ISSN: 2490-3442

UNIVERSITY OF EAST SARAJEVO
FACULTY OF AGRICULTURE

Agrofor

AGROFOR International Journal

AGROFOR International Journal, Vol. 7, Issue No. 1, 2022

agrofor.ues.rs.ba

AGROFOR International Journal

PUBLISHER
University of East Sarajevo, Faculty of Agriculture
Vuka Karadzica 30, 71123 East Sarajevo, Bosnia and Herzegovina
Telephone/fax: +387 57 340 401; +387 57 342 701
Web: agrofor.ues.rs.ba; Email: agroforjournal@gmail.com

Frequency: 3 times per year
Number of copies: 300
ISSN 2490-3434 (Printed)
ISSN 2490-3442 (Online)

CONTENT

Review paper
10.7251/AGRENG2201005E
UDC 338.43.1(662.6)

AGRICULTURE EXTENSION AND ADVISORY SERVICES IN BURKINA FASO AND NIGER

Hamid EL BILALI[1]*, Lawali DAMBO[2], Imaël Henri Nestor BASSOLE[3], Jacques NANEMA[3]

[1]International Centre for Advanced Mediterranean Agronomic Studies (CIHEAM-Bari), Valenzano (Bari), Italy
[2]Abdou Moumouni University, Niamey, Niger
[3]Joseph KI Zerbo University, Ouagadougou, Burkina Faso
*Corresponding author: elbilali@iamb.it

ABSTRACT

Agriculture still plays an important socio-economic role in Burkina Faso and Niger. However, the agriculture sector faces several difficulties and both countries still suffer from food insecurity and malnutrition. Therefore, there is an urgent need to develop the agricultural sector. Extension and advisory services are widely recognised as an essential instrument for agriculture development. Therefore, the present review analyses the state of research on extension and advisory services in Burkina Faso and Niger. It draws upon a search performed in June 2021 on the Web of Science. The analysis of the scholarly literature suggests a gap in the research field. The modernisation of the extension system implied the co-existence of different extension approaches, from the public training and visit (T&V) extension program to various participatory advisory approaches such as Farmer Field Schools (FFS), with different levels of involvement of the private sector and NGOs. Modern advisory services stress the centrality of farmers' participation and experiential and social learning. A prominent feature of modern advisory services is their focus on innovation development rather than the linear transfer of knowledge and technologies. The modernisation of extension services has implied an increase in ICT use. Proposals to improve advisory services performance include building extension staff's capacity, increasing funding, creating an enabling institutional environment and fostering farmers' participation. Strengthening the extension system is essential to foster the sustainable development of agriculture in Burkina Faso and Niger in the face of climate change, which, in turn, is vital to achieving sustainable food and nutrition security.

Keywords: *agriculture, rural development, extension services, training & visit, farmer field school, West Africa.*

INTRODUCTION

Burkina Faso (BF) and Niger are two developing, landlocked countries in Sahelian West Africa. Agriculture still plays an important socio-economic role in both countries. Recent data from the World Bank show that agriculture, forestry and fishing contribute to 20.2% of the gross domestic product (GDP) in Burkina Faso and 37.8% in Niger, while employment in agriculture is at 73% in Niger and 26% in BF. Furthermore, 83% of the population in Niger and 70% in BF lives in rural areas (World Bank, 2021). While agriculture is still central in the livelihoods of rural populations in both countries, it is generally extensive, poorly mechanized and almost entirely reliant on the variable summer rainfall, making it vulnerable to climate change (USAID, 2017). Agriculture is also important for the food security of the population. However, according to the State of Food Security and Nutrition in the World 2020 (FAO et al., 2020), the prevalence of undernourishment in the total population is still high in BF at 19.2% over 2017–19, while it is even worse when considering the prevalence of moderate or severe food insecurity in the total population, which was at 47.7% over the same period. Challenges relating to rural livelihoods and food security show the urgent need to develop the agricultural sector in both countries.

Extension and advisory services are widely recognised as essential for agriculture development (Cook et al., 2021). According to Leeuwis and van den Ban (2004), *"Extension [is] a series of embedded communicative interventions that are meant, among others, to develop and/or induce innovations which supposedly help to resolve (usually multi-actor) problematic situations".* The public extension system has been the most common source of information for farmers in developing countries (Eicher, 2007). Nevertheless, there is a widespread concern that the public extension system is underperforming (Cook et al., 2021; FAO et al., 2017). This has led to the promotion of farmer-driven extension systems. Indeed, to improve the efficiency of extension services, most developing countries have attempted to move from supply-driven, public extension to demand-driven, pluralistic advisory services but results have been rather mixed (Cook et al., 2021; Meena & Singh, 2013). Mapiye et al. (2021) show that different extension approaches co-exist nowadays viz. technology transfer-based extension (e.g. ministry-based or public extension, training and visit extension), commodity specialized extension approach, participatory agricultural extension approaches (e.g. farmer field school, project extension, farming systems research–extension), cost-sharing extension approach, and education institution extension approach. Also in West Africa, advisory services are increasingly considered as a promising alternative to the Training and Visit extension system (Moumouni et al., 2011). The impacts of the reform and restructuring of public extension services on their performance and plurality change from a country to another. In this context, the present review analyses the state of research on extension and advisory services in Burkina Faso and Niger.

MATERIALS AND METHODS

The paper draws upon a systematic review of all documents indexed in the Web of Science (WoS) and follows the PRISMA guidelines (Preferred Reporting Items for Systematic Reviews and Meta-Analyses) (Moher et al., 2009). A search was performed on 11 June 2021 using the following Title-Abs-Key search query: *(extension OR advisory OR advice) AND (agriculture OR agricultural OR farming OR farmer OR crop OR animal OR fish) AND (Burkina OR Niger OR "West* Africa" OR Sahel).* The initial search on WoS yielded 288 documents. The selection of the documents to be included in the systematic review was informed by the methodology suggested by El Bilali (2021). Table 1 describes the selection steps and process. Particularly, three inclusion/eligibility criteria were considered: geographical coverage (viz. the document deals with Burkina Faso, Niger or broader West Africa/Sahel); thematic focus (viz. the document deals with extension and advisory services in agriculture and/or rural areas); and document type (viz. only research articles, chapters or conference papers were selected; editorial materials – letters to editors, commentaries and/or notes – as well as reviews were discarded).

Table 1. Articles selection process.

Selection steps	Number of selected documents	Number of documents excluded and exclusion reasons
Search on WoS	288	--
Screening of documents based on titles	288	107 documents excluded because they deal with other countries than Burkina Faso and/or Niger e.g. Bangladesh, Benin, Brazil, Cameroon, Gabon, Gambia, Ghana, Guinea, India, Indonesia, Ivory Coast, Kenya, Mali, Mauritania, Morocco, Nepal, Nigeria, Rwanda, Senegal, South Africa, Sudan, Tanzania, Togo, Uganda and USA
Screening of documents based on abstracts	181	157 documents excluded: • 32 documents that do not deal with Burkina Faso and/or Niger • 8 documents that do not deal with agriculture • 117 documents that do not address extension and advisory services
Scrutiny of full-texts	24	5 documents excluded: • 2 documents that do not deal with Burkina Faso and/or Niger • 1 document that does not address extension and advisory services • 2 reviews

Selection steps	Number of selected documents	Number of documents excluded and exclusion reasons
Inclusion of documents in the systematic review	19	--

Following the screening of titles, 107 documents were excluded as they do not refer to Burkina Faso and/or Niger; these mainly refer to other West African and/or Sahelian countries (especially Nigeria). At this step, documents covering wider geographical areas (e.g. West Africa, Sahel, Sub-Saharan Africa) or those where the geographical scope is not specified in the title were kept for further analysis. Further 157 documents were excluded following the scrutiny of abstracts mainly because they do not address extension and advisory services. Additionally, 5 documents were discarded following the analysis of full-texts, including 2 reviews (Hansen et al., 2019; Nkiaka et al., 2019). Consequently, only 19 documents resulted eligible and were included in the systematic review (Table 2).

Table 2. List of the eligible articles.

Publication year	References
2021*	Bakker et al. (2021)
2020	Zossou et al. (2020); Bacci et al. (2020)
2019	Sylla et al. (2019)
2018	Boafo et al. (2018); Siddo et al. (2018); Faure et al. (2018); Maredia et al. (2018)
2015	Ihm et al. (2015)
2011	Faure et al. (2011); Le Gal et al. (2011); van Paassen et al. (2011); Settle and Garba (2011)
2005	Riise et al. (2005)
1999	Evenson and Siegel (1999)
1997	Bindlish and Evenson (1997)
1994	Okali et al. (1994)
1993	Shapiro et al. (1993)
1992	Schaefer (1992)

* As of 11 June 2021.

RESULTS AND DISCUSSION

The low number of the selected articles suggests a gap in research on extension and advisory services in BF and Niger. This is particularly true in Niger as most of the

selected documents deal with BF. The majority of the selected articles address extension services for crop production while animal production is generally overlooked. Among crops, there is more attention to commercial ones such as cotton (Boafo et al., 2018; Settle & Garba, 2011) and rice (Zossou et al., 2020). However, some papers address general and transversal extension services such as those relating to meteorological information (Bacci et al., 2020).

Different extension approaches coexist in Burkina Faso and Niger. The Training and Visit (T&V) system (Bindlish & Evenson, 1997; Evenson & Siegel, 1999) has been the dominant agricultural extension program in developing countries for decades. Despite recurring criticisms of the T&V system, data from Kenya and Burkina Faso suggest that it helped to support agricultural growth, increase productivity and close the yield gap. However, evidence shows that T&V management affects the effectiveness of the extension services and the development of the agricultural sector (Bindlish & Evenson, 1997). There is an ongoing, yet slow, transition from T&V to advice for family farms. In the latter are included various advisory approaches such as Farmer Field Schools (FFS) (Bakker et al., 2021; Settle & Garba, 2011) and Management Advice for Family Farms (MAAF) (Faure et al., 2018). The results of the reform of the public agricultural services have been mixed and change from a country to another. In their analysis of the perceptions of the stakeholders about the outcomes of the reforms of the cotton sector in Ghana and Burkina Faso, Boafo et al. (2018) found that the assessment of Ghanaian stakeholders generally highlighted only negative outcomes (e.g. lack of farmer's voice in extension services, corrupt field agents of private companies, conflicting extension policies of cotton companies), while Burkinabe ones perceived both negative (e.g. side aging extension agents) and positive (e.g. regular training and support services, development of better-quality extension services) outcomes.

Community-based and participatory extension approaches have become increasingly prominent in developing countries as a consequence of the decline of investments by governments in public extension services in the 1980s–1990s period as a result of the Structural Adjustment Programs (SAPs) promoted by the World Bank and International Monetary Fund (IMF). In this respect, Faure et al. (2011) stress the need for continuous adaptation of the methods of extension and advice taking into consideration the local context as well as resources (financial, human) available for providing advisory services. This also implies a better understanding of the drivers of knowledge acquisition, learning and technology adoption in agriculture and rural areas (Shapiro et al., 1993; Zossou et al., 2020) in order to use it in the design of effective extension and advisory programs. Zossou et al. (2020) show that a range of factors affects farmers' knowledge such as household size, training, access to knowledge sources and socioeconomic status. They also stressed the central role of the experiences of farmers themselves or that of fellow ones in the adoption of new technologies (Zossou et al., 2020). Therefore, effective extension programs should promote both first-order (experiential) learning and second-order (social) learning (van Paassen et al., 2011).

The modernisation of extension services has implied an increase in the use of information and communication technologies (ICT) (Ihm et al., 2015; Maredia et al., 2018). This has determined a change in the communication media used by extension staff to convey extension messages to the farmers. In this respect, Maredia et al. (2018) found that animated videos shown on mobile phones, a versatile and economical method, were as effective as the traditional extension method (i.e. live demonstration) in Burkina Faso. Animated videos were also effective in inducing the adoption of innovative technologies by Burkinabe farmers but less effective than live demonstration (Maredia et al., 2018). However, Ihm et al. (2015) argue that the use of Information and communication technologies for development (ICT4D) should take into consideration the different media environments and preferences of target populations within countries. This encompasses the digital divides within countries and target population preferences as well as the disparities among extension agents and farmers in media ownership, access and ICT skills.

The governance mechanisms to steer the advisory services affect not only the content of the advice but also the overall performance of the advisory services (Faure et al., 2011). Governance is often linked to the funding of extension and advisory services. Indeed, Faure et al. (2011) argue that "*the funding mechanisms are pivotal in defining the rules of governance*" (p. 364). Nowadays, extension and advisory services are no more only public and there is a growing participation of the private sector (Sylla et al., 2019) and NGOs (Evenson & Siegel, 1999). Interestingly, Sylla et al. (2019), in their analysis of the perceptions of Burkinabe farmers about the quality of public and private agricultural extension, found that farmers were satisfied with the quality of both types of providers of extension services (e.g. facilitating market access, technical support, facilitating access to credit, facilitating input provision), but private advisory services were rated better. This finding supports the privatisation of extension services.

A prominent feature of modern advisory services is their focus on innovation development rather than the linear transfer of knowledge and technologies (Bakker et al., 2021). In this context, the literature describes different attempts to involve farmers in the co-design of advice content and tools (Le Gal et al., 2011). Bakker et al. (2021) show that even when it comes to participatory methods such as FFS their effect on farmers' innovation and change processes depends on the level of their participation. Indeed, the authors suggest that the innovation of farmers in pest management (e.g. use of bio-pesticides), organic and mineral fertilization (e.g. use of compost) and legume cropping practices (e.g. intercropping, rotation) in Burkina Faso and Togo was much better when farmers' participation in FFS was collaborative (i.e. they participated actively in developing the FFS curricula and structure) than when merely consultative (i.e. farmers have low, limited participation in developing the FFS curricula and structure) (Bakker et al., 2021). This clearly shows that no matter how the advisory approach is named, the real system performance depends on how it is implemented at the community, local level. Meanwhile, Zossou et al. (2020) argue that the "*key policies for*

strengthening the innovation systems are those that help farmers access both formal and informal knowledge sources, credit services, better welfare and information and communication tools" (p. 291).

Gender issues are generally overlooked in studies on extension and advisory services in BF and Niger, with a few exceptions. For instance, Evenson and Siegel (1999) analyse the effects of government (Training and Visit) and non-government (NGO) extension programs on the role of women as farm managers in Burkina Faso. They concluded that women's access to extension services strengthens their involvement in the management of farms and has a positive impact on crop yields.

Scholars made several proposals to improve the performance of extension and advisory services in BF and Niger. These include building the capacity of extension staff (Bakker et al., 2021; Faure et al., 2011, 2018), increasing and better targeting financing (Faure et al., 2011, 2018), reinforcing collaboration between different stakeholders (Faure et al., 2011) as well as fostering farmers' participation in the design and implementation of extension programs (Bakker et al., 2021; Faure et al., 2018; Okali et al., 1994; van Paassen et al., 2011). Faure et al. (2011) suggest that the functioning and performance of advisory services in Burkina Faso and Benin are affected by "*the financing mechanisms, the governance mechanisms put in place, the quality of the human resources delivering advice, and the characteristics of the advisory method*" (p. 364). This clearly shows the central role of human resources in determining the performance of extension and advisory services. Indeed, the authors posit that "*the nature and the quality of advisory activities are closely related to the skills of advisors and managers of advisory services*" (p. 364) (Faure et al., 2011). As for the private advisory services, creating an enabling business environment seems crucial. Referring to BF, Sylla et al. (2019) put that the "*government can promote private participation in extension delivery by creating a good business environment for the private systems to operate*" (p. 1). Institutional environment also matters in the case of extension services based on adult learning such as Management Advice for Family Farms (MAAF). Faure et al. (2018) found that the main constraint to scaling MAFF is institutional and recommended giving producers' organisations a greater say in the governance of the advisory system not only to improve its effectiveness but also to reduce operating costs and strengthen its sustainability. A further proposal regards increasing the use of ICTs in the design and delivery of extension services (Ihm et al., 2015; Maredia et al., 2018).

CONCLUSIONS

This article provides a comprehensive review of the state of research on extension and advisory services in BF and Niger. The analysis of the scholarly literature suggests a gap in the research field, especially in Niger. It also suggests that the extension system focuses mainly on crop production while other agriculture sub-sectors (e.g. animal production) as well as rural development are generally overlooked. Different extension approaches coexist in BF and Niger. These include the T&V extension system as well as various advisory approaches such as Farmer

Field Schools (FFS) and Management Advice for Family Farms (MAAF). Nowadays, extension and advisory services are no more only public and there is a growing participation of the private sector and NGOs. The results of the reform of the public agricultural services have been mixed and change from a country to another. Community-based and participatory extension approaches have become increasingly prominent as a consequence of the decline of investments by the government in public extension services. Modern advisory services support continuous adaptation of the methods of extension and advice taking into consideration the local context as well as resources (financial, human) available for providing advisory services. They also stress the central role of the participation of farmers and the importance of experiential and social learning. Extension is a complex, highly dynamic process that cannot be based on generalized science-based knowledge, but should take into account the context-specific knowledge of farmers as well as values and perceptions of the different local stakeholders. A prominent feature of modern advisory services is their focus on innovation development rather than the linear transfer of knowledge and technologies. The modernisation of extension services has implied an increase in the use of ICT. However, the use of ICT should take into consideration the different media environments and preferences of target populations. The governance mechanisms affect not only the content of the advice but also the overall performance of the advisory services; no matter how the advisory approach is named the real system performance depends on how it is governed and implemented. Proposals to improve the performance of extension and advisory services in BF and Niger include building the capacity of the extension staff, increasing funding and improving its targeting, reinforcing collaboration between the different stakeholders in the agricultural knowledge and innovation system (AKIS), creating an enabling institutional environment as well as fostering the participation of farmers in the design and implementation of advisory programs. Moreover, more attention should be paid to gender issues to meet the differentiated needs of women farmers. Building the capacity of the extension staff is essential for festering the sustainable development of agriculture in BF and Niger in the face of climate change. This, in turn, is vital to achieve sustainable food and nutrition security.

ACKNOWLEDGMENTS

This work was carried out within the project SUSTLIVES (*SUSTaining and improving local crop patrimony in Burkina Faso and Niger for better LIVes and EcoSystems*) of the DeSIRA initiative (Development Smart Innovation through Research in Agriculture) financed by the European Union (contribution agreement FOOD/2021/422-681).

REFERENCES

Bacci, M., Ousman Baoua, Y., & Tarchiani, V. (2020). Agrometeorological Forecast for Smallholder Farmers: A Powerful Tool for Weather-Informed

Crops Management in the Sahel. *Sustainability*, *12*(8), 3246. https://doi.org/10.3390/su12083246

Bakker, T., Dugué, P., & de Tourdonnet, S. (2021). Assessing the effects of Farmer Field Schools on farmers' trajectories of change in practices. *Agronomy for Sustainable Development*, *41*(2), 18. https://doi.org/10.1007/s13593-021-00667-2

Bindlish, V., & Evenson, R. E. (1997). The Impact of T&V Extension in Africa: The Experience of Kenya and Burkina Faso. *The World Bank Research Observer*, *12*(2), 183–201. https://doi.org/10.1093/wbro/12.2.183

Boafo, Y. A., Balde, B. S., Saito, O., Gasparatos, A., Lam, R. D., Ouedraogo, N., Chamba, E., & Moussa, Z. P. (2018). Stakeholder perceptions of the outcomes of reforms on the performance and sustainability of the cotton sector in Ghana and Burkina Faso: A tale of two countries. *Cogent Food & Agriculture*, *4*(1), 1477541. https://doi.org/10.1080/23311932.2018.1477541

Cook, B. R., Satizábal, P., & Curnow, J. (2021). Humanising agricultural extension: A review. *World Development*, *140*, 105337. https://doi.org/10.1016/j.worlddev.2020.105337

Eicher, C. K. (2007). *Agricultural Extension in Africa and Asia*. Department of Agricultrural Economics, Michigan State University.

El Bilali, H. (2021). Organic food and farming in West Africa: A systematic review. *Landbauforschung – Journal of Sustainable and Organic Agricultural Systems*, *70*(2), 94–102. https://doi.org/10.3220/LBF1611507579000

Evenson, R. E., & Siegel, M. (1999). Gender and Agricultural Extension in Burkina Faso. *Africa Today*, *46*(1), 75–92. https://doi.org/10.1353/at.2003.0075

FAO, IFAD, UNICEF, WFP, & WHO. (2020). *The State of Food Security and Nutrition in the World 2020*. FAO. https://doi.org/10.4060/ca9692en

FAO, IFPRI, & OECD. (2017). *Information and Communication Technology (ICT) in Agriculture - A Report to the G20 Agricultural Deputies*. http://www.fao.org/3/a-i7961e.pdf

Faure, G., Rebuffel, P., & Violas, D. (2011). Systemic analysis of management advice for family farms in West Africa. *Cahiers Agricultures*, *20*(5), 364–369. https://doi.org/10.1684/agr.2011.0507

Faure, G., Toillier, A., & Moumouni, I. (2018). How to scale Management Advice for Family Farms in Africa. *Canadian Journal of Development Studies / Revue Canadienne d'études Du Développement*, *39*(4), 515–532. https://doi.org/10.1080/02255189.2018.1447912

Hansen, J. W., Vaughan, C., Kagabo, D. M., Dinku, T., Carr, E. R., Körner, J., & Zougmoré, R. B. (2019). Climate Services Can Support African Farmers' Context-Specific Adaptation Needs at Scale. *Frontiers in Sustainable Food Systems*, *3*. https://doi.org/10.3389/fsufs.2019.00021

Ihm, J., Pena-Y-Lillo, M., Cooper, K. R., Atouba, Y., Shumate, M., Bello-Bravo, J., Malick Ba, N., Dabire-Binso, C. L., & Pittendrigh, B. R. (2015). The Case for a Two-Step Approach to Agricultural Campaign Design. *Journal of*

Agricultural & Food Information, *16*(3), 203–220. https://doi.org/10.1080/10496505.2015.1033529

Le Gal, P.-Y., Andrieu, N., Dugué, P., Kuper, M., & Taher Sraïri, M. (2011). Simulation tools for supporting crop-livestock farmers in their strategic decision-making. *Cahiers Agricultures*, *20*(5), 413–420. https://doi.org/10.1684/agr.2011.0509

Leeuwis, C., & van den Ban, A. (2004). *Communication for Rural Innovation: Rethinking Agricultural Extension*. Blackwell Publishing Ltd.

Mapiye, O., Makombe, G., Molotsi, A., Dzama, K., & Mapiye, C. (2021). Towards a Revolutionized Agricultural Extension System for the Sustainability of Smallholder Livestock Production in Developing Countries: The Potential Role of ICTs. *Sustainability*, *13*(11), 5868. https://doi.org/10.3390/su13115868

Maredia, M. K., Reyes, B., Ba, M. N., Dabire, C. L., Pittendrigh, B., & Bello-Bravo, J. (2018). Can mobile phone-based animated videos induce learning and technology adoption among low-literate farmers? A field experiment in Burkina Faso. *Information Technology for Development*, *24*(3), 429–460. https://doi.org/10.1080/02681102.2017.1312245

Meena, M. S., & Singh, K. M. (2013). *Pluralistic Agricultural Extension System in India: Innovations and Constraints*. https://mpra.ub.uni-muenchen.de/48324/1/MPRA_paper_48324.pdf

Moher, D., Liberati, A., Tetzlaff, J., & Altman, D. G. (2009). Preferred Reporting Items for Systematic Reviews and Meta-Analyses: The PRISMA Statement. *PLoS Medicine*, *6*(7), e1000097. https://doi.org/10.1371/journal.pmed.1000097

Moumouni, I., Nouatin, G. S., & Baco, M. N. (2011). From training and visits to advice for family farms in Benin: Break or continuity? *Cahiers Agricultures*, *20*(5), 376–381. https://doi.org/10.1684/agr.2011.0514

Nkiaka, E., Taylor, A., Dougill, A. J., Antwi-Agyei, P., Fournier, N., Bosire, E. N., Konte, O., Lawal, K. A., Mutai, B., Mwangi, E., Ticehurst, H., Toure, A., & Warnaars, T. (2019). Identifying user needs for weather and climate services to enhance resilience to climate shocks in sub-Saharan Africa. *Environmental Research Letters*, *14*(12), 123003. https://doi.org/10.1088/1748-9326/ab4dfe

Okali, C., Sumberg, J. E., & Reddy, K. C. (1994). Unpacking a Technical Package: Flexible Messages for Dynamic Situations. *Experimental Agriculture*, *30*(3), 299–310. https://doi.org/10.1017/S0014479700024406

Riise, J. C., Permin, A., & Kryger, K. N. (2005). Strategies for developing family poultry production at village level - Experiences from West Africa and Asia. *World's Poultry Science Journal*, *61*(1), 15–22. https://doi.org/10.1079/WPS200437

Schaefer, K. C. (1992). A Portfolio Model for Evaluating Risk in Economic-Development Projects, with an Application to Agriculture in Niger. *Journal of Agricultural Economics*, *43*(3), 412–423. https://doi.org/10.1111/j.1477-9552.1992.tb00235.x

Settle, W., & Garba, M. H. (2011). Sustainable crop production intensification in the Senegal and Niger River basins of francophone West Africa. *International*

Journal of Agricultural Sustainability, *9*(1), 171–185. https://doi.org/10.3763/ijas.2010.0559

Shapiro, B. I., Sanders, J. H., Reddy, K. C., & Baker, T. G. (1993). Evaluating and adapting new technologies in a high-risk agricultural system—Niger. *Agricultural Systems*, *42*(1–2), 153–171. https://doi.org/10.1016/0308-521X(93)90073-B

Siddo, S., Moula, N., Hamadou, I., Issa, M., Issa, S., Hamani, M., Leroy, P., & Antoine-Moussiaux, N. (2018). Q method to map the diversity of stakeholder viewpoints along agricultural innovation systems: a case study on cattle genetic improvement in Niger. *Archives Animal Breeding*, *61*(1), 143–151. https://doi.org/10.5194/aab-61-143-2018

Sylla, A. Y., Mahama Al-Hassan, R., Egyir, I. S., & Anim-Somuah, H. (2019). Perceptions about quality of public and private agricultural extension in Africa: Evidence from farmers in Burkina Faso. *Cogent Food & Agriculture*, *5*(1), 1685861. https://doi.org/10.1080/23311932.2019.1685861

USAID. (2017). *Climate Change Risk in West Africa Sahel: Regional Fact Sheet*. https://www.climatelinks.org/sites/default/files/asset/document/2017 April_USAID ATLAS_Climate Change Risk Profile - Sahel.pdf

van Paassen, A., de Ridder, N., & Stroosnijder, L. (2011). Role of an explorative model for learning about sustainable agricultural development in Burkina Faso. *International Journal of Agricultural Sustainability*, *9*(2), 310–321. https://doi.org/10.1080/14735903.2011.582360

World Bank. (2021). *World Bank Open Data*. https://data.worldbank.org

Zossou, E., Arouna, A., Diagne, A., & Agboh-Noameshie, R. A. (2020). Learning agriculture in rural areas: the drivers of knowledge acquisition and farming practices by rice farmers in West Africa. *The Journal of Agricultural Education and Extension*, *26*(3), 291–306. https://doi.org/10.1080/1389224X.2019.1702066

Original Scientific paper
10.7251/AGRENG2201016A
UDC 637.1

EVALUATION OF MILK YIELD AND MILKING CHARACTERISTICS OF DIFFERENT GENOTYPES DURING LACTATION IN ORGANIC FARMS

Lina ANSKIENĖ[1], Renata JAPERTIENĖ[1], Giedrius PALUBINSKAS[1], Sigitas JAPERTAS[2]

[1]Department of Animal Breeding, Lithuanian University of Health Sciences, Faculty of Animal Sciences, Tilžės 18, LT-47181, Kaunas, Lithuania
[2]Public Institution Practical training and trial center of Lithuanian University of Health Sciences. Akacijų str. 2, Giraitės village, LT-54310, Kauno distr., Lithuania
*Corresponding author: lina.anskiene@lsmuni.lt

ABSTRACT

Dairy cattle on organic farms are still selected on the basis of information from conventional systems. Productivity of cows differs between organic and conventional herds, therefore, the ability of cows to adapt to an organic production environment has been questioned. The aim of this study was to investigate the milk yield and milk ability traits during lactation of Lithuanian black & white and Holstein cows with different genotypes in organic farm. The research was carried out in organic farm in 2020 with dairy cows (n=364) of different genotype of Lithuanian black and white cattle population. The milk yield (MY), milking speed (MS), highest milk flow (HMF), milking time (MT) were evaluated. Investigated traits were measured with DeLaval electronic milk meters, "Apro Windows" software. All records were between five and 305 days of lactation. The statistical analysis of data was performed using the SPSS 20.0 (SPSS Inc., Chicago, IL, USA) software package. A probability of less than 0.05 was considered significant ($P<0.05$). We observed that a highest MY and HMF in organic farm was detected in cows with a genotype of Lithuanian Black and White breed (LB&W, LB&WxH and LB&WxHxLB&W) during all stages of lactation. MT of these cows during the first two stages of lactation was longer, compared to cows of other genotypes. We estimated that of all fixed effects the biggest influence on MY, MS, MT was by stage of lactation ($P<0.001$); a genotype showed a highest impact on MT($P<0.001$) and MY($P<0.01$).

Keywords: *genotype, milk yield, milk flow, milking time.*

INTRODUCTION

The global debate on climate change and environmental issues on sustainable food production systems, development of adapted to different farming conditions, (Ahlman, 2010; Röös *et al*., 2018), and the need for healthy products on the

market, owners of dairy farms are increasingly choosing organic farming. Productivity of cows differs between organic and conventional herds, therefore, the ability of cows to adapt to an organic production environment has been questioned. (Pryce *et al*., 2004, Ahlman, 2010). Dairy cattle on organic farms are still selected on the basis of information from conventional systems (Algers *et al*., 2009; Ahlman, 2010; Ahlman *et al*., 2011 m.; Horn *et al*., 2012, Rodríguez-Bermúdez *et al*., 2019). The ability of high-yielding cow breeds to adapt to the organic environment, which is often associated with lower energy and protein content in feed and limited use of antibiotics, can be difficult task (Verhoog *et al*., 2004, Nauta *et al*., 2006a). Not only the productivity of cows but also their functional characteristics are important in cattle breeding (Ahlman *et al*., 2014).

Holstein cattle have been used for many decades for the breeding of other dairy cattle in order to increase milk production. Schaeffer et al. (2011) Studies in a conventional dairy farm have shown that crossbred cows in Canada produce less milk than purebred Holstein. Dairy herds in Europe and North America were selected for high milk production under intensive farming conditions. Even under optimal management conditions, selection for increase of milk yield has reduced dairy cattle health and reproductive efficiency worldwide (Brotherstone and Goddard, 2005; Oltenacu and Broom, 2010). This raised doubts are these high-cost genotypes, are suitable for organic farming systems (Nauta *et al*.,2006b; Horn *et al*., 2012).

The effect of the stage of lactation on milking characteristics was analysed by Sandrucci et al. (2007), who estimated significantly higher average milk flow rate in Holstein dairy cows in the first half of lactation - up to 150 days.

The aim of this study was to investigate the milk yield and milking characteristics during lactation (different stage of lactation) of Lithuanian black & white and Holstein cows with different genotypes in organic farm.

MATERIALS AND METHODS

The research was carried out in organic farm in 2020 with dairy cows (n=364) of different genotype of Lithuanian black and white cattle population.

The milk yield (MY), milking speed (MS), highest milk flow (HMF), milking time (MT) were evaluated. The milking characteristics and milk yield of dairy cows were measured with DeLaval electronic milk meters installed on milking sites; the data was processed with DeLaval "Apro Windows" software. All records were between five and 305 days of lactation, with average 2.14±0.245 lactation. All cow's had two milk-recording events per test day (morning and evening).

Genotype has been estimated according to a records of cows with complete 3 ancestor's generations pedigree information estimated from the data base of State Enterprise Agricultural Information and Rural Business Center. Cows of different genotypes were investigated. Breed of cow: Lithuanian Black and White (LB&W), (n=174) and Holstein (H) (n=190). Genotypes: mother breed x father breed: Holstein x Holstein (HxH), (n=140) and Lithuanian Black and White x Holstein (LB&WxH), (n=125); mother breed x father breed x mother's father breed:

Holstein x Holstein x Holstein (HxHxH), (n=106), Holstein x Holstein x Lithuanian Black and White (HxHxLB&W), (n=18), Lithuanian Black and White x Holstein x Holstein (LB&WxHxH), (n=65), Lithuanian Black and White x Holstein x Lithuanian Black and White (LB&WxHxLB&W), (n=18). Differences in the number of cows between groups were due to an unknown breed of father or mother father.

Lactation of cows was divided into stages: stage 1st - early period - up to 100 days. During this period, the amount of milk increases rapidly from three to six weeks after calving. At this stage, the cow should be re-fertilized. Stage 2 - mid-lactation - 100-200 days. In mid-lactation, the goal is to maintain the maximum amount of milk for as long as possible, because cows are already pregnant and some of the nutrients are used by the body for the needs of the fetus. Stage 3 - late period - 200 to 305 days, when the lactation of the cow is coming to an end.

Statistical characteristics of the sample (n) – arithmetic mean (M), standard error (SE), P – value (P) – were calculated using the SPSS 20.0 (SPSS Inc., Chicago, IL, USA) software package. Data analysis was performed by using Student-t and Chi-Square statistical significance tests. The impact of single factor (genotype, stage of lactation, milk yield) on milking characteristics was evaluated using Post Hoc - Tukey test. The differences were considered as significant at $P<0.05$.

Data of cows were analysed by using a linear model of ANOVA:

$Y_{ij}=\mu+G_i+L_j+ + GL_{ij} +e_{ij}$

Where: Yij=dependent variables (MY, MS, HMF, MT); μ=general mean, Gi – genotypes of cows (8 genotypes: LB&W, H, HxH, LB&WxH, HxHxH, HxHxLB&W, LB&WxHxH, LB&WxHxLB&W), Lj – lactation stage (1st - early period - up to 100 days, 2 - mid-lactation - 100-200 days,3 - late period - 200 to 305 days), eij - residual error.

RESULTS AND DISCUSSION

Organic farmers have realized that many of the traditional commercial cow breeds are not well adapted to organic farm situations and a stronger constitution is needed for cows that can perform well in an ecological environment to achieve acceptable longevity and productivity.

Therefore, the researchers are investigating the current diversity of organic dairy breeds to identify the cow breeds and their genotypes that would best meet the goals of breeding in organic herds. Choosing a breed it is recommended to take into account the ability of the animals adapt to local conditions, their viability and their resistance to disease, however, most dairy farms that have transitioned to ecosystems maintain the same high-productivity cow breeds such as Holstein (Peeters and Wezel, 2017).The analysis of milk yield and milking characteristics of Lithuanian Black and White (LB&W) and Holstein (H) cows of different genotypes showed, that high-yielding cow breeds are kept on the farm, which need to adapt to the environment, which are often associated with lower energy and protein levels in feed and limited use of antibiotics, can be challenging. In

investigated organic farm purebred Holstein cows accounted for 52.2% (χ^2=58.78, df = 1, P<0.001).

Milking characteristics of dairy cows are very important economic indicators that determine health of cow's udder and milk cost. The scientific literature focuses on production characteristics and animal health, and research on cow milking characteristics in organic farm is still scarce. Juozaitienė *et al*. (2016) found that cows with a higher degree of Holstein breed had higher milk yield in conventional dairy farms, which resulted in higher milk flow, but in our research, cows with LB&W breed blood had the highest milk yield and higher milk flow (Figure 1).

The results show, that the highest milk yield of all genotypes of cows was estimated in all stages of lactation. The milk yield of LB&W cows' was 4.34% (P<0.05), milking speed – 3.24% and high milk flow - 5.98% (P>0.05) higher compared to genotype of H cows' in organic farm on first stage of lactation (Figure 1).

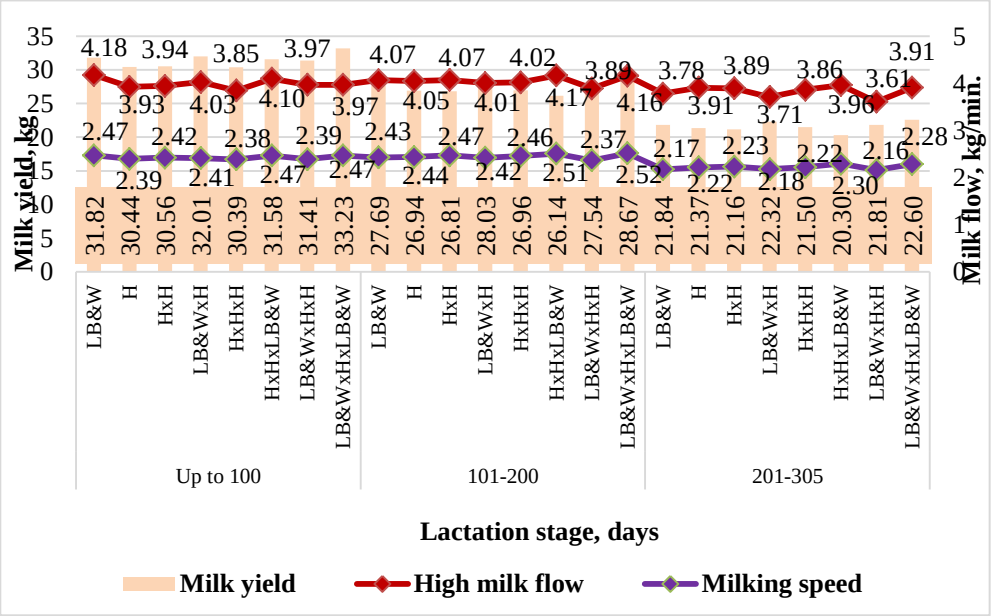


Figure 1. Average of daily milk yield and milk flow of cows during lactation.

The milk yield on the second and on the last stage of lactation of LB&W cows was 2.71% and 2.15% higher than of purebred H cows', but milking speed of H cows' were not significant (0.41% and 2.25% higher compared to LB&W cows). The high milk flow of LB&W cows on the second stage of lactation was 0.49% higher, but on the last stage of lactation - 3.33% lower than of H cows'.

The purebred Holstein cows produced 4.53% (HxH) and 5.06% (HxHxH) lower milk yield; high milk flow - 2.23% (HxH) and 4.47% (HxHxH) lower than a crossbred LB&WxH; while the milking speed of genotype HxH cows was was 0.41% higher, and genotype HxHxH cows was 1.25% lower than genotype

LB&WxH, and these results also were not statistically significant (P>0.05). Juozaitiene *et al*. (2016) studies with different genotypes of cows, reveals that cows with a higher Holstein breed degree had a higher milk yield, which leads to a greater milk flow.

Statistical analysis of various genotypes of crossbred cows shows that the higher milk yield was estimated in genotypes with higher LB&W breed blood, but had no significant effects (P>0.05).

The daily milking time of the first and second stages of lactation depended on the milk yield (Figure 2). The milking time of cows with higher milking yield was longer. On the last stage of lactation cows with more LB&W blood in their genotype, milk yield was higher, and a shorter milking time compared to purebred H (P>0.05).

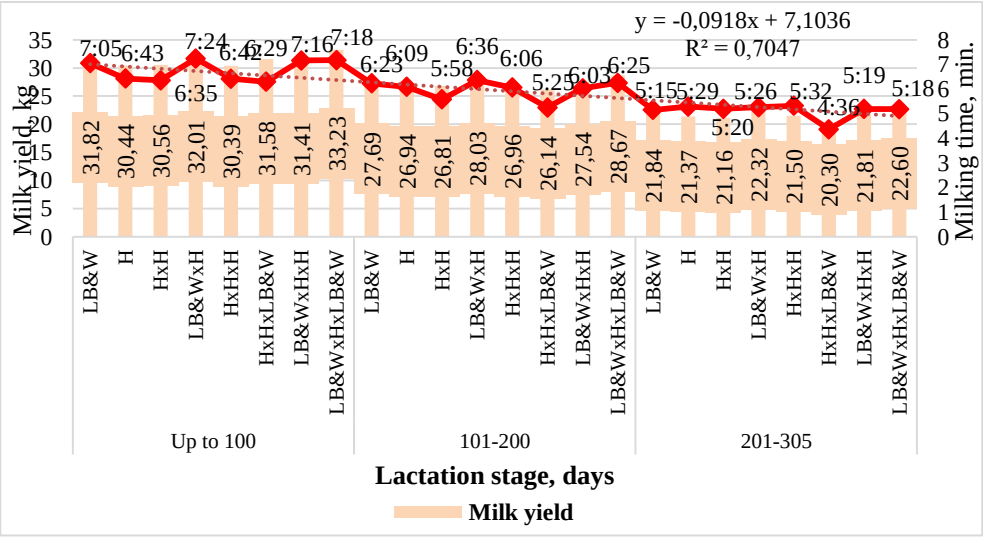


Figure 2. Average of daily milk yield and daily milk time of cows during lactation.

As we can see from the data (Figure 2), the daily milking time of cows tends to decrease on average by 0.09 min with increasing of stage of lactation (R^2=0.705). Also the results show, that the highest milking time of all genotypes of cows was estimated in first stage of lactation (up to 100 days). The daily milking time of LB&W cows was 0:22 min. higher compared Holstein cows; the daily milking time of purebred Holstein -HxH-0:49 min; and HxHxH-0:41 min. (P >0.05) cows in organic farm was lower compared to genotype LB&WxH. The results of Bobić *et al*. (2014) showed that the average milking time of Holstein cows in conventional farm was slightly longer in Holstein cows (7.26 min.), compared to our results.

Statistical analysis of various genotype crossbred Lithuanian Black and White and Holstein cows shows that the milking time of genotype HxHxLB & W cows 0:49 min. higher than of genotype LB & WxHxLB & W and 0:47 min. higher than of

genotype LB & WxHxH cows, however these results also were not statistically significant (P>0.05).

Our previous research (Japertiene *et al*., 2018) related to the production and milking characteristics of Lithuanian dairy cows during the transition period from conventional to organic farms showed, that the performance of ecologically managed dairy cows differs from conventionally managed cows. During lactation the highest milk yield, milk flow and the lowest milking time were estimated when the farm was conventionally managed. The lowest milk yield and high milk flow were when transitional period was started, milking speed and milking time - when the farm was at transitional period. The cows, which produced higher milk yield, had the faster milking speed. The lowest productivity traits were, when the farm maintain the status organic.

We estimated that of all fixed effects the biggest influence on MY, MS, MT was by stage of lactation (P<0.001); a genotype showed a highest impact on MT(P<0.001) and MY(P<0.01), (Table 1). These results correspond to Strapák *et al*., (2011), were the milk flow rate was also significantly influenced by the lactation stage (P<0.01).

Table 1. Investigation of milk yield (MY), milking speed (MS), highest milk flow (HMF), milking time (MT) affecting factors influence

Source	**MY**	**MS**	**HMF**	**MT**
G_i	**0.002**	0.820	0.653	**0.000**
L_j	**0.000**	**0.000**	**0.028**	**0.000**
GL_{ij}	0.999	0.923	1.000	1.000
Model	R Squared = 0.998 (Adjusted R Squared =0.735)	R Squared = 0.995 (Adjusted R Squared = 0.461)	R Squared =0.337 (Adjusted R Squared = 0.330)	R Squared = 0.995 (Adjusted R Squared = 0.487)

The research results of Bobić *et al*. (2014) indicate that the parity significantly affected the milking characteristics and milk yield. Many studies (Strapák *et al*., 2009; Strapák *et al*., 2011; Schaeffer *et al*., 2011; Muller *et al*., 2011) with Holstein cows in conventional dairy farms showed that the milk yield of cows is related to milk flow and milking time, and a shorter milking time may negatively affect the total milk yield.

Studies with small samples have shown a trend, that crossbreds with local breeds are more adapted to organic farming, therefore, further studies with a larger sample of different breeds of cows are needed in order to determine which genotypes best suites in organic farming systems.

CONCLUSIONS

The highest milk yield in organic farm was obtained from genotypes LB&W, LB&WxH and LB&WxHxLB&W cows at all stages of lactation. The milking duration of these cows in the first two stages of lactation (1 st - up to 100, 2 nd - 100-200 days) was longer than that of cows of other genotypes. The milking

duration of the studied cows during lactation decreased along with the decreasing amount of milk.

Differences in milk flow indicators of cows with different genotypes at different stages of lactation were small, but cows with LB&W blood in the genotype had a higher milk flow.

Although most of the results of the study were not statistically reliable, but they confirmed the statements of other researchers, and it is important not only to choose the breed that best adapts to the conditions of organic farming, but also to plan the selection of pairs according to the animal genotype.

According to our results, local breeds are well-adapted and more suitable for organic farming, but further studies should be carried out in order to identify best genotypes of dairy cows for organic farming.

REFERENCES

Ahlman T. (2010). Organic Dairy Production. Herd Characteristics and Genotype by Environment. Doctoral Thesis Swedish University of Agricultural Sciences, Uppsala, p.p. 9–44.

Ahlman T., Berglund B., Rydhmer L., Strandberg E. (2011). Culling reasons in organic and conventional dairy herds and genotype by environment interaction for longevity. Journal of Dairy Science, vol. 94, pp. 1568–1575.

Ahlman T., Ljung M., Rydhmer L., Röcklinsberg H.,Strandberg E., Wallenbeck A.(2014). Differences in preferences for breeding traits between organic and conventional dairy producers in Sweden. Journal of Livestock Science, vol. 162, pp. 5–14.

Algers B., Bertoni G., Broom D., Hartung J., Lidfors L., Metz J., Munksgaard L., Pina T.N., Oltenacu P., Rehage J., Rushen J. (2009). Effects of farming systems on dairy cow welfare and disease. Annex to the EFSA Journal, vol. 1143, pp. 27–28.

Brotherstone S. and Goddard M. (2005). Artificial selection and maintenance of genetic variance in the global dairy cow population. Philosophical Transactions of the Royal Society of London. Series B., Biological Sciences 360, pp. 1479–1488.

Bobić T., Mijić P., Vučković G., Gregić M. (2014). Breed differences of dairy cows. Journal Mljekarstvo, vol. 64 (2), pp.71–78.

Horn M., Steinwidder A., Podstatzky L., Gasteiner J., Zollitsch W. (2012). Comparison of two different dairy cow types in an organic, low input milk production system under Alpine conditions. Journal of Agriculture and Forestry Research, vol. 362 (3), pp.22–325.

Japertienė R., Anskienė L., Japertas S. (2018). Evaluation of selected traits of Lithuanian dairy cows before and starting the transition process and become an organic farm. Journal of Veterinarija ir zootechnika, vol.76 (98), pp.33–38.

Juozaitienė V., Anskienė L., Japertienė R., Juozaitis A., Stankevičius R., Černauskienė J., Žymantienė J., Žilaitis V. (2016). Dependence of Dairy Cows

Milkability Traits On Genotype. Journal of Veterinarija ir zootechnika, vol. 73 (95), pp. 32–36.

Muller A.B., Rose-Meierhofer S., Ammon C., Brunsch R. (2011). Comparison of the effects of quarter individual and conventional milking systems on milkability traits. Journal of Archiv Tierzucht, vol.54, pp.360–373.

Nauta W.J., Veerkamp R.F., Brascamp E.W. & Bovenhuis H. (2006). Genotype by environment interaction for milk production traits between organic and conventional dairy cattle production in The Netherlands. Journal of Dairy Science, vol. 89, pp. 2729-2737.

Oltenacu P.A. and Broom D.M. (2010). The impact of genetic selection for increased milk yield on the welfare of dairy cattle. Journal of Animal Welfare, vol. 19, pp. 39–49.

Peeters A. and Wezel A. (2017). Agroecological principles and practices for grass-based farming systems. In Wezel A (ed), Agroecological Practices for Sustainable Agriculture. World Scientific, London, chapter 11, pp. 293–354.

Pryce J.E., Conington J., Sørensen, P. Kelly H.R.C. & Rydhmer L. (2004). Breeding strategies for organic livestock. In: Vaarst, M., Roderick, S., Lund, V. and Lockeretz, W. (eds) Animal Health and Welfare in Organic Agriculture. CAB International, Oxon, UK, pp. 357-388.

Rodríguez-Bermúdez R., Miranda M., Baudracco J., Fouz R., Pereira V., López-Alonso M. (2019). Breeding for organic dairy farming: what types of cows are needed? Journal of Dairy Research, vol. 86, pp. 3–12.

Röös E., Mie A., Wivstad M., Salomon E., Johansson B., Gunnarsson S., Wallenbeck A., Hoffmann R., Nilsson U., Sundberg C., Watson C.A. (2018). Risks and opportunities of increasing yields in organic farming. A review. Journal of Agronomy for Sustainable Development, vol. 38, pp.14–21.

Schaeffer L.R., Burnside E.B., Glover P., Fatehi J. (2011). Crossbreeding results in Canadian dairy cattle for production, reproduction, and conformation. The Open Agriculture Journal, vol. 5, pp.63–72.

Sandrucci A., Tamburini A., Bava L., Zucali M. (2007). Factors affecting milk flow traits in dairy cows: results of a field study. Journal of Dairy Science, vol. 90, pp.1159–1167.

Strapák P., Antalík P., Szencziová I. (2011). Milkability evaluation of Holstein dairy cows by Lactocorder. Journal of Agrobiology, vol. 27, pp. 139–146.

Strapák P., Súkeníková Z., Antalík P. Milkability in Holstein cows. (2009). Journal of Central European Agriculture, vol. 10, pp. 207–21

Original Scientific paper
10.7251/AGRENG2201024M
UDC 630:582.632

LONG-TERM EFFECTS OF OAK DECLINE ON SHRUB INDIVIDUAL'S OCCURRENCES IN AN HUNGARIAN OAK FOREST

Tamás MISIK*, Imre KÁRÁSZ

Department of Environmental Sciences and Landscape Ecology, Eszterházy Károly University, Hungary
*Corresponding author: misiktom@gmail.com

ABSTRACT

Information about the occurrence of shrubby individual's relation with oak decline is fundamental to developing knowledge from forest stand. This paper focuses on the following questions: (1) how are the shrub's occurrences changed after oak decline? (2) Which shrubs have the highest occurring in the subplots? The mixed oak stand is located in the Bükk Mountains of Hungary. The monitoring plot (48 m × 48 m) was subdivided into 144 permanent subplots; the measured parameter was observed in the period 1972-2017. The shrub layer was divided into low (< 1.0 m in height) and high (≥ 1.0 m) layer. High shrub specimens were randomly distributed and had become more homogeneous by 2017; there was no subplot with 10 or more specimens in the last decade. In the subplots the policormon forming shrubs were present with a higher occurrence. Correlation analysis showed that occurrence of *Acer tataricum*, *Cornus mas* and *Euonymus verrucosus* in the high and *Cornus sanguinea* and *Ligustrum vulgare* in the low shrub layer changed significantly after the oak decline. High shrubs with the highest occurrence were *E. verrucosus* and *C. mas*. The most occurrent low shrub species were *E. verrucosus* and *L. vulgare*. Our results suggest that after the oak decline the most shrubs' occurrence decreased considerably and the distribution was more homogeneous.

Keywords: S*hrub community, oak decline, occurrence, subplots*.

INTRODUCTION

Oak decline has been described as a widespread and complex phenomenon in many countries (Tomiczek, 1993; Sonesson and Drobyshev, 2010). An increase in the death of oak trees has been observed in many regions of Hungary since 1978 (Igmándy, 1987). In the Síkfőkút forest stand species composition of the canopy was stable until 1979 and the healthy *Quercus petraea* Matt. L. (sessile oak) and *Quercus cerris* L. (Turkey oak) also remained constant. Oak decline was first reported in 1979–80 and by 2017, 62.9% of the oaks had died.

Relatively few studies deal with shrub layer dynamics after oak death and the possible relation between trees and shrubs (Légaré *et al.,* 2002). Understory and

overstory relationships are complex and mutual but are dominated by the canopy structure and condition (Burrascano *et al*., 2011; Burton *et al*., 2011; Cutini *et al*., 2015). Shrub layers of forest ecosystems change dynamically and respond sensitively to the environmental changes (Chipman and Johnson, 2002; Rees and Juday, 2002). They are strongly related to the composition and structure of the overstory (Klinka *et al.,* 1996; Palik and Engstrom, 1999). Shrub species play a major role in the cycles of some essential nutrients, including the dynamics of nitrogen, potassium and carbon (Gilliam, 2007). The shrub layers are directly contributes to forest biodiversity (Kerns and Ohmann, 2004; Čermá *et al.,* 2009), including compositional and structural diversity, enhancing the aesthetics of forest ecosystems and helping to protect watersheds from erosion (Alaback and Herman, 1988; Halpern and Spies, 1995; Muir *et al.,* 2002). The distribution of shrubs is strongly influenced by environmental conditions, such as climate (Pedley, 1979; Westman, 1991; Kienast *et al.,* 1998). Chemical and physical soil properties and biotic interactions play a major role in influence the distribution of shrub species (Pedley, 1979). Importance of shrub patch characteristics against other abiotic factors driving the occurrence of shrub species is also poorly studied (Gavilán *et al.,* 2002).

Misik *et al*. (2013) described the possible responses of understory shrub layer's cover, basal area and diversity to the remarkable changes in stand density. Misik *et al*. (2014) reported the dynamics behind the increase in the sizes of woody species and the structure of the new subcanopy layer below the canopy. This paper focuses on the following questions: (1) how are the shrub's occurrences changed after oak decline? (2) Which shrubs have the highest occurring in the subplots? (3) Finally, is a strong relation between occurrences and densities of shrub species?

MATERIAL AND METHODS

Study area The reserve research site (Síkfõkút Project) was established in 1972 by Jakucs (1985) and is located in the Bükk Mountains (47°552 N, 20°462 E) in the north-eastern part of Hungary at an altitude of 320-340 m a.s.l. (Figure 1A). Mean annual temperature is 9.9 °C and mean annual precipitation typically ranges from 500 to 600 mm. Descriptions of the geographic, climatic, soil conditions and vegetation of the forest were reported in detail by Jakucs (1985, 1988). The common forest association in this region is *Quercetum petraeae-cerridis* (Soó, 1963) (sessile oak-Turkey oak) with a dominant canopy of *Q. petraea* and *Q. cerris*; the long-term dynamics of understory shrub layer dynamics are described among others in works of Misik *et al*. (2013, 2014, 2017, 2020). The plots under study were made up of evenly aged temperate, mixed species deciduous forest that was at least 110 years old and had not been harvested for more than 55 years.

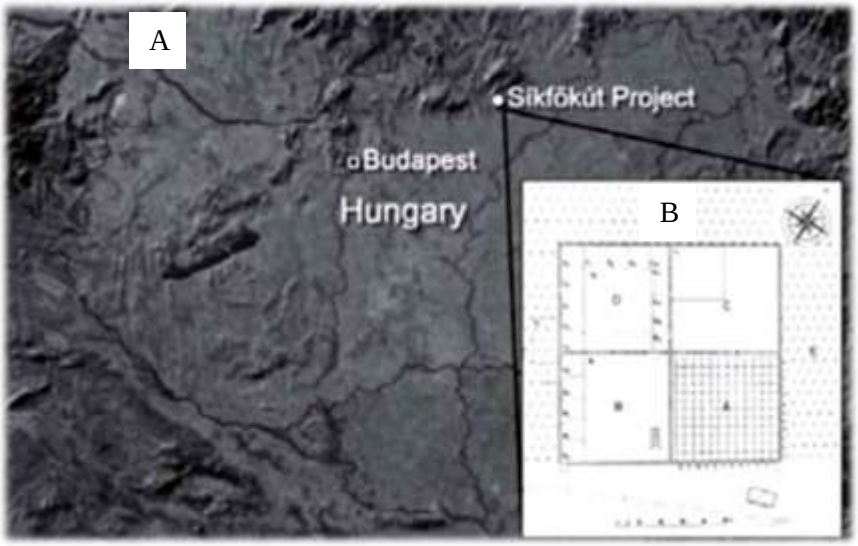


Figure 1.A. Location of the study area in Hungary. B. Study site location with plots.

Sampling and data analysis The structural condition of the shrub layer was monitored on an "A" plot at the research site, 48 m × 48 m in size; the plot was subdivided into 144 4 m × 4 m permanent subplots (Figure 1B).

The subplots were established in 1972; the understory occurrences data collected at subplots measured in the period of 1972-2017 vegetation season on site. Repeated shrub inventories took place in every 4-5 years period. The shrub layer was divided into low (< 1.0 m in height) and high (≥ 1.0 m) layer. Shrub specimens of the vegetation lower than 1.0 m in height were categorized as low understory; higher specimens were categorized as high understory. The occurrence of specimens of each shrub species (occurrence % in subplots of the monitoring plot) was determined as a number expressing in what percentage of the 144 subplots they occurred. Presented frequency values in this paper represent the occurrence of the species (in percentage) in the shrub layer.

Linear regression was used to analyse the relationship between shrub densities and shrub occurrences. The experimental data were analysed by correlation analysis to investigate the possible effects by the occurrences of shrubs on oak tree density (SPSS Statistics 19, Tulsa, USA). Statistical analysis was performed using the PAST statistical software and significant differences for all statistical tests were evaluated at the level of $^{*}P < 0.05$; $^{**}P \leq 0.01$; $^{***}P \leq 0.001$. There was no significant correlation found between the test variables at $^{n.s.}P \geq 0.05$.

RESULTS AND DISCUSSION

Most species were less occurring in 2017 compared with 1972. The occurrence of shrub species change between 0.7 - 98.6% in the period of 1972-2017. Mean values of occurrence changed between 22.4% and 57.6% in the high and between 42.0%

and 94.6% in the low shrub layer. High shrubs with the highest mean occurrence were *Acer campestre* L. (field maple) (57.6%) and *Cornus mas* L. (European cornel) (52.1%) in the last decades. The most occurring low shrub species with the highest mean values were *Euonymus verrucosus* Scop. (spindle tree) (94.6%) and *Ligustrum vulgare* L. (wild privet) (70.8%). The distribution of *Q. petraea* seedlings and saplings occurrence fluctuated between 16.7% and 81.9% on the 144 subplots in the past 45 years. The most frequent species of the shrub community was *E. verrucosus*; followed them *Q. petraea* and *A. campestre* (Table 1).

Table 1. Frequency of occurrence (%) of most common species in shrubs between 1972 and 2017.

species	**high shrub layer**							
	1972	**1982**	**1988**	**1993**	**2002**	**2007**	**2012**	**2017**
A. campestre	61.8	66.7	58.3	59.0	56.9	52.8	52.1	50.0
A. tataricum	31.9	27.8	26.4	22.2	24.3	19.4	14.6	12.5
C. mas	70.8	57.6	59.7	47.9	46.5	46.5	43.8	40.3
E. verrucosus	0.7	36.1	50.7	39.6	61.8	54.2	47.2	54.9
species	**low shrub layer**							
	1972	**1982**	**1988**	**1993**	**2002**	**2007**	**2012**	**2017**
C. sanguinea	93.1	67.4	72.2	25.7	13.9	37.5	18.1	16.0
E. verrucosus	95.1	94.4	96.5	89.6	91.7	98.6	91.7	95.8
L. vulgare	97.9	91.0	88.9	74.3	52.1	63.9	43.1	45.8
Q. petraea	98.6	16.7	63.2	19.4	41.0	41.7	81.9	78.5

Correlation analysis showed a significantly positive relationship between oak tree density and the occurrence frequency of Acer tataricum L. (Tatar maple) ($r = 0.82^*$), C. mas ($r = 0.84^{**}$) and E. verrucosus ($r = 0.91^{**}$) high shrub species and the Cornus sanguinea L. (common dogwood) ($r = 0.84^*$) and L. vulgare ($r = 0.73^*$) low shrub species in the subplots. There was a statistically non-significant interaction ($p > 0.05$) between occurrence values on A. campestre high and E. verrucosus, Q. petraea low shrub species and long-term trend of the canopy oaks density (Table 2).

Table 2. Long-term relationship between canopy oak trees density and occurrences of dominant low and high shrub species on the Síkfőkút mixed oak forest (*N*=81).

high shrub	**oak trees density ind. ha^{-1}**									**correlation**	
layer species	**1972**	**1982**	**1988**	**1993**	**1997**	**2002**	**2007**	**2012**	**2017**	***p***	***F***
A. campestre										0.057$^{n.s.}$	5.50
A. tataricum										0.012*	12.41
C. mas	816	651	408	372	304	324	323	305	303	0.009**	14.17
E. verrucosus										0.002**	27.01
low shrub	**oak trees density ind. ha^{-1}**									**correlation**	
layer species										***p***	***F***
C. sanguinea										0.018*	12.16
E. verrucosus	816	651	408	372	304	324	323	305	303	0.930$^{n.s.}$	0.008
L. vulgare										0.039*	6.91
Q. petraea										0.244$^{n.s.}$	1.74

Regression analysis resulted a highly significant relation ($p < 0.001$) between density and frequency of occurrence by *A. tataricum* and *C. mas* high and *C. sanguinea* and *L. vulgare* low shrub species. This association is lower ($p < 0.05$) by *A. campestre* and *E. verrucosus* high shrub species. This relationship was not significant ($p \geq 0.05$) in the cases of *E. verrucosus* and *Q. petraea* in the low shrub layer (Table 3).

The effect of competition between trees was examined by Szwagrzyk (1990) in terms of the spatial distribution of tree individuals. It was found that competition did not affect the distribution of trees. Skov (2000) investigated the effect of neighbouring individuals on tree distribution in forest communities. In his research, he found that the main influencing factor of the distribution is the size and density of open areas, open lanes along roads and the species diversity of neighbouring stocks. Maestre and Cortina (2005) paper resulted than relative importance of shrub size, species identity and abiotic factors as determinants of shrub species occurrence.

Table 3. Long-term relationship between densities and occurrences of dominant shrub species on the Síkfőkút mixed oak forest (*N*=144) (SD=standard deviation).

high layer shrub species	**min. density ind. ha^{-1}**	**max. density ind. ha^{-1}**	**mean density ind. ha^{-1}±SD**	**linear regression**		
				r	***p***	***t***
A. campestre	543	1905	932±360	0.77	0.016*	3.16
A. tataricum	87	430	226±93	0.94	0.15^{-3}***	7.40
C. mas	373	2335	857±453	0.95	8.89^{-5}***	8.03
E. verrucosus	4	1263	663±314	0.87	0.002**	4.73
low layer shrub species	**min. density ind. ha^{-1}**	**max. density ind. ha^{-1}**	**mean density ind. ha^{-1}±SD**	**linear regression**		
				r	***p***	***t***
C. sanguinea	655	13673	3762±3561	0.92	4.38^{-4}***	6.22
E. verrucosus	8098	22967	13471±2761	0.46	0.218$^{n.s.}$	1.35
L. vulgare	1432	21059	8124±5353	0.93	2.62^{-4}***	6.76
Q. petraea	417	47354	9791±10710	-0.18	0.651$^{n.s.}$	-0.47

CONCLUSIONS

The consequences of serious oak decline cause notable changes in the light and stand thermal conditions of forest community which led to structural changes of the shrub layer (Chapman *et al.*, 2006). Our results confirm that the decreasing canopy density led to the occurrence condition changes of the shrubs. The most occurring low shrub species were *E. verrucosus* and *Q. petraea*; the highest frequency of occurrence values have got *A. campestre* and *E. verrucosus* shrub species in the high shrub layer. The mean occurring values changed between 22.37% and 94.60% in the shrub community on the basis of the 45-years long dataset. Our results from 1972 suggest that some dominant shrub species in the understory responded very differently and counter to the oak decline; the occurrence of *A. tataricum* and *C. mas* high shrubs decreased considerably after the oak decline. In parallel in this layer the occurrence of *E. verrucosus* increased many times in a short time. We found highly significant interaction between occurrence of *C. mas* and *E. verrucosus* high shrubs and oak trees density. This relationship is lower or non-significantly by other shrub species. Our results suggest that were a highly significant impact of shrub density on shrub occurrence by *A. tataricum*, *C. mas* high and *C. sanguinea*, *L. vulgare* low shrub species. A better understanding of forest structure and shrub species occurrence in temperate ecosystems may provide useful further information on the shrub layer dynamics of the community.

REFERENCES

Alaback P.B., Herman F.R. (1988). Long-term response of understory vegetation to stand density in *Picea-Tsuga* forests. Canadian Journal of Forest Research, 18: 1522-1530.

Burrascano S., Sabatini F.M., Blasi C. (2011). Testing indicators of sustainable forest management on understorey composition and diversity in southern Italy

through variation partitioning. Plant Ecology, 212: 829-841. DOI: 10.1007/s11258- 010-9866-y

Burton J.I., Mladenoff D.J., Clayton M.K., Forrester J.A. (2011). The roles of environmental filtering and colonization in the fine-scale spatial patterning of ground-layer plant communities in north temperate deciduous forests. Journal of Ecology, 99: 764-776. DOI: 10.1111/j.1365-2745.20 11.01807.x

Chapman R.A., Heitzman E., Shelton M.G. (2006). Long-term changes in forest structure and species composition of an upland oak forest in Arkansas. Forest Ecology and Management, 236: 85-92.

Chipman S.J., Johnson E.A. (2002). Understory vascular plant species diversity in the mixedwood boreal forest of western Canada. Ecological Applications, 12: 588-601.

Cutini A., Chianucci F., Giannini T., Manetti M.C., Salvati, L. (2015). Is anticipated seed cutting an effective option to accelerate transition to high forest in European beech (*Fagus sylvatica* L.) coppice stands? Annals of Forest Sciences, 72: 631-640. DOI: 10.1007/s13595-015-0476-7

Gavilán R.G., Sánchez-Mata D., Escudero A., Rubio A. (2002). Spatial structure and interspecific interactions in Mediterranean high mountain vegetation (Sistema Central, Spain). Israel Journal of Plant Sciences, 50: 217-228.

Gilliam F.S. (2007). The ecological significance of the herbaceous layer in temperate forest ecosystems. BioScience, 57: 845-858.

Halpern C.B., Spies T.A. (1995). Plant species diversity in natural and managed forests of the Pacific Northwest. Ecological Applications, 5: 913-934.

Jakucs P. (ed.) (1985). Ecology of an oak forest in Hungary. Results of „Síkfőkút Project" I. Akadémia Kiadó, Budapest.

Jakucs P. (1988). Ecological approach to forest decline in Hungary. Ambio, 17: 267-274.

Kerns B.K., Ohmann J.L. (2004). Evaluation and prediction of shrub cover in coastal Oregon forests (USA). Ecological Indicators, 4: 83-98.

Kienast F., Wildi O., Brzeziecki B. (1998). Potential impacts of climate change on species richness in mountain forests–an ecological risk assessment. Biological Conservation, 83: 291-305.

Klinka K., Chen H.Y.H., Wang Q.L., de Montigny L. (1996). Forest canopies and their influence on understory vegetation in early-seral stands on West Vancouver Island. Northwest Science, 70: 193-200.

Légaré S., Bergeron Y., Paré D. (2002). Influence of forest composition on understory cover of boreal mixedwood forests of western Quebec. Silva Fennica, 36: 353-366.

Maestre F.T., Cortina J. (2005). Remnant shrubs in Mediterranean semi-arid steppes: Effects of shrub size, abiotic factors and species identity on understorey richness and occurrence. Acta Oecologica, 27: 161-169. DOI: 10.1016/j.actao.2004.11.003

Misik T., Varga K., Veres Zs., Kárász I., Tóthmérész B. (2013). Long-term response of understorey cover, basal area and diversity to stand density in a

mixed oak forest on the Síkfõkút plot in Hungary. Journal of Forest Science, 59: 319-327.

Misik T., Kárász I., Tóthmérész B. (2014). Understory development in an oak forest in Northern-Hungary: the subcanopy layer. Acta Silvatica et Lignaria Hungarica, 10: 9-21. DOI: 10.2478/aslh-2014-0001

Misik T., Kotroczó Zs., Kárász I., Tóthmérész B. (2017). Long-term oak deedling Dynamics and regeneration ability in a deciduous forest in Hungary. Baltic Forestry, 23: 595-602.

Misik T., Kárász I. (2020). Long-term relationship between oak decline and shrub growth dynamics in an Hungarian oak forest, 1972-2017. Agrofor, 5/3: 47-54.

Muir P.S., Mattingly R.L., Tappeiner J.C., Bailey J.D., Elliott W.E., Hager J.C., Miller J.C., Peterson E.B., Starkey E.E. (2002). Managing for biodiversity in young Douglas-fir forests of Western Oregon. Biological Science Report. US Geological Survey, Forest and Rangeland Ecosystem Science Center: Corvallis, OR. p. 76.

Palik B., Engstrom R.T. (1999). Species composition. In: Hunter, M.J. (ed.) Maintaining Biodiversity in Forest Ecosystems. Cambridge University Press, Cambridge, UK, pp. 65-94.

Pedley, L. (1979). A revision of *Acacia* Mill. in Queensland. Austrobaileya, 1: 235-337.

Rees D.C., Juday G.P. (2002). Plant species diversity on logged versus burned sites in central Alaska. Forest Ecology and Management, 155: 291-302.

Sonesson K., Drobyshev I. (2010). Recent advances on oak decline in southern Sweden. Ecological Bulletins, 53: 197-207.

Tomiczek C. (1993). Oak decline in Austria and Europe. Journal of Arboriculture, 19: 71-73.

Westman W.E. (1991). Measuring realized niche spaces: climatic response of chaparral and coastal sage scrub. Ecology, 72: 1678-1684.

Original Scientific paper
10.7251/AGRENG2201032T
UDC 633.32:665.9

FOLIAR APPLICATION OF ZINC IN THE PRODUCTION OF RED CLOVER SEED ON ACID SOIL

Dalibor TOMIĆ[1*], Vladeta STEVOVIĆ[1], Dragan ĐUROVIĆ[1], Milomirka MADIĆ[1], Đorđe LAZAREVIĆ[2], Jasmina KNEŽEVIĆ[3], Miloš MARJANOVIĆ[1], Nenad PAVLOVIĆ[1], Anđelka POPADIĆ[1]

[1]University of Kragujevac, Faculty of Agronomy, Cara Dušana 34, 32000 Čačak, Serbia
[2]Institute of Forage Crops, +38137251 Globoder, Kruševac, Serbia
[3]University of Priština, Faculty of Agriculture, 38219 Lešak, Serbia
*Corresponding author: dalibort@kg.ac.rs

ABSTRACT

Zinc is an important microelement that enters into the composition of hundreds of enzymes and thousands of proteins in the plant and animal world. The aim of the study was to analyze the effect of foliar application of zinc in acid soil on the most important seed yield components and seed yield of selected red clover (*Trifolium pratense* L.) genotypes from Serbia and Bulgaria. The experiment was established in 2012 in Čačak (Serbia) on loessivized vertisol soil type with acid reaction (pH_{H2O} 4.8). Sowing was done at a distance of 70x40 cm. There were used four different genotypes of red clover, three of which were isolated from the local population and variety of K-39. For the analysis was used the second cut in the second year of cultivation. Two treatments were used: control - no fertilization and foliar treatment with zinc in the form of $ZnSO_4$ x $7H_2O$, at a concentration of 0.2%, using 1000 L ha^{-1} of water, at the stage of intensive plant growth. Foliar application of zinc had a positive effect on the number of stems per plant, the number of inflorescences per stem, the number of inflorescences per plant and the number of flowers per inflorescence. The negative effect of foliar application of zinc has been reported on the thousand seeds weight and the fertility of flowers at the genotypes originating from Bulgaria. A significant increase in seed yield per plant, under the influence of foliar application of zinc, was observed only in the variety K-39 (for 11.4 g $plant^{-1}$), which otherwise had the highest seed yield.

Key words: *foliar fertilization, red clover, seed yield, zinc.*

INTRODUCTION

Red clover (*Trifolium pretense* L.) is one of the most important legumes for the production of high quality forage. It grows in wild forms in meadows throughout Europe and Asia. Red clover seed yield is mainly determined by the genetic basis of the variety, the ecological conditions of the area, the time of mowing, the presence of pollinator insects, as well as the interaction of the genotype and

environment (Steiner et al., 1995). The high variability and genetic plasticity of the species are the result of the extremely xenogamous character of fertilisation and entomophilous pollination (Taylor and Smith, 1979). Due to the high variability and adaptability to the various environmental conditions, natural selection has produced a large number of local ecotypes, superior to the given growing conditions (Helgadottir, 1996).

Zinc (Zn) is one of the elements that has an important metabolic role in plant growth and development and therefore it is called a necessary trace element or microelement. It has a very important physiological role in all living systems such as maintaining the structural and functional integrity of biological membranes, energy production, facilitating protein synthesis and gene expression (Mosuavi et al., 2013). Zinc is part of hundreds of enzymes and thousands of proteins in plants and animals. Therefore, the quantitative and qualitative yield of crops depends on the supply of plants with zinc. Lack of zinc in nutrition of domestic animals and humans leads to the severe health complications, including impairments of growth and the immune system combined with increased risk of infections, DNA damage and cancer development (Poblaciones et al., 2017).

In Southeast Europe, red clover (*Trifolium pratense* L.) seed crop is commonly established on acidic soils where certain macro- and micro-nutrients are less available to the plants. Zinc is absorbed from the soil in the form of Zn^{2+}. According to Mosuavi et al. (2013) the solubility of zinc in acidic soils is generally high, and therefore there may be a loss by leaching, leading to its deficiency in plants. However, according to Kadović and Knežević (2002), only a small part of the total Zn in the soil is accessible to plants, only a few mg kg^{-1} of soil, and often less than one mg kg^{-1} of soil. Zn adsorption increases with increasing pH and the presence of $CaCO_3$ in the soil. McBride and Blasiak (1979) indicate that the solubility of zinc in acid soils can be reduced by the presence of competing cations, primarily Al, Cu, and Fe. The solubility of zinc in acidic soil also depends on other factors such as the content of different organic fractions, calcium carbonate, phosphorus, clay minerals and temperature (Mosuavi et al., 2013). Therefore, one reaction mechanism probably cannot explain the solubility of Zn on acidic soil, but it is conditioned by numerous factors in each individual case.

The effect of zinc fertilization on growth and yield of many plants such as alfalfa, wheat, maize, barley, cotton and potato were investigated in numerous researches and increasing in yield with zinc application was observed (Kinaci and Kinaci, 2005; Mosuavi et al., 2007; Galavi et al., 2011; Xi-Wen et al., 2011; Efe and Yarpuz, 2011; Mosuavi et al., 2013).

The aim of this study was to analyze the impact of foliar application of zinc on acidic soil on the most important yield components and seed yield of selected genotypes of red clover from Serbia and Bulgaria.

MATERIALS AND METHODS

A field trial was conducted in Čačak in Serbia (43°54'39.06" N, 20°19'10.21" E, 243 m a.s.l.) in the period 2012-2013. The experiment was established on a leached vertisol (pH 4.8), which contained 3.18% organic matter, 0% $CaCO_3$, 22.08 mg extractable P 100 g^{-1} soil, 30.0 mg K 100 g^{-1} soil and 82 mg Zn kg^{-1} of soil. Prior to seeding and in autumn, 45 kg ha^{-1} N, 45 kg ha^{-1} P_2O_5, and 45 kg ha^{-1} K_2O were incorporated into the soil.

Two factorial experiment was set up according to a completely randomised block design in five replications (with 20 plants per plot at a plant spacing of 70x40cm). A total of four red clover genotypes, including the cultivar K-39 was used. Besides the K-39 cultivar (G_1), two genotypes were extracted from local populations from Bulgaria, Petrič (G_2) and Rozova dolina (G_3), as well as one genotype (G_4) isolated from the local populations in Serbia, vicinity of Aleksandrovac (Central Serbia). Two treatments were applied: control - without fertilization and foliar treatment with zinc in the form of $ZnSO_4$ x $7H_2O$, in a concentration of 0.2%, using 1000 L ha^{-1} water in the phase of intensive plant growth (BBCH 34-36). The experiment was set up according to a randomized block system with five replicates. Weeds were controlled mechanically. No irrigation was employed.

The mean annual air temperature in 2012 and 2013 was 13.12°C and 12.99°C respectively and amount of annual rainfall 463.5 mm and 582.7 mm respectively. The average annual air temperature for the multi-year period (1992-2002) was 11.97°C, and the average amount of annual rainfall 680.3 mm.

The second growth in the second year (2013) was evaluated under field conditions for the following: stem number per plant, inflorescence number per stem, and inflorescence number per plant, using a sample of five plants per plot. Laboratory evaluation included determination of: flower number per inflorescence and seed number per inflorescence (using ten randomly selected inflorescences). Fertility (ratio between seed number and total flower number per inflorescence) and thousand seed weight (based on the seeds extracted from the same sample) were calculated. Seed yield components (inflorescence number per plant, seed number per inflorescence, thousand seed weight) were used to determine seed yield per plant which was calculated as g $plant^{-1}$.

The obtained results were subjected to a two-factor analysis of variance using SPSS software (1995). The significance of differences between mean values was tested by LSD test.

RESULTS AND DISCUSSION

Foliar application of zinc affected a significant increase in the number of stems per plant only in G3 (significance of the variety / foliar application interaction) (Table 1). The genotypes did not differ significantly in the control variant in terms of the number of stems per plant, while in the zinc variant a significantly higher number of stems per plant was recorded in G3 compared to G2 and G4. The positive effect of foliar treatment may be related to the fact that the metabolism of plant hormones

such as auxins decreases in the state of zinc deficiency. Zinc is an element necessary for the synthesis of tryptophan, and this is a prerequisite for the formation of auxin, and the amount of auxin is reduced in the absence of zinc (Pedler et al., 2000). According to Derakhshani et al. (2011) foliar application of zinc in the amount of 1 and 3 g of L^{-1} affected the growth of stems in *Chrysanthemum balsamita* L., while there were no significant differences between the stated concentrations. In this experiment, foliar treatment with zinc affected the increase in the number of inflorescences per stem only in G1. In the control variant, the varieties did not differ significantly in terms of the number of inflorescences per stem. In the zinc variant, a significantly higher number of inflorescences per stem was observed in G1 and G2 compared to G4. Thanks to the cumulative effect on the number of stems per plant and the number of inflorescences per stem, foliar application of zinc also affected the increase in the number of inflorescences per plant in all genotypes except G4. Varieties did not differ significantly in the number of stem per plant in the control variant.

The average number of flowers per inflorescence was 93.9, and it ranged from 77.1-124.1.

Foliar fertilization with zinc affected a significant increase in the number of flowers per inflorescence only in G3. In the control variant, a significantly higher number of flowers per inflorescence was recorded in G2 compared to G3, while in the variant with zinc G3 had the highest number of flowers per inflorescence. According to Ali et al. (2011), zinc fertilization in the amount of 0.75 kg ha^{-1} increased significantly the number of flowers in cotton, which later affected a significant increase in yield. Foliar application of zinc in this experiment did not have a positive effect on flower fertility, and in G3 it even had a negative effect. In the control variant, the cultivars did not differ significantly in terms of fertility, while in the treatment with zinc G1 had significantly higher flower fertility compared to G2 and G3, as a result of of the negative effect of zinc in G2 and G3. The content of amino acids in plant tissues and protein synthesis decreases due to zinc deficiency. One of the places of protein synthesis is the pollen tube, in the top of which the amount of zinc is up to 150 micrograms per gram of dry matter. Thus, zinc contributes to pollination by influencing pollen tube formation (Outten and O'Halloran, 2001; Pandei et al., 2006). However, when the amount of zinc is higher than optimal, toxicity can occur in plants (Mosuavi et al., 2013). Foliar treatment with zinc, generally observed, had no effect on the number of seeds per inflorescence. However, the results of the foliar treatment / genotype interaction indicate that in the control treatment the cultivars did not differ significantly in this trait, while in the zinc treatment a significantly higher number of seeds per inflorescence was recorded in G1 compared to G2. This indicates that foliar treatment had a minimal impact on this trait in some varieties. Jevtić et al. (2007) and Wilczek and Ćwintal (2008) also show that the presence of insect pollinators has a major impact on the number of seeds per inflorescence. Also, large amounts of rainfall during flowering can significantly reduce fertility and harvest yield in relation to the potential seed yield. The negative effect of zinc was also observed

on thousand seed weight in G2 and G3. The mentioned genotypes had a significantly higher thousand seed weight on the control variant compared to G1 and G4. In the variant with zinc, the genotypes did not differ significantly in terms of the weight of a thousand seeds.

Table 1. Seed yield and seed yield components of the red clover genotypes depending on foliar application of zinc (Ø without zinc, foliar treatment with zinc)

Genotype	Treatment	SP	IS	IP	FI	SI	F	TSW	SYP
G 1	Ø	34,0ab	5,0bc	172b	77,1bc	30,8ab	40,4ab	1,385b	8,46b
	Zn	42,8abc	7,44a	308a	89,4b	39,8a	44,9a	1,525b	19,84a
G 2	Ø	33,4bc	5,74abc	191b	86,3b	30,9ab	36,6ab	1,907a	11,9b
	Zn	32,0bc	6,34ab	197a	84,1bc	25,1b	29,8bc	1,318b	6,41b
G 3	Ø	30,2bc	5,36abc	182b	59,3c	24,1b	41,7ab	1,878a	7,6b
	Zn	52,4a	5,58abc	268a	124,1a	27,5ab	22,3c	1,494b	8,59b
G4	Ø	33,4bc	4,2bc	139b	81,2bc	35,6ab	43,8ab	1,459b	7,07b
	Zn	23,6c	4,0c	93b	78,0bc	28,7ab	36,8ab	1,584b	4,42b

SP – stems per plant, IS – inflorescences per stem, IP – inflorescences per plant, FI – flowers per inflorescence, SI – seeds per inflorescence, TSW – thousand seed weight (g), F - fertility (%), SYP – seed yield per plant (g); Values followed by different small letters within columns are significantly different ($P<0.05$) according to the LSD test.

The average seed yield in this experiment was 9.3 g per plant. Herrmann et al. (2006) state that the average seed yield per plant in two genotypes of red clover was 5.72 g, with the variation interval of 0.71-11.31g. According to Vasiljević et al. (2000), the coefficients of genetic correlation show that seed yield per plant mostly depended on the number of seeds per inflorescence, the number of flowers per inflorescence and the number of productive stems per plant. The authors also claim that there were significant positive genetic correlations between the number of inflorescences and the number of internodes per stem and green matter yield, as confirmed by Steiner and Alderman (2003). Foliar application of zinc in this experiment had a significant positive effect on seed yield per plant only in G1. In the control variant, the genotypes did not differ significantly in terms of seed yield per plant, while in the treatment with foliar application of zinc, significantly higher seed yield per plant was recorded in G1 compared to all other genotypes. A possible reason for the different response of genotypes to foliar treatments with zinc in terms of seed yield and seed yield components may be the different proportion of leaves at the time of treatment (author's observations). According to numerous studies, zinc has a great influence on the basic life processes of plants, such as: metabolism and nitrogen intake; photosynthesis and chlorophyll synthesis, carbonic anhydrase activity; resistance to abiotic and biotic stresses; protection against oxidative damage (Potarzicki and Grzebisz, 2009; Cakmak, 2008; Mousavi, 2011). Zinc enters into the composition of over 300 plant enzymes (Ramezani et al., 2016). Due to the mentioned unsubstituted effects, zinc plays a crucial role in plant growth and development, so it can greatly influence the yield components

and seed yield of cultivated plants (Derakhshani et al., 2011; Farnia and Khodabandehloo, 2015). According to the results of Ramezani et al. (2016) foliar application of zinc in alfalfa in a concentration of 0.3% led to an increase in its concentration in plants, which led to an increase in dry matter yield. Farnia and Khodabandehloo (2015) state that the application of the chelated form of zinc in maize in the phase of 4-6 leaves affected the increase in the number of seeds per cob and the thousand seed weight, which led to an increase in seed yield and harvest index. Poblaciones et al. (2017) indicate that zinc as a heavy metal antagonist, foliar application in legumes, may reduce the uptake of heavy metals from contaminated soils. The authors indicate that foliar application of 0.25% w/v $ZnSO_4 7H_2O$ decreased stem Cd concentration in *L. rigidum* and *T. subterraneum* grown in Cd-contaminated soil and ameliorated the adverse effects of Cd exposure on root growth, mainly in *T. subterraneum*.

CONCLUSION

Foliar application of zinc had a positive effect on the number of stems per plant, the number of inflorescences per stem, the number of inflorescences per plant and the number of flowers per inflorescence in certain red clover genotypes. The negative impact of foliar application of zinc was observed on the thousand seed weight and flower fertility in red clover genotypes originating from Bulgaria. A significant increase in seed yield per plant under the influence of foliar application of zinc was recorded only in the variety K-39, which otherwise had the highest yield. The analyzed genotypes of red clover from natural populations had significantly lower seed yield in the treatment with zinc compared to the variety K-39, but they had higher values for certain yield components. As such, they can be used in selection work.

ACKNOWLEDGEMENTS

The research was financed by the Ministry of Education, Science and Technological Development, Republic of Serbia, project ref. number 451-03-9/2021-14.

REFERENCES

Ali L., Ali M., Mohyuddin Q. (2011). Effect of foliar application of zinc and boron on seed cotton yield and economics in cotton-wheat cropping pattern. Journal of Agricultural Research, 49(2): 173-180.

Cakmak I. (2008). Enrichment of cereal grains with zinc: Agronomic or genetic biofortification? Plant and Soil, 302: 1-17.

Derakhshani Z., Hassani A., Sadaghiani M.H.R., Hassanpouraghdam M.B., Khalifani H.B., Dalkani M. (2011). Effect of Zinc Application on Growth and Some Biochemical Characteristics of Costmary (*Chrysanthemum balsamita* L.). Communications in Soil Science and Plant Analysis, 42: 2493–2503.

Efe L., Yarpuz E. (2011). The effect of zinc application methods on seed cotton yield, lint and seed quality of cotton (*Gossypium hirsutum* L.) in east

Mediterranean region of Turkey. African Journal of Biotechnology, 10: 8782-8789.

Farnia A., Khodabandehloo S. (2015). Changes in Yield and its Components of Maize (*Zea mays* L.) to Foliar Application of Zinc Nutrient and Mycorrhiza under Water Stress Condition. International Journal of Life Sciences, 9(5): 75-80.

Galavi M., Yosefi K., Ramrodi M., Mousavi S.R. (2011). Effect of bio-phosphate and chemical phosphorus fertilizer accompanied with foliar application of micronutrients on yield, quality and phosphorus and zinc concentration of maize. Journal of Agricultural Science, 3(4): 22-29.

Helgadottir A. (1996). Legumes in Icelandic agriculture. Grassland and land use systems. Grassland science in Europe, 1: 741-745.

Herrmann D., Boller B., Studer B., Widmer F., Kolliker R. (2006). QTL analysis of seed yield components in red clover (*Trifolium pratense* L.), Theoretical and applied genetics, 112 (3): 536-545.

Jevtić G., Radović J., Lugić Z., Sokolović D., Valić T. (2007). Influence of honey bee (*Aphis mellifera* L.) and sugar syrup on alfalfa and red clover seed yield. Proceedings, IX Symposium of Forage Plants of the Republic of Serbia, 44(1): 99-106.

Kadović R., Knežević M. (2002). Heavy metals in forest ecosystems of Serbia, University of Belgrade, Faculty of Forestry, Ministry of Natural Resources and Environmental Protection of the Republic of Serbia, Belgrade.

Kinaci G., Kinaci E. (2005). Effect of zinc application on quality traits of barley in semi arid zones of Turkey. Plant, Soil and Environment, 51(7): 328–334.

McBride B.M., Blasiak J.J. (1979). Zinc and Copper Solubility as a Function of pH in an Acid Soil. Soil Science Society America Journal, 43: 866-870.

Mousavi R.S., Galavi M., Rezaei M. (2013). Zinc (Zn) Importance for Crop Production – A Review. International journal of Agronomy and Plant Production, 4(1): 64-68.

Mousavi S.R. (2011). Zinc in crop production and interaction with phosphorus. Australian Journal of Basic and Applied Sciences. 5: 1503-1509.

Mousavi S.R., Galavi M., Ahmadvand G. (2007). Effect of zinc and manganese foliar application on yield, quality and enrichment on potato (*Solanum tuberosum* L.). Asian Journal of Plant Sciences, 6: 1256-1260.

Outten E., O'Halloran T.V. (2001). Femtomolar sensitivity of metalloregulatory protein controlling Zn homeostasis. Science, 292: 2488-2492.

Pandey N., Pathak G.C., Sharma C.P. (2006). Zinc is critically required for pollen function and fertilisation in lentil. Journal of Trace Elements in Medicine and Biology, 20: 89-96.

Pedler J.F., Parker D.R., Crowley D.E. (2000). Zinc Deficiency-induced phytosiderophore release by the Triticaceae is not consistently expressed in solution culture. Planta, 211:120-126.

Poblaciones J.M., Damon P., Rengel Z. (2017). Foliar zinc biofortification effects in *Lolium rigidum* and *Trifolium subterraneum* grown in cadmium-contaminated soil. PLoS ONE, 12(9): 1-11.

Potarzycki J., Grzebisz W. (2009). Effect of zinc foliar application on grain yield of maize and its yielding components. Plant, Soil and Environment, 55(12): 519–527.

Ramezani A., Sajedi A.N., Yarahmadi M.H. (2016). The Effect of Foliar application of Iron, Zinc, and Selenium on Nutrient Uptake and Yield of Alfalfa. International Research Journal of Applied and Basic Sciences, 10(5): 557-562.

SPSS.Inc. 1995.STATISTICA for Windows (Computer Program Manual). Tulsa. OK.

Steiner J.J., Alderman C.S. (2003). Red clover seed production: VI. Effect and Economics of Soil pH Adjusted by Lime Application, Crop Science, 43: 624-630.

Steiner J.J., Leffel J.A., Gingrich G., Aldrich-Markham S. (1995). Red clover seed production: III Effect of herbage removal time under varied environments. Crop Science, 35: 1667-1675.

Taylor N.M., Smith R.R. (1980). Red clover breeding and genetics. Advances in Agronomy, 31: 125-154.

Vasiljević S., Šurlan-Momirović G., Katić S., Mihailović V., Lukić D., Živanović T. (2000). Relationships between green forage and seed yield components in genotypes of red clover (*Trifolium pratense* L.), Genetika, 32(1): 37-43.

Wilczek M., Ćwintal M. (2008). Effect of the methods of additional feeding with microelements (B,Mo) on the yield structure and seed yield of red clover. Electronic Journal of Polish Agricultural Universities, http://www.ejpau.media.pl/volume11/issue4/abs-05.html.

Xi-Wen Y., Xiao-Hong L., Xin-Chun T., William G.J., Yu-Xian C. (2011). Foliar zinc fertilization improves the zinc nutritional value of wheat (*Triticum aestivum* L.) grain. African Journal of Biotechnology, 10: 14778-14785

Scientific Original paper
10.7251/AGRENG2201040S
UDC 591.3:632.95.02

TERATOGENICITY TESTING OF CHLORPYRIFOS AND TEBUCONAZOLE IN CHICKEN EMBRYOS AFTER SIMULTANEOUS ADMINISTRATION

Rita SZABÓ[1]*, László MAJOR[1], József LEHEL[2], Nadhirah Binti SAIDON[1], Péter BUDAI[1]

[1]Hungarian University of Agriculture and Life Sciences, Georgikon Campus, Institute of Plant Protection, H-8360 Keszthely, Deák F. u. 16., Hungary
[2]University of Veterinary Medicine, Department of Food Hygiene, H-1078 Budapest, István u. 2., Hungary
*Corresponding author: Szabo.Rita@uni-mate.hu

ABSTRACT

The objective of this study was to determine the single and simultaneous toxic effects of chlorpyrifos containing insecticide formulation (Pyrinex 48 EC) and tebuconazole containing fungicide formulation (Mystic 250 EW) on the development of chicken embryos. Amount of 0.1 ml of 1% Pyrinex 48 EC and of 0.4% Mystic 250 EW was alone and concomitantly injected into the air chamber of eggs on the first day prior to incubation. The chicken embryos were examined on day 19 of incubation for the followings: number of embryonic deaths, body, liver and heart weight of the embryos, and type of developmental anomalies. The body, liver and heart weight data were evaluated statistically by One-Way ANOVA, Tukey and Dunnett tests, the embryonic mortality and the developmental abnormalities were analysed by Fisher's exact test. The combined administration of Pyrinex 48 EC and Mystic 250 EW pesticides on the chick embryo had shown to be embryotoxic to the chick. The rate of mortality and the incidence of developmental anomalies were increased due to the simultaneous application of them. The body and liver weight were significantly reduced. Our teratogenicity study revealed that the combined administration of both chlorpyrifos (Pyrinex 48 EC) and tebuconazole (Mystic 250 EW) pesticides on the chick embryo had shown to be embryotoxic to the chick. They had a slight addition effect on the rate of embryonic mortalities, however, the toxic interaction of both pesticides on developmental anomalies, liver and body weight was not proven.

Keywords: *chlorpyrifos, tebuconazole, teratogenicity, chicken embryo, developmental anomalies.*

INTRODUCTION

Pesticide has received great popularity in agricultural sector in enhancing the production by its purpose of eliminating and preventing pest infestation that may

cause great losses to the yield. Principally, pesticides are allowed to be registered if after demonstration, it will not remain or persist in the environment exceeding their period of its intended use. Generally, pesticides residue can be found abundantly in the natural environment. The degradation of these pesticides through microbial transformation such as oxidation and hydrolysis had caused contamination mainly in the water source and reservoir (Fenner *et al*., 2013).

Majority of pesticide show high degree of toxicity due to their key function to eliminate certain organism consequently creating some risk of harm. Humans may accidentally expose to pesticides by consuming contaminated foods and drinks or substantial exposure around the housholden vironment. Pesticides caused adverse effects to the environment (water, soil, and air) from leaching, runoff and spray drift while also caused detrimental effects on wildlife, fish, plants, and other non-target organisms. Thus, the main concern is not only on human health, but also negative impacts on wildlife such as avian species as one of the non-target organisms encompassing the sensitive ecosystems (Damalas and Eleftherohorinos, 2011). Organophosphate (OP) and carbamate (CB) are anti-cholinesterase (anti-ChE) insecticides that is generally more toxic to avian than to mammals. Narrowing down to animal or wildlife exposure, many research has shown that bird as one of the bio-monitoring organism for chemical toxicity in the environment, has been detected dangerous levels of heavy metals, pesticides and chemicals sourcing from water bodies and food sources for both humans and animals (Lightfoot and Yeager, 2008). There are vast records of accidental poisoning and mortality of non-target avian species caused by OP (Bartkowiak and Wilson, 1995). Many of the frequently used azole fungicides might cause endocrine disruption *in vivo*, although the profile of action *in vivo* varies (Taxvig *et al*., 2007). The residue of tebuconazole along with other plant protection products are also detected in grey partridge eggs, a gamebird in French cereal ecosystem (Bro *et al*., 2016). Eggs exposed to pesticide in laboratory setting is determined as a relevant exposure scenario in risk assessment procedure because of the potential to affect the reproductive success of wild bird in natural ecosystems (Ortiz-Santaliestra *et al*., 2020).

The aim of this study was to reveal the individual and possible common embryotoxic effects of organophosphate insecticide (Pyrinex 48 EC) and tebuconazole fungicide (Mystic 250 EW) evidently found in the environment which may jeopardize the ecosystems and living organisms.

MATERIALS AND METHODS

Test Materials

- Control: Distilled water
- 1% emulsion of Pyrinex 48 EC in distilled water, based on plant protection practice in the field. This pesticide is an organophosphate insecticide containing 44.4 ± 2m/m% chlorpyrifos as an active ingredient and assigned to marketing category I. It is used for the control of a wide range of insect pests on arable land as well as in orchards.

- 0.4% emulsion of Mystic 250 EW in distilled water, based on plant protection practice in the field. This pesticide is a triazole fungicide containing 26.0 ± 1.6 m/m% tebuconazole as an active ingredient and assigned to marketing category I. It is used to combat fungal diseases on grapes, cherries, almonds, cereals, and rapeseed or canola.

Experimental animals

The experiment was conducted on the eggs of a mixed-use hen breed called Farm (Gallus gallus f. domesticus) obtained from incubation plant of a local poultry farm, Goldavis Ltd. (Sármellék, Hungary). A total of 160 fertile hen eggs were used in the experiment.

Treatments

Prior to the treatments, the eggs were randomly divided into four different groups which were made as homologous as possible with respect to egg size and weight. Forty eggs were used in each group for each treatment. In the individual treatments, emulsions made from test chemicals in 0.1–0.1 ml end-volume were used while in the joint treatments, 0.2 ml of the chemical agents were injected into the air chambers of eggs in each combination.

Methods

On the first day prior to incubation, the blunt end of the eggs was disinfected, a hole was drilled in the calcic eggshell to inject test material into the air chamber using micro-pipette (Fejes et al., 2002; Budai et al., 2003; Szabó et al., 2019). After injection, the hole was sealed with paraffin. The same manner was applied for control group but the test material was replaced with distilled water (Várnagy et al., 2003).

All eggs were placed into the incubator (Ragus type table incubator Vienna, Austria). They were incubated for 19 days with the incubation temperature of 37-38°C, relative humidity 65-75% and the eggs were turned three times a day until the day of final processing.

Processing

The assessment was made on day 19th of incubation (two days before hatching). The eggs were open and examined based on the following criteria (parameters):

- the body, liver, and heart weight of the embryo
- number and type of development anomalies, if any, and
- number and day of embryonic death

The identification or estimation day of embryonic death were determine based on detailed description on chick embryo development stages by Hamburger and Hamilton (Darnell and Schoenwolf, 2000).

Statistical evaluation

The body, liver, and heart weight data were evaluated statistically by the One-Way ANOVA after controlling of their distribution using Comparison-Quantile Plot. Comparative evaluation of the results of the different groups was carried out by Tukey and Dunnett tests. The statistical analysis of the results of embryo-mortality and development abnormalities were performed by Fisher's exact test.

RESULTS AND DISCUSSION

Embryonic mortalities

Lowest number of embryonic mortality (4) was observed in the control group after 19 days of incubation (Table 1). Significant number of embryonic death was recorded for embryos with single and combined administration of Pyrinex 48 EC and Mystic 250 EW compared to the control group ($p<0.001$). Embryo treated with 0.4% of tebuconazole fungicide (Mystic 250 EW) recorded the lowest number of dead embryos (21) among the groups of embryo treated with the pesticides (Table 1).

Embryos in the group with single administration of chlorpyrifos insecticide (Pyrinex 48 EC) at concentration of 1% shown second highest rate of embryonic death (25) (Table 1). Combined administration of 1% chlorpyrifos containing insecticide (Pyrinex 48 EC) and 0.4% tebuconazole pesticide (Mystic 250 EW) gave the highest rate of embryonic mortalities (31). The increase of embryonic mortality rate after combined administration was also significant ($p<0.05$) compared to the embryo treated with Mystic 250 EW (Table 1).

Developmental anomalies

There was no malformation in the control group (Table 1). Out of 15 alive embryos from the group treated with 1% chlorpyrifos (Pyrinex 48 EC), three embryos (20%) were observed with developmental anomalies. This rate was significant ($p<0.05$) compared to control group (Table 1). The embryos were shown with malformations on the legs and beak from the evaluation.

Leg deformation was observed on embryo (5.26%) in the group with single treatment of 0.4% tebuconazole (Mystic 250 EW). The rate was, however, not significant ($p<0.05$) compared to control group (Table 1).

The group with combined administration of 1% chlorpyrifos (Pyrinex 48 EC) and 0.4% tebuconazole (Mystic 250 EW) were observed to have developmental anomalies (open abdomen and beak malformation) on two embryos (22.22%), which was also the highest number of embryos to be malformed per number of alive embryo and had significant rate of developmental anomalies ($p<0.05$) compared to normal embryo in control group (Table 1).

Pathological processing

Body Weight

All groups with pesticides treatments gave significant reduction in body weight compared to control group. The body weight of embryo from single administration of 1% chlorpyrifos (Pyrinex 45 EC) was significantly different ($p<0.01$) from the control (Table 2). Embryo treated with tebuconazole fungicide (Mystic 250 EW) at concentration of 0.4% weighed significantly less compared to embryo in control group ($p<0.05$) (Table 2).

Combined administration of 1% chlorpyrifos (Pyrinex 45 EC) and 0.4% tebuconazole fungicide (Mystic 250 EW) resulted with the lowest body weight and was significantly differ ($p<0.01$) compared to embryo in the control group (Table 2). The mean weight was also lowest compared to embryo with single administration of chlorpyrifos or tebuconazole.

Liver Weight

Embryos from the control group had the highest liver weight (Table 2). Embryos that were administered with 1% chlorpyrifos (Pyrinex 45 EC), 0.4% tebuconazole (Mystic 250 EW) and the combination of both on the first day prior to incubation had caused significant reduced in liver weight.

Single administration of chlorpyrifos containing insecticide (Pyrinex 45 EC) at concentration of 1% resulted in significant reduced of mean liver weight compared to the control ($p<0.01$) (Table 2).

Embryos in the group that had been administered with tebuconazole fungicide (Mystic 250 EW) at concentration of 0.4% shown the lowest liver weight among all groups with significant decrease compared to control group ($p<0.01$) (Table 2).

Combined administration of both pesticides Pyrinex 45 EC and Mystic 250 EW at concentration of 1% chlorpyrifos and 0.4% tebuconazole respectively on the first day prior to incubation resulted in significant decrease of liver weight compared to control group ($p<0.01$) (Table 2).

Heart Weight

The heart weight was rather sporadic among all groups. Lowest mean heart weight was recorded for the embryos from the group that had been administered with tebuconazole fungicide (Mystic 250 EW) at a concentration of 0.4% but, it was not significant compared to control group (Table 2). Combined administration of chlorpyrifos containing insecticide (Pyrinex 40 EC) at concentration of 1% and tebuconazole fungicide (Mystic 250 EW) at concentration of 0.4% resulted in insignificant higher heart weight compared to control group (Table 2).

Results obtained from this teratogenicity study shown that single administration of chlorpyrifos containing insecticide (Pyrinex 48 EC) at concentration of 1% had induced the toxicity to the embryo resulting in developmental deformities and increase the mortality. It also caused significant reduction in body and liver weight compared to the embryo in control group.

Findings from this study on the toxic effect of Pyrinex 48 EC can further support the findings from the experiment conducted by using the same chlorpyrifos insecticide on chick embryo which had caused significant reduction in body weight and increase rate of embryonic mortality (Budai *et al*., 2015; Lehel *et al*., 2014; Szabó *et al*., 2016).

In another experiment, the combination of insecticide (chlorpyrifos and cypermethrin) induced explicit alterations in the embryonic growth and development and resulted in malformations particularly to the axial and appendicular skeletal structures of chick embryos after it was administered as a single dose (0.005, 0.001, 0.01, 0.05, 0.1, or 0.5 μg) on day '0' of incubation (Uggini *et al*., 2012). The metabolites of chlorpyrifos is proved to be embryotoxic after it was evaluated using the chick embryo and were administered to 3-day embryos by the air cell method (Muscarella *et al*., 1984).

Fungicide (Mystic 250 EW) containing active ingredient tebuconazole was applied at 0.4% concentration as single administration on the chick embryo was proved to be embryotoxic. It increased the rate of mortality compared to normal embryo and

had cause embryo with developmental anomalies. The fungicide also caused significant reduction of body weight and liver weight of the embryo.

Similar experiment by using immersion method of the eggs treated with 0.1% tebuconazole pesticide (Mystic 250 EW) together with single and combined administration of 0.01% cadmium sulphate and 0.01% lead acetate shown significant reduction in body weight. Embryonic mortalities were higher in all treated groups except for the group treated with Mystic 250 EW with sporadic rate of developmental anomalies (Szemerédy *et al*., 2018).

An experiment was also conducted to observe the effect of tebuconazole on birds. Captive partridges were fed with 0%, 20% or 100% of tebuconazole application rate during 25 days in late winter. Birds fed with high dose of tebuconazole has reduction of hatching rate by 23% and the brood size is 1.5 times smaller compared with controls (Lopez-Antia *et al*., 2021).

Table 1. Mortality and developmental anomalies of embryos on day 19 of incubation

Treatment	No of embryos showing abnormality/No of live embryos	Death No/Total eggs
Control	0/36	4/40
Pyrinex 48 EC	3/15[c]	25/40[a]
Mystic 250 EW	1/19	21/40[a]
Pyrinex 48 EC + Mystic 250 EW	2/9[c]	31/40[a; b]

a=significant decrease compared to the control group ($p<0.001$)

b=significant decrease compared to the group treated with Mystic 250 EW alone ($p<0.05$)

c=significant decrease compared to the control group ($p<0.05$)

Table 2. Embryonic body, liver and heart weights (g) on day 19 of incubation

	Control	Pyrinex 48 EC	Mystic 250 EW	Pyrinex 48 EC + Mystic 250 EW
Number of embryos (n)	36	15	19	9
Average body weight (g)	25.42	22.24[b]	24.54[a]	20.97[b]
Average liver weight (g)	0.451	0.390[b]	0.373[b]	0.400[a]
Average heart weight (g)	0.141	0.144	0.126	0.157

a=significant decrease compared to the control group ($p<0.05$)

b=significant decrease compared to the control group ($p<0.01$)

CONCLUSION

Our teratogenicity study revealed that the combined administration of both chlorpyrifos (Pyrinex 48 EC) and tebuconazole (Mystic 250 EW) pesticides on the chick embryo had shown to be embryotoxic to the chick. It increased the rate of

embryonic mortality and the incidence of embryonic developmental anomalies. The body and liver weight were also significantly reduced. Pyrinex 48 EC and Mystic 250 EW had a slight addition effect on the rate of embryonic mortalities, however, the toxic interaction of both pesticides on developmental anomalies, liver and body weight was unjustified. Further investigation is recommended to study the toxic effects on the developing embryo and the hatching rate. The chick may allow to mature in order to evaluate its functional normality after the treatments during the embryo stage. Beside the injection treatment method applied during the studies it would be advisable to perform complete examinations with immersion treatment that can represent better the exposure realized in the environment and compare the results achieved from different treatment methods.

ACKNOWLEDGEMENT

The publication was supported by the EFOP-3.6.3-VEKOP-16-2017-00008 project. The project was co-financed by the European Union and the European Social Fund.

REFERENCES

Bartkowiak D. J., Wilson B. W. (1995). Avian plasma carboxylesterase activity as a potential biomarker of organophosphate pesticide exposure, Environmental Toxicology and Chemistry, 14(12), 2149-2153. doi: 10.1002/etc.5620141219

Bro E., Devillers J., Millot F., Decors A. (2016). Residues of plant protection products in grey partridge eggs in French cereal ecosystems, Environmental Science and Pollution Research, 23(10), 9559-9573.

Budai P., Fejes S., Várnagy L., Somlyay I. M., Kulcsár-Szabó Z. (2003). Teratogenicity test of dimethoate containing insecticide formulation and Cd-sulphate in chicken embryos after administration as a single compound or in combination, Communications in Agricultural and Applied Biological Sciences, 68, 795-798.

Budai P., Grúz A., Várnagy L., Kormos E., Somlyay I., Lehel J., Szabó R. (2015). Toxicity of chlorpyriphos containing formulation and heavy elements (Cd, Pb) to chicken embryos, Commun. Agric. Appl. Biol. Sci, 80(3), 393-396.

Damalas C. A., Eleftherohorinos I. G. (2011). Pesticide exposure, safety issues, and risk assessment indicators, International journal of environmental research and public health, 8(5), 1402-1419.

Darnell D. K., Schoenwolf G. C. (2000). The chick embryo as a model system for analyzing mechanisms of development, Developmental Biology Protocols (pp. 25-29): Springer.

Fejes S., Budai P., Várnagy L., Molnár T., Szabó R., Fáncsi T. (2002). Toxicity of a mancozeb containing fungicide formulation and CU-sulphate to chicken embryos after administration as single compounds or in combination, Mededelingen: Rijksuniversiteit te Gent Fakulteit van de Landbouwklundige en Toegepaste Biologische Wetenschappen, 67(2), 105-109.

Fenner K., Canonica S., Wackett L. P., Elsner M. (2013). Evaluating pesticide degradation in the environment: blind spots and emerging opportunities, Science, 341(6147), 752-758.

Lehel J., Szabó R., Gajcsi D., Jakab C., Grúz A., Kormos E., Budai P. (2014). Investigation of toxic interaction of copper sulphate and chlorpyriphos on chicken embryo, Magyar Állatorvosok Lapja, 136(8), 494-500.

Lightfoot T. L., Yeager J. M. (2008). Pet Bird Toxicity and Related Environmental Concerns, Veterinary Clinics of North America: Exotic Animal Practice, 11(2), 229-259. doi: https://doi.org/10.1016/j.cvex.2008.01.006

Lopez-Antia A., Ortiz-Santaliestra M. E., Mougeot F., Camarero P. R., Mateo R. (2021). Birds feeding on tebuconazole treated seeds have reduced breeding output, Environ Pollut, 271, 116292. doi: 10.1016/j.envpol.2020.116292

Muscarella D. E., Keown J. F., Bloom S. E. (1984). Evaluation of the genotoxic and embryotoxic potential of chlorpyrifos and its metabolites in vivo and *in vitro*, Environ Mutagen, 6(1), 13-23. doi: 10.1002/em.2860060103

Ortiz-Santaliestra M. E., Alcaide V., Camarero P. R., Mateo R., Mougeot F. (2020). Egg Overspray with Herbicides and Fungicides Reduces Survival of Red-Legged Partridge Chicks, Environmental Science & Technology, 54(19), 12402-12411. doi: 10.1021/acs.est.0c04203

Szabó R., Kántor A., Kormos É., Grúz A., Somody G., Szemerédy G., Lehel J., Budai P. (2016). Study of the toxic effects of an insecticide and copper sulphate on chicken and pheasant embryos, Növényvédelem, 52(10), 489-494.

Szabó R., Budai P., Kormos É., Buda I., Grúz A., Szemerédy G., Lehel J., Fáth R. (2019). Model study to investigate the toxic interaction between glyphosate and copper sulphate on chicken embryos, AGROFOR International Journal, 4(3), 76-82.

Szemerédy G., Kormos E., Somody G., Buda I., Szabó R., Lehel J., Budai P. (2018). Teratogenicity test of individual and combined toxic effects of tebuconazole fungicide and lead-acetate on chicken embryos, Acta Agraria Kaposváriensis, 22(2), 25-32.

Taxvig C., Vinggaard A. M., Hass U., Axelstad M., Metzdorff S., Nellemann C. (2007). Endocrine-disrupting properties in vivo of widely used azole fungicides, International Journal of Andrology, 31, 170–177.

Uggini G. K., Patel P. V., Balakrishnan S. (2012). Embryotoxic and teratogenic effects of pesticides in chick embryos: a comparative study using two commercial formulations, Environ Toxicol, 27(3), 166-174. doi: 10.1002/tox.20627

Várnagy L., Budai P., Fejes S., Susan, M., Fáncsi T., Keserű M., Szabó R. (2003). Toxicity and degradation of metolachlor (Dual Gold 960 EC) in chicken embryos, Communications in Agricultural and Applied Biological Sciences, 68, 807-811.

Review paper
10.7251/AGRENG2201048K
UDC 631-027.541(620)

URBAN AND PERI-URBAN AGRICULTURE IN EGYPT

Islam Mohamed KAMEL, Hamid EL BILALI*

International Centre for Advanced Mediterranean Agronomic Studies – Mediterranean Agronomic Institute of Bari (CIHEAM-Bari), Valenzano (Bari), Italy
*Corresponding author: elbilali@iamb.it

ABSTRACT

Around the world, urban and peri-urban agriculture (UPA) has evolved into a new socio-political manifestation that can endorse social solidarity, environmental education, and leisure activities. It is also a way to support the urban poor in middle and low-income counties and ensure food sovereignty and self-sufficiency. Furthermore, global shocks, pandemics, and crises (e.g., food crisis 2008, COVID-19, climate change) have illustrated the vulnerability of the global food supply chain, as well as the need for resilience in cities' long-term food security, shedding more light on UPA's multiple functions in densely populated areas, offering an alternative land use and greater genuine value. Considering the present worldwide governmental push to promote urban agriculture and contemplate its consequences on urban dwellers and their environs, it is vital to investigate Egypt as one of the world's most populous countries, with densely packed cities and significant poverty rates. Using a systematic literature review, this article studies the impact of UPA in Egypt. Data were gathered using the Scopus database and supplemented with information from grey literature. The findings demonstrate that UPA can perform a wide range of socio-economic and environmental roles, including aesthetic urban design, waste management, circular economy, energy use efficiency, microclimate control, preservation of cultural heritage, biodiversity conservation, and health and well-being promotion. However, there is possible apprehension concerning soil erosion, extensive use of fertilizers and pesticides, contamination from wastewater resulting from the poor implementation. Finally, while UPA can make a beneficial difference in Egypt, socio-political, cultural, and technical hurdles may stymie its growth.

Keywords: *urban agriculture, sustainability, food security, sustainable cities, Egypt.*

INTRODUCTION

Ancient Egyptian and Roman civilizations managed to build self-sufficient and sustainable cities that have lasted for thousands of years (Groening, 2016). Unfortunately, nowadays the majority of cities especially in developing countries confront several challenges in terms of urban planning and development. These concerns have a major influence on their socio-economic and environmental performance. Egypt's cities are no exception since they have been experiencing

rapid urbanization and industrialization in recent years (Hegazy, Seddik and Ibrahim, 2017). Egypt's current population is 104,412,590, of which 43.0% live in urban areas. Cairo is the most populated city in Egypt with about 21,322,750 inhabitants. Nearly all of the country's population resides in Cairo, Alexandria, or other cities near the Nile River and the Suez Canal. Cairo and other large cities in Egypt are among the world's most densely inhabited areas (WPR, 2021). Moreover, air pollution resulting from industrial activity and the huge number of cars (estimated to exceed 2.3 million in Cairo alone), inadequate waste management, and high concentrations of hazardous and radioactive substances (e.g., lead and promethium) have grown (Zakey *et al.*, 2008).

Egypt's poverty rate is estimated to cross 29.3% in 2021 (Statista, 2021). Besides, unemployment has reached 37% among youth in 2016; this figure is expected to be even higher considering the impact of the COVID-19 pandemic. Egyptian youth are still misled and confused; demands for equality, social justice, and better livelihood are the most pressing issues confronting the Egyptian people (Mansour, 2016; Nasr-Allah et al., 2019). Food insecurity, malnutrition, gender-based inequality, and climatic shocks are among Egypt's long-standing development difficulties (WFP, 2021). Furthermore, within the upcoming 15 years, the present phenomenon of urban growth will confront tremendous demand on lands, putting urban and rural communities under unprecedented strain. The present policies and approaches cannot deal with the future challenges regarding the direction of urban development. Many researchers, on the other hand, argue that distinctive innovations might have a positive impact on urban planning that should be examined and incorporated within the Egyptian setting (Mahmoud and Divigalpitiya, 2015).

Many studies have suggested that adopting agricultural practices within urban and peri-urban areas can contribute to addressing urban food insecurity, poverty, malnutrition, and health problems, and mitigate different environmental and social challenges (Zezza and Tasciotti, 2010). UPA is a process involving horticulture, animal husbandry, aquaculture, and other activities for generating fresh food or other agricultural goods in urban districts and their surroundings (peri-urban). It is defined as "*an industry that produces, processes and markets food and fuel, largely in response to the daily demand of consumers within a town, city or metropolis, on land and water dispersed throughout the urban and peri-urban area, applying intensive production methods, using and reusing natural resources and urban wastes, to yield a diversity of crops and livestock"* (UNDP, 1996). Moreover, it encompasses all persons, groups, activities, sites, and economies that focus on primary production in a spatial environment in addition to its positive externalities and co-benefits on the environment and society (Skar *et al.*, 2020). Notwithstanding the importance of UPA, few studies have investigated its importance in the Egyptian context, yet it did not touch all its dimensions or provide a comprehensive view of UPA and its impacts. Therefore, the objective of this paper is to assess the current situation of UPA in Egypt and investigate its impact on mitigating the current sustainability challenges in Egyptian cities.

MATERIAL AND METHODS

Data were collected through a systematic literature review on the Scopus database on the 15th of June, using different search keywords to grasp every piece of information about UPA in Egypt. Different sets of keywords were used such as Egypt AND "urban agriculture"; Egypt AND "urban farming"; Egypt AND "urban gardening"; Egypt AND "urban horticulture"; Egypt AND "vertical farming"; Egypt AND "urban animal husbandry"; Egypt AND "urban aquaponics"; Egypt AND "hydroponic". After removing duplicates, the search yielded 177 publications, which were filtered by title and abstract according to their relevance to the study as illustrated in figure 1. Finally, 23 publications including original research papers and book chapters were selected. Furthermore, data from grey literature (e.g., institutional statistics and reports) were included.

The following two sections will discuss the main results, conclusions, and recommendations for the different forms of UPA including aquaponics, roof gardening, livestock, and dairy production besides, their pros and cons within the Egyptian cities.

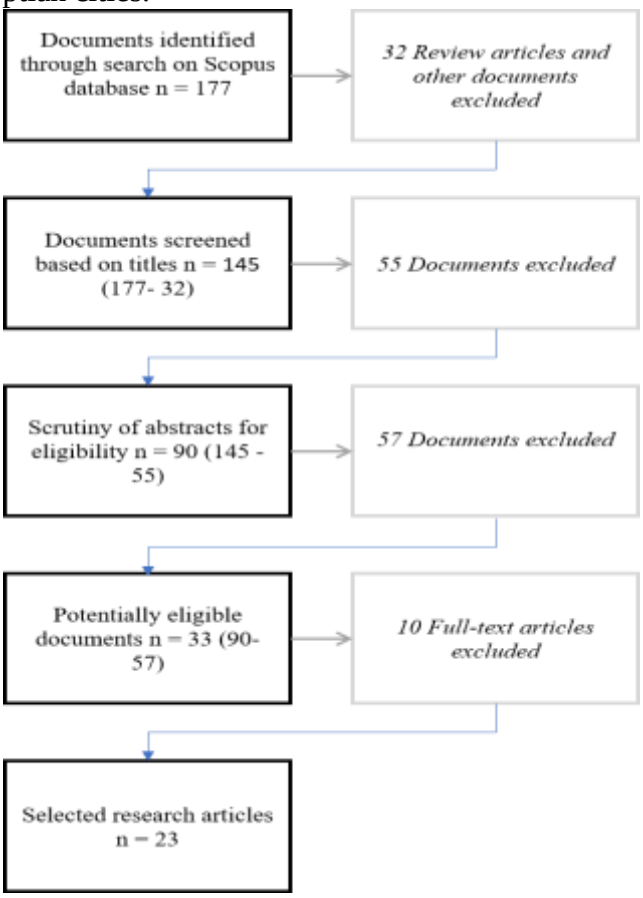


Figure 1. Process and steps of the systematic review.
Source: Adapted from Moher *et al.* (2009).

RESULTS AND DISCUSSION

Major worldwide changes are taking place rapidly, altering people's wellbeing, productivity, and livelihood. Challenges that represent a threat for Egypt include water shortages and food insecurity, combined with a substantial increase in population especially in the major cities as a result of migration from rural to urban areas. That has resulted in the extensive increase of informal settlements in urban areas. Cities such as Cairo, Alexandria, Kafr Elsheikh, Sohag, Asyut, and El-Beheira are experiencing rapid population growth and are showing a keen interest in lateral population expansion, which is causing loss of agricultural land and increasing the threat of food insecurity (Elbeih et al., 2011; Mahmoud and Divigalpitiya, 2015; Nassar and Elsayed, 2018; Afifi and Darwish, 2018; Mohamed and Yacout, 2019; Salem et al., 2019). Moreover, this expansion is jeopardizing the infrastructure and deteriorating the cities' ability to absorb the rising population (Mohamed and Yacout, 2019). It also impacts the agri-food production and consumption market and poses major complications to the country's agricultural sector (El-Essawy, Nasr, and Sewilam, 2019).

The revolutionary implementation of Egypt's new cities is based on achieving three key aspects: housing, employment, and providing services to meet people's needs. However, this is not enough, as Egypt faces numerous sustainability problems; growing air, water, and soil pollution, nonrenewable energy usage, and water shortages (Sheweka, 2012). Indeed, these disasters are a consequence of the last three decades' misleading and corrupted Egyptian national policies. In this sense, the most often discussed subject in developing cities throughout the world is sustainability (Hegazy, Seddik, and Ibrahim, 2017). Several schemes have shown that UPA can change our living conditions into one that is both environmentally sustainable and healthful. Moreover, it could contribute to energy use efficiency, natural resource conservation, ecological biodiversity, and cost savings in urban and peri-urban management (Ibrahim and Elariane, 2018). Urban agriculture takes various forms, and it is frequently a component of city planning authorities' responsibilities. It includes gardening in schoolyards, public roadside and community gardens, urban farms, rooftops and balcony gardens, hydroponic, aquaculture, and vertical gardening, keeping small livestock (e.g., hens, rabbits), beekeeping, greenhouses, permaculture design in parks, edible landscaping, public orchards or food forests, and agriculturism (Martin and Wagner, 2018).

Systems like aquaponics are becoming increasingly essential as vertical agriculture is getting more fashionable and feasible in urban areas. It's a low-input, low-waste, and sustainable food production system that uses circular economy concepts and a biomimetic natural system to decrease waste. An aquaponic system is a blend of two primarily farming systems: circular aquaculture, which involves the farming of fish in a tank, and hydroponic agriculture, which involves the growing of vegetables in a soil-less medium. Each of these techniques is popular throughout the world for its production, quality, and food safety. It is an efficient mechanism that perfectly integrates intensive agriculture with sustainable growth (Wirza and Nazir, 2021). Aquaponics is a long-term resolution to several challenges since it

offers new business and start-up opportunities while also displaying promising results for urban food supply especially for developing countries like Egypt (El-Essawy, Nasr and Sewilam, 2019). The awareness among youth about urban farming is increasing since many entrepreneurial activities in Egypt are taking place by initiatives such as "Grow Your Own" in Cairo governorate, "Your Roof Is Your Paradise" in Beheira governorate, "Shagara Roofs" in El Abour City, and the "Egyptian Switchers Community" to encourage urban gardening (Groening, 2016).
Giro et al., (2016) investigated the impact of a simplified hydroponic system to produce vegetables for the locals at the city of the Dead called Al Quarafa, one of the poorest slums in Cairo, whose people are suffering from malnutrition and extreme living conditions. The soil in that area is polluted with heavy metals as well as airborne impurities that can contaminate the produce. The research team advised the locals to use palm boxes, which are available materials in the area for cultivation, as a good compromise between efficiency and production cost. The study showed that aquaponics can provide an output free of contaminants since it does not use the polluted urban soil that is prevalent in slum areas. It also can provide vegetables, scarce food items due to their perishability and high cost. Residents of Al Quarafa consume a Mediterranean diet rich in grains and legumes, thus including vegetables in their diet will help them overcome malnutrition. According to the findings, hydroponics can help cities better their condition and satisfy the needs of individuals living in disadvantaged areas. While aquaponics offers more economic and ecosystem services than drawbacks, there are technical and behavioral restrictions that must be taken into account when adopting it. The most significant issue is the high requirement for surveillance, control, and technical expertise. Aquaponics' initial startup expenses are excessive when compared to traditional agriculture and land reclamation, making it difficult for entrepreneurs and start-ups to compete with large investors. Furthermore, aquaponics is hampered by a scarcity of qualified specialists and laborers on the market (El-Essawy, Nasr, and Sewilam, 2019). In addition, the reuse of polluted, untreated irrigation water from urban streams poses a health concern. UPA's heavy use of fertilizers, pesticides, and fungicides may result in agrochemical residues in crops or groundwater. The danger is most prevalent in places where commercial urban farming is practiced (Chatterjee, Debnath, and Pal, 2016).
Another example of UPA is animal husbandry, which may take many different production forms whether for livestock fattening or dairy processing. Production systems are diversified, with a variety of players making a living at various places along the value chain. Urban livestock is typically maintained in backyards or allowed to scavenge due to land scarcity, while peri-urban farms may be extensive and very commercial. The most common type of agriculture in Egypt is polyculture-livestock family farming, which accounts for the majority of agricultural output, with a considerable amount produced in urban or peri-urban regions. Simultaneously, commercial agriculture is expanding, both for domestic consumption and exportation (Daburon *et al.*, 2018).

A survey was done in two low-income neighborhoods in Egypt; Zabaleen area, an existing informal settlement in Agouza city, and Masaken Osman in the 6th of October city; to study the feasibility of urban animal husbandry (Ibrahim and Elariane, 2018). Poultry and lambs are raised by 50% of Zabaleen inhabitants while pigs are raised by 31.3%. Moreover, about 76.2% of those at Masaken Osman, on the other hand, rear chicken. In Zabaleen and Masaken Osman, accordingly, 68.8% and 95.2 % raise animals for personal use. Moreover, for those who do not own any type of animal or poultry, there are a few options; about 34.6% believe they have negative effects, while 44.2% believe they have good ones, 59.6% support raising them versus 34.6% that do not. Despite the contradiction of opinions, urban animal husbandry can be a viable option since it frequently produces profitable goods. Furthermore, for the reason that some species sustain themselves by scavenging, they may be kept even without specific territory, such as in backyards. This type of production is generally done in parallel with other activities and involves tiny creatures that are inexpensive to buy, sell, multiply fast, and maybe by fed domestic waste (Moekti, 2020).

However, animals can cause issues such as odor, risk of infection linked with poor sanitary conditions, contamination of rivers, and waste from abattoirs, or neighbor disputes when they enter and destroy gardens, causing the unpleasant smell, dust, and noise, as well as devastation to ornamental plants. Furthermore, concerns about overgrazing of metropolitan gardens, space requirements, wayward animals and traffic issues, animal welfare, production of a small portion of total dietary needs, unregulated marketing, aesthetic preferences, and fears about property value are all issues that need to be addressed (Mantovani, 2004). Despite their benefits in supplying food and guaranteeing improved income levels for impoverished urban households, there are no official regulations involving urban agriculture and animal husbandry in Cairo during the colonial era or later in the independence period (Ibrahim and Elariane, 2018). This would have an impact on the future sustainability of various urban farming techniques that should be encouraged to combat urban expansion, which divides huge open spaces, lowering agricultural land by nearly 80% by 2030 (Robson *et al.*, 2012). Thus, highlighting the critical need of developing regulations and monitoring procedures to minimize any negative consequences of such activities while still maintaining their economic relevance is crucial (Robson *et al.*, 2012). Appropriate governance at the local level is required so that national environmental plans can have long-term effects and offer a coherent strategic vision for green technology diffusion and urban development. Moreover, before creating a project, it is critical to address the environmental factors and conduct an ecological life cycle assessment. It is also central to involve the public in environmental choices to focus on public health, poverty alleviation, and ecological sustainability (Hegazy, Seddik, and Ibrahim, 2017). Furthermore, it is critical to develop new programs or alter current ones, to set new priorities and dedicate resources to sustainable causes as well as raising environmental consciousness among Egyptians through education and the mass

media. Improved waste management and the use of biodegradable products that have a lower environmental impact are critical (Sheweka, 2012).

CONCLUSION

The growing world population is increasing the pressure on natural resources and food insecurity has become one of the major threats to the existence of the human race especially in developing counties. Egypt is one of the most populated nations in the world, with heavily populated metropoles and extreme poverty rates. This study aimed to investigate the impact of UPA as a way to mitigate socio-economic and environmental issues in Egypt. The study showed that there are some entrepreneurial models of UPA in Egypt including urban farms, rooftop and balcony gardens, hydroponic, aquaculture, dairy, and animal production. These models have demonstrated strong ability in supporting poor and marginalized communities, ensuring food security and self-sufficiency, health, and wellbeing. UPA can also provide attractive urban design, better waste management, green economy, energy usage efficiency, and biodiversity conservation. However, there may be concerns about soil degradation resulting from overgrazing and limited land area, the heavy use of mineral fertilizers, and pesticides. Nevertheless providing appropriate local governance and long-term policies as well as a coherent strategic vision for green technology dissemination and urban development is necessary to prevent any negative repercussions of such activities while retaining their economic relevance. Finally, while the UPA can make a positive effect in Egypt, sociopolitical, cultural, and technological barriers may impede its expansion.

REFERENCES

Afifi, A. A. and Darwish, K. M. (2018) 'Detection and impact of land encroachment in El-Beheira governorate, Egypt', *Modeling Earth Systems and Environment*, 4(2), pp. 517–526. doi: 10.1007/s40808-018-0462-9.

Chatterjee, A., Debnath, S. and Pal, H. (2016) 'Implication of Urban Agriculture and Vertical Farming for Future Sustainability', in Ruano, P. et al. (eds) *Urban Horticulture - Necessity of the Future urbanization*. IntechOpen: London. Available at: https://www.intechopen.com/books/advanced-biometric-technologies/liveness-detection-in-biometrics.

Daburon, A. *et al.* (2018) 'Urban and peri-urban agriculture, the dairy farms of Cairo, Egypt', in Bosc, P.-M. et al. (eds) *Diversity of Family Farming Around the World: Existence, Transformations and Possible Futures of Family Farms*. Springer: Netherlands, pp. 29–42. doi: 10.1007/978-94-024-1617-6_4.

El-Essawy, H., Nasr, P. and Sewilam, H. (2019) 'Aquaponics: a sustainable alternative to conventional agriculture in Egypt – a pilot scale investigation', *Environmental Science and Pollution Research*, 26(16), pp. 15872–15883. doi: 10.1007/s11356-019-04970-0.

Elbeih, S. F., Belal, A. A. B. and Zaghloul, E. S. A. (2011) 'Hazards mitigation and natural resources evaluation around Sohag - Safaga highway, Eastern Desert,

Egypt', *Egyptian Journal of Remote Sensing and Space Science*, 14(1), pp. 15–28. doi: 10.1016/j.ejrs.2011.01.001.

Giro, A., Ciappellano, S. and Ferrante, A. (2016) 'Vegetable production using a simplified hydroponics system inside City of Dead (Cairo)', *Advances in Horticultural Science*, 30(1), pp. 23–29. doi: 10.13128/ahs-18698.

Groening, G. (2016) 'Urban horticulture – Gardens as elements of an urbanizing world', *European Journal of Horticultural Science*, 81(6), pp. 285–296. doi: 10.17660/eJHS.2016/81.6.1.

Hegazy, I., Seddik, W. and Ibrahim, H. (2017) 'Towards green cities in developing countries: Egyptian new cities as a case study', *International Journal of Low-Carbon Technologies*, 12(4), pp. 358–368. doi: 10.1093/ijlct/ctx009.

Ibrahim, A. A. M. and Elariane, S. A. (2018) 'Feasibility tools for urban animal husbandry in cities: case of greater Cairo', *Urban Research and Practice*, 11(2), pp. 111–138. doi: 10.1080/17535069.2017.1298000.

Mahmoud, H. and Divigalpitiya, P. (2015) 'Part II Innovative Technologies: Modeling Future Land Use and Land-Cover Change in the Asyut Region Using Markov Chains and Cellular Automata', in Bisello, A. et al. (eds) *Smart and Sustainable Planning for Cities and Regions*. Springer: Switzerland, pp. 3–13. doi: 10.1007/978-3-319-44899-2_1.

Mansour, A. M. H. (2016) 'Sustainable youth community development in Egypt', *Alexandria Engineering Journal*, 55(3), pp. 2721–2728. doi: 10.1016/j.aej.2016.05.019.

Mantovani, A. (2004) *Urban Veterinary Hygiene in Developing Countries*. Available at: https://www.vin.com/apputil/content/defaultadv1.aspx?pId=11181&catId=30088&id=3852227 (Accessed: 15 June 2021).

Martin, W. and Wagner, L. (2018) 'How to grow a city: Cultivating an urban agriculture action plan through concept mapping', *Agriculture and Food Security*, 7(1), pp. 1–9. doi: 10.1186/s40066-018-0186-0.

Moekti, G. R. (2020) 'Industrial livestock production: A review on advantages and disadvantages', *IOP Conference Series: Earth and Environmental Science*, 492(1), pp. 1–22. doi: 10.1088/1755-1315/492/1/012094.

Mohamed, M. Z. and Yacout, D. M. M. (2019) 'Assessing the impact of urban encroachment on agricultural land in Kafr El-sheikh governorate using GIS and remotely sensed data', *Current Applied Science and Technology*, 19(1), pp. 57–65. doi: 10.14456/cast.2019.5.

Moher, D. *et al.* (2009) 'Preferred reporting items for systematic reviews and meta-analyses: The PRISMA statement', *PLoS Medicine*, 6(7), pp. 1–6. doi: 10.1371/journal.pmed.1000097.

Nasr-Allah, A. *et al.* (2019) 'Employment generation in the Egyptian aquaculture value chain: implications for meeting the Sustainable Development Goals (SDGs)', *Aquaculture*, 520, pp. 1–60. doi: 10.1016/j.aquaculture.2020.734940.

Nassar, D. M. and Elsayed, H. G. (2018) 'From Informal Settlements to sustainable communities', *Alexandria Engineering Journal*, 57(4), pp. 2367–2376. doi: 10.1016/j.aej.2017.09.004.

Robson, J. S. *et al.* (2012) 'Spatial disintegration and arable land security in Egypt: A study of small- and moderate-sized urban areas', *Habitat International*, 36(2), pp. 253–260. doi: 10.1016/j.habitatint.2011.10.001.

Salem, M., Tsurusaki, N. and Divigalpitiya, P. (2019) 'Analyzing the driving factors causing urban expansion in the peri-urban areas using logistic regression: A case study of the greater Cairo region', *Infrastructures*, 4(1), pp. 1–14. doi: 10.3390/infrastructures4010004.

Sheweka, S. M. (2012) 'New Egypt with a new hybrid skin: A new hybrid architecture vision for the Egyptians' development corridor', *Energy Procedia*, 18, pp. 449–457. doi: 10.1016/j.egypro.2012.05.056.

Skar, S. L. G. *et al.* (2020) 'Urban agriculture as a keystone contribution towards securing sustainable and healthy development for cities in the future', *Blue-Green Systems*, 2(1), pp. 1–27. doi: 10.2166/bgs.2019.931.

Statista (2021) *Egypt_ projected poverty rate 2018-2021*. Available at: https://www.statista.com/statistics/1237041/poverty-headcount-ratio-in-egypt/ (Accessed: 15 July 2021).

UNDP (1996) *Urban agriculture: Food, jobs and sustainable cities*. New York: UNDP.

WFP (2021) *Egypt | World Food Programme*. Available at: https://www.wfp.org/countries/egypt (Accessed: 15 July 2021).

Wirza, R. and Nazir, S. (2021) 'Urban aquaponics farming and cities- a systematic literature review', *Reviews on Environmental Health*, 36(1), pp. 47–61. doi: 10.1515/reveh-2020-0064.

WPR (2021) *Cairo Population 2021 (Demographics, Maps, Graphs)*. Available at: https://worldpopulationreview.com/world-cities/cairo-population (Accessed: 15 July 2021).

Zakey, A. S. *et al.* (2008) 'Seasonal and spatial variation of atmospheric particulate matter in a developing megacity, the Greater Cairo, Egypt', *Atmosfera*, 21(2), pp. 171–189.

Zezza, A. and Tasciotti, L. (2010) 'Urban agriculture, poverty, and food security: Empirical evidence from a sample of developing countries', *Food Policy*, 35(4), pp. 265–273. doi: 10.1016/j.foodpol.2010.04.007.

Original Scientific paper
10.7251/AGRENG2201057A
UDC 635.8:639.16(497.17)

RADIOACTIVITY LEVELS IN SOME MUSHROOM SPECIES COLLECTED FROM MACEDONIA AND CONSEQUENT DOSES

Angeleska ALEKSANDRA*, Radmila CRCEVA NIKOLOVSKA, Elizabeta DIMITRIESKA STOJKOVIK, Katerina BLAGOEVSKA, Biljana DIMZOSKA STOJANOVSKA, Risto UZUNOV, Vasilka POPOSKA TRENEVSKA

Faculty of Veterinary Medicine, Food Institute, University "Ss. Cyril and Methodius" – Skopje, North Macedonia
*Corresponding author:e-mail:mizasandra@yahoo.com

ABSTRACT

Mushrooms are bioindicators in the environment that accumulate radionuclides, therefore they can pose a radiological threat. In the Republic of Macedonia, mushrooms are widely consumed as a part of the diet, for this reason their radiological control is required. In this research, the activity concentrations in different types of mushrooms (Boletus edulis, Marasmius oreades, Morchela conica, Lactarius deliciosus) were determined. The mushrooms were collected from six different locations in the Republic of Macedonia, and information on the radiation dose for the general population were obtained with this measurement. The analyses were performed by means of HPGe gamma spectrometry, i.e. an instrument - gamma spectrometer (Canbera Packard) with a high purity germanium detector. The obtained spectra from the measurement were analyzed by using the GENIE 2000 program. On the basis of the performed tests, the mean values for the activity concentrations in the mushrooms were as follows 41.9±1.2 Bq kg − 1 for 226Ra, 40.3±1.8 Bq kg − 1 for 232Th, 83.75±6.2 Bq kg − 1 for 40K, and 2.34±0.24 Bq kg − 1 for 137Cs. The mean value of the radiation risk index H_{eks} is lower than the maximum allowed value which is <1 for H_{eks}. The value of the radium equivalent activity Ra_{eq} is below the maximum recommended limit, i.e. 370 Bq kg-1. Significant variations were not observed in regard to the activity concentrations of 137Cs and 40K in the same mushroom species from different sampling points. High activity concentrations of 137Cs activity were not detected in any of the mushrooms. The researches in this study show that all mushroom samples pose no biological threat, i.e. it was found that the analyzed radionuclides do not pose a health risk and the levels are below the international standards.

Key words: *mushrooms, gamma-ray spectrometry, natural radioactivity, food safety.*

INTRODUCTION

It is essential to know the concentration of radioactivity in our environment when estimating the dose that accumulates in the population and also in forming the basis for assessing the degree of radioactive contamination or environmental pollution in the future. Radioactive contamination of plant organisms is formed by dynamic continuous common interaction of the atmosphere, the pedosphere and the hydrosphere. From the above-mentioned spheres of life, the influence of the pedosphere is dominant, given that the main part of the mineral matter, which makes up the plant body, originates from the soil. Considering that radionuclides can cause undesired effects on the human organism, it is necessary to control their content in the environment, and on the basis of the obtained results, to calculate the dose that the human receives. Mushrooms as one of the most important components of the forest ecosystem, are considered excellent bioindicators of environmental pollution, because they have the ability to accumulate radionuclides from the soil and the atmosphere (Kalač, 2010). In the Republic of Macedonia, mushrooms are widely consumed in the diet because they contain a large amount of protein and minerals, and for that reason their radiological control is required (Agrahar-Murugkar and Subbulakshmi, 2005). Many researches and studies have been conducted, in order to assess the possible threat to human health from consuming wild mushrooms that contain higher levels of radionuclides (Gast et al., 1988). Most studies have shown that the content of radionuclides in wild mushrooms depends on several parameters, such as the type of mushroom, the soil type, the depth of the mycelium, the climate and the bioavailability of the radionuclide. It was also found that mushrooms collected in coniferous forests are characterized by higher content of radionuclides compared to samples of mushrooms collected in deciduous forests (Čipáková, 2004). In Macedonia, large quantities of wild mushrooms are collected which are consumed in the diet and at the same time large quantities are exported from the country to European countries such as France, Switzerland, Germany, Belgium, the Netherlands, Spain, Sweden. In order to export these products abroad from the country it is necessary to submit a certificate of analysis for the presence of radionuclides in mushrooms. The aim of the research itself was to determine the level of concentration of ^{226}R, ^{232}Th, ^{40}K and ^{137}Cs in different species of wild mushrooms collected from several different locations, in order to determine the behavior of mushrooms as indicators of radioactive contamination in the environment and to calculate radiation doses for the general population on the basis of the obtained results.

MATERIALS AND METHODS

Sampling

The samples of wild mushrooms were collected in 2020 from different locations in the Republic of Macedonia (Table 1). All samples were homogenized, and after removing the inedible parts, they were placed in Marinelli beakers that had the same geometry as the one for the reference materials by which the measuring

equipment was calibrated. The capacity of the Marinelli beaker was 0.5 L for gamma spectrometry analysis.

Table 1. Mushroom species examined in the study

Mushroom Species	Family	Local Name	Growing	Edilebility
Boletus edulis	Boletaceae	Vrganj	Wild	Edible
Lactarius deliciosus	Russulaceae	Rujnica	Wild	Edible
Morchella conica	Morchellaceae	Smrcka	Wild	Edible
Marasmius oreades	Marasmiaceae	Livadarka	Wild	Edible

Instrument

The samples were measured with an instrument - gamma spectrometer (Canbera Packard) with a high purity germanium detector. The measurement was carried out in beakers that were hermetically sealed so that ^{222}Rn produced by the decomposition of ^{226}Ra would not result in gas leakage. After ensuring a time balance between the successors of the ^{238}U and the ^{232}Th series, these sealed samples were prepared for analysis. GENIE 2000 software was used for data acquisition and analysis. The specific activity of ^{226}Ra is calculated for the energy line of 186.1 (keV) and ^{232}Th through its decay descendant ^{228}Ac (second in the decay chain), that is, through its three gamma decay energy lines which occur at 338.4; 911.07 and 968.9 (keV).

The activities of ^{40}K were determined from its γ-line of 1460 keV, while the activities of ^{137}Cs were determined by means of an estimation of the γ-line at 661.66 (keV). The time interval for calculation (counting) was 108000 seconds.

Specific activity

The specific activity (A) is determined according the equation

$$A = \frac{\frac{N}{t} - \frac{N_0}{t_0}}{\varepsilon \cdot \gamma \cdot m} \quad (Bq \cdot kg^{-1})$$

Where, N is clean surface of peak accumulated from a specific radionuclide in analysis of a specific sample (number of readings), N_0 is clean surface of peak accumulated from the spot of a specific radionuclide without an analysis of sample (number of readings), t is live time of accumulation of the sample spectrum (s), t_0 is live time of accumulation of the phone spectrum (s), ε is detector efficiency for a given energy (for a specific peak), γ is intensity of gamma transition in radioactive decay for a respective radionuclide (%), and m is sample mass (kg).

Absorbed dose rate in air (D)

The natural decay of radionuclides is one of the major sources of human radiation exposure. The rate of absorbed dose in the air for radionuclides at a height of 1 m above the Earth's surface was calculated on the basis of data provided by (UNSCEAR, 2000)

$D\ (nGy / h) = 0{,}462\ A_{Ra} + 0{,}604\ A_{Th} + 0{,}0417\ A_k + 0{,}030\ A_{Cs}$

where; D is the dose rate at 1 meter above ground, ARa, ATh, AK and ACs are activity concentrations of ^{226}Ra, ^{232}Th, ^{40}K, ^{137}Cs in the samples, respectively. Conversion factors of ^{226}Ra, ^{232}Th, ^{40}K, ^{137}Cs are 0,462, 0,604, 0,0417 and 0,030 nGy/h per Bq/kg, respectively (Kurnaz et al., 2007).

Radium equivalent activity (Raeq)

The calculation of the radium equivalent activity (Raeq) is a quantity for comparing the specific activities of the samples with different contents of ^{226} Ra, ^{232}Th and ^{40}K. The uniformity with respect to radiation exposure was defined in terms of the radium equivalent activity (Raeq) in Bq/kg in order to compare the specific activity of the materials containing different amounts of ^{226}Ra, ^{232}Th and ^{40}K. It is assumed that 370 Bq/kg of ^{226}Ra, 259 Bq/kg of ^{232}Th and 4810 Bq/kg of ^{40}K produce the same gamma-ray dose rate. It is calculated by using the following ratio $Ra_{eq}(Bq/kg) = A_{Ra} + 1.43A_{Th} + 0.07A_k$ (Beretka and Methew, 1985) A_{Ra}, A_{Th}, A_k – specific activiites (Bq/kg) of ^{226}Ra, ^{232}Th and ^{40}K, respectively. The value of the radium equivalent activity of 370 Bq/kg corresponds to the maximum allowed dose for a population of 1 mSv.

External and internal hazard index

In order to assess the equivalent average of the annual effective dose imposed to the residents of each area, the external hazard index for the soil samples was calculated.

$H_{eks} = A_{Ra}/370 + A_{Th}/259 + A_k/4810 \leq 1$

A_{Ra}, A_{Th}, A_k-specific activites (Bq/kg) ,$^{226}Ra^{232}$ Th and ^{40}K, respectively (Kurnaz et al., 2007)

Annual gonadal dose equivalent (AGDE)

The activity bone marrow and the bone surface cells are considered the organs of interest by UNSCEAR (1988). Therefore, the AGDE due to the specific activities of 226Ra, 232Th and 40K was calculated using the following formula (MamontCiesla et al., 1982),

AGDE (μSv/year) = 3.09 ARa + 4.18 ATh + 0.314 AK (Kurnaz et al., 2007)

Annual effective dose equivalent (AEDE)

In order to estimate the annual effective doses, one has to take into consideration the conversion coefficient from the absorbed dose in the air to effective and the outdoor occupancy factor. In the UNSCEAR from 2000 reports, a value of 0.7 Sv/Gy was used for the conversion coefficient from absorbed dose in air to

effective dose received by adults, and 0.2 for the outdoor occupancy factor. The annual effective dose equivalent was calculated from following equation:
AEDE (μSv/year) = D(nGy/h) × 8760(h/year) × 0.2 ×0.7(Sv/Gy) ×10-3 (Kurnaz et al., 2007).

RESULTS AND DISCUSSION

The concentration of activity of **^{40}K** in all types of mushrooms is greater than the one of ^{232}Th and ^{26}Ra which is in accordance with the literature data. In the radioisotope ^{40}K, the highest level of activity is determined, with an average value of 83.7±6.2 Bq/kg. Since potassium is a basic nutrient, its range of variation is limited. Also no correlation was observed between ^{40}K and ^{137}Cs, although cesium is a chemical analogue of potassium, which suggests different uptake mechanisms for these two elements (Mietelski et al., 1994).

Table 2. ^{232}Th, ^{26}Ra , ^{40}K and ^{137}Cs activity concentrations of mushroom samples

	Specific activity* (Bq/kg – 1)			
	^{26}Ra	^{232}Th	^{40}K	^{137}Cs
S1	19.4±0.5	17.2±0.9	140.6 ± 6.8	<4.8
S2	33.5±3.9	28,6±0,3	46.9 ± 10.7	<1.0
S3	21.7±0.9	16.2±0.5	124.8± 10.4	0.5 ± 0.1
S4	44.3±0.5	31.5±0.5	52.1 ± 5.2	0.4 ± 0.1
S5	31.2±0.4	26.5±0.5	49.7 ± 3.0	<0.5
S6	28.7±0.3	19.8±0.2	68.3 ± 5.0	<1.0
M1	11.7±0.9	27.1±5.0	47.4±6.8	<1.0
M2	10.2±0.5	21.7±5.2	41.6±6.8	<1.0
M3	9.2±0.2	16.3±3.0	44.2±6.0	<0.5
M4	11.5±0.9	21.8±2.0	39.1±6.0	<0.5
M5	10.7±0.5	17.0±2.5	40.3±5.0	<0.5
M6	17.6±0.8	31.3±2.0	47.7±6.2	<0.5
D1	74.3±2.0	75.3±1.0	98.1 ± 5.0	3.6 ± 0.1
D2	71.5±3.0	66.2±0.5	124.7 ± 5.8	2.4 ± 0.3
D3	82.7±2.0	71.5±0.5	96.1 ± 3.8	5.1 ± 0.3
D4	66.3±0.5	59.2±0.1	115.3 ± 5.0	0.4 ± 0.1
D5	79.1±0.5	77.1±1.0	100.6 ± 6.8	2.8 ± 0.2
D6	72.0±0.5	71.7±0.9	142.3 ± 2.2	3.4 ± 0.1
E1	55.0±2.5	51.2±0.3	124.7 ± 11.5	0.8 ± 0.2
E2	49.0±0.5	46.0±0.5	102.1 ± 5.0	0.5 ± 0.1
E3	49.7±0.5	38.2±0.5	111.76 ± 6.5	2.68 ± 0.5
E4	57.4±2.0	44.3±0.2	89.5 ± 5.0	<1
E5	49.9±2.5	42.7±0.2	68.4 ± 2.5	<1
E6	51.2±2.0	49.0±0.1	92.9 ± 12.1	5.6 ± 0.8

*S1-S6 (Boletus edulis): M1-M2 (Marasmius oreades): D1-D6 (Morchela conica): E1-E6 (Lactarius deliciosus)

The mean specific activity of **^{226}Ra** is 41.9 ± 1.2 Bq kg-1, while the one of ^{232}Th is 40.3±1.8 Bq kg-1 (Table 3). It can be noticed that these two radionuclides occur with nominal concentrations.

Table 3. ^{232}Th, ^{26}Ra , ^{40}K and ^{137}Cs activity concentrations of mushroom samples mean values

Radionuclides	Bq kg – 1
^{26}Ra	41.9±1.2
^{232}Th	40.3±1.8
^{40}K	83.7±6.2
^{137}Cs	2.35±0.2

The highest content of ^{137}Cs was found in Morchela conica (5.1 ± 0.3) Bq/kg f.m., while Marasmius oreades had the lowest level of ^{137}Cs (Table 2). There are several factors that affect the content of ^{137}Cs in mushrooms. First, the amount deposited on the soil is closely related to the content range, especially the maximum content (UNSCEAR, 2000). The consequences of Chernobyl were inhomogeneous in all affected countries and, therefore, in a specific country there can be areas with different radioactive contamination (Mietelski et al., 1996). The content of ^{137}Cs also varied from one type of mushroom to another, depending on the type of the nutritional mechanism and the habitat of the mycelium (Yoshida and Muramatsu, 1994). The analyzes revealed very low levels of ^{137}Cs if compared to the specific activity limits determined with the international legislation of 600 Bq/kg for ^{137}Cs.

Table 4. D, Raeq, Hex, AGDE, AEDE, Risk of lifetime cancer values of mushroom

Parameters	Family	[1]WAV
D (nGy/h)	47.25	55
Raeq (Bq/kg)	90.07	370
Hex	0.28	/
AGDE (μSv/year)	207.42	2398
AEDE (μSv/year)	57.94	70

[1]WAV: The world average value

From the specific activities, the doses were calculated and compared with other researches. The absorbed dose rate was calculated and it amounted to 47.25nGy/hour (Table 4). This value is lower than the values of the eastern desert in Egypt (488 nGy/h) (Arafa, 2004) and Istanbul, Turkey (49 nGy/h)). (Karahan and Bayulken, 2000) Our score was lower than the international recommended value (55 nGy/h) (UNSCEAR, 1988).

The radium equivalent activity (Raeq) was calculated and amounts to 90.07 Bq/kg respectively. Our results were lower than those surveyed in the Firtina Valley, Turkey (166.3 Bq/kg) (Kurnaz et al., 2007) and the Eastern Desert of Egypt (493.8 Bq/kg) (Arafa, 2004). In addition, our score was lower than the international recommended value (370 Bq/kg), (UNSCEAR, 2000) for Raeq (UNSCEAR.

2000). The external hazard index (Hex) is calculated and it amounts to 0.28, the value of which is lower than other countries Egypt (Eastern Desert) (2.03) (Arafa, 2004) The average value of the Radiation Risk Index H_{eks} shows that there is no significant radiation risk to the population. The values of the external hazard index obtained in this study, regardless of the location, did not exceed the safety limits, noting the negligible radiation hazard arising from naturally occurring terrestrial radionuclides.

The annual gonadal dose equivalent (AGDE) is calculated with a value 207.42 μSv/year, which is lower than other studies (246.4, 214.9 μSv/year, (Yılmaz et al., 2016) and (2398 μSv/year) for the Eastern Desert in Egypt. (Arafa, 2004)

The annual effective dose rate (AEDE) is calculated and amounts to 57.94 mSv/year, respectively. The world average annual effective dose equivalent (AEDE) from outdoor terrestrial gamma-radiation is 70 mSv/year (UNSCEAR, 1988). Hence, our values are lower than the world average value and the Istanbul value (69.8 mSv/year) (Karahan and Bayulken, 2000).

CONCLUSION

It can concluded that even after many years since the Chernobyl nuclear accident, the levels of cesium in mushrooms in the region of the Republic of Macedonia are very low compared to the limit set by international law. The small differences observed between different types of mushrooms may be due to the different contamination at the selected sampling site, the mycelial depth, the climate and the radionuclide bioavailability. However, the data from this research provide useful information about the environmental risk of the studied area and can be further used for radiological imaging.

REFERENCES

Agrahar-Murugkar D, Subbulakshmi G. (2005). Nutritional value of edible wild mushrooms collected from the Khasi hills of Meghalaya. Food Chem., 89(4): 599-603.

Arafa W. (2004). Specific activity and hazards of granite samples collected from the Eastern Desert of Egypt. J. Environ. Radioact., 75: 315–327.

Yılmaz, A., Yıldız, S., Çelik, A., Çevik, U. (2016). Determination of Heavy Metal and Radioactivity in Agaricus campestris Mushroom Collected from Kahramanmaraş and Erzurum Proviences. Turkish Journal of Agriculture - Food Science and Technology, 4(3): 208-215.

Čipáková, A. (2004). Cs-137 content in mushrooms from localities in eastern Slovakia. Nucleonika, vol. 49, suppl. 1, p. 25-29.

Gast C.H., Jansen E., Bierling J., Haanstra L. (1988). Heavy metals in mushrooms and their relationship with soil characteristics. Chemosphere. 17: 789–799. doi:10.1016/0045-6535(88)90258- 5

J.Beretka, P.J.Methew (1985). Natural radioactivity of Australian building materials, industrial wastes and by-products, Health Physics 48, pp. 87-95.

Kalač P. (2010). Trace element contents in European species of wild growing edible mushrooms: A review for the period 2000 – 2009. Food Chemistry. 122(1): 2–15.

Karahan G, Bayulken A. (2000). Assessment of gamma dose rates around Istanbul, Turkey. Environ. Radioact., 47: 213–221.

Kurnaz A, Küçükömeroğlu B, Keser R, Okumusoglu NT, Korkmaz F, Karahan G, Çevik U. (2007). Determination of radioactivity levels and hazards of soil and sediment samples in Fırtına Valley (Rize, Turkey). Applied Radiation and Isotopes. 65: 1281–1289.

Mamont-Ciesla K, Gwiazdowski B, Biernacka M, Zak A. (1982). Radioactivity of building materials in Poland. Vohra G. Pillai KC. Sadavisan S (Eds.), Natural Radiation Environment. Halsted Press. 551

Mietelski, J. W., Jasinska, M., Kozak, K., & Ochab, E. (1996). The method of measurement used in the investigation of radioactive contamination of forests in Poland. *Applied Radiation and Isotopes*, *47*, 1089–1095.

Mietelski, J. W., Jasinska, M., Kubica, B., Kozak, K., & Macharski, P. (1994). Radioactive contamination of polish mushrooms. *Science of the Total Environment*, *157*, 217–226. doi:10.1016/0048-9697(94)90582-7

UNSCEAR (1988). United Nations Scientific Committee on the Effect of Atomic Radiation, Sources, effects and risk of ionizing radiation, United Nations, New York

UNSCEAR (2000). United Nations Scientific Committee on the Effect of Atomic Radiation, Sources, effects and risk of ionizing radiation, United Nations, New York.

Yoshida, S., Muramatsu, Y. (1994). Accumulation of radiocesium in basidiomycetes collected from Japanese forests. *Science of the Total Environment, 157*, 197–205.

Professional paper
10.7251/AGRENG2201065D
UDC 633.872

CERTIFICATION SYSTEM FOR SUSTAINABLE FOREST MANAGEMENT OF CORK TREE (*QUERCUS SUBER* L.) FORESTS

Ignacio J. DIAZ-MAROTO*

Department of Agroforestry Engineering, University of Santiago de Compostela, Spain
*Corresponding author: ignacio.diazmaroto@usc.es

ABSTRACT

Sustainable Forest Management (SFM) seeks to make sure a performance of forest ecosystems environmental and socioeconomically just. The cork oak forest management presents a complex problem because silvicultural peculiarities. The aim of the SFM, to guarantee a functioning of forest ecosystems environmental and socioeconomically adequate, is difficult to achieve in the Mediterranean forests, because they have a limited capacity to reply to the systematic changes, human impacts, wide climatic, edaphic, and biological variability, and a complicated socioeconomic environment. Given its heterogeneity, the management of these ecosystems represents a multifaceted problem, being particularly significant in the cork oaks because of their silvicultural features. The key aspect of the cork oak silviculture is the production of cork, which is separated regularly without cutting down the trees. Cork oak stands need a SFM to resolve their major problems: scarce natural regeneration, ageing of stands, quality loss, severe pruning, and cork oak decline ("seca"). Cork is the outer bark of the cork oak (*Quercus suber* L.), an evergreen tree species belonging to the *Fagaceae* family. Its chemical composition is different from other lignocellulosic raw materials. It is light, squeezable, and impermeable; it has low thermal conductivity, energy-absorbing capacity, and high friction resistance. The best sheets are used for manufacturing natural stoppers, vital in the aging process of "great" wines. The cork oak is a Mediterranean species covering a global area of about 2,2 million hectares; Portugal and Spain being the countries where it occupies a larger area. Our goal was to consider possible options for carrying out a forest certification system in small stands of cork oak with a lower area of 25 hectares. These forests are essential agents of sustainability and a driving force for sustainable rural development. They play a crucial role in the world's ecological balance, fighting climate change and desertification and maintaining biodiversity.

Keywords: *Mediterranean western, Quercus suber L., Cork oak stands, Properties.*

INTRODUCTION

Cork is a secondary protection layer of cork oaks (*Quercus suber* L.) and from viewpoint anatomical is a part of the periderm (Pereira, 2007). It can be obtained for the first time after the 20-25 years of growth of the cork oaks; this bark is known as "bornizo" or "virgem" cork in Spain and Portugal, respectively. It is broken, cracked and of low quality, so it is intended for crushing. Between nine and twelve years after the first uncorking, the "segundero" or "secundeiro" cork have a better quality, nevertheless still deficient. Cork sheets do not reach optimum quality until the next uncork, about 9-12 years later (3rd uncorking), when the cork tree is approximately 60 years old. The best cork sheets are mostly used for the manufacture of stoppers (Paulo *et al*., 2015). Thereafter, the cork produced every 9-15 years is well-known as reproduction cork. Uncorking is a sustainable process that does not harm the tree (Faias *et al*., 2018). Cork is composed of suberin, lignin, holocellulose, and extractives. Suberin is the principal component, accounting for 30-50% of cork's cell walls, and it is stored on the secondary walls. The lignin percentage has been found to range between 20-25%, and the polysaccharide concentration is relatively small (Song *et al*., 2017). Given the structure of the cell wall, the chemical composition and the lignocellulosic materials, cork performance it is outstanding compared to other materials. It is light and impermeable and has many exceptional properties including of little thermal conductivity, energy-absorbing aptitude, extraordinary friction resistance, and excellent sound insulation, between other (Pereira, 2007; Shiqian *et al*., 2018).

Cork is a 100% natural, sustainable, and recyclable material. Cork oaks have a unique ability to absorb CO_2 from the atmosphere. It is estimated that the cork oak forests can absorb up to 14 million tons of CO_2 per year. Their relationship over three centuries with wine guarantees its privileged position as a cultural reference. The natural cork stopper is the closure chosen for the best quality aging wines (Gil, 2014; APCOR, 2015). Wineries pay a high price for them and require a guaranteed tightness and absence of defects aroma, increasing demand for quality controls for the cork industry (Diaz-Maroto and Sylvain, 2016). This must have rapid and efficient analysis systems that allow it to select cork in the forest and monitor their evolution until obtaining the stoppers, following a strict traceability program (PEFC, 2012).

The annual world wine production is about 275 million hectolitres (292 Mhl in 2018) (OIV, 2019), and 90% of bottle wines are stopped by corks. In the manufacture (about 300 thousand tons of cork are used per year), and the cork waste is 25% of the raw material. Different cork wastes can be found depending on their characteristics, density, moisture, granulometry, size, ash content, and tannin concentration (Reis *et al*., 2019). The cork is also employed for a wide variety of products, including sealing materials and gaskets, heat and sound insulating materials, construction materials, and all types of ornaments. Among the bio-based materials that give satisfactory results in sound protection we can find black agglomerated cork with composite panels (APCOR, 2016) (Figure 1).

Figure 1. "Saca" –uncorking– of the cork oak [Source: Las Provincias, July 20th, 2012]

The present work was developed to assess the opportunity for applying a certification system for small cork oak stands, area under 25 hectares. The process must be leaded by the Regional Groups of Forest Certification and Chain of Custody. The certification system used is mostly the Programme for the Endorsement of Forest Certification (PEFC). As specified by the multifunctional forestry employed in these ecosystems, its main use is the production of natural cork stoppers for the best quality aging wines.

MATERIAL AND METHODS

Study framework and kind of cork oak forests

Cork oak forests are located along the occidental Mediterranean basin, focusing on the Iberian Peninsula. *Quercus suber* is the dominant tree species, in many stands unique, being an evergreen tree belonging to the *Fagaceae* family. The cork oak woodlands cover a worldwide area of 2,139,942 ha (Gil, 2014, APCOR, 2016). In Spain occupy more than half a million hectares and are set in the southwest (Extremadura and Andalucía) and west (Cataluña). The annual output is 340,000 tons of natural cork. Spain is the second largest world producer with 30%,

surpassed only by Portugal with 61%. In both countries they are around 300 companies linked to the cork, highlighting the stopper industry, 85% of the turnover of the sector (MAPA, 2019). These forests are vital ambassadors of environmental and socioeconomic sustainability and a powerful force for sustainable development. They play a crucial role in the ecological balance, fighting climate change and desertification, and maintaining biodiversity. *Quercus suber* is normally found in forests or open woodlands as the main tree species (Houston *et al*., 2016) (Figure 2).

Figure 2. Cork forests and open cork woodlands [Source: El Mundo, March 8th, 2009].

Cork oak woodlands in Spain and Portugal can be differentiated into cork oak forests (higher density) according to environmental, silvicultural, and socioeconomic characteristics and open cork oak forests (low tree density, "dehesas" and "montados", respectively) (Torres and Montero, 2000; Pereira, 2007) (Figure 2). Cork oak forests have a higher tree density and an understory of shrubs such as *Arbutus unedo* L., *Juniperus* spp., *Ulex* spp., *Cistus* spp., and aromatic species, among other (Torres and Montero, 2000). Open cork oak forests have a canopy cover of 10 to 60% and well-developed annual grasslands in the understory; the main production is cork extraction, but also provide grazing for domestic and wild livestock. In addition, the variety of productions linked to the species, such as: cork, firewood, acorns, biodiversity, and landscape diversity, give cork tree a privileged position among forest species in the Iberian Peninsula (Montero and Cañellas, 2003).

Open cork oak forests: growth patterns and competition with understory

Growth patterns can provide to the forest management decision making process so long as give stand development forecasts. Interspecific competition between trees and understory may affect stand growth patterns, along with intraspecific competition and forest structure, depending on the species, environment, and forest management. The understory may be positive for some soil process as it

contributes to the nutrient concentration and increase natural regeneration development (Faias *et al*., 2018). Specially, the growth models for dominant trees are highly suitable in the management of open cork oak forests (Sánchez-González *et al*., 2005). However, ecological factors, silvicultural treatments, and productive characteristics of these woodlands are not the same in Spanish and Portugal, "dehesas" and "montados", so growth behaviour could also be different.

RESULTS AND DISCUSSION

Structure of Regional Model of Forest Certification

In Spain have been certified around 125,000 hectares of cork oak forests and 20 companies in the sector have received certification of the Chain of Custody, 2015 data, through the Forest Stewardship Council (FSC) and the Programme for the Endorsement of Forest Certification Systems (PEFC). This process must be leaded by the Regional Groups of Forest Certification and Chain of Custody, main regional certification organisms (Figure 3). The certification system managed by those is mostly the Programme for the Endorsement of Forest Certification (PEFC). As specified by the multifunctional forestry applied in the *Quercus suber* forests, its main use is the production of cork stoppers for the best quality aging wines. PEFC is a non-profit, non-governmental organization dedicated to promoting SFM by independent third-party certification (PEFC, 2012).

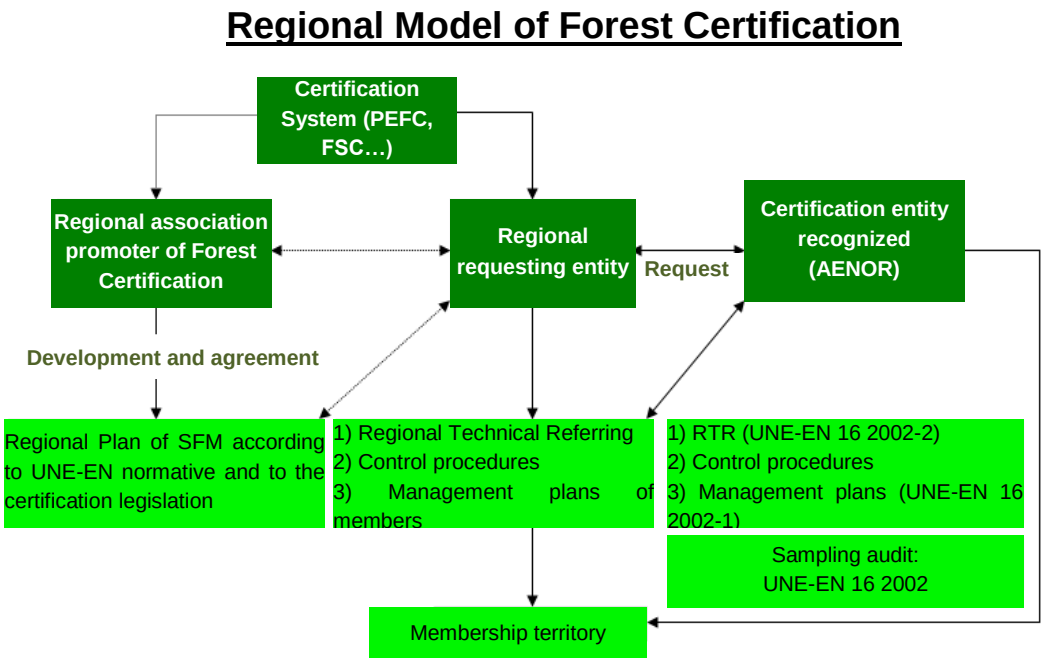


Figure 3. General scheme of Regional Model of Forest Certification
[*RTR: Regional Technical Referring].

Variability of bark, sapwood and heartwood depending on age

All members of the system plots (plots that have received acceptance notification and the membership reference code), the "saca" should be done considering the following (PEFC, 2012; Robak et al. 2012):

1) No delivery orders will be issued for certified cork oak plots whose date of registration in the application for license of "saca" is before the date of been membership of the system.

2) It's needed to submit a request for authorization by cadastral reference and properly complete all fields of the form. In addition to reflect all relevant observations, it is important to note the following data: i) complete cadastral reference; ii) total area of the plot to be uncorked (ha), wooded total area (ha), and forest total area (ha); iii) species to profit different to cork oak (if any); iv) number of trees; v) tons of cork to be extract (m^3).

3) Joined to the authorization of the Forestry Service, the uncorking must have all other legally required permits that would be applicable (water, patrimony, environment, etc.).

4) Companies engaged to the uncorking plots must comply with the provisions in this regard in the Manual of Good Practice of the Regional Groups of Forest Certification and Chain of Custody. Therefore, they must have sent the form entitled "Commitment Compliance of the Good Practices Manual", signed, and sealed before carrying out the first uncorking of a certified plot.

CONCLUSIONS

Sustainable Forest Management developed quickly. However, the implementation of its strategic framework has been delayed and some significant components progressing slowly. Greater time and effort obtaining transparency of roles and support from all stakeholders at the SFM stages, slowing the early stages, could have led to more progress. Meanwhile, the forestry industry suffered increasing pressure from the markets and public opinion to show that it had implemented sustainable practices. However, the private forest sector, which is moving in this way despite the difficulties caused by the small exploitations and fragmented ownership, has begun to understand that the SFM implementation, including the widespread application of forest certification systems, could reduce some of forest sector problems. Since some positive outcomes linked with administration's strategy have been achieved by private initiatives in a brief period of time, there are reasons to be optimistic.

REFERENCES

APCOR (2015). Manual Técnico: Tapón de corcho. Cultura, naturaleza, futuro [Technical Guide: Cork Stoppers. Culture, nature, future], Associação Portuguesa da Cortiça, Santa Maria de Lamas, Portugal.

APCOR (2016). O Anuário de Cortiça 2016 [The Cork Yearbook 2016], Associação Portuguesa da Cortiça, Santa Maria de Lamas, Portugal.

Diaz-Maroto I.J., Sylvain T. (2016). Analysis of physical properties of wood in three species of Galician oaks for the manufacture of wine barrels. Part I: Wood infradensity. Wood Research, vol. 61, pp. 683-696.

Faias S.P., Paulo J.A., Palma J.H.N, Tomé M. (2018). Understory effect on tree and cork growth in cork oak woodlands. Forest Systems, vol. 27, pp. 11967.

Gil (2014). Cork: A strategic material. Frontiers in Chemistry, vol. 2, pp. 1-2.

Houston T., de Rigo D., Caudullo G. (2016). *Quercus suber* in Europe: distribution, habitat, usage, and threats. In: San-Miguel-Ayanz J., de Rigo D., Caudullo G., Houston T., Mauri A. (Eds.), European Atlas of Forest Tree Species, Publ. Off. EU, Luxembourg.

International Organization of Vine and Wine (OIV) (2019). Statistical Report on World Vitiviniculture, International Organization of Vine and Wine, Paris, France.

MAPA (2019). Anuario de Estadística Forestal 2016 [Forest Statistics Yearbook 2016], Madrid, Spain.

Montero G., Cañellas I. (2003). Selvicultura de los alcornocales en España [Forestry of the cork oaks in Spain]. Silva Lusitana, vol. 11, pp. 1-19.

Paulo J.A., Palma J.H.N., Gomes A.A., Faias S.P., Tomé J., Tomé M. (2015). Predicting site index from climate and soil variables for cork oak (*Quercus suber* L.) stands in Portugal. New Forests, vol. 46, pp. 293-307.

Pereira H. (2007). Cork: Biology, production and uses. Elsevier Sci., Amsterdam, The Netherlands.

Programme for the Endorsement of Forest Certification (PEFC) (2012). Sustainable timber: a guide to procurement for the public sector, PEFC Council Information Register, Geneva, Switzerland.

Robak E, Aboal J, Picos J. (2012). Sustainable Forest Management in Galicia (Spain). In: Martin-Garcia, J., Diez, J.J. (Eds.), Lessons Learned, Sustainable Forest Management – Case Studies, Intech, London, U.K.

Reis S.F., Lopes P., Roseira I., Cabral M., Mateus N., Freitas V. (2019). Recovery of added value compounds from cork industry by-products. Industrial Crops and Products, vol. 140, pp. 111599.

Sánchez-González M., Tomé M., Montero G. (2005). Modelling height and diameter growth of dominant cork oak trees in Spain. Annals of Forest Science vol. 62, pp. 633-643.

Shiqian W., Song X., Yafang L., Mingqiang Z. (2018). Characterizations and properties of torrefied *Quercus variabilis* cork. Wood Research, vol. 63, pp. 947-958.

Song X., Lingyan Z., Shiqian W., Yafang L. (2017). Structural and mechanical properties of cork cell walls from *Quercus variabilis* Blume (*Fagaceae*). Wood Research, vol. 62, pp. 873-882.

Torres E., Montero G. (2000). Los alcornocales del Macizo del Aljibe y Sierras del Campo de Gibraltar [Cork oak forests of the Massif of El Aljibe and Mountains of the Campo de Gibraltar], MAPA, Madrid, Spain.

Original Scientific paper
10.7251/AGRENG2201072Y
UDC 004.738.5:[641/642:658.562

CONSUMERS' PERSPECTIVES ON ONLINE SHOPPING AND FOOD SAFETY DURING THE COVID-19 PANDEMIC

Aysun YENER ÖGÜR*, Mustafa DİKMEN, Mehmet Emin YARDIMCI

Selcuk University, Faculty of Agriculture, Department of Agricultural Economics, Konya, Turkey
*Corresponding author: aysunyener@selcuk.edu.tr

ABSTRACT

The coronavirus disease (COVID-19) pandemic, which emerged in 2019, is a serious respiratory disease. "Stay at home", "good hygiene" and "social distance" campaigns have been launched to prevent the spread of the virus worldwide. However, these measures were not enough to prevent the devastating effect of the virus, and these new conditions affected the purchasing behavior of consumers. The study, consumers' perspectives on online shopping and food safety during the COVID-19 pandemic were determined. In the study, the sample size was calculated using the proportional sample size formula and was determined as 150. The Covid-19 epidemic has changed the consumption and purchasing attitudes of people in our country as well as in the world. With the change in purchasing behavior, there is an increase in the level of awareness about food safety. According to the results of the research, it has been determined that consumers have a positive attitude towards online shopping and will continue to shop online after the pandemic. In addition, during the pandemic period, consumers should be aware of the expiration date of the food, the hygiene of the place where they buy food, the proper storage of food at home, the reading of the content on the label, gloves, masks, aprons, etc. It has been determined that they pay attention to clothing, brand, additives, food, safety and quality standards, production date, glass packaging. Manufacturers need to pay attention to food safety.

Keywords: *COVID-19, Food Safety, Online Shopping.*

INTRODUCTION

In recent years, drug-resistant infectious diseases have begun to emerge worldwide. For example, in 2003, outbreaks of severe acute respiratory syndrome (SARS-CoV) in Guangdong, China, followed by Middle East respiratory syndrome (MERS-CoV) outbreaks in Saudi Arabia in 2012 (Peiris et al., 2003; Zaki et al., 2012; Jung et al., 2016). Available coronavirus disease 2019 (COVID-19) pandemic, first of all is a serious respiratory disease caused by China's Wuhan city declared in the SARS-COV-2. And the virus has been found to be transmitted through direct exposure to respiratory droplets of an infected person or through

direct contact with infected surfaces (Wu et al., 2020; Ma et. al., 2021). And before the WHO classified COVID-19 as a pandemic, On March 16, 2020, some policy tools were created by the scientific committee to prevent the spread of the virus. All education and training institutions and public gathering places were closed, the country's borders were controlled, domestic travel was restricted, and curfews were imposed across the country. Citizens to encourage them to help stop the spread of the virus "stay at home", "good hygiene" and "social distance" campaigns were initiated (Copper, 2020; Bostan et al, 2020; Seyahi et al, 2020). However, these measures were not enough to prevent the devastating effect of the virus. These new conditions have affected the purchasing behavior of consumers (Cranfield, 2020; Górnicka et al., 2020; Grashuis et al., 2020; Pulighe and Lupia, 2020; Sheth, 2020; Sidor and Rzymski, 2020). Examples of changes in consumer behavior include stocking and rushing purchases of essential products during homeownership, postponing purchasing behavior of certain products, and shifting from physical experiences to virtual experiences (Donthu and Gustafsson, 2020; Kirk and Rifkin, 2020; Kohli et al., 2020; Sheth, 2020). Although there is no evidence that the epidemic can be transmitted through food, COVID-19 has an economic impact on the food and agriculture sector. Countries have re-examined their food and agriculture policies to ensure that food supplies remain available to the public and affordable (Ma et al., 2021). The effects of the pandemic on the food supply chain are increasing day by day. According to the United Nations report, food insecurity increased from 23.3% in 2014 to 26.4% in 2018. Before COVID-19 in September 2019, approximately 821 million people, or more than 10% of the world's population, were starving. (FAO; IFAD; UNICEF; WFP; WHO, 2019) It impacted the food supply for an additional 260 million people by the end of 2020 (Anthem, 2020; FAO, 2020). Availability, access, use, and stability need to be addressed in food safety during the pandemic (Zurayk, 2020). During the COVID-19 epidemic, food safety is endangered by the disruptions in the distribution of food products (Galanakis, 2020; Vallianatos et al., 2010). In the study, consumers' perspectives on online shopping and food safety during the COVID-19 epidemic were examined.

MATERIAL AND METHOD

The primary data of this research consists of the survey data conducted with individuals who shop online in Konya, Karapınar (Turkey) in 2020. Secondary data were compiled from public institutions, local and foreign scientific studies, journals, and various publications related to the subject. The number of questionnaires used in the study was calculated by using the proportional sample size formula, which is also used in many studies (Çobanoğlu et al., 2003; Armağan and Akbay, 2007; Büyükbay Oruç et al., 2009) in order to reach the maximum sample size is limited populations (Newbold, 1995). The questionnaires were conducted face-to-face with randomly selected people. The formula for determining the number of surveys is given below. Survey questions consist of

multiple-choice, open-ended, and Likert questions. The survey was conducted taking into account the Covid-19 rules and no problems were encountered.

$$n = \frac{N\ p\,(1-p)}{(N-1)\,\sigma^2_{px} + p(1-p)}$$

In the formula; n: Sample volume, N: Total number of people in the sampling frame, p: Number of women living in rural and urban areas (based on 50% assumption), σ^2_{px}: variance of the ratio (with a table value of 1.65 and 5% margin of error at 90% confidence interval to reach the maximum sample volume). Since the characteristics of the consumers constituting the main population were not known at the beginning, p=0.5 was taken to maximize the sample size. The sample volume was taken as p=q=0.5 and calculated as 150 in order to reach the 95% confidence interval, 10% margin of error, and maximum sample volume in the formula. Whether or not the consumers who participated in the survey agreed with some values was measured with a 5-point Likert scale (1: Completely Disagree 2: Disagree 3: Undecided 4: Agree 5: Totally Agree). The Likert scale is one of the attitude measurement methods and is included in the group of graduated scales. The scale value of the person is found with the scores calculated for the degree of participation. With this value obtained, the individual's attitude towards an event or the factors that determine his attitude is rated (Erdoğan, 1997).

RESULTS AND DISCUSSION

In order to examine the consumers' perspectives on online shopping and food safety in the COVID-19 period, it is necessary to examine some demographic characteristics of consumers. When socio-demographic characteristics are examined in the study, 70.6% of consumers are male and 29.4% are female. Considering the ages of the participants, 34.6% are between the ages of 31-40. 28% are between the ages of 21-30. and 19.4% are between the ages of 41-50. The educational status of the individuals participating in the survey is 31.3% at the undergraduate level. 28% at primary education level. 24% at high school level. and 15.3% at associate degree level. Considering the income level of the participants. 35.4% of them are between 2501-3500 TL; 32.6% of it is between 3501-5000 TL; It is seen that 21.4% of it is between 1001-2500 TL. 63.3% of the participants are married and 36.4% are single. Looking at the professions of the participants 24% of them are farmers. The second place is followed by tradesmen with 20% and students with 17%. 4% of the participants are retirees. It was determined that 54.6% of the consumers have a personal computer and 45.4% do not have a personal computer. It was determined that 86% of the individuals who participated in the survey had an internet connection at their place of residence and 14% did not have an internet connection. When looking at the frequency of connecting to the Internet, it was determined that 76% did not connect every day, 11.3% did not connect 2-3 times a week, 4% did not connect several times a month, and 8.7% did not connect regularly.

Table 1. Attitudes towards online shopping

Variables	Mean
Having detailed content about the products increases my shopping desire.	3.94
Being able to get after-sales support increases my desire to shop.	3.57
Having comments on products increases my shopping desire	3.48
The arrival of the product with a secure cargo company increases my shopping desire.	3.34
Referrals (SSL) applications that increase the reliability of the website increase my willingness to shop	3.28
Having special campaigns for members increases my desire to shop.	3.08
Receiving informative e-mails specific to my interests increases my desire to shop.	3.06
The fact that the delivery date of the products I want to buy is certain increases my interest in the product.	3.03
Having different payment options other than credit cards increases my desire to shop.	2.94
Even though I don't need the products to be on the home page of the website, it causes me to be interested in the product.	2.91
Receiving informational e-mails about products with discounts or campaigns increases my desire to shop.	2.91
Having credit card installment opportunities increases my desire to shop.	2.90
Instant or daily discounts on products increase my desire to shop.	2.84
The fact that the sites I shop online are easy to use increases my desire to shop.	2.73

5: Strongly agree 4: Agree 3: Undecided 2: Disagree 1: Strongly Disagree

*Source: Author s' elaboration based on the questionnaire survey results.

According to Table 1, consumers' attitudes towards the factors that are thought to affect their online shopping behavior are given. According to the attitudes of the consumers, it has been determined that the detailed content about the products and the institution that can receive after-sales support increase their shopping desires.

Table 2. Consumers' attitudes towards food safety

Variables	Mean
I pay attention to the expiration date on foods.	4.44
I pay attention to the hygiene of the place where I buy food	4.39
I take care to store food inappropriate conditions at home.	4.26
I read the content section on the label	4.07
The gloves and masks of the employees at the place where I buy food. I make sure that they are wearing aprons etc.	4.01
I pay attention to the brand.	4.00

I'll look into additives.	3.97
I make sure that it has food safety and quality standards.	3.88
I pay attention to the production date of the food.	3.85
I prefer to buy products with glass packaging	3.74
I call the authorities for the problem food product	3.72
On behalf of the manufacturer and packaging company. I pay attention to the address and the place where it was produced.	3.39
I buy open food	1.92

5: I definitely pay attention 4: I pay attention 3: I'm indecisive 2: I don't pay attention 1: I definitely don't pay attention

*Source: Author s' elaboration based on the questionnaire survey results.

The increasing number of conscious consumers in society has increased the importance of the concept of food safety. The dangers arising from food and the change in consumers' perception of quality have led to more emphasis on reliable food production. Along with the COVID 19 pandemic process, consumer behavior has also undergone a rapid and great change (Baltacı and Akaydın, 2020). According to the table 2, during the pandemic period, consumers should be aware of the expiration date of the food, the hygiene of the place where they buy food, the proper storage of food at home, the reading of the content on the label, gloves, masks, aprons, etc. It has been determined that they pay attention to the wearing, brand, additives, food, safety and quality standards, production date, and glass packaging. In some studies, it has been determined that since viruses can be found in environments where we live for a very long time, consumers should also follow general hygiene rules and pay attention to gloves and masks in order to be protected (Akın and Akın, 2020; Aslan, 2020; EUFIC, 2020; FAO and WHO 2020).

CONCLUSION

The study, consumers' perspectives on online shopping and food safety during the COVID-19 pandemic were determined. The Covid-19 epidemic has changed the consumption and purchasing attitudes of people in our country as well as in the world. With the emergence of the pandemic crisis and its increasing impact, the way consumers shop has begun to change. Market in general before the epidemic. purchases made in shopping places such as stores. With the increase of the epidemic, it has started to shift to online shopping more. With the change in purchasing behavior, there is an increase in the level of awareness about food safety. According to the results of the study, during the pandemic period, consumers should be aware of the expiration date of the food, the hygiene of the place where they buy food, the proper storage of food at home, the reading of the content on the label, gloves, masks, aprons, etc. It has been determined that they pay attention to the wearing, brand, additives, food, safety and quality standards,

production date, and glass packaging. Because online shopping is more advantageous than face-to-face shopping, consumers are shifting to online shopping. However, there are some concerns about food safety in online shopping. Manufacturers need to pay attention to food safety.

REFERENCES

Akın M.B., Akın M.S. (2020). Food Safety and COVID-19. Harran University, Journal of Engineering, *5*(3), 268-277.

Anthem P. (2020). Risk of hunger pandemic as coronavirus set to almost double acute hunger by end of 2020. World Food Programme Insight. https://insight.wfp.org/co vid-19-will-almost-double-people-in-acute-hunger-by-end-of-2020-59df0c4a8072 (Accessed 6 July 2021).

Armağan G., Akbay C. 2007. An Econometric Analysis of Urban Households' Animal Products Consumption in Turkey, Applied Economics, 1-8.

Aslan D. (2020). On New Coronavirus Disease (COVID-19), Food Health and Safety. HASUDER Public Health Professionals Association. https://www.istabip.org.tr/koronavirus/Haberler/5660/covid-19- gida-sagligi-ve-gida-guvencesi-uzerine

Baltacı A., Akaydın H. (2020). The effect of the COVID-19 pandemic process on the consumers' purchasing behavior of food products: A literature review. Yüksek İhtisas University Journal of Health Sciences, *1*, 57-64.

Bostan S., Erdem R., Öztürk Y.E., Kılıç T., Yılmaz A. (2020). "The effect of COVID-19 pandemic on the Turkish Society", Electronic Journal of General Medicine, Vol. 17 No. 6, pp. 1-8.

Büyükbay Oruç E., Sayılı M., Uzunöz M. (2009). The Relationship Between Socio-Economic Characteristics of Consumers and Their Consumption of Tomato Paste: The Case of Tokat Province, Electronic Journal of Food Technologies Vol: 4, No: 1, 2009 (17).

Çobanoğlu F., Konak K., Bozkurt M. (2003). Economic Analysis and Marketing Status of Broiler Chicken Farms in Aydın Province. Journal of Akdeniz University Faculty of Agriculture, 15, Antalya.

Cranfield J.A.L. (2020). "Framing consumer food demand responses in a viral pandemic", Canadian Journal of Agricultural Economics, Vol. 68 No. 2, pp. 151-156.

Donthu N., Gustafsson A. (2020). "Effects of COVID-19 on business and research", Journal of Business Research, Vol. 117 No. 9, pp. 284-289.

Erdoğan İ. (1997). Behavior in Business, Istanbul University Faculty of Business Publication Vol : 272, İstanbul.

EUFIC (The European Food Information Council) (2020). Food and coronavirus (COVID-19): what you need to know. https://www.eufic.org/tr/page/food-and-coronavirus-covid-19-what-you-need-to-know

FAO (2020). COVID-19 global economic recession: Avoiding hunger must be at the centre of the economic stimulus. Food and Agriculture Organization of the United Nations, Rome. http://www.fao.org/documents/card/en/c/ca8800en

FAO, IFAD, UNICEF, WFP, WHO, (2019). Safeguarding against economic slowdowns and downturns. Food and Agriculture Organization of the United Nations, Rome.

FAO, WHO (2020). COVID-19 and Food Safety: Guidance for Food Businesses: interim guidance. WHO reference number: WHO/2019-nCoV/Food_Safety/2020.1. https://apps.who.int/iris/bitstream/handle/10665/331705/WHO-2019-nCoV-Food_Safety-2020.1- eng.pdf

Galanakis C.M. (2020). "The food systems in the era of the coronavirus (COVID-19) pandemic crisis", Foods, Vol. 9 No. 523, pp. 1-10.

Górnicka M., Drywień M.E., Zielinska M.A., Hamułka J. (2020). Dietary and lifestyle changes during COVID-19 and the subsequent lockdowns among Polish adults: a cross-sectional online survey PLifeCOVID-19 study. *Nutrients*, *12*(8), 2324.

Kirk C.P., Rifkin L.S. (2020). "I'll trade you diamonds for toilet paper: consumer reacting, coping and adapting behaviors in the COVID-19 pandemic", Journal of Business Research, Vol. 117 No. 9, pp. 124-131.

Kohli S., Timelin B., Fabius V., Veranen S.M. (2020), "How COVID-19 is changing consumer behavior-now and forever, McKinsey&Company", available at: https://www.mckinsey.com/ industries/retail/our-insights/how-covid-19-is-changing consumer-behavior-now-and-forever (Accessed 7 July 2021).

Ma N.L., Peng W., Soon C.F., Hassim M.F.N., Misbah S., Rahmat Z., Sonne C. (2021). Covid-19 pandemic in the lens of food safety and security. *Environmental research*. *193*. 110405.

Newbold P. (1995). Statistics for Business and Economics. Prentice Hall International Editions.

Peiris J.S.M., Lai S.T., Poon L.L.M., Guan Y., Yam L.Y.C., Lim W., Nicholls J., Yee W.K.S., Yan W.W., Cheung M.T., Cheng V.C.C., Chan K.H., Tsang D.N.C., Yung R.W.H., Ng T.K., Yuen K.Y. (2003). Coronavirus as a possible cause of severe acute respiratory syndrome. Lancet 361 (9366), 1319–1325.

Pulighe G., Lupia F. (2020). "Food first: COVID-19 outbreak and cities lockdown a booster for a wider vision on urban agriculture", Sustainability, Vol. 12 No. 5012, pp. 1-4.

Seyahi E., Poyraz B.C., Sut N., Akdogan S., Hamuryudan V. (2020). "The psychological state and changes in the routine of the patients with rheumatic diseases during the coronavirus disease (COVID-19) outbreak in Turkey: a web-based cross-sectional survey", Rheumatology International, Vol. 40 No. 8, pp. 1229-1238.

Sheth J. (2020). "Impact of Covid-19 on consumer behavior: will the old habits return or die?", Journal of Business Research, Vol. 117 No. 9, pp. 280-283.

Sidor A., Rzymski P. (2020). "Dietary choices and habits during COVID-19 lockdown: experience from Poland", Nutrients, Vol. 12 No. 1657, pp. 1-13.

Vallianatos M., Azuma A.M., Gilliland S., Gottlieb R. (2010). Peer reviewed: food access, availability, and affordability in 3 Los Angeles communities, Project CAFE, 2004-2006. Prev. Chronic Dis. 7 (2), A27.

Wu Y.C., Chen C.S., Chan Y.J. (2020). The outbreak of COVID-19: An overview. J. Chin. Med. Assoc. 83 (3), 217–220.

Zaki A.M., Van Boheemen S., Bestebroer T.M., Osterhaus A.D.M.E., Fouchier R.A.M. (2012). Isolation of a novel coronavirus from a man with pneumonia in Saudi Arabia. N. Engl. J. Med. 367 (19), 1814–1820.

Zurayk R. (2020). Pandemic and food security. J. Agric. Food Syst.Community Dev. 9, 1–5.

Original Scientific paper
10.7251/AGRENG2201080A
UDC 66

IN VITRO CHARACTERIZATION OF FIXED OIL CONTENTS FROM CALLUS CULTURES OF JOJOBA (*SIMMONDSIA CHINENSIS* L.)

Muhammad AKRAM[1,2]*, Faheem AFTAB[2]

[1]Department of Botany, Govt. Postgraduate College Sahiwal-57000, Pakistan
[2]Institute of Botany, University of the Punjab, Lahore-54590, Pakistan
*Corresponding author: akram.botany@pu.edu.pk

ABSTRACT

Objective of the present study was to investigate and determine fixed oil (lipids) contents from *in vitro* induced calluses of leaf, cotyledon and intermodal explants of jojoba (*Simmondsia chinensis* L.). The leaf and internodal explants were from micropropagated *in vitro* plants whereas mature seeds were used for the acquisition of cotyledons. Sterilized explants were cultured on different concentrations (1, 2, 4, 6, 8 or 10 µM) of either 2, 4-Dichlorophenoxyacetic acid (2, 4-D) or α-Naphthaleneacetic acid (NAA) alone or in combination (1+1, 2+1, 4+1, 6+2, 8+2 or 10+2 µM) with 6-Benzylaminopurine (BAP) or Kinetin (Kin) for callus induction. Highly proliferating callus lines (CLs) growing on 8 µM 2, 4 - D (CL-1), 10 µM 2, 4-D (CL-2) and 2, 4-D (10 µM) + 2 µM BAP (CL-3) were selected for the determination of fixed oil contents by distillation method. Highest rate of callus induction (100%) as well as 6.87 gm fresh weight was obtained from leaf explant on CL-3 after 99 days of culture. Amount of fixed oil content (290mg/6gm FW), acid value (0.320), free fatty acid (0.160) and saponification number (91) were highest from calluses of cotyledons on CL-1 as compared to other callus lines CL-2 or CL-3. The present investigation demonstrated an efficient method for determination of the fixed oil contents and related biochemical parameters from calluses of jojoba.

Keywords: *Callus, fixed oil, free fatty acid, jojoba, liquid wax.*

INTRODUCTION

Simmondsia chinensis L. is commonly known as 'jojoba' belongs to the family Simmondsiaceae, deer nut or wild hazel (Steven, 2000). This is a small semi-hardwood 2-20 feet high shrub. Jojoba is wide spread over 18,500 hectares and the demand of its oil is 6400-200,000 tons per year throughout the world (Hussain *et al.* 2011). Jojoba is also known as desert shrub, drought resistant crop cultivated in waste and dry places in different regions of Pakistan of Bahawalpur, D.I. Khan, Karachi and Quetta (Aftab *et al*., 2008).

Secondary metabolites primarily serve as reserve stored food material in plants. Such chemicals provide protection to plants as well as contribute to the preparation of life-saving medicines, confectionaries, cosmetic and health promoting activities (Tietela *et al.,* 2021). Fixed oil is an important metabolite of jojoba (Aftab *et al*. 2008) contained 50% lipids of its dry seed weight. Oil quantity in jojoba seed is twofold the amount of other oil yielding crops (Kumar *et al.,* 2012). Jojoba oil is golden in color "unsaturated liquid wax with odorless greasy feel" (Kumar *et al.,* 2012). Jojoba lipids are straight chain monoesters of alcohol and acids (Wisniak, 1987). It has different uses for synthesis of high-pressure lubricants, renewable energy source, preparation of cosmetics, fire retardants, food, transformer oils, electrical insulators and pharmaceuticals (Wisniak, 1987).

Oil yield may be enhanced by introducing improved verities and disease-free plant production. For this purpose, plant tissue culture technology is usually employed for the improvement of plant health, vigor and rapid multiplication. Cells of callus tissue are totipotent that may have ability to regenerate into complete plant. It is therefore prerequisite to harvest whole callus tissue acquainted with its oil yielding capacity for subsequent commercial application. Thus, aim of the present work was to induce callus and selection of suitable callus lines having improved growth for the determination of fixed oil contents of jojoba.

MATERIALS AND METHODS

Plant material and culture conditions

Jojoba seeds were procured from PU Seed Centre, University of the Punjab, Lahore, Pakistan. Seeds were thoroughly washed under running water followed by 0.1% $HgCl_2$ for 10 min, and finally decontaminated with 6% (v/v) active chlorine in the form of NaOCl (Robin Bleach, Reckitt Benckiser, Pakistan) for another 15 min. Thoroughly rinsed seeds with autoclaved distilled water under sterile conditions were cultured on MS (Murashige & Skoog 1962) medium + 0.5 µM 6-Benzylaminopurine (BAP) for 25 days for the establishment of axenic seedlings at 25 ± 2 °C and 16h photoperiod (35 µmol $m^{-2}s^{-1}$).

Callus induction and growth

There were 36 treatments to test callus induction from cotyledon, internode and leaf explants. Axenic seedlings were cut to prepare cotyledons, internode and leaf explants (0.5 – 1.0 cm^2) and cultured on MS medium supplemented with different concentrations (1, 2, 4, 6, 8 or 10 µM) of either 2, 4-Dichlorophenoxyacetic acid (2, 4-D) or α-Naphthaleneacetic acid (NAA) alone or in combination (1+1, 2+1, 4+1, 6+2, 8+2 or 10+2 µM) with 6-Benzylaminopurine (BAP) or Kinetin (Kin) for callus induction under complete dark conditions at the temperature mentioned above. The data for callus induction were taken after 49 days of initial culture.

Selection of callus lines and fresh weight (FW)

Cultures with rapid and healthy callus growth were categorized as callus lines (CL). Such lines were growing on 2,4-D 8 µM (CL-1), 2,4-D 10 µM (CL-2), 2,4-D 10 µM + 2 µM BAP (CL-3). Calluses of these lines were further cultured on the

respective fresh medium. Fresh weights (FW) of the calluses were then taken after 14, 63 and 99 days of re-culture.

Extraction of total lipids from CL

Total lipids were extracted by immersing 6g FW callus tissue from selected CL in distillation flask fitted with condenser containing 100 ml n-hexane. Flask was placed on the heating bath to heat the solvent at 60 °C. The same process was used for lipids extraction for all other experimental tissues. After 60 min, condenser was removed and n-hexane containing lipid contents were evaporated at 50 °C by agitating. Weight of the remaining evaporated material was recorded and calculated total lipids contents by the following formula.

$$\text{Lipid contents (\%)} = \frac{\text{Wt. of extracted lipids}}{\text{Wt. of callus tissue}} \times 100$$

Biochemistry

For biochemical investigation, the modified method of Akubugwo *et al*. (2008) was followed.

Acid value

Acid value was determined by dissolving 50 mg oil (W) in equal proportion (1:1 v/v) of 1.5 ml ethyl alcohol: diethyl ether solvent. This was then titrated by stirring against 0.1N NaOH (V) and phenolphthalein was used as indicator. Acid value was determined by using the following formula.

$$\text{Acid value} = \frac{56.1 \times N \times V}{W}$$

Free fatty acid (%FFA) was determined by multiplying acid value with factor 0.503 as follows: %FFA = 0.503 × acid value

Saponification number:

Saponification number was determined by taking 50 mg oil in conical flask and dissolved in 1 ml of 0.5% ethanolic KOH and refluxed on water bath for 30 min. Then few drops of phenolphthalein indicator were added and the hot mixture was titrated against 0.5 N HCl taken in the burette. A blank reading was recorded and determined the saponification number by using the following formula.

$$\text{Saponification number} = \frac{56.1N\ (V1-V2)}{W}$$

N= Normality of HCl

V_1= Volume of HCl used in test

V_2= Volume of HCl used in blank

W= Weight of oil used

Data analyses

Statistically data were analyzed by ANOVA using the function Duncan's multiple range test (DMRT) for comparison of means. Mean values of fresh weight of calluses were compared using the function box plot. All analyses were carried out at $p<0.05$ probability level as determined by SPSS v. 16.0.

RESULTS AND DISCUSSION

Callus induction

The rate of callus induction was significantly improved by increasing the concentration of 2,4-D alone from cotyledon explants (Table 1). Highest (100%) callus induction was obtained at the both concentrations of 8µM or 10µM 2,4-D from cotyledon explants after 49 days of initial culture (Table 1). Similarly, 100% callus induction was also observed at 10 µM 2,4-D + 2 µM BAP from leaf explants. Callus induction at lower concentrations of NAA alone was good enough while higher levels seemed detrimental, when BAP or Kin was used along with NAA; the harmful effect vanished.

Authors observed a little callus from internode on all media types that may be due to the hard texture of an explant. Fig. 1 showed the trend of callus induction frequency from different explants on different media formulation. Cotyledon was most responsive explant as compared to leaf or internode.

Table 1. Effect of various treatments involving MS medium supplemented with various growth regulators on callus induction from different explants of jojoba after 49 days

Tr. No.	PGRs (µM)				Callus induction (%)		
	2,4-D	NAA	BAP	Kin	Cotyledon	Internode	Leaf
1	1				90.22 ± 6.55^{abc}	50.55 ± 4.51^{fgh}	20.21 ± 2.60^{ijk}
2	2				92.38 ± 4.33^{abc}	66.66 ± 3.55^{ef}	60.82 ± 3.21^{ef}
3	4				93.21 ± 4.02^{ab}	50.22 ± 4.22^{fgh}	66.63 ± 6.51^{def}
4	6				93.82 ± 5.01^{ab}	51.25 ± 4.45^{fgh}	70.25 ± 7.33^{cde}
5	8				100^{a}	81.33 ± 5.55^{cd}	100^{a}
6	10				100^{a}	95.21 ± 5.21^{ab}	100^{a}
7		1			81.55 ± 6.66^{cd}	25.21 ± 2.52^{ij}	0
8		2			75.33 ± 3.31^{cde}	25.21 ± 4.52^{ij}	88.33 ± 3.21^{bc}
9		4			66.61 ± 4.51^{def}	33.33 ± 3.21^{hij}	66.66 ± 6.32^{def}
10		6			66.61 ± 6.65^{def}	0	25.42 ± 4.24^{ijk}
11		8			40.01 ± 4.32^{d}	40.41 ± 4.25^{hi}	40.05 ± 4.25^{ij}
12		10			41.22 ± 3.32^{d}	40.36 ± 2.15^{hi}	41.33 ± 1.25^{ij}
13	1		1		92.25 ± 7.33^{abc}	50.21 ± 3.33^{fgh}	75.55 ± 5.21^{cd}
14	2		1		93.15 ± 6.21^{ab}	53.63 ± 5.55^{fg}	76.51 ± 4.37^{cd}
15	4		1		97.25 ± 3.20^{ab}	55.68 ± 5.61^{fg}	75.75 ± 4.25^{cd}
16	6		2		97.15 ± 7.33^{ab}	80.82 ± 6.45^{cd}	81.37 ± 3.40^{bc}
17	8		2		98.01 ± 6.33^{ab}	89.37 ± 7.55^{b}	88.25 ± 4.44^{b}
18	10		2		100^{a}	98.45 ± 6.45^{a}	100^{a}
19	1			1	66.25 ± 3.33^{def}	40.44 ± 4.25^{hi}	50.51 ± 5.55^{fij}
20	2			1	66.37 ± 4.21^{def}	42.25 ± 3.21^{hi}	60.02 ± 3.33^{ef}
21	4			1	71.91 ± 4.25^{de}	48.37 ± 3.00^{gh}	62.55 ± 4.22^{ef}
22	6			2	75.55 ± 5.21^{cde}	50.51 ± 4.21^{fgh}	68.25 ± 4.01^{def}
23	8			2	76.51 ± 6.66^{cde}	55.33 ± 3.33^{fg}	68.37 ± 7.33^{def}
24	10			2	80.25 ± 5.51^{cd}	60.21 ± 2.11^{efg}	71.25 ± 3.21^{cde}
25		1	1		21.22 ± 2.01^{hi}	15.99 ± 3.21^{ij}	27.13 ± 4.10^{ijk}
26		2	1		25.33 ± 3.21^{hi}	19.21 ± 3.25^{ij}	30.21 ± 2.11^{ijk}
27		4	1		26.25 ± 2.33^{hi}	21.27 ± 4.02^{ij}	30.25 ± 2.11^{ijk}
28		6	2		38.19 ± 4.17^{gh}	35.02 ± 4.12^{hij}	35.29 ± 3.33^{ijk}
29		8	2		41.21 ± 2.11^{gh}	40.25 ± 2.21^{hi}	45.21 ± 2.22^{ij}
30		10	2		49.23 ± 4.27^{fg}	41.20 ± 4.21^{hi}	47.22 ± 5.55^{ij}

31	1	1	60.21 ± 5.77^{efg}	94.88 ± 6.45^{ab}	66.66 ± 4.32^{def}
32	2	1	62.71 ± 3.11^{efg}	83.45 ± 4.51^{cd}	41.24 ± 2.24^{ij}
33	4	1	62.61 ± 4.21^{efg}	75.45 ± 6.51^{bc}	53.45 ± 5.21^{fij}
34	6	2	64.66 ± 5.63^{def}	41.21 ± 4.25^{hi}	60.66 ± 6.32^{ef}
35	8	2	66.33 ± 5.63^{def}	35.33 ± 2.41^{hij}	65.23 ± 6.32^{def}
36	10	2	79.14 ± 5.63^{cd}	35.42 ± 3.63^{hij}	71.46 ± 6.32^{cde}

The observed values given in the columns are the mean of three replicates of three independent experiments. Mean values (±SE) indicated with small alphabetical different letters are significantly different as per DMRT (Duncan's multiple range test $p \leq 0.05$).

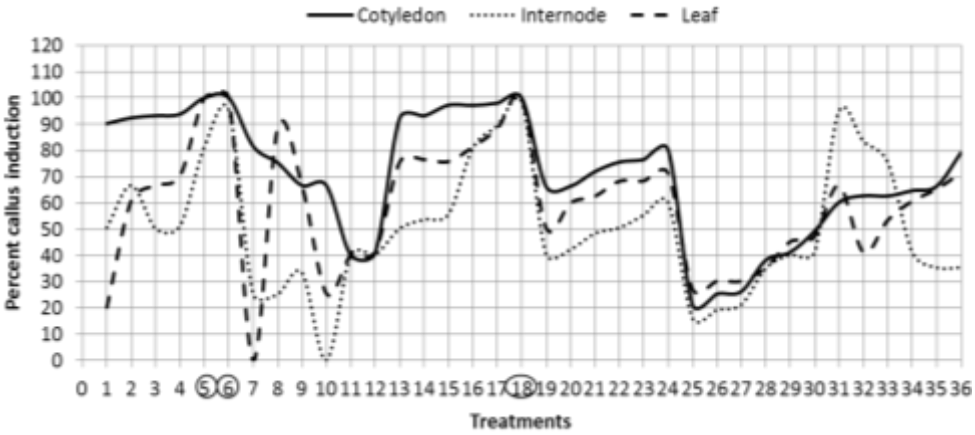


Fig. 1. Comparison of values and trend percent callus induction of each treatment from different explants of 49-day old cultures of jojoba. Encircled treatments indicate CL-1, CL-2 and CL-3, respectively

Selection of callus lines (CL)

There were three treatments (2, 4-D 8 µM or 10 µM and 2, 4-D 10 µM + 2 µM BAP) that formed 100% callus on both cotyledon and leaf explants. Color of calluses was different of white and milky appearance observed at the periphery of the excised cotyledon at 8 µM 2, 4-D after 14 days of initial culture (Fig. 2a). Such calluses proliferated further and formed huge amount of translucent friable mass with leathery and grayish appearance after 21 days (Fig. 2b). Calluses obtained with 10 µM 2, 4-D + 2 µM BAP from leaf explants were soft textured observed after 63 days of culture (Fig. 2c). Friable calluses were observed with 2 µM NAA + 2 µM BAP (Fig. 2d). It was therefore such calluses formed on these treatments selected and designated as CL. The FW of CL was significantly increased with the passage of time after 14, 63 and 99 days of culture (Fig. 3). Highest FW (6.87gm) was obtained from leaf explants followed by 6.65 gm from cotyledon with CL-3 after 99 days.

Quantity and characteristics of total lipid contents

Highest amount of total lipids (290 mg) was obtained from cotyledons cultured on CL-1 followed by CL-3 (250 mg). In case of leaf explants, amount of lipid was higher (120 mg) with CL-3 as compared to 110 mg of other callus lines (Table 2).

Callus tissue from internodal explants produced 110 mg lipids with CL-3 followed by CL-2 (90 mg) and CL-1 (100 mg).

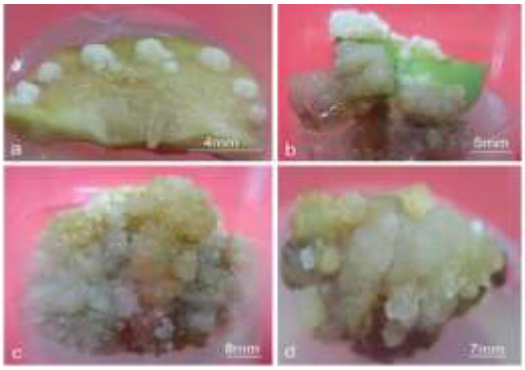


Fig. 2. Callus induction and proliferation in jojoba. a) Callus initiation at the periphery of cotyledon at 8 µM 2, 4 -D after 14 days. b) Translucent and grayish callus proliferation on cotyledonary explant at 10 µM 2, 4 -D after 21 days. c) 63-day old multicolored mass of callus induced with 2, 4-D 10 µM + BAP (2 µM). d) Friable callus induction at 2 µM NAA + 2 µM BAP after 63 days

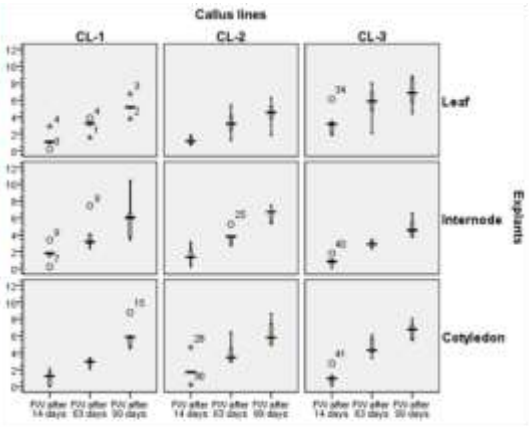


Fig. 3. Fresh weight (FW) of callus lines (CL-1: 2, 4-D 8 µM, CL-2: 2, 4-D 10 µM, CL-3: 2, 4-D 10 µM + 2 µM BAP) of different explants of jojoba. Box plot indicates upper and lower quartile range of fresh weight.

Table 2. Total lipid contents from callus lines of different explants of jojoba

Callus lines	Total lipid contents (mg/6gm FW)		
	Cotyledon	Internode	Leaf
CL-1	290 ± 0.01^{a} (4.66)	100± 0.01^{a} (0.50)	110± 0.01^{b} (1.69)
CL-2	210 ± 0.02^{c} (3.23)	90 ± 0.01^{b} (0.45)	100± 0.01^{b} (1.69)
CL-3	250 ± 0.02^{b} (3.84)	110± 0.02^{a} (0.46)	120 ± 0.02^{a} (1.84)

Oil contents (%) = weight of oil / weight of callus used for extraction × 100, Values in parenthesis are the percentages of oil contents, CL-1: 2, 4-D 8 µM, CL-2: 2, 4-D 10 µM, CL-3: 2, 4-D 10 µM + 2 µM BAP

Mean values (±SE) indicated with small alphabetical different letters are significantly different as per DMRT (Duncan's multiple range test $p \leq 0.05$).

The present study demonstrated that medium composition of CL-3 was most effective for callus induction and callus FW production from leaf explants. Whereas, CL-1 produced highest amount of lipid contents (290 mg/6gm FW of callus) from cotyledon explants. Chemical characteristics of lipids in terms of acid value (0.320), percent free fatty acids (0.160) and saponification number (91) were highest from cotyledonary explant grown at CL-1 followed by CL-2 and CL-3 (Table 3). The amount of acid value was ranged from 0.320-0.201, FFA 0.160-0.10 and saponification number 91 to 88 in the present study. We observed that callus induction from internodal explant had smallest amount of all parameters that decreased from CL-1 to CL-3. In the contemporary literature, information vis-à-vis indigenous determination of oil in callus cultures is completely lacking in jojoba.

Table 3. Chemical properties of callus oil from different explants of jojoba

Callus source		Acid value (Meq Kg^{-1})	%Free Fatty Acid (FFA)	Saponification number
Cotyledon	CL-1	0.320 ± 0.110^{a}	0.166 ± 0.045^{a}	91 ± 4.25^{a}
	CL-2	0.290 ± 0.013^{b}	0.150 ± 0.041^{ab}	91 ± 3.25^{a}
	CL-3	0.250 ± 0.045^{c}	0.131 ± 0.025^{ab}	89 ± 6.33^{ab}
Internode	CL-1	0.210 ± 0.045^{cd}	0.112 ± 0.036^{abc}	89 ± 4.25^{ab}
	CL-2	0.201 ± 0.033^{d}	0.101 ± 0.045^{bc}	89 ± 6.55^{ab}
	CL-3	0.201 ± 0.021^{d}	0.101 ± 0.025^{bc}	88 ± 7.88^{ab}
Leaf	CL-1	0.220 ± 0.041^{cd}	0.115 ± 0.074^{abc}	89 ± 6.56^{ab}
	CL-2	0.215 ± 0.036^{cd}	0.110 ± 0.042^{abc}	89 ± 6.22^{ab}
	CL-3	0.217 ± 0.085^{cd}	0.112 ± 0.036^{abc}	88 ± 4.66^{ab}

Mean values (±SE) indicated with small alphabetical different letters are significantly different as per DMRT (Duncan's multiple range test $p \leq 0.05$).

Secondary metabolites are reserve food materials stored within the plant organs such as leaves, stems, roots or seeds. Parenchymatous tissues are the key components where these metabolites accumulate in the form of various chemical constituents of lipids or carbohydrates. A callus is an amorphous parenchymatous mass of cells gathers metabolites as predisposition until regenerated. Growth regulators, specifically auxins control the cell division activity and intricate metabolites production. Whereas gamma radiations have also been reported for increased wax oil contents in calluses of jojoba (El-Shabrawi *et al.* 2019). We obtained highest callus growth on 2, 4-D (8 or 10 µM) supplemented media. Farhadi *et al*. (2017) also achieved similar results by using 2, 4-D in *Allium hirtifolium*. On the other hand, El-Shabrawi *et al.* (2019) obtained highest callus induction of jojoba with 0.5 mg/l kinetin + 6% sucrose. Similar to our results, Kumar *et al*. (2012) reported 97.3% callus induction from field collected leaf explants of jojoba on MS medium supplemented with 2 mg/L 2,4-D + 0.5 mg/L BAP + 100 mg/L casein hydrolysate after 22 days of initial culture. In the present study, all concentrations of NAA induced callus albeit the higher levels 8 µM or 10 µM seemed detrimental. The negative effect of NAA on callus growth has been reported in *Zingiber officinale* (El-Nabarawy *et al*. 2015). Leaf explants produced proliferating calluses similar to Arce and Jordan (1988) with 5 mg/L NAA + 0.1 mg/L BAP. The explants containing one node and a shoot tip seemed vital for invigorating the axillary shoots of jojoba cultured on MS medium supplemented with BA or Kin alone (10 mg/L) or in combination with 0.2 mg/L IBA (Rossi 1996). Similarly, 2 mg/L BAP induced callus formation after 14 days and sub-cultured on B_5 + 1 mg/L 2, 4-D + 0.1 mg/L Kin as compared to the callus induction from leaf explants on B_5 + 1mg/L 2, 4-D + 0.1 mg/L Kin (Jabeen 1999). These reports showed explant's response similar to our findings albeit we used MS medium + 8 µM 2, 4-D or 2 µM BAP.

Authors demonstrated that cotyledon was good enough for callus induction whereas nodal explant seemed recalcitrant. Generally, calluses obtained on the combination of both auxin and cytokinin has much more potential for metabolite production (Verma *et al*. 2016) and hard tissues are less responsive for callus induction under in vitro conditions (Asakuraa and Hoshino 2017).

Cotyledonary structures of somatic embryos have been reported for the determination of liquid wax likely that of jojoba seeds (Lee and Thomas 1985). We extracted lipids from calluses of seedling tissues and greenish cotyledons of germinating seeds, whereas Aftab *et al*. (2008) used 6 to 1-year-old plant tissues and mature cotyledons of jojoba. They reported higher fixed oil contents (1502 mg) from calluses of cotyledons as compared to us. This may be due to the difference of satiated plant material at the time of collection. Palmer *et al*. (1994) support this phenomenon that older calluses retained maximum amount of oil than fresh ones. Explant collection from different sources plays fundamental role on the production of lipid contents (Palmer *et al*., 1994). In contrast, traces of lipid contents from the older callus tissues have been reported in the present study. It seems that the proliferating calluses from different explants usually keep the capacity to produce maximum amount of lipids to that of intact plants.

FFA and acid value are important indicators for the quality of the jojoba oil. We obtained higher value of the biochemical parameters of jojoba callus cultures. High acid value demonstrated that oil has more carbon residue resulted more combustion potential in the diesel engines. FFA composition, acid value, palmitic acid and linoleic acid have been reported from callus cultures of *Ajuga* (Sahakyan *et al.*, 2010). Identification of high amount of FFA and other parameters in the present study demonstrated that jojoba oil may become a renewable energy source and substitute of conventional diesel (Durrett *et al*., 2008; Correa and Atehortúa, 2012).

CONCLUSION

It was observed that 2, 4-D was the best auxin for high yielding callus production from green cotyledons of jojoba. Moreover, such calluses may also provide the basis for subsequent regeneration for improved and high yielding crop production. High amount of FFA in the oil of jojoba provides the evidence for an alternative source of energy. This is first report demonstrating the determination of biochemical parameters from callus tissues of jojoba. A very simple and reproducible method for high amount of callus production for sustainable agricultural practices.

ACKNOWLEDGEMENT

Punjab Higher Education Commission (PHEC), Lahore, Pakistan is gratefully acknowledged for the award of travel grant to first author to present this research in the Symposium “Agrosym 2021” at Bosnia Herzegovina.

REFERENCES

Aftab F., Akram, S., Iqbal J. (2008). Estimation of fixed oils from various explants and *in vitro* callus cultures of jojoba (*Simmondsia chinensis*). Pakistan Journal of Botany, vol. 40, pp. 1467-1471.

Arce P., Jordan M. (1988). Evaluation of regenerative responses in explant of jojoba (S*immondsia chinensis* Link) cultured *in vitro* using cuttings. Simiente, vol. 58, pp. 1-2.

Asakuraa I., Hoshino Y. (2017). Evaluation of plant regeneration ability of different explants and establishment of an efficient regeneration system using immature seeds in *Actinidia kolomikta*, a cold-hardy kiwifruit relative. Scientia Horticulturae, vol. 220, pp. 275-282.

Correa S.M., Atehortúa L. (2012). Lipid profile of *in vitro* oil produced through cell culture of *Jatropha curcas*. Journal of AOAC International, vol. 95, pp. 1162-1169.

Durrett T.P., Benning C., Ohlrogge J. (2008). Plant triacylglycerols as feedstocks for the production of biofuels. Plant Journal, vol. 54, pp. 593-607.

El-Shabrawi H.M., Wahba H.E., Gabr A.M., El-Morsy S.I., Saber M.A., Bekheet S.A. (2019). Improvement of Wax Oil Content of Embryonic Callus of Jojoba Using Gamma Radiation. Plant Tissue Culture & Biotechnology, vol. 29, pp. 207-217.

El-Nabarawy M.A., El-Kafafi S.H., Hamza M.A., Omar M.A. (2015). The effect of some factors on stimulating the growth and production of active substances in *Zingiber officinale* callus cultures. Annals of Agricultural Sciences, vol. 60, pp. 1-9.

Farhadi N., Panahandeh J., Azar A.M., Salte S.A. (2017). Effects of explant type, growth regulators and light intensity on callus induction and plant regeneration in four ecotypes of Persian shallot (*Allium hirtifolium*). Scientia Horticulturae, vol. 218, pp. 80-86.

Hussain G., Bashir M.A., Ahmad M. (2011). Brackish Water Impact on Growth of Jojoba (*Simmondsia Chinensis*). Journal of Agricultural Research, vol. 49, pp. 591-596.

Jabeen F. (1999). *In vitro* regeneration of jojoba (*Simmondsia chinensis*). M.Sc. thesis, University of the Punjab, pp. 1-86.

Kumar S., Mangal M., Dhawan AK., Singh N. (2012). Biotechnological advances in jojoba [*Simmondsia chinensis* (Link) Schneider]: recent developments and prospects for further research. Plant Biotechnology Reports, vol. 6, pp. 97-106.

Lee C.W., Thomas J.C. (1985). Jojoba embryo culture and oil production. Horticulture Sciences, vol. 20, pp. 762-764.

Murashige T., Skoog F. (1962). A revised medium for rapid growth and bioassay, with tobacco tissue culture. Physiologia Plantarum, vol. 15, pp. 473-497.

Palmer C.E., Keller W.A., Vasil I.K. (1994). *In vitro* culture of oil seeds. In: Thorpe, T. A. (eds) Plant Cell and Tissue Culture, Springer Netherlands, pp. 413-455.

Rossi C. (1996). Rooting and acclimatization of micropropagated jojoba seedlings. Proceedings of the 9th International Conference on new industrial crops and products, pp. 25-30.

Verma S.K., Das A.K., Cingoz G.S., Uslu E., Gurel E. (2016). Influence of nutrient media on callus induction, somatic embryogenesis and plant regeneration in selected Turkish *Crocus* species. Biotechnology Reports, vol. 10, pp. 66-74.

Sahakyan N., Petrosyan M.T., Popov G.Y., Volodin V.V., Matistov N.V., Gruzdev I.V., Shirshova T.I. (2010). Content of neutral lipids and fatty acids in callus cultures and leaves of intact plants of *Ajuga genevensis* and *Ajuga chia*. Biotechnology and Biotechnology Equipments, vol. 24, pp. 87-90.

Steven J.P. (2000). A Natural History of the Sonoran Desert. In: Patricia Wentworth Comus (eds.), University of California Press, pp. 256-257.

Tietel Z., Kahremany S., Cohen G., Ogen-Shtern N. (2021). Medicinal properties of jojoba (Simmondsia chinensis). Israel Journal of Plant Sciences. http://dx.doi.org/10.1163/22238980-bja10023.

Wisniak J. (1987). The Chemistry and technology of jojoba oil, American Oil Chemist's Society, Champaign, IL, USA.

Original Scientific paper
10.7251/AGRENG2201090E
UDC 630*323:582.632

IMPROVEMENT OF SEED GERMINATION AND SEEDLING RESISTANCE OF BEECH (*FAGUS SYLVATICA*) BY GROWTH REGULATORS

Dina ELISOVETCAIA[1*], Raisa IVANOVA[1], Natalia MASCENCO[1], Jan BRINDZA[2]

[1]Institute of Genetics, Physiology and Plant Protection, Republic of Moldova
[2]Slovak University of Agriculture in Nitra, Department of Genetics and Plant Breeding, Slovak Republic
*Corresponding author: dina.elis.s@gmail.com

ABSTRACT

Beech seeds are characterized by a strong exogenous and deep physiological dormancy. They need a long period of stratification, but even through stratification, seed germination is greatly extended in time. The aim of this study was to accelerate the seeds germination and improve the resistance of seedling by application of different growth regulators (gibberellic acid, capsicoside and genistifolioside). The concentration of growth regulators has been modified from 0.1% to 0.001%. The mean daily germination, mean germination time, speed of germination and total germination of seeds were evaluated. The best results of increase in daily germination (up to 18.5%) were obtained under influences of capsicoside and genistifolioside at concentration of 0.001%. Moreover, the time of total seeds germination was reduced by 20-22 days, which allowed for an earlier sowing of germinated seeds. The beneficial effect of treatment with growth regulators was also established when the germinated seeds were transferred to the soil by better adaptation and development of seedlings. The emergence rate and survival rate of beech seedlings increased 2.5-3.3 times. The use of growth regulators affected the morphometric parameters. The leaves from control seedlings were significantly smaller than in the variants with capsicoside and genistifolioside treatment – shorter by 1.7-2.9 cm and narrower by 0.3-1.3 cm. The leaves from seedlings treated with gibberellic acid were 1.5-1.9 times narrower than in the others variants. The relative chlorophyll index determined in the phase of three pairs leaves on seedlings treated with capsicoside and genistifolioside significantly exceeded the control (by 9.2-24.1 g/m^2).

Key words: *Fagus sylvatica, seed germination, seedlings adaptation, capsicoside, genistifolioside.*

INTRODUCTION

The European beech *Fagus sylvatica* L. is one of the main forest-forming trees in the Republic of Moldova. In order to help preserve clean air and ecology, forests should be at least 15% of the entire territory. At the same time, the forest fund in the republic has been rapidly decreasing over the past decades: only 6.7% of forests remain in the southern zone of the Republic of Moldova, 7.2% in the north, and 13% of the entire territory in the center (http://www.moldsilva.gov.md). The main reason for the decline in the forest fund is the rapidly growing deforestation. Another important factor that negatively affects beech forests is climatic changes, which entail an increase in the average annual temperature and a decrease in precipitation (Republic of Moldova Forest Policy Note, 2014). Forest beech is one of the most vulnerable forest-forming species in terms of temperature parameters and is picky about high moisture content in the ambient air (Dolschak *et al.*, 2019). Currently, serious measures are being taken in the Republic of Moldova to preserve forest lands: a draft law on a three-year moratorium on deforestation has been developed (The draft law "On the establishment of a moratorium on timber harvesting", 2020). However, limiting deforestation and natural regeneration of beech forests is not enough. It is necessary to ensure the availability of sufficient quantities of beech seeds for planting in nurseries and for increasing seedling production.

Fagus sylvatica seeds are deeply dormant seeds (Kolařova *et al*., 2010). Most authors agree that in order to successfully interrupt the dormant period, beech seeds need cold stratification (+ 3 - 4 ºC) at a moisture content of 28-30% for at least 12 weeks, which can last up to 17-20 weeks. However, prolonged cold stratification can also lead to a loss of seed viability. Therefore, the problem of reducing the dormancy period of beech seeds is one of the most important tasks at the present stage, which scientists from many European countries are trying to solve (Staszak *et al*., 2019)

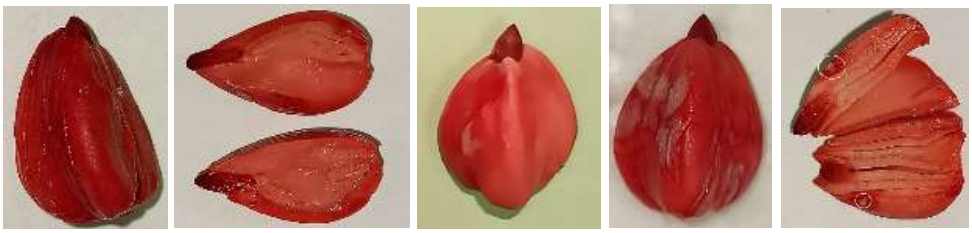
The purpose of our research was to study the effect of natural growth bioregulators (genistifolioside and capsicoside) on increasing the germination of European beech seeds and the survival of seedlings in a greenhouse.

MATERIALS AND METHODS

The experiments were carried out in the Laboratory of Natural Bioregulators of the Institute of Genetics, Physiology and Plant Protection, Republic of Moldova during 2020. The seeds of European beech *Fagus sylvatica* L. (Fagaceae) were collected in the autumn of 2019 from beech stand in the Slovak Republic (Tribeč Mountains, Western Carpathians). The beech seeds were dried at ambient temperature and humidity until they reached a moisture content of 8-10% (fresh weight basis) and were stored at the temperature of $+4\pm1^{o}$C in polyethylene bags, placed in plastic containers.

The viability of seeds was determined by two tests using the 2.3.5-triphenyltetrazolium chloride (2.3.5-TTC) solution (Kerkez *et al.*, 2018) and hydrogen peroxide (HP) solution (Sharma and Sibi, 2020). In each test, 4 replicates

were used – in the TTC test 25 seeds, in the HP test – 30 seeds per replicate. In the TTS test, the germination percentage or viability was calculated on the basis of the total number of viable (complete staining of the cotyledons and radicle in bright red color) and conditionally viable (at least 2/3 of the basal part of the cotyledon stained with stained radicle) seeds (Figure 1). In the HP test viability was calculated on the basis of the sum of all germinated seeds – both with a radicle of equal and more than 2 mm (Figure 2).



a b c d e

Figure 1. Staining *Fagus sylvatica* seeds in the TTC test: a) completely viable seed with bright red staining; b) completely viable fully stained seed in horizontal cut; c, d) partially stained seeds that may produce either normal or abnormal seedling; e) partially stained seeds in horizontal cut – the circles indicate the infection sites.

a b c

Figure 2. The degree of germination of radicle in *Fagus sylvatica* seeds in the hydrogen peroxide test (a – radicle more than 2 mm; b, c – radicle equal to 2 mm).

The germination test (four replicates of 80 seeds each) were carried out in accordance with the recommendations of the International Seed Testing Association (ISTA, 2006). The germination of *Fagus sylvatica* seeds was stimulated by their treatment with bioregulators - 0.1-0.001% solutions of capsicoside and genistifolioside for 22-24 hours. Genistifolioside (the sum of iridoid glycosides) and capsicoside (the sum of steroid glycosides) were obtained from the aerial part of the *Linaria genistifolia* (L.) Mill and from the seeds of the *Capsicum annuum* L. according to the described techniques (Mascenco *et al*., 2015; Borovskaia *et al.*, 2020). Distilled water was used as a control, and 0.02% solution of gibberellic acid was used as a standard. Seed stratification was carried out at moisture content of 30% and temperature of +4±1 °C until germination. The appearance of radicles was defined as germination (Figure 3). The daily germination counts were made on the seeds until no further germination occurred (for up to 112

days). The following observations: total germination percentage, mean daily germination, germination rate index were made (Al-Ansari and Ksiksi, 2016). Data are presented as the means and standard deviation of four biological replicates. The relationship between particular parameters was examined using Pearson's correlation coefficient analysis.

a b c

Figure 3. The degree of radicle development in different variants of *Fagus sylvatica* seeds treatment for the same period of time: a – control; b – 0.001% capsicoside; c – 0.02% gibberellic acid.

Seedling emergence studies were carried out in a greenhouse conditions, namely seasonal fluctuation of temperature, natural ventilation and drip irrigation. A mixture of white peat with a pH of 5.5 and soil 1:1 was used as a substrate. Seeds were sown as they were germinated.

The assessment of the total proportion of emergent seedlings and development of plants continued until the appearance of the first and second pairs of true leaves. During the growing season two foliar treatments with aqueous solutions of bioregulators (0.1-0.001%) were applied to the seedlings (in the growth phase of the second pair of true leaves and one month after the first treatment). Seedlings treatment with water and gibberellic acid solution (0.02%) was used as control and standard, respectively.

Relative chlorophyll content was quantified as the chlorophyll content index (CCI), measured for the first and second pair of true leaves with the FIELD SCOUT CM1000TM Chlorophyll Meter (Spectrum Technologies, Inc., USA). For each leaf, ten measurements on different locations were taken, avoiding the mid-vein (Van Wittenberghe, 2012).

RESULTS AND DISCUSSION

The viability of seeds of *Fagus sylvatica* L., determined by TTC and PH tests, was on average 69.50% and 61.68%, respectively. Mathematical analysis of the data showed a high degree of correlation between the viability established by the TTC and total germination of seeds (Pearson correlation coefficient was equal 0.9892).

The highest rates of seed germination were obtained by treating seeds with solutions of capsicoside and genistifolioside at a concentration of 0.001%. Treatment with bioregulators at higher concentrations, despite the positive effect – a decrease in the proportion of infected seeds, still leads to a decrease in seed germination compared to a concentration of 0.001%. It was noted that pretreatment of beech seeds with 0.001% capsicoside and genistifolioside contributed to a significant increase in daily seed germination (up to 18.5%) in laboratory

conditions compared to the control and standard (Table 1). Considering that the germination time of a large batch (1000 and more) of beech seeds is rather long and amounts to 90-140 days (Bonner and Leak, 2008), stimulation of daily seed germination from 0.54 (control) and 0.57 (gibberellic acid) up to 0.64 and 0.66 seeds (capsicoside and genistifolioside, respectively) leads to a significant reduction in the total period of seed germination by 20-22 days (in comparison with gibberellic acid and control). This significant reduction in germination time is very important for planting. Moreover, treatment with growth regulators depending on the duration of the stratification period, increases seed germination by 13-56% (of the total number of germinated seeds) in comparison with gibberellic acid and control.

Table 1. Effects of pretreatment on seed germination, adaptation and survival of *Fagus sylvatica* seedlings

Variant	In laboratory conditions			In greenhouse conditions	
	Mean daily germination	Total germination, %	Germination rate index	Adapted seedlings, %	Survival rate of seedling, %
Control	0.54	84.51	1.23	12.77	10.64
Gibberellic acid, 0.02%	0.57	76.12	0.89	16.33	12.24
Capsicoside, 0.001%	0.64	87.01	1.18	32.79	31.15
Genistifolioside, 0.001%	0.66	87.34	1.39	19.64	35.29

During seed stratification, it was noted that treatment with gibberellic acid 0.02% often led to lengthening and thinning of the radicle, and also promoted the germination of infected seeds (Figure 3c), which died after sowing.

The beneficial effect of treatment with capsicoside and genistifolioside 0.1-0.001% was also established during the adaptation of germinated seeds sown in a greenhouse (Figure 4). The highest rates of seedling survival were obtained from seeds and seedlings treated with solutions of capsicoside and genistifolioside at a concentration of 0.001%. The seedling emergence rate was 31.15 and 35.29%, which 2.5-2.9 times higher than in the variant with gibberellic acid, and 3.0-3.3 times higher than in the control (Table 1). In the hot summer period, when the temperature in the greenhouse sometimes reached to 48 ºC, the number of seedlings decreased in all variants. However, the proportion of seedlings that survived after foliar treatment with bioregulators capsicoside and genistifolioside 0.001 % was on average 2.7-3.0 times higher than in the variants with gibberellic acid and control (Table 1). It was revealed that the treatment with gibberellic acid leads to an increase in growth. Thus, it was found that the seeds treated with 0.001% capsicoside and genistifolioside become more resistant during the periods

of adaptation to sowing and growth in suboptimal conditions. In addition, the seedlings obtained in variants with bioregulators were distinguished by bright color of leaves, strong stem and rapid development. Thus, the presented results show the advantage of processing by these natural growth regulators, which turned out to be much more effective than gibberellic acid.

a b c

Figure 4. Germination and development of *Fagus sylvatica* in a greenhouse, treatment with 0.001% genistifolioside (a – seedling emergence, b – unfolding of the cotyledons, c – development of first pair of true leaves).

It was found that the use of bioregulators and a growth stimulator of gibberellic acid affects the morphometric parameters of beech seedlings, in particular, the leaf shape (Figure 5). Thus, in the control, the leaves were significantly smaller than in the variants with bioregulators treatment – shorter by 1.7-2.9 cm and narrower by 0.3-1.3 cm. On average, the length and width of leaves were 3.9 ± 1.5 x 2.9 ± 1.3 cm in the control, 5.1 ± 0.7 x 3.5 ± 0.4 cm and 5.3 ± 0.9 x 3.8 ± 0.9 cm in the capsicoside and genistifolioside, correspondingly.

a b c d

Figure 5. Leaf shape in different variants of treatment: a, b - gibberellic acid (seedling and leaf in autumn); c, d –capsicoside (seedling and leaf in autumn).

The gibberellic acid treatment not only promoted seed germination, but also accelerated the growth of beech seedlings by stretching internodes (Figure 5a). Moreover, gibberellic acid led to a significant change in the leaf shape, the leaves were 1.5-1.9 times narrower than the others variants (Figure 5b). The ratio of length-to-width increased 1.5 times and reached 2.3±0.6 cm instead of 1.5±0.2 cm (control and treatment with bioregulators).

Relative chlorophyll index in the phase of three pairs of true leaves from seedlings in the variants capsicoside and genistifolioside 0.001% was 144.9±3.5 and 145.6±3.7 g/m^2 respectively, that significantly exceeded the control (135.7±3.2 g/m^2) and the variant with gibberellic acid (121.5±2.9 g/m^2).

CONCLUSION

The pre-germination treatment of European beech seeds with natural growth regulators capsicoside and genistifolioside in concentration of 0.001% significantly increased the mean daily germination, total germination of seeds, adaptation and survival of *Fagus sylvatica* seedlings.

ACKNOWLEDGEMENT

The research was carried out in frame of State programs 20.80009.7007.07 „Determining the parameters that characterize the resistance of plants with the different level of organization to the action of extreme temperatures in order to reduce the effects of climate change" by financial support of the National Agency for Research and Development of the Republic of Moldova (www.ancd.gov.md). The authors gratefully acknowledge colleagues from the beech nursery "Foreste" (Nitra region, Slovak Republic), who helped us to obtain European beech seeds from the Slovak Republic.

REFERENCES

Al-Ansari F., Ksiksi T. (2016). A quantitative assessment of germination parameters: the case of Crotalaria persica and Tephrosia apollinea. The Open Ecology Journal, Vol. 9, pp. 13-21. DOI: 10.2174/1874213001609010013

Bonner F.T., Leak W.B. (2008). Fagaceae – Beech family. Fagus L. beech. In: The Woody Plant Seed Manual. United States Department of Agriculture, Forest Service, Agriculture Handbook No. 727. Editors Bonner F. T., Karrfalt R.P., editorial coordinator Nisley R.G. Washington, USDA Forest Service. pp. 520-524.

Borovskaia A.D., Mascenco N.E., Ivanova R. (2020). The effectiveness of protective-stimulating complexes (bioregulators) of plant origin. In the monograph: Ecologization of the plant protection to preserve the biodiversity of insects and pollinators. Resp. ed.: R. A. Ivanova, J. Brindza. Nitra: Slovak Agricultural University in Nitra, pp. 100-134. ISBN: 978-80-552-2278-3, doi: https://doi.org/10.15414/2020.9788055222783 (In Russian).

Dolschak K., Gartner K., Berger T. W. (2019). The impact of rising temperatures on water balance and phenology of European beech (Fagus sylvatica L.) stands. Modeling Earth Systems and Environment 5:1347–1363, https://doi.org/10.1007/s40808-019-00602-1

http://www.moldsilva.gov.md/pageview.php?l=ro&idc=180&t=/Fondul-forestier-national/Resursele-forestiere&

ISTA (International Seed Testing Association). (2006). International Rules for Seed Testing, Edition 2006/1, Bassersdorf, Switzerland.

Kerkez I., Pavlovic S., Lucic A., Devetakovic J., Sijacic/Nikolic M., Popovic V. (2018). Different methods for beech seed quality testing. Sustainable forestry. Collection 77-78, Institute of Forestry, Belgrade, pp. 1-10.

Kolařova P., Bezděčková L., Prochazkova Z. (2010). Effect of gibberellic acid and ethephon on the germination of European beech dormant and chilled beechnuts. Journal of Forest Science, Vol. 56(9), pp. 389-396.

Mascenco N., Gurev A., Lupascu G., GorincioI E. (2015). Iridoid glycosides from Linaria genistifolia (L.) Mill in biological control of soil-borne fungal pathogens of wheat and some structure consideration. Chemistry Journal of Moldova. General, Industrial and Ecological Chemistry, Vol. 10 (1). pp. 57-63. ISSN 1857-1727.

Republic of Moldova Forest Policy Note. Report No: ACS11526. (2014). Europe and Central Asia Region, Environment and Natural Resources Global Practice. International Bank for Reconstruction and Development/The World Bank. 44 P.

Sharma S., Sibi G. (2020). Seed germination and maturation under the influence of hydrogen peroxide-a review. Journal of Critical Reviews, Vol. 7, Issue 1, pp. 6-10. ISSN- 2394-5125

Staszak A.M., Pers-Kamczyc E., Klupczyńska E.A., Pawłowski T.A. (2019). Expression of abscisic and gibberellic acid signalling factors in Fagus sylvatica L. seeds during dormancy breaking and germination. Dendrobiology, Vol. 81, pp. 22-30. http://dx.doi.org/10.12657/denbio.081.003

The draft law "On the establishment of a moratorium on timber harvesting". Registration date: 6/5/2020. Authors: Ivanov V., Vitiuc V., Fotescu V., Himici I., Apostolova R., Ulanov D. http://parlament.md/ProcesulLegislativ/Proiectedeactelegislative/tabid/61/LegislativId/5080/language/en-US/Default.aspx

Van Wittenberghe S., Adriaenssens S., Staelens J., Verheyen K., Samson R. (2012). Variability of stomatal conductance, leaf anatomy, and seasonal leaf wettability of young and adult European beech leaves along a vertical canopy gradient. Trees, Vol. 26, pp. 1427–1438. DOI 10.1007/s00468-012-0714-7

Original Scientific paper
10.7251/AGRENG2201098P
UDC 582.736.3

SELECTION AT ULTRA-LOW DENSITY OF SECOND GENERATION LINES OF BEAN CULTIVARS UNDER WATER STRESS

Fokion PAPATHANASIOU[1*], Ioannis MYLONAS[2], Ilias AVDIKOS[3], Olga PETIDOU[1], Elisavet NINOU[4], Fotini PAPADOPOULOU[1], Ioannis PAPADOPOULOS[1]

[1]University of Western Macedonia, Department of Agriculture, Terma Kontopoulou, 53100 Florina, Greece
[2]Hellenic Agricultural Organization-Demeter, Institute of Plant Breeding and Plant Genetic Resources, Thermi, GR-57001 Thessaloniki, Greece
[3]Aristotle University of Thessaloniki, Laboratory of Genetics and Plant Breeding, School of Agriculture, 54124 Thessaloniki, Greece
[4]International Hellenic University, Department of Agriculture, Sindos, 57400 Thessaloniki, Greece
*Corresponding author: fpapathanasiou@uowm.gr

ABSTRACT

Selection in the absence of competition under ultra-low density has been proposed as a mean to develop new cultivars serving the needs of a sustainable agriculture under diverse climatic conditions. In 2018, individual high-yielding plants of the determinate type bean varieties (*Phaseolus vulgaris* L.) Iro and Pirgetos were selected in R21 honeycomb design trials under normal and deficit irrigation treatments. This led to 14 high-yielding second generation lines from cultivar Iro and four from cultivar Pirgetos which together with the original varieties were evaluated again in 2019 under the same ultra-low density and irrigation conditions. Water stress affected total chlorophyll content measured in selected individual plants from start of flowering until physiological maturity with some of the second generation lines showing similar or higher chlorophyll concentrations from the original varieties especially during the seed filling stage. Significant differences between normal and deficit irrigation were also shown on CO_2 assimilation rate for all genotypes, but there were no significant differences between the evaluated progenies and their respective ancestors. Compared to the original variety Iro, four of the second generation lines had similar or higher yield plant^{-1} (up to 15%) and exhibited the highest stability under both normal and water deficit conditions, whereas for the variety Pirgetos two progenies outperformed the original cultivar (by 5,5 to 27%). Further research is under way for a final evaluation of the selected progenies under farming density and water stress.

Keywords: *Ultra-low plant density, Water stress, Chlorophyll concentration, CO_2 assimilation rate.*

INTRODUCTION

Common bean (*Phaseolus vulgaris* L) is one of the most significant legume crops for human consumption due to its high protein content, fiber and other essential minerals (Graham and Ranalli, 1997). In Greece, beans are the most important pulses with increased cultivated areas in recent years (Kargiotidou *et al.,* 2019). They are cultivated during spring and summer and the most prominent area is in the north of Greece, specifically at relatively high altitudes and cool temperatures. Modern agriculture depends by far on uniform crop varieties in order to meet a growing demand for food by the world's population, and in most cases several landraces have progressively been replaced by elite cultivars satisfying the farmers and consumer's needs (Mavromatis *et al.,* 2007). The existence of genetic heterogeneity in Greek genotypes is offered for plant selection with new breeding strategies and main criterion plant yield while such germplasms could broaden the genetic base of commercial beans for developing high-yielding varieties with an improved performance under different environmental constraints (Papadopoulos *et al.,* 2007).

Honeycomb breeding has been suggested as an effective technique to exploit the genetic variability of gene pools like heterogeneous landraces or even early released varieties (Tokatlidis, 2015). This has been experimentally validated in single-plant progeny lines derived from two dry bean landraces of indeterminate type that were proved to be tolerant to heat stress and improved in yield capacity up to 38% (Tokatlidis *et al.,* 2010), as well as in a lentil landrace grown under low-input conditions (Kargiotidou *et al.,* 2014).

Water scarcity raises a soaring concern in the Mediterranean region, as higher temperatures and more frequent drought events are projected to occur due to climate change (Cammarano *et al.,* 2019). Water deficit is a major limiting factor for crop productivity worldwide resulting in significant seed yield reductions across 60% of global bean production areas (Soureshjani *et al*., 2019). Reduced water availability affects plant physiological responses resulting in a reduction in photosynthesis and transpiration rate, intercellular carbon dioxide concentration and activates stomatal closure by the accumulation of abscisic acid (ABA) causing growth inhibition and reduced plant productivity (Mathobo *et al.,* 2017; Soureshjani *et al.,* 2019). Chlorophyll content of common bean is also reduced as a result of the degradation caused by drought conditions (Beede *et al*., 2013), and is directly related to biomass accumulation. These responses depend on the intensity of the stress, the plant genotype, and the plant developmental stage at stress incidence, among other factors (Beebe *et al.,* 2013). The necessity to tackle this challenge has led to breeding and developing new varieties adapted to a continuously changing environment either exploiting intraspecific variability or by transferring genes from closely related wild species adapted to low irrigation (Martinez *et al.,* 2007). Extensive evidence exists to show that genetic resources for drought tolerance have potential for breeder programs (Andrade *et al.,* 2016; Farooq *et al.,* 2017).

The main objective of the present study was to evaluate high-yielding second generation lines of two bean varieties selected at ultra-low density under water deficit conditions during anthesis and seed-filling developmental stage. The range of variation in agronomic and physiological parameters that could exist would be utilized in further experiments under farmer's densities for identifying and developing improved genotypes which could perform better under adverse conditions.

MATERIALS AND METHODS

Plant material and experimentation

In 2018, individual high-yielding plants of the determinate type bean varieties (*Phaseolus vulgaris* L.) Iro and Pirgetos, developed originally by the Hellenic Industrial and Fodder Crops Institute, were selected in R21 honeycomb design trials under normal and deficit irrigation treatments in the main farm of the University of Western Macedonia in Florina as described in previous work (Papathanasiou *et al*., 2019). This led to 14 high-yielding second generation lines from cultivar Iro (coded IR1 to IR14) and four from cultivar Pirgetos (coded PIR1 to PIR4) which together with the original varieties and an imported one Great-northern type (GNTY) were evaluated again in 2019 under the same ultra-low density and irrigation conditions. Approximately 50 plants per second generation line were assessed in two R21 honeycomb design trials under normal and deficit irrigation treatments respectively. The experiments were sown on 11^{h} of May in the experimental farm of the University of W. Macedonia in Florina Greece (40°46′ N, 21°22′E, 707 m asl), in a sandy loam soil with pH 6.5, organic matter content 14.0 g kg^{-1}, N-NO_3 100 mg kg^{-1}, P (Olsen) 50.3 mg kg^{-1} and K 308 mg kg^{-1} and water holding capacity 21.8% (0 to 30 cm depth). The ultra-low density of 1.2 plants/m^2 was used i.e. single-plant hills were spaced 100 x 100 cm apart. Two or three seeds were sown in each hill and later thinned to obtain single-plant hills. A total of 400 Kg/ha 0-20-0 and 200 Kg/ha 11-15-15 fertilizers were applied at planting, while additional N (50 g per plant of a 27-0-0 fertilizer) was top-dressed when plants had reached the appropriate developmental stage. Complete weed control was obtained by tilling and hand.

Irrigation treatments

The normal irrigation received a full irrigation treatment, while deficit irrigation was 50% of the normal to simulate drought stress. The irrigation treatments are fully described in Papathanasiou *et al.*, (2019).

Chlorophyll and gas-exchange measurements

Total chlorophyll content was measured with a hand-held dual-wavelength meter (SPAD 502, Chlorophyll meter, Minolta Ltd., Japan) at five 10-day intervals from start of anthesis until physiological maturity (SPAD1 to SPAD5) in six plants of each genotype in normal and deficit irrigation conditions. A portable

photosynthesis system that measures CO_2 uptake (LI-6400 XT, Li-Cor, USA) equipped with a square (6.25 cm^2) chamber was used for determinations of CO_2 assimilation rate (A), transpiration rate (E) and stomatal conductance to water vapour (g_s) during the seed filling period. Leaf gas exchange was measured in the middle leaflet of a fully expanded trifoliate leaf close to the top of the plants. Measurements were performed on the same six plants of each genotype that chlorophyll measurements were taken from 09:00-12:00 in the morning to avoid high vapor-pressure deficit and photoinhibition at midday.

Harvest and statistical analysis

Plants were harvested individually and seed yield was measured at the physiological maturity stage and recorded at a per-plant basis for both normal and deficit irrigation treatments. Comparison of means was conducted by Least Significance Difference Test (LSD) after analysis of Variance (ANOVA), for completely random design.

RESULTS AND DISCUSSION

Grain yield $plant^{-1}$, change% compared to the original genotype and stability% at ultra-low density under normal and deficit irrigation for the eighteen second generation lines and the controls are presented in Table 1. Deficit irrigation significantly reduced the yield $plant^{-1}$ in all the second generation lines and their original genotypes but with different intensity. In the normal irrigation conditions the genotypes IR1 with 134,2 g yield $plant^{-1}$, IR8 with 159,2 g and an increase compared to the control of 14,2%, IR7 with 136,2 g and 100% stability and the genotype IR2 with 139,4 g exactly the same yield as the original genotype Iro and 80,5% stability where the ones that stood out. Under the water stress conditions the 2nd generation lines that outperformed the original variety Iro in terms of yield $plant^{-1}$ were the genotypes IR12 with 63,2 g and an increase compared to the control 15,5% while its stability ranged at 94,0%, the IR4 with 60,5 g and 10,6% increase in yield $plant^{-1}$, IR8 with 60,4 g, 10,5% increase and stability 83,7%, IR2 with a yield of 56,1 g, IR1 with 55,8 g, and also the genotype IR7 which had a yield $plant^{-1}$ of 55,5 g, higher by 1,5% compared to the yield of the original variety and stability 82,8%. Considering the behavior of the 2^{nd} generation lines originating from the variety Iro in both irrigation treatments we see that the genotypes IR1, IR2, IR7 and IR8, stood out for further evaluation. Similarly, the 2^{nd} generation lines that outperformed the original variety Pirgetos either in yield $plant^{-1}$ or in stability where the genotype PIR1 with 159,2 g and an increase of 27% compared to the control in normal irrigation and 67,8 g, higher 8% than the original variety under deficit irrigation and the genotype PIR3 with a yield $plant^{-1}$ of 119,1 g and a high stability of 97,8% under the normal irrigation conditions and 66,2 g, higher by 5.5%

compared to the yield of the original variety Pirgetos and 100% stability under the water stress conditions (Table 1). This is in agreement with other studies where under adverse conditions such as high temperatures and increased biotic stress second generation sister lines of bean and/or other legumes such as lentils, outperformed the original genotypes under ultra-low density (Papadopoulos *et al.*, 2004; Kargiotidou *et al.*, 2014; Vlahostergios *et al*., 2018).

Table 1. Grain yield plant^{-1} (g), change compared to the original genotype (Change%) and stability (%) at ultra-low density under normal and deficit irrigation for the eighteen second generation lines and the controls evaluated.

	Normal Irrigation			**Deficit Irrigation**		
Second generation lines	**Yield g plant^{-1}**	**Change %**	**Stability %**	**Yield g plant^{-1}**	**Change %**	**Stability %**
IR-Control	**139,4**		**79,0**	**54,7**		**75,6**
IR1	134,2	-3,7	94,1	55,8	+2,0	83,4
IR2	139,4	0,0	80,5	56,1	+2,5	78,0
IR3	112,9	-19,0	82,1	43,8	-19,9	82,2
IR4	129,9	-6,8	69,4	60,5	+10,6	84,1
IR5	101,5	-27,2	81,9	43,3	-20,9	64,6
IR6	113,8	-18,4	67,3	43,9	-19,7	82,3
IR7	136,2	-2,3	100	55,5	+1,5	82,8
IR8	159,2	+14,2	80,5	60,4	+10,5	83,7
IR9	118,2	-15,2	91,8	52,3	-4,3	93,1
IR10	130,4	-6,4	79,1	54,4	-0,6	80,3
IR11	133,7	-4,1	74,1	54,6	-0,2	65,3
IR12	135,3	-3,0	72,6	63,2	+15,5	94,0
IR13	129,3	-7,3	71,7	47,2	-13,8	87,9
IR14	84,8	-39,2	69,8	52,8	-3,5	82,5
PIR-Control	**125,3**		**83,2**	**62,8**		**90,2**
PIR1	159,2	+27,0	96,7	67,8	+8,0	91,6
PIR2	140,7	+12,3	84,9	55,0	-12,4	86,3
PIR3	119,1	-4,9	97,8	66,2	+5,5	100
PIR4	98,4	-21,4	82,3	46,0	-26,7	85,2
GNTY-Control	**113,9**		**86,7**	**62,3**		**88,5**

Physiological parameters such as mean chlorophyll content, assimilation rate A, stomatal conductance g_s and transpiration rate E under normal and deficit irrigation are shown in Table 2 for all genotypes evaluated. Reduction in water supply was associated with decreased chlorophyll content (SPAD) during the seed filling stage in all genotypes evaluated in both irrigation treatments. In normal irrigation there were no statistically significant differences between the second generation lines and their original genotypes for SPAD1 and SPAD4 measurements. Genotype IR2 showed a statistically significantly lower chlorophyll

concentration at the level of P <0,05 (38,2) in the SPAD1 measurement compared to the initial variety Iro (44,2). Also, the lines PIR2 and PIR4 had a statistically significant reduction in SPAD1 under reduced irrigation compared to the original variety Pirgetos. At the beginning of seed filling there were no statistically significant differences in the reduced irrigation between the 2nd generation lines and the original variety Iro. Genotype PIR2 had a significantly reduced chlorophyll concentration (29,7) compared to the original Pirgetos variety (40,1). Chlorophyll content has been proposed as a good indicator of green color and the stay green characteristic under water stress is a commonly observed phenomenon (Fotonat *et al.*, 2007). All the physiological parameters measured differed statistically significant between the two irrigation treatments as shown by the ANOVA analysis (data not shown). Differences in assimilation rate, stomatal conductance and transpiration rate were observed between the 2nd generation lines and their original varieties in both normal and reduced irrigation conditions but did not differ statistically significant (Table 2).

Table 2. Mean chlorophyll content (SPAD1 and 4) during start of anthesis and early seed-filling stage, assimilation rate A (μmol CO_2 m^{-2} s^{-1}), stomatal conductance g_s (mol of H_2O m^{-2} s^{-1}) and transpiration rate E (mol of H_2O m^{-2} s^{-1}) at ultra-low density under normal and deficit irrigation for selected plants of the eighteen second generation lines and the controls evaluated.

	Normal Irrigation					**Deficit Irrigation**				
Second generation lines	**SPAD1**	**SPAD4**	**A**	**g_s**	**E**	**SPAD 1**	**SPAD 4**	**A**	**g_s**	**E**
IR-Control	**41,1**	**37,0**	**15,00**	**0,16**	**2,82**	**44,4**	**36,7**	**16,87**	**0,16**	**2,99**
IR1	41,2	34,8	17,13	0,20	3,38	42,6	31,9	12,64	0,10	2,07
IR2	47,2	44,2	20,96	0,27	4,22	38,2 *	33,1	10,95	0,12	2,15
IR3	40,6	36,3	14,27	0,15	2,89	41,7	34,8	10,32	0,07	1,40
IR4	43,5	34,5	16,78	0,19	2,89	41,4	30,3	10,83	0,10	1,92
IR5	40,1	38,7	13,92	0,13	2,55	44,7	34,5	12,40	0,10	2,22
IR6	43,1	36,6	19,66	0,28	4,00	43,7	31,1	12,45	0,11	2,35
IR7	45,9	34,9	16,64	0,16	2,79	42,7	38,4	15,10	0,15	2,67
IR8	43,0	34,5	16,27	0,27	3,65	43,6	30,4	14,04	0,15	2,73
IR9	37,6	34,7	18,99	0,28	4,27	41,9	32,2	15,46	0,13	2,94
IR10	40,3	31,1	16,22	0,15	2,77	41,8	31,6	13,29	0,12	2,24
IR11	37,7	39,6	18,95	0,24	4,15	43,6	32,1	10,50	0,12	2,28
IR12	41,7	34,0	21,88	0,31	3,89	43,9	31,6	12,80	0,11	2,54
IR13	45,0	33,4	18,27	0,22	3,34	44,7	32,5	8,67	0,05	1,32
IR14	45,3	35,5	17,92	0,23	3,53	42,4	34,3	14,69	0,17	2,75
PIR-Control	**42,8**	**32,8**	**19,67**	**0,33**	**3,85**	**46,2**	**40,1**	**14,38**	**0,14**	**2,50**
PIR1	44,5	30,5	21,67	0,35	4,46	43,1	33,6	10,49	0,09	1,82
PIR2	40,6	38,8	21,00	0,26	4,43	42,2 *	29,7 *	11,16	0,09	2,19

PIR3	43,2	34,0	17,93	0,26	3,71	43,2	30,6	14,14	0,15	2,83
PIR4	42,0	40,0	17,80	0,25	3,59	42,3 *	31,2	14,19	0,13	2,16
GNTY-Control	**46,7**	**37,3**	**15,82**	**0,16**	**2,87**	**45,9**	**43,0**	**12,98**	**0,11**	**2,21**

*, Denotes significant difference to the mother landrace (t test for independent means and different standard deviations at the level P<0,05)

CONCLUSION

The results of this study demonstrate that there is intracultivar variation mainly on seed yield under normal and deficit irrigation during anthesis and seed filling stage within second generation sister lines. Compared to the original variety Iro, four of the second generation lines had similar or higher yield plant^{-1} (up to 15%) and exhibited the highest stability under both normal and water deficit conditions, whereas for the variety Pirgetos two progenies outperformed the original cultivar (by 5,5 to 27%). Further research is under way for a final evaluation of the selected progenies under farming density and water stress.

ACKNOWLEDGEMENT

Financial support of this project (project code 80164) by the Special Account for Research Grants of the University of Western Macedonia, Greece is gratefully acknowledged.

REFERENCES

Andrade E.R., Ribeiro V.N., Azevedo C.V.G., Chiorato A.F., Williams T.C.R., Carbonell S.A.M. (2016). Biochemical indicators of drought tolerance in the common bean (*Phaseolus vulgaris* L.). Euphytica 210, 277–289.

Beebe S.E., Rao I.M., Blair M.W., Acosta-Gallegos J.A. (2013). Phenotyping common beans for adaptation to drought. Frontiers in Physiology 4, 1–20.

Cammarano D., Ceccarelli S., Grando S., Romagosa I., Benbelkacem A., Akar T., Al-Yassin A., Pecchioni N., Francia E., Ronga D. (2019). The impact of climate change on barley yield in the Mediterranean basin. European Journal of Agronomy 106, 1-11.

Farooq M., Gogoi N., Barthakur S., Baroowa B., Bharadwaj N., Alghamdi S.S., Siddique K.H.M. (2017). Drought stress in grain legumes during reproduction and grain filling. Journal of Agronomy and Crop Science 203, 81–102.

Fotovat R., Valizadeh M., Toorchi M. (2007). Association between water-use efficiency components and total chlorophyll content (SPAD) in wheat (*Triticum aestivum* L.) under well-watered and drought stress conditions. J. Food Agric. and Environment 5, 225-227.

Graham P.H., Ranalli, P (1997). Common bean (*Phaseolus vulgaris* L.). Field Crops Research 53, 131–146.

Kargiotidou A., Chatzivassiliou E., Tzantarmas C., Sinapidou E., Papageorgiou A., Skaracis, G., Tokatlidis I. (2014). Selection at ultra-low density identifies plants

escaping virus infection and leads towards high-performing lentil (*Lens culinaris* l.) varieties. The Journal of Agricultural Science, 152(5), 749-758.

Kargiotidou A., Papathanasiou F. Baxevanos D., Vlachostergios D.N., Stefanou S., Papadopoulos I. (2019). Yield and Stability for agronomic and seed quality traits of common bean genotypes under Mediterranean conditions. Legume research, 42(3), 308-313.

Martinez J.P., Silva H., Ledent J.F., Pinto M. (2007). Effect of drought stress on the osmotic adjustment, cell wall elasticity and cell volume of six cultivars of common beans (*Phaseolus vulgaris* L.). European Journal of Agronomy 26, 30–38.

Mathobo R., Marais D., Steyn J.M. (2017). The effect of drought stress on yield, leaf gaseous exchange and chlorophyll fluorescence of dry beans (*Phaseolus vulgaris* L.). Agricultural Water Management, 180, 118-125.

Mavromatis A.G., Arvanitoyannis I.S., Chatzitheodorou V.A., Khah E.M., Korkovelos A.E., Goulas C.K. (2007). Landraces versus commercial common bean cultivars under organic growing conditions: A comparative study based on agronomic performance and physicochemical traits. European Journal of Horticultural Science, 72, 214-219.

Papadopoulos I., Tokatlidis I., Tamoutsidis, E. (2004). Environmental effects on phenotypic expression are blunted in greenhouse compared to open field. In the Proceedings of the 4th International Crop Science Congress, 2004 Brisbane, Australia.

Papadopoulos I.I., Tokatlidis I.S., Koutika-Sotriou M., Tamoutsidis E., Kouroubas S. (2007). Crop yield potential estimated under too low density effectively predicts performance under dense stand in dry bean. International Journal of Plant Breeding and Genetics, 1(2), 75-81.

Papathanasiou F., Papadopoulou F., Mylonas I., Ninou E., Papadopoulos I. (2019). Single-plant selection at ultra-low density of first generation lines of three bean cultivars under water stress. Agriculture and Forestry, 65(4), 27-34.

Soureshjani H.K., Nezami A., Kafi M., Tadayon M. (2019). Responses of two common bean (*Phaseolus vulgaris* L.) genotypes to deficit irrigation. Agricultural Water Management, 213, 270-279.

Tokatlidis I.S., Papadopoulos I.I., Baxevanos D., Koutita O. (2010). GxE effects on single-plant selection at low density for yield and stability in climbing dry bean. Crop Science, 50, 775–783.

Tokatlidis I.S. (2015). Conservation breeding of elite cultivars. Crop Science, 55, 2417-2434.

Vlahostergios D.N., Tzantarmas C., Kargiotidou A., Ninou E., Pankou C., Gaintatzi C., Mylonas I., Papadopoulos I., Foti. C., Chatzivassiliou E., Sinapidou A., Lithourgidis A., Tokatlidis I.S. (2018). Single-plant selection within lentil landraces at ultra-low density: a short-time tool to breed high yielding and stable varieties across divergent environments. Euphytica, 214 (58).

Original Scientific paper
10.7251/AGRENG2201106F
UDC 636.23:637.1

PRODUCTIVITY DYNAMICS AND RELATIONSHIP BETWEEN QUANTITATIVE AND QUALITATIVE INDICATORS IN COWS OF THE HOLSTEIN BREED

Valentin FOKSHA[1], Alexandra KONSTANDOGLO[1*], Vera GRANATCH[1], Igor AKBASH[2], Vasily KURULYUK[1]

[1]Scientific and Practical Institute of Biotechnologies in Zootechny and Veterinary Medicine, Republic of Moldova
[2]Society of limited liability „Doksancom" v. Tomay, ATU Gagauzia, Republic of Moldova
*Corresponding author: aliek55@mail.ru

ABSTRACT

The article presents the results of a study of the milk productivity of Holstein cows in the dynamics of lactations, correlation between the level of milk yield, fat content, and the amount of milk fat, live weight and milk ratio. The studies were carried out in the herd of the breeding farm of SLL (Society of limited liability), „Doksancom" on Holstein cows in the dynamics of 4 lactations. A comparative analysis of the yield of cows in the dynamics of lactations showed that during the second lactation, milk productivity was 934 kg of milk, the third - 959 kg of milk, the fourth - 1527 kg of milk more than in the first lactation, the difference being significant at $P < 0.01$, $P < 0.05$ and $P < 0.001$, respectively. The lactation curve of all analyzed cows of the Holstein breed of the herd of SLL „Doksancom" changed with a certain regularity and had a leveled character, characterized by a high stable type. The relationship between milk yield and the percentage of fat in milk of cows of first-fourth lactation was in negative correlation from weak (-0.187, second lactation), moderate (-0.338 – -0.486, first-fourth lactation) to noticeable (-0.557, third lactation). A negative correlation in the entire analyzed animal population was found between milk ratio and live weight, ranging from mild -0.018 to moderate -0.334. This will allow further selection of highly productive cows in the herd with an optimal live weight for the Holstein breed.

Key words: *milk productivity, fat content, lactation, live weight, correlation*.

INTRODUCTION

In modern conditions, one of the priorities is to increase the productivity of cattle, and not the increase of their number. Industrial production of milk requires a certain concentration of animals on farms, its narrow specialization, a high level of mechanization and automation of animal services. At the same time, a great attention is paid not only to production technology, but also to the quality of the livestock used for production (Gorelik *et al.*, 2014). In the dairy cattle breeding of

the developed countries of the world, the leading place is occupied by the highly productive Holstein breed. The determining factor in the qualitative transformation of cattle breeding in the Republic of Moldova is the further development of livestock breeding, the presence of highly productive dairy cattle, adapted to modern technologies, climatic and feed conditions of the republic, as well as improving the food supply and the creation of advanced technologies for keeping animals. For the last ten years, the Holstein breed of cattle began to be imported into the republic of the Dutch, German, French and other breeding, as well as cattle of a double direction of productivity - Simmental breed. As it is known, the milk productivity of cows is an important economically useful sign, one of the main indicators of animal husbandry. As a biological feature, it fluctuates depending on a number of factors, such as: season of the year, lactation in a row, membership in genealogical lines, generation and types. When breeding cows of dairy breeds and especially Holstein breed, it is taken into account the complex of economically useful traits, it is paid attention to the productivity and quality of milk - milk fat and protein milk. Thus, under optimal feeding and keeping conditions, milk yield of Holstein cows in the breeding herd of SLL "Doksancom" amounted to 8980 kg of milk (first lactation), 10082 kg (second lactation) and 10530 kg (third lactation) milk per lactation (Foksha and Konstandoglo, 2019). The realization of the genetic potential for milk production was higher for the third lactation and amounted to 108.3%, which is on average by 7.1% more than at the animals of the first two lactations. Milk productivity of cows is the result of the interaction of a complex of physiological processes of the body, which are controlled by many gene systems and determine the hereditary status of the breed. The study of the relationship between economic and useful traits has a great importance for breeding and pedigree work, as these dependencies can be used in the selection of animals of the desired types in the process of creation (Nicoro *et al*., 1968). In most cases, according to Belyaev (1966), correlations between characters arise on the basis of the pleiotropic effect of not one, but many genes that make up the gene systems that have developed during the evolution of animal species and breeds. Therefore, depending on the genotypes of individuals, the selection of pairs, changes are observed in the correlation between the characters. Correlations due to the pleiotropic effects of genes are called genetic correlations, and all other cases of correlations are called phenotypic correlations. The relationship between the signs is measured by the correlation coefficient, while the correlation is observed between both quantitative and qualitative signs (Stenkin and Mulyanov, 2014; Abrompolsky and Abylkasymov, 2005; Gaidukova and Tyutyunikov, 2013). In dairy cattle breeding, the most important is the identification of the nature and magnitude of correlation between the level of milk yield and the mass fraction of fat in milk. The correlation between milk yield and the mass fraction of fat and protein in milk is usually negative (Ruzsky, 1982; Osipenko *et al*., 1985; Yeghiazaryan and Braginets, 2010; Smith and Omoas, 1984; Sonderegger, 1986; Vleck, 1985; Abrompolsky and Abylkasymov, 2005). Therefore, in each individual case, it is necessary to determine the form, direction and degree of correlation

(Dautbaev, 1995; Egiazaryan and Braginets, 2010; Stenkin and Mulyanov, 2014). The data of many scientists confirm the positive relationship between milk productivity and live weight, and the fact that bigger animals have greater milk productivity (Kutrovsky, 2006, 2007; Brilling, 1985; Ratheises, 1972). According to many scientists and practitioners of livestock science, the relationship between milk yield, qualitative indicators of milk with age is positive (Shmeleva and Basonov, 2014; Wilver, 2015).

The aim of our research is to study the milk productivity of Holstein cows in the dynamics of lactation, the correlation between the level of milk yield, fat content, and the amount of milk fat, live weight and milk ratio.

MATERIAL AND METHODS

The studies were carried out in the herd of the SLL "Doksancom" breeding farm on Holstein cows imported from Holland. The main data on the milk production of animals were taken from forms of zootechnical and pedigree accounting. All the analyzed number of cows was kept in optimal conditions of feeding and keeping in accordance with the basic zootechnical and hygiene requirements. Were used zootechnical research methods with biometric processing of materials by the method of variation statistics according to Plohinsky (1978) and Merkurieva (1983): arithmetic mean (X), arithmetic mean error (Sx), coefficient of variability (Cv), correlation coefficient (r) and correlation coefficient error (m). Lactation curves of cows were constructed, and was calculated the milk coefficient (MC) proposed by Startsev (1965) using the formula: MC = MY / LW, where MC is the milk coefficient, kg; Y- milk yield for 305 days of lactation, kg; LW - live weight, kg. The relationship between milk productivity indicators and milk quality, live weight was determined by calculating the correlation coefficient using Microsoft Excel, the reliability of the indicators was determined by Student.

RESULTS AND DISCUSSION

Indicators of milk productivity, fat content in the milk of cows of the breeding farm of SLL "Doksancom" in the dynamics of four lactations are presented in Table 1.

Table 1. The dynamics of milk production of cows' of SLL "Doksancom" (X ± Sx)

Indicators	Lactation			
	first	second	third	fourth
The number of cows, n	117	65	38	26
Live weight, kg	632±3.4	667±3.6	692±2.9	685±2.8***
Milk yield, kg	10159±142	11093±302.6**	11118±378*	11686±425***
Cv	15.2	21.9	20.9	18.6
Fat, %	3.90±0.01	3.9±0.01	3.85±0.02	3.85±0.02
Fat, kg	393±5.5	429.4±11.5	426.5±13.2	432±22.3
Cv	15,2	21.5	19.2	26.3
Milking ratio, kg	1612±24	1665±45.2	1593±56.2	1705±61.7
Cv	16.0	21.9	21.7	18.5

Note: * P <0.05; ** P <0.01; *** P <0.001

It was established that cows in the first lactation had milk productivity at the level of 90.5% of full-aged cows (at a rate of 70-75%), which averaged 11217 kg of milk with a fat content of 3.87%. A comparative analysis of the yield of cows in the dynamics of lactations showed that during the second lactation, milk productivity was by 934 kg of milk, the third - by 959 kg of milk, the fourth - by 1527 kg of milk more than in the first lactation, the difference is significant at P <0.01, P <0.05 and P <0.001, respectively, second - fourth lactations. Studies have shown that cow yields increase with increasing live weight and a maximum milk yield - 11686 kg or 109% of the average is obtained with a live weight of 685 kg. Cows of fourth lactation in live weight exceeded the data obtained on average (657 kg) for the analyzed animal population by 28 kg, the difference was significant (P <0.001). The number of kilograms of milk received per 100 kg of live weight (milk yield coefficient) in the entire analyzed number of cows exceeds the norm, (the norm is up to 1000 kg). The highest milk ratios have the cows of the fourth lactation – 1705 kg of milk, the average for the sample - 1632 kg of milk. Relatively high indicators of milk ratio indicate the level of their productivity and express of the milk type. By the analysis of the variability of signs was established that the greatest variability at a positive correlation is observed between milk yield and the amount of milk fat, live weight and the amount of milk fat (Cv = 26.3; fourth lactation), also at a high positive correlation between milk yield and milk coefficient, between the amount of milk fat and milk coefficient (Cv from 16.0 to 21.9). Consequently, the highest coefficients of variability are noted for milk yield, milk fat and live weight. As it is known, the milk productivity of cows during lactation is subjected to significant fluctuations. After calving, the daily milk yield of cows' increases, reaching a maximum at 2–3 months of lactation, then gradually decreases (Katmakov, 2004), this process is graphically reflected by the lactation curve. The nature of the lactation curve depends on the maximum daily milk yield, the subsequent degree of its decrease and the duration of lactation. High yielding cows within each breed are characterized by a large increase in productivity in the second or third month of lactation and its slow decrease in the subsequent. The change in milk yield at highly productive cows of the herd of the SLL "Doksancom" is shown in the following figures of the lactation curves, Figures 1-4.

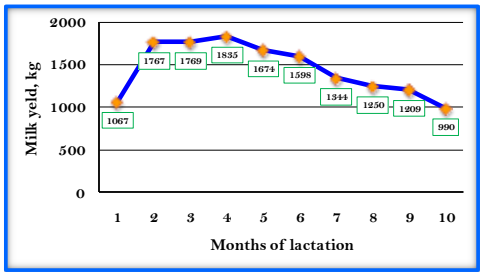


Figure 1. Lactation curve of the cow no 7320, fourth lactation, milk yield 14513 kg of milk

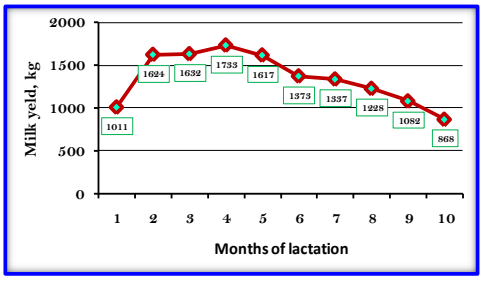


Figure 2. Lactation curve cow no 7582, third lactation, milk yield 13422 kg of milk

As it is seen from the figures, at cows no 7320 and no 7582 the lactation curve rises by the 2nd month of lactation and within 2-3 months of lactation stabilizes, by the fourth month rises to the peak of lactation. Then it gradually decreases until the end of lactation by an average of 9.6% with fluctuations from 4.5 to 18.1% (no. 7320) and by 10.6% with fluctuations from 6.6 to 19.8% (no 7582).

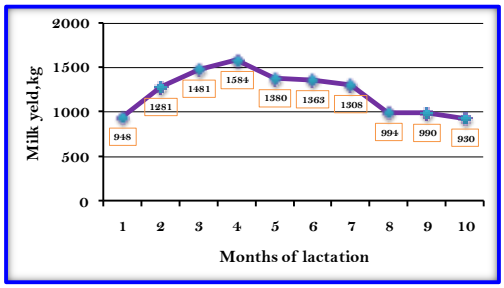


Figure 3. Lactation curve cow no 1667, fourth lactation, milk yield 12556 kg of milk

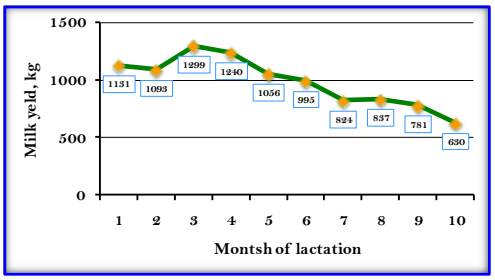


Figure 4. Lactation curve cow no 2967, first lactation, milk yield 9886 kg of milk

At cow no 1667 from the first to 4 months lactation takes place a gradual increase in the average monthly milk yield, the peak of the lactation curve falls on 4 month of lactation, then over the next months the curve gradually and smoothly decreases until the end of lactation. The lactation curve of cow no 2967 is somewhat different from the rest in that the average monthly milk yield for the second month of lactation is slightly less (by 38 kg) of the first month of lactation. The peak lactation occurs in the third month, followed by a gradual decrease until the end of lactation. It should be noted that the results of our studies are consistent with the data (Nekrasov *et al*., 2011), which also recorded the maximum average monthly milk yield for 2-3-4 months of lactation and the conclusion (Devyatov, 1983; Aldrich, 1987) that the lactation curve, regardless of productivity, has a certain optimal form with balanced feeding.

Thus, the lactation curve at all analyzed cows of the Holstein breed of the herd of SLL "Doksancom" changes with a certain regularity and has a leveled character, characterized by a high stable type, which is characteristic to animals with a strong constitution, having high milk productivity.

Of great importance in breeding work with dairy cattle has the correlation between economically useful signs. For example, the variability of the mass fraction and amount of fat in milk, as well as live weight, depend on the variability of cows' milk yield per lactation. The results of studying of the correlation between productivity indicators in herd of SLL "Doksancom" are shown in Table. 2.

The relationship between milk yield and the percentage of fat in milk of cows of the first-fourth lactation was in negative correlation from weak (-0.187, second lactation), moderate (-0.338 – -0.486, first-fourth lactation), to a noticeable one (-0.557, third lactation).

Table 2. Correlation between productivity indicators, live weight, r ± m

No	Correlated sign	first lactation	second lactation	third lactation	fourth lactation
1.	milk yield – fat, %	-0,338±0,08	-0,187±0,12	-0,557±0,14	-0,486±0,14
2.	milk yield – fat, kg	+0,891±0,02	+0,981±0,02***	+0,982±0,03*	0,721±0,02
3.	live weight – milk yield	+0,044±0,09	+0,049±0,12	-0,093±0,16	+0,089±0,2
4.	live weight – fat, %	+0,06±0,09	-0,197±0,12	+0,156±0,16	+0,109±0,2
5.	live weight – fat, kg	+0,03±0,09	+0,020±0,12	-0,065±0,16	+0,071±0,2

Note: *** P <0.001

As it is seen, unilateral selection by the level of milk yield led to an increase of the negative relationship between these signs. The presence of a negative relationship between the level of milk yield and fat content in milk makes it difficult to conduct a successful selection and indicates the need for simultaneous selection for milk yield and fat content in milk. It should be noted a high correlation between the signs of milk yield - the amount of milk fat, which in the first lactation is +0.891, second – +0.981, third – +0.982 and the fourth lactation – +0.721. By a comparative analysis of the results of the relationship between milk yield and the amount of milk fat is established a high reliable positive relationship between the second and the first (P <0.001), between the third and first lactations (P <0.05). As a result of studying the correlation between the live weight of cows and their milk productivity, a weak positive relationship was revealed for the first (+0.044), second (+0.049) and fourth (+0.089) lactations, and a weak negative (-0.093) – for the third lactation. Low correlation coefficients between milk yield for all lactations and live weight indicate the non-linear nature of the relationships between them, which characterizes the herd's uniformity in live weight. The correlation between live weight and percentage of fat, as well as the amount of milk fat was mainly positive, weakly expressed. An exception is the revealed negative relationship between live weight and the percentage of fat (-0.197) at cows of second lactation and between live weight and the amount of milk fat (-0.065) – cows of third lactation. The data of the results of studying the correlation between the milk coefficient and productivity indicators are given in Table 3. As it can be seen, the correlation between milk yield and milk ratio was positive and very high with fluctuations in lactation from 0.905 to 0.994.

Table 3. The correlation between the coefficient of milk and productivity indicators, r ± m

No	Correlated sign	first lactation	second lactation	third lactation	fourth lactation
1.	milk ratio – milk yield	+0,905±0,01	+0,980±0,01	+0,963±0,04	+0,994±0,02
2.	milk ratio – fat, %	-0,149±0,09	-0,145±0,12	-0,598±0,13**	-0,502±0,17
3.	milk ratio – fat, кg	+0,812±0,03	+0,972±0,03	+0,932±0,06	0,717±0,14
4.	milk ratio – live weight	-0,334 ±0,03	-0,148±0,12	-0,194±0,16	-0,018±0,2

Note: ** P <0.01

As it can be seen, the correlation between the mass fraction of fat and the milk ratio is negative: weak (-0.149; -0.145) – first and second, noticeable (-0.598; -0.502) - third and fourth lactations, respectively. It is noticed a significant difference between the third and the second (-0.598), third and first lactations (-0.598) at P <0.01. Therefore, selection aimed at creating a highly productive herd of Holstein cattle at SLL "Doksancom" has led to a decrease in the milk fat content of cows. The correlation between the amount of milk fat and the milk ratio is high positive (+0.717, +0.812) – fourth and first lactations and very high - (+0972, +0.932) - second and third lactations, respectively. A negative correlation was found between the coefficient of milk yield and live weight in the entire analyzed animal population, which varies from weak -0.018 (fourth lactation) – -0.194 (third lactation), to moderate – -0.334 (first lactation). This will allow further selection of highly productive cows in the herd with the optimal live weight for the Holstein breed. Thus, the established negative relationship between the mass fraction of fat and milk yield, the mass fraction of fat and the milk ratio indicates that further selection according to one of these characteristics in the herd of SLL "Doksancom" must be carried out taking into account the other.

CONCLUSIONS

The milk productivity of the cows of the herd of SLL "Doksancom" for the second lactation was by 934 kg of milk, the third was by 959 kg of milk, the fourth was by 1527 kg of milk more than for the first lactation, the difference was significant at P <0.01, P < 0.05 and P <0.001, respectively. Relatively high indicators of milk ratio indicate the level of productivity of cows and the express of the milk type. Cows of the fourth lactation have the highest milk yield coefficient - 1704.8 kg of milk, and the average milk yield coefficient in the sample was 1632 kg of milk. The lactation curve of the Holstein cows of the herd of SLL "Doksancom" changes with a certain regularity and has a leveled character, characterized by a high stable type. Low correlation coefficients between milk yield for all lactations and live weight indicate the non-linear nature of the relationships between them, which characterizes the homogeneity of the herd of SLL "Doksancom" by live weight.

REFERENCES

Abrampolsky, N.F., Abylkasymov D.A. (2005). Assessment of body type of cows and its relationship with milk productivity. "Modern technological and breeding aspects of the development of livestock in Russia", Scientific. tr All-Russian Institute of Livestock, Dubrovitsy. issue 63, vol. 1, pp. 128-132.

Aldrich J. M. (1987). How you can use lactation curves. May 25, USA.

Belyaev D.K. (1966). Genetics and problems of animal breeding. Genetics. No 10, pp. 36-48.

Brilling W. (1985). Restrictions or continue with H.F. Topagraz. No 12, pp. 6-7.

Dautbaev J.K. (1995). Efficiency of intensive cultivation and early calving of black-motley cows in the Southern Urals: Dissertation to obtain the Doctoral Degree…of Agricultural Sciences. Troitsk. 15 p.

Devyatov P.N. (1983). The use of lactationalcurves in the improvement of black-motleycattle. Scientific works All-Union Agricultural Institute of Distance Education: Ways to improve the breeding and productive qualities of cattle. Moscow. pp. 66-71.

Gaidukova E.V., Tyutyunikov A.V. (2013). Correlation of milk productivity of Kholmogory cows with the duration of the service period. Zootechnics. 9, pp. 22 - 23.

Gorelik V.S., Gorelik O.V., Rebezov M.B., Mazaev A.N. (2014). Milk productivity of cows, depending on origin. Young scientist. No 9, pp. 88-91.

Katmakov P.S., Gavrilenko V.P., Katmakova N.P. (2004). Assessment of lactationalactivity of cows. Zootechnics. No 7, pp. 22-24.

Kutrovsky V.N., Ivanova N.I., Puretsky V.M., Emelyanov V.I. (2006). The technology of growinghighly productive cows. Achievements and prospects of selection and technological AIC in the Non-Black zone of the RussianAcademy of Agricultural Sciences. Moscow, pp. 478-482.

Kutrovsky V.N. (2007). Intensive rearing of youngcattle. Scientific and technologicalprogress in animal husbandry. Machine-technologicalmodernization of the industry. Moscow, vol. 17, part 2, pp. 169-176.

Merkuryev E.K., Shangin-Berezovsky G.N. (1983). Genetics with the basics of biometrics. Moscow: Kolos.400 p.

Nekrasov R.V., Sivkin N.V., A.V. Golovin A.V, et al. (2011). Lactationalcurve of cows as a tool for workingwith the herd. Achievements of science and technology of the agro-industrialcomplex. No. 11, pp. 58-60.

Nikoro Z.S., Stakan G.A., Kharitonova N. et al. (1968). Theoretical Foundations of Breeding. Moscow: Kolos. 430 p.

Osipenko G., Romanenko N., Dmitrieva V. (1985). The use of Ayrshirecattle to increase the geneticpotentialduringcrossbreeding. Methods of increasing the geneticpotential in dairycattlebreeding. All-Union Research Institute of Genetics and Animal Breeding, Pushkin-Leningrad, pp. 116-123.

Plokhinsky N.A. (1978). Mathematical methods in biology. Moscow: Moscow State University. 265 p.

Ratheises N. (1972). Beziehungen zwischen dem Lebendgewicht der Milch und Fettleistung und der Persistenz bei Erstlingskühen des österreichischen Fleckviehs. Bodenkultur. No 4, pp. 23-25.

Ruzsky S. (1982). Selection and geneticparameters of record holders of the Kostroma and Simmental breeds. Livestock. No 4, pp. 51-53.

Shmeleva E.V., Basonov O.A. (2014). Characterization and interconnection of economicallyusefulsigns of Holstein cows of black-motleybreedcows of the Nizhny Novgorod region. Bulletin of the Ulyanovsk State Agricultural Academy: Scientific and theoretical journal. Ulyanovsk. No 4 (28), October-December, pp. 152-154.

Smith S.P., Omoas R. (1984). Productive lifespan of bull progeny groups: Failure time analysis. J. Dairy Sc. Vol. 67, no12.

Sonderegger H. (1985). Influence de l'a Faffoura genent sur le profit de la courbe de lactation at al tereur du lait. Tachetee rouge Simmental. No 4.

Startsev D.I. (1965). Direction of pedigree workwhenbreedinglivestock of Simmental breed in foreign countries. Agriculture abroad. Livestock. No 6, pp. 11-16.

Stenkin N.I., Mulyanov G.M. (2014). The relationship of milk production of highly productive Bestuzhevcowswithvariouseconomicallyuseful traits. Bulletin of the Ulyanovsk State Agricultural Academy. No 2, pp. 126–129.

Foksha, V., Konstandoglo, A. (2019). Dairy productivity of Holstein cows and realization of their genetic potential. Bulgarian Journal of Agricultural Science. 25 (Suppl. 1), pp. 31-36.

Vleck D.V. (1985). Should you be selection for triple plus bulls. Hoard's Dairyman. Vol. 30, no 5, 269 p.

Wilver, D.S. (2015). Milk productivity of cows of black-motley breed and the relationship of economically useful signs. News of the Orenburg State Agrarian University. Orenburg. No 1 (51), pp. 107-109.

Yeghiazaryan, A., Braginets, C. (2010). Interrelation of economicallyuseful traits atcowswithdifferentlevels of milk production. Dairy and beefcattlebreeding. No 2, pp. 8-10.

Original Scientific paper
10.7251/AGRENG2201115K
UDC 633.541

FEATURES OF CHEMICAL COMPOSITION AND TECHNOLOGICAL CHARACTERISTICS OF ROOT OF SUGAR BEET GENOTYPES

Oksana KLIACHENKO[1*], Larysa PRYSIAZHNIUK[2]

[1]National University of Life and Environmental Sciences of Ukraine, Ukraine
[2]Ukrainian Institute for Plant Variety Examination, Ukraine
*Corresponding author: klyachenko@ukr.net

ABSTRACT

Sugar beet breeding aimed at improving root processability involves the selection of breeding genotypes for the content of harmful non-sugars − K^+, Na^+, non-protein nitrogen − directly affecting the technological white sugar yield. The relevant are the researches on the chemical composition of plant organs of sugar beet breeding genotypes, including the content and patterns of ash, macro- and micronutrients, nitrogenous and insoluble substances distribution in order to improve beet processability. As a result of vegetation and field experiments, significant genotypic differences of the pectin substances content in sugar beet roots of Ukrainian and foreign breeding were found. The dynamics of accumulation of various forms of pectin substances in ontogenesis and the phase of development of sugar beet with the maximum accumulation of pectin and protopectin in roots were detected. The studied sugar beet genotypes were cultivated in different soil-climatic zones of the Forest-Steppe of Ukraine. Dependence of the increase in the content of pectin substances in roots from the northwest to the southeast was found. The regularities of changes in technological quality indicators during long-term storage of roots and their affection by phytopathogenic microorganisms were studied. It was proved that hybrids of foreign breeding accumulated significantly more reducing sugars and soluble nitrogenous substances during storage, which negatively affected the juice purity and increased the loss of sucrose in molasses. It was shown that varieties and hybrids of Ukrainian breeding and the hybrid Taltos (Belgian breeding) were the most resistant to affection by phytopathogenic microorganisms. Hybrids of German and Swedish breeding were characterized by a high content of rotten mass.

Keywords: *sugar beet, roots storage, technological quality.*

INTRODUCTION

In the processing of roots, sugar losses in molasses are caused mainly by soluble nitrogenous substances (α-amine nitrogen), potassium, and sodium, which inhibit the crystallisation of a certain part of sucrose (1.5−2.0%) (Yapo *et al*., 2005;

Askarova *et al*., 2017). Beet processability is also negatively affected by calcium, magnesium, trace elements, nitrate forms of nitrogen, and pectin. The latter forms hydrophilic colloids and impair the filtration of beet juice (Chee, 2008; Zaidel *et al*., 2013; Zheryakov *et al*., 2016) and storage conditions of roots at beet collection points (root loss, phytopathogenic damage). At sugar factories, short-term (2−10 days) storage of roots in adverse conditions along with simultaneous drying causes considerable sugar loss (0.15% daily) due to enzymatic sucrose splitt followed by an increased content of monosaccharides, which reduces sugar content and beet processability indicators (Balakhontsev, 1979; Boiko, 2015).

Beet processability is significantly influenced by the varietal characteristics of sugar beet and the factors that modify these indicators (Dautova & Alimgafarov, 2013). Efficient from the agro-chemical point of view beet cultivars are characterized by high nutrient absorption from fertilisers and soil, physiologically balanced systems of adsorption, ion transport and metabolism, stability of homeostasis, rational expenditure of absorbed ions for the synthesis of organic matter in photosynthetic and storage organs. Varietal charactristics are attributed to breeding and genetic factors, which, in contrast to the environmental factors and together with existing breeding methods, significantly improve the components of beet quality (processability) (Boiko, 2015).

Sugar beet plants absorb nitrogen from the soil mainly in the form of nitrates, which are subsequently converted in the process of metabolism into organic compounds (amino acids, proteins, etc.). In the process of assimilation reduction of NO_3^-, glutamine (nitrogen acceptor) is formed from ammonia and glutamate. Glutamine, transported to the roots, is the main source of amine nitrogen. Many researchers place a high emphasis on the ratio of sugar-soluble carbohydrates and nitrogen-containing compounds in beet leaves as one of the regulatory mechanisms that trigger the aging process (Kliachenko, & Funina, 2002; Roik, 2003).

Sugar beet breeding aimed at improving root processability involves the selection of breeding genotypes for the content of harmful non-sugars − K^+, Na^+, non-protein nitrogen − directly affecting the technological white sugar yield. Today, hybrid heterosis breeding of sugar beet for improving root processability is aimed at overcoming genetically determined physiological limit of sucrose accumulation in roots and a planned change in the chemical composition of plants (Kliachenko, 2002; Kliachenko, 2006; Kliachenko, 2015). It was shown that the inheritance of the content of K^+ and Na^+ ions related to the gene effects of multigerm pollinator lines with low general combination ability (Combo *et al*., 2013.

Therefore, still relevant are the researches on the chemical composition of plant organs of sugar beet breeding genotypes, including the content and patterns of ash, macro- and micronutrients, nitrogenous and insoluble substances distribution in order to improve beet processability.

The goal of the research was to evaluate the most common in modern sugar beet production monogerm varieties, di- and triploid hybrids of Ukrainian and foreign breeding for productivity, pectin content, root processability indicators as well as their resistance to phytopathogenic organisms during long-term storage.

MATERIALS AND METHODS

Vegetation experiments were carried out by the method of soil culture in 14-kg Wagner vessels at 60% of full soil water capacity. The objects of research were Ukrainian and foreign sugar beet varieties and hybrids: Bilotserkivskyi Odnonasinnyi 45, Bilotserkivskyi Odnonasinnyi 50, Yaltushkivskyi Odnonasinnyi 64, Yuvileinyi, Ivanivskyi CMS 33, Ukrainskyi CMS 70, Slovianskyi CMS 94, Reno (Germany), Tsermo (Germany), Matador (Sweden). Countings were carried out in six replications on plots of an area of 100 m^2. Determination of the sugar content in roots was performed by the polarimetric method of cold digestion according to Pochynko (Yakovets *et al*., 2007). Pectin substances in roots were determined by the volumetric method according to Rayk (Vasilieva, 2002; Hlevaskyi, 2015).

Determination of beet processability indicators was performed according to generally accepted methods (Dautova & Alimgafarov, 2013). Root samples in mesh bags were stored in stationary root storage. They were analyzed for damage by rot and reduction of technological indicators after 70 days of storage. The roots affected during vegetation were not taken to mesh bags. All experiments were conducted during 2015-2018 at experimental plots and laboratories of National University of Life and Environmental Sciences of Ukraine (Kyiv, Ukraine).

Statistical processing of the obtained experimental data was carried out using the Excel Data Analysis package.

RESULTS AND DISCUSSION

It is known that pectins are accumulated in sugar beet roots in the form of water-soluble pectin, Ca-Mg pectic acid, and protopectin, which significantly increases the accumulation of colloids (araban) and calcium salts during sugar beet processing and eventually deteriorate juice filtration and reduces quality. Particularly undesirable for sugar production is the high content of water-soluble pectin, which completely goes into diffusion juice, prevents the optimal course of the technological process, and increases sugar loss in molasses. Water-insoluble protopectin, which together with other macromolecular components constitutes a cell wall, has a high ability to swell and only partially (0.04−0.25%) passes into diffusion juice. The content of pectin substances in sugar beet roots is greatly influenced by the conditions of mineral nutrition, water availability, soil, climate, and other factors (Stepovyi & Rodionova, 2015).

As a result of our experimental studies, it was found that studied sugar beet varieties and CMS hybrids differed more in terms of the content of water-soluble pectin in the roots than protopectin. Noticeably, there was no clear relationship between the level of pectin accumulation in roots and the sugar content. Low content of pectin substances was observed both in low sugar content cultivars Uladivskyi odnonasinnyi 35, Ukrainskyi CMS 70 and high sugar content cultivar Slovianskyi CMS 94.

Table 1. The content of pectin substances and sugar in roots of sugar beet varieties and hybrids

Variety/ hybrid	Water-soluble pectin	Protopectin	Total pectin	Sugar content (% wet mass)
	(% dry matter)			
Bilotserkivskyi Odnonasinnyi 45	1.006	2.389	3.395	18.1
Yaltushkivskyi Odnonasinnyi 64	0.869	3.303	4.172	18.5
Yuvileinyi	0.988	2.951	3.939	18.2
Ivanivskyi CMS 33	0.985	2.804	3.789	18.9
Ukrainskyi CMS 70	0.666	2.929	3.595	17.9
Slovianskyi CMS 94	0.676	2.596	3.272	20.4
$LSD_{0,5}$	0.027	0.101	-	0.30

It was revealed that the processes of biosynthesis and accumulation of pectin substances in sugar beet roots intensively continues until the end of the growing season (Table 2). Regardless of the cultivar, there was an increase in the content of pectin substances, mainly due to the increase in the content of water-soluble pectin.

Table 2. The content of pectin substances in the roots of different sugar beet varieties and hybrids as affected by the harvest date

Variety/ hybrid	Water-soluble pectin	Protopectin	Total pectin	Water-soluble pectin	Protopectin	Total pectin
(% dry matter)						
	30 August			10 October		
Bilotserkivskyi Odnonasinnyi 45	1.604	2.393	3.997	2.502	2.791	5.293
Bilotserkivskyi Odnonasinnyi 64	0.867	2.883	3.750	1.765	3.280	5.045
Yaltushkivskyi Odnonasinnyi 30	0.875	3.415	4.290	1.774	3.812	5.586
Yuvileinyi	0.991	2.975	3.966	1.889	3.375	5.264
Ivanivskyi CMS 33	0.989	2.875	3.864	1.888	3.272	5.160
Ukrainskyi CMS 70	0.673	2.989	3.662	1.571	3.387	4.958
Slovianskyi CMS 94	0.689	2.631	3.320	1.588	3.030	4.618
$LSD_{0,5}$	0.031	0.115		0.043	0.131	

The level of pectin accumulation in sugar beet roots is significantly affected by harvest time. To illustrate, in late August, the content of water-soluble pectin on average, regardless of cultivar, amounted to 20−25% of the total pectin, while in

early October, the content reached 30−37% with a simultaneous slight increase in the level of protopectin.

The results of our studies of the pectin content in the roots of different sugar beet cultivars can be used in practical breeding to produce hybrids with improved root processability. The increase in the content of pectin substances in roots detected by us at late harvest dates can serve as substantiation for earlier harvest time.

One of the significant reserves for further increase in the production of sugar beet and sugar in Ukraine is to provide the industry with highly productive disease-resistant competitive monogem sugar beet hybrids (Roik, 2003). Researchers found that the yield potential of new Ukrainian hybrids is 80−90 t/ha. Alongside, sugar yield potential is not less than 18% (Roik & Kornieieva, 2010). However, recently the seeds of KWS, Dutch, Danish and Swedish breeding gained demand. Roots of foreign breeding become affected by root rot and have low sugar content when grown in different sugar beet growing regions of Ukraine (Roik & Lytvyniuk, 2010; Yakovets *et al*., 2007; Strausbaugh *et al*. 2010).

In our comparative evaluation of the most common in contemporary production monogerm diploid sugar beet varieties and hybrids of Ukrainian and foreign breeding in terms of productivity, root processability, and disease resistance during storage, the highest root yield (60.5 t/ha) and sugar yield (11.67 t/ha) was demonstrated by a Belgian hybrid Rima. Slightly lower values (57.5 t/ha and 11.37 t/ha, respectively) had German hybrid Reno. Among Ukrainian varieties and hybrids, high root and sugar yields were demonstrated by variety Yaltushkivskyi Odnonasinnyi 64, hybrids Ivanivskyi CMS 33, Lyhovsko-Verkhniatskyi CMS 21, Ukrainskyi CMS 70, and Yuvileinyi. Root yield in these cultivars ranged between 47.8 and 55.0 t/ha and sugar yield between 9.21 and 10.19 t/ha. Compared to other varieties, Uladivskyi Odnonasinnyi 35 (sugar yield 7.06 t/ha) was significantly inferior in the studied sugar beet collection. Determination of the chemical composition and technological indicators of the roots harvested in different periods revealed high-quality cultivars: hybrids 'Reno', Taltos, Ivanivskyi CMS 33, Ukrainskyi CMS 70; variety Yaltushkivskyi Odnonasinnyi 64. In these cultivars, sugar content varied with a range of 18.70−19.83%, juice purity 92.3−93.43%, sugar yield 15.60−16.91% **(**Table 3). It should be emphasized that at harvest, and especially during storage, hybrids Reno and Tsermo (Germany), Matador (Sweden) accumulated much more reduced sugars and soluble nitrogenous substances compared to Ukrainian varieties and CMS hybrids, which negatively affected juice purity and increased sugar loss in molasses.

Table 3. Chemical composition and technological indicators of different sugar beet varieties and hybrids

Variety/ hybrid	Sugar content (%)	Reduced substances (%)	K + Na	*α-amine N*	Juice purity (%)	Sugar loss in molasses (%)	Sugar yield (%)
			(Mmol/100 g of wet root mass)				

Bilotserkivskyi Odnonasinnyi 64	19.30	0.146	4.50	1.79	92.60	1.84	16.20
Yaltushkivskyi Odnonasinnyi 45	18.20	0.123	5.82	3.61	91.70	2.01	15.11
Yuvileinyi	17.40	0.148	6.07	3.05	90.80	2.13	14.14
Ivanivskyi CMS 33	18.90	0.139	4.17	2.50	93.00	1.78	15.71
Ukrainskyi CMS 70	18.70	0.106	5.05	2.28	92.30	1.90	15.60
Taltos	19.10	0.131	4.20	1.56	92.90	1.77	16,11
Reno	19.83	0.335	4.23	2.71	93.43	1.72	16.91
Tsermo	19.12	0.319	4.58	3.42	91.92	1.99	15.93
Matador	17.89	0.319	4.34	4.26	91.48	2.01	14.68
$LSD_{0,5}$	0.50	0.09	1.02	0.96	4.5	0.56	1.03

It is known that the storage stability of sugar beet is determined by the degree of damage to roots by clamp rot and decrease in technological indicators. In stable beet cultivars, the level of rotten mass after storage does not exceed 0.1%, and the purity of the clarified juice does not decrease by more than 1%, which corresponds to an increase in sugar loss in molasses by 0.2% (Yakovets *et al*., 2007; Piskureva, 2012). Our research results revealed the most resistant to phytopathogenic microorganisms cultivars: variety Yaltushkivskyi Odnonasinnyi 64 and hybrid Ukrainskyi CMS 70, in which the share of rotten mass reached 0.3−0.4%, and rotten roots 8.30−15.90% (Table 4).

Table 4. Preservation of roots of different sugar beet varieties and hybrids after storage for 70 days

Variety/ hybrid	**Roots (mass %)**		**Rotten mass (%)**
	sprouted	**rotten**	
Yaltushkivskyi Odnonasinnyi 30	42.9	11.2	0.3
Yaltushkivskyi Odnonasinnyi 64	23.8	18.2	0.8
Bilotserkivskyi Odnonasinnyi 45	51.6	20.6	1.0
Yuvileinyi	49.3	21.2	0.6
Ukrainskyi CMS 70	21.4	15.9	0.3
Ivanivskyi CMS 33	25.7	28.9	1.5
Taltos	32.8	20,6	0.6
Reno	63.2	53.4	5.6

Tsermo	48.3	47.8	7.0
Matador	41.6	33.2	3.6
$LSD_{0,5}$			0.11

The decrease in the juice purity made up 1.3−1.9%. Sugar loss in molasses was 0.17−0.18% (Table 5). The hybrids of foreign breeding, except for Taltos, were significantly inferior to Ukrainian cultivars in terms of resistance to rot (Table 5). Thus, the share of rotten mass in Ukrainian cultivars ranged between 3.6 and 7.0%, with the share of rotten roots reaching 33.2−53.4%, syrup purity decreasing by 5.02−5.25%, and sugar loss in molasses increasing by 1.03−1.23%.

Table 5. Chemical composition and technological indicators of sugar beet roots after storage for 70 days

Variety/ hybrid	Sugar content (%)	Reduced substances (%)	K + Na	α-amine N	Juice purity (%)	Sugar loss in molasses (%)	Sugar yield (%)
			(Mmol/100 g of wet root mass)				
Yaltushkivskyi Odnonasinnyi 64	17.80	0.352	5.28	1.94	90.90	2.10	14.54
Bilotserkivskyi Odnonasinnyi 45	17.00	0.403	5.01	3.54	90.00	2.23	13.68
Uladivskyi Odnonasinnyi 35	15.30	0.309	5.98	4.07	88.90	2.42	12.18
Yuvileinyi	16.10	0.296	5.40	3.67	89.50	2.31	12.78
Ivanivskyi CMS 33	17.20	0.355	4.56	2.45	91.10	2.08	13.92
Ukrainskyi CMS 70	17.70	0.257	4.73	1.86	91.40	2.03	14.50
Taltos	18.30	0.264	4.55	2.36	91.90	1.93	15.17
Reno	18.54	0.840	4.96	4.56	88.18	2.92	14.42
Tsermo	17.00	0.957	4.85	3.83	86.90	3.22	12.58
Matador	16.70	0.740	7.04	5.70	86.34	3.04	12.46
$LSD_{0,5}$	1.50	0.021	0.24	0.15	4.3	0.11	0.68

Thus, a comparative evaluation showed that of the studied sugar beet cultivars, foreign hybrids do not have advantages over Ukrainian varieties and hybrids. This became especially evident after storage (70 days). Therefore, varieties and hybrids of Ukrainian breeding can fully provide the sugar industry of Ukraine. They all are well-adapted to the zonal variations of the Ukrainian intensive technology of sugar beet cultivation and are quite competitive providing adherence to the technology.

New generation Ukrainian hybrids Bilotserkivskyi CMS 51, Bilotserkivskyi CMS 57, and Oleksandria will help to overcome the crisis in beet cultivation.

From the literature, it is known that chemical elements in plant organisms have certain physiological functions in metabolism, which are crucial for their growth and productivity. This is the difficulty of the breeding aimed at increasing quality, i.e. the breeder has to combine not only high sugar content breeding genotypes with low potassium, sodium, and α-amine nitrogen content but also those with high root yield. This contradicts the existing correlations between these traits (that is, in roots with high sugar content, the content of chemical elements is reduced and vice versa). However, these correlations are not so strict to give a breeder no chance for success. It should be noted that there are some limits to improve the technological quality of roots through breeding, as the quality indicators are significantly affected by environmental conditions and agronomic practices. Nevertheless, breeding sets the borders within which the agronomic practices can be effective. Given that the indicators of technological quality used in this comparative study can be easily measured or determined, under similar conditions they should be considered in the tasks of breeding for quality.

CONCLUSIONS

The studied hybrids of foreign breeding accumulated much more reducing sugars and soluble nitrogen substances during storage, which negatively affected juice purity and increased sugar loss in molasses. The most resistant to phytopathogenic microorganisms are varieties and hybrids of Ukrainian breeding and hybrid Taltos of Belgian breeding. The studied hybrids of German and Swedish breeding demonstrated a high content of rotten mass during storage.

REFERENCES

Askarova, G., Orynbekov, D., & Asanova, G. (2017). Phytoremediation of saline soil of Kyzylorda region with the use of sugar beet. *International Journal of Applied and Fundamental Research*, *6*(1), 79−82. [in Russian]

Balakhontsev, E. (1979). Influence of the mineral nutrition regime and climate on the accumulation of polysaccharides in sugar beet roots. In: Mineral nutrition, metabolism, and productivity of sugar beet. Ufa. [in Russian]

Boiko, I. (2015). Productivity and technological qualities of different biological forms of sugar beets. *Agrobiology*, *2*, 32−36. [in Ukrainian]

Chee, K. S., Williams, P. A., Steve, W. C, & Qi, W. (2008). Characterization of the surface -active components of sugar beet pectin and the hydrodynamic thickness of the adsorbed pectin layer. *J. Agric. Food Chem*, 56, 8111−8120**.**

Combo, A., Aguedo, M., Quievy, N., Danthine, S., Goffin, D., Jacquet, N., Blecker, C., Devaux, J., & Pagout, M. (2013). Characterization of sugar beet pectin-derived oligosaccharides obtained by enzymatic hydrolysis. *International Journal of Biological Macromolecules*, 52, 148−156. dx.doi.org/10.1016.

Dautova, Z. & Alimgafarov, R. (2013). Chemical composition of sugar beet root. *Contemporary research-intensive technologies*, 9, 12−13. [in Russian]

Hlevaskyi, V. (2015). Quality of sugar beet roots of domestic and foreign breeding after storage. *Agrobiology*, *2*(121), 28−32. [in Ukrainian]

Kliachenko, O. (2002). Distribution of macronutrients in the tissues of sugar beet roots in connection with the localization of sucrose. *Physiology and biochemistry of cultivated plants*. *34*(6), 481−490. [in Russian]

Kliachenko, O. (2006). The effect of shading on the chemical composition and productivity of different genotypes of sugar beet (*Beta vulgaris* L.). *Bulletin of Agrarian Science*, *2*, 44−47. [in Ukrainian]

Kliachenko, O. (2015). Variety-specific features of pectin substance accumulation in sugar beet roots. Scientific Bulletin of NAU, 84, 264−269. [in Ukrainian]

Kliachenko, O. & Funina, I. (2002). Chemical composition of sugar beet roots depending on their varietal differences. *Scientific Bulletin of NAU*, *47*, 100−104. [in Ukrainian]

Piskureva, V. (2012). Dynamics of pectin substance accumulation in beet (*Beta vulgaris* L.) roots in ontogenesis. Cand. Sc. (Agr.) thesis. Orel. [in Russian]

Roik, M. & Kornieieva, M. (2010). Sugar beet breeding. In: Special breeding of field crops. Bila Tserkva: BTs NAU, 276−314. [in Ukrainian]

Roik, M. & Lytvyniuk, V. (2010). Implementation and maintenance of monogerm varieties of Yaltushkiv breeding in production. *Scientific papers of IBCSB*, *11*, 126−132. [in Ukrainian]

Roik, M. (2003). Complex scientific support for the development of modern technology of the breeding process. *Bulletin of Ukr. Society of Geneticists and Breeders*, *1*, 17−37. [in Ukrainian]

Stepovyi, A., Rodionova, L. (2015). Improving beet pulp pre-treatment to obtain food pectin extract. *Young researcher*, *5*(85). [in Russian]

Strausbaugh, C., Eujayl, I. & Foote, P. (2010). Sugar beet cultivar evaluation for bacterial root rot. *Journal of Sugar Beet Research*, *47*(1−2), 51−64.

Vasilieva, I. (2000). The use of sugar beet in the development of technology and recipes for catering products. Cand. Sc. (Tech.) thesis, St. Petersburg. [in Russian]

Yakovets, V., Lytvyniuk, V. & Yakovets, H. (2007). Method of complex selection of breeding genotypes of sugar beets for resistance to root rot. *Scientific papers of IBCSB*, *9*,113−117. [in Ukrainian]

Yapo, B., Robert, C., Etienne, I., Wathelet, B. & Paquot, M. (2007). Effect of extraction conditions on the yield, purity and surface properties of sugar beet pulp pectin extracts. Food Chemistry, 100, 1356−1364. doi^10.1016/j.foodchem.2005.12.012.

Zaidel, D., Norulfairuz, A. & Meyer, A. (2013). Oxidative enzymatic gelation of sugar beet pectin for emulsion stabilization. Research Journal of biotechnology, *8*(7), 81−86.

Zheryakov, E. V., Breducheva, E. S., Kotlov S. A., Rozhkov, S. S. (2016). Technological qualities of sugar beet roots in the forest-steppe conditions of the middle Volga region. *International research journal*, *12*(54), 130−132. [in Russian]

Original Scientific paper
10.7251/AGRENG2201124K
UDC 633.11:581.1

EVALUATION OF GREEK BREAD AND DURUM WHEAT CULTIVARS FOR DROUGHT TOLERANCEUSING POLYETHYLENE GLYCOL

Maria KARAGIANNI, Maria MOSCHOU, Theano B. LAZARIDOU*

University of Western Macedonia - Florina, School of Agricultural Sciences 53100 Florina, Hellas
*Corresponding author: thelazaridou@yahoo.gr

ABSTRACT

In order to study the effect of drought stress seven bread wheat (*Triticum aestivum* L.) cultivars (Acheloos, Apollonia, Generoso, Doirani, Nestos, Strimonas, and Orpheas) and seven durum wheat (*Triticum durum* L.) cultivars (Sifnos, Anna, Elpida, Thraki, Papadakis, Athos, Mexikali 81) were evaluated at three levels of drought treatment using polyethylene glycol in a randomized complete block design with 10 replications. For this purpose, seedlings of the aforementioned genotypes were cultured in pots with three different concentrations of polyethylene glycol (5, 10% and 15% PEG 8000) whereas irrigation without PEG was used as control. During the experiment, the length of spike with awns, the length of spike, the plants height, the number of tillering per plant, and the concentration of proline in the plants treated were measured. The drought treatment reduced the length of spike and the plant height of the cultivars studied, whereas it increased the length of spike with awns in bread wheat cultivars. It was concluded that there are considerable differences in drought resistance between the genotypes studied. The drought stress, caused by the polyethylene glycol, increased the concentration of proline in the cultivars tested. The highest value of proline was estimated in plants of bread wheat cultivar Strimonas and in plants of durum wheat cultivar Sifnos. So consequently, we can conclude that Strimonas and Sifnos were the most tolerant cultivars of the two species. Orpheas (bread wheat) and Thraki (durum wheat) had the lowest concentration of proline, so Orpheas and Thraki were considered the most sensitive cultivars.

Keywords: *drought stress, tolerance, polyethylene glycol, proline*

INTRODUCTION

Drought is the major abiotic stress factor that reduces the productivity of cultivated plants. This reduction depends on the stage of plant development, the intensity of the stress and the resistance of the genotype. Furthermore, durum and bread wheat, two of the most important crops for global economy are affected by drought, with regard to their productivity and quality (Singh and Chaudhary, 2006). In addition,

in order to face the drought stress, the breeding of many cultivated plants nowadays is based on the creation and use of varieties with high yield potential and resistance to biotic and abiotic stresses (Acevedo and Fereres, 1994).Thus, the evaluation of the existing genetic material characterized by tolerance to drought is the only approach that could decisively contribute to the problem. Under water stress conditions, plants react with a number of morphological, physiological and biochemical adaptations in order to maintain the basic metabolic activity. Among the effects of a decrease in water potential are the loss of turgescence, a decrease in cell wall composition, a decrease in the rate of protein synthesis, a decrease in the biosynthesis of chlorophyll, an increase in the biosynthesis of abscisic acid, an increase in the rate of respiration, the accumulation of osmotically active substances, and the final drying of the plant. The phenotype remains the basic criterion for the selection of resistant genetic material and is based on the morphological and physiological characteristics of plants in drought conditions, and on yield (Monneveux *et al*., 2012). Biochemical analysis including mannitol, glycine, betaine, and proline has been proposed as an effective method for selecting drought tolerant genotypes (Mwadzingeni *et al*., 2016). The amino acid proline is associated with various osmotic protection roles, including osmotic adaptation (Zadehbagheri *et al*., 2014), membrane stabilization (Hayat *et al*., 2012) and signaling for the activation of antioxidant enzymes (de Carvalho *et al*., 2013).

Polyethylene glycol (PEG), a chemical that causes osmotic stress, is often used to control and select early-stage drought-resistant seedlings in the laboratory. Several studies have shown that PEG of high molecular weights can be used to modify the osmotic potential of a culture medium and thus induce water deficiency in a controlled manner (Zhu *et al*., 1997), and simulate plant stress in water scarcity in a manner similar to soil drought. Therefore, it is an effective and simple enough method in order to evaluate a large number of genotypes for drought tolerance (Hassanein, 2010). Lazaridou and Xynias, (2017) evaluated *in vitro* sixteen bread and durum wheat cultivars using polyethylene glycol and found considerable differences in drought resistance between the genotypes studied.The objective of this study was to evaluate the resistance to drought stress of fourteen Greek bread and durum wheat cultivars using polyethylene glycol 8000.

MATERIALS AND METHODS

For the purpose of the study seven bread wheat (*Triticum aestivum* L.) cultivars (Acheloos, Apollonia, Generoso, Doirani, Nestos, Strimonas, and Orpheas) and seven durum wheat (*Triticum durum* L.) cultivars (Sifnos, Anna, Elpida, Thraki, Papadakis, Athos, Mexikali 81) developed at the Cereal Institute of Thessaloniki (Cereal Institute, 1985) were used. The effect of drought stress was measured *in vivo* in a greenhouse (plants in pots) at four different osmotic potential levels (four concentrations of Polyethylene glycol PEG 0%, 5%, 10% and 15%). The stress applied to mature plants before and after flowering. The experimental design was split-plot in randomized complete block design (RCB). Ten replications were used for each treatment, so 560 experimental plots were used (14 varieties x 4 treatments

x 10 replications = 560 pots). During the growing period, phenotypic as well as biochemical parameters were evaluated. The length of spike with awns, the length of spike, the plants height and the concentration of proline in the plants, as a response to the drought, were measured. The concentration of proline (mmol/gr dry weight) in the plant tissues was measured using the spectrophotometer according to Bates method (Bates *et al*., 1973). Data were statistically analyzed and the means were compared according to LSD test at p=0.05.

RESULTS AND DISCUSSION

Significant differences were recorded between the examined cultivars in all morphological traits and the concentration of proline. Significant differences were recorded also between the different treatment (0, 5, 10 and 15% PEG) in all morphological traits and the concentration of proline.

Table 1. Height, length of spike with awns, length of spike, and concentration of proline after 4 treatments with PEG (0, 5, 10 and 15%) of seven bread wheat cultivars

PEG	Height cm	Length of spike without awns	Length of spike with awns	Proline mmol/gr DW
0%	56.93 a	4.93 a	8.06 b	0.10 c
5%	53.42 b	4.59 b	8.14 b	0.24a
10%	50.62 c	3.70 c	9.67 a	0.21 b
15%	50.68 c	3.49 d	9.70 a	0.22 ab

Means in columns followed by different letters, are significantly different at p<0.05 by LSD test.

The length of spike as well as the height of the plants of bread wheat decreased with the increase of the concentration of polyethylene glycol, which caused an increase in water stress in plants (Table 1). The same was reported by Aboughadareh *et al*., (2020) who found that water stress caused a significant reduction, among other agro-morphological characteristics, in the height of the plant, and the length of the spikes of 17 durum wheat genotypes. Reduction in plant height was noticed also by Khamssi and Najaphy (2011) who studied the effect of drought stress in fourteen bread wheat cultivars. Singh *et al*., (2014) came to the same conclusion in terms of spike length when they evaluated 10 bread wheat genotypes from India under drought stress, caused by treatment with polyethylene glycol (PEG). On the contrary the present work showed that the water stress caused by the treatment with polyethylene glycol led to an increase in the spike length with awns, giving the highest length with the highest concentration of polyethylene glycol (15%). This could be because, according to Evans *et al*. (1971), the presence of awns in the spike is related to the yield of cereals under water stress conditions.

Table 2. Height, length of spike with awns, length of spike, and concentration of proline of seven bread wheat cultivars after the treatments with PEG

A/A	Genotype	Height cm	Length of spike without awns	Length of spike with awns	Proline
1	**ACHELOOS**	53.77 ab	4.08 b	10.95a	0.16 c
2	**APOLLONIA**	50.97 c	4.15 b	11.32 a	0.17 c
3	**GENEROZO**	47.94d	4.04 b	4.76 d	0.18 c
4	**DOIRANI**	54.29 ab	3.35 c	8.88 b	0.21 b
5	**NESTOS**	52.30bc	5.41a	6.18 c	0.16 c
6	**STRYMONAS**	56.14a	4.20 b	9.26 b	0.31 a
7	**ORPHEAS**	54.98 ab	4.01 b	10.90 a	0.15 c

Means in columns followed by different letters, are significantly different at $p<0.05$ by LSD test.

The drought stress, caused by the polyethylene glycol, increased the concentration of proline in the cultivars tested compared to the control. Increased concentration of proline was also recorded by Alvarez *et al*., (2008), in corn under water stress conditions. Similar results were recorded in wheat by Saeedipour (2013). The same conclusion was reached by Singh *et al*., (2014) who recorded an increase in proline content after treatment with PEG in 10 wheat genotypes from India. In the present study the highest value of proline was recorded in the plants of Strimonas (0.31 mmol/gr DW), whereas the lowest concentration of proline (0.15 mmol/gr DW) had the plants of Orpheas (Table 2). Lazaridou and Xynias, (2017) evaluated *in vitro*, using polyethylene glycol, Greek varieties of bread wheat, and found that the cultivars Acheron, Acheloos and Apollonia were the most tolerant ones. The results of the present study do not agree with the above researchers because Strimonas had the highest value of proline, so it was considered the most resistant variety.

Table 3. Height, length of spike with awns, length of spike, and concentration of proline after 4 treatments with PEG (0, 5, 10 and 15%) of seven durum wheat cultivars

PEG	Height cm	Length of spike without awns	Length of spike with awns	Proline mmol/gr DW
0%	61.24a	4.00a	12.06 a	0.11d
5%	55.26b	3.50b	11.82 a	0.18c
10%	53.78b	2.79c	11.75 b	0.25b
15%	51.57c	2.54d	11.44 c	0.29a

Means in columns followed by different letters, are significantly different at $p<0.05$ by LSD test.

The length of spike with awns, the length of spike without awns, as well as the height of the plants of durum wheat decreased with the increase of the concentration of polyethylene glycol (Table 3). Regarding the proline concentration, in the present study it was found that drought stress led to an increase in the concentration of proline in the treated plants, compared to the control and the highest value was measured in the plants treated with 15% PEG.

Table 4. Height, length of spike with awns, length of spike, and concentration of proline of seven durum wheat cultivars after the treatments with PEG

A/A	GENOTYPE	Height cm	Length of spike without awns	Length of spike with awns	Proline
1	**ANNA**	54.65bc	3.38a	11.98a	0.27b
2	**SIFNOS**	57.27ab	3.51a	11.99a	0.31a
3	**ATHOS**	52.27c	2.76c	11.68b	0.24c
4	**MEXIKALI 81**	55.04 b	3.39a	10.86c	0.17d
5	**PAPADAKIS**	55.68 b	3.37a	11.67b	0.23 c
6	**ELPIDA**	58.68 a	3.12b	12.06a	0.17d
7	**TRHAKI**	54.65bc	2.91b	12.12 a	0.08e

Means in columns followed by different letters, are significantly different at $p<0.05$ by LSD test.

Significant differences were observed between the durum wheat genotypes regarding the plant height, the length of spike, the length of spike with awns and the concentration of proline (Table 4). Similar results were recorded by Mirbahar *et al*., (2009) who studied the effect of different water stresses at different stages of development of 25 wheat varieties.

In the present study the highest value of proline had the plants of Sifnos (0.31 mmol/gr DW) whereas the lowest concentration of proline (0.08 mmol/gr DW) had the plants of Thraki (Table 4). Lazaridou and Xynias (2017) evaluated *in vitro*, Greek durum wheat varieties for drought tolerance and found that Thrace and Anna were the most tolerant varieties to drought, while Papadakis and Mexikali 81 were the most sensitive ones. In the present study the highest value of proline had Sifnos followed by variety Anna. In addition, Thraki according to the concentration of proline was the most sensitive variety.

CONCLUSION

There are considerable differences in drought resistance between the genotypes studied. The presence of PEG, or otherwise the drought stressing conditions reduced the height of plants, the length of spike and increased the concentration of proline in bread and durum wheat cultivars. Among the genotypes studied Strimonas and Sifnos were the most tolerant cultivars of the two species. Orpheas (bread wheat) and Thraki (durum wheat) were considered the most sensitive cultivars. However, further research, is needed to confirm the results of the present study.

REFERENCES

Acevedo E., Fereres E. (1994). Resistance to abiotic stresses. In Hayward, M. D., Bosemark, N. O. and Romagosa, I. (eds) Plant breeding: principles and prospects. Chapman and Hall, London. pp. 406-421.

Aboughadareh A.P., Mohammadi R., Etminan A., Shooshtari L., Maleki-Tabrizi N., Poczai P. (2020).Efects of Drought Stress on Some Agronomic and Morpho-Physiological Traits in Durum Wheat Genotypes. Sustainability, 12, 5610; doi:10.3390/su12145610.

Alvarez S., MarshE.L., SchroederS.G., Schachtman D.P. (2008). Metabolomic and proteomic changes in the xylem sap of maize under drought. Plant Cell Environ.31, pp. 325–340.

Bates L., Waldren R.P., Teare I.D. (1973). Rapid determination of free proline for water– stress studies. Plant and soil 39, pp. 205-207. doi.org/10.1007/BF00018060.

Cereal Institute of Thessaloniki. (1985). Cereal Varieties, Hellenic Ministry of Agriculture, Thessaloniki (in Hellenic).

de Carvalho K., de Campos M.K.F., Domingues D.S., Pereira L.F.P., Vieira L.G.E. (2013). The accumulation of endogenous proline induces changes in gene expression of several antioxidant enzymes in leaves of transgenic Swingle

citrumelo. Mol. Biol. Rep. 40, pp. 3269–3279. doi: 10.1007/s11033-012-2402-5.

Evans L.T., Bingham J., Jackson P., Sutherland J. (1971). Effect of awns and drought on the supply of photosynthate and its distribution within wheat ears Annals of Applied Biology pp. 67-76.

Hassanein A. (2010). Establishment of Efficient *in vitro* Method for Drought Tolerance Evaluation in Pelargonium. Journal of Horticultural Science & Ornamental Plants 2(1): 08-15.

Hayat S., Hayat Q., Alyemeni M.N., Wani A.S., Pichtel J., Ahmad A.(2012). Role of proline under changing environments. Plant Signal. Behav. 7, pp. 1456–1466.doi:10.4161/psb.21949.

Khamssi N.N., Najaphy A. (2011).Agro-morphological and phenological attributes under irrigated and rain-fed conditions in bread wheat genotypes. Pakistan Journal of Botany, 40 (4) pp. 1777-1781.

Lazaridou T.B., Xynias I.N., (2017) In vitro screening of durum and bread wheat genotypesfor drought resistance using polyethylene glycol. Proceedings of VII International Agricultural Symposium "AGROSYM", Jahorina, 5-8 October 2017, Bosnia and Herzegovina. pp 276-281, 2017

Monneveux P., Jing, R.,Misra S. (2012). II. 1.2 Phenotyping wheat for adaptation to drought using physiological traits. Front. Physiol. 3. pp. 429. doi: 10.3389/fphys.2012.00429.

Mwadzingeni L., Shimelis H., Tesfay S.,TsiloT.J.(2016) Screening of Bread Wheat Genotypes for Drought Tolerance Using Phenotypic and Proline Analyses, Frontiers in Plant Science, vol.7ISSN=1664-462X. doi=10.3389/fpls.2016.01276.

Saeedipour S. (2013). Relationship of Grain Yield, ABA and Proline Accumulation in Tolerant and Sensitive Wheat Cultivars as Affected by Water Stress. Front. Plant Sci.7. Pp. 1276. doi: 10.3389/fpls.2016.01276.

Singh G., Chaudhary H. (2006). Selection parameters and yield enhancement of wheat (*Triticum aestivum* L.) under different moisture stress condition. Asian Journal of Plant Sciences, 5. pp. 894-898.

Singh N.P., Pal P.K., Vaishali S.K. (2014).Morpho-physiological characterization of Indian wheat genotypes and their evaluation under drought condition. African Journal of Biotechnology.DOI:10.5897/AJB2013.13486. ISSN 1684-5315.

Zadehbagheri M., Azarpanah A., Javanmardi S. (2014). Proline metabolite transport an efficient approach in corn yield improvement as response to drought conditions. Nature 566. Pp. 76–485. doi:10.5829/idosi.aejaes.2014. 14.05.12328.

Zhu J.K., Hasegawa P.M., Bressan R.A. (1997). Molecular aspects of osmotic stress in plants: Critical Rev. Plant Sci. 16. pp. 253-277.

INDEX OF AUTHORS

INSTRUCTIONS FOR AUTHORS

AGROFOR International Journal publishes scientific and expert papers on agriculture, rural development, environmental protection and management of natural resources, animal husbandry and veterinary medicine, forestry, and biology. It is the endeavor of the Journal to give place to papers of high scientific quality and international interest, authored by international scientist in order to stimulate contacts and exchange of knowledge fostering scientific productivity

Manuscripts, submitted via electronic journal web system should be prepared in Microsoft Word **(Times New Roman font, 11 pt)** and submitted in format 17 x 24 cm **(File / Page setup / Paper / Width = 17 cm; Height = 24 cm)**, with single line spacing **(Format / Paragraph / Line spacing = Single)**, 2 cm margins all around **(File / Page setup / Margins / Top = 2 cm; Bottom = 2 cm; Left = 2 cm; Right = 2 cm)**, that is approximately 44 lines per page in this format. All technical details are available also on AGROFOR Internatinal Journal website: http://www.agrofor.rs.ba/page.php?id=8

Manuscripts are published in English. Papers that have been published elsewhere, in whole or extracts (excerpts) of their important findings, will not be accepted. A manuscript should not exceed 10 pages. Exceptions can be made if content and quality of the paper justify it (at the discretion of the Editor).

Full research papers should include the following sections:

- Title and author/s name/s

The author/s name/s should be placed below the title with affiliations. Author/s affiliation should indicate name and address of institution, including the e-mail address of the corresponding author. Title should provide a concise but also an informative synthesis of the study (recommended not more than 100 characters including spaces). Ensure that the title contains the most important words that relate to the topic.

- Abstract

The summary, in English language, should provide basic data on the problem that was treated and the results obtained. It should be brief, preferably one paragraph only, up to 250 words, but sufficient to inform the reader of the character of the work, its results and its conclusions.

- Key words

Keywords should provide 4-6 words or compound words, suitable for an information retrieval system. Choose the appropriate keywords and phrases for your article. Think of a phrase of 2-4 words that a researcher might search on to find your article. Repeat your keywords and phrases 3-4 times throughout the abstract in a natural, contextual way.

- INTRODUCTION

The introduction should answer the questions what was studied, why was it an important question, what was known about it before and how the study will advance our knowledge.

- MATERIAL AND METHODS

Material and methods explain how the study was carried: the organism(s) studied; description of the study site, including the significant physical and biological features, and the precise location (latitude and longitude, map, etc); the experimental or sampling design; the protocol for collecting data; how the data were analyzed. In this section also should be provided a clear description of instruments and equipment, machines, devices, chemicals, diagnostic kits, plants/animals studied, technology of growing/housing, sampling sites, software used, etc.

- RESULTS and **DISCUSSION**

Results and Discussion should be combined into a single section.

The results objectively present key results, without interpretation, in an orderly and logical sequence using both text and illustrative materials (tables and figures).

The discussion interpret results in light of what was already known about the subject of the investigation, and explain new understanding of the problem after taking results into consideration.

The International System of Units (SI) should be used.

- CONCLUSIONS

The conclusion should present a clear and concise review of experiments and results obtained, with possible reference to the enclosures.

- ACKNOWLEDGMENTS

If received significant help in designing, or carrying out the work, or received materials from someone who did a favour by supplying them, their assistance must be acknowledged. Acknowledgments are always brief and never flowery.

- REFERENCES (LITERATURE)

References should cover all papers cited in the text. The in-text citation format should be as follows: for one author (Karaman, 2011), for two authors (Erjavec and Volk, 2011) and for more than two authors (Rednak *et al*., 2007). Use semicolon (Rednak *et al*., 2012; Erjavec and Volk, 2011) to separate multiple citations. Multiple citations should be ordered chronologically. The literature section gives an alphabetical listing (by first author's last name) of the references. Please see "INSTRUCTIONS FOR AUTHORS" / Bibliographic style on the web page of the Journal for more details: http://www.agrofor.rs.ba/page.php?id=8

Short communication should include the following sections: Title, Abstract, Key words, Main text, Acknowledgments, References, Tables and Figures with captions.

SUPPLY OF ARTWORK, PHOTOS: Diagrams and graphs should be provided as finished black and white line artwork or colour images. Electronic graphics included in your manuscript should be either inserted in the word document or as .gif or .jpg formats. Please check with the editor if you wish to submit any other type of graphic for conversion suitability. Photos should be supplied un-screened in original form or in electronic form. All illustration (diagrams, graphs, tables, photos) must be fully captioned. When there are a number of illustrations, the author should endeavour to reduce the amount of text to accommodate the illustrations in the limited space available for any article.

THE REVIEW PROCESS: Submitted manuscripts are reviewed anonymously by 2 international referees (duble blind review). All tracking of manuscripts and reviewers is done by the Editor. All attempts will be made to ensure review process done within three months after the submission. Manuscripts will be returned to the coresponding authors when each review is completed.


Agrofor