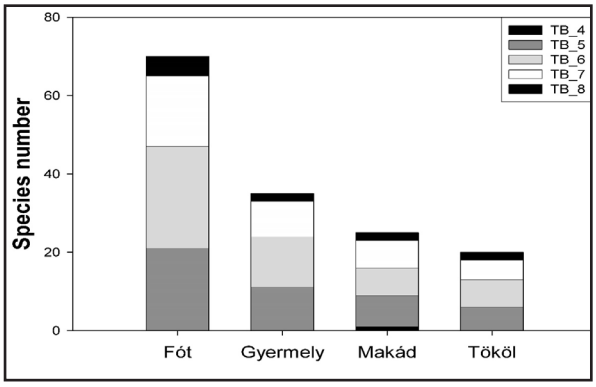


Figure 2. Distribution of Borhidi's relative temperature figures occurring on the studied sample areas. Categories: TB_4 (montane needle-leaved forest or taiga belt); TB_5 (montane mesophilous broad-leaved forest belt); TB_6 (submontane broad-leaved forest belt); TB_7 (thermophilous forest or woodland belt); TB_8 (submediterranean woodland and grassland belt)

WB (relative moisture figures)

Looking at relative moisture figures, types between xero-indicators (WB_2) and plants of wet soils (WB_9) were present. (Figure 3). Plants of semihumid habitats (WB_5) were dominant in 3 stands, but in Fót plants of semidry habitats (WB_4), plants of semihumid habitats (WB_5) and the xero-tolerants (WB_3) were present. Xero-indicators (WB_2) only here were found.

NB (nitrogen figures)

On the studied areas mostly categories between habitats very poor in N (NB_2) and hyperfertilized soils (NB_9) were found except for the area of Fót where 5 species were present indicating soils

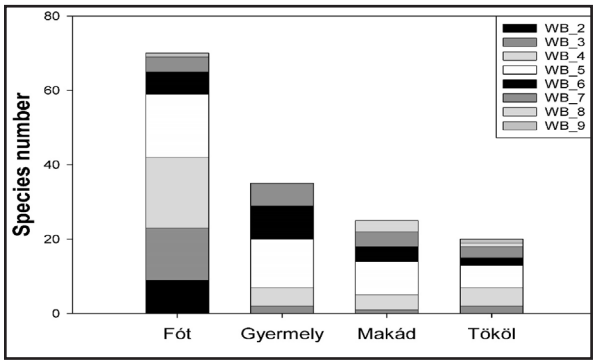


Figure 3. Distribution of Borhidi's relative moisture figures occurring on the studied sample areas. Categories: WB_2 (xero-indicators on habitats with long dry period); WB_3 (xero-tolerants, but eventually occuring on fresh soils); WB_4 (plants of semidry habitats); WB_5 (Plants of semihumid habitats, under intermediate conditions); WB_6 (plants of fresh soils); WB_7 (plants of moist soils not drying out and well aerated); WB_8 (plants of moist soils tolerating short floods); WB_9 (plants of wet, not well aerated soils)

extremely poor in mineral N (NB_1). This area has been proven to be diverse in relative nitrogen demand as all occurring categories were presented by more species, and no significant number of species in either could be observed. Most species (15) were plants of soils rich in mineral nitrogen (NB_7). In Gyermely and Tököl plants had mostly medium (NB_5) or higher relative N demand, and in Makád plants of submesotrophic habitats (NB_4) were rather typical.

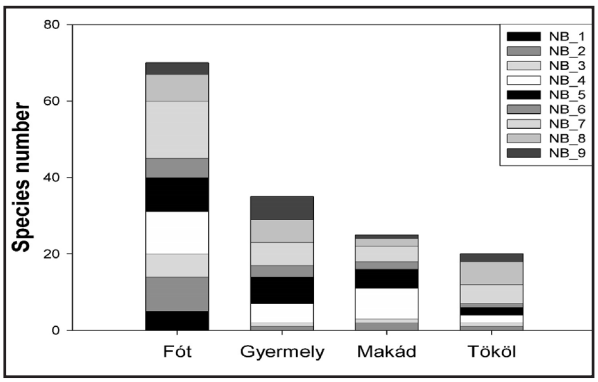


Figure 4. Distribution of Borhidi's nitrogen figures occurring on the studied sample areas. Categories: NB_1 (only in soils extremely poor in mineral nitrogen, plants); NB_2 (plants of habitats very poor in nitrogen); NB_3 (plants of moderately oligotrophic habitats); NB_4 (plants of submesotrophic habitats); NB_5 (plants of mesotrophic habitats); NB_6 (plants of moderately nutrient rich habitats); NB_7 (plants of soils rich in mineral nitrogen); NB_8 (N-indicator plants of fertilized soils); NB_9 (plants only on hyperfertilized soil, extremely rich in mineral nitrogen (indicating pollution, manure deposition)

DOI: 10.18380/SZIE.COLUM.2017.4.1.15

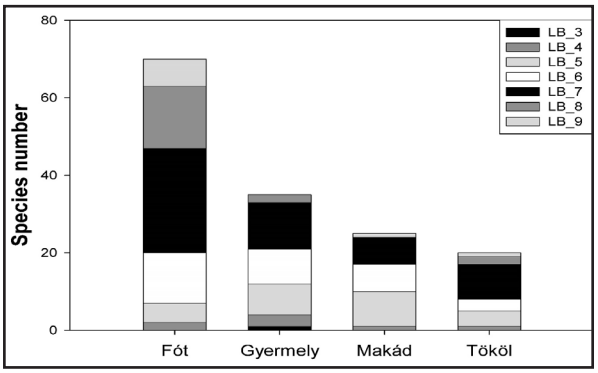


Figure 5. Distribution of Borhidi's light figures occurring on the studied sample areas. Categories: LB_3 (shadow plants; photosynthetic minimum under 5% relative light intensity, but survive more illuminated places); LB_4 (shadow-halfshadow plants; photosynthetic minimum between 5 and 10% rel. light intensity); LB_5 (halfshadow plants receiving more than 10% but less than 100% rel. light intensity); LB_6 (halfshadow-halflight plants; photosynthetic minimum between 10 and 40% rel. light intensity); LB_7 (halflight plants; mostly living in full light but also shadow tolerants); LB_8 (light plants; photosynthetic minimum above 40% rel. light intensity, less only in exceptional cases); LB_9 (full light plants of open habitats not receiving less than 50% of rel. light intensity)

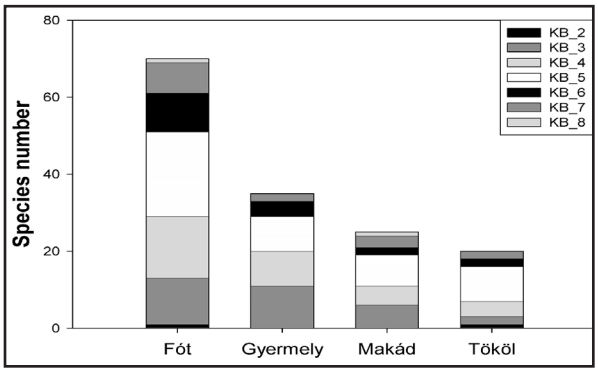


Figure 6. Distribution of Borhidi's continentality figures occurring on the studied sample areas. Categories: KB_2 (oceanic species, mainly in West Europe and western Central Europe); KB_3 (oceanic-suboceanic species, are in whole Central Europe); KB_4 (suboceanic species, mainly in Central Europe but reaching to East); KB_5 (intermediate type with slight suboceanic-subcontinental character); KB_6 (subcontinental, main area in eastern Central Europe); KB_7 (continental-subcontinental species main area in East-Europe); KB_8 (continental species reaching only eastern part of Central Europe)

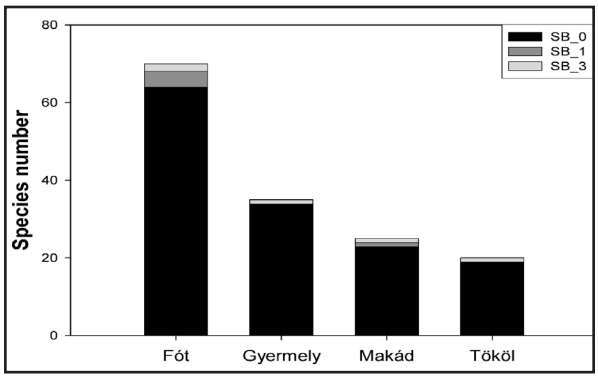


Figure 7. Distribution of Borhidi's salt figures occurring on the studied sample areas. Categories: SB_0 (Halophob species non occurring in salty or alkalic soils); SB_1 (salt tolerant plants but living mainly on non-saline soils); SB_3 (Beta-mesohalin plants living on soils of intermediate chloride content)

LB (light figures)

In each area 6-6 relative category of light figures are present from the shadow plants (only in Gyermely) to the full light plants (Figure 5). In general it can be said that quadrats are characterized by halfshadow plant, halfshadow-halflight plant and halflight plant species.

KB (continentality figures)

Among groups characteristic for continentality we could find species between oceanic (only in Fót and Tököl) and continental (only in Fót and Makád) categories (Figure 6). In the highest proportion intermediate, oceanic-suboceanic and suboceanic species occurred.

SB (salt figures)

Regarding the salt resistance, studied areas showed a very similar picture (Figure 7). Total of 3 categories are present. Most of the species are considered as halophob, but we found on all of the four areas Beta- mesohaline species, furthermore salt tolerant plant species on Fót and Makád.

Life form

Species found on the studied areas could be classified to 12 life form categories (Figure 8). From these, on Fót hemikryptophyta and therophyta, on Gyermely hemikryptophyta and phanerophyta, on Makád phanerophyta, and on Tököl hemikryptophyta species were dominant. According to this, on Makád mostly woody- stemmed, and on the other three areas mostly herbaceous species could be found in the herb layer.

DOI: 10.18380/SZIE.COLUM.2017.4.1.15

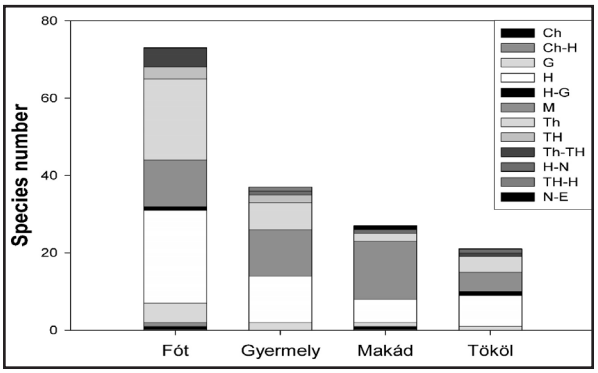


Figure 7. Distribution of Raunkiaer's life forms occurring on the studied areas. Categories: Ch (Chamaephyta); G (Kryptophyta); H (Hemikryptophyta); M (Phanerophyta); Th (Therophyta); TH (Hemitherophyta); E (Epiphyta)

Conclusions

Most of the examined ecological indicators (e.g. SBT, WB, NB, LB, KB, life forms) were represented in a significant number in most of the study areas, which may indicate the habitat transforming effect of spreading *Ailanthus altissima*. For example its falling foliage that increases N content of the soil (Csiszár 2012) can be one of the reasons why this many categories are represented in the studied stands.

In comparison of the studied areas based on the number of species (76) and occurring social behavior, continentality, relative nitrogen demand and life-form types, it can be concluded that the herb layer of Fóti-Somlyó proved to be much more diverse than the others, which is probably explained by this tree of heaven stocks' lower closure, by this habitats' more mosaic nature and by meeting of the highland and plain flora here. Most of the species being present here, are disturbance tolerant or generalist, halflight (according to this tree of heaven did not close above that), but shadow tolerant, with intermediate continentality, halophob herbaceous plants which indicate semidry or semihumid habitats that is rich in nutrients, and belongs to relative temperature demand of submontane broad-leaved forests. Regarding the number of occurring species (37) Gyermely area is the second one. We encountered here mostly generalists and disturbance tolerant, halflight but shadow tolerants with oceanic-suboceanic continentality, halophob herbaceous and woody-stemmed plants which indicate semihumid or fresh habitat that is richer in nutrients, and belongs to temperature demand of submontane broad-leaved forests.

Less species (28) than the above mentioned were found in Makád. On this sample area we found mostly generalist, halfshadow plants with intermediate continentality, halophob woody-stemmed plants which indicate semihumid habitat that is submesotrophic or richer in nutrients and belongs to temperature demand of submontane broad-leaved forests. From our sample areas this is the lowest, and the closest to Danube, which assumes more favorable hydrological circumstances. The latter is confirmed by the highest proportion of WB7's and WB8's categories.

The least number of species (21) was found in herb layer of Tököl area. The bigger part of these consists of disturbance tolerants and alien competitors, which are halflight plants, with mostly intermediate continentality, halophob herbaceous plants. They indicate semihumid or fresh habitat that is richer in nutrients and belongs to temperature demand of submontane broad-leaved forests.

Acknowledgements

We need to say thanks to Csépányi Péter chief engineer of production and nature conservation of Pilisi Parkerdő Zrt. for his professional help and advice and to his colleagues Kazi Róbert, Limp Tibor and Tóth Péter who helped in the selection of the research fields.

The publication was supported by Research Centre of Excellence project (1476-4/2016/FEKUT) of Faculty of Agricultural and Environmental Sciences of Szent István University.

References

Borhidi, A.(1995): Social Behaviour Types, the naturalness and relative ecological indicator values of the higher plants in the Hungarian flora. – Acta Botanica Hungarica 39:97-181.

Csiszár, Á. (2007): Özönnövénnyé vált a sátoros felleng. – Erdészeti Lapok 142: 78-80.

Csiszár, Á. (szerk.) (2012): Inváziós növényfajok Magyarországon. – Nyugat-magyarországi Egyetem Kiadó, Sopron, 364 pp.

DAISIE (2006): 100 of the worst. http://www.europealiens.org/speciesFactsheet.do?speciesId=16970

Demeter, A., Kovács, E., Trenyik, P., Csákvári, E., Czóbel, Sz. (2016): Economic evaluation of two invasive plant species - preliminary results. 33 p., – In: Agrillo, E., Attorre, F., Spada, F. & Casella, L. (eds.) Book of abstracts, 25th International Workshop of the European Vegetation Survey, Rome (Italy), 6-9 April 2016, 115 pp.

Demeter, A. & Czóbel, Sz. (2016): A mirigyes bálványfa (Ailanthus altissima (Mill) Swingle) hazai kutatásainak áttekintése és inváziójának mértéke a hazai élőhelyeken. – Természetvédelmi Közlemények 22:20-32.

Kowarik, I. – Böcker, R. (1984): Zur Verbreitung, Vergesellschaftung und Einbürgerung des Götterbaumes (Ailanthus altissima (Mill.) SWINGLE) in Mitteleuropa. – Tuexenia 4: 9-29.

Magyar Állami Természetvédelem (2007): Özönnövények fajlistája. (List of invasive plant species) http://www.termeszetvedelem.hu/index.php?pg=sub_643

Mihók, B., Pataki, Gy., Kovács, E., Balázs, B., Ambrus, A., Bartha, D., Czirák, Z., Csányi, S., Csépányi, P., Csőszi, M., Dudás, Gy., Egri, Cs., Erős, T., Gőri, Sz., Halmos, G., Kopek, A., Margóczi, K., Miklay, G., Milton, L., Podmaniczky, L., Sárvári, J., Schmidt, A., Sipos, K., Siposs, V., Standovár, T., Szigetvári, Cs., Szemethy, L., Tóth, B., Tóth, L., Tóth, P., Török, K., Török, P., Vadász, Cs., Varga, I. & Báldi, A. (2014): A magyarországi természetvédelem legfontosabb 50 kutatási kérdése a következő 5 évben. – Természetvédelmi Közlemények 20:1-23.

Udvardy, L. (1997): Fásszárú adventív növények Budapesten és környékén – Kandidátusi értekezés. Kertészeti és Élelmiszeripari Egyetem Növénytani Tanszék és Soroksári Botanikus Kert, Budapest, 126 pp.

Udvardy, L. (1998a): Budapest környéki bálványfa (Ailanthus altissima) állományok florisztikai-cönológiai vizsgálata. Kitaibelia 3: 343–346.

Udvardy, L. (1998b): Spreading and coenological circumstances of tree of heaven, [Ailanthus altissima (MILL.) SWINGLE] in Hungary. Acta Botanica Hungarica 41: 299–314.

Udvardy, L. 2004: Bálványfa. – In: Mihály, B. & Botta-Dukát, Z. (szerk.): Özönnövények. – TermészetBÚVÁR Alapítvány Kiadó, Budapest, 408 pp.

Udvardy, L. & Facsar, G. (1995): Weed vegetation of Budapest as an indicator of changes in environments's quality. 9th EWRS (European Weed Research Society) Symposium Budapest 1995: „Challenges for Weed Science in a Changing Europe. Perspektiven für die Unkrautforschung im veranderten Europa." 10-12 July 1995 Proceedings vol. 1: 107-112.

Udvardy, L. & Zagyvai, G. (2012): Mirigyes bálványfa (Ailanthus altissima [Mill.] Swingle). – In: Csiszár, Á. (szerk.): Inváziós növényfajok Magyarországon. – Nyugat-magyarországi Egyetem Kiadó, Sopron, 364 pp.

 DOI: 10.18380/SZIE.COLUM.2017.4.1.15

Examination the health state with instrumental measurements and the diversity of sessile oak stands in Zemplén mountains

Petra TRENYIK − Csilla FICSOR − András DEMETER − Dominika FALVAI − Szilárd CZÓBEL

Department of Nature Conservation and Landscape Ecology, Faculty of Agricultural and Environmental Sciences, Szent István University, H-2100 Páter Károly u. 1. Gödöllő, Hungary. Email: trenyikpetra@gmail.com

Abstract: Researching the naturalness of forests has been becoming more and more pronounced in Hungary, since the forest's ecosystems are also concerned by the biosphere crisis, that is a global degradation of the biotic environment. This deterioration could be observed not only through the decrease of the extention of the forests, but also through the altering of their structures.

FAKOPP 3D Acoustic Tomograph have been used and coenological surveys have been carried out in 3 different layers. The examinations have been accomplished in sessile oak stands of the Zemplén Mountains, within 5 different age groups. According to the results of the instrumental measurements – except the youngest age group – the highest value of the rottenness is located in the closest layer to the topsoil, then upwards on the trunk it shows decreasing tendency. Among the stands the 80 years old age group proved to be the healthiest, while the 20 years old group had the worst values. Based on the Shannon and Simpson diversity, the most diverse age group in the ground layer is the 60 years old group, while in the canopy is the 80 years old group. The rottenness measured in the younger stands concerned the higher parts of the trunks and it has presumably evolved because of the frost cracks. The diversity values of the shrub and the ground layer followed the same tendency.

Keywords: sessile oak, Zemplén Mountains, FAKOPP 3D Acoustic Tomograph, health state

Introduction

The reactions of the living world could be totally different within various dimensions causing the biggest difficulty for the climate research. In the interest of achieving more and more accurate prognosis of temporal and spatial changes, numerous researches are needed (Walther et al. 2002, Parmesan & Yohe 2003, Root et al. 2003, Parmesan 2006).

According to several studies the climate zones will give the most sensitive reaction (e.g. Risser 1995). Besides the increasing temperature, the alteration of the actually observed and predicted precipitation is the most hazardous for the ecosystems of the Carpathian Basin (Czóbel et al. 2010). However the responses of the ecosystems to these changes have been slightly explored so far (Czóbel et al. 2008).

According to the scientists besides the rising temperature the presence of drought periods are becoming more often. These periods are considered to be serious threats because of the deterioration of the health state of the stands during those periods which could lead to thinning or to even entire destruction (Csóka et al., 2007; Csóka et al., 2009). Some prognosis predict the shrinkage of the optimal climate range (niche) of sessile oak, according to Czúcz et al. (2013) the extent of this progression could be 80-100% by 2050.

Sessile oak forests cover approximately 160 000 hectares in Hungary, since not only the climatic conditions, but also the natural resources are suitable for them. This is the reason for the huge distribution of forests dominated by sessile oak. The continental xero- and mezophilic oak forests extend around the plains following the border of the Hungarian Mid-Mountains. The typically shallow topsoil of the Central Mountains and the often extremely drought regions are no longer favourable for closed-canopy stands, since the minimumfactor of these associations is the water (Mátyás et al. 1997). Parallel to the drought periods the desvastation of the dominant tree species had been also observed, among those the deterioration of sessile oak (*Quercus petraea*) has seriously appeared. The reason of its decay has been investigated by many researchers as Igmándy et al. (1985), Jakucs et al. (1988) and

DOI: 10.18380/SZIE.COLUM.2017.4.1.21

Berki (1991, 1995). Eventually, Vajna (1989, 1990) assigned a reason to this rather complex issue. According to Vajna the primal responsible for the oak's destruction could be the years with drought weather conditions when the parasitic fungi and the herbivorous insects have occurred in large quantities on those trees which were weakened due to the lack of water. Afterwards, it was observed that in those stands where the climate is pushing the limits of the tolerability of the stands, weakening of the vitality of the tree species is typical (Mészáros et al. 2012).

We have used FAKOPP 3D Acoustic Tomograph that is an instrument to determine the extent of the deterioration in tree trunks, developed in Hungary. This instrument measures the speed of sound propagation in the tree matter, since rotten and healthy tissues conduct sound differently. The theory behind this measurement is that sound propagates better in healthy tree tissue than in the decaying material. Every species has its optimal value; the deviation from that - in this case the decreasing of the value - refers to the rot inside of the trunk. The wood decay is caused by the white-rot and the brown-rot fungi; out of them the latter is the responsible for the decomposition of the cellulose content of the wood as well as for the propagation of the sound. Measurements in case of living trees are typically conducted in parks for the purpose of landscape architecture (Divós et al. 2005, Divós et al. 2008, Molnár 2011).

In order to create a complete picture of the stands investigating sessile oak individuals within the given stands are not sufficient; surveying the species pool and the structure are required too.

Since the forestry with the various forest management practices has great influence on the species composition, on the structure and on the processes of forest dynamics (Rubio et al. 1999, Bengtsson et al. 2000). Species needs to adapt to the regularly disturbances which affect them, and to the situation that their habitat have been continuously narrowing or fragmenting. The generalist species are better able to comply with these changing conditions rather than the specialist species of the forest ecosystems (Hermy et al. 1999). The herbaceous taxa play an important role in the diversity of forests, as they take part in the carbon storage and nutrient supply; moreover have a great impact on the primary production (Whigham 2004). In addition these species are proved to be appropriate indicators of the environmental changes because of their relative quick life cycle (Standovár et al. 2006). The different types of disturbance have direct or indirect influences on the species composition and on the structure of the given layers (Brunet et al. 1996, Decocq et al. 2004, 2005, Van Calster et al. 2008, von Oheimb & Härdtl 2009). The development state of the canopy and the ground layer has direct effect on the amount of light reaching to the latter layer, so that on the species composition and their coverage ratio in forest floor (Barbier et al. 2008, Tinya et al. 2009).

The following aims are set during the research:

What is the degree of the rottenness of the selected age groups of sessile oak in different layers?

How the Shannon and the Simpson diversity indices vary in the layers of the investigated age groups?

Could it be possible to observe some tendency during the analysis of the results of the instrumental measurements and the diversity values?

Materials and methods

The examinations have been carried out in the stands of sessile oak in Zemplén Mountains. In order to obtain data from every development state of the forest five age groups have been marked out. The age groups have set out in every 20 years, thus 20, 40, 60, 80 and 100 years old stands have been examined (Table 1.). The areas of all age groups are part of the Natura 2000 network.

With the purpose of collecting representative data corresponding areas have been determined according to standard parameters. These parameters are the followings: the elevation

 DOI: 10.18380/SZIE.COLUM.2017.4.1.21

Table 1. The selected subcompartments containing the age groups

Age groups	Subcompartments
20	Nagyhuta 109 A
40	Nagyhuta 109 B
60	Komlóska 53 D
80	Makkoshotyka 15 A
100	Háromhuta 101 D

(~400m), the relief (~15°), the southern exposure, furthermore the sessile oak (*Quercus petraea*) as the dominant tree species (minimum 70% of the canopy). The detailed descriptions of the given subcompartment have been provided by the local competent foresty.

In each selected subcompartment two 20×20 m quadrats have been selected in the way that the sample trees were the closest sessile oak trees to the corner of the quadrats and the closest sessile oak tree to the middle point. Therefore 5 sample trees have been investigated in each quadrats and 10 specimens in each age groups. The trunk diameter at breast height (DBH) (1,3 m) was measured at every sample trees, furthermore the presence of the frost crack was recorded. To determine the health state of the age groups FAKOPP 3D Acoustic Tomograph has been applied. The evaluation of data has been made by computers, that besides the measurements of the tree takes the species into consideration.

During the field measurements specifically developed detectors have been installed on the trees horizontally. In the younger stands 6 detectors have been used because of the small diameter of the trunks, while in the older stands 8 detectors have been applied. In order to receive accurate measurements of the degree of the decay and its evolution between the layers the trunks have been examined in total at 5 different heights above the soil surface (40 cm, 80 cm, 120 cm, 160 cm, 200 cm). The place of the first detector of every layer have been painted on the trunks to achieve repeatable measurements. The investigations have been carried out in the spring of 2015. In all 50 sessile oak trees have been measured regarding the health state of all 5 layers in each case. The collected data accordingly to this method has been quantified with the FAKOPP software and statistically evaluated with the Microsoft Office Excel. While the software expresses the degree of the rottenness in percentage, it is important to evaluate the stem diameter of each age groups.

The coenological surveys have been carried out in 20×20 meters quadrats in the way that the canopy, the shrub layer and the ground layer have been examined separately. At first each species in the quadrats have been recorded, then the cover-abundance values have been estimated in percentage, moreover the closeness of the canopy layer has been estimated too (Veperdi 2008).

The Shannon and the Simpson diversity values have been calculated by the Microsoft Office Excel, based on the field data (Tóthmérész 2002).

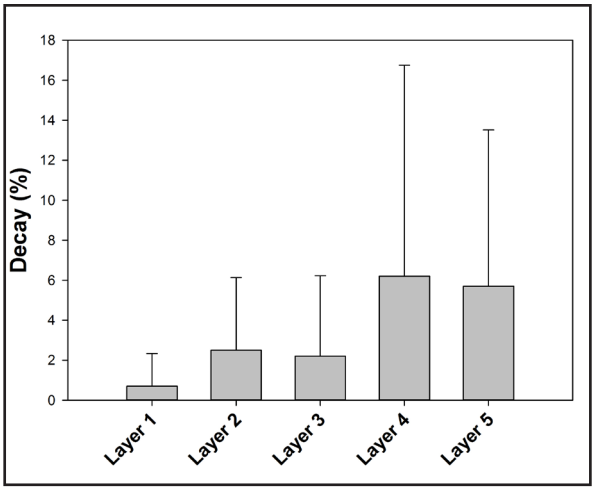
The levels of the significance of the examined layers within certain age groups and between certain age groups have been checked with the T-test during the statistical analysis. T-test has been used to determine the significance level between certain layers during the calculating of the two diversity indices.

Results

The results of the health state examination

Within the 20 years old group the degree of decay has been increased in higher and higher layers (Fig 1). The layer 4 (160 cm) had the

Figure 1. State of wood-decay in the measured layers (0.4 - 2 m). 20 year-old sessile oak stand (Zemplén Mountains, 2015).



worst health state. The rot part of this cross-section is 6.2%, which is considered to be high regarding that it is the youngest stand. In the 5th layer the average value of deterioration was 5.7%. The upper two layers had the biggest standard deviation value too; in the 4th layer it was 10.55 and the 5th layer had the value of 7.82%. Presumably it could be interpreted as the most of the specimens had appropriate health state, regularly these specimens were entirely healthy or had only 1-2% of decay in case of both layers. However two sample trees have badly damaged and the consequence of this they possessed bigger destruction. In the 4th layer the two extreme values of decay were 11% and 34%, while in the 5th layer these values were 11% and 24%.

In the first three layers the degree of degradation and the values of standard deviation had lower values too. In most cases some specimens had low decay values varying from 0 to 3%. In case of the 6th specimen the degree of deterioration in the 2nd and the 3rd layers was 11%; the 5th sample tree had higher value (5%) in the 1st and 2nd layers.

Within the 40 years old group the average and the standard deviation values seemed to be less unsteady (Fig 2). The degree of decay was the smallest in the two layers closest to the soil surface, while in the upper three layers had bigger deterioration. The latter layers had similar decay value; the 5th layer had the lowest with 1.8% and the 3rd layer had the highest with 2.2%. Low average values have belonged to low standard deviation values. Among some specimens the highest decomposition value was 8% that is considered to be corresponding regarding the age of the stand. Compare to this the first two layers had even lower deterioration. In the cross-section of these layers 0.8% of decay has measured equally. The values of standard deviation were also lower than in case of the upper 3 layers. The 3rd sample tree had the worst health state, 4 of its layers had higher decomposition value than 6%. Besides this higher deterioration values have been measured than 7% with the 6th and 7th specimens. Within this age group decay have not been detected in 64% of the examined layers.

In case of the 60 years old group the so far experienced trend has changed. Instead of the former linear growing values of decomposition, some layers are located through a curve in the way that a decrease could be seen until the 3rd layer which is followed by an increase until the 5th layer (Fig 3). The average value of deterioration could be divided into two groups: the 1st, 2nd and 5th layer possess the 3 highest values, while the health state of the 3rd and 4th layers considered to be better (0.5% and 1.1%). This stand had the lowest degree of decomposition among the 5 age groups. The health state has been experienced to be equal between the layers as well as with the standard deviation values. The standard deviation values varied within a narrow range from 2.29% to

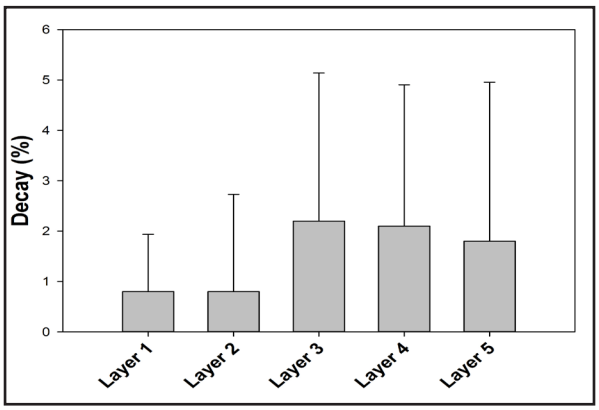


Figure 2. State of wood-decay in the measured layers (0.4 - 2 m). 40 year-old sessile oak stand (Zemplén Mountains, 2015).

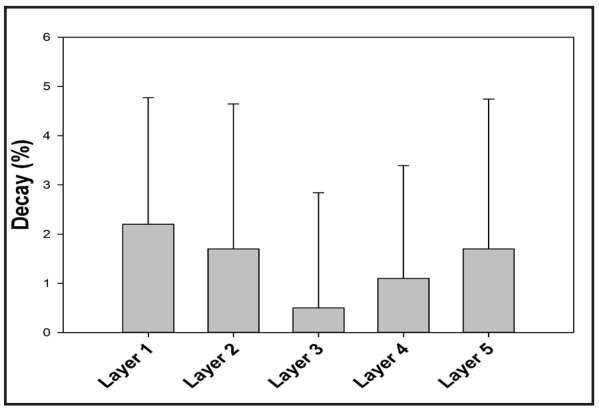


Figure 3. State of wood-decay in the measured layers (0.4 - 2 m). 60 year-old sessile oak stand (Zemplén Mountains, 2015).

DOI: 10.18380/SZIE.COLUM.2017.4.1.21

3.04%. The two thirds of the investigated layers of the stand were totally healthy, 22% showed 0-5% of damage, 12% seemed to have bigger than 5% of decomposition so that the highest decay value was 10%. Among the 10 sample trees there was only one which had all of the 5 measured layers entirely healthy. There were 5 specimens with one damaged layer, 2 specimens with 2 deteriorated layers and with the rest 2 specimens there were 3 and 4 decayed layers, respectively.

The trend of the 80 years old group has changed dramatically so that it became the opposite as we experienced in the 20 and 40 years old group. From the first layer – measured at 40 cm height – continuous reduction can be seen towards the 5th layer located at 200 cm height (Fig 4). The closest layer to the soil surface showed higher degree of deterioration (with the average of 4.1%). Even though among the 10 specimens 7 were entirely healthy in this layer, 2 had the decomposition of 3%, but the rot of the 5th specimen had been measured 35%. Better health state had been experienced in the 2nd layer, since its decay was only 1%. Altogether 2 specimens presented deteriorated health state, in one case the rot of the cross-section reached 8%, while the other specimen had 2% of decay. From the 3rd layer decomposition could be hardly experienced, the 3rd layer had 0.6% and the 5th layer had only 0.2%. For the latter ones the highest value of decay was 3% and the 73.3% of the layers were totally healthy. The standard deviation was the highest (6.75%) in the first layer, while the other layers showed far lower standard deviation values (1.97% és 2.81%).

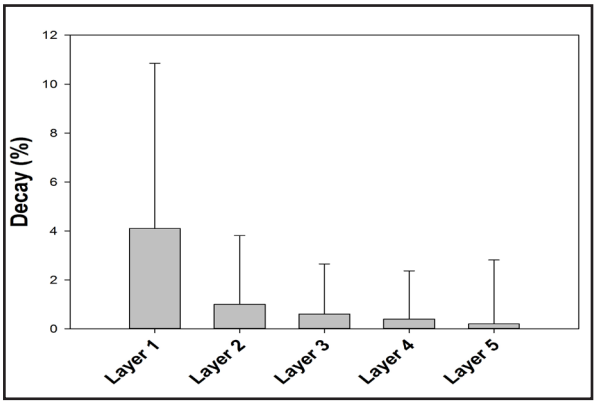


Figure 4. State of wood-decay in the measured layers (0.4 - 2 m). 80 year-old sessile oak stand (Zemplén Mountains, 2015).

The 100 years old group followed the same trend that we formerly used to experience with the 80 years old group (Fig 5). The lowest layer possessed the worst health state; the degree of decomposition decreased linearly towards the 5th layer. The standard deviation values have been observed to be high in the first 2 layers (8.46% and 5.86%). Compared to them the upper 3 layers had less fluctuation and the standard deviation values varied from 1.78 to 2.65. Within this group 3 sessile oak tree with worse health state have been measured too. Within certain age groups the examined layers showed no significant deviation. Compare to that the layers of the second youngest age group (40 years) and the layers of the two oldest age groups (80 and 100 years) indicated statistically proved significant deviation ($p<0.05$). In case of the age groups are evaluated separately, the value of the rottenness expressed by the software in percentage is suitable for comparing certain layers

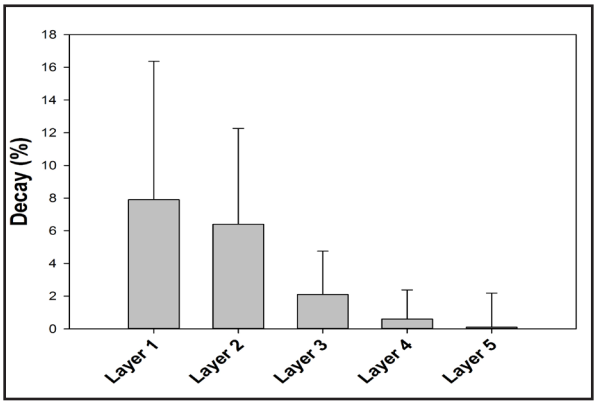


Figure 5. State of wood-decay in the measured layers (0.4 - 2 m). 100 year-old sessile oak stand (Zemplén Mountains, 2015).

or sample trees, evaluating the presented trends among them. However, comparing the age groups with this method is not possible because there is appreciable difference among the trunk diameters which concerns the extension of the rottenness.

The Figure 6. shows the values of the rottenness of the examined age groups in percentage and in cm2. The Exponential growth (single, 2-parameters) had the most strictly, significant

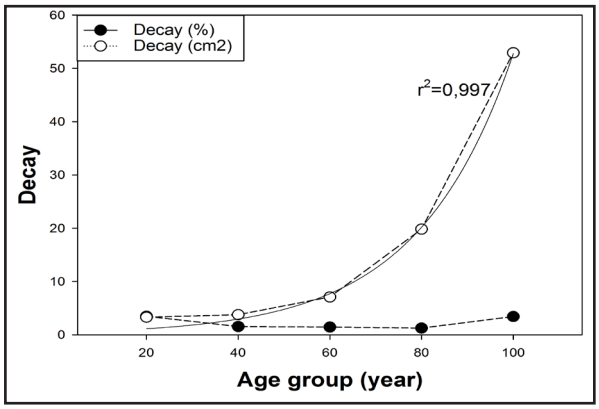


Figure 6. The rottenness of certain age groups expressed in percentage and in layer's extensions

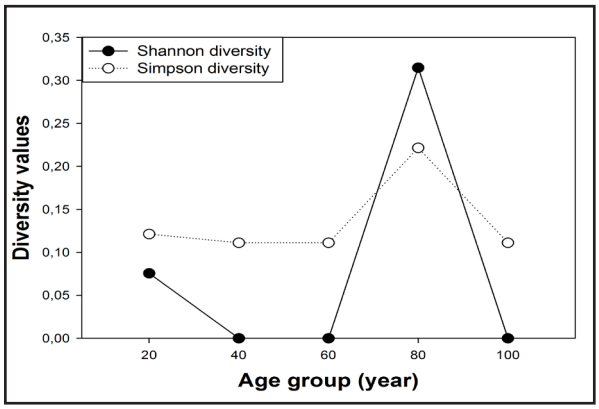


Figure 7. Shannon and Simpson-diversity values in the studied age groups of canopy layer (Zemplén Mountains, 2015).

($p<0.001$) correlation among the rottenness based on the layer's extension, expressed in cm^2.

The diversity of the canopy

The forestry has the biggest effect on the species composition of the canopy, since the forest managements have also influence on the species pool and on the cover values through the future forestry practices. The natural resources and the climatic factors play an important role in the combination of species, as the possibly dominant species are determined by them.

Table 2. The distribution of the closeness of the canopy in certain age groups

Age groups	Closeness (%)
20	70
40	70
60	70
80	75
100	72,5

The closeness of the canopy of certain age groups are presented in Table 2. Significant deviation is not observed in the closeness among certain age groups, so thus this has no influence on the diversity values.

The 80 years old group had the biggest diversity value from that the other groups lagged behind (Fig 7). The diversity of the 40, 60 and 100 years old stands were similar to each other, compare to them the diversity of the 20 years old group was slightly higher. These differences could predict that the other tree species could not be able to find their essential necessities due to the process of forestation.

The biggest distinctions have been realised between the Shannon and Simpson diversity indices in some groups when the canopy had consisted only of sessile oak. This phenomenon could be explained with the shallow topsoil of the Zemplén Mountains and the bad water management of the soils. Within these stands the Shannon index regularly has the value of 0, while the Simpson index has the value of 1.11 (Fig 7).

Based on the different sensitivity of the indices the Simpson diversity had higher values, since this index is rather sensitive for wide range tolerance and abundant species. In the 80 years old group where specialist species have been presented the Shannon index showed the higher value.

The diversity of the shrub layer

Besides the canopy the forestry management practices has strong effect on the combination of species of the shrub layer and its cover values too. The shrub layer has often been deliberately removed, or sometimes the managements of the canopy have such a huge disturbance so that the coverage of some species decreases. Furthermore the ratios within the combination of species could be shifted towards the disturbance tolerant species due to the influence of the disturbances.

In the case of the shrub layer bigger diversity was expected in the younger stands too that statement has seemed to be verified by the

 DOI: 10.18380/SZIE.COLUM.2017.4.1.21

surveys. Despite the higher species diversity in the younger stands less coverage values have been recorded probably caused by the disturbances.

Within this layer between the two indices have significantly higher difference than in the case of the canopy (Fig 8). Generally the values of

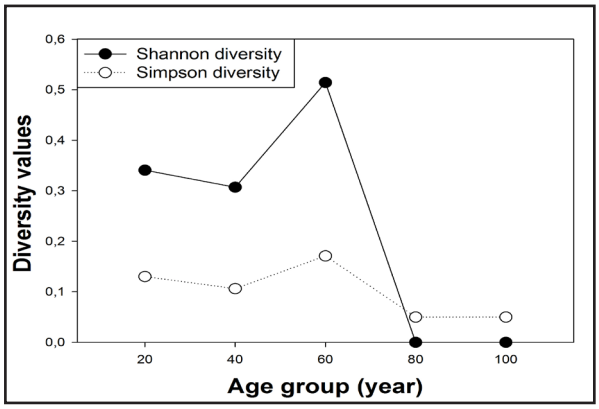


Figure 8. Shannon and Simpson-diversity values in the studied age groups of shrub layer (Zemplén Mountains, 2015).

the Shannon index are two times higher than the value of the Simpson index. Within the shrub layer there are also two groups where only one species has been presented, so that the value of the Shannon diversity is zero like in the former case.

The diversity of the ground layer

In the ground layer of the investigated stands 55 species have been surveyed. The values of the two diversity indices markedly differ from each other, but following the same trend in cases of

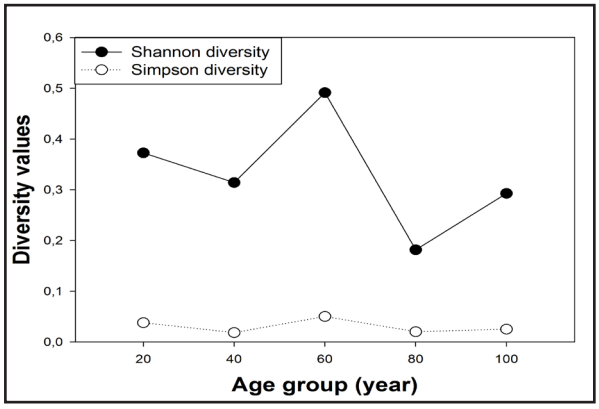


Figure 9. Shannon and Simpson-diversity values in the studied age groups of ground layer (Zemplén Mountains, 2015).

given age groups. The values of the Shannon diversity are always higher, indicating that the examined stands have high diversity, and are seminatural with several protected species. The most commonly appeared dicotyledon species were *Viola sylvestris, Campanula sp.* and *Gallium odoratum.* Because of the shallow topsoil of the examined stands of the Zemplén Mountains relatively low coverage values have been estimated, however this has not affected significantly the number of species (Fig 9).

There are no significant deviation in the case of both indices of the canopy and the shrub layer. On the other hand, in the case of both indices of the canopy and the ground layer indicated significant deviation ($p<0.5$), while among the shrub and the ground layer only the Simpson diversity had significant deviation ($p<0.5$).

Discussion

Our measurements complete the ongoing EVH I status survey since 1988. This survey measures the health state of the stands located in the intersections of the16x16 km grid considering 8 types of damages (game, insect, fungi, abiotic, anthropogenic, fire, other damage, degradation) (Hirka et al 2015).

The expediency of instrumental measurements is confirmed by the fact that accomplishing the measurements not only the accurate pictures of the degree of decomposition is achieved, but also the reasons of decay could be revealed. So thus the responses of the stands due to the extremities in climate parameters could be determined more precisely.

Among the stands the 80 years old age group proved to be the healthiest, while the 20 years old group had the worst values, but the decay of all age groups were less than 5%.

In this examination two facts can be for responsible for the evolution of deterioration. The observed decay in the younger stands that typically concerned the upper layers. The reason of this could be the evolution of the frost cracks since the rottenness and the recorded frost cracks located in the same places. The deterioration of

these trees could be seen by visual observations too, and they have been extracted during the later thinning. Hence this type of decomposition do not affects the older stands. In those stands the large-scale devastation of the lower layers is typically continuously reducing towards the higher layers. This type of decay concerns the coppice trees, since the trunk of the formerly extracted trees started to decompose that proceeded towards the coppice too.

According to the coenological evaluations and the diversity indices the examined stands have high diversity and are seminatural ones. Despite the fact that the aspect of nature conservation receives bigger and bigger emphasis on the field of forestry, the latter often negatively affects the diversity of some layers. This statement has been verified by the diversity values too. The diversity values of the shrub layer and the ground layer followed the same trend. The shrub layers of low diversity belong to similarly low diversity of ground layer, and this correlation could be observed in case of high diversity too. Presumably, this could be explained with the fact that when the disturbance influenced the shrub layer, it had affected collaterally the ground layer too. Furthermore, the closeness and the density of the canopy directly influence the amount of light reaching to the deeper layers, thus it has an effect on the diversity and on the coverage values too.

Acknowledgements

Hereby, I would like to express my acknowledgement to the forestry director and to the acting manager of Forestry of Sárospatak, to Zsolt Sándor and Márk Vitányi for the supplying of data, moreover for the help during the field researches.

Furthermore, I would like to thank to professor Ferenc Dívós for making available to me the FAKOPP 3D Acoustic Tomograph.

The publication was supported by Research Centre of Excellence project (1476-4/2016/FEKUT) of Faculty of Agricultural & Environmental Sciences of Szent István University.

References

Barbier, S., Gosselin, F., Balandrier, P. (2008): Infl uence of tree species on understory vegetation diversity and mechanisms involved – A critical review for temperate and boreal forests. Forest Ecology and Management 254, 1–15.

Berki, I. (1995): Az északi-középhegységi kocsánytalan tölgypusztulás néhány okának vizsgálata. Kandidátusi értekezés KLTE, Debrecen

Berki, I. (1991): Research into the cause of oak decay decline in Hungary. Proceedings of International Symposium on Ecological Approaches of Environmental Chemicals. Debrecen, 8–14.

Brunet, J., Falkengren-Grerup, U., Tyler, G. (1996): Herb layer vegetation of south Swedish beech and oak forests – eff ects of management and soil acidity during one decade. Forest Ecology and Management 88, 259–272.

Czóbel, Sz., Horváth, L., Szirmai, O., Balogh J., Pintér, K., Németh, Z., Ürmös, Zs., Grosz, B., Tuba Z. (2010): Comparison of N2O and CH4 fluxes from Pannonian natural ecosystems. European Journal of Soil Science 61: 671-682

Czóbel, Sz., Szirmai, O., Nagy, J., Balogh, J., Ürmös, Zs., Péli, E.R., Tuba Z. (2008): Effects of irrigation on the community composition, and carbon uptake in Pannonian loess grassland monoliths. Community Ecology 9: 91-96.

Czúcz, B., Gálhidy, L., Mátyás, Cs. (2013): A bükk és a kocsánytalan tölgy elterjedésének szárazsági határa. Erdészettudományi közlemények 3:39-53

Csóka, Gy.; Koltay, A.; Hirka, A., Janik, G. (2007): Az aszályosság hatása kocsánytalan tölgyeseink és bükköseink egészségi állapotára. In: Mátyás Cs. és Vig P. (szerk.): Erdő és klíma V. kötet, Sopron, 229–239 p.

 DOI: 10.18380/SZIE.COLUM.2017.4.1.21

Csóka, Gy.; Koltay, A.; Hirka, A., Janik, G. (2009): Az aszályosság hatása kocsánytalan tölgyeseink és bükköseink egészségi állapotára. KlÍMA-21 füzetek, 57: 64–73.

Decocq, G., Aubert, M., Dupont, F., Alard, D., Saguez, R., Wattez-Franger, A., De Foucault, B., Deleis-Dusollier, A., Bardat, J. (2004): Plant diversity in a managed temperate deciduous forest: understorey response to two silvicultural system. Journal of Applied Ecology 41, 1065–1079.

Decocq, G., Aubert, M., Dupont, F., Bardat, J., Wattez-Franger, A., Saguez, R., De Foucault, B., Alard, D., Deleis-Dusollier, A. (2005): Silviculture-driven vegetation change in a European temperate deciduous forest. Annals of Forest Science 62, 313–323.

Divos, F.; Denes, L.; Iniguez, G. (2005), Effect of cross-sectional change of a board specimen on stress wave velocity determination, Holzforschung, 2005 Vol. 59, no.: 2. http://www.fakopp.com

Divos F., Divos P., Divos Gy.: (2008) Acoustic Technique ues from Seedling to Wooden Structures, , Proceedings of the 15th International Symposium on Nondestructive Testing of Wood Duluth, MN, Sept. 10–12, 2007 (2008),

Hermy, M., Honnay, O., Firbank, L., GrashofBokdam, C., Lawesson, J.E. (1999): An ecological comparison between ancient and other forest plant species of Europe, and the implications for forest conservation. Biological Conservation 91, 9–22.

Hirka, A., Koltay, A., Kolozs, L., Szőcs, L., Csóka, Gy., (2015): Erdeink egészségi állapota 2014-ban. Erdészeti lapok, 150 (9): 258-261

Igmándy, Z.; Pagony, H.; Szontagh, P., Varga F. (1985): A kocsánytalan tölgy (Quercus petraea MATT./Lieb) pusztulása hazánkban. Növényvédelem, 21(7): 311–311.

Jakucs, P. (1988): Ecological approach to forest decay in Hungary. Ambio, 17(4): 267–274.

Mátyás Cs. (1997): Erdészeti ökológia. Mezőgazda Kiadó, Budapest pp. 312

Mészáros, I., Fenyvesi, A. Cs., Láposi, R., Oláh, V., és Veres, Sz.,(2012): Középhegységi zonális fafajok klímaérzékenységének ökofiziológiai vizsgálata, különös tekintettel a vízforgalmi sajátosságokra; Ecophysiological study of trees species of climate-zonal forests covering Hungarian central range, with special focus on water budget. Munkabeszámoló. OTKA.

Molnár, S. (2011): 2011 az erdők éve, Örök társunk a fa. Nyugat-magyarországi Egyetem Kiadó pp. 56

Parmesan, C. (2006): Ecological and Evolutionary responses to recent climate change. Annu. Rev. Ecol. Evol. Syst. 37: 637-669.

Parmesan, C., Yohe, G. (2003): A globally coherent fingerprint of climate change impacts across natural systems. Nature 421: 37–42.

Risser, P.G. (1995): The status of the science examining ecotones. BioScience 45: 318–325.

Root, T.L., Price, J.T., Hall, K.R., Schneider, S.H., Rosenzweig, C., Pounds, J.A. (2003): Fingerprints of global warming on wild animals and plants. Nature 421: 57–60

Rubio, A., Gavilán, R., Escudero, A. (1999): Are soil characteristics and understorey composition controlled by forest management? Forest Ecology and Management 113, 191–200.

Standovár, T., Ódor, P., Aszalós, R., Gálhidy, L. (2006). Sensitivity of ground layer vegetation diversity descriptors in indicating forest naturalness. Community Ecology 7, 199–209.

Tinya, F., Márialigeti, S., Király, I., Németh, B., Ódor, P. (2009): The eff ect of light conditions on herbs, bryophytes and seedlings of temperate mixed forests in Őrség, Western Hungary. Plant Ecology 204, 69–81.

Tóthmérész B. 2002: A diverzitás jellemzésére szolgáló módszerek evolúciója. In: Salamon-Albert éva (szerk.): Magyar botanikai kutatások az ezredfordulón. PTE Növénytani Tanszék és MTA Pécsi Akadémiai Bizottság, Pécs, pp. 607–638.

Vajna, L. (1989): A kocsánytalan tölgy pusztulásának kórok- és járványtani kérdései. Erdô, 38: 169–175.

Vajna, L. (1990): Fungi associated with oak-decline. EPPO Bulletin, 20: 3.

Van Calster, H., Baeten, L., Verheyen, K., Keersmaeker, D.L., Dekeyser, S., Rogister, J.E., Hermy, M. (2008): Diverging eff ects of overstorey conversion scenarios on the understorey vegetation in a former coppice-with-standards forest. Forest Ecology and Management 256, 519–528.

Veperdi G., 2008: Erdőbecsléstan, Sopron.

von Oheimb, G., Westphal, C., Tempel, H., Härdtle, W. (2005): Structural pattern of a nearnatural beech forest (Fagus sylvatica) (Serrahn, North-east Germany). Forest Ecology and Management 212, 253–263.

Walther, G.R., Post, E., Convey, P., Menzel, A., Parmesan, C., Beebee, T.J.C., Fromentin, J.M., Hoegh-Guldberg, O.H., Bairlein, F. (2002): Ecological responses to recent climate change. Nature 416: 389–395.

Whigham, D.F. (2004): The ecology of woodland herbs in temperate deciduous forests. Annual Review of Ecology, Evolution, and Systematics 35, 583–621.

 DOI: 10.18380/SZIE.COLUM.2017.4.1.21

Preliminary results of SSR based characterization of sour (*Prunus cerasus* L.) and sweet cherry (*Prunus avium* L.) genotypes cultivated in Hungary

Eren BARIS* – Janka BEDŐ* – Ogboro Samson EDOSA – Erzsébet KISS – Anikó VERES

Szent István University, Faculty of Agricultural and Environemental Sciences Institute of Genetics, Microbiology and Biotechnology H-2100 Páter Károly u. 1. Gödöllő, Hungary. Email: veres.aniko@mkk.szie.hu

* These two authors equally contributed to the article.

Abstract: Cherry cultivation in the Carpathian basin area began more than 100.000 years ago. Adapting to the basin specific ecological conditions resulted in high degree of genetic variability among the cherry cultivars. The SSR (Simple Sequence Repeat) markers allow the discrimination of the cultivars and determination their specific DNA fingerprints. Due to the high degree of polymorphism of microsatellite markers, generally only six SSR loci are enough to differentiate the varieties. Microsatellite markers are used not only for cultivar identification but also for the verification of synonyms and homonyms. Owing to their locus specificity and Mendelian codominant inheritance, parentage can be clearly identified, primary and secondary relationships between the cultivars can be discovered.

The aim of this research was to characterize 29 sour cherry (*Prunus cerasus* L.), and 38 sweet cherry *(Prunus avium* L.*)* genotypes cultivated in Hungary to establish their DNA fingerprints in 6 SSR loci by allele numbers and sizes.

Keywords: Microsatellite, *Prunus*, *Prunus avium* L.*, Prunus cerasus* L.*,* parent-progeny analysis

Introduction

Both sour and sweet cherries are important economic dicot stone fruit plants, belonging to the the *Rosaceae* family within the *Prunoideae* subfamily.

Sour cherry (*Prunus cerasus* L.) is an allopolyploid, spontaneous hybrid species originating from West Asia and South-Eastern Europe. It is supposedly formed from the cross between the diploid sweet cherries (*Prunus avium* L., 2n=2x=16) and the tetraploid Mongolian cherries (*Prunus fruticosa* Pall, 2n=4x=32) (Beaver et al. 1995). Presently, sour cherry still grows wild in various parts of Europe, from Scandinavia and the north of Turkey to the south and shows great genetic diversity.

Sweet cherries (*Prunus avium* L.) are diploid plants (2n=2x=16). Primary centers of origin are Pre-Asia (Caspian Sea, Black Sea region) as well as Western China mountains. Europe is considered to be the secondary center of origin (Pór and Fabula 1982; Tropicos 2015).

Hungary is an important sour cherry producing country because of its traditions, varieties, technology, and market opportunities (Nyéki et al. 2003). Towards the end of the 19th century, Pándy and Cigány varieties dominated in sour cherry production. The quality of Pándy is considered as a standard; it is a unique Hungarian variety (Nyéki et al. 2003).

Sweet cherry production has been doubled in the world since 2001, the average yield in Hungary is 5-6 t/ha. In Hungary ripening period of sweet cherry begins in the third decade of May and ends in the middle of July. According to experts Germersdorfi óriás, Bigarreau Burlat, Van, Margit, Katalin, Linda were the most used cultivars in Hungary in 2012. However, other hybrid species have also become popular such as Carmen, Vera, Rita and other Canadian, American and Italian cultivars (Radóczné 2012).

Microsatellites or simple sequence repeats **(**SSRs**)** are co-dominant, abundant, multi-allelic, as well as uniformly distributed over the genome, and can be detected by simple reproducible assays (Powell et al. 1996). Its length ranges from 1- 6 nucleotides (Van Oppen et al. 2000) and can be classified as mono-, di-, tri-, tetra-, penta- and hexanucleotide repeats. With the minimum repeat length of 12 base-pairs they are tandemly repeated usually 5-20 times in the genome (Goodfellow 1992; Vaughan and Lloyd 2003; Ellegren 2004; Prajapati et al. 2017). As a result of their quickness, simplicity, rich polymorphism and stability SSR markers

are highly popular in genetic diversity analysis (Turkoglu et al. 2013; Gürcan et al. 2015; Batnini et al. 2016), construction of fingerprints (Cantini et al. 2001; Rojas et al. 2008; Klabunde et al. 2014; Turet-Sayar et al. 2012; Ivanovych et al. 2017), genetic purity test (Spann et al. 2010), molecular map construction and gene mapping (Ogundiwin et al. 2009; Olukolu et al. 2009; Fan et al. 2010; Pacheco et al. 2014; Rowland et al. 2014; Wang et al. 2014; Eduardo et al. 2015), utilization of heterosis, especially in the identification of species that are genetically related. Microsatellite markers have also been used in several studies to define conserved regions among related species (Decroocq et al. 2003; Martínez-Gómez et al. 2003; Maghuly and Laimer 2011; Alisoltani et al. 2016) for both plants and animals genome mapping (Weising et al.1998).

Gustavsson (2014) and Lacis (2014) were of the opinion that SSR markers should be compoulsorily used to provide molecular profiles for the cultivars thus detecting duplicates and mislabelling in germplasm collections. In order to ensure that data are compatible with international data bases, there is a marker set which the ECPGR (European Cooperative Programme for Plant Genetic Resources) Prunus WG recommended.

Table 1. Studied sour cherry genotypes and their parents, origin and self(in)compatibility status (SI, SC)

Genotypes	Parents	Origin	Self-compatibility
3/48 9	Csengődi x Érdi bőtermő	Hungary	
Cigány 59 12	Clone of Cigány	Hungary	SC (S6, S9, Sa, Sb)
Cigány 7 13	Clone of Cigány	Hungary	SC
Cigány C404 9	Clone of Cigány	Hungary	SC
Csengődi	traditional cultivar/landrace	Hungary	SC
Csengődi 11	Clone of Csengődi	Hungary	SC
Debreceni bőtermő	traditional cultivar/landrace	Hungary	SC
Érdi bőtermő	Pándy x Nagy angol	Hungary	SC (S4, S6m, Sa)
Érdi bőtermő 13	Clone of Érdi bőtermő	Hungary	SC
Érdi jubileum	Pándy x Eugénia	Hungary	SC
Érdi nagygyümölcsű	Hankovszky x unknow	Hungary	SI (S1, S12, Sc, Sd)
Favorit	Pándy x Montreulli	Hungary	SC
Kántorjánosi	traditional cultivar/landrace	Hungary	SC
Kántorjánosi 3	Clone of Kántorjánosi	Hungary	SC
Korai pipacs	Pándy x Császár	Hungary	SC
Kőrösi korai	traditional cultivar/landrace	Hungary	SC
Maliga emléke	Pándy x Eugénia	Hungary	SC
Meteor korai	Pándy x Nagy angol	Hungary	SC
Oblacsinszka	traditional cultivar/landrace	Yugoslav region	SC
Pándy 279	Clone of Pándy	Hungary	SI
Pándy 279 11	Clone of Pándy	Hungary	SI
Pándy 48	Clone of Pándy	Hungary	SI
Pándy 48 10	Clone of Pándy	Hungary	SI
Pándy BB 119	Clone of Pándy	Hungary	SI
Pándy Bb119	Clone of Pándy	Hungary	SI
Paraszt	synonym of cigány?	Hungary	SC
Pipacs 14	unknown origin Hybrid	Hungary	SC
Piramis	M221 x Meteor korai	Hungary	partially SC
Újfehértói fürtös	traditional cultivar/landrace	Hungary	SC (S4, Sd, Se)

DOI: 10.18380/SZIE.COLUM.2017.4.1.31

Table 2. Studied sweet cherry genotypes, their parents, origin and self(in)compatibility status (SI, SC)

	Parents	Origin	Selfcompatible
Aida	Moldvai fekete (Trusenszkaja 40) x H 236	Hungary	SI (S_6S_{12})
Alex	Van x John Innes	Hungary	SC ($S_3S_{3'}$)
Anita	Trusenszkaja 2 x H 3	Hungary	SI (S_3S_6)
Bigarreau Burlat	traditional cultivar/ landrace	France	SI (S_3S_9)
Botond	German, H 264	Hungary	SI (S_3S_4)
Canada giant	unknown	Canada	SI (S_1S_2)
Carmen	Yellow Dragan x H 203	Hungary	SI (S_4S_5)
Colney	unknow, UK Norfolk, John Innes Centre	United Kingdom	SI (S_5S_6)
Germersdorfi clone 1	clone of Germersdorfi	Hungary	SI (S_3S_{12})
Germersdorfi clone 3	clone of Germersdorfi	Hungary	SI (S_3S_{12})
Germersdorfi clone 45	clone of Germersdorfi	Hungary	SI (S_3S_{12})
Giorgia	ISF 123 x Caccianese	Germany	SI (S_1S_3)
Hedelfingeni Óriás	traditional cultivar/ landrace	Germany/HU	SI (S_3S_4)
Jaboulay	traditional cultivar/landrace	France	SI (S_6S_9)
Katalin	Germersdorfi óriás x Podjebrad yellow	Hungary	SI (S_4S_{12})
Kavics	Germersdorfi óriás x Budakalász	Hungary	SI (S_4S_{12})
Krupnoplodnaja	Napoleon Blanc open pollination	Ukraine	SI (S_5S_9)
Linda	Hedelfingeni óriás x Germersdorfi óriás 3	Hungary	SI (S_3S_{12})
Margit	Germersdorfi seedling	Hungary	SI (S_4S_{12})
Merchant	traditional cultivar/landrace	United Kingdom	SI (S_4S_9)
Münchenbergi korai	Flamentier x Márki korai	Germany	SI (S_3S_4)
Octavia	Schneiders Spate Knorpelkirsche x Rube	Germany	
Pál	Bigarreau Burlat x Stella	Hungary	SC (S_4S_9)
Péter	Bigarreau Burlat x Stella	Hungary	SC ($S_3S_{4'}$)
Regina	Schneiders Spate Knorpelkirsche x Rube	Hungary	SI (S_1S_3)
Rita	Germersdorfi x Szomolyai fekete	Hungary	SI (S_5S_{22})
Samba	2S-84-10 (=Stella 35A) x Stella 16.A.1	Canada	SI (S_1S_3)
Sándor	Bigarreau Burlat x Stella	Hungary	SC (S_4S_9)
Solymári gömbölyű	traditional cultivar/landrace	Hungary	SI (S_3S_4)
Stella	Lambert x St. John Innes 2420	Canada	SC ($S_3S_{4'}$)
Sunburst	Van x Stella	Canada	SC ($S_3S_{4'}$)
Sylvia	Van x Sam	Canada	SI (S_1S_4)
Szomolyai fekete	traditional cultivar/ landrace	Hungary	SI (S_2S_4)
Tünde	Yellow Dragan x Bigarreau Burlat	Hungary	SI (S_3S_5)
Valeri Cskalov	Rozovaja species open pollination	Romania-Ukraine	SI (S_1S_9)
Van	Imperatrice Eugenie seedling	Canada	SI (S_1S_3)
Vega	Bing x Victor	Canada	SI (S_2S_3)
Vera	Ljana (Truzenszkaja 6) x Van	Hungary	SI (S_1S_3)

SSRs or microsatellites (Schueler et al. 2003; Höltken and Gregorius 2006) have been developed and successfully applied both in sour and sweet cherries (Downey and Iezzoni 2000; Cantini et al. 2001; Pedersen 2006; SSR markers of *Prunus* origin are polymorphic and transferable within *Prunoideae* (Cipriani et al. 1999; Downey & Iezzoni 2000; Dirlewanger

et al. 2002; Wunsch & Hormaza 2004; Lacis et al. 2009) thus using these SSR markers it was possible to distinguish the cultivars and accessions studied. Our aim was to determine the SSR fingerprints of sour and sweet cherry genotypes to expand databases set by the Institute of Genetics, Microbiology and Biotechnology.

Materials and methods

Plant material

The study included 29 sour cherry (*Prunus cerasus* L.) and 38 sweet cherry (*Prunus avium* L.) genotypes (landraces, clones, hybrids, open pollinated variety) developed in Hungary and common cultivars of foreign origin. The plant material was collected at the Fruit Research Institute in Érd, Hungary, and National Food Chain Safety Office NÉBIH, Hungary (*Table 1*, *2*).

DNA isolation

Young leaves were collected from a single tree for each genotype, stored at -70°C. Total genomic DNA was extracted using the modified CTAB protocol (Van der Beek et al. 1992). The DNA was dissolved in distilled water to a final volume of 200 µl and its concentration was checked with a Nanodrop spectrophotometer.

Amplification conditions

PCR in a volume of 10 µL was done in an iCycler equipment (BioRad). The components of the reaction mixture were: 20 ng of template DNA, 0.6 U of WTB-Taq polymerase (WestTeam Biotech, Pécs), 0.1 mM dNTP mix, 0.75 µM of each forward and reverse primer, and 1.25 mM $MgCl_2$ in 1X PCR buffer. For the amplification with the SSR primers we performed touchdown PCR, which consisted of an initiation cycle at 94C for 2 min; 10 cycles of denaturation at 94°C for 30 seconds, primer annealing at 65°C for 30 seconds and extension at 72°C for 1 minute, where the annealing temperature was decreased by 1°C at each cycle. This was followed by 24 cycles of denaturation at 94°C for 30 seconds, annealing at 56°C for 30 seconds and extension at 72°C for 1 minute. The reaction was completed with a post-polymerization extension cycle at 72°C for 5 minutes.

The amplified products were separated on 6% polyacrylamide gel (ReproGel™, GE Healthcare, AP Hungary Ltd) in a vertical system (ALF-Express II., Amersham Biosciences, AP Hungary Ltd, Budapest). Fragments were detected by Cy-5 fluorescent labelling of the forward primer. The precise sizes of the amplified SSR regions were determined by applying DNA molecular weight standards and ALFwin Fragment Analyser 1.0 software. Dendrograms (Average Linkage) Between Groups were constructed based on the SSR data using SPSS 22 statistical program.

Markers

The same 6 SSR primer pairs were used in both sour and sweet cherry (*Table 3*).

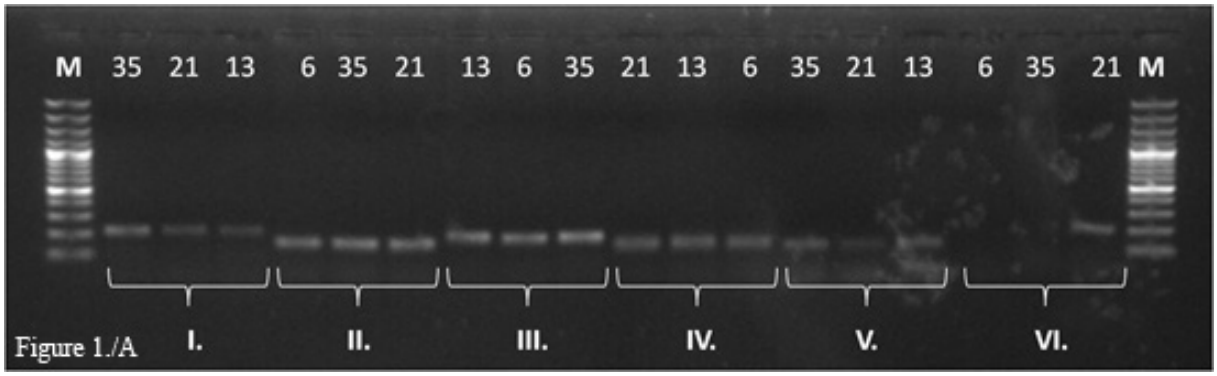
Table 3. Six *Prunus* microsatelliteprimer pairs used in the marker analyses

Locuscode	Primer sequences (5'-3')	References
BPPCT 041 F	CAATAAGGCATTTGGAGGC	Dirlewanger et al. (2002)
BPPCT 041 R	CAGCCGAACCAAGGAGAC	
BPPCT 030 F	AATTGTACTTGCCAATGCTATGA	Dirlewanger et al. (2002)
BPPCT 030 R	CTGCCTTCTGCCACACC	
BPPCT 002 F	TCGACAGCTTGATCTTGACC	Dirlewanger et al. (2002)
BPPCT 002 R	CAATGCCTACGGAGATAATAGAC	
UDP 96 005 F	GTAACGCTCGCTACCACAAA	Cipriani et al. (1999)
UDP 96 005 R	CCTGCATATCACCACCCAG	
UDP 96 001 F	AGTTTGATTTTCTGATGCATCC	Cipriani et al. (1999)
UDP 96 001 R	TGCCATAAGGACCGGTATGT	
UCDCH 17 F	TGGACTTCACTCATTTCAGAGA	Struss et al. (2003)
UCDCH 17 R	ACTGCAGAGAATTTCCACAACCA	

DOI: 10.18380/SZIE.COLUM.2017.4.1.31

Results and Discussion

The modified CTAB DNA extraction procedure (Van der Beek et al. 1992) resulted in sufficient amount of DNA (40-80 ng/µl). The PCR products tested on agarose gel and the size of the amplified SSR fragments determined with ALFwin Fragment Analyser 1.0 software can be seen in *Figure 1*. The precise sizes of the amplified SSR fragments are shown in *Table 4, 5, 6* and *7*.



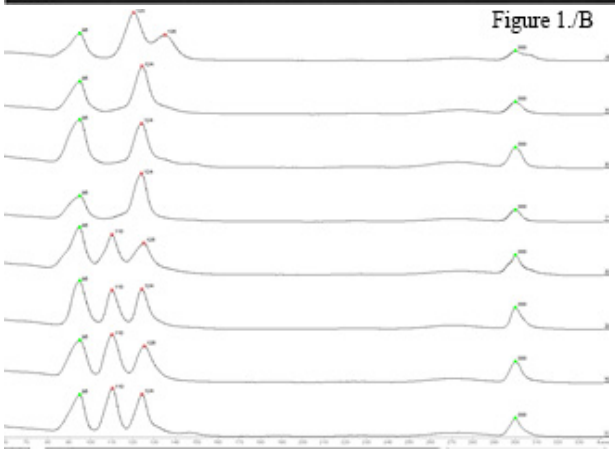


Figure 1. ***A***: Agarose gel electrophoresis results with six SSR markers on sweet cherry genotypes **M**: GeneRuler 100bp+; **I**: BPPCT 041, **II**: BPPCT 030, **III**: BPPCT 002, **IV**: UDP 96 005, **V**: UDP 96 001, **VI**: UCD-CH 17; **6**: Carmen, **13**: Kavics, **21**: Regina, **35**: Conley

B: ALFwin Fragment Analyser 1.0 software results; Samples order (row number, primer, name of the samples) **4**: UDP 96 005 Jaboulay, **5**: UDP 96 001 Hedelfingeni óriás, **6**: UDP 96 001 Katalin, **7**: UDP 96 001 Krupnoplodnaja, **8**: UDP 96 001 Münchenbergi korai, **9**: UDP 96 001 Margit, **10**: UDP 96 001 Sunburst, **11**: UDP 96 001 Van

All of the 6 SSR markers displayed polymorphic pattern. Because of the allopolyploid origin of sour cherry we got 1-3 different alleles, however 4 different alleles were not found, alleles represent 2 heterozygous and 1 homozygous alleles, meaning that one of the loci was heterozygous while the other homozygous. In absence of reference data we were not able to decide which allele belongs exactly to the homozygous locus. We could calculate the allelic frequency from the numbers of alleles (*Table 4*). The number of the alleles were 2-5 and the frequency of the alleles were between 2.3%-85.3%. In the case of UDP 96 005 primer there was a rare allele (118 bp) which can be observed only in two cultivars (Maliga emléke, Piramis).

We found only one unique allele formule in the case of BPPCT 002, Maliga emléke has a 183 bp homozygous form. Only four of the 6 SSR primer-pairs generated alleles proved to be suitable to distinguish the studied sweet cherry genotypes. Their frequencies have ranged from 1.32% to 100%. A total of 456 polymorphic alleles were amplified and the number of the alleles per locus ranged from 1 to 4 (*Table 6*).

UDP96 001 and UCDCH 17 primers resulted in 2 different alleles, both in homozygous or heterezygous form. Four alleles were identified in cherry genotypes using UDP96 005 locus-specific primers (*Table 6*). Two alleles were common and two were rare. Cultivars Botond, Regina and Octavia had unique combinations of these alleles and they could be identified by using this marker. In the case of BPPCT 002 primer we got an allele (179 bp) in high fequency, the almost monomorphic pattern was broken only in 2 cultivars, Antia and Hedelfingeni óriás contained a rare allele (183 bp) (*Table 7*).

We observed the same range of alleles like

Table 4. Microsatellite loci, alleles and their frequency in sour cherry genotypes cultivated in Hungary

UDP 96 001		**UDP 96 005**		**BPPCT002**		**BPPCT030**		**BPPCT041**		**UCDCH 17**	
Fragment size bp	Frequency %	Fragment size bp	Frequency %	Fragment size bp	Frequency %	Fragment size bp	Frequency %	Fragment size bp	Frequency %	Fragment size bp	Frequency %
101	14.3	106	22.4	167	41.8	140	48.3	201	85.3	178	40.8
115	59.2	110	22.4	179	23.9	158	33.3	229	14.7	182	14.1
125	26.5	118	2.3	183	34.3	162	18.4			188	14.1
		120	34.1							198	31
		136	18.8								

Table 5. SSR fingerprint of the 29 sour cherrygenotypes with 6 *Prunus* primer pairs

No	**VARIETIES**	**UDP 96 001**	**UDP 96 005**	**BPPCT002**	**BPPCT030**	**BPPCT041**	**UCDCH 17**
1	Csengődi	115	110/120/136	167/183	140/158	201	178/198
2	Érdi bőtermő	101/115/125	106/120/136	167/183	140/162	201	178/198
3	Pándy 279	115	106/110/120	167/179/183	140/158	201	178/182/198
4	Pándy 48	115	106/110/120	167/179/183	140/158	201	178/182/198
5	Pándy BB 119	115	106/110/120	167/179/183	140/158	201	178/182/198
6	Újfehértói fürtös	115	106/110/120	167/179/183	140/158	201	178/182/198
7	Pipacs	115	106/110/120	167/183	140/162	201	178/188
8	Érdi nagygyümölcsű	115	120/136	167/183	140/162	201	178/188
9	Hybrid 3/48 9	115/125	106/120/136	167/183	140/162	201	178/188/198
10	Csengődi 11	115	110/120/136	167/183	140/158	201	178/198
11	Maliga emléke	115/125	106/120/136	**183**	140/162	201	178/198
12	Korai pipacs	101/115/125	106/120/136	167/183	140/162	201	182/188
13	Érdi jubileum	101/115/125	120/136	167/183	140/158/162	201	182/198
14	Meteor korai	115	106/**118**/120	167/183	140/158/162	201	182/198
15	Kántorjáncsi 10	115	106/110/120	167/179/183	140/162	201	178/182/198
16	Cigány C404	115/125	110/120/136	167/179	140/158	201/229	178/188
17	Cigány 7 13	115/125	110/120/136	167/179	140/158	201/229	178/188
18	Debreceni bőtermő	101/115	106/110/120	167/179/183	140/158	201	178/182/198
19	Piramis	115/125	106/**118**/120	167/183	140/158	201	178/188/198
20	Kántorjáncsi 3	101/115/125	106/110/120	167/179/183	140/158	201	178/182/198
21	Paraszt	115/125	110/120/136	167/179	140/158	201/229	178/188
22	Kőrösi korai	115	110/120/136	167/179	140/158	201	178/198
23	Cigány 59 12	115/125	110/120/136	167/179	140/158	201/229	178/188
24	Pándy Bb119 14	115	106/110/120	167/179/183	140/158	201	178/182/198
25	Favorit	115	106/120/136	167/183	140/162	201	178/198
26	Pándy 279 12	115	106/110/120	167/179/183	140/158	201	178/182/198
27	Pándy 48 13	115	106/110/120	167/179/183	140/158	201	178/182/198
28	Oblecsinszka	101/115/125	110/120/136	167/179	140/158	201/229	178/188/198
29	Érdi bőtermő 13	101/115/125	106/120/136	167/183	140/162	201	178/198

DOI: 10.18380/SZIE.COLUM.2017.4.1.31

Table 6. Microsatellite loci, alleles and their frequency in sweet cherry genotypes cultivated in Hungary

UDP 96 001		**UDP 96 005**		**BPPCT002**		**BPPCT030**		**BPPCT041**		**UCDCH 17**	
Fragment size bp	Frequency %	Fragment size bp	Frequency %	Fragment size bp	Frequency %	Fragment size bp	Frequency %	Fragment size bp	Frequency %	Fragment size bp	Frequency %
110	27.6	110	1.4	179	96.1	140	100	201	100	188	32.9
125	72.4	118	3.9	183	3.9					198	67.1
		120	42.1								
		136	52.6								

Table 7. SSR fingerprint of the 38 sweet cherry genotypes with 6 *Prunus* primer pairs

No	**VARIETIES**	**UDP 96 001**	**UDP 96 005**	**BPPCT002**	**BPPCT030**	**BPPCT041**	**UCDCH 17**
1	**Anita**	125/125	120:120	**179:183**	140:140	201:201	198:198
2	Aida	110/125	120:120	179:179	140:140	201:201	198:198
3	Alex	110/125	136:136	179:179	140:140	201:201	188:188
4	Bigarreau Burlat	110/125	120:136	179:179	140:140	201:201	188:198
5	**Botond**	125/125	**110/120**	179:179	140:140	201:201	188:188
6	Carmen	125/125	120:136	179:179	140:140	201:201	188:188
7	Germ.3	110/125	120:136	179:179	140:140	201:201	188:198
8	Germ. 45	110/125	120:136	179:179	140:140	201:201	188:198
9	**Hed. Óriás**	125/125	120:136	**183:183**	140:140	201:201	188:188
10	Kavics	110/125	120:136	179:179	140:140	201:201	188:188
11	Katalin	125/125	136:136	179:179	140:140	201:201	188:188
12	Krupnoplodnajaa	125/125	120:136	179:179	140:140	201:201	198:198
13	Linda	125/125	120:136	179:179	140:140	201:201	188:198
14	Münch. Korai	110/125	136:136	179:179	140:140	201:201	198:198
15	Margit	110/125	120:136	179:179	140:140	201:201	188:188
16	Pál	125/125	120:136	179:179	140:140	201:201	188:188
17	Péter	110/125	120:136	179:179	140:140	201:201	188:188
18	**Regina**	110/125	**118/136**	179:179	140:140	201:201	188:198
19	Rita	110/125	120:136	179:179	140:140	201:201	188:198
20	Sándor	110/125	136:136	179:179	140:140	201:201	188:198
21	Solymári gömb.	125/125	120:136	179:179	140:140	201:201	188:188
22	Stella	125/125	120:136	179:179	140:140	201:201	188:188
23	Sunburst	110/125	120:136	179:179	140:140	201:201	188:188
24	Szom. Fekete	125/125	120:136	179:179	140:140	201:201	188:188
25	Tünde	110/125	120:136	179:179	140:140	201:201	198:198
26	Val. Eskavol	110/125	120:136	179:179	140:140	201:201	198:198
27	Van	110/125	120:136	179:179	140:140	201:201	188:188
28	Vega	110/125	136:136	179:179	140:140	201:201	188:188
29	Vera	125/125	120:136	179:179	140:140	201:201	188:198
30	Canada giant	125/125	120:136	179:179	140:140	201:201	188:188
31	Germ. 1	110/125	120:136	179:179	140:140	201:201	188:198
32	Conley	125/125	136:136	179:179	140:140	201:201	188:188
33	Sylvia	110/125	120:136	179:179	140:140	201:201	188:188
34	Samba	125:125	120:136	179:179	140:140	201:201	188:188
35	**Octavia**	125:125	**118:118**	179:179	140:140	201:201	198:198
36	Merchart	125:125	120:136	179:179	140:140	201:201	198:198
37	Jabolay	110:125	120:136	179:179	140:140	201:201	188:188
38	Giorgia	110:125	120:136	179:179	140:140	201:201	188:188

Note. – numbers in bold are unique allele combinations.

DOI: 10.18380/SZIE.COLUM.2017.4.1.31

Table 8. The origin, and the observed allele sizes of the 6 SSR primers

Primer	Origin	Reference	Allele size range in sour cherry (bp)	Allele size range in sweet cherry (bp)	Allele size range according to our results (bp)	
					sour cherry	sweet cherry
UDP 96 001	peach	Cipriani et al. (1999)	99-113 (Turkoglu et al. 2010)	97-125 (Öz et al. 2013) 105-125 (Turkoglu et al. 2010)	101-125	110-125
UDP 96 005	peach	Cipriani et al. (1999)	115-135 (Turkoglu et al. 2010)	109-135 (Öz et al. 2013) 115-135 (Turkoglu et al. 2010)	106-136	110-136
BPPCT 002	peach	Dirlewanger et al. (2002)	168-182 (Antonius et al. 2011)	179–185(Dirlewanger et al. 2002)	167-183	179-183
BPPCT 030	peach	Dirlewanger et al. (2002)		140(Dirlewanger et al. 2002)	140-162	140
BPPCT 041	peach	Dirlewanger et al. (2002)		201(Dirlewanger et al. 2002)	201-229	201
UCD-CH17	sweet cherry	Struss et al. (2003)	178-202 (Turkoglu et al. 2010)	186-190 (Struss et al. 2003) 186-214 (Öz et al. 2013) 180-202 (Turkoglu et al. 2010)	178-198	188-198

in the litereture (*Table 8*), we found only 1-2 bp differences, but it is general because of the different gelelectrophoresis methods (acrylamid or capillary). Dendrograms constructed based on the SSR data of 29 sour cherry and 38 sweet cherry cultivars are shown in *Figure 2* and *3*.

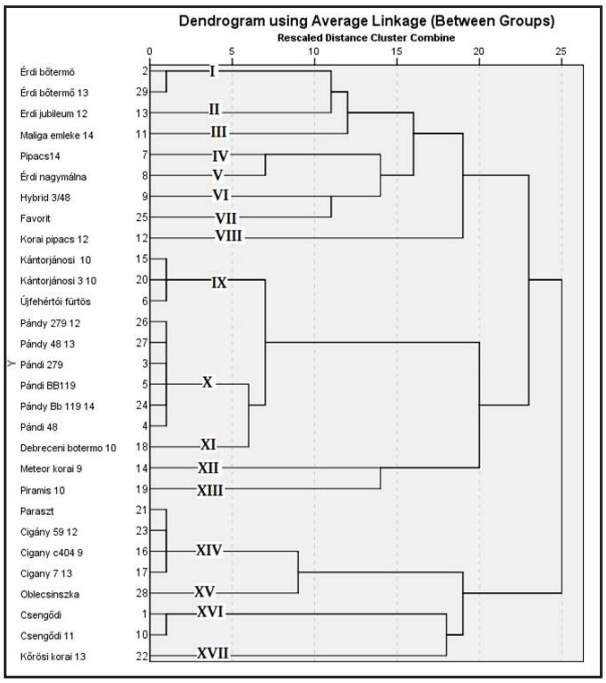


Figure 2. The SSR results of the analyzed 29 sour cherry genotypes

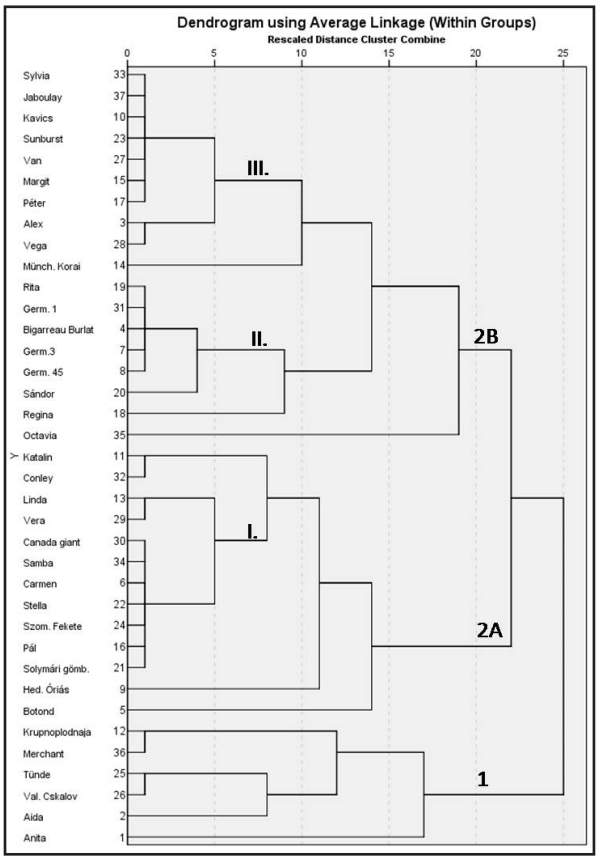


Figure 3. The SSR results of the analysed 38 sweet cherry genotypes

DOI: 10.18380/SZIE.COLUM.2017.4.1.31

Table 9. Parent-progeny relationship of Van, Stella and Sunburst

Primers	Van	Sunburst	Stella
BPPCT 041	201	201	201
BPPCT 030	140	140	140
BPPCT 002	179	179	179
UDP 96 005	120:136	120:136	120:136
UDP 96 001	110:125	110:125	125
UCDCH 17	188	188	188

The SSR results of sour cherry

The dendrogram (*Figure 2*) displays two major groups. The first large group includes I-XIII clusters and the second small group contains the XIV-XVII clusters with the Cigány clones, the cultivars Csengődi, Paraszt, Oblecsinszka and Kőrösi korai. The Oblecsinszka of Serbian origin is close to Cigány. The larger group consists of two small ones: the first contains the I-VIII clusters while the other the IX-XIII clusters. The Érdi varieties (Érdi bőtermő, Érdi jubileum, Érdi nagymálna), the Pipacs, Korai pipacs, Maliga emléke, Hybrid 3/48 and Favorit are in the first group. These cultivars have the same parent or grandparent which was the Pándy. Clones of Pándy, Debreceni bőtermő, Piramis, Meteor korai the Kántorjánosi and the Újfehértói fürtös are in the second group. These cultivars originate from natural and landscape selection. The Kántorjánosi clones and the Újfehértói fürtös could not be differentiated. These two cultivars were selected by the staff of Research and Extension Centre for Fruit Growing Ltd.

Based on the dendrogram it can be seen that the 6 SSR primers could discriminate the

Table 10. Parent-progeny relationship of Pándy (P1) and its offsprings Érdi jubileum, Érdi bőtermő, Maliga emléke, Korai pipacs, Favorit

Primers	Cultivars/ Clones	P1	Primers	Cultivars /Clones	P1
Primers	**Érdi jubileum**	**Pándy**	**Primers**	**Korai pipacs**	**Pándy**
BPPTC002	167:183	167:179:183	BPPTC002	167:183	167:179:183
BPPCT030	140:158:162	140:158	BPPCT030	140:162	140:158
BPPCT041	201	201	BPPCT041	201	201
UDP96-001	101:115:125	115	UDP96-001	101:115:125	115
UDP96-005	120:136	106:110:120	UDP96-005	106:122:136	106:110:120
UDC-CH017	182:198	178:182:198	UDC-CH017	182:188	178:182:198
Primers	**Érdi bőtermő**	**Pándy**	**Primers**	**Favorit**	**Pándy**
BPPTC002	167/183	167/179/183	BPPTC002	167183	167/179/183
BPPCT030	140/162	140/158	BPPCT030	140/162	140/158
BPPCT041	201	201	BPPCT041	201	201
UDP96-001	101115/125	115	UDP96-001	115	115
UDP96-005	106/122/136	106/110/120	UDP96-005	106/122/136	106/110/120
UDC-CH017	178/198	178/182/198	UDC-CH017	178/198	178/182/198
Primers	**Maliga emléke**	**Pándy**			
BPPTC002	183	167:179/183			
BPPCT030	140/162	140/158			
BPPCT041	201	201			
UDP96-001	115/125	115			
UDP96-005	106/122/136	106/110/120			
UDC-CH017	178/198	178/182/198			

cultivars except Kántorjánosi and Újfehértói fürtös. The clones gave the same microsatellite fingerprints. Because of the tetraploidy of the sour cherry we have to use more SSR primers to distinguish all cultivars as this has been confirmed by the participants of FA 1004 COST action. The Cigány clones and the Paraszt were indistinguishable at the chosen 6 SSR loci. According to several references these two cultivars might be synonyms. To answer this question either more polymorphic SSRs (about 32) are necessary for a better resolution of the relationships or e.g. SNP markers should be applied since according to Fernandez et al. (2009) SNPs are more efficient tools for cultivar fingerprinting and identification than SSRs.

The SSR results of sweet cherry

On the dendrogram (*Figure 3*) 3 main groups could be observed. The group (1) consists of Krupnoplodnaja, Merchant, Tünde, Valerij Cskalov, Aida and Anita. Tünde, Aida and Anita are Hungarian cultivars, Sándor Brózik and János Apostol were their breeders. One of the parents of Aida and Anita is a Trusenszkaja clone which was brought about in the former Soviet Union. Valerij Cskalov derives from open pollination of Rozojna and it was made in the former Soviet Union, too. Krupnoplodnaja originates from open pollination of Napoleon Blanc in Ukraine. This group (1) could be classified into the Slavic ancestor group except Merchant (which originates from the United Kingdom) and Tünde (Hungarian cultivar).

The remaining two groups (2A, 2B) contain mostly the Van (III), Germersdorfi (II) and Stella offsprings. with a few exceptions. There are cultivars (Münchenberg korai, Regina, Octavia, Hedelfingeni óriás, Botond) forming separate individual groups outside the main group.

Though Sándor, Pál as well as Péter have the same parents, only Sándor and Péter are in the same group (II) together with one of their parents (Bigarraue Burlat). Pál got in an other group (I) with the other parent, Stella.

As it can be seen on the dendrogram there are varieties which could not be distinguished from each other (Katalin-Conley, Valerij Cskalov-Tünde, Péter-Margit-Van-Kavics-Sunburt-Sylvia-Jaboulay, Alex-Vega, Canada giant-Samba-Carmen-Stella-Szomolyai fekete-Pál-Solymári gömbölyű). We need to include more polymorphic SSR loci in order to be able to discriminate them.

Parent-progeny relationship

Due to their Mendelian codominant inheritance, microsatellites can be used for pedigree identification of cultivars. The parent-progeny relationships may be clearly identified even if the actual or assumed crossing partners are heterozygous in the given microsatellite locus, because the progeny will receive one allele from one parent and the other allele from the other one.

Table 11. Parent-progeny relationship Hedelfingeni óriás, Germersdorfi óriás 3 and Linda

Primers	Hedelfingeni óriás	Linda	Germersdorfi óriás
UDP 96 005	120/136	120/136	120136
UDP 96 001	125	125	**110:125**
UCDCH 17	188	188/198	188/198

Table 12. Parent-progeny relationship of Van, Stella and Sunburst

Primers	Van	Sunburst	Stella
UDP 96 005	**120/136**	**120/136**	**120/136**
UDP 96 001	110/125	110/125	125
UCDCH 17	188	188	188

Table 13. Parent-progeny relationship of Bigarreau Burlatand Stella and their offsprings: Péter; Sándor, Pál

Primers	Bigarreau Burlat	Péter	Stella
UDP 96 005	120/136	120/136	120/136
UDP 96 001	110/125	110/125	125
UCDCH 17	188/198	188	188
Primers	**Bigarreau Burlat**	**Sándor**	**Stella**
UDP 96 005	120/136	136	120/136
UDP 96 001	110/125	110/125	125
UCDCH 17	188/198	188/198	188
Primers	**Bigarreau Burlat**	**Pál**	**Stella**
UDP 96 005	120/136	120/136	120/136
UDP 96 001	110/125	125	125
UCDCH 17	188/198	188	188

DOI: 10.18380/SZIE.COLUM.2017.4.1.31

Table 14. Parent-progeny relationship of Stella and its offspring, Samba

Primers	P1	Offspring
	Stella	Samba
UDP 96 005	120/136	120/136
UDP 96 001	125	125
UCDCH 17	188	188

Table 15. Parent-progeny relationship of Bigarreau Burlat and its offspring, Tünde

Primers	P1	Offspring
	Bigarreau Burlat	Tünde
UDP 96 005	120/136	120/136
UDP 96 001	110/125	110/125
UCDCH 17	188/198	198

Table 16. Relationship between Yellow Dragan's offsprings (Tünde; Carmen)

Primers	Tünde	Carmen
UDP 96 005	120/136	120/136
UDP 96 001	110/125	125
UCDCH 17	198	188

The following tables (*Table 9-15, 17, 18*) present the parent-progeny results of the sour and sweet cherry cultivars. In some cases we could examine only one of the putative parents (*Table 16 and 19*).

The SSR data obtained at 6 loci do not contradict the putative parentage of the following cultivars: Linda, Sunburst, Péter, Sándor, Pál, Samba, Tünde, Carmen, Germersdorfi clones (3 clones), Margit, Rita, Alex, Vera, Sylvia, Regina, Octavia (*Table 11-19*). However, exact determination would require to add more (~30) microsatellite loci to the analysis.

Conclusion

Five (UDP 96 001, UDP 96 005, BPPCT 002, BPPCT030, BPPCT041) of the 6 SSR primers were developed for peach. They could be applied in sour and sweet cherry genotyping together with the sweet cherry-specific UCD-CH 17. While the sour cherry varietes could be distinguished (expect Kántorjánosi and Újfehértói fürtös), several sweet cherry varietes

Table 17. Analysis of Germersdorfi offsprings' SSR fingerprints clones and seedlings (Margit), Germersdorfi x Szomolyai fekete

Primers	Germersdorfi clone 3	Germersdorfi clone 1	Germersdorfi clone 45	Margit
UDP 96 005	120:136	120:136	120:136	120:136
UDP 96 001	110:125	110:125	110:125	110:125
UCDCH 17	188:198	188:198	188:198	188
Primers	P1	Offspring	P2	
	Szomolyai fekete	Rita	Germersdorfi clone 1	
UDP 96 005	120:136	120:136	120:136	
UDP 96 001	125	110:125	110:125	
UCDCH 17	188:198	188:198	188:198	

Table 18. Parent-progeny relationship of Van and its offsprings

Primers	P1	Offspring	Offspring	Offspring
	Van	Alex	Vera	Sylvia
UDP 96 005	120/136	136	120/136	120/136
UDP 96 001	110/125	110/125	125	110/125
UCDCH 17	188	188	188:198	188

Table 19. Schneiders Spate Knorpelkirsche and Rube offsprings' realationship

Primers	Offspring	Offspring
	Regina	Octavia
UDP 96 005	118/136	118
UDP 96 001	110/125	110/125
UCDCH 17	188/198	198

DOI: 10.18380/SZIE.COLUM.2017.4.1.31

had the same SSR patterns. BPPCT primers described by Dirlewangeret al. (2002) did not give appropriate polymorphisms in sweet cherry despite these primers were more discriminative in sour cherry.There are two BPPCT primers which produced monomorphic pattern in the case of sweet cherry, while these primers amplified 2-4 different alleles in sour cherry. The UDP primers presented satisfactory level of polymorphism both in sweet and sour cherry. According to our results more UDP primers with known map position are advisable to use for discriminating the non-distinguishable varieties.

Acknowledgements

This study was supported by the Grants of Research Centre of Excellence 1476-4/2016/ FEKUT/SZIU.

References

Agarwal, M., Neeta, S., (2008): Advances in molecular marker techniques and their applications in plant sciences. Plant Cell Reports. **27**: 617–631. **DOI**: 10.1007/s00299-008-0507-z

Ahmad, R.; Potter, D.; Southwick, S. M. (2015): Identication and characterization of plum and pluot cultivars by microsatellite markers. The Journal of Horticultural Science and Biotechnology. **79**: 1. 164-169. **DOI**:10.1080 /14620316.2004.11511743

Alisoltani, A., Ebrahimi, S., Azarian, S., Hematyar, M., Shiran, B., Jahanbazi, H., Fallahi, H., Mousavi-Fard, S., Rafiei, F. (2016): Parallel consideration of SSRs and differentially expressed genes under abiotic stress for targeted development of functional markers in almond and related *Prunus* species. Scientia Horticulturae. **198**: 462-472. **DOI**: 10.1016/j.scienta.2015.10.020

Antonius, K., Aaltonen, M., Uosukainen, M., Hurme, T. (2011): Genotypic and phenotypic diversity in Finnish cultivated sour cherry (*Prunus cerasus* L.). Genetic Resources and Crop Evolution. **59:** 3. 375-388. **DOI**: 10.1007/ s10722-011-9688-3

Apostol, J. (2000): Breeding resistant sour cherry varieties in Hungary. Hungarian Agricultural Research Hungary.

Batnini, M. A., Krichen, L., Bourguiba, H., Trifi-Farah, N., Ruiz, D., Martínez-Gómez, P., Rubio, M. (2016): Comparative analysis of traditional and modern apricot breeding programs: A case of study with Spanish and Tunisian apricot breeding germplasm. Spanish Journal of Agricultural Research. **14:** 3. e0706. **DOI**: 10.5424/ sjar/2016143-8638

Beaver, J.A, Iezzoni,A.F., Ramn, C. (1995): Isozyme diversity in sour, sweet and ground cherry. Theoretical and Applied Genetics. **90**: 847-852. **DOI**: 10.1007/BF00222021

Cantini, C., Iezzoni,A. F., Lamboy,W. F., Boritzki,M.,Struss,D. (2001): DNA fingerprinting of tetraploid cherry germplasm using simple sequence repeats. Journal of the American Society for Horticultural Science **126**: 2. 205-209.

Cipriani, G., Lot, G., Huand, W.-G., Marrazzo, M. T., Peterlunger, E., Testolin, R. (1999): AC/GT and AG/CT microsatellite repeats in peach (*Prunus persica* (L) Batsch): isolation, characterization and cross-species amplification in *Prunus*. Theoretical and Applied Genetics. **99**: 65-72. **DOI**: 10.1007/s001220051209

Decroocq, V., Fave, M. G, Hagen, L., Bordenave, L., Decroocq, S. (2003): Development and transferability of apricot and grape EST microsatellite markers across taxa. Theoretical and Applied Genetics. **106:** 5. 912-922. **DOI**: 10.1007/s00122-002-1158-z

Dirlewanger, E., Cosson, P., Tavaud, M., Aranzana, M.J., Poizat, C., Zanetto, A., Arus, P., Laigret, F.(2002): Development of microsatellite markers in peach (*Prunus persica* (L.) Batsch) and their use in genetic diversity analysis in peach and sweet cherry. Theoretical and Applied Genetics. 105: 127–138. DOI: 10.1007/s00122-002-0867-7

Downey, S. L.,Iezzoni, A. F. (2000): Polymorphic DNA markers in black cherry (*Prunus serotina*) are identified using sequences from sweet cherry, peach, and sour cherry. Journal of the American Society for Horticultural Science. 125: 1. 76-80.

 DOI: 10.18380/SZIE.COLUM.2017.4.1.31

Eduardo, I., Picañol, R., Rojas, E., Batlle, I., Howad, W., Aranzana, M. J., Arús, P. (2015): Mapping of a major gene for the slow ripening character in peach: co-location with the maturity date gene and development of a candidate gene-based diagnostic marker for its selection. Euphytica. 205: 2. 627-636. DOI: 10.1007/s10681-015-1445-9

Ellegren, H (2004): Microsatellites: simple sequences with complex evolution. Nature Reviews Genetics. 5: 435-445. DOI:10.1038/nrg1348

Fan, S., Bielenberg, D. G,. Zhebentyayeva, T. N, Reighard, G. L.,. Okie, W. R, Holland, D., Abbott, A. G. (2010): Mapping quantitative trait loci associated with chilling requirement, heat requirement and bloom date in peach (*Prunus persica*). New Phytol. 185: 4. 917-930 DOI: 10.1111/j.1469-8137.2009.03119.x

Goodfellow, P. N. (1992): Variation is now the theme. Nature.359: 777-778.

Gustavsson (2014): Cost action FA1104 on sustainable production of high-quality cherries for Europe market: The WG 1 meeting on the use of molecular markers for diversity studies: 03-05 March 2014, Budapest.

Gürcan, K., Öcal, N., Yılmaz, K. U., Ullah, S., Erdoğan, A., Zengin, Y. (2015): Evaluation of Turkish apricot germplasm using SSR markers: Genetic diversity assessment and search for Plum pox virus resistance alleles. Scientia Horticulturae. 193: 155-164. DOI: 10.1016/j.scienta.2015.07.012

Haussmann, B. I., Hess, D. E., Omanya, G. O., Folkertsma, R. T., Reddy, B. V., Kayentao, M, Welz, H. G., Geiger, H. H. (2004): Genomic regions influencing resistance to the parasitic weed *Striga hermonthica*in two recombinant inbred populations of sorghum. Theoretical and Applied Genetics. 9: 1005–1016. DOI: 10.1007/s00122-004-1706-9

Höltken, A. M., Gregorius,H.-R. (2006): Detecting local establishment strategies of wild cherry (*Prunus avium* L.). BMC Ecology. 6: 1. 13. DOI: 10.1186/1472-6785-6-13

http 1 (inspection: 2017.03.18.) [online] : http://www.resinfru.hu/media/uploads/cseresznye/cseresznye_fajtaleirasok.pdf

Ivanovych, Y. I., Udovychenko, K., Bublyk, M., Volkov, R. (2017): ISSR-PCR fingerprinting of Ukrainian sweet cherry (*Prunus avium* L.) cultivars. Cytology and Genetics. 51: 1. 40-47. DOI: 10.3103/S0095452717010066

Joshi, N. K., Biddinger, D., Rajotte, E. G. (2011): A survey of apple pollination practices, knowledge and attitudes of fruit growers in Pennsylvania, in 10th International Pollination Symposium (Puebla, Mexico).

Kállay, E., Apostol, J., Vermes-Thuranszky, E. (1999): Anniversary conference of the Hungarian sweet cherry breeding. Budapest, Hungary, June 17-19, 1998:[proceedings]

Klabunde, G. H. F., Dalbó, M. A., Nodari, R. O. (2014): DNA fingerprinting of Japanese plum (*Prunus salicina*) cultivars based on microsatellite markers. Crop Breeding and Applied Biotechnology. 14: 3. 139-145. DOI: http://dx.doi.org/10.1590/1984-70332014v14n3a21

Lacis, G. (2014): Cost action FA1104 on Sustainable production of high-quality cherries for Europe market: The WG 1 meeting on the use of molecular markers for diversity studies: 03-05 March 2014, Budapest

Liu, Y. G., Biyashev, R. M., Saghai-Maroof, M. A. (1996): Development of simple sequence repeat DNA markers and their integration into a barley linkage map. Theoretical and Applied Genetics. 93: 869–876. DOI: 10.1007/BF00224088

Maccaferri, M., Sanguineti, M.C., Natoli, E., Araus-Ortega, J.L., Salem, M. B., Bort, J., Chenenaoui, S., Deambrogio, E., Garcia, D.M.L., De Montis, A. (2006): A panel of elite accessions of durum wheat (*Triticum durum* Desf.) Suitable for association mapping studies. Plant Genetic Resources. 4: 79–85. DOI: https://doi.org/10.1079/PGR2006117

Maghuly, F., Laimer, M. (2011): Microsatellite variability between apricot and related *Prunus* species. Paper read at BMC Proceedings DOI: 10.1186/1753-6561-5-S7-P10

Martínez-Gómez, P., Arulsekar, S., Potter, D., Gradziel, T. M. (2003): Relationships among peach, almond, and related species as detected by simple sequence repeat markers. Journal of the American Society for Horticultural Science. 128: 5. 667-671.

Morgante, M., Rafalski, A., Biddle, P., Tingey, S., Oliveri, A. M. (1994): Genetic mapping and variability of seven soybean simple sequence repeat loci. Genome. 37: 763–769. DOI: https://doi.org/10.1139/g94-109

Nayak, S. N., Zhu, H., Varghese, N., Datta, S., Choi, H. K., Horres, R. (2010): Integration of novel SSR and gene-based SNP marker loci in the chickpea genetic map and establishment of new anchor points with *Medicago truncatula* genome. Theoretical and Applied Genetics. 120: 1415–1441. DOI: 10.1007/s00122-010-1265-1

Nyéki, J.,Szabó, Z., Soltész, M. (2003): Sweet cherry (*Prunus avium* L.). In: Kozma, P. – Nyéki, J. – Soltész, M. – Szabó, Z.: Floral Biology, Pollination and Fertilisation in Temperate Zone Fruit Species and Grape. Akadémiai Kiadó. Budapest. 341-358.

Ogundiwin, E. A., Peace, C. P., Gradziel, T. M., Parfitt, D. E., Bliss, F. A., Crisosto, C. H. (2009): A fruit quality gene map of *Prunus.* BMC Genomics. 10: 587. DOI: 10.1186/1471-2164-10-587

Olukolu, B. A., Trainin, T., Fan, S., Kole, C., Bielenberg, D. G., Reighard, G. L., Abbott, A. G., Holland, D. (2009): Genetic linkage mapping for molecular dissection of chilling requirement and budbreak in apricot (*Prunus armeniaca* L.). Genome. **52**: 10. 819-828. **DOI**: 10.1139/g09-050

Öz, M. H.;Vurgun, H.;Bakir, M.;Buyuk, I.;Yuksel, C.;Unlu, H. M.;Cukadar, K., Karadogan, B., Kose, O., Ergul, A. (2013): Molecular analysis of East Anatolian traditional plum and cherry accessions using SSR markers. Genetics and Molecular Research. **12**: 4. 5310-20. **DOI**: http://dx.doi.org/10.4238/2013

Pacheco, I., Bassi, D., Eduardo, I., Ciacciulli, A., Pirona, R., Rossini, L., Vecchietti, A. (2014): QTL mapping for brown rot (*Monilinia fructigena*) resistance in an intraspecific peach (*Prunus persica* L. Batsch) F1 progeny. Tree Genetics & Genomes. **10:** 5. 1223-1242. **DOI**: 10.1007/s11295-014-0756-7

Pedersen, B. H. (2006): DNA fingerprints of 51 sweet and sour *Prunus* accessions using simple sequence repeats. The Journal of Horticultural Science and Biotechnology. **81**: 118-124. **DOI**: http://dx.doi.org/10.1080/14620316.2006.11512037

Pór, J., Faluba, Z. (1982): Cseresznye és meggy. Budapest, Mezőgazdasági Kiadó. 31-33, 108-112.

Powell, W., Machray, G., Provan, J., (1996): Polymorphism revealed by simple sequence repeats. Trends in Plant Science **1**: 215–222. **DOI**: https://doi.org/10.1016/1360-1385(96)86898-1

Prajapati, B., Gupta, J., Pandey, D., Parmar, G., Chaudhari, J. (2017): Molecular markers for resistance against infectious diseases of economic importance. Veterinary World. **10**: 1. 112. **DOI**: 10.14202/vetworld.2017.112-120

Radóczné Kocsis Teréz (2012): A cseresznyetermesztés helyzete Magyarországon. Agrofórum, 2012. September

Roder, M. S., Korzun, V., Wendehake, K., Plaschke, J., Tixer, M. H., Leroy, P., Ganal, M. W. (1998): A microsatellite map of wheat. Genetics. **149**: 2007–2023.

Rojas, G., Méndez, M. A., Muñoz, C., Lemus, G., Hinrichsen, P. (2008): Identification of a minimal microsatellite marker panel for the fingerprinting of peach and nectarine cultivars. Electronic Journal of Biotechnology. **11:** 5. 4-5. **DOI**: http://dx.doi.org/10.4067/S0717-34582008000500004

Rowland, L. J., Ogden, E. L., Bassil, N., Buck, E. J., McCallum, S., Graham, J., Brown, A., Wiedow, C., Campbell, A. M., Haynes, K. G., Vinyard, B. T. (2014): Construction of a genetic linkage map of an interspecific diploid blueberry population and identification of QTL for chilling requirement and cold hardiness. Molecular Breeding. **34:** 4. 2033-2048. **DOI**: 10.1007/s11032-014-0161-9

Schueler, S., Tusch, A., Schuster, M., Ziegenhagen, B. (2003): Characterization of microsatellites in wild and sweet cherry (*Prunus avium* L.) markers for individual identification and reproductive processes. Genome. **46**: 1. 95-102. **DOI:** https://doi.org/10.1139/g02-107

Senior, M. L., Chin, E. C. L., Lee, M., Smith, J. S. C. (1996): Simple sequence repeat markers developed from maize sequences found in the GenBank database: map construction. Crop Science. **36**: 1676–1683. **DOI**: doi:10.2135/cropsci1996.0011183X003600060043x

 DOI: 10.18380/SZIE.COLUM.2017.4.1.31

Spann, D., Möller, O., Büchele, M., Xuan, H., Ding, Y., Neumüller, M. (2010): Microsatellite markers (SRR) as a tool to assist in identification of European plum (*Prunus domestica*). Paper read at XXVIII International Horticultural Congress on Science and Horticulture for People (IHC2010): III International Symposium on 918.

Struss, D., Ahmas, R., Southwich,S.M. (2003): Analysis of sweet cherry (*Prunus avium* L.) cultivars using SSR and AFLP markers. Journal of the American Society for Horticultural Science, **128**: 904-909.

Temnykh, S. V., Park, W. D., Ayres, N., Cartinhour, S., Hauik, N., Lipovich, L. (2000): Mapping and genome organization of microsatellite sequences in rice (*Oryza sativa* L.). Theoretical and Applied Genetics. **100**: 697–712. **DOI**: 10.1007/s001220051342

Tropicos (2015) Inspection: 2015. August 28. [online]: http://www.tropicos.org/Name/27801088

Turet-Sayar, M., Turkec, A., Demir, T. (2012): Identification of Sweet Cherry Cultivars (*Prunus avium* L.) and Analysis of Their Genetic Relationship Using Microsatellite DNA Fingerprinting. Journal of Agricultural Science. **4:** 8. 134 **DOI:** http://dx.doi.org/10.5539/jas.v4n8p134

Turkoglu, Z., Bilgener, S., Ercisli, S., Bakir, M., Koc, A., Akbulut, M., Gercekcioglu, R., Gunes, M., Esitken, A. (2010): Simple sequence repeat-based assessment of genetic relationships among *Prunus* rootstocks. Genetics and Molecular Research. **9**: 4. 2156-2165. **DOI:** 10.4238/vol9-4gmr957.

Turkoglu, Z., Bilgener, S., Ercisli, S., Yildirim, N. (2013): Simple sequence repeat (SSR) analysis for assessment of genetic variability in wild cherry germplasm. Journal of Applied Botany and Food Quality. **85:** 2. 229.

Van-Der-Beek, J. G, Verkerk, R., Zabel, P., Lindhout, P. (1992): Mapping strategy for resistance genes in tomato based on RFLPs between cultivars: Cf9 (resistance to *Cladosporium fulvum)* on chromosome 1. Theoretical and Applied Genetics. **84**: 1-2. 106-112. **DOI**: 10.1007/BF00223988

Van Oppen, M. J., Rico, C., Turner, G.F., Hewitt, G.M. (2000): Extensive homoplasy, nonstepwise mutations, and shared ancestral polymorphism at a complex microsatellite locus in Lake Malawi cichlids. Molecular Biology and Evolution. **17**: 489-498. **DOI**: https://doi.org/10.1093/oxfordjournals.molbev.a026329

Vaughan, V., Lloyd, A. M. (2003): An analysis of microsatellite loci in *Arabidopsis thaliana*: mutational dynamics and application. Genetics. **165**: 1475-1488.

Wang, H., Walla, J. A., Magnusson, V. A., Zhong, S., Dai, W. (2014): Construction of genetic linkage maps and QTL mapping for X-disease resistance in tetraploid chokecherry (*Prunus virginiana* L.) using SSR and AFLP markers. Molecular Breeding. **34:** 1. 143-157. **DOI**: 10.1007/s11032-014-0025-3

Weising, K., Winter, P., Huttel, B., Kahl, G. (1998): Microsatellite markers for molecular breeding. Journal of Crop Protection. **1**: 113–143. **DOI**: http://dx.doi.org/10.1300/J144v01n01_06

Winter, P., Pfaff, T., Udupa, S., Huttel, B., Sharma, P. C., Sahi, S. (1999): Charecterization and mapping of sequence tagged microsatellite sites in chickpea (*Cicer arietinum*L.). Molecular and General Genetics MGG. **262**: 90–101. **DOI**: 10.1007/s004380051063

Wu, K. S., Tanksley, S. D. (1993): Abundance, polymorphism and genetic mapping of microsatellites in rice. Molecular and Genetics Genetics MGG. **241**: 225–235. **DOI**: 10.1007/BF00280220

Yang, G. P., Saghai Maroof, M. A., Xu, C. G., Zhang, Q., Biyshev, R. M. (1994): Comparative analysis of microsatellite DNA polymorphism in landraces and cultivars of rice. Molecular and General Genetics. 245: 2. 187–194. DOI: 10.1007/BF00283266

Characteristics and regulation of anthocyanin biosynthesis in pepper-review

Zsófia KOVÁCS[1] – Gábor CSILLÉRY[2] – Antal SZŐKE[1] – Erzsébet KISS[1] – Anikó VERES[1]

1: Szent István University, Faculty of Agricultural and Environemental Sciences Institute of Genetics, Microbiology and Biotechnology H-2100 Páter Károly u. 1. Gödöllő, Hungary. Email: veres.aniko@mkk.szie.hu

2: Budakert Ltd., Budapest, Hungary

Abstract: Pepper is an important horticultural crop due to its culinary as well as ornamental applications. Some *Capsicum* varieties build up anthocyanins in their different organs. The biosynthesis of these pigments – beside genetic determinism – depends on diverse factors such as the environment, developmental stage and type of tissue. Though anthocyanin biosynthetic pathway has been first described in the 1800s and from then on it has been well established even in species belonging to *Solenaceae*, information on the pathway is scarce in case of *Capsicum* spp. This review comprises the current knowledge on the biochemistry and molecular biology of the anthocyanin biosynthetic pathway.

Keywords: anthocyanin, biosynthetic pathway, *Capsicum*

Anthocyanins

Anthocyanins comprise a major branch of the phenylpropanoid pathway, thus they are found ubiquitously in nature. These pigments are responsible for a range of colours – from red to purple according to pH - in various fruits, vegetables and flowers and to a lesser extent in mosses. They colour different organs and tissues at their different stages of development and their accumulation is environmentally dependent. Besides colouring these water soluble pigments serve a wide variety of functions, e.g.; since they contribute to other organoleptic or nutritional qualities of plants such as flavour, moreover, they exhibit pharmaceutical applications to human health, anthocyanins are termed as nutraceutical compounds. As for their medical applications, studies showed that these secondary metabolites induce apoptosis, promote DNA repair and they also play a role in the protection against oxidative stress and in the inhibition of tumor cell proliferation (Winkel-Shirley, 2001a; Wang and Stoner, 2008; Welch et al., 2008; Kocic et al., 2011; Benvenuti et al., 2016). Furthermore polyphenols also possess cardio-protective effects besides their anti-inflammatory, analgesic, bactericidal, fungicidal, spasmolytic properties and antioxidant qualities (Cisowska et al., 2011; Wallace 2011; Yousuf et al., 2016). In the case of plants, anythocyanins support them in preventing ultra violet and oxidative light stresses and in attracting insect pollinators, thus they contribute to seed dispersal as well. They could also play a role in male fertility in the case of some plant species, signalling during nodulation, in auxin transport, and in defense mechanisms against antimicrobial agents, and in feeding deterrents (Holton and Cornish, 1995; Winkel-Shirley, 2002; Falcone Ferreyra Mí et al., 2012; Kumar and Pandey, 2013).

Anthocyanins emerge from a diverse family of aromatic molecules, called flavonoids. Flavonoids comprise six major subgroups in higher plants beside anthocyanins; chalcones, flavones, flavonols, flavandiols, condensed tannins (or proanthocyanidins) and aurones (Winkel-Shirley, 2001a). Specialized forms of flavonoids are also synthesized by some plant species, e.g.: isoflavonoids are synthesized almost exclusively by some leguminous plants and polymerized forms of phlobaphenes are synthesized by some maize, gloxinia and sorghum varieties (Winkel-Shirley, 2001a; Falcone Ferreyra Mí et al., 2012). To date, more than 9,000 flavonoid compounds have been found in different plants, therefore forming one of the largest families of natural products (Wang et al., 2011).

As of *Capsicum annuum* L., its fruit colour is mainly due to the mixture of different carotenoids

DOI: 10.18380/SZIE.COLUM.2017.4.1.47

Figure 1. Mutants showing different nodal colouration

and chlorophylls, thus its fruit rarely contains anthocyanins. Although there are good examples for the co-occurence of anthocyanins and carotenoids in the fruits of *Solanaceae* e.g. the tamarillo and tomato (Sadilova et al., 2006). There are various species of *Capsicum spp*. which show anthocyanin pigmentation not only in their fruits but in the flowers and foliage as well (Aza-González et al., 2012), however flower organs are rarely coloured (Chaim et al., 2003). Although, in a mutant collection comprising of almost 400 mutations maintained by Gábor Csilléry (*Figure 1*) different vegetative and generative organs show anthocyanin colouration (*Figure 2*) (Csilléry, 2016b, a).

Ripe fruit colour varies from yellow to red and it can even be brown as a combination of red and green colour. The colour of unripe pepper fruit however can vary from ivory to nearly black including yellow, different shades of green, lilac and purple (Anderson, 2006). Foliar pigmentation together with the lengthy maturation period with its purple and black

Figure 2. Mutants showing different level of anther colouration

DOI: 10.18380/SZIE.COLUM.2017.4.1.47

colours provides ornamental interest of pepper as well as culinary applications (Lightbourn et al., 2008). Different analytical experiments showed that the main and only anthocyanidin found in the fruit, foliage and in the flower of pepper is delphinidin-3-p-coumaroyl-runtinoside-5-glucoside (Sadilova et al., 2006; Aza-González and Ochoa-Alejo, 2012).

Genetic background of anthocyanin biosynthesis

Genetic background of anthocyanin pigmentation has been studied extensively since the early work of Mendel on pea (*Pisum sativum* L.) flower colour in the 1800s. By then anthocyanin biosynthesis pathway has been well established in the case of maize (*Zea mays)*, petunia (*Petunia x hybrida)*, snapdragon (*Antirrhinum majus*) and lately Arabidopsis (*Arabidopsis thaliana)* is also used as a model plant (Holton and Cornish, 1995; Winkel-Shirley, 2001b; Shi and Xie, 2014). Pigmentation pattern is diverse despite of the biosynthetic pathway in the listed plants share many common reactions. The discrepancies can either be explained by the different regulation of structural genes, or that some structural genes are not expressed in plants. In addition, some genes encode enzymes with different substrate specificity, for e.g. a major difference is that petunia does not produce pelargonidin pigments, while snapdragon and maize are incapable of synthesizing delphinidin. This can be explained by the substrate specificity, since petunia dihydroflavonol reductase enzyme does not use dihydrokaempferol as substrate (Holton and Cornish, 1995).

Locus *A* is responsible for the anthocyanin biosynthesis in the immature fruit, flower and in the foliage in *Capsicum spp* (*Figure 4)*. This *A* locus is incompletely dominant and is responsible for the violet and black colour in the flower, foliage and immature fruit. Another gene; *MoA* (Deshpande, 1933; Daskalov and Poulos, 1994), in the presence of *A* intensifies the purple colouration of the tissues. Number of genes (*al-1, al-2, al-3, al-4*, *al-5,* and *al-6, al-7* in *Capsicum chinense* furthermore, *al-8* in *C. chacoense*) are responsible for the development of anthocyanin-less tissues (*Figure 4)* (Csillery, 1980; Csillery, 1983).

Purple colouration can also occur in the style and in the filament even in the abscence of *A*, in this case, purpe colour is determined by *Asf*, whilst *As* in the abscence of *A* or *Asf* will colour the style purple (*Figure 4)* (Odland, 1960; Lippert et al., 1966).

Anther is the only tissue where the anthocyanin pigmentation is not controlled by *A*, locus *Fc* is responsible for the purple pigmentation of anther filament (Borovsky et al., 2004). Mapping of genes related to anthocyanin biosynthesis has been reported in the family of *Solanaceae* family including pepper. In the case of pepper, genes controlling anthocyanin biosynthesis have been mapped to chromosome 10. Both *A* and *Fc* were mapped in the same position of chromosome 10, suggesting that they are allelic (Chaim et al., 2003).

Genomes of species belonging to *Solanaceae* – especially tomato (*Solanum lycopersicum*), petunia (*Petunia x hybrida*), potato (*Solanum tuberosum*), eggplant (*Solanum melongena*) and pepper (*Capsicum annuum* L.*)* - share extensive co-linearity of gene order although there are several chromosomal rearrangements in their genomes, meaning that if a gene position has been determined in one of the species, its position may coincide in the others (De Jong et al., 2004).

Such orthologous loci are the *A* on pepper chromosome 10 in a region that coincides with the chromosomal region of potato *F* and *I* – controlling flower and tuber skin colour - *fap10.1* and other major genes for anthocyanin present in eggplant and tomato *ag* (*anthocyanin gainer*), or *Aft* (*Anthocyanin fruit)* a dominant mutation introgressed from *S. chilense*.

Further mapping of genes showed that petunia *An2* is located in the same site on chromosome 10 where *A* was mapped in pepper (Borovsky et al., 2004; De Jong et al., 2004; Paran and van der Knaap, 2007; Lightbourn et al., 2008).

Regulatory and structural elements of the pathway

Regulatory as well as structural genes are necessary for the anthocyanin biosytnhesis (Aza-Gonzalez et al., 2013). Enzymes of the biosynthetic pathway have already been characterized and plenty of genes coding for these enzymes have been cloned and they showed high sequence similarity among species (Stommel et al., 2009).

Enzymes involved in pepper anthocyanin biosynthesis are; phenylalanine ammonia-lyase (*PAL*), cinnamate 4-hydroxylase (*C4H*), 4-coumarate: CoA ligase (*4CL*), chalcone synthase (*CHS*), chalcone isomerase (*CHI*), flavanone 3-hydroxylase (*F3H*), flavonoid 3′,5′-hydroxylase (*F3′5′H*), dihydroflavonols 4-reductase (*DFR*), anthocyanin synthase (Quattrocchio et al., 1993), and UDP-glucose flavonoid 3-glucosyltransferase (*UFGT*). Additional enzymes which are not directly linked to anthocyanin biosythetic pathway are the anthocyanin permease (*ANP*) and gluthaione S-transferase (Thorup et al., 2000) both act in sequestration of anthocyanin in the vacuole. Additionally 3/5-O-glycosyltransferases (*3GT/5GT*), rhamnosyl transferase (*RT*) and O-methyltransferase (*OMT*) or anthocyanin methyltransferase can further modify flavonoids to produce anthocyanins (*Figure 4)* (Holton and Cornish, 1995; Borovsky et al., 2004; Stommel et al., 2009; Aza-Gonzalez et al., 2013; Aguilar-Barragán and Ochoa-Alejo, 2014).

The first enzyme of the anthocyanin biosynthetic pathway is *CHS* that forms tetrahydroxychalcone by using malonly-CoA and 4-coumaroyl CoA as substrate. Then, tetrahydroxychalcone will be isomerized by *CHI* to naringenin, which will be converted to dihydrokaempferol by *F3H. F3'5'H* will then hydroxylate the dihydrokaepmferol to form colouress dihydroflavonols. These will be converted to coloured anthocyanins by *DFR, ANS* and *UFGT.*

Enzymes of anthocyanin biosynthesis pathway can be grouped based on either being coded by early or late structural genes. Early structural genes (EBG) of the pathway are the *CHI, F3H, F3'5'H* the late genes (LBG) are the *DFR, ANS, UFGT* and *RT* (*Figure 4)* (Zhang et al., 2015). Just as in the case of the Petunia, the early genes of the pathway in pepper are expressed independently of regulatory genes whereas late genes are *A*-dependent (Quattrocchio et al., 1993). Genomic comparison of *A* revealed that there is no sequence difference in the coding region in green, - or purple fruited peppers, thus colour differences are due to the variations in the promoter regions (Borovsky et al., 2004).

In the case of pepper the regulation of the pathway is controlled by three types of transcription factor (TF) families: bHLH MYC, R2R3-MYB and WD40 repeat proteins (Stommel et al., 2009). Although in Arabidopsis, at least six transcription factors belonging to MYB, bHLH, WD40, WRKY, zinc finger, and MADS box proteins are involved in the pathway (Terrier et al., 2009).

Most plant MYB proteins are characterized by two imperfect repeats; R2 and R3 making R2R3-MYB the largest TFs gene family in plants. There are two other subfamilies of MYB TFs, the MYB 1R and MYB 3R (Li et al., 2011). The smallest subfamily is the 4R MYB group comprises of R1 R2 repeats (Dubos et al., 2010). These subfamilies differ in the number of their imperfect repeats of the conserved MYB DNA-binding motif.

The repeats encode three α-helices of 50 to 53 amino acids, and the second and the third helices form a helix-turn-helix (HTH) structure when they bind to the DNA (Hichri et al., 2011; Aguilar-Barragán and Ochoa-Alejo, 2014). R2R3-MYB TFs are involved

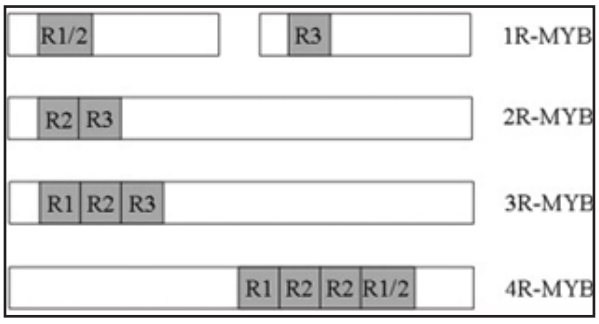


Figure 3. Imperfect repeats of MYB transcription factor

DOI: 10.18380/SZIE.COLUM.2017.4.1.47

in diverse physiological and biochemical processes including the regulation of secondary metabolism, control of cell morphogenesis, regulation of meristem formation, floral and seed development and the control of the cell cycle. Some of them are also involved in various defense and stress responses and in light and signal transduction pathways (Du et al., 2009; Berenschot and Quecini, 2014). Anthocyanin-related R2R3-MYB proteins are encoded by *colorless1 (C1)* - for seed pigmentation - and *Pl* - for the pigmentation of other plant tissues –multigene families were first described in maize (Gonzalez et al., 2008; Stommel et al., 2009). MYB proteins together with its MYC partners act both as the activators of the structural genes, and MYB alone activates the gene coding for the bHLH transcription factor (Liu et al., 2015).

Another widespread transcription factor is the basic helix-loop-helix (bHLH) protein also known as MYC. These proteins contain a conserved bHLH domain in their C-terminus region, which consists of a DNA-binding basic region followed by two helices (Zhao et al., 2013; Aguilar-Barragán and Ochoa-Alejo, 2014). Plant MYC proteins were also first described in maize where these R-like proteins interact with C1 MYB proteins and by binding to the promoters of the biosynthesis genes they activate anthocyanin synthesis in maize (Stommel et al., 2009).

WD40 or WDR (WD repeat) proteins are characterized by a peptide motif of 44–60 amino acids, typically delimited by a core region that contains the glycine-histidine (GH) dipeptide on the N-terminal side (11–24 residues from the N-terminus) and the tryptophan-aspartate dipeptide at the C-terminus (Stommel et al., 2009; Hichri et al., 2011). The WD motif is tandemly repeated 4 to 16 times thus forming the WDR protein, which then acts as a platform to facilitate diverse protein-protein interactions (Stommel et al., 2009; Aguilar-Barragán and Ochoa-Alejo, 2014).

At least one member of each of the transcription factor families is required to control tissue, - and developmental stage specific expression of anthocyanin structural genes (Li et al., 2011). Different varieties of species have been investigated and showed that the anthocyanin pathway is activated by similar MYB, MYC and WD40 proteins, suggesting that their function is conserved (Quattrocchio et al., 2006).

As discussed anthocyanin pigmentation in *Capsicum annuum* is influenced by an incompletely dominant gene, *A* which encodes Myb_a transcription factor, which is absent from genotypes that do not accumulate anthocyanins (Lightbourn et al., 2007).

If the *A* locus is present, TFs form a complex (MBW) which then interacts with the promoters of the structural genes of the anthocyanin biosynthetic pathway in order to modulate their expression. Before or after its assembly of the WD40 and MYC, the bHLH protein binds to a specific amino acid sequence: (DE) Lx2(RK)x3Lx6Lx3R on helices 1 and 2 of the R3 repeat of MYB. When it is coupled, the MYB will bind to the recognition element of the consensus sequence AACCTA of the anthocyanin biosynthesis structural gene promoter and MYC will be bound to the E-box of the promoter containing consensus sequence CAGCTG (Lightbourn et al., 2007).

As mentioned above structural genes can be grouped whether they are early, or late biosynthetic genes. Interaction of MBW complexes lead to an increase of the expression of the LBGs; *DFR, ANS, UFGT* and *RT* which are *A*-dependent. Separation pattern of the early and late genes varies according to plant species, for example: anthocyanin biosynthesis of Arabidopsis is regulated at *F3´H*, while the pathway of petunia is regulated at the *DFR* step (Quattrocchio et al., 1993; Gonzalez et al., 2008).

Different expression studies have been carried out that rectify the regulation of *A*-dependent genes. For example, a study investigated a purple fruited and a green fruited genotype. In the purple fruited anthocyanins became visible 10 days post-anthesis, reached their maximum level around day 20 and they disappeared upon ripening. Anthocyanins could not be detected

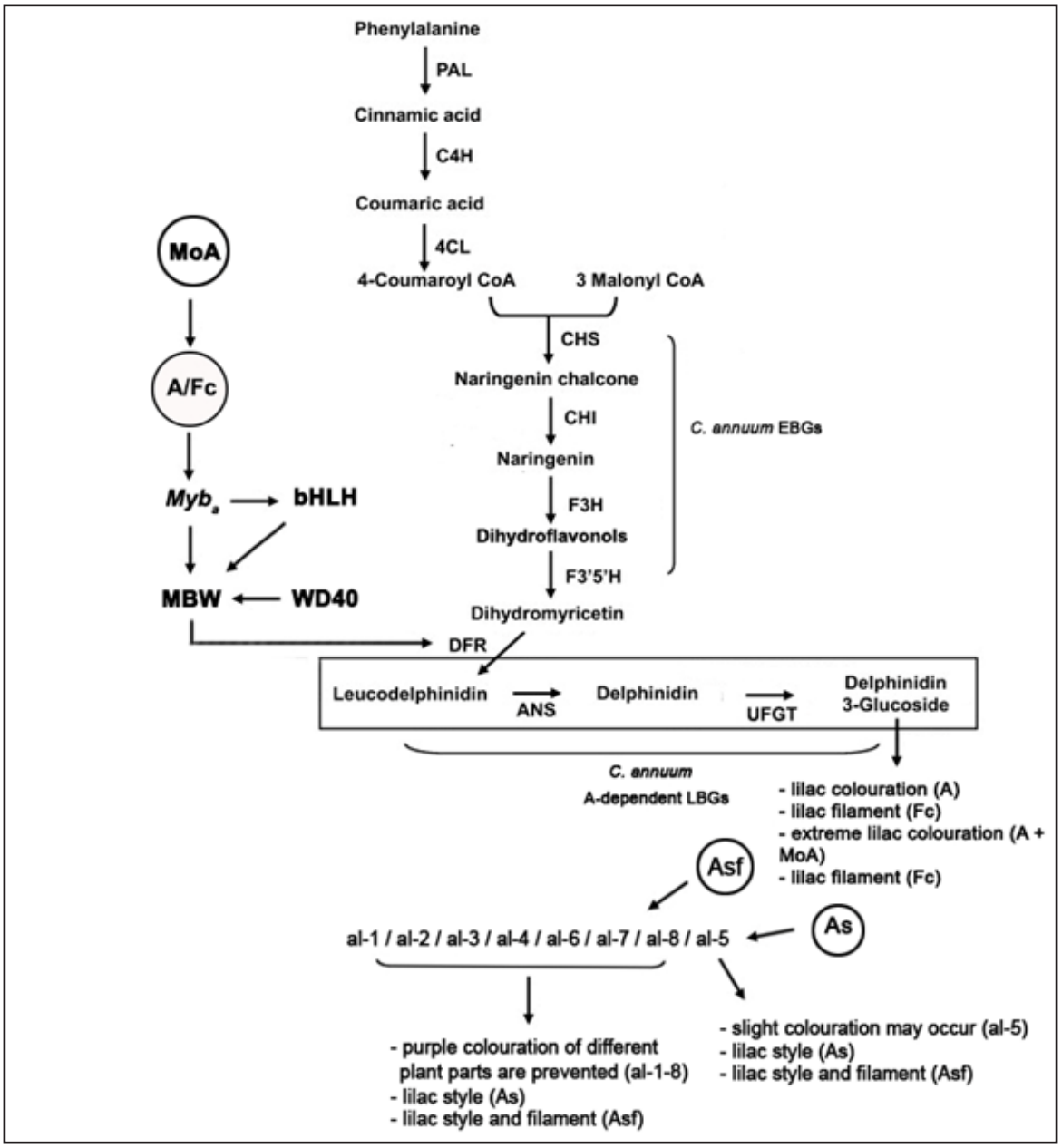


Figure 3. Simplified anthocyanin biosynthetic pathway

at any stage of fruit development in the green fruited plants. Northern analysis showed that in the purple fruited plant transcript accumulation was detected at all stages of fruit development, except for the ripe fruit and in flower petals and leaves, while no transcripts could be detected in the green fruited genotype, which can be explained by the lack of expression of *A.* PCR amplification of the *A* gene showed that the length of the PCR products from both genotypes are indistinguishable. Expression analysis of different structural genes of the pathway showed results that the early genes (*CHI, CHS)* were present in all tissues of both genotypes. On the other hand, late genes, such as *DFR* and *ANS* could only be detected in the purple fruited and not in the green fruited genotype (Borovsky et al., 2004).

Regulatory and structural genes were also examined in order to investigate how they influence tissue-specific expression. Two related genotypes of common pedigree were selected; one with purple flowers and black fruit and foliage, the other with white flowers and green fruit and foliage. Though the second genotype lacked anthocyanin in its fruit, flower or foliage,

 DOI: 10.18380/SZIE.COLUM.2017.4.1.47

it contained some in its internodes, meaning that the lack of anthocyanin in its other tissues could be a result of differential tissue-specific gene expression. Expression *of CHS, DFR, ANS* from the biosynthetic genes and *Myc, Myb*$_a$ and *Wd* genes were analyzed in the flower, fruit, and foliar tissues of both anthocyanin-pigmented and non pigmented genotypes. Transcript levels of structural genes were higher in anthocyanin pigmented tissues than in the non pigmented genotype. *Wd* transcript levels did not differ significantly between the genotypes. *Myb*$_a$ and *Myc* transcript levels – consistent with the higher levels of structural gene transcripts – were higher in the floral and fruit tissues of the anthocyanin-pigmented genotype. However, in leaf tissue there was no significant difference in either of the regulatory transcript levels between the two genotypes. The *Myb*$_a$ and *Myc* transcript levels of the leaf tissue were significantly lower in the case of the anthocyanin-pigmented genotype than in the flower or in the fruit tissue. This suggests that other mechanisms contribute to the anthocyanin regulation of the foliage (Stommel et al., 2009).

Factors affecting purple colouration

Post-transcriptional gene silencing by miRNAs could explain the differential expression. These microRNAs are endogenous single-stranded approximately 20-24 nucleotide long RNAs that are associated with the RNA-induced silencing complex (RISC). They play important regulatory roles in both animals and plants by targeting mRNAs for cleavage or translational repression. They are likely to influence the output of many protein-coding genes. The majority of these genes codes for transcriptional factors. Beside regulation of gene expression, they also play role in plant development, signal transduction, protein degradation, response to environmental stress and pathogen invasion, and regulate their own biogenesis (Bartel, 2004; Zhang et al., 2006; Li et al., 2007). Different studies suggest that miRNAs could be involved in the regulation of the anthocyanin biosynthetic pathway. In Arabidopsis, increased miRNA156 activity enhanced the accumulation of anthocyanins, whereas reduced miRNA156 activity resulted in high levels of flavonols. It was also suggested that one of the miRNA156 targets SQUAMOSA PROMOTER BINDING PROTEIN LIKE 9 (SPL), negatively regulates anthocyanin accumulation by directly preventing expression of biosynthetic genes through the destabilization of a MBW transcriptional complex. Studies of Litchi (*Litchi chinensis* Sonn.) and Chinese radish (*Raphanus sativus* L.) indicated the same, i.e. several target genes for the miRNAs encode TFs involved in anthocyanin biosynthesis, including MYB, bHLH, WD40 repeat, SPL, auxin response factor, ethylene insensitive 3, WRKY and MADS-box proteins (Gou et al., 2011; Liu et al., 2016; Sun et al., 2017). A study targeting tomato miRNAs brought into light that miRNA858 regulates anthocyanin biosynthesis by modulating the expression of two R2R3-MYB transcription factors (Jia et al., 2015). Virus induced gene silencing (VIGS) vector systems have been developed from both RNA and DNA plant viral sources to specifically silence target genes in plants (Lange et al., 2013). This VIGS technique has been employed successfully in silencing of the R2R3-MYB transcription factor in *Capsicum spp.* (Kim et al., 2017). Silencing of the MYB also altered MYC and WD40 transcript levels in the *CaMYB* silenced leaves.

Expression of flavonoid pathway genes were also altered in the silenced plants (Zhang et al., 2015). In another study similar results were found when Tobacco rattle virus (TRV) constructs were used for VIGS in *Capsicum eximium.* Chili pepper fruits were transformed with TRV2-MYB and TRV2-WD40 constructs. Compared to control, these plants demonstrated reduced accumulation of anthocyanins in their fruits. This reduction both included the structural and the TF genes. Plants transformed with TVR2-MYB constructs exhibited decreased expression of *CHS, CHI, F3'5'H, DFR* and *3GT* genes, whereas there was no decrease in the level of *F3H.* Chilies infected with the TRV2-WD40 construct displayed reduction in *CHS, F3H , F3'5'H, DFR* and *3GT* but not in *CHI* in their fruit (Aguilar-Barragán and Ochoa-Alejo, 2014).

Even environmental conditions have an impact on the degree of anthocyanin pigmentation. Numerous articles describe the effect of light on anthocyanin biosynthesis (Mancinelli, 1985; Takos et al., 2006; Cominelli et al., 2008; Albert et al., 2009; Nakatsuka et al., 2009). Temperature is another well known factor which effects anthocyanin accumulation. Although a study demonstrated that anthocyanin content of leaves from *C. annuum* was not influenced by temperature whether the plants were grown under either low or high light conditions. High light positively influenced *CHS, DFR* and *ANS* expression both at low and high temperature. As of regulatory genes, they had a constant level of expression under all circumstances, except that low temperature – high light condition triggered a higher Myb_a expression (Lightbourn et al., 2007). However, both structural and regulatory gene transcript levels increased under low-temperature treatment of *Zea mays* L. seedlings (Christie et al., 1994).

Nutrient deficiency can also trigger anthocyanin colouration. The most affecting one is phosphorus (P) availability, a distinctive symptom of a plant suffering from P shortage is anthocyanin pigmentation (Jiang et al., 2007), although nitrogen deficiency can also lead to anthocyanin accumulation. Boron, magnesium, sulphur and zinc deficiencies were too reported to enhance anthocyanin accumulation (Chalker-Scott, 2002). Nutrient shortage generates similar responses to oxidative stress response as a result of high light conditions, thus the photo-protective role of these molecules could also be relevant for stress caused by nutrient deficiency (Henry et al., 2012). Nitrogen starvation affects the photosynthesis of plants via the interruption of the photosynthetic membrane due to starch accumulation, resulting in an increased light sensitivity. To prevent oxidative damage, the plant will produce an elevated amount of anthocyanins and flavonols which serve as photo-protective pigments (Stewart et al., 2001). A relationship is hypothesised between nutritional deficiencies, anthocyanin build-up and water stress. Foliar pigmentation caused by P stress is speculated to play role in the osmoregulation of water stress induced by low P levels (Chalker-Scott, 2002). Different environmental conditions such as flooding or cold soil conditions can both result in a decreased P uptake (Steyn et al., 2002) due to the relative shortage of P, thus leading to anthocyanin pigmentation. Symptoms caused by this relative shortage are transient and mainly affect young seedlings. Upon warmer soil and aerial conditions the colouration will gradually disappear (http1).

Another argument supporting the research of anthocyanin content of different pepper varieties is from the breeder's point of view. Thanks to the ever detailed genetic map of *C. annuum* linkage groups of different economically important genes have been described (Prince et al., 1993; Yi et al., 2006; Lee et al., 2009; Wu et al., 2009; Cheng et al., 2016). For example: *anthocyaninless* gene could possibly be linked to the L^3 gene of TMV resistance (Csilléry and Ruskó, 1980; Zatykó and Moór, 1998), linkage of *A* to a major quantitative trait locus for fruit shape index *fs10.1* – or locus *O* that is responsible for the round shaped fruit - has been described both in pepper and in potato (Peterson, 1959; Chaim et al., 2003)). The *anthocyaninless* gene is also used as a marker in producing hybrid lines, where anthocyaninless, male-sterile plants are used as female crossing partners (Csillery et al., 1986). Investigation of further linkages between different economically important genes and the use of molecular markers could help the breeders in the selection of desirable trait combinations in the early cotyledonous stage.

Acknowledgements

This study was supported by the Grants of Research Centre of Excellence *1476-4/2016/FEKUT/SZIU.*

 DOI: 10.18380/SZIE.COLUM.2017.4.1.47

References

Aguilar-Barragán, A., and Ochoa-Alejo, N. (2014). Virus-induced silencing of MYB and WD40 transcription factor genes affects the accumulation of anthocyanins in chilli pepper fruit. *Biologia plantarum* **58**, 567-574.

Albert, N. W., Lewis, D. H., Zhang, H., Irving, L. J., Jameson, P. E., and Davies, K. M. (2009). Light-induced vegetative anthocyanin pigmentation in Petunia. *Journal of experimental botany* **60**, 2191-2202.

Anderson, N. O. (2006). "Flower breeding and genetics: issues, challenges and opportunities for the 21st century," Springer Science & Business Media.

Aza-Gonzalez, C., Herrera-Isidrón, L., Núñez-Palenius, H., De La Vega, O. M., and Ochoa-Alejo, N. (2013). Anthocyanin accumulation and expression analysis of biosynthesis-related genes during chili pepper fruit development. *Biologia Plantarum* **57**, 49-55.

Aza-González, C., Núñez-Palenius, H. G., and Ochoa-Alejo, N. (2012). Molecular biology of chili pepper anthocyanin biosynthesis. *Journal of the Mexican Chemical Society* **56**, 93-98.

Aza-González, C., and Ochoa-Alejo, N. (2012). Characterization of anthocyanins from fruits of two Mexican chili peppers (Capsicum annuum L.). *Journal of the Mexican Chemical Society* **56**, 149-151.

Bartel, D. P. (2004). MicroRNAs: genomics, biogenesis, mechanism, and function. *cell* **116**, 281-297.

Benvenuti, S., Bortolotti, E., and Maggini, R. (2016). Antioxidant power, anthocyanin content and organoleptic performance of edible flowers. *Scientia Horticulturae* **199**, 170-177.

Berenschot, A. S., and Quecini, V. (2014). A reverse genetics approach identifies novel mutants in light responses and anthocyanin metabolism in petunia. *Physiol Mol Biol Plants* **20**, 1-13.

Borovsky, Y., Oren-Shamir, M., Ovadia, R., De Jong, W., and Paran, I. (2004). The A locus that controls anthocyanin accumulation in pepper encodes a MYB transcription factor homologous to Anthocyanin2 of Petunia. *Theor Appl Genet* **109**, 23-9.

Chaim, A. B., Borovsky, Y., De Jong, W., and Paran, I. (2003). Linkage of the A locus for the presence of anthocyanin and fs10.1, a major fruit-shape QTL in pepper. *Theor Appl Genet* **106**, 889-94.

Chalker-Scott, L. (2002). Do anthocyanins function as osmoregulators in leaf tissues? *Advances in Botanical Research* **37**, 103-127.

Cheng, J., Zhao, Z., Li, B., Qin, C., Wu, Z., Trejo-Saavedra, D. L., Luo, X., Cui, J., Rivera-Bustamante, R. F., and Li, S. (2016). A comprehensive characterization of simple sequence repeats in pepper genomes provides valuable resources for marker development in Capsicum. *Scientific reports* **6**.

Christie, P. J., Alfenito, M. R., and Walbot, V. (1994). Impact of low-temperature stress on general phenylpropanoid and anthocyanin pathways: enhancement of transcript abundance and anthocyanin pigmentation in maize seedlings. *Planta* **194**, 541-549.

Cisowska, A., Wojnicz, D., and Hendrich, A. B. (2011). Anthocyanins as antimicrobial agents of natural plant origin. *Nat Prod Commun* **6**, 149-56.

Cominelli, E., Gusmaroli, G., Allegra, D., Galbiati, M., Wade, H. K., Jenkins, G. I., and Tonelli, C. (2008). Expression analysis of anthocyanin regulatory genes in response to different light qualities in Arabidopsis thaliana. *J Plant Physiol* **165**, 886-94.

Csillery, G. (1980). Gene mapping of the pepper needs more initiatives (Contribution to the gene list). *Eucarpia. Proc. IV Mtg. Capsicum Working Group, Wageningen, The Netherlands*, 27-28.

Csillery, G. (1983). New Capsicum mutants found on seedling, growth type, leaf, flower and fruit. *In* "Proc. 5th Eucarpia Meeting of Capsicum and Eggplant Working Group", pp. 127-130.

Csilléry, G. (2016a). Paprikamutánsok. *Kertészet és szőlészet* **65.**, 6-9.

Csilléry, G. (2016b). Pepper mutant collection.

Csillery, G., Quagliotti, L., and Rota, A. (1986). Natural cross-pollination experiment on pepper (Capsicum annuum L.) in Piedmont, Italy, in 1986. *Capsicum Newsletter* **28**, 38-39.

Csilléry, G., and Ruskó, J. (1980). The control of a new Tobamovirus strain by a resistance linked to anthocyanin deficiency in pepperC. annuum. *Synopses IVth Meet. Eucarpia Capsicum Working Group, Wageningen*, 40-43.

Daskalov, S., and Poulos, J. (1994). Updated Capsicum gene list. *Capsicum and Eggplant Newsletter* **13**, 15-26.

De Jong, W. S., Eannetta, N. T., De Jong, D. M., and Bodis, M. (2004). Candidate gene analysis of anthocyanin pigmentation loci in the Solanaceae. *Theor Appl Genet* **108**, 423-32.

Deshpande, R. (1933). Studies in Indian chillies. 3. The inheritance of some characters in Capsicum annuum L. *Indian Jour. Agr. Sci* **3**, 219-300.

Du, H., Zhang, L., Liu, L., Tang, X.-F., Yang, W.-J., Wu, Y.-M., Huang, Y.-B., and Tang, Y.-X. (2009). Biochemical and molecular characterization of plant MYB transcription factor family. *Biochemistry (Moscow)* **74**, 1-11.

Dubos, C., Stracke, R., Grotewold, E., Weisshaar, B., Martin, C., and Lepiniec, L. (2010). MYB transcription factors in Arabidopsis. *Trends Plant Sci* **15**, 573-81.

Falcone Ferreyra Mí, L., Rius Sá, P., and Casati, P. (2012). Flavonoids: biosynthesis, biological functions, and biotechnological applications. *Front Plant Sci* **3**.

Gonzalez, A., Zhao, M., Leavitt, J. M., and Lloyd, A. M. (2008). Regulation of the anthocyanin biosynthetic pathway by the TTG1/bHLH/Myb transcriptional complex in Arabidopsis seedlings. *The Plant Journal* **53**, 814-827.

Gou, J. Y., Felippes, F. F., Liu, C. J., Weigel, D., and Wang, J. W. (2011). Negative regulation of anthocyanin biosynthesis in Arabidopsis by a miR156-targeted SPL transcription factor. *Plant Cell* **23**, 1512-22.

Henry, A., Chopra, S., Clark, D. G., and Lynch, J. P. (2012). Responses to low phosphorus in high and low foliar anthocyanin coleus (Solenostemon scutellarioides) and maize (Zea mays). *Functional Plant Biology* **39**, 255-265.

Hichri, I., Barrieu, F., Bogs, J., Kappel, C., Delrot, S., and Lauvergeat, V. (2011). Recent advances in the transcriptional regulation of the flavonoid biosynthetic pathway. *J Exp Bot* **62**, 2465-83.

Holton, T. A., and Cornish, E. C. (1995). Genetics and biochemistry of anthocyanin biosynthesis. *The Plant Cell* **7**, 1071.

Jia, X., Shen, J., Liu, H., Li, F., Ding, N., Gao, C., Pattanaik, S., Patra, B., Li, R., and Yuan, L. (2015). Small tandem target mimic-mediated blockage of microRNA858 induces anthocyanin accumulation in tomato. *Planta* **242**, 283-93.

Jiang, C., Gao, X., Liao, L., Harberd, N. P., and Fu, X. (2007). Phosphate starvation root architecture and anthocyanin accumulation responses are modulated by the gibberellin-DELLA signaling pathway in Arabidopsis. *Plant physiology* **145**, 1460-1470.

Kim, J., Park, M., Jeong, E. S., Lee, J. M., and Choi, D. (2017). Harnessing anthocyanin-rich fruit: a visible reporter for tracing virus-induced gene silencing in pepper fruit. *Plant Methods* **13**, 3.

Kocic, B., Filipovic, S., Nikolic, M., and Petrovic, B. (2011). Effects of anthocyanins and anthocyanin-rich extracts on the risk for cancers of the gastrointestinal tract. *J buon* **16**, 602-8.

Kumar, S., and Pandey, A. K. (2013). Chemistry and Biological Activities of Flavonoids: An Overview. *ScientificWorldJournal* **2013**.

Lange, M., Yellina, A. L., Orashakova, S., and Becker, A. (2013). Virus-induced gene silencing (VIGS) in plants: an overview of target species and the virus-derived vector systems. *Methods Mol Biol* **975**, 1-14.

Lee, H.-R., Bae, I.-H., Park, S.-W., Kim, H.-J., Min, W.-K., Han, J.-H., Kim, K.-T., and Kim, B.-D. (2009). Construction of an integrated pepper map using RFLP, SSR, CAPS, AFLP, WRKY, rRAMP, and BAC end sequences. *Molecules and cells* **27**, 21-37.

 DOI: 10.18380/SZIE.COLUM.2017.4.1.47

Li, J.-G., Li, H.-L., and Peng, S.-Q. (2011). Three R2R3 MYB transcription factor genes from Capsicum annuum showing differential expression during fruit ripening. *African Journal of Biotechnology* **10**, 8267-8274.

Li, P. W., Lu, X. Y., Li, C. Z., Fang, J., and Tian, Y. (2007). [Advances in the study of plant microRNAs]. *Yi Chuan* **29**, 283-8.

Lightbourn, G. J., Griesbach, R. J., Novotny, J. A., Clevidence, B. A., Rao, D. D., and Stommel, J. R. (2008). Effects of anthocyanin and carotenoid combinations on foliage and immature fruit color of Capsicum annuum L. *J Hered* **99**, 105-11.

Lightbourn, G. J., Stommel, J. R., and Griesbach, R. J. (2007). Epistatic interactions influencing anthocyanin gene expression in Capsicum annuum. *Journal of the American Society for Horticultural Science* **132**, 824-829.

Lippert, L., Smith, P., and Bergh, B. (1966). Cytogenetics of the vegetable crops. garden pepper, capsicum sp. *The Botanical Review* **32**, 24-55.

Liu, J., Osbourn, A., and Ma, P. (2015). MYB Transcription Factors as Regulators of Phenylpropanoid Metabolism in Plants. *Molecular Plant* **8**, 689-708.

Liu, R., Lai, B., Hu, B., Qin, Y., Hu, G., and Zhao, J. (2016). Identification of MicroRNAs and Their Target Genes Related to the Accumulation of Anthocyanins in Litchi chinensis by High-Throughput Sequencing and Degradome Analysis. *Front Plant Sci* **7**, 2059.

Mancinelli, A. L. (1985). Light-dependent anthocyanin synthesis: a model system for the study of plant photomorphogenesis. *The Botanical Review* **51**, 107-157.

Nakatsuka, A., Yamagishi, M., Nakano, M., Tasaki, K., and Kobayashi, N. (2009). Light-induced expression of basic helix-loop-helix genes involved in anthocyanin biosynthesis in flowers and leaves of Asiatic hybrid lily. *Scientia horticulturae* **121**, 84-91.

Odland, M. (1960). Inheritance of flower color in Capsicum annuum L. *In* "Proceedings. American Society for Horticultural Science", Vol. 76, pp. 475-81.

Paran, I., and van der Knaap, E. (2007). Genetic and molecular regulation of fruit and plant domestication traits in tomato and pepper. *Journal of Experimental Botany* **58**, 3841-3852.

Peterson, P. A. (1959). Linkage of Fruit Shape and Color Genes in Capsicum. *Genetics* **44**, 407-419.

Prince, J. P., Pochard, E., and Tanksley, S. D. (1993). Construction of a molecular linkage map of pepper and a comparison of synteny with tomato. *Genome* **36**, 404-417.

Quattrocchio, F., Verweij, W., Kroon, A., Spelt, C., Mol, J., and Koes, R. (2006). PH4 of Petunia is an R2R3 MYB protein that activates vacuolar acidification through interactions with basic-helix-loop-helix transcription factors of the anthocyanin pathway. *Plant Cell* **18**, 1274-91.

Quattrocchio, F., Wing, J. F., Leppen, H. T., Mol, J. N., and Koes, R. E. (1993). Regulatory genes controlling anthocyanin pigmentation are functionally conserved among plant species and have distinct sets of target genes. *The Plant Cell* **5**, 1497-1512.

Sadilova, E., Stintzing, F. C., and Carle, R. (2006). Anthocyanins, colour and antioxidant properties of eggplant (Solanum melongena L.) and violet pepper (Capsicum annuum L.) peel extracts. *Z Naturforsch C* **61**, 527-35.

Shi, M. Z., and Xie, D. Y. (2014). Biosynthesis and Metabolic Engineering of Anthocyanins in Arabidopsis. *Recent Pat Biotechnol* **8**, 47-60.

Stewart, A., Chapman, W., Jenkins, G., Graham, I., Martin, T., and Crozier, A. (2001). The effect of nitrogen and phosphorus deficiency on flavonol accumulation in plant tissues. *Plant, Cell & Environment* **24**, 1189-1197.

Steyn, W., Wand, S., Holcroft, D., and Jacobs, G. (2002). Anthocyanins in vegetative tissues: a proposed unified function in photoprotection. *New Phytologist* **155**, 349-361.

Stommel, J. R., Lightbourn, G. J., Winkel, B. S., and Griesbach, R. J. (2009). Transcription factor families regulate the anthocyanin biosynthetic pathway in Capsicum annuum. *Journal of the American Society for Horticultural Science* **134**, 244-251.

Sun, Y., Qiu, Y., Duan, M., Wang, J., Zhang, X., Wang, H., Song, J., and Li, X. (2017). Identification of anthocyanin biosynthesis related microRNAs in a distinctive Chinese radish (Raphanus sativus L.) by high-throughput sequencing. *Mol Genet Genomics* **292**, 215-229.

Takos, A. M., Jaffé, F. W., Jacob, S. R., Bogs, J., Robinson, S. P., and Walker, A. R. (2006). Light-Induced Expression of a MYB Gene Regulates Anthocyanin Biosynthesis in Red Apples. *Plant Physiology* **142**, 1216-1232.

Terrier, N., Torregrosa, L., Ageorges, A., Vialet, S., Verries, C., Cheynier, V., and Romieu, C. (2009). Ectopic expression of VvMybPA2 promotes proanthocyanidin biosynthesis in grapevine and suggests additional targets in the pathway. *Plant Physiol* **149**, 1028-41.

Thorup, T. A., Tanyolac, B., Livingstone, K. D., Popovsky, S., Paran, I., and Jahn, M. (2000). Candidate gene analysis of organ pigmentation loci in the Solanaceae. *Proc Natl Acad Sci U S A* **97**, 11192-7.

Wallace, T. C. (2011). Anthocyanins in cardiovascular disease. *Adv Nutr* **2**, 1-7.

Wang, L. S., and Stoner, G. D. (2008). Anthocyanins and their role in cancer prevention. *Cancer Lett* **269**, 281-90.

Wang, Y., Chen, S., and Yu, O. (2011). Metabolic engineering of flavonoids in plants and microorganisms. *Applied Microbiology and Biotechnology* **91**, 949.

Welch, C. R., Wu, Q., and Simon, J. E. (2008). Recent Advances in Anthocyanin Analysis and Characterization. *Curr Anal Chem* **4**, 75-101.

Winkel-Shirley, B. (2001a). Flavonoid Biosynthesis. A Colorful Model for Genetics, Biochemistry, Cell Biology, and Biotechnology. *Plant Physiol* **126**, 485-93.

Winkel-Shirley, B. (2001b). It Takes a Garden. How Work on Diverse Plant Species Has Contributed to an Understanding of Flavonoid Metabolism. *Plant Physiol* **127**, 1399-404.

Winkel-Shirley, B. (2002). Biosynthesis of flavonoids and effects of stress. *Curr Opin Plant Biol* **5**, 218-23.

Wu, F., Eannetta, N. T., Xu, Y., Durrett, R., Mazourek, M., Jahn, M. M., and Tanksley, S. D. (2009). A COSII genetic map of the pepper genome provides a detailed picture of synteny with tomato and new insights into recent chromosome evolution in the genus Capsicum. *Theoretical and applied genetics* **118**, 1279-1293.

Yi, G., Lee, J. M., Lee, S., Choi, D., and Kim, B.-D. (2006). Exploitation of pepper EST–SSRs and an SSR-based linkage map. *Theoretical and Applied Genetics* **114**, 113-130.

Yousuf, B., Gul, K., Wani, A. A., and Singh, P. (2016). Health Benefits of Anthocyanins and Their Encapsulation for Potential Use in Food Systems: A Review. *Crit Rev Food Sci Nutr* **56**, 2223-30.

Zatykó, L., and Moór, A. (1998). Linkage between an al gene (anthocyanin less) and the L3 gene of TMV resistance in pepper. *In* "10th EUCARPIA Meeting on Genetics and Breeding on Capsicum and Eggplant, Avignon (France)", pp. 249.

Zhang, B., Pan, X., Cobb, G. P., and Anderson, T. A. (2006). Plant microRNA: a small regulatory molecule with big impact. *Dev Biol* **289**, 3-16.

Zhang, Z., Li, D. W., Jin, J. H., Yin, Y. X., Zhang, H. X., Chai, W. G., and Gong, Z. H. (2015). VIGS approach reveals the modulation of anthocyanin biosynthetic genes by CaMYB in chili pepper leaves. *Front Plant Sci* **6**, 500.

Zhao, L., Gao, L., Wang, H., Chen, X., Wang, Y., Yang, H., Wei, C., Wan, X., and Xia, T. (2013). The R2R3-MYB, bHLH, WD40, and related transcription factors in flavonoid biosynthesis. *Funct Integr Genomics* **13**, 75-98.

http1: https://www.agroinform.hu/kerteszet_szoleszet/ezert-a-paprikabogyo-lila-foltosodasa-24191, accessed on: 2017.03.07.

 DOI: 10.18380/SZIE.COLUM.2017.4.1.47

Role of agrotechnical elements in sustainable wheat and maize production

Péter PEPÓ

University of Debrecen, Agronomy Faculty, Institute of Crop Sciences, Debrecen, Hungary;
E-mail: pepopeter@agr.unideb.hu

Abstract: In conventional crop production the yield increasing was based on the huge industrial chemical inputs and new genotypes of cereals. This extreme external input use could modified the resilience, adaptive capacity and sustainability of different crop models. The effects of different crop management factors were studied in long-term experiments on chernozem soil in Eastern Hungary. The fertilizer responses of wheat varieties depended on the crop year (6.5-8.2 t ha^{-1} in 2011-2014 years) and the genotype (in 2012 the difference was 2.7 t ha^{-1} among genotypes). The optimum N(+PK) dose varied between 30-150 kg ha^{-1} in different crop years. The N-doses over optimum caused NO_3-N accumulation in chernozem soil (from 32 mg kg^{-1} to 170 mg kg^{-1}). In sustainable maize production the fertilization resulted high yield surpluses in average (2.0-4.1 t ha^{-1}) and rainy (2.1-5.4 t ha^{-1}) crop year in our long-term experiment (in 1986-2014 years). The yield increasing of irrigation were agronomically effective only in dry crop years (3.3-4.9 t ha^{-1}) and they were very limited in average (1.2-1.3 t ha^{-1}) and rainy (0-0.2 t ha^{-1}) crop year (in 1986-2014 years) The optimum fertilization could improve WUE in maize production.

Keywords: crop rotation, fertilization, irrigation, WUE, NO_3-accumulation

Introduction

Significant yield increases of cereals (mainly wheat and maize) have been achieved from the 1970's years in the developed and partly in the developing countries. These yield increasements were based on the huge industrial, chemical inputs (fertilizers, pesticides, gasoline etc.) and new genotypes of cereals. This "industry-like" crop production resulted high yield and enormous harmful environmental effects and less agronomy and energy efficiency (Austin 1999, Pepó 2007, Olesen et al. 2011). Traditional cereal production uses a lot of external inputs to achieve high yields (Hole et al. 2005). Hungarian crop production is cereal-oriented one. Proportion of cereals (small grains and maize) takes about 70% of Hungarian arable land. In sustainable cereal production nutrient supply, fertilization is a key agrotechnical element (Jordan et al. 1997, Oehl et al. 2004, Keller et al. 2012), but the crop rotation, irrigation, plant density, weed control (Berzsenyi et al. 2000, Vad et al. 2007) have important role, too. The yield-losses and yield fluctuation of cereals caused by crop year (climate change) depended on soil conditions, the stress-tolerance of genotypes and the agrotechniques. According to literature (Shen et al. 1999, Pepó 2009) the yield decreases of cereals varied between 2-55%. Because of climate change the water saving crop management and water use efficiency are especially important in arable crop production. Graymore and Wallis (2010) built up a conceptual model of the factors impacting on water use of different users, including drivers and barriers to water saving.

The main aim of this study was to evaluate the long-term experimental data on chernozem soil in Eastern-Hungary to show the effects of ecological factors (crop year) and agrotechnical elements (fertilization, crop rotation, irrigation) and genotypes on the yields of winter wheat and maize. Our further aim was to show how can modify the applied input level the resilience and adaptive capacity of different cereal crop models and how we can keep the sustainability of cereal crop production.

Materials and methods

Our study was based on long-term experiments on chernozem soil in Eastern Hungary.

Location

The long-term experiments were set up in Látókép Experimental Station on calcorous chernozem soil in 1983 year. Geographical location is N 47°33' and 21°27'.

Experimental site

Soil type is chernozem which has nearly neutral

pH (pH_{KCl} = 6.46). The original chemical traits of soil are as the following: humus content 2.76% (0-0.2 m upper soil layer), thickness of humus layer 0.8 m, AL-P_2O_5 content 130 mg kg^{-1}, AL-K_2O content 240 mg kg^{-1} of plowing layer). Chernozem soil has excellent water husbandry.

The long-term experimental site can be characterized by continental climatic conditions. The average yearly precipitation is 565 mm and average yearly mean temperature is 9.84 °C.

Treatments of long-term experiments

Fertilizer response testing of winter wheat genotypes experiment which includes 2 factors (i = fertilization, control and N = 30 kg ha^{-1}, P_2O_5 = 22.5 kg ha^{-1}, K_2O = 26.5 kg ha^{-1} and 2-, 3-, 4-, 5-folds of the basic dose; ii = genotypes [15-20 varieties]). The experimental design is split-split-plot with 4 replications. The plot-size is 10 m^2. (Long-term experiment 1 = LTE1)

Polyfactorial long-term experiment of cereal crop models which includes 3 factors (i = crop rotation: mono-, bi- and triculture, ii = fertilization: control and N = 60 kg ha^{-1}, P_2O_5 = 45 kg ha^{-1}, K_2O = 45 kg ha^{-1} and 2, 3, 4-folds, iii = water supply [rainfed and irrigated]). The experimental design is split-split-plot with 4 replications. The plot-size is 46 m^2. (Long-term experiment 2 = LTE2)

Measurements and observations in the long-term experiments

At the harvest the yields and seed moisture determined. In certain years we measured the soil NO_3-N, AL-soluble P_2O_5 and K_2O contents (in 0-3 m soil profile in every 0.2 soil layers) after harvest.

Statistical analyses of data

The experimental data analysed with SPSS 13.0 statistical software package.

Results and discussions

The basic element of sustainable cereal production is to select the suitable, adaptable genotypes into agroecological and agrotechnical conditions. The nutrient supply and fertilization have the key-role in the sustainable wheat production because on the one hand fertilization directly and indirectly modifies all other agrotechnical factors (crop protection etc.) and the other hand the over-optimum fertilization causes different harmful effects (NO_3-N accumulation in different soil layers etc.). Our long-term experimental results (LTE1) proved that weather conditions (mainly the rainfall quantity and its distribution) strongly modified the yields of winter wheat genotypes even on chernozem soil characterized by excellent water- and nutrient husbandry. In the average of wheat varieties and crop years the yield was 7508 kg ha^{-1} but the yields varied depending on the crop years (Table 1). The minimum yield was in 2013 (6514 kg ha^{-1})

Table 1. Fertilizer response of winter wheat genotypes in different crop years (Debrecen, chernozem soil, 2011-2014)

Variety	2011(N_{opt})	2012(N_{opt})	2013(N_{opt})	2014(N_{opt})	Average
GK Öthalom	6819 $_{(150)}$	6175 $_{(150)}$	5983 $_{(150)}$	8713 $_{(30)}$	6923
Pannonikus	8123 $_{(90)}$	8139 $_{(150)}$	6576 $_{(150)}$	7996 $_{(30)}$	7684
Euclide	9586 $_{(150)}$	8919 $_{(150)}$	7590 $_{(150)}$	-	8698
GK Csillag	-	7263 $_{(150)}$	6562 $_{(150)}$	8350 $_{(60)}$	7392
Bitop	-	6075 $_{(150)}$	6089 $_{(120)}$	6663 $_{(30)}$	6276
GK Békés	-	7917 $_{(150)}$	6281 $_{(120)}$	7915 $_{(30)}$	7371
Average	*8176*	*7415*	*6514*	*7927*	*7508*
Yield interval, t/ha	6.8-9.6	6.1-8.9	6.0-7.6	6.7-8.4	6.4-8.6
Min-Max, %	83-117	82-120	92-117	84-105	85-115
Interval of yield fluctuation, %	34	38	25	21	30
Interval of N_{opt} kg ha^{-1}	90-150	120-150	120-150	30-60	90-128
$LSD_{5\%}$	457	355	600	674	-

DOI: 10.18380/SZIE.COLUM.2017.4.1.59

and we got the maximum yield in 2011 (8176 kg ha^{-1}). The winter wheat genotypes could differently adapt to the crop year. According to our long-term experimental data we could state that the differences among the varieties were about 3 t ha^{-1} in the same agrotechnical conditions (in 2012 the yields varied between 6075-8919 kg ha^{-1}). The crop year (mainly the water supply during the vegetation period) can modify the optimum N+PK doses, too. In crop year characterized by average water supply the optimum N+PK doses varied between N=90-150 kg ha^{-1} +PK and in crop year after very mild winter the N_{opt} +PK dropped down to N=30-60 kg ha^{-1} +PK (because of very high mineralization of organic matter in the chernozem soil).

The winter wheat is one of the best fertilizer-responding field crops. Our long-term experimental data (LTE1) proved that the fertilization of wheat resulted good yield surpluses on chernozem soil characterized by excellent natural nutrient stock (*Table 2*). The yield surpluses of wheat varied between 940 kg ha^{-1} (2002/2003 crop year) and 4858 kg ha^{-1} (2012/2013 crop year). The yields of control treatment proved the excellent natural nutrient avaibility of chernozem soil (1816 kg ha^{-1} and 5897 kg ha^{-1}). The other meteorological parameters could modify the yield surplus of wheat genotypes (in 2010 extra rainfall caused high lodging and leaf-, stem- and ear infections, in 2013 the strong and long frosting period in March decreased the yields, in 2014 the very mild winter period accelerated the N-mineralization in chernozem soil).

The using of N-fertilization over optimum level can cause NO_3-N accumulation in different soil layer. Our long-term experimental data proved (LTE1) that N-fertilization exceeding the agroecological demand of winter wheat genotypes increased the NO_3-N accumulation zone in chernozem soil (*Table 3*), so this fertilization method could decrease the sustainability of wheat crop models. The usage of N-doses over the optimum level caused NO_3-N accumulation in chernozem soil. The NO_3-N content of soil increased (from 32 mg kg^{-1} to 170 mg kg^{-1} NO_3-N) and its accumulation zone moved down to the deeper soil layer (from 0.8-1.0 m to 1.0-2.5 m). So if we want to convert the

Table 2. Effect of crop year on the control and maximum yield of winter wheat (Debrecen, 1999-2014) (average of varieties)

Crop year	Control yield kg ha^{-1}	Maximum yield kg ha^{-1}	Yield-surplus kg ha^{-1}	Rainfall in veg. period (mm)	Rainfall deviation from 30 year average (mm)
1998/1999	4042	6598	2556	470.4	+69.5
1999/2000	4041	8296	4250	312.9	-88,0
2000/2001	3193	7226	4033	430.2	+29,3
2001/2002	4466	6555	2091	184.6	-216,3
2002/2003	3447	4387	940	279.3	-121,6
2003/2004	4713	8573	3860	376.5	-24,4
2004/2005	4539	8098	3559	410.4	+9,5
2005/2006	3949	7016	3067	476.5	+37,6
2006/25007	3402	6893	3491	208.6	-192.3
2007/2008	5138	7218	2080	484.9	+84.0
2008/2009	3775	7696	3921	329.8	-71.1
2009/2010	3618	5539	1921	630.5	+229.6
2010/2011	4023	8043	4020	340.9	-60.0
2011/2012	3906	7303	3397	320.7	-80.2
2012/2013	1816	6674	4858	480.2	+79.3
2013/2014	5897	8556	2659	284.0	-116.9

DOI: 10.18380/SZIE.COLUM.2017.4.1.59

Table 3. Effect of nitrogen fertilization on the NO_3-N content of chernozem soil in long-term experiment (Debrecen, 1988-2003)

Year	Maximum NO_3-N content (mg kg^{-1})	Width of NO_3-N accumulation zone (m)	Years from set up the long-term experiment
1988	32	0.8-1.0	5
1992	120	0.8-1.3	9
1996	150	1.0-1.6	13
1999	170	1.4-2.0	16
2001	270	1.2-2.4	18
2003	170	1.0-2.5	20

conventional cereal production based on huge industrial inputs (Austin 1999, Pepó 2007, Mayer et al. 2015) we have to use optimum genotype selection and optimum agrotechnical elements (focusing on fertilization).

Maize is a sensitive field crop to agroecological and agrotechnical factors. Our multifactorial long-term experimental data (LTE2) proved that the effects of fertilization were different depending on the crop rotation and the weather of crop year. In Eastern Hungary characterized by continental climate the precipitation quantity and its distribution are the decisive agroecological factors on chernozem soil. The effects of crop year were significant on the yields of maize in every crop rotation (*Table 4*). We obtained the strongest effect of crop year in monoculture, so sustainability needs diversified crop rotation. The efficiency of fertilization was modified by crop year and crop rotation. The yield surpluses of maize were low (891-1315 kg ha^{-1}) in dry crop years and they were much bigger in average (1998-4145 kg ha^{-1}) and in rainy crop years (2117-5399 kg ha^{-1}), respectively. The biggest fertilization effects were in monoculture and lowest ones were in triculture (*Table 4*) because of high control yields. So the appropriate crop rotation can reduce the N+PK fertilizer doses (in mono- N_{180} +PK, in bi- N_{120} +PK, in triculture N_{60} +PK) and can promote the sustainability in maize production.

Our long-term research data (LTE2) proved that the using optimum fertilizer doses (N+PK) can increase the water use efficiency (WUE = kg yield/1 mm rainfall in vegetation period) of maize both in dry and average crop years (*Table 5*). In different crop rotations the WUE of control varied between 9.5-23.7 kg mm^{-1} in dry and 20.8-30.6 kg/mm in average crop years, respectively. In optimum N+PK treatment the

Table 4. Effect of crop year, crop rotation and fertilization on the yield of maize in long-term experiment (Debrecen, chernozem soil, 1986-2014)

Crop rotation	Yield (kg ha^{-1})					
	Dry crop year 11 years (38%)		Average crop year 12 years (41%)		Rainy crop year 6 years (21%)	
Monoculture						
Control	3743 e	1315*	6397 e	4145*	7190 c	5399*
N_{opt} +PK	5058 d		10 542 bc		12 589 a	
Biculture						
Control	7279 bc	924*	9289 d	2825*	9963 b	2117*
N_{opt} +PK	8203 a		12 114 a		12 080 a	
Triculture						
Control	6708 c	891*	9451 cd	1998*	10 023 b	2355*
N_{opt} +PK	7599 ab		11 449 ab		12 378 a	

*yield surplus of fertilization (kg ha^{-1})

a, b, c, d, e Letters are significantly different at $P \leq 0.05$ level

DOI: 10.18380/SZIE.COLUM.2017.4.1.59

Table 5. Water use efficiency (WUE) of maize in different crop years (Debrecen, chernozem soil, non irrigated)

Crop rotation	Fertilizer treatment	Dry crop year	Average crop year
		yield kg/1 mm rainfall in vegetation period	
Monoculture	Control	9.5 d	20.8 d
	N_{opt} +PK	15.2 c	39.1 a
Biculture	Control	22.1 b	28.4 c
	N_{opt} +PK	27.2 ab	35.8 ab
Triculture	Control	23.7 ab	30.6 bc
	N_{opt} +PK	28.2 a	40.4 a

a, b, c, d Letters are significantly different at $P \leq 0.05$ level

Table 6. Effect of crop year, crop rotation and irrigation on the yield of maize in long-term experiment (Debrecen, chernozem soil, N_{opt} +PK, 1986-2014)

Crop rotation	Yield (kg ha^{-1})					
	Dry crop year		Average crop year		Rainy crop year	
Water supply	11 years (38%)		12 years (41%)		6 years (21%)	
Monoculture						
non irrigated	5039 d	4858*	10 536 d	1323*	11 662 c	-38*
irrigated	9897 b		11 859 bc		11 624 c	
Biculture						
non irrigated	8182 c	3341*	12 019 bc	1276*	11 723 bc	98*
irrigated	11 523 a		13 295 a		11 821 bc	
Triculture						
non irrigated	7619 c	3466*	11 547 c	1284*	12 071 ab	197*
irrigated	11 085 ab		12 831 ab		12 268 a	

*yield surplus of irrigation (kg ha^{-1})

a, b, c, d Letters are significantly different at $P \leq 0.05$ level

WUE values were much higher (15.2-28.2 kg mm^{-1} and 35.8-40.4 kg mm^{-1}, respectively).

The most efficient agrotechnical element against drought is irrigation. The effect of irrigation depended on the meteorological situation of crop years (*Table 6*). During 29 years of our long-term experiment the proportion of crop years was the following: 38% dry, 41% average and 21% rainy crop year, respectively. The yield surpluses were fairly big in dry crop years to obtain good irrigation response of maize (3341-4858 kg ha^{-1}). In average and rainy crop years the yield surpluses of irrigation were very limited (1276-1323 kg ha^{-1} and -38-197 kg ha^{-1}, respectively).

In sustainable maize production fertilization, irrigation and crop rotation have decision role on the yields. The scientific findings of Berzsenyi et al. (2000) and Vad et al. (2007) showed the crop rotation, fertilization and irrigation have main effects on the yields of maize according to our long-term experimental results.

Long-term experiments with a range of different cropping systems, fertilization treatments, genotype testing are a central component of research to develop more sustainable agricultural systems including different crop models. Monitoring agricultural sustainability requires different indicators (Barrios and Sarte, 2008).

References

Austin, R.B. (1999): Yield of wheat in the United Kingdom: recent advances and prospects. Crop Science. 39, 6: 1604–1610. doi:10.2135/cropsci1999.3961604x

Barrios, E.B., Sarte, G.Ma.F. (2008): Monitoring sustainable agriculture in Southeast Asia. International Journal of Sustainable Development & World Ecology, 15, 2: 95-102. http://dx.doi.org/10.1080/13504500809469774

Berzsenyi, Z., Győrffy, B., Lap, DQ. (2000): Effect of crop rotation and fertilisation on maize and wheat yields and yield stability in a long-term experiment. European Journal of Agronomy. 13, 2-3: 225-244. http://citeseerx.ist.psu.edu/viewdoc/download?doi=10.1.1.464.2995&rep=rep1&type=pdf

Graymore, M.L.M., Wallis, A.M. (2010): Water savings or water efficiency? Water-use attitudes and behaviour in rural and regional areas. International Journal of Sustainable Development & World Ecology. 17, 1: 84-93. http://dx.doi.org/10.1080/13504500903497249

Hole, D.G., Perkins, A.J., Wilson, J.D., Alexander, H.I., Grice, P.V., Evans, A.D. (2005): Does organic farming benefit biodiversity? Biological Conservation. 122: 113–130. doi:10.1016/j.biocon.2004.07.018

Jordan, V.W.L., Hutcheon, J.A., Donaldson, G.V., Farmer, D.P. (1997): Research into and development of integrated farming systems for less-intensive arable crop production: experimental progress (1989–1994) and commercial implementation. Agriculture Ecosystems and Environment. 64, 2: 141–148. http://journals.sagepub.com/doi/pdf/10.1177/003072709802700303

Keller, M., Oberson, A., Annaheim, K.E., Tamburini, F., Maeder, P., Mayer, J., Frossard, E., Buenemann, E.K. (2012): Phosphorus forms and enzymatic hydrolyzability of organic phosphorus in soils after 30 years of organic and conventional farming. Journal of Plant Nutrition and Soil Science. 175, 3: 385–393. DOI: 10.1002/jpln.201100177

Maye, J., Gunst,, L., Mäder, P., Samson, M-F., Carcea, M., Narducci, V., Thomsen, I., Dubois, D. (2015): Productivity, quality and sustainability of winter wheat under long-term conventional and organic management in Switzerland. European Journal of Agronomy. 65: 27-39. DOI 10.1016/j.eja.2015.01.002

Oehl, F., Frossard, E., Fliessbach, A., Dubois, D., Oberson, A. (2004): Basal organic phosphorus mineralization in soils under different farming systems. Soil Biology and Biochemistry. 36: 667–675. DOI: 10.1016/j.soilbio.2003.12.010

Olesen, J.E., Trnka, M., Kersebaum, K.C., Skjelvag, A.O., Seguin, B., Peltonen-Sainio, P., Rossi, F., Kozyra, J., Micale, F. (2011): Impacts and adaptation of European crop production systems to climate change. European Journal of Agronomy. 34, 2: 96–112. doi:10.1016/j.eja.2010.11.003

Pepó, P. (2007): The role of fertilization and genotype in sustainable winter wheat (Triticum aestivum L.) production. Cereal Research Communications. 35, 2: 917-920. http://dx.doi.org/10.1556/CRC.35.2007.2.188

Pepó, P. (2009): Effects of water supply as an abiotic stress on the yields and agronomic traits of winter wheat (Triticum aestivum L.) on chernozem soil. Cereal Research Communication, 37, Supplement: 29-32. https://www.cabdirect.org/cabdirect/abstract/20103103995?freeview=true

Shen, S.H., Gao, W.Y., Li, B.B. (1999): Water consumption and its impact on the yield of winter wheat in Xifeng. Journal of Nanjiing Institute of Meteorology, 22, 1: 88-94.

Vad, A., Zsombik, L., Szabó, A., Pepó, P. (2007): Critical crop management factors in sustainable maize (Zea mays L.) production. Cereal Research Communications. 35, 2: 1253-1256. http://dx.doi.org/10.1556/CRC.35.2007.2.272

 DOI: 10.18380/SZIE.COLUM.2017.4.1.59

Source of the graphics

Front cover:
Gallo-Roman harvesting machine, called Vallus. Source: U. Troitzsch - W. Weber (1987): Die Technik : Von den Anfangen bis zur Gegenwart

Rear cover:
Portrait of Columella, in Jean de Tournes, Insignium aliquot virorum icones. Lugduni: Apud Ioan. Tornaesium 1559. Centre d'Études Supérieures de la Renaissance - Tours

POSTA Katalin, editor-in-chief

DSc /agric/, dean of the the Faculty of Agricultural and Environmental Sciences of the Szent István University, Gödöllő, Hungary, member of the Soil Science, Water Management and Crop Production Committee of the Hungarian Academy of Sciences. Professional fields: soil microbiology, arbuscular mycorrhizal fungi, plant protecton by soil microorganisms.

Lucius Junius Moderatus Columella

(AD 4 – 70) is the most important writer on agriculture of the Roman empire. His De Re Rustica in twelve volumes has been completely preserved and forms an important source on agriculture. This book was translated to many languages and used as a basic work in agricultural education until the end of the 19th Century.