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AGRO-BIODIVERSITY IN NATIONAL PATHWAYS FOR FOOD SYSTEM TRANSFORMATION: CASE OF WEST AFRICA

Hamid EL BILALI[1,*], Gianluigi CARDONE[1], Abdel Kader NAINO JIKA[2], Eleonora DE FALCIS[2], Susanna ROKKA[3], Ali Badara DIAWARA[4], Bassirou NOUHOU[5], Andrea GHIONE[6]

[1]International Centre for Advanced Mediterranean Agronomic Studies (CIHEAM-Bari), Valenzano (Bari), Italy
[2]Alliance Bioversity International – CIAT, Rome, Italy
[3]Natural Resources Institute Finland (Luke), Jokioinen, Finland
[4]Afrique Verte Burkina Faso (APROSSA), Ouagadougou, Burkina Faso
[5]Afrique Verte Niger (AcSSA), Niamey, Niger
[6]Italian Agency for Development Cooperation (AICS), Ouagadougou, Burkina Faso
*Corresponding author: elbilali@iamb.it

ABSTRACT

The challenges relating to biodiversity loss, food insecurity and climate change show the urgent need to make transition towards sustainable food systems in West Africa. To bring about such a transition worldwide, the United Nations' Food Systems Summit was held in September 2021. One of the main outcomes of the Summit was the national pathways to sustainable food systems. This review analyses whether and how agro-biodiversity is addressed in the food system transformation pathways submitted by West African countries in the framework of the Summit. The content analysis suggests that agro-biodiversity is not a central topic in the national transformation pathways. In fact, it is completely overlooked in some pathways documents, and rather marginal in others. Some national documents (cf. Burkina Faso, Ghana, Niger, Nigeria) refer to the promotion of the diversity of crops and farm animals as a means of adapting to climate change, improving livelihoods and diversifying diets thus contributing to nutrition security. Moreover, only a few measures and actions dealing with the valorisation of the neglected and underutilised species (NUS) and traditional crop varieties are included in the national transformation pathways (cf. Guinea, Liberia, Niger, Sierra Leone). The conservation, management and restoration of agro-biodiversity and agro-ecosystems are crucial to boost the transition towards nature-positive food systems in the region. Therefore, a paradigm change is needed in policy, research and practice to conserve the natural resource base and contribute to sustainable development by addressing, inter alia, food insecurity and malnutrition, rural poverty and climate change challenges.

Keywords: *biodiversity conservation, nature-based solutions, Food Systems Summit, orphan crops, transition pathways*.

INTRODUCTION

Biodiversity loss is one of the most pressing challenges that humanity faces (IPBES, 2019). Therefore, biodiversity conservation is considered vital for sustainable development and addressed in several Sustainable Development Goals (SDGs) such as SDG 2 "Zero Hunger" and SDG 15 "Life on Land" (United Nations, 2015). Biodiversity loss is mainly driven by habitat and land-use change, overexploitation of natural resources and ecosystems, climate change, pollution and invasive alien species (Millennium Ecosystem Assessment, 2005). The loss of agro-biodiversity is exacerbated by the abandonment of a wide range of edible plants, the so-called neglected and underutilised species (NUS) (Chivenge et al., 2015; Padulosi et al., 2013). Biodiversity loss is particularly a challenge in regions where the anthropogenic and natural pressure on fragile ecosystems as well as the reliance on natural resources and ecosystems are high such as the Sahel and West Africa regions. Evidence shows that the impacts of climate change will be high in Sub-Saharan Africa in general (Baarsch et al., 2020; Bakshi et al., 2019; Hassan, 2010; Lokonon et al., 2019) and the Sahel and West Africa regions in particular (Baarsch et al., 2020; Lokonon et al., 2019). West Africa is still highly dependent on agriculture for the food security and livelihoods of its population (Egbebiyi et al., 2019). Agriculture, which is predominantly rain-fed, is highly vulnerable to climate fluctuations and droughts. In this respect, Sultan and Gaetani (2016) point out that "*West Africa is known to be particularly vulnerable to climate change due to high climate variability, high reliance on rain-fed agriculture, and limited economic and institutional capacity to respond to climate variability and change*". Meanwhile, food insecurity and malnutrition are still big challenges in West Africa. Indeed, the prevalence of undernourishment in the total population is still high in the region averaging 14.8%; it ranged from 38.9% in Liberia to 6.1% in Ghana over the period 2018–20. The situation is even worse when considering the prevalence of moderate or severe food insecurity in the total population that reached 57.8% region-wide over the same period, ranging from 83.9% in Sierra Leone to 35.1% in Cabo Verde (FAO et al., 2021). Challenges relating to biodiversity loss, food insecurity and climate change show the urgent need to make transition towards sustainable and resilient food systems in the region.

To bring about such a transition, the United Nations' Food Systems Summit (FSS) was held in September 2021 to unleash the power of food systems in the realisation of the 2030 Agenda for Sustainable Development and the achievement of the SDGs worldwide. It aimed to deliver more sustainable, equitable, and healthier food systems (United Nations, 2021a) by working on five action tracks/areas viz. ensuring access to safe and nutritious food for all; shifting to sustainable consumption patterns; boosting nature-positive production at sufficient scales; advancing equitable livelihoods; and building resilience to vulnerabilities, shocks and stress (United Nations, 2021b). Biodiversity and ecosystems are addressed in the action track 3 "Boosting nature-based solutions" (Hodson et al., 2021). In preparing for the Summit, within the Food Systems Summit Dialogues (FSSDs), a diverse range of stakeholders were invited to identify the most powerful ways to

make food systems more sustainable. Indeed, the focus of the Summit was on the transformation of food systems. Therefore, one of the main outcomes of the FSSDs was the articulation of national pathways to sustainable, equitable and resilient food systems where national FSSDs were consolidated into clear visions of what governments and other stakeholders expect of national food systems by 2030 and worked together on exploring challenges and options to transform their food systems in the coming decade (Anonymous, 2021a). The articulation of national pathways took into consideration different factors, particularly (Anonymous, 2021a): clarifying the expectations of national food systems in the coming decade and identifying changes needed for national food systems to meet the defined expectations by 2030.

Analyses of the role of agro-biodiversity in the processes of agro-food sustainability transitions in developing countries, in general, and Sub-Saharan ones, in particular, are hard to find (El Bilali, 2019). In this context, the present article analyses whether and how agro-biodiversity is addressed in the food systems transformation pathways in West Africa. In particular, the paper analyses the measures included in the pathway documents to address the conservation and management of agro-biodiversity as well as the valorisation of orphan crops (cf. NUS).

METHODS

Cohen- Shacham et al. (2016) defined the term Nature- based Solutions (NbS), an overall concept that was used for nature- positive food systems in the context of the Food Systems Summit accordingly. It is based on three pillars: protection, sustainable management and restoration of (agro)ecosystems (Figure 1). According to Hodson et al. (2021), "*Nature- positive food systems are characterized by a regenerative, non- depleting and non- destructive use of natural resources. It is based on stewardship of the environment and biodiversity as the foundation of critical ecosystem services, including carbon sequestration and soil, water, and climate regulation. Nature Positive Food Systems refer to protection, sustainable management and restoration of productive system. Finally, nature positive food systems cover the growing demand for food in a sufficient way and include sustainable and healthy nutrition*" (p. 4). The themes covered by national pathway documents regarding the action area 3 "Boosting nature-based solutions" include agrobiodiversity; agroecology; aquatic/blue foods; halting deforestation and conversion from agricultural commodities; land management; nature-positive innovation; restoring grasslands, shrublands and savannahs; soil health; sustainable livestock; sustainable productivity growth and water (United Nations, 2022).

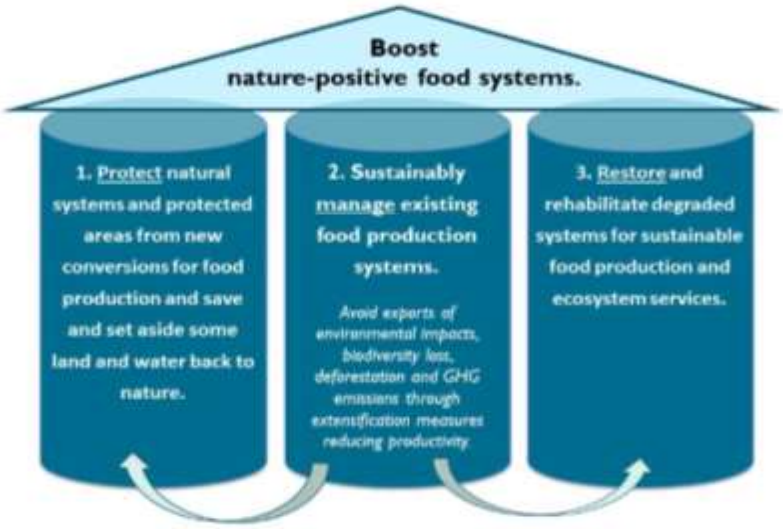

Figure 1. The three pillars of nature- positive food systems: protection, sustainable management and restoration.
Source: Adapted by Hodson et al. (2021) from Cohen- Shacham et al. (2016).

The present article focuses on the West Africa region viz. Benin, Burkina Faso, Cape Verde/Cabo Verde, the Gambia, Ghana, Guinea, Guinea-Bissau, Ivory Coast/Côte d'Ivoire, Liberia, Mali, Mauritania, Niger, Nigeria, Senegal, Sierra Leone and Togo. A content analysis was performed to see how biodiversity, one of the themes of the action area on boosting nature-based solutions, is addressed in the pathways submitted by West African countries in the framework of the UN Food Systems Summit (Table 1).

Table 1. National pathways for food systems transformation submitted by West African countries.

West African country*	Transformation pathway document title**/***	Reference
Burkina Faso	*Feuille de route nationale en vue de la transformation des systèmes alimentaires pour soutenir l'Agenda 2030*	Anonymous (2021c)
Gambia	Articulating National Pathways for Food Systems Transformation in Support of the 2030 Agenda: A Strategic National Pathway Document	Ministry of Agriculture – The Gambia (2021)
Ghana	Pathways to Ghana's food systems transformation	Anonymous (2021h)

West African country*	Transformation pathway document title**/***	Reference
Guinea	*Feuille de route nationale de transformation des systèmes alimentaires pour l'atteinte des Objectifs de Développement Durable en Guinée*	Anonymous (2021b)
Liberia	Liberia's food systems dialogues outcomes & pathways	Anonymous (2021e)
Mauritania	*Note explicative et de justification du contenu de la feuille de route*	Anonymous (2021g)
Niger	*Feuille de Route pour opérationnaliser les voies de transformation des Systèmes Alimentaires pour une Alimentation Saine à l'horizon 2030 au Niger*	Anonymous (2021d)
Nigeria	Nigeria national pathways to food systems transformation	Anonymous (2021f)
Senegal	*Projet de feuille de route du Sénégal pour le Sommet mondial sur les systèmes alimentaires durables*	Ministère de l'Agriculture et de l'Equipement Rural – Sénégal (2021)
Sierra Leone	United Nations Food System Summit: Pathways to attain sustainable access to safe and nutritious foods for all in Sierra Leone	FSS Technical Working Group (2021)

* The following West African countries have no profiles on the website of the Food Systems Summit 2021 (https://summitdialogues.org/explore-countries): Cape Verde, Guinea-Bissau and Togo.
** No transformation pathways were submitted as of 20 April 2022 by Benin, Ivory Coast and Mali.
*** In case different pathway versions have been submitted, only the most recent one was considered.

The methodology used was informed by that utilised by WWF (2021) in their analysis of the action track 3 "Boosting nature-based solutions" in the documents submitted by member states (viz. national dialogues feedback forms, national pathways, pre-summit statements, summit statements). In particular, during the content analysis on agro-biodiversity, the following keywords were used (translated into French, if any): agrobiodiversity, genetic diversity, crop diversity, diversification, forgotten crop, neglected crop, traditional crop, indigenous crop, neglected species, underutilised species, NUS, gene bank, seed bank, variety, cultivar, breed.

RESULTS AND DISCUSSION

In *Nigeria*, the recommendations from the dialogues have been grouped into 6 solution clusters (Anonymous, 2021f). Cluster 4 "Increase demand for, and consumption of adequate, nutritious, and healthy foods, including in humanitarian contexts" contemplates promoting homestead food production and the use of traditional vegetables (Table 2). Meanwhile, cluster 6 "Linking research, innovation, and extension for a sustainable food system" focuses on breeding for resilience (Anonymous, 2021f). In *Niger*, the conservation and promotion of agro-biodiversity are foreseen in the context of the third pathways towards sustainable food systems[1] concerning the promotion of priority value chains of food products with high nutritional and commercial potential, particularly those of fruits and vegetables, legumes (cowpea), fish, milk and dairy products, meat, dry cereals (millet, sorghum) and non-timber forest products (e.g. moringa). Within this pathway, diversification of plant and animal productions as well as the sustainable management of the environment and natural resources are foreseen in the framework of the action plan 2021-2025 of the initiative 3N (I3N – Nigeriens Nourish Nigeriens) (Anonymous, 2021d). *Burkina Faso* highlights in its national pathway document the importance of agro-ecology, agro-forestry and organic farming for improving the diversity of not only agro-ecosystems but also diets. It also calls for land conservation and the reclamation of degraded lands as well as the valorisation of local breeds. The country also highlights the need for close collaboration between producer organizations and research actors to have improved seeds and high-yielding varieties that resist attacks from pests and are resilient to climate change (Anonymous, 2021c). While such efforts might allow valorising the local varieties, they might also lead to the erosion of the local crop patrimony if imported varieties are promoted to the detriment of local, traditional ones.

Table 2. Agro-biodiversity in national pathways for food systems transformation in West Africa.

Country	Measures and actions related to agro-biodiversity-	Source
Burkina Faso	Enhancement of the genetic potential of local breeds	Anonymous (2021c)
Ghana	Developing a comprehensive strategy towards ensuring seed and breed security, achieving food sovereignty and enhancing biodiversity Research on recipes that cover indigenous crops, fruits, vegetables and other local products.	Anonymous (2021h)

[1] Other pathways concern: improving the governance and financing of food systems; promoting administrative and legislative reforms in food systems; strengthening research and innovation for sustainable food systems; supporting resilience building and recovery; making quality statistical data available and strengthening sector information and monitoring-evaluation systems.

Country	Measures and actions related to agro-biodiversity-	Source
Niger	Promotion of priority value chains of food products with high nutritional and commercial potential	Anonymous (2021d)
Nigeria	Promoting homestead food production and animal husbandry with improved varieties for household consumption Promoting the use of traditional/local vegetables for the preparation of meals for school feeding programmes, hospitals, military, and other public institutions Developing resilient crop seed varieties, livestock, and aquaculture	Anonymous (2021f)

In the case of the *Gambia*, the 3rd milestone of the national food system transformation pathway is about "Sustainable exploitation of the country's natural resource base" but apart from a broad reference to mainstreaming conservation agriculture in the agricultural policies and enacting soil and water regulations – which can have positive externalities in terms of biodiversity conservation and management – there is no specific action or measure on agro-biodiversity in the country. It is even more surprising that biodiversity is not considered a part of the natural resource base in the Gambia. *Sierra Leone* identifies some expectations relating to agro-biodiversity such as increasing agricultural production and productivity (intensification and diversification) among the rural poor smallholders through a variety of support measures, and establishing supply chains for inputs such as high-yielding seed varieties and livestock breeds (FSS Technical Working Group, 2021) but there are no specific measures in the national pathway regarding agro-biodiversity. *Senegal* considers that agro-silvo-pastoral and fisheries production systems are moderately sustainable due, in particular, to practices that degrade natural resources, disrupt ecosystems and reduce biodiversity (Ministère de l'Agriculture et de l'Equipement Rural – Sénégal, 2021) but apart from the initiation of an integrated program for the recovery of degraded land and the promotion of agroecology, which might contribute to the restoration of agro-ecosystems, there is no specific intervention on agro-biodiversity. Likewise, *Guinea* highlights that sustainable agriculture can reduce deforestation and promote healthy terrestrial ecosystems, and lists encouraging reforestation and protection of degraded areas as one of the priority axes in its national pathway (Anonymous, 2021b) but with no specific reference to agro-biodiversity. *Mauritania* refers to the promotion of agro-pastoral practices that respect the environment and organic farming (Anonymous, 2021g) without providing further details.

Many countries in the region (e.g. Burkina Faso, Ghana, Senegal, Niger) highlight the need to promote *agroecological practices* to reap their multiple benefits

(Anonymous, 2021h, 2021d, 2021c; Ministère de l'Agriculture et de l'Equipement Rural – Sénégal, 2021). For example, Ghana (Anonymous, 2021h) puts that "*Agroecology, in practice, encompasses conservation, regenerative and climate-smart agriculture as well as biodiversity conservation and sustainable land management practices which involve minimal use of external agrochemical inputs*". Similarly, some countries (e.g. Ghana, Senegal) refer to the food sovereignty concept (Anonymous, 2021h; Ministère de l'Agriculture et de l'Equipement Rural – Sénégal, 2021) also when addressing the issue of the availably of seeds at national and local levels.

The findings are in line with those of the analysis of the feedback forms of the member state dialogues, which shows that there is a recognition that food systems generate environmental impacts as well the urgency of ensuring food and nutrition for all while reducing the environmental footprint of the current food consumption and production patterns (United Nations, 2021c, 2021e, 2021d). The dialogues outcome documents stress the importance of valuing nature to consider the real, full cost of food. They also highlight that nature – both ecosystem services and biodiversity – should be valued properly and managed sustainably with an appropriate, balanced mix of preservation/conservation and regeneration/renaturation measures. This calls for well-functioning and effective policies and regulatory frameworks – at international, regional, national and local levels – that can, among others, promote the development of seed banks, payments for producers' contributions to nature, and appropriate changes in legislation on land use (United Nations, 2021e).

Some *NUS* featured prominently in the summit dialogues and, consequently, in the national transformation pathways of West African countries such as cassava in *Liberia* (Anonymous, 2021e), sweet potato in *Sierra Leone* and *Guinea* (Anonymous, 2021b; FSS Technical Working Group, 2021), moringa in *Guinea* and *Niger* (Anonymous, 2021b, 2021d). Limited access to high-yield cassava varieties was identified as one of the hindrances that farmers face to expand cassava production in *Liberia* (Anonymous, 2021e). *Sierra Leone* posits the limited availability of innovations for improving livestock breeds and crops varieties, bio-fortification (e.g. orange-fleshed sweet potatoes) and usage of agricultural biodiversity (e.g. traditional leafy green vegetables) among the major challenges of its food system (FSS Technical Working Group, 2021) but there is no specific measure regarding the NUS. Furthermore, different countries such as Liberia, Nigeria, Mauritania, Guinea, Niger and Burkina Faso highlight the importance of growing and sourcing food locally (Anonymous, 2021e, 2021g, 2021f, 2021b, 2021d, 2021c), which might contribute to the promotion of NUS. For instance, *Nigeria* considers producing blended foods from local food sources for the prevention and management of moderate acute malnutrition (Anonymous, 2021f).

CONCLUSIONS

This review paper analyses whether and how agro-biodiversity is addressed in the transformation pathways submitted by West African countries within the UN Food

Systems Summit. The analysis suggests that agro-biodiversity is not a central topic in the national transformation pathways. In fact, it is completely overlooked in some pathways documents, and rather marginal in others. Some pathways refer to the promotion of the diversity of crops and farm animals as a means to adapt to climate change, improve livelihoods and living conditions of rural communities as well as diversify diets thus contributing to nutrition security. While the productivism-inspired approach of West African countries, focusing on the increase of agricultural production and productivity, might be understandable in the short term, as these countries suffer from malnutrition and food insecurity, it might result short-sighted and unsustainable in the long run. Indeed, agriculture intensification might lead to the degradation and depletion of the natural resource base, including local crop and animal resources. A growing body of evidence suggests that there can be no sustainable and resilient food systems without the conservation, sustainable management and restoration of agro-biodiversity and agro-ecosystems. Therefore, a change of paradigm with the promotion of agro-ecological practices and methods and nature-positive solutions in agriculture and food systems is needed in policy, science and practice realms. In this context, the valorisation of NUS is crucial since these neglected and underutilised crops can contribute not only to food and nutrition security, especially in poor households and remote communities, but also to food system sustainability and resilience. This, in turn, requires investments in research, innovation and dissemination of knowledge and good practices (in, inter alia, production, conservation/storage, processing, use) of NUS with the involvement of all actors of the agricultural knowledge and innovation system (AKIS) in West Africa.

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SUSTAINABILITY OF NEGLECTED AND UNDERUTILISED SPECIES (NUS): TOWARDS AN ASSESSMENT MATRIX FOR CROP SPECIES

Hamid EL BILALI[1,*], Gianluigi CARDONE[1], Susanna ROKKA[2], Abdel Kader NAINO JIKA[3], Eleonora DE FALCIS[3], Ali Badara DIAWARA[4], Bassirou NOUHOU[5], Andrea GHIONE[6]

[1]International Centre for Advanced Mediterranean Agronomic Studies (CIHEAM-Bari), Valenzano (Bari), Italy
[2]Natural Resources Institute Finland (Luke), Jokioinen, Finland
[3]Alliance Bioversity International – CIAT (Centro Internacional de Agricultura Tropical), Rome, Italy
[4]Afrique Verte Burkina Faso (APROSSA), Ouagadougou, Burkina Faso
[5]Afrique Verte Niger (AcSSA), Niamey, Niger
[6]Italian Agency for Development Cooperation (AICS), Ouagadougou, Burkina Faso
*Corresponding author: elbilali@iamb.it

ABSTRACT

Neglected and underutilised species (NUS) are widely claimed to contribute to sustainability and sustainable development. Verifying such a claim implies the use of a scientifically sound assessment tool. In this context, the present article aims to suggest a matrix for the assessment of the environmental, economic and social sustainability of NUS. In particular, the paper provides a set of indicators and metrics to assess the different sustainability dimensions. It draws upon a search carried out on the Web of Science in May 2022 that returned 126 records. Eligible documents underwent two steps: in the first step, indicators, metrics and criteria regarding sustainability were identified; in the second step, expert knowledge was used to systematise the identified indicators and metrics according to the three sustainability dimensions and group them into themes. Agronomic aspects were included in the environmental dimension while nutrition, health and cultural aspects were included in the social dimension. One of the main results of the analysis is that there is a dearth of quality scholarly documents dealing with the assessment of NUS sustainability. Furthermore, economic and social indicators and metrics are hard to find. The operationalisation of the proposed indicators requires their contextualisation taking into consideration the conditions in each country/territory as well as the NUS concerned. A further important step to operationalise the proposed matrix implies the identification of a sustainability threshold and an assessment scale for each indicator. Apart from sustainability assessment, the proposed assessment matrix can allow selecting the NUS that have the highest potential and whose promotion can contribute to the sustainable development of the concerned countries and territories.

Keywords: *orphan crops, environmental sustainability, economic sustainability, social sustainability, sustainability assessment.*

INTRODUCTION

Neglected and underutilised species (NUS) – also known as orphan, minor, abandoned or lost crops (Padulosi, 2017) – represent tens of thousands of plant species (Chivenge et al., 2015). NUS are widely claimed to contribute to sustainability and sustainable development, thus sustainable food systems (SFS). Indeed, NUS offer the potential to diversify not only the human diets, but also farming systems, thus enabling more resilient and sustainable agri-food systems. They can help addressing various challenges such as food and nutrition insecurity, water scarcity, environmental degradation, poverty and climate change (Mabhaudhi et al., 2019). NUS contribute to climate-resilient food systems and offer opportunities to reduce greenhouse gas (GHG) emissions from agriculture (Mabhaudhi et al., 2019). Furthermore, NUS are critical for the conservation of agro-biodiversity and agro-ecosystems (Padulosi et al., 2013). NUS can also reduce environmental contamination from agriculture as they often tolerate diseases and grow on low-quality soils thus requiring lower levels of chemical inputs (Mabhaudhi et al., 2019). They play an important role in achieving food and nutrition security since millions of people, especially in developing countries, rely on NUS as their primary source of food (Mabhaudhi et al., 2019; Padulosi et al., 2013; Ulian et al., 2020). Certain NUS were also reported to have health protection properties (Tadele, 2018). NUS can also improve the livelihoods of rural people as they can enhance income from agriculture (Kour et al., 2018; Padulosi et al., 2013). Given all the above-mentioned benefits of NUS, Mabhaudhi et al. (2016) argue that their promotion could contribute to the achievement of the Sustainable Development Goals (SDGs).

Sustainability has been central in the current debate on food systems and their role in sustainable development, as shown by the United Nations' Food Systems Summit held in September 2021 (United Nations, 2021). There are also many regional initiatives on SFS; for instance, the Farm to Fork strategy in the European Union (EU) aims at fostering transition towards sustainable, resilient and inclusive food systems (European Commission, 2020). Nevertheless, in a systematic review on sustainable agri-food systems (AFS), El Bilali et al. (2021) show an increasing interest in AFS but suggest that while environmental aspects are sufficiently addressed, social, economic, and political ones are generally overlooked. Over the last decades, different frameworks have been developed to assess sustainability in agriculture and food systems such as the SAFA (Sustainability Assessment of Food and Agriculture systems) approach (FAO, 2013, 2014). Such frameworks are based on the use of different indicators and metrics. However, Adinolfi et al. (2015) point out that the sustainability assessment focus (product, diet, food supply chain, food system) and geographical coverage (local, territorial, national, regional) should be clearly defined for the selection of appropriate indicators. This clearly shows that appropriate indicators should be developed ad-hoc for each sustainability

assessment purpose depending on whose sustainability is assessed. Therefore, while sustainability assessment indicators exist for different levels of the food system and supply chain, to the best of our knowledge, no assessment matrix deals specifically with NUS. To address this gap, the present paper aims to suggest a matrix for the assessment of the environmental, social and economic sustainability of NUS.

MATERIAL AND METHODS

The present paper is based on a systematic literature review that follows the PRISMA guidelines (Preferred Reporting Items for Systematic Reviews and Meta-Analyses) (Moher et al., 2009; Page et al., 2021). The paper draws upon a search of all documents indexed in the Web of Science (WoS) carried out on May 4th, 2022, using the following search string: *(sustainability OR sustainable) AND (indicator OR criteri* OR metric OR index OR evaluation OR assessment) AND ("neglected and underutilised species" OR NUS OR "neglected species" OR "underutilised species" OR "neglected and underutilized crop" OR "neglected crop" OR "underutilized crop" OR "abandoned crop" OR "abandoned species" OR "alternative crop" OR "alternative species" OR "local crop" OR "local species" OR "lost crop" OR "lost species" OR "minor crop" OR "minor species" OR "niche crop" OR "niche species" OR "orphan crop" OR "orphan species" OR "traditional crop" OR "traditional species" OR "underdeveloped crop" OR "underdeveloped species")*. The search on WoS returned 126 documents. Two eligibility criteria were considered: NUS and sustainability assessment. Only the documents that meet both eligibility criteria were included in the systematic review.

In total, 114 documents were excluded following the screening of titles and abstracts as well as the scrutiny of full-texts, as they weren't eligible. Out of these, 84 documents were excluded because they do not deal with NUS. For instance, some documents refer to some forest tree species, such as *pinus*, or the National University of Singapore (NUS), Nigerian University System (NUS), non-uniform sampling (NUS), norm-based user selection (NUS) or number of undeveloped seeds (NUS), rather than neglected and underutilised species (NUS). Some documents deal with major commercial crops such as wheat, potato, maize, hazelnut, corn/maize, grapevine, citrus, sugarcane, cotton and sugar beet. Also, documents referring to local and minor arthropod and insect species as well as animal species (e.g. buffalo) were discarded. Further 30 documents were excluded because they do not address sustainability assessment. Some articles address sustainability assessment but in relation to livelihoods or farms/farming systems rather than NUS. Consequently, only 12 documents resulted eligible and were included in the systematic review: Georgiadis (2022), Kakabouki et al. (2021), Eissler et al. (2021), Mugiyo et al. (2021), Mwangi et al. (2020), Ibrahim Bio Yerima et al. (2020), Pande et al. (2018), DeHaan et al. (2016), Balemie and Singh (2012), Manos et al. (2008), Schmidt et al. (2008) and Scott (2003).

The analysis of the eligible documents was structured in two different steps. In the first step, indicators, metrics and criteria regarding the sustainability, as well as the selection and/or prioritisation of NUS, were identified. In the second step, expert knowledge was used to systematise the identified indicators and metrics according to the three sustainability dimensions (environmental, social and economic). During this step, preference was given to indicators and metrics to the detriment of criteria. The grouping of indicators and metrics into themes was informed by the SAFA approach (FAO, 2013, 2014). For the purpose of the present work, agronomic aspects were included in the environmental dimension; nutrition, health and cultural aspects in the social dimension.

RESULTS AND DISCUSSION

Table 1 provides an overview of indicators and metrics proposed in the selected articles/documents for the assessment of the sustainability of NUS. It also includes criteria suggested by different scholars to perform the selection or prioritisation of NUS to be included in the domestication programs or in different endeavours aiming at enhancing NUS and their products as well as developing their value chains. The table specifies for each source the context of reference (country/region) as well as the NUS or botanical groups considered.

Georgiadis (2022) documents traditional ecological knowledge and reports ethnobotanical uses of 125 plant taxa by an indigenous Karen community in Northern Thailand. The author ranks the cultural significance of the reported species in the community based on different indices such as the cultural importance index (Tardío & Pardo-de-Santayana, 2008) and the cultural value index (Reyes-García et al., 2006). Kakabouki et al. (2021) evaluate the potential contribution of seven alternative crops (viz. quinoa, teff, tritordeum, camelina, nigella, chia, and sweet potato) to climate change mitigation in the EU and examine the factors that might determine their successful integration in the Mediterranean area. They conclude that the limiting factors for crop establishment include soil properties, environmental and climatic parameters, and crop performance and dynamics. A good alternative crop should have high adaptability to different soil types, reduced water demands, reduced fertilization needs, reduced CO_2 emissions, reduced agrochemical inputs, reduced tillage and multiple uses, and increased employment. Eissler et al. (2021) use a sustainable intensification (SI) assessment framework – developed by the Feed the Future Innovation Lab for Collaborative Research on Sustainable Intensification (SIIL) (Musumba et al., 2017), that incorporates five measurable domains (productivity, economic, environmental, human condition, and social) – to assess the current uses of NUS as well as the perceived benefits and challenges to their use and management in northwestern Cambodia. They show a wide range of values and benefits associated with NUS that compose wild gardens. Mugiyo et al. (2021) develop land suitability maps for selected NUS – sorghum (*Sorghum bicolor*), cowpea (*Vigna unguiculata*), amaranth and taro (*Colocasia esculenta*) – in South Africa. They distinguish between natural or biophysical factors (e.g. rainfall, temperature and soil fertility) that directly affect

the growth of crops, and social and economic factors that do not directly affect crop growth, but influence land use degree of appropriateness. They found that sorghum was highly suitable and rainfall was the most critical variable and the criterion with the highest impact on land suitability of the NUS.

Mwangi et al. (2020) use the FAO's seed security framework (FAO, 2016) to assess seed security among smallholder sweet potato producers in Kenya. The framework is based on four parameters namely availability, accessibility, varietal suitability and seed quality. They show that smallholder producers experienced mild seed insecurity and seed access is the most critical element influencing food security. Ibrahim Bio Yerima et al. (2020) assess the phenotypic variability of 180 accessions of fonio from West Africa (Guinea, Mali, Burkina Faso, Niger, Benin) using 20 agro-morphological descriptors, including both qualitative and quantitative traits. They found significant differences among fonio accessions for most of the quantitative traits. Furthermore, highly significant correlations were found between grain yield and harvest index, thousand seeds weight, flowering and maturity times.

Pande et al. (2018) examine the economic sustainability of alternative agri-horticultural systems – drumstick (*Moringa oleifera*)- and aonla (*Emblica officinalis*)-based agri-horticulture trees with *Phaseolus radiatus* and *Foeniculum vulgare* crops – on reclaimed ravine lands in Gujarat, western India. The analysis shows the financial viability of the alternative cropping systems on the marginal lands, but the sensitivity analysis pointed out market and yield risks in crop components that need to be taken into account before recommending the alternative agri-horticultural system to farmers. DeHaan et al. (2016) suggest a pipeline approach to increase the success in contemporary domestication of new grain crops. They list criteria for ranking domestication species and discuss strategies to prioritize initial research efforts once the candidates have been selected. The domestication pipeline consists of three phases: (1) screening of plant species to discover candidates; (2) developing each candidate according to one of three general development strategies designed to produce a partially domesticated species usable as a new crop (viz. addressing the primary limitations, building on strengths, breeding to improve quantitative traits); and (3) integrating strategies to develop a commodity crop. Balemie and Singh (2012) survey diversity in a range of local crops (especially wheat and tef - *Eragrostis tef*) in the Lume and Gimbichu districts of Ethiopia and analyse local people's knowledge regarding crop uses, socio-economic importance, conservation and management. They found that agronomic performance (yield and pest resistance), market demand, and nutritional and use diversity attributes of the crop varieties were the most important criteria for making decisions regarding crop planting and maintenance.

Manos et al. (2008) evaluate tobacco alternatives [aromatic and medicinal crops: oregano, mountain tea, basil, mint, thyme, lavender, camomile; energy crops: sunflower, sugar beet, oilseed rape, anise; organic crops: wheat, barley, maize, alfalfa, and vetch; fruit trees: cherries, plums, pears, pomegranates] in Greece and classified them according to different criteria, mainly economic ones such as

profitability, in the context of the reform of Common Agricultural Policy (CAP) for the tobacco sector with phasing out of the subsidy payment for tobacco cultivation. This exercise led to the drafting of a list of the most suitable alternative crops for every region (Thessaly and Central Macedonia). They concluded that the most profitable alternatives are aromatic and medical crops. Schmidt et al. (2008) carried out farmer interviews and a literature review to prepare a 'masterlist' of promising NUS in China, Cambodia, north-eastern Thailand and northern Vietnam. Promising NUS underwent an initial pre-selection to narrow down the list then a multi-criteria and trans-disciplinary assessment involving different stakeholders (e.g. scientists, farmers, NGOs, policymakers). The process allowed identifying the most promising NUS for each country. The criteria for the evaluation of NUS were adapted from Padulosi et al. (1999). Scott (2003) highlights the importance of considering the commercial/market potential of minor/lost crops. Referring to the example of quinoa in the Andes region, he suggests a practical, low-cost procedure to evaluate the market prospects and procedures for these crops. According to Scott (2003), "*The principal steps involved in evaluating the commercial viability of processing and marketing new or improved agricultural products can be summarized as follows: Initial assessment. Evaluation of market competition and consumer demand. Input supply analysis. Analysis of costs and returns. Development of a marketing strategy*" (p. 207).

Table 1. Synthesis of documents considered in the systematic review.

Source	Country/region	NUS/crops considered	Indicators, metrics and criteria
Georgiadis (2022)	Thailand	125 plant taxa	Cultural importance index; Cultural value index
Kakabouki et al. (2021)	European Union	Quinoa, teff, tritordeum, camelina, nigella, chia, and sweet potato	Soil properties: texture, pH value, salinity, and sodicity (sodium adsorption ratio); Environmental and climatic parameters: temperature, altitude, latitude, photoperiod; Crop performance and dynamics: water demand (water use efficiency), fertilization needs (nitrogen use efficiency, nitrogen agronomic efficiency), light (growing degree days) and heat requirements

Source	Country/region	NUS/crops considered	Indicators, metrics and criteria
Eissler et al. (2021)	Cambodia	Various NUS including chaya (*Cnidoscolus aconitifolius*), galangal (*Alpinia galanga*), lemongrass (*Cymbopogon citratus*)	Productivity: ability to improve household consumption, ability to increase crop production, ability to diversify production; Economic: ability to increase income, ability to increase diversified income, ability to save on investments, ability to save time for labor; Environmental: ability to increase species diversity [biodiversity], reduce needs for chemical inputs, promote the use of natural composts; Human condition: ability to improve access to nutrition and nutritional diversity, ability to improve food security, ability to improve health [medicinal]; Social: gender, social cohesion, collective action
Mugiyo et al. (2021)	South Africa	Sorghum, cowpea, amaranth and taro	Climatic: temperature, rainfall, length of the growing season, reference evapotranspiration; Topographic: altitude, slope; Land use: land cover; Social/economic: distance to road
Mwangi et al. (2020)	Kenya	Sweet potato	Seed security: seed availability, seed access, varietal suitability and seed quality
Ibrahim Bio Yerima et al. (2020)	West Africa	Fonio	Qualitative: vigour at seedling, phenotypic grain colour; Quantitative: plant height, days to 50% flowering, days to 50% maturity, panicle length, dry biomass yield, grain yield, harvest index, thousand seeds weight
Pande et al. (2018)	India	Drumstick and aonla	Price; Net revenue
DeHaan et al. (2016)	Various countries/regions	Grain crops (maximilian sunflower,	Domestic morphology and phenology; ease of breeding and genetics; easily harvestable; high

Source	Country/region	NUS/crops considered	Indicators, metrics and criteria
		rice grass, chickpea, wild rice, sweet white lupin)	yield; grain similar to that of current crops; high-value product; high nutrition and quality attributes; available genetic resources; broadly adapted or adaptable; low input requirements; enhanced ecosystem services; culturally tenable; knowledge of the candidate's disease and pest risk; low potential to become invasive or contaminate the gene pool of a native species.
Balemie and Singh (2012)	Ethiopia	Wheat, tef, field pea, grass pea, fenugreek, lentil	Agronomic performance: yield and pest resistance; Use diversity; Nutritional and ethno-medicinal importance; Market demand; Socioeconomic importance
Manos et al. (2008)	Greece	Aromatic and medicinal plants	Income; Gross margin; Variable costs; Labour requirement
Schmidt et al. (2008)	China, Cambodia, Thailand and Vietnam	Various NUS e.g. Chinese white olive, taro, cashew, star goosbery	Economic and agronomic competitiveness: potential generated income, changing abiotic and biotic conditions (e.g. climate, pests), attractive traits; Local and national use, cultural acceptance; Traditional knowledge: knowledge on cultural practices, propagation techniques, knowledge on uses; Scientific Knowledge: research on genetic diversity, propagation techniques, knowledge on uses; Policy & legislation: extension and research activities by government and NGO's, favourable policies or government support; Opportunities for national/export niche market: availability of

Source	Country/region	NUS/crops considered	Indicators, metrics and criteria
			existing or potential future markets in the region, neighbouring countries or overseas
Scott (2003)	Andes	Quinoa	Consumer demand; Availability of inputs; Production costs; Returns

The analysis of the indicators, metrics and criteria proposed in the selected documents led to the drafting of the matrix for the assessment of the sustainability of NUS reported in Table 2. In the above-mentioned table, data from different sources have been merged and collated. Preference was given to indicators and metrics to the detriment of criteria that are hardly evaluated or for which no straightforward, simple evaluation method has been suggested. The analysis of the results reported in the table suggests that there are ways more environmental metrics and indicators than social and economic ways. Furthermore, some metrics need further elaboration in order to make them ready to use. In fact, not all metrics proposed satisfy all conditions to be considered SMART (Specific, Measurable, Achievable, Relevant and Time-bound) indicators. However, many general sustainability indicators are feasible in terms of the analysis of NUS as well.

Table 2. Proposed preliminary matrix for the assessment of the environmental, social and economic sustainability of NUS.

Sustainability dimension	Sustainability Theme	Proposed indicator/ metric	Source(s) informing proposal
Environmental	Environmental integrity	Fertiliser/nitrogen requirement	Kakabouki et al. (2021); Eissler et al. (2021); DeHaan et al. (2016)
		Pesticide requirement	Eissler et al. (2021); DeHaan et al. (2016)
		Water demand	Mugiyo et al. (2021); Kakabouki et al. (2021)
		Reference evapotranspiration	Mugiyo et al. (2021)
		Genetic diversity[2]	Eissler et al. (2021)
	Agronomic performance and productivity	Yield	Mugiyo et al. (2021); Eissler et al. (2021); Ibrahim Bio Yerima et al. (2020); DeHaan et al. (2016); Balemie and

[2] This metric might refer to the number of known varieties.

Sustainability dimension	Sustainability Theme	Proposed indicator/ metric	Source(s) informing proposal
			Singh (2012)
		Length of the growing season / Time to maturity	Mugiyo et al. (2021); Ibrahim Bio Yerima et al. (2020)
		Growing degree days	Kakabouki et al. (2021)
		Level of tolerance to salinity/sodicity	Kakabouki et al. (2021); DeHaan et al. (2016); Schmidt et al. (2008)
		Level of tolerance to high temperatures	Kakabouki et al. (2021); DeHaan et al. (2016)
		Level of tolerance/resistance to pests and diseases[3]	DeHaan et al. (2016); Balemie and Singh (2012); Schmidt et al. (2008)
		Seed security - Availability	Mwangi et al. (2020); DeHaan et al. (2016)
		Seed security - Varietal suitability	Mwangi et al. (2020)
		Seed security - Seed quality	Mwangi et al. (2020)
Social	Cultural significance and relevance	Number of documented uses[4]	Georgiadis (2022); DeHaan et al. (2016); Balemie and Singh (2012); Schmidt et al. (2008)
	Nutritional quality and diversity	Content of bioactive and health-promoting compounds	Eissler et al. (2021); DeHaan et al. (2016); Balemie and Singh (2012)
		Protein content	
	Employment	Labour requirement	Eissler et al. (2021); Manos et al. (2008)
	Equity and fair accessibility	Seed security - Access	Mwangi et al. (2020)
Economic	Competiveness	Price	Pande et al. (2018)
		Market demand	Balemie and Singh

[3] This metric might refer to the number of key pests and diseases.
[4] Human food, technology, medicinal, firewood, animal feed, symbolic uses, other.

Sustainability dimension	Sustainability Theme	Proposed indicator/ metric	Source(s) informing proposal
			(2012); Schmidt et al. (2008); Scott (2003)
		Production cost	Pande et al. (2018); Manos et al. (2008); Scott (2003)
	Profitability	Gross margin	Pande et al. (2018); Manos et al. (2008)
		Income	Eissler et al. (2021); Manos et al. (2008); Schmidt et al. (2008)

CONCLUSIONS

One of the main results of this analysis is that there is a dearth of quality scholarly documents that deal with the assessment of the sustainability of NUS. This is rather surprising and largely unexpected given the ongoing rhetoric on the enhancement and development of NUS and their value chains to address different challenges such as biodiversity loss, climate change, food insecurity and malnutrition, poverty and livelihoods vulnerability. This, in turn, clearly shows that the present work is timely and highly needed. Furthermore, economic and, especially, social indicators and metrics are hard to find. The operationalisation of the proposed indicators requires their contextualisation taking into consideration the conditions in each country/territory as well as the NUS concerned. Such a contextualization as well as the overall validation of the proposed assessment matrix should involve local stakeholders; which is foreseen in the framework of SUSTLIVES project that is being implemented in Burkina Faso and Niger[5]. A further important step to operationalise the proposed matrix, to make it functional for sustainability assessment, implies the identification of a sustainability threshold and an assessment scale for each indicator. The proposed assessment matrix can have different uses. In fact, apart from sustainability assessment, it can also guide initiatives for the selection and prioritisation of NUS to be included in the different programmes and initiatives aiming at the valorisation and enhancement of NUS and the development of their value chains. Given the limited resources, efforts should be concentrated on the most promising NUS; the proposed matrix allows selecting the NUS that have the highest potential and whose development can have the highest environmental, social and economic impacts thus contributing to the sustainable development of the concerned countries and territories.

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[5] https://www.sustlives.eu

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RESEARCH ON LIVE FOOD PRODUCTION FOR FISH

Daniela Cristiana ALEXANDRESCU[1*], Mioara COSTACHE[2], Loredana Beatrice NEAGU FRĂSIN[1]

[1]University Valahia of Targoviste, Faculty of Environmental Engineering and Food Science, Romania
[2]Fish Culture Research and Development Station Nucet, Romania
*Corresponding author: alexdanaa@yahoo.com

ABSTRACT

In Romania, although the importance of live food in fish feeding is known, unfortunately, the results obtained so far have not materialized through their application in fish production units, the realization of such crops being a sporadic activity and concentrated almost exclusively at the level of fish research units. The main objective was to identify native species of protozoa, rotifers, copepods, cladoceres and aquatic philopods that are suitable for intensive cultivation, in order to achieve crops for biomass production and the development of cultivation technologies accessible to production units that have as specific production of juveniles from various species of fish. The research did not explicitly aim at inventing new techniques or cultivation facilities, the literature being very rich in this respect and most of them already having, internationally, an industrial character, but the use and adaptation of existing ones to native species and to the technological conditions in Romania. The advantages of creating zooplankton culture systems are numerous, of which the most important can be mentioned: independence from weather conditions, the possibility of scheduling biomass production, depending on technological needs, the possibility to correlate the size of the chicks with that of the live food provided. Internationally, intensive crops of organisms that serve as food for fish larvae and chicks are widely practiced, in recent years developing a branch of aquaculture that deals only with them.

Keywords: *live food, fish, feeding, Romania*

INTRODUCTION

Feeding fish larvae and chicks during the first period of rearing (after switching to active feeding) is a problem that is largely dependent on the entire fish farming activity. If in the case of post-embryonic development in soil ponds there is a technology to stimulate the development of zooplankton (protozoa, rotifers, copepods, cladoceres), which, if successful, can ensure feeding and breeding up to a certain size of chicks, with good survival (Pillay, T.V.R., 2005). In the case of post-embryonic development in closed systems, the production and provision of live food is a relatively difficult problem (Falconer, D.S., Mackay, T.F.C., 1996).

Although there is currently a wide range of special feeds (starter, prestarter type) for the post-embryonic growth period, it is known that the best results are obtained when feeding live zooplankton, which, among other benefits, poses fewer problems with water quality alteration in growing facilities (Oprea, L., Rodica, G., 2000). The advantages of making zooplankton culture systems are numerous, of which the most important can be mentioned:

- ✓ independence from weather conditions
- ✓ the possibility of scheduling biomass production, depending on technological needs
- ✓ the possibility to correlate the size of the chicks with that of the live food provided.

Internationally, intensive crops of organisms that serve as food for fish larvae and chicks are widely practiced, in recent years developing a branch of aquaculture that deals only with them.

In Romania, although the importance of live food in fish feeding is known, unfortunately, the results obtained so far have not materialized through their application in fish production units, the realization of such crops being a sporadic activity and concentrated almost exclusively at the level of fish research units.

The main objective was to identify native species of protozoa, rotifers, copepods, cladoceres and aquatic philopods that are suitable for intensive cultivation, in order to achieve crops for biomass production and the development of cultivation technologies accessible to production units that have as specific production of juveniles from various species of fish (Midlen, A., Redding, T., 1998).

The research did not explicitly aim at inventing new techniques or cultivation facilities, the literature being very rich in this respect and most of them already having, internationally, an industrial character, but the use and adaptation of existing ones to native species. and to the technological conditions in Romania.

The work on the preparation of cultures of zooplankton organisms has been directed in two directions:

- ✓ Obtaining and using *Artemia salina philopodine* wolves to feed *Polyodon spathula* larvae in the first days of active feeding, using resistance eggs produced and packaged by the Dutch company Artemia Systems or Original Great Salt Lake " U.S.A
- ✓ obtaining and using intensive cladocere crops (*Daphnia longispina* or *Daphnia magna*), outdoors, in concrete basins and in ground basins.

MATERIAL AND METHODS

Artemia salina is one of the best choices in terms of live food, it is very nutritious and suitable for fish that are preparing to lay eggs, fish that have laid eggs and fish that are more demanding of food.

Artemia hawthorns have a high fat content, 23% of their body weight, this huge amount of fat is used for metamorphosis in adults. Once mature, *Artemia* has less fat and much more protein, respectively 63%. The culture of *Artemia* can be prepared by almost anyone because it is not very difficult, you just need to know a few basic questions about the biology of these crustaceans. One of the few disadvantages of *Artemia* is that a crop is rapidly depleted and to feed fish or

juveniles more crops are needed, installed on a different day, most often 3-4 crops are used in parallel but can be achieved and more if necessary.
Artemia salina, is the most used species of *Artemia* in our country, it is a small crustacean species, which at maturity reaches a length of only 1,5 cm. It can be found in salt lakes, lakes such as Techirghiol (Romania). At hatching, *Artemia* has a larval stage called nalupi, which are used as food for juvenile fish, especially in the case of species whose chicks are small. *Artemia* hawks are about 0,5 mm long, they are attracted to light but adults avoid light. After about 8 days the *Artemia* nalupes become adults and thus are able to lay eggs and the cycle resumes (Piper, R.G., 1983).

Fig.1. *Artemia* -SERA (crustaceans living in salt water)

Production of *Artemia salina* nauplies
Artemia salina nauplies (Fig. 2) are needed to feed fish larvae in the first three days of active feeding.

Fig. 2 Artemia salina nauplies

Adapted Zug-Weiss incubators (capacity 8 liters) are used to obtain the nauplii, by closing the basal orifice and introducing through the upper part a central aerator, with a role in oxygenating the water and in keeping the *Artemia* eggs in the water mass. 2 g of dried eggs / l of culture medium were used for incubation. The incubation temperature was 27^{o}C, with aeration and permanent lighting (with 40 W fluorescent tube). The incubation period was 27-28 hours.

Depending on the number of chicks hatched in each batch, the number of eggs incubated is staggered so that the ad libitum feeding of the fish larvae for 72 hours can be ensured.

Harvested nauplies are harvested by siphoning them in a fresh solution of NaCl 30 g / l, in which they are kept, with moderate aeration, at room temperature, until they are administered as food (maximum 4 hours).

Prior to administration, the nauplii are filtered through a 25µ mesh nytal cloth and wash with pool water.

A Sartorius-type electric balance was used for weighing, and a Kolkwitz-type chamber was used for counting eggs and nauplies. The nauplies were counted on a binocular magnifier and an MC-5 research microscope (I.O.R.).

To assess the density of the nauplies in the samples, three counts were performed in known volumes (0,5 - 1 ml), the resulting average being extrapolated to the total volume of the medium.

RESULTS AND DISCUSSION

Carrying out intensive cladocere cultures (*Daphnia longispina* and *Daphnia magna*)

For the culture of cladocera species (*Daphnia longispina* and *Daphnia magna*), two stages have been provided:

- ✓ establishment and maintenance of laboratory
- ✓ cultures carrying out intensive crops for biomass production

Laboratory cultures have been initiated since winter (January), being made in 2 plexiglass carafes with a volume of 230 l each.

The water temperature in the culture vessels in the first 2 months was in the range of 10 – 15^{0} C, the cultures being moderately aerated.

As a culture medium, pond water was used, filtered through 25 µ nytal mesh cloth. Two nytal sleeves filled with alfalfa hay were immersed in the culture pots and seeded with resistance eggs (epiphytes).

After 10 days (at an interval of 3-5 days), brewer's yeast was administered, in amounts of 10 mg / l dry matter, on culture medium. The vessels were placed in front of the window, but received additional artificial light, the lighting being done with 20 W fluorescent lamps, for 8 hours / day (from 8^{00} – 16^{00}).

After about a month, the first cladocers appeared, which were further fed with yeast. At the beginning of March, the temperature in the culture vessels was gradually increased to 18–22^{0}C, using electric heaters for this purpose.

At the same time, the artificial lighting period was extended to 12 hours / day. The amount of food administered was increased to 20 mg / day of dry matter per liter of

culture medium in 24 hours. After about 25 days, the density in the culture vessels increased to 300 - 500 ex / l (parthenogenetic females in various stages of development). Specimens of *Daphnia sp*. thus obtained, they were used to populate the 2 concrete basins, intended for the cultivation of zooplankton organisms. The culture was initially carried out in the 2 external concrete basins, with a volume of about 32 m³, fed with water from the Ilfov stream (Romania), filtered through a nytal sieve with a = 1,25 mm.

The preparation of the basin went through several technological sequences: flooding, the creation of the culture medium by administering 300 kg of fermented horse manure (in 2 ballasted nytal mattresses), the population of the pools with parthenogenetic females of *Daphnia longispina* and *Daphnia magna*, (fig. 3) at a density of 2 ex / l. Two successive cultures were carried out in each basin, the duration of a culture cycle being 25-30 days.

Fig. 3 Specimens of *Daphnia sp*. from live food culture made in the laboratory

Crop maintenance consisted of the administration of ammonium nitrate and superphosphate, in order to maintain the N-NO3- concentration around 5 mg / l and an N / P ratio of 10: 1.

Feeding was initially done with dry feed yeast (as long as it was marketed), administered daily in quantities of 5 mg / l in the first 7-10 days, 15 mg / l in the last 5-10 days, after which, Bakery yeast 30 mg / l was used daily until the fall of the cladocere culture.

From the 15th day onwards, 0,5-2 kg zooplankton biomass were harvested daily from each pool, quantities that were used to feed the polyiodon and to inoculate other crops, initiated in 2 other land basins, with an area of 0,2 ha. each.

Feeding

The growth of larvae to the shape of adult-like phenotypic characters is done according to a feeding scheme that involves feeding that is based on the administration of live food in the first 7-8 days of growth followed by a mixed feeding based on natural food and feed (Oprea, L., Rodica, G., 2000).

Feeding exclusively with natural food consists in the administration of planktonic and / or benthic organisms. Cladoceres are administered: *Daphnia sp*., *Moina sp*. and *Bosmina sp* .; the philopod Artemia salina; tubifex spigot. Natural food is collected directly from the natural environment or from laboratory crops (De Silva, S.S., Anderson, T. A., 1995).

The natural food combinations practiced are the following:

- ✓ 90% zooplankton and 10% *Tubifex sp*.
- ✓ 50% sweet or saline zooplankton 50% *Tubifex sp.*

Both types of food (plankton and benthos) before administration are subjected to a prophylactic treatment with a 1% solution of methylene blue in the case of the oligocet *Tubifex sp*. and with a solution of 23 mg / l potassium permanganate in the case of sweet zooplankton.

The food ration is determined daily according to the weight of the batch.

The ration administered in 24 hours is calculated as a percentage of 100% of the weight of the batch on that day (Goddard, S., 1996).

Equal fractions of this ration are distributed every 3 hours, both during the day and at night.

Feeding the larvae using live natural food in a mixed diet with feed will involve the application of the following feeding scheme that will allow the phased transition to a feed based exclusively on feed:

- ✓ first 5 days: 85% zooplankton, 5% benthos, 10% feed
- ✓ the following 5 days: 60% zooplankton, 20%, benthos, 25% feed
- ✓ the following 5 days: 50% zooplankton, 5%, benthos, 50% feed
- ✓ the following 5 days: 10% zooplankton, 5%, benthos, 85% feed
- ✓ the next feeding period will be provided only by feed.

With the exclusive switch to feed, the daily ration will be determined according to consumption, the percentage varying between 4-20% of the weight of the lot.

It is recommended to use fodder with a protein content of 35 -45%.

The amount of feed administered, as well as its granulation, will mainly take into account the size of the chicks and the temperature of the technological water.
The percentage of feed in sleeping chicks will be set at 10% of the total weight of the batch / day for specimens with a body mass of 0,5 g, provided that the technological water temperature is 25^0C. This percentage will be reduced with increasing temperature. If the batch has a body mass of 1–3 g / ex. the ration will be set at 2,5 -3,5% of the lot weight. The time interval between meals will be 4-6 hours.
In the conditions in which the lot will become heterogeneous, it will be absolutely necessary to be regrouped by weight categories, situation in which the ration will be adjusted according to biomass, and the granulation of the administered feed will be correlated with the size of the biological material.
The growth increase to be obtained after 45 days, until the outline of the phenotypic characters similar to adults is 3-4 g / ex.

CONCLUSIONS

Artemia salina is one of the best choices in terms of live food, it is very nutritious and suitable for fish that are preparing to lay eggs, fish that have laid eggs and fish that are more demanding of food.
Artemia hawthorns have a high fat content, 23% of their body weight, this huge amount of fat is used for metamorphosis in adults. Once mature, *Artemia* has less fat and much more protein, respectively 63%. The culture of *Artemia* can be prepared by almost anyone because it is not very difficult, you just need to know a few basic questions about the biology of these crustaceans. One of the few disadvantages of *Artemia* is that a crop is rapidly depleted and to feed fish or juveniles more crops are needed, installed on a different day, most often 3-4 crops are used in parallel but can be achieved and more if necessary. *Artemia salina*, is the most used species of *Artemia* in our country.

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PRODUCTION OF FERTILIZERS FROM BIOWASTES FOR ORGANIC AGRICULTURE

Monika JAKUBUS*

Department of Soil Science and Microbiology, Poznan University of Life Sciences, ul. Szydłowska 50, 60 – 656 Poznań, Poland
*Corresponding author: monika.jakubus@up.poznan.pl

ABSTRACT

Biowastes (garden and park wastes, food and kitchen wastes from households and restaurants) are a group of municipal wastes, which mass has recently increased significantly. Currently due to the rapid increase in the mass of municipal solid waste, it must be perceived as a multidimensional global problem. Thus proper waste management must be developed to attain such goals as prevention of waste generation and reduction of its volume, as well as encouraging its reuse and raw material recovery. Biowaste composting is a popular and cheap solution, in line with the circular economy concept and principles of sustainable management of organic mass. Such composts can be important organic fertilizers for use in organic agriculture. However, the quality of composts must be valorized and verified, the more so as they are made of waste of different origins. In this study, the quality of composts and vermicompost prepared from various biowastes was assessed in terms of their use for organic agriculture. In the analyzed materials the content of macro- and micronutrients essential for plants, heavy metal amounts as well as the quantity and quality of humic compounds were determined. On the basis of the obtained results it can be concluded that both vermicompost and composts showed good quality, serving as a valuable source of organic matter and nutrients for plants and thus they can be used for organic agriculture. Moreover, the content of heavy metals in these organic materials did not exceed the permissible standards.

Keywords: *organic wastes, vermicompost, compost, macro- micronutrients, humic compounds*

INTRODUCTION

Maintaining soil fertility and health in organic farming is based on the use of natural fertilizers, which include mainly manure, slurry, green manure and composts. Chemical composition of manure or slurry in the case of animal fertilizers mainly depends on the species, age, sex, breeding direction, feed and fertilizer storage conditions. Composts as fertilizers of plant origin are produced as a result of the composting process, which can take place at different rates and for which various plant raw materials can be used. As a result, composts differ in their chemical composition, and hence in their potential impact on the soil environment and plants. Currently, in many European countries the principles of circular

economy are being implemented (Jakubus, 2020), which indicates the legitimacy and necessity of reusing waste as a valuable raw material. Composting of selectively collected biomass waste fits with this type of ideas and principles. Moreover, such a method of recycling biodegradable waste is positively perceived both by the inhabitants and local authorities (Jakubus and Michalak-Oparowska, 2021). Among educational campaigns, home composting of household waste is promoted and the obtained fertilizers are used by residents for their own purposes, mainly fertilizing garden plants or planting potted plants. Additionally, in Poland there is a well-developed network of composting plants producing compost from biowaste for commercial purposes. Composts are an excellent alternative to manure, especially when it is unavailable on the local market. However, as previously mentioned, composts can be of different quality due to the variety of raw materials and composting conditions. Therefore, in this study a qualitative assessment was conducted for 3 composts obtained on the basis of selectively collected biowaste, but composted in a different manner.

MATERIALS AND METHODS

According to the list of waste referred to Article 7 of Directive 2008/98/EC, used wastes belong to the same group of municipal wastes, code 20 (food and kitchen wastes from households and biowastes from the garden). These wastes collected separately by inhabitants were used in the composting process. The detailed information concerning used biowastes and conditions of the composting process are given in Table 1.

Table 1 Raw materials and technology used for individual composts

Organic fertilizer	Raw materials	Technology
VC	Food and kitchen wastes from households	Vermicomposting process was carried out in the household in a vermicomposter. A mixture of apple pomace with earthworms (*Eisenia fetida)* was used as an initial input material for the vermicomposting process. The biowaste for the vermicomposter was delivered with a varying frequency and in different amounts, which depended on the activity of the household.
C1	Mixture of yard trimmings as plant residues except for mowed grass clippings	C1 was prepared by the aerobic method as a fertilizer for their home gardens by private home owners. The composting process was carried out in home composters made of thermoplastic. The temperature of the composting process depended on weather conditions. The organic material was successively collected in containers without

		any mixing of the bulk volume. Under such conditions the organic waste mixture was kept for a year. After this time, the whole mass was mixed to homogenize it and then transferred to dark plastic bags to complete the maturation stage.
C2	Mixed wood, forestry and agricultural residue, garden and park wastes, food and kitchen wastes from households and restaurants	The composting process under controlled bioreactor conditions in a composting plant until the completion of the cooling phase of composting. The maturation stage of compost run in an open shed and lasting 2 months depending on weather conditions.

The chemical analyses were conducted on dried samples. Organic matter (OM) in composts (C1, C2) and vermicompost (VC) was determined by the loss-on-ignition test (dry combustion for 6 hours at a temperature of 550^{o}C). The obtained ashes of composts were used to assess the amounts of macronutrients (K, Mg), micronutrients (Mn, Zn, Cu) and heavy metals (Pb, Cr) after extraction in 6 mol·dm^{3} HCl (Ostrowska *et al.*, 1991) using atomic absorption spectrophotometry in a Varian Spectra AA 220 FS apparatus. In the same extracts phosphorus (P) content was measured colorimetrically by the vanadium–molybdenum method. Total organic carbon (TOC), nitrogen (N) and sulphur (S) contents were determined using Vario Max CNS. Humus fractionation was determined according to the method proposed by Kononova and Bielczikova (Dziadowiec and Gonet, 1999), in which humic substances were determined in a mixture of 0.1 mol·dm^{-3} $Na_4P_2O_7$ + 0.1 mol·dm^{-3} NaOH solution. Optical density ($Q_{4/6}$) of the obtained fractions was determined at 465 nm and 665 nm. The fulvic acid fraction (FA) was separated after precipitation of humic acids at pH 1.5 (HA). Carbon in the obtained fractions (C_{HS} and C_{FA}) was oxidized by 0.1 mol·dm^{-3} $KMnO_4$ in the H_2SO_4 medium. Humic acid carbon (C_{HA}) was calculated by subtracting C_{FA} from C_{HS}. On the basis of C_{HA} and C_{FA} degree of polymerisation (DP), was calculated for VC and C1 and C2 according to equation: DP = C_{HA}:C_{FA} The labile carbon (LC) was assessed by $KMnO_4$ oxidation (Łoginow *et al.*, 1987). All the assays determining the amounts of individual elements in the tested samples were performed in six replications and the presented results are their mean values. The obtained results were subjected to formal evaluation by the analysis of variance using the F test at the significance level $p \leq 0.95$. The least significant differences were calculated using the Tukey test at the significance level $\alpha \leq 0.05$ and then uniform groups within the factor level were established.

RESULTS AND DISCUSSION

In general, VC was characterized by statistically higher amounts of OM, CHS and $Q_{4/6}$ (Table 2) as well as macronutrient contents (N, P, K, S, Mg) (Figure 1) with the lowest amounts of micronutrients (Cu, Zn, Mn) (Table 3). Compost No. 2 was characterized by significantly higher contents of LC (Table 2) as well as higher amounts of micronutrients (Mn, Zn, Cu) and Pb content (Table 3). Generally, for C1 the smallest amounts of macronutrients (Figure 1), OM, CHS, LC (Table 2) were determined. The contents of Mn, Zn and Cu in C1 did not differ significantly from those determined for VC (Table 3). The amounts of Cr were the same for C1 and C2. The mentioned composts had the same $Q_{4/6}$ values (Table 2). Compost No. 1 characterized by the lowest Pb amount (Table 3). In Poland VC and Cs dedicated to agricultural use have to meet specific threshold amounts of N, P, K, organic matter and heavy metals given in the Regulation of the Minister of Agriculture and Rural Development (2008). According to this the content of OM must be at least 30% d.m., the amount of potassium (K_2O) and phosphorus (P_2O_5) should be more than 0.2% d.m., while the total N value should be min. 0.3% d.m. The tested fertilizers met the criteria for N, P, K, but the one required OM content was not found only for C1. At this point, it should be emphasized that the raw materials used and the composting technology applied had a major impact on the abundance in nutrients of composts and vermicompost. In literature one can find various contents of macro- and micronutrients, both similar, lower and higher than in this study (Sciubba *et al*., 2015, Ibrahim *et al*., 2019, Jakubus, 2020, Singh *et al*., 2020, Jakubus and Michalak-Opraowska, 2022). According to the above-mentioned Regulation vermicompost or compost cannot exceed, among other things, 100 mg · kg^{-1} Cr and 140 mg·kg^{-1} Pb. The EU guidelines (Commission Regulation 2008) in this regard are more restrictive, because the amount of Pb in composts cannot be higher than 45 mg·kg^{-1}, and Cr greater than 70 mg·kg^{-1} d.m. Nevertheless, the tested fertilizers were characterized by much smaller amounts of heavy metals. It is worth emphasizing this fact because many authors (Jakubus, 2020, Ibrahim *et al*., 2019, Rodrigues *et al*., 2020) gave significantly higher amounts of Cr and Pb for composts. It should be noticed that composts are a significant source of organic matter, including carbon compounds, especially humic substances. Humic substances mainly consist of fulvic and humic acids. Fulvic acids are compounds weakly polymerized and relatively readily undergoing chemical and microbiological changes, which results in their considerable solubility and mobility. In turn, HAs are generally recognized as being non-degradable or sparsely degradable compounds with a strongly polymerized structure (Yu *et al.* 2019). Generally immature composts contain a high FA content, while HA dominates in mature composts. According to this the obtained data indicate that the maturation degree was favorable in the case of C2. Although the amounts of SH for CV and C2 did not differ statistically, the proportion of FAs and HAs was different as shown in Figure 2 and FAs (57%) predominated in the HS of CV, while the HAs (65%) predominated in HS of C2. The percentage share of FAs and HAs for C1 was comparable, accounting for 49.5% and 50.5%, respectively. When assessing

the quality of vermicompost and composts the evaluation of their stability and maturity is also essential. It is an extremely important element of composting, because an unstable and immature organic material may have adverse effects on plant growth and the environment. The assessment of composts in this respect can be made based not only on the HS composition, but also on the value of C:N. In these studies, the values of C:N were the same regardless of the tested fertilizer (Table 2). Jakubus and Michalak–Oparowska (2022) cited various authors indicating that the C:N ratio for the matured compost should range at 9-20:1. Taking this criterion into account, the tested composts can be considered mature and usable for soil fertilization. The $Q_{4/6}$ ratio is negatively related to the aromatic polycondensation degree and molecular weight of humic substances. High $Q_{4/6}$ values imply the presence of low molecular weight aromatic molecules, which in contrast to $Q_{4/6}$ low values indicate high contents of large molecular weight molecules, such as humic–like compounds, usually present in well-matured organic materials (Alvarenga *et al*., 2016). In the present study the analyzed vermicompost and composts also showed comparable values of optical density expressed as $Q_{4/6}$; however, slightly lower values were found for C1 and C2. Compost maturation can be also assessed on the basis of C_{HA}:C_{FA} (DP). Azim *et al*. (2018) based on a literature review stated that the correct threshold value of the polymerization degree needs to be greater than 1. Taking into account that threshold it may be assumed that all analyzed organic fertilizer meet this criterion, because the values were 1.0, 1.1 and 1.2 for C1, VC and C2, respectively (Table 2). In terms of the quality of carbon connections, one should also interpret the amounts of labile carbon (LC), which were the highest for C2 (1.18 $g \cdot kg^{-1}$) and the smaller for C1 (0.38 $g \cdot kg^{-1}$) (Table 2). Labile carbon represents the most active pool of organic carbon that is susceptible to microbial degradation. Taking into account the fact that the LC is more susceptible to decomposition it can theoretically be assumed that they may play an important role in the transformation of VC and Cs in the soil. The rapid degradation of easily mineralizable LC can contribute to enhancing the microbial activity of the soil, as well as releasing N and S from easily mineralizable combinations.

Table 2. The amounts of organic matter (OM), humic substances (CHS), labile carbon (LC) as well as values of degree of polymerisation (DP) and optical density ($Q_{4/6}$) obtained for analysed organic fertilizers

Organic fertilizer	OM	CHS	LC	C:N	DP	$Q_{4/6}$
	$g \cdot kg^{-1}$					
VC	494.9a	34.4a	0.97b	10:1	1.1	8.12
C1	192.9b	23.9b	0.38c	10:1	1.0	6.92
C2	357.9b	31.3a	1.18a	10:1	1.2	6.92

Table 3. The mean content of metals in analysed organic fertilizers (mg·kg^{-1})

Organic fertilizer	Mn	Zn	Cu	Pb	Cr
VC	90.9b	5.1b	16.5b	11.5b	7.6a
C1	103.4b	7.5b	18.7b	2.7c	4.4b
C2	182.4a	22.6a	36.1a	22.4a	4.4b

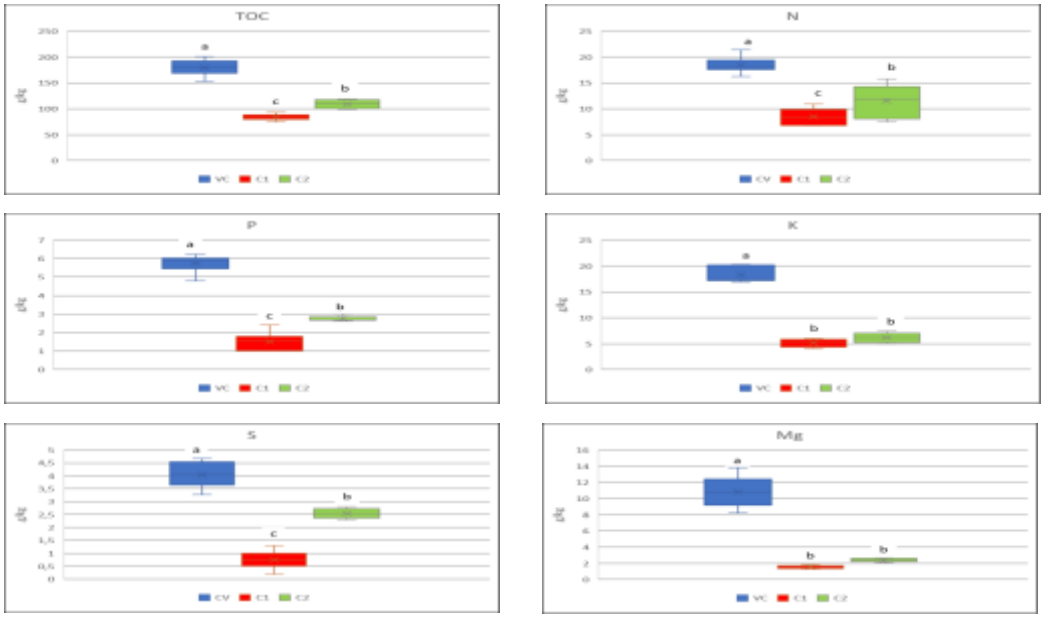

Fig.1 The mean macronutrient contents in organic fertilizers

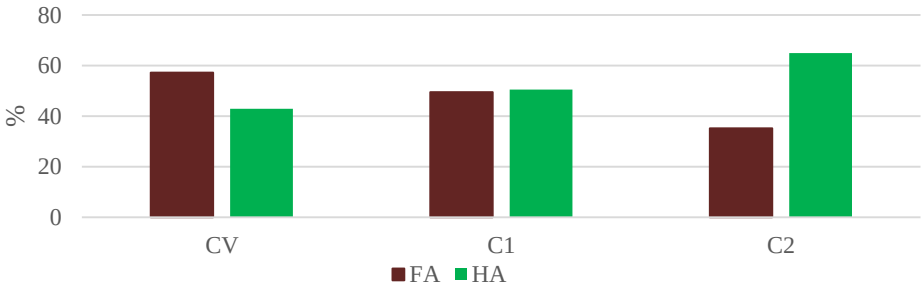

Fig.2 The percentage share of FA and HA in humic substances of organic fertilizers

CONCLUSION

The conducted research has shown that the quality of composts is the result of both different raw materials and the composting processes carried out. The assessed parameters allowed to evaluate the fertilising value of composts. The worst quality was found for compost No. 1 prepared using a backyard composting method only from green waste. Vermicomposting of kitchen waste resulted in favorable amounts of OM, CHS and macronutrients. On the other hand, compost No. 2 produced in professional installations was characterized by higher amounts of micronutrients and favorable values of humification parameters. Since the content of heavy metals in VC and Cs did not exceed the permissible standards, high safety of their soil application should be underlined. Regardless of the differences shown, the tested organic fertilizers can be used by individual households for their own purposes, including gardening and agricultural purposes, for soil fertilization, which clearly underlines the importance of recycling biowaste both at the household level and on an industrial scale. At the same time, it should be remembered that when using composts in organic agriculture, they should undergo a certification process based on applicable standards.

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DOES SOIL TILLAGE BEFORE AFFORESTATION CONTRIBUTE TO HIGHER CARBON STOCKS?

Gediminas SURVILA, Iveta VARNAGIRYTĖ-KABAŠINSKIENĖ*

Lithuanian Research Centre for Agriculture and Forestry, Kaunas district, Lithuania
*Corresponding author: iveta.kabasinskiene@lammc.lt

ABSTRACT

Afforestation of former agricultural land contributes to the higher carbon (C) stocks in aboveground biomass and dead organic matter. Soils contain major C stocks and are of great importance for C sequestration. Soil preparation prior to planting tree seedlings, especially if deep soil cultivation is applied, causes significant disturbances in the soil profile. Therefore, the afforestation can alter organic C budgets, both in soil and forest biomass. This study analyzed the influence of deep soil tillage up to 40–60 cm depth on the SOC stocks in Arenosols (20 years post afforestation) and Planosols (10 years post afforestation) of Scots pine plantations in comparison to non-ploughed soils in naturally regenerated Scots pine stands. The samples of forest floor and mineral soil in different layers up to 80 cm depth were analyzed. The results showed no differences in forest floor C contents between deep ploughing and non-ploughed sites. The SOC stocks increased at deeper mineral soil layers in relation to the upper mineral soil layers. The total SOC stocks, calculated by summing the SOC values obtained in forest floor and mineral soil layers up to 80 cm depth, were higher in deep ploughing sites than in non-ploughed sites. Therefore, this demonstrated the positive effect of deep soil tillage not only on SOC retaining in the deeper soil layers but also showed the continuous SOC accumulation in the new topsoil. Potentially, soil C sequestration in the deeper layers for a longer period could also be important in the context of climate change.

Keywords: *deep ploughing, organic carbon, carbon concentration, carbon stock.*

INTRODUCTION

The discussions on more intensive land use management in the context of climate change are initiating an assessment of the carbon (C) balance in terrestrial ecosystems at various levels. The management of CO_2 emissions from the energy and transport sectors and the C sequestration following different land use categories and land-use change practices, including afforestation, are among the main challenges for the sustainable management of environmental systems in Europe (IPCC, 2007; Ellison *et al*., 2011). The C sequestration in both plant biomass and terrestrial soils reduces atmospheric CO_2 concentrations over time

(Lal, 2004; 2010; Smith *et al*., 2013). Existing research recognizes that the stocks of soil organic carbon (SOC) are about three times higher than in terrestrial plant biomass, and soil is exclusively valued as a long-term SOC stock in the biosphere (Guggenberger, 2010; Palosuo *et al*., 2016; FAO, 2017). Additionally, the higher SOC stocks positively contribute to climate change mitigation (UNFCCC, 1997; IPCC, 2007). The existing studies show that C sequestration and SOC stock management are related to various land use management practices (Mayer et al., 2020). It is obvious, that the afforestation of former agricultural land leads to higher C stocks in the aboveground plant biomass and a higher amount of dead organic matter. However, at the ecosystem level, the response of soil chemical composition to afforestation, especially when different soil cultivation techniques are applied prior to tree planting, is likely to be a more complex process.

For higher environmental sustainability and biodiversity, the afforestation of abandoned and former agricultural lands is one of the priorities in increasing overall forest cover in Lithuania. The previous studies identified the increased or unchanged SOC stocks in infertile sandy soils during the 30 years after afforestation (Bárcena *et al.*, 2014; Varnagirytė-Kabašinskienė *et al*., 2021). The different afforestation techniques could potentially have a stronger positive effect on climate change management for a long time. When agricultural land is evaluated as a potential land for afforestation, a layer of compacted soil could potentially inhibit tree growth by slowing down root penetration and water penetration (Malinauskas and Urbaitis, 2008). When the deep soil ploughing practice is used, the soil is deeply mixed throughout the profile and the upper carbon-rich soil horizon is buried in deeper soil layers (Schneider *et al*., 2017). Usually, deep soil ploughing is performed up to 50–60 cm in depth as compared to ordinary soil ploughing up to 20 cm. A slower decomposition of additional SOC inputs in the subsoil compared to the topsoil was indicated in the previous studies (Wordell-Dietrich, 2016). This was explained by a lower density of decomposing microorganisms followed by a lower possibility of SOC mineralization (Don *et al.*, 2013). The higher SOC stability in the subsoil was also demonstrated due to lower oxygen concentration in the subsoil and lower changes in humidity and temperature regimes (Rumpel and Kögel-Knabner, 2011). The development of new forest plantations leads to new carbon input from an annual litter of leaves or needles, dead wood and roots slowly entering the subsoil. In this study, we analyzed the influence of deep soil ploughing up to 55–60 cm depth on the SOC stocks in Arenosols 20 years after afforestation and in the Planosols 10 years after afforestation with *Pinus sylvestris* plantations in comparison to naturally regenerated *P. sylvestris* stands on non-ploughed soils. We presumed that the deep soil ploughing could be considered when afforestation is planned on low fertility sandy soils.

MATERIAL AND METHODS

For this study, two experimental regions were selected: the first, the Druskininkai region located in southern Lithuania (54°40’ N; 23°39’ E), and the second, the

Širvintos region located in eastern Lithuania (54°91' N; 24°93' E). For estimation of SOC stocks under different soil cultivation techniques, 20 years old Scots pine (*Pinus sylvestris* L.) sites on Arenosols and 10 years old Scots pine sites on Planosols were sampled in August of 2020 and 2021, respectively. In the Druskininkai region, the sites represented four treatments: deep soil ploughing up to 60 cm depth; soil cultivation by 40 cm × 40 cm microsites when the surface soil layer is removed; soil ploughing by making furrows up to 20 cm depth; and naturally regenerated forest stand on non-ploughed Arenosols. This study region was established after the application of different soil cultivation methods prior to afforestation in 2000. In the Širvintos region, there were two treatments, including deep soil ploughing up to 55 cm depth; and naturally regenerated forest stand on non-ploughed Planosols, established in 2012.

For mass determination, the forest floor was sampled with a metal frame of 25 cm × 25 cm. The four samples of the forest floor were composed of five subsamples in the field; then dried at 105 °C to a constant mass and weighed in the laboratory. The mineral soil was sampled with a metallic soil auger from the 0–10 cm, 10–20 cm, 20–40 cm, and 40–80 cm soil layers. The four composite samples were combined from five subsamples, collected systematically at each site of each study region. For the assessment of bulk density ($g \cdot cm^{-3}$) of fine (<2 mm) mineral soil, the four composite samples from the 0–10 cm, 10–20 cm, 20–40 cm, and 40–80 cm layers were taken from five subsamples using a metal cylinder. Then, the samples were passed through a 2 mm sieve to remove stones and gravel and dried at 105 °C to a constant mass (ISO 11272:1998). The organic C concentration was determined in forest floor and mineral soil samples using a dry combustion method with a total carbon analyzer Analytic Jena multi EA 4000 Germany (ISO 10694:1995). The SOC stocks in the forest floor were calculated by multiplying the concentrations by the forest floor mass. The SOC stocks in the mineral soil layers were calculated according to the methodology given by Vesterdal et al. (2008), which included the value of bulk density, the thickness of the soil layer, and the SOC concentration of the layer. The total SOC stocks for the mineral soil profile up to 80 cm depth were obtained by summing the values in all mineral soil layers.

The data were analyzed for differences between the sites representing different soil cultivation methods by Kruskal–Wallis analysis of variance (ANOVA). The STATISTICA 12.0 (StatSoft. Inc, Tulsa, OK, USA, 2007) software with a level of significance of $p < 0.05$ was used.

RESULTS AND DISCUSSION

The SOC concentrations obtained in the forest floor were different from the remaining soil layers for both Arenosols and Planosols (Table 1). In Arenosols, the SOC concentrations in the forest floor found for the sites afforested after deep soil ploughing were comparable to non-ploughed sites but slightly lower than for the sites afforested after making furrows. In Planosols, the SOC concentrations in the sites of deep ploughing were lower than in the naturally regenerated Scots pine stand.

Table 1. The concentrations of soil organic carbon (SOC) ($g \cdot kg^{-1}$) in the forest floor and mineral soil layers at the sites representing different treatments on Arenosols and Planosols. Different letters indicate statistically significant differences in mean concentrations of mineral soil layers between the sites within the same soil type ($p < 0.05$).

Treatment	Forest floor	Mineral soil layer (cm)			
		0–10	10–20	20–40	40–80
Arenosols					
Afforested, deep ploughing	227.7 ± 24.0	6.2±0.2ab	5.5±0.3a	7.2±0.6b	6.3±0.4ab
Afforested, microsites	214.3 ± 12.5	8.4±1.0c	6.4±0.5b	4.6±0.3ab	4.1±0.2a
Afforested, furrows	271.8 ± 17.6	9.8±0.6b	8.3±0.6b	7.0±0.2ab	5.7±0.5a
Naturally regenerated, non-ploughed	220.1 ± 31.4	9.1±0.9c	7.0±0.4b	5.1±0.5a	4.7±0.5a
Planosols					
Afforested, deep ploughing	204.1±8.3	4.5±1.3a	5.0±0.4ab	7.7±0.6b	4.6±1.5a
Naturally regenerated, non-ploughed	227.4±20.9	9.8±1.9c	7.0±1.5a	5.9±1.5ab	3.0±0.1a
Croplands, furrows	-	6.6±0.4b	6.7±0.3b	6.4±0.6ab	4.2±0.3a

The estimated SOC concentrations varied among the mineral soil layers up to 80 cm in depth for both study regions (Table 1). In deeply ploughed soils, the SOC concentration in the 20–40 cm layer was slightly higher for the Arenosols and by 1.5-1.7 times for the Planosols compared with the remaining soil layers. In the Arenosols, relatively similar SOC concentrations of 6.2–6.3 g kg^{-1} were found in the upper 0–10 cm and the deepest 40–80 cm layers but lower concentrations were found in the 10–20 cm layer. The SOC concentrations in the remaining study sites showed a consistent decreasing trend from the upper soil layer to a profile depth of 80 cm for both soil types.

In this study, we compared the SOC stocks in different mineral soil layers in both soil types (Figure 1). The comparison of different treatments showed an uneven distribution of SOC stocks in the soil profile. The highest SOC stock values in the Arenosols were obtained in the deepest 40–80 cm mineral soil layers, which varied from approximately 20 $t \cdot ha^{-1}$ in the sites afforested by making microsites to 34 $t \cdot ha^{-1}$ in the deeply ploughed sites (Figure 1A). The SOC values obtained in the upper 0–10 cm and 10–20 cm soil layers were from 2.3–2.6 to 4.3–4.8 times lower than in the 20–40 cm and 40–80 cm layers, respectively. These data demonstrated a clear trend for higher carbon accumulation in deeper soil layers following deep soil ploughing. Not as clearly expressed as in the sites with deep ploughing, but still, quite a similar trend was obtained in the sites afforested after making furrows. However, in the non-ploughed sites, the SOC stocks were similarly distributed among the 0–10 cm, 10–20 and 20–40 cm layers with the higher values found in the 40–80 cm layer.

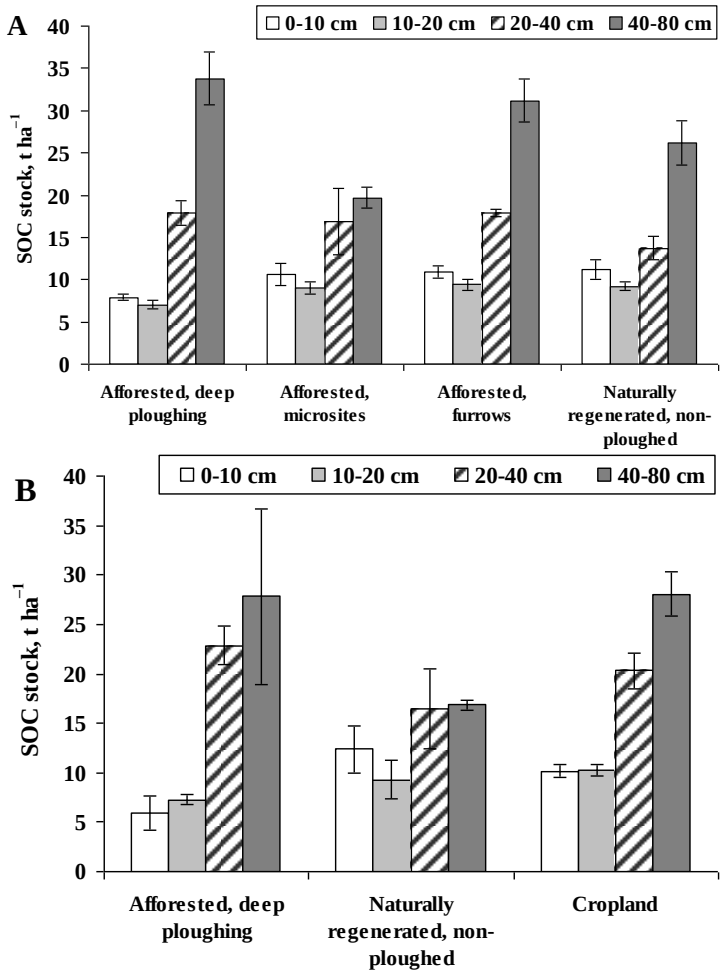

Figure 1. The stocks of soil organic carbon (SOC, $t \cdot ha^{-1}$) in the mineral soil layers in the different treatments on Arenosols (A) and Planosols (B)

In the Planosols, the comparable SOC values of 23–28 $t \cdot ha^{-1}$ were obtained for the 20–40 cm and 40–80 cm layers of afforested soil at deeply ploughed sites (Figure 1B). The distribution of the SOC stocks in the soil profiles representing different treatments was different. The deep soil ploughing caused lower SOC stocks for the topsoil layers and higher SOC stocks for the subsoil layers. There were no significant differences among the SOC values in different layers of the non-ploughed sites. While the SOC stocks in different soil layers of the croplands were distributed as follows: 10 $t \cdot ha^{-1}$ in both 0–10 cm and 10–20 cm layers; 20 $t \cdot ha^{-1}$ in the 20–40 cm layer, and 28 $t \cdot ha^{-1}$ in the 40–80 cm layer.

In comparison to non-ploughed sites, the total SOC stocks for the whole mineral soil profile up to 80 cm depth showed from 7 $t \cdot ha^{-1}$ to 9 $t \cdot ha^{-1}$ higher SOC stock in the deep ploughed Planosols and Arenosols, respectively.

The most obvious finding to emerge from the analysis was that deep soil ploughing significantly increased the SOC stocks in the 20–40 cm layer while it did not facilitate SOC sequestration to a significant extent for the whole soil profile. Comparable trends were obtained for both studied soil types – Arenosols and Planosols, which were 20 and 10 years, respectively, after afforestation with *P.sylvestris*. This finding was contrary to some previous studies which have suggested that intensive ploughing resulted in the SOC loss (Lal 2007) or showed that the SOC stocks in the former plough layer decreased in the first decade after afforestation, and only later increased (Smal et al. 2019). More often, the use of deep soil ploughing prior to afforestation is highlighted as an effective tool for long-term SOC sequestration (Alcántara et al. 2016). Furthermore, the added value of this soil ploughing is that the topsoil carbon enters the deeper layers and remains for a longer time in a non-fully mineralized matter, additionally, the SOC begins to accumulate as a new litter layer after afforestation (Alcántara et al. 2016). Several studies outline, that deep soil ploughing prior to afforestation facilitates deep rooting, therefore, the SOC stocks can be expected to increase over time. These findings suggest a higher potential to increase the SOC sequestration after the application of deep ploughing for a longer period at least in the soils of lower fertility, therefore, it could be useful in the context of climate change.

CONCLUSIONS

This study has discussed the effect of deep soil ploughing up to 55–60 cm depth on the SOC stocks in the Arenosols and Planosols two and one decade, respectively, after afforestation with *Pinus sylvestris* plantations in comparison to non-ploughed soils and other land uses. We found no differences in the SOC content in the forest floor layer between deep ploughing and non-ploughed sites. However, the SOC stocks increased in the subsoil layers at a depth of 40–80 cm in comparison to the mineral topsoil layers up to 20 cm in depth. The deep ploughing induced 7–9 $t \cdot ha^{-1}$ higher SOC stock in the entire mineral soil profile up to 80 cm depth, compared to the non-ploughed sites. Overall, we assumed that there is a positive effect of deep soil ploughing on the SOC retaining in the deeper soil layers with a potential to accumulate new SOC in the litter layer after afforestation of former agricultural land.

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IRANIAN KISHK AS A SOURCE OF LACTIC ACID BACTERIA PRODUCING EXOPOLYSACCHARIDE

Mohammad Reza EDALATIAN DOVOM[1]*, Paria RAHNAMA VOSOUGH[1], Mohammad B. HABIBI NAJAFI[1], Ali JAVADMANESH[2], Baltasar MAYO[3]

[1]Food Science and Technology Department, Faculty of Agriculture, Ferdowsi University of Mashhad (FUM), Mashhad, Iran
[2]Animal Science Department, Faculty of Agriculture, Ferdowsi University of Mashhad (FUM), Mashhad, Iran
[3]IPLA, CSIC, Asturias, Spain
*Corresponding author: edalatian@um.ac.ir

ABSTRACT

Exopolysaccharides are high molecular weight polymers composed of sugar subunits. Produced exopolysaccharides by lactic acid bacteria (LAB) play a significant role in improvement of organoleptic properties of fermented dairy products such as yogurt. Diversely, the probiotic function of these bacteria and the prebiotic properties of their produced biopolymers promote consumer's health. For this purpose, a traditional dairy product known as "Kishk" was selected. 143 strains of lactic acid bacteria were isolated from Iranian Kishk in Khorasan Province and cultured in formulated MRS mediums with different sugars such as glucose, fructose, sucrose and, lactose (40 g/L) and incubated in anaerobic conditions at 30 and 37°C for 48 hours. The microscopic features of the isolates were assessed and the production of exopolysaccharide in the culture medium was evaluated by disk and ruthenium red methods. The phenol-sulfuric and weight method were used to quantify exopolysaccharide production. Results showed pH of Kishk samples ranged from 3.60 to 4.08 and the average of total mesophilic count and LAB count of samples were 6.50 and 5.89 log CFU/g, respectively. Analysis of data exhibited 79 out of 143 lactic acid bacteria isolates were exopolysaccharide producer and 70% of them were cocci. The average of maximum and minimum production by weight method were 2.61 g/L and 0.08 g/L, respectively. The average of highest and the lowest amount of exopolysaccharide by phenol sulfuric method were measured 1.87 g/L and 0.06 g/L, respectively. This study indicates the potential of exopolysaccharide production by Iranian native species from dairy products.

Keywords: *Exopolysaccharide, Lactic acid bacteria, Kishk.*

INTRODUCTION

The increased demand for natural polymers in various industrial applications during recent years has led to a renewed interest in exopolysaccharide (EPS) production by microorganisms. Many microorganisms have an ability to synthesize extracellular polysaccharides and excrete them out of cell (Suresh Kumar *et al*., 2007). New microbial polysaccharides might have innovative uses as gelling agent, emulsifier, stabilizer or texture enhancing agent (Sutherland, 2001).

Eff orts have been made to use LAB as microbial cell factories for the production of industrially interesting metabolites either to be used as purified compounds or to be produced *in situ* in fermented foods (Boguta *et al*., 2014). As a traditional dairy product, Kishk is mostly produced from sheep's milk. Firstly, raw milk is boiled and then cooled and inoculated with traditional yoghurt made earlier as starter culture. The butter is separated from sour yoghurt by Mashk, which is made from hide (sheepskin) and is used for butter making. Then buttermilk is boiled and sieved by cloth bag. Finally the thick whitish semi-solid part of buttermilk, which is sieved, is shaped in form of conic or cubic balls and then sun-dried for 3–4 days (Iranmanesh *et al*., 2018). A wide variety of carbon sources, used to produce microbial exopolysaccharides, include sucrose, glucose, lactose, maltose, mannitol, sorbitol, whey, starch, sugar concentrates. The type of carbon source influences the yield of exopolysaccharide. The size of the exopolysaccharide may also vary with the carbon source (Suresh Kumar *et al*., 2007). Exopolysaccharide concentration is estimated as neutral carbohydrate content usually determined by the phenol sulfuric acid method or by weighting the polymer dry matter (Vaningelgem *et al*., 2004, Dubois *et al.*, 1956).

This study demonstrates the effect of type of carbon source on exopolysaccharide yield in EPS producing lactic acid bacteria isolated from Iranian Kishk.

MATERIALS AND METHODS

Chemical and Microbiological analyses:

The pH value of the Kishk samples was measured using a pH meter. The total number of mesophilic aerobic bacteria were enumerated on Nutrient Agar (NA) incubated at 35±2°C for 24 h. MRS agar was used for counting Lactic acid bacteria (Kirdar and Advances, 2012).

Isolation of Lactic acid bacteria:

Kishk samples were taken from eight regions in Khorasan province according to Iranian National Standard No. 326. For the isolation of LAB strains from Kishk samples, 10 gr of samples were taken aseptically and transferred to 90 ml skim milk followed by the preparation of serial dilutions. Streak culture method on MRS (de Man, Rogosa and Sharpe) agar was done and the plates incubated at 30°C and 37°C for 24 h. Colonies with typical characteristics of lactic acid bacteria were selected from MRS and tested for Gram stain, cell morphology, oxidase and catalase reaction (Ispirli and Dertli, 2017).

Screening of EPS producer isolates:

EPS synthesis was performed in MRS agar medium with disc and ruthenium red methods. In the disc method, MRS culture medium was formulated with 40% of 4 kinds of sugars (sucrose, fructose, lactose and glucose) instead of glucose in the main formula and the paper discs were inoculated with inoculum, incubated at 30 and 37°C for 48 h (Paulo *et al*., 2012). In ruthenium red method, streak culture method was performed by overnight cultures on MRS-RR medium (0.08 g/l ruthenium red). After 48 h of incubation at 30 and 37°C, ruthenium red stains the bacterial cell wall, producing pink colonies for non-EPS producers and white colonies for producers(Hongpattarakere *et al*., 2012).

Isolation of EPS and determination of EPS:

For the isolation of EPS, all strains were grown in formulated MRS broth, inoculated at 1% (v/v) with an overnight culture then incubated at 30 and 37°C for 2 d anaerobically. Then centrifuged at 10,000 ×g for 10 min to remove the cells. Three volumes of 96% (v/v) cold ethanol were added to the supernatant and stored overnight in 4°C to precipitate. The culture was then centrifuged again (10,000× g for 10 minutes). After removing the supernatant, the precipitate was mixed with a sevag reagent (chloroform: n-butanol = 4: 1) and then centrifuged. The dialysis (cut-off 8000-12000Da) of the resulting supernatant with distilled water was performed at 4°C for 48 hours. The produced EPS was stored for 24 hours in -80°C freezer and then lyophilized. Determination of EPS was performed via phenol-sulfuric acid method using glucose as standard and weight method by measuring the dry weight of EPS at 60°C in an oven (Lin and Chien, 2007; Jeong *et al*., 2017; Dubois *et al*., 1956).

Statistical analysis:

Each test was performed in duplicate. Data from each test were subjected to SPSS (version 22.0.0.0) for analysis of variance. Duncan's multiple range test was used to determine any significant difference ($p<0.05$) among treatments.

RESULTS AND DISCUSSION

Chemical and Microbiological characteristic:

The results concerning chemical and microbiological properties (mean values and standard deviation) of the Kishk samples were shown in Table 1. The mean value of pH ranged from 3.62-4.08 in Kishk samples from different region of Khorasan province in Iran. Noori *et al* (2013) reported the pH of Kishk samples were in a range of 3.85-4.11 (Noori *et al*., 2013). According to Gadallah *et al* (2019) in Kishk samples pH ranged between 4.39–4.84 (Gadallah and Hassan, 2019). The average value of total aerobic mesophilic bacteria and lactic acid bacteria were 6.50 and 5.89 log CFU/g in Kishk samples, respectively. The results of Kirdar *et al* (2012) showed the average values of total bacterial count, *Lactobacillus* sp., *Lactococcus* sp. and *Enterobacteria* in Kishk samples were 8.24±0.95, 7.63±0.99, 7.52±0.96 and 2.62±1.13, respectively. These findings were higher than that of this study. A high number of TAMB can be explained by sufficient change in the environmental conditions which occur during Kishk storage and which allows for

the growth of microorganisms (Kirdar and Advances, 2012). The total count and Lactic acid bacterial count of Kishk samples in different researches have been reported in a range of 3.46-7.4 and 3.65-4.89 log cfu/g, respectively (Tamime and Robinson, 2007).The total bacterial counts of different Kishk samples in work of Gadallah *et al* (2019) were ranged from 5.15 to 7.50 log cfu/g and Lactic acid bacterial counts were ranged from 6.04 to 7.88 log cfu/g (Gadallah and Hassan, 2019). There was a significant positive correlation between total aerobic mesophilic bacteria and lactic acid bacteria ($r=0.93$, $P\leq0.05$) (Figure 1). These results were in agreement with the findings of Kirdar and Advances, 2012.

Table1- pH and microbiological properties of Kishk samples

Code of Samples	**pH** *	**The total lactic acid bacteria*(TLAB)**	**The total aerobic mesophilic bacteria (TAMB)***
Q	$3.66\pm0.014^{c**}$	6.08 ± 0.38^{c}	7.02 ± 0.4^{b}
K	3.675 ± 0.007^{c}	5.45 ± 0.35^{e}	5.85 ± 0.27^{e}
T	4.04 ± 0.056^{a}	7.2 ± 0.41^{a}	7.8 ± 0.42^{a}
M	3.62 ± 0.028^{d}	5.36 ± 0.22^{f}	6.03 ± 0.32^{d}
S	3.85 ± 0.07^{b}	5.64 ± 0.41^{d}	5.8 ± 0.2^{e}
N	4 ± 0.035^{ab}	5.38 ± 0.3^{ef}	5.68 ± 0.41^{f}
R	3.85 ± 0.07^{b}	5.9 ± 0.3^{c}	6.75 ± 0.43^{c}
F	4.08 ± 0.042^{a}	6.14 ± 0.34^{b}	7.1 ± 0.35^{b}

Q: Sarab Region, K: Nasr Region, T: Suran Region, M: Darsoufian Region, S: Rayab Region, N: Chenesh Region, R: Akhlamad, F: Azghad Region.

*Values are reported as (mean ± sd)

**The letters in each column show statistical differences

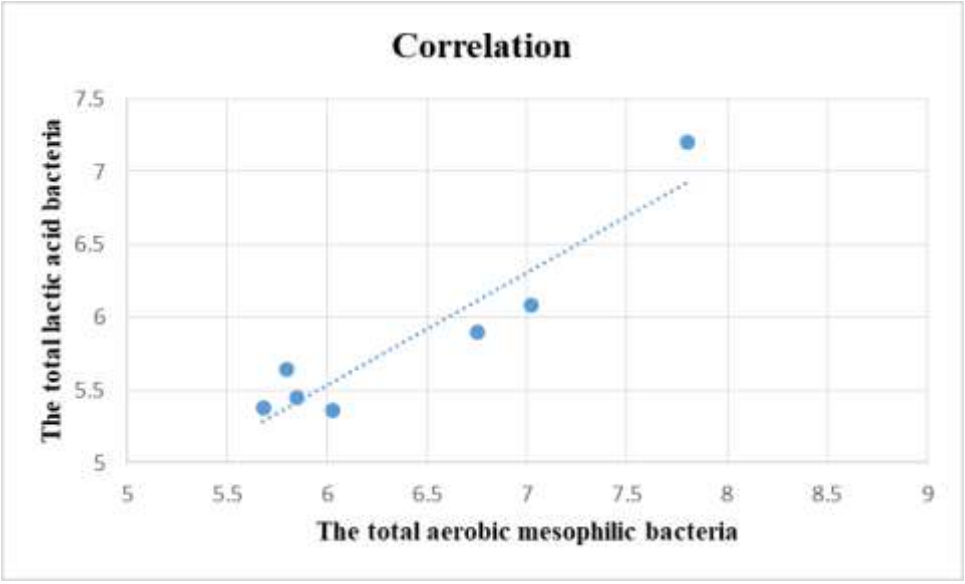

Figure 1-Correlation between the number of total aerobic mesophilic bacteria and total lactic acid bacteria

Isolation and identification of EPS producers and quantification of EPS:

The findings revealed that 79 out of 143 Gram-positive, non-spore forming, catalase and oxidase negative isolates were detected as putative EPS positive with both disk and ruthenium red methods. Amount of EPS was determined by phenol-sulfuric method and weight method. These isolates had a mucoid character. Ruas-Madiedo *et al* (2007) demonstrated 92% of their isolates were mucoid. It was in accordance with our results which states mucoid phenotype is dominant phenotype (Ruas-Madiedo *et al*., 2007). According to our findings, the average of maximum EPS production in 4 culture media (containing 4 kinds of sugars) by weight method was, 2.61 g/L (*E. faecium* R114) and minimum production 0.08 g/L (isolate T229 with cocci phenotype), and the greatest amount of exopolysaccharide by phenol sulfuric method was 1.87 g/L (*E. faecium* R114) and the lowest amount was 0.06 g/L (isolate T229). In this work, 3 isolates were selected and identified for high EPS production. They were identified as *E. faecium* (code T52), *E. faecium* (code R114) and *E. durans* (code K48). The partial 16S rRNA sequences of the identified strains in this study were deposited in GenBank under accession numbers MT437248- MT437250 (Table 2). The amount of EPS by weight and phenol sulfuric methods in them measured, 2.39; 1.70, 2.61; 1.87 and 2.17; 1.55 g/l, respectively (Table 2). The amount of EPS production in different references is different. *Streptococcus thermophilus* CC30 produce 1.95 g/L of EPS when grown in skim milk lactose medium at 30°C (Kanamarlapudi and Muddada, 2017). The EPS produced by the wild and mutant *L. delbrueckii* ranged from 5570.34 – 5910.62 mg/L (Adebayo-Tayo and Fashogbon, 2020). A high molecular weight EPS was recovered and purified to a yield of 2.8 ± 0.5 g/L from *Lb. plantarum* BR2 (Sasikumar *et al*., 2017). The highest EPS producing strains isolated from boza, yielding 2.39 ± 0.49 and 1.98 ± 0.23 g/L of EPS, respectively (Heperkan *et al*., 2014). *Lactobacillus kefiranofaciens* DN1 produced EPS, using glucose and lactose, and EPS yield rose to 2.2 g/L in modified MRS broth (60 g/L glucose) (Jeong *et al*., 2017).

The reported yields of HePS range from 50 to 350 mg/L for *Strep. thermophilus*, 60 to 150 mg/L for *Lb. delbrueckii* spp. *bulgaricus*, 25 to 600 mg/L for *Lc. lactis* spp. *cremoris*, and from 50 to 60 mg/L for *Lb. casei* (Ruas-Madiedo and De Los Reyes-Gavilán, 2005).

Table 2. Results of 16S rRNA sequencing of three highest EPS producer and GenBank accession number(s).

Code	16S rRNA sequencing results	Gen Bank accession number(s)	EPS amount by weight Method (g/L)	EPS amount by phenol sulfuric methods (g/L)
K48	*Ent. Durans*	MT437248	2.17	1.55
R114	*Ent.faecium*	MT437249	2.61	1.87
T52	*Ent.faecium*	MT437250	2.39	1.7

a

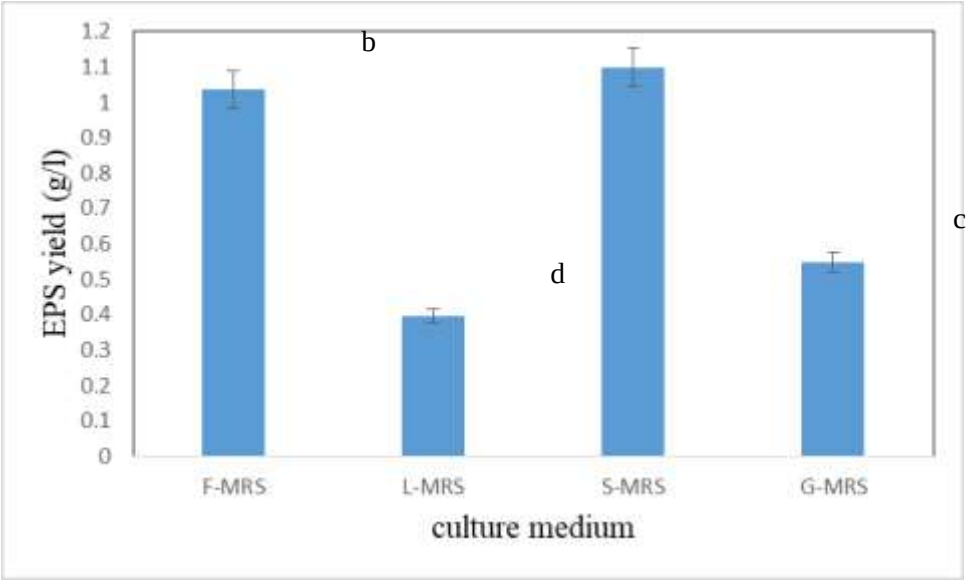

Figure 2- effect of carbon source on EPS yield

Analysis of data showed MRS broth medium formulated with sucrose had great effect on EPS production, but MRS broth formulated with lactose had a low impact on EPS synthesis. Kanmani *et al* (2013) showed that the production of EPS from *E. faecium* MC13 in the sucrose medium was higher than in lactose, glucose and fructose media which was 11.33 g/l (Kanmani *et al*., 2013). In other work Kanmani *et al* (2011) expressed the maximum yield of EPS from *Streptococcus phocae* PI80 (11.75 and 12.14 g/L) was obtained in the presence of lactose and yeast extract at a concentration of 20 g/l (Kanmani *et al*., 2011). Knowledge of the effect of the sugar source on EPS production and the activities of biosynthetic enzymes provides information about the mechanisms of regulation of the synthesis of EPS which can contribute to improve polymer production (Mozzi *et al*., 2001).

CONCLUSION

Food industry is looking for the multifunctional strains of LAB that contribute to the organoleptic, technological, nutritional and health properties of fermented dairy products. EPS producing lactic cultures have tremendous potential as functional starters, which can be better substituted to many commercial additives in use. However, EPS producing character is plasmid associated in LAB and may be lost over generations. Further it varies from strain to strain. Hence, selection of promising strains that retain EPS producing characteristic over a long period, may give opportunities to food manufacturer to produce various low-fat products. It is concluded that among these three enterococcus isolates, strain R114 identified as *Ent. faecium* produced the highest EPS amount in both methods of EPS measurement including weight and phenol sulfuric methods 2.61 and 1.87 g/L,

respectively. Fermented dairy products, especially made by traditional method in rural areas, are the potential reservoir for isolation of EPS producing cultures.

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NUTRITIONAL VALUES ANALYSIS OF SANDY GRASSLANDS ALONG THE DANUBE FROM THE PANNONIAN REGION TO THE ROMANIAN PLAIN

Attila FŰRÉSZ[1]*, Ferenc PAJOR[2], Szilárd SZENTES[2], Tímea KISS[3], Károly PENKSZA[1]

[1]Hungarian University of Agriculture and Life Sciences, Institute of Agronomy, Department of Botany, Gödöllő Hungary
[2]Hungarian University of Agriculture and Life Sciences, Institute of Animal Husbandry, Gödöllő, Hungary
[3]John von Neumann University, Faculty of Horticulture and Rural Development, Department of Horticulture, Kecskemét, Hungary
*Corresponding author: furesz.attila.zoltan@phd.uni-mate.hu

ABSTRACT

Grazing livestock farming has a long tradition in Hungary. The most valuable feed for grazing animals is provided by plants of grasslands. Supplementation of feed rations with fiber increases the saturation of digestive tract, thus making the animals calmer and improving animal welfare. Grasses can be useful supplements as they contain a lot of digestible fiber. Our aim is to find out about the grassland management values of sandy grasslands dominated by *Festuca* species along the Danube. Cut samples were made along the Danube, beginning in the northwestern part of the Little Hungarian Plain, across the central sandy plains of the Carpathian Basin to the southernmost part of the Basin at Deliblato, Serbia. The last samples were made beyond the Carpathians on the Romanian Great Plain and Bulgaria. Weende analysis of the cut samples was carried out in the laboratory of MATE. Their original dry matter, crude protein, crude fat and crude fiber content were analysed, and fiber fractions (NDF, ADF, ADL) were measured. Based on the results, contents of absolute dry matter, crude fiber and NDF were high in all samples. The five samples of the analysed *Festuca* species showed significant differences between dry matter and crude fiber. *Festuca wagneri* had the highest dry matter content. The highest crude protein contents were found in *Festuca vaginata, Festuca wagnerii* and *Festuca rupicola* samples, the highest crude fiber content in *Festuca tomanii* samples.

Keywords: *feed value, fescue.*

INTRODUCTION

As in Europe, biodiversity is declining in Hungarian Pannonian grasslands, both in agricultural fields and in semi-natural vegetation types (Bakker and Berendse, 1999; Bischoff et al., 2005; Valkó et al., 2011; Tasi et al., 2013, 2014; Halász et al., 2016). Biodiversity loss is caused by anthropogenic impacts, but also by improper use of grassland and lack of land management (Fischer and Stöcklin, 1997; Bischoff et al., 2005). Grazing pressure is important for pastures, but overgrazing or a lack of grazing can cause a decline, resulting in changes of phytomass conditions in many grasslands (Guo 2007; Kelemen et al, 2013; Szentes et al, 2009; Penksza et al, 2013; Antal and Huzsvai, 2007; Antal and Juhász, 2008; Cornwell and Grubb, 2003; Gillman and Wright, 2006; Mittelbach et al, 2001; Précsényi, 1975). Species of the genus *Festuca* are an important group of grassland species for the Pannonian vegetation, and are also the dominant species of the vegetation, because they can survive in habitats where conditions are too extreme for most plant species. In addition to their importance for grassland management, they also have a high natural value. As climate changes, dry habitats are becoming increasingly important. Biomass studies on sandy grasslands following the Danube have been carried out at regional level in several countries (Rácz et al., 2021), and investigations of the nutritional value of grasslands have also become interesting. In the description of the feed materials used for feeding and of the forages made from them, the crude fiber content is the most frequent indicator of the nutritional value. Crude fibre is a chemical composition of residues that are generated after cooking in dilute acid and alkali. Neutral detergent fibre (NDF), acid detergent fibre (ADF), acid detergent lignin (ADL) and the group of non-starch polysaccharides (NSP), which have an important role, and their component polymers can be defined (Orosz, 2015; Halász et al., 2022). Introducing fibre into the feed rations enhances the saturation of the digestive system, which makes the animals calmer and thus increases animal welfare. The addition of grasses can be a convenient supplement as they contain a lot of digestible fibre. One of the biggest challenges of feeding is to maximise the available genetic potential at high production and sustainable costs (Orosz, 2017; Orosz and Mézes, 2007).

The main purpose of the present study is to find out how the nutritional values of grasslands by dominated *Festuca* species along a geographical gradient.

MATERIALS AND METHODS

Sample collection

Cut samples for our studies were collected from 37 sample areas following the Danube from the northwestern part of the Carpathian Basin, starting from the Little Hungarian Plain, through the central Carpathian Basin's extensive sandy ridge, to the southernmost Deliblato, to the Romanian Plain and Bulgaria (Figure 1).

In addition to the separated geographical units, the following *Festuca* species were dominant: *Festuca vaginata, F. pseudovaginata, F. wagneri, F. tomanii* (new to the Hungarian flora), *F. javorkae* and *F. rupicola*, which were combined, because only after the ploid level analysis can it be confirmed which species is concerned.

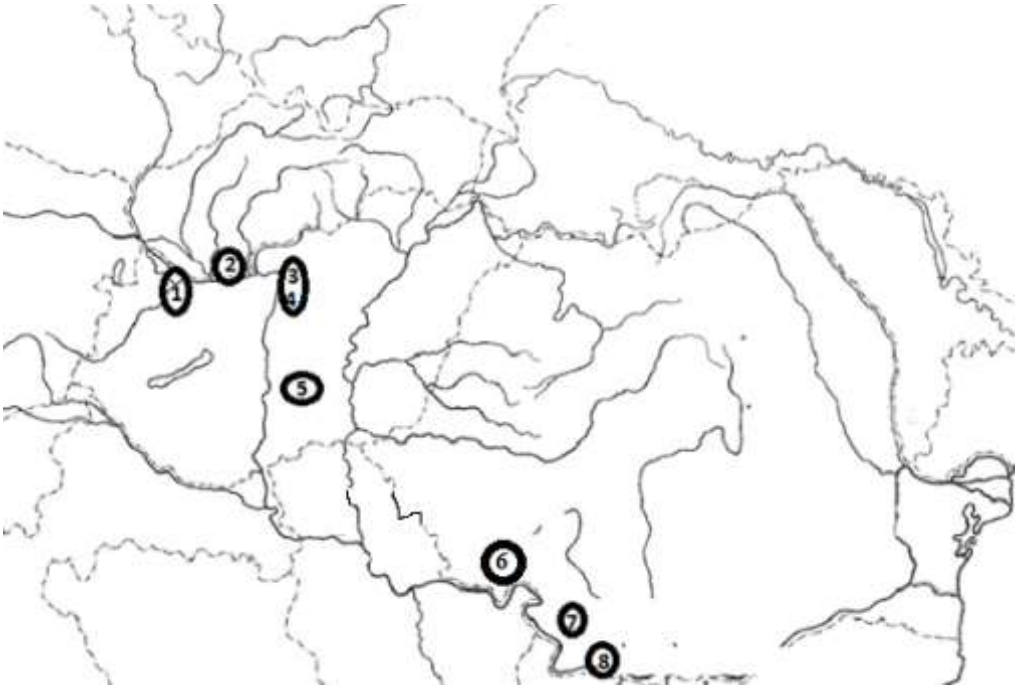

Figure 1. Sampling areas along the Danube River (1: Gönyű, 2: Cenkov, 3: Szigetmonostor, 4: Homoktövis TT, 5: Bugac, 6: Deliblato,7: Balta Verde, 8: Vidin)

Nutritional values analysis

The nutritional values of the cut samples were defined by Weende analysis, including the original dry matter content, crude protein, crude fat and crude fibre content according to MSZ EN ISO 6865, Harris et al. (1972), and NRC (1989), plus the fibre fractions (NDF, ADF, ADL). Samples were classified according to the dominant *Festuca* species, within this, mixed samples with co-occurring *Festuca* species (Schmidt, 1993; Schmidt et al., 2000; Orosz, 2015).

RESULTS AND DISCUSSION

Differences were observed for the nutritional values.

The mixed *Festuca vaginata* values were high in all areas with high absolute dry matter content, but there was a slight increase towards the southern area, the highest values being observed in the sample from Balta Verde (BvFv), which was 897.54 mg/kg. Crude protein values were low, never reaching the 100 mg/kg value. Crude fat ranged only between 18 and 25 mg/kg. The highest values of the fibre fraction were found in the NDF data, around 500 mg/kg. The amount of ADF fibre did not change a lot, the ADL sample from Deliblato (DFv) had the lowest value with 298.22 mg/kg.

The values of mixed *Festuca pseudovaginata* were characterised by high absolute dry matter content in all samples, increasing towards the south. Crude protein values showed minimal variation, not reaching the 100 mg/kg value in any case. Crude fat ranged between 22 and 24 mg/kg. The highest crude fibre content was measured in Bugac (BFp), which was 366,68 mg/kg. There were differences in the ratios of the fibre fraction. The highest values of the fibre fraction were in NDF, around 500 mg/kg. The amount of ADF fibre decreased towards the southern area. The amount of ADL showed a smaller increase in the sample from Bugac (BFp).

The absolute dry matter content was high for the mixed *Festuca tomanii* samples. Crude protein was low with 72.20 mg/kg for the sample from Újpest Homoktövis Természetvédelmi Terület/Újpest Sea-buckthorn Nature Reserve (HFt) but 57.13 mg/kg for the sample from Szigetmonostor. Crude fat ranged from 22 to 23 mg/kg. There was no significant difference in the amount of crude fibre. Fibre fraction ratios: highest values of fibre fraction were found in NDF above 500 mg/kg, ADF showed higher value in the sample from Szigetmonostor (SFtX), while ADL showed lower value for fibre.

Based on the measurements of mixed *Festuca wagneri* values, the absolute dry matter content was high in all samples, but showed an increase towards the southern area: the highest values were observed in the sample from Vidin (VFw), which was 882.37 mg/kg. Crude protein values were low, not reaching 100 mg/kg in any case. Crude fat ranged from 20 to 32 mg/kg. There were no significant differences in the crude fibre content, which was around 320 mg/kg. The highest values of the fibre fraction were found in the NDF data, above 500 mg/kg, while the amount of ADF fibre did not show significant differences. ADL showed an increasing value towards the south.

The absolute dry matter content was high in the mixed *Festuca javorkae/rupicola* samples, 384.54 mg/kg in the samples from Gönyű (GFjrX) and 666.02 mg/kg in the samples from Cenkov (CFjrX). Crude protein values were low, not exceeding 100 mg/kg in either case. The crude fat content did not show significant differences, ranging from 24 to 26 mg/kg. There was no significant difference in the amount of crude fibre, which was around 350 mg/kg. The highest values of the fibre fraction were found in the NDF data, 535.71 mg/kg at Gönyű (GFjr) and 498.13 mg/kg at Cenkov. The ADF fibre showed higher values than Cenkov (CFrjX) and ADL than Gönyű (GFrjX) (Table 1).

Table 1. Data of content values of the mixed samples according to sampling areas

	Absolute dry matter	Crude protein	Crude fat	Crude fibre	Crude ash	NDF	ADF	ADL
	g/kg tak.	g/kg sz.a.						
mixed *Festuca vaginata*								
Gönyű (GFvX)	460,13	96,28	25,75	334,69	58,03	541,68	397,52	36,12
Szigetmonostor (SzFvX)	511,58	96,36	23,02	355,45	49,37	551,30	403,31	36,03
Homoktövis TT (HFvX)	487,34	85,33	21,28	342,95	47,96	524,09	370,49	37,37
Bugac (BFvX)	554,50	93,30	21,57	287,11	73,84	543,09	386,78	33,36
Deliblato (DFvX)	631,28	69,91	18,71	371,25	39,55	512,67	400,86	29,82
Balta Verde (BvFvX)	897,54	73,13	21,29	321,47	60,23	515,06	402,24	31,51
mixed *Festuca pseudovaginata*								
Szigetmonostor (SzFpX)	457,45	79,28	23,52	360,43	62,79	533,68	391,61	32,88
Homoktövis TT (HFpX)	532,57	87,33	22,75	352,02	58,44	506,95	376,28	32,45
Bugac (BFpX)	530,57	77,15	23,00	366,68	87,20	519,47	355,23	28,68
mixed *Festuca tomanii*								
Szigetmonostor (SzFtX)	677,71	57,13	22,51	370,75	29,25	528,77	400,84	35,88
Homoktövis TT (HFtX)	619,18	72,20	22,46	365,60	48,45	537,06	364,85	29,74
mixed *Festuca wagneri*								
Bugac (BFwX)	482,01	97,08	31,90	320,67	69,04	533,88	394,95	30,29
Deliblato (DFwX)	767,37	93,97	25,63	318,95	54,78	566,15	386,01	34,06
Balta Verde (BvFwX)	615,45	86,03	20,17	325,54	60,41	500,05	361,83	31,49
Vidin (VFwX)	882,37	91,85	20,05	324,99	60,99	528,96	380,78	32,78
mixed *Festuca javorkae/rupicola*								
Gönyű (GFjrX)	384,54	87,66	25,66	340,92	75,26	535,71	376,31	33,03
Cenkov (CFjrX)	666,02	92,31	24,07	354,48	54,34	498,13	393,62	30,70

CONCLUSION

The analysed grassland samples have typically high crude fibres and NDF values, as well as high dry matter content. However, the crude protein values seem to be low.

It can be concluded that the amount of crude protein in the samples decreases with increasing dry matter ($r=-0.29$; $P<0.10$; $r=-0.24$; $P<0.10$).

The amount of crude protein decreases with increasing crude fibre content in the dominant *Festuca* species ($r=-0.46$; $P<0.05$).

Festuca wagneri has the highest dry matter value according to the research, and these samples are typically from Great Hungarian Plain, Kiskunság and Balkan regions. The lowest dry matter value was found in *Festuca rupicola* samples, which were derived from Little Hungarian Plain and Slovakia. *Festuca tomanii*

samples had the lowest crude protein value. These samples were mostly from Kiskunság. The samples with the highest crude protein value were *Festuca vaginata, Festuca wagneri* and *Festuca rupicola*. For crude fibre, the highest value was found in *Festuca tomanii* and the lowest in *Festuca vaginata*.

We could not detect any differences between the samples for cell wall components, but available values compared with literature (Schmidt et al., 2000) show medium quality.

In total, *Festuca vaginata* and *Festuca rupicola* samples had the highest nutritional value, while *Festuca tomanii* samples were considered to have the lowest nutritional value.

From the studied vegetation types, low quality hay from meadows can be expected. Based on the presented results, it could be a potential feed source for the small ruminant livestock sector. The most suitable application of the investigated grasslands is mainly for sheep production (Schmidt, 1993).

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THE IMPACT OF PESTICIDES ON OCCUPATIONAL HEALTH, THE HEALTH OF THE GENERAL POPULATION, AND THE ENVIRONMENT

Vesna KRSTOVIĆ SPREMO*, Sanja MARIĆ

University of East Sarajevo, Faculty of Medicine, Foča, Studentska 5, 73300 Foča
Republic of Srpska, Bosnia and Herzegovina
*Corresponding author: vesnakspremo@gmail.com

ABSTRACT

According to Food and Agriculture Organization (FAO) of the United Nations pesticide means any substance, or mixture of substances of chemical or biological ingredients intended for repelling, destroying or, controlling any pest, or regulating plant growth. However, it is known that the increasing food demands of the world population necessarily mean the increasing use of pesticides in agriculture, forestry and other activities. Today, a huge number of chemical substances are used as pesticides, and their use inevitably leads to negative consequences for human health and the environment. The goal of our research was to analyze the impact of pesticides on occupational health, the health of the general population and the environment. A research performed in May 2022 included documents, and data from scholarly literature, and relevant literature from medical journals, the electronic databases Pubmed, data of European Commission, International Labour Organization, World Health Organization (WHO), and content on the website that deals with the topic of the final paper. Authors paid special attention to the legal regulation of the use of pesticides in the Republic of Srpska and Bosnia and Herzegovina (B&H). Also, authors analyzed the international regulations related to pesticides. About 63 of them were analyzed, but 38 of them were included in the systematic review. Most of the selected documents show that production, use, and exposure to pesticides lead to the occurrence of occupational diseases, accidental poisoning of the population, as well as environmental pollution. The analysis suggests that permanent control of the production and use of pesticides through legislation is needed, in order to achieve the best possible protection against these agents.

Keywords: *pesticides, occupational diseases, environmental pollution.*

INTRODUCTION

The term pesticide is derived from the Latin words *pestis*, which means plague, and *occidere*, which means to kill. Since ancient times, people have faced various diseases and had great economic losses due to various pests. Also, since ancient

times people have faced various diseases and had great economic losses due to various pests, which is why they found different ways to control them. Even the ancient Romans used sulfur to protect the vine grape. During the17th century, nicotine was widely used to protect plants in France. The "pesticide revolution" involves the use of DDT insecticide since the 1940s, when this pesticide was first used in southern Italy against mosquitoes (Savić, 1997).

The types of exposure to pesticides are dermal, inhalation, and ingestion. The most frequent route for occupational exposure is dermal. A high percentage of pesticides can be absorbed across intact human skin because of their high lipid solubility. Inhalation exposure derives from the aerosols during the process of application and some inadequate proceedings in agriculture. Ingestion occurs in low percentages when the users eat, smoke, or drink in places that contain pesticides or if they do not wash their hands after usage of pesticides. The active substances that structure pesticides are very different, so there are several divisions of pesticides. They are most often divided according to purpose, chemical composition, toxicity and mechanism of action (FAO, 2014).

Classification of Pesticide (Bulat, 2014):

Pesticides according to purpose

- Insecticides – insects;
- Herbicides – plants:
- Rodenticides – rodents (rats & mice);
- Bactericides – bacteria;
- Fungicides – fungi;
- Larvicides – larvae.

Pesticides according to the chemical composition

- Organophosphate: Most organophosphates are insecticides (malathion, parathion, chlorofos, etc.).
- Carbamate (carbaril,bendiocarb).
- Organochlorine insecticides: (DDT, chlordane, and toxaphene).
- Pyrethroid: These are a synthetic version of pyrethrin, a naturally occurring pesticide, found in flower.
- Sulfonylurea herbicides: The sulfonylureas herbicides have been commercialized for weed control such as: sulfosulfuron, rimsulfuron, nicosulfuron, nicosulfuron,ethoxysulfuron, azimsulfuron, and amidosulfuron.
- Biopesticides: The biopesticides are certain types of pesticides derived from such natural materials as animals, plants, bacteria, and certain minerals.

Insecticides, as one of the most commonly used groups of pesticides, can be divided into natural and synthetic, whereby synthetics are divided into organic and inorganic, while natural pesticides are divided into plant-based and mineral oil-based pesticides (Figure 1), (Kaur et al.,2019).

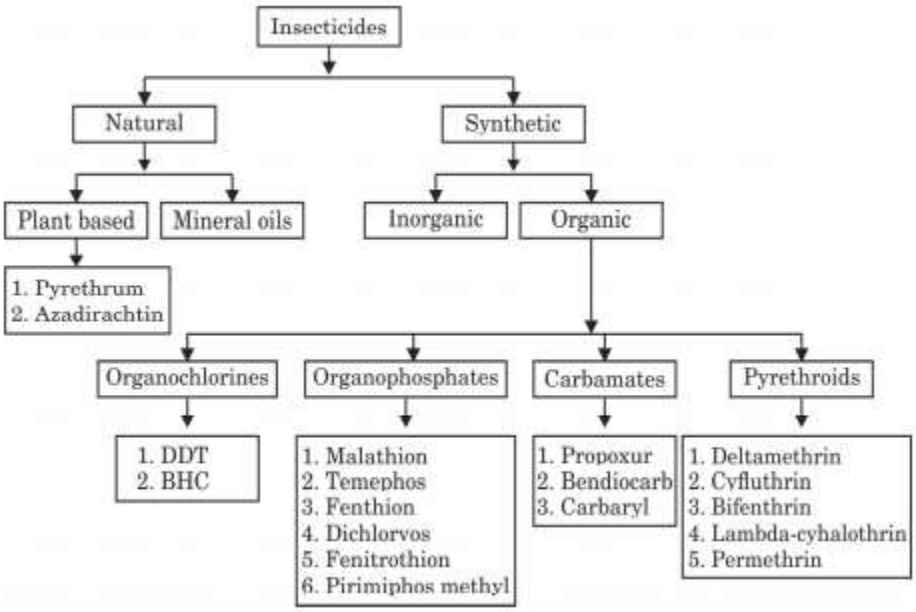

Figure 1. Classification of pesticide

*Source: Kaur et al. (2019).

Toxicity characterization of pesticide indicates that the most of them are related to at least one health effect according to databases of EPA, IARC, and WHO. Some of these effects are: acute and chronic poisoining, carcinogenic, endocrine and reproductive damages. Toxicity of a pesticide refers to the ability of it to produce its harmful effects. These effects may occur from slight symptoms to severe symptoms like convulsions or death. Pesticides cause reversible or irreversible damage. Toxicity of pesticide work by altering normal body functions. Toxicity can be classified into two types as acute and chronic. Acute toxicity with systemic damage may be a result of exposure to relatively large amounts of the pesticide. Chronic toxicity is result of effects produced by long-term, and low-level exposure to pesticides (Relf, 1996).

Acute toxicity of a pesticide is determined by subjecting laboratory animals to different dosages or concentrations of the active ingredient. Tests are also conducted to assess the impact through the skin, through inhalation, and orally. Test results are then used to classify pesticides into one of four toxicity categories (Table 1), (WHO, 2010).

Table 1. Acute toxicity of pesticides according to WHO classification

Class	Classification	LD_{50} for the rat (mg/kg b.w.)			
		Oral		Dermal	
		Solids	Liquids	Solids	Liquids
Ia	Extremely hazardous	<5	<20	<10	<40
Ib	Highly hazardous	5-50	20-200	10-100	40-400
II	Moderately hazardous	50-500	200-2,000	100-1,000	4004,000
III	Slightly hazardous	>501	>2,001	>1,001	>4,001
U	Unlike to present acute hazard	>2,000	>3,000	–	–

Source: WHO. International Code of Conduct on the Distribution and Use of Pesticides: Guidelines for the Registration of Pesticides. World Health Organization; Rome, Italy: 2010.

According to EPA: Toxicity Category I–All pesticide products shall bear on the front panel the signal word "Danger." If the product is assigned a Toxicity Category I on the basis of its oral, inhalation, or dermal toxicity, the word "Poison" shall appear in red on a contrasting background colour, and the skull and crossbones shall appear in immediate proximity to the word "Poison."
Toxicity Category II – All pesticide products shall bear on the front panel the signal word "Warning." Toxicity Category III – All pesticide products shall bear on the front panel the signal word "Caution." Toxicity Category IV – All pesticide products shall bear on the front panel the signal word "Caution" (Table 2.) (EPA, 2009).

Table 2. Acute toxicity of pesticides according to the EPA classification.

Class	Signal words	Acute toxicity to rat		
		Oral LD_{50} (mg/kg)	Dermal LD_{50} (mg/kg)	Inhalation LC_{50} (mg/L)
I	DANGER	<50	<200	<0.2
II	WARNING	50-500	200-2,000	0.2-2.0
III	CAUTION	500-5000	2,000-20,000	2.0-20
IV	CAUTION (optional)	>5,000	>20,000	>20

Source: EPA *Registering Pesticides* 2009. Available online: http://www.epa.gov/pesticides/regulating/re-gistering/index.htm

The purpose of this paper is to present and discuss adverse effects of pesticides on human health and the environment, as well as their impact on occupational health.

MATERIAL AND METHODS

A research was performed in May 2022, and the article is based on a systematic review of all documents, and data from scholarly literature, relevant literature from medical journals, the electronic databases Pubmed, data of European Commission, International Labour Organization, World Health Organization, and content on the website that deals with the topic of the final paper. We paid special attention to the legal regulation of the use of pesticides in the Republic of Srpska, and B&H. Also, we analyzed the international regulations related to pesticides. About 63 of them were analyzed, but 38 of them were included in the systematic review.

RESULTS AND DISCUSSION

The risk of pesticides to human health has been investigated since the 1970s. Most of the early works was conducted in the USA after 1980s, with rapid growth in the number of studies in the 1990s. Although the use of pesticides is necessary in many human activities, these chemical substances can have very serious consequences on human health. However, pesticide overuse and pollution have increased. Global pesticide use has continued growth to 4.1 million tonnes per year in 2017, an increase of nearly 80% from 1990. (FAO, 2019). Currently, around two million tonnes are used per year on a global basis, most of which are herbicides (50%), followed by insecticides (30%), fungicides (18%) and other types such as rodenticides and nematicides (Sharma et al., 2019).

According to the other studies, herbicides (over 40%), insecticides (over 30%), and fungicides (over 20%) are used the most, while other pesticides are used to a slightly lesser extent. The major pesticides for human poisonings were highly toxic organophosphorus pesticides, which accounted for 86.02% of the total cases (Zhang et al., 2011).

Professional exposure to pesticides is seen in workers who work on the formulation of pesticides, agricultural workers who work on pesticide application, as well as employees in agricultural pharmacies and warehouses. The most exposures and poisoning occur in workers involved in agricultural operations such are: mixing, loading, applying, and flagging. Mixers and loaders are exposed to high concentration of pesticide because the leaking or poorly equipment may fail and produce overexposures with any type of device including the closed mixing-loading system. Workers who work on the processes of disinsection and pest control can also be victims of pesticide poisoning. Poisoning by pesticides is severe public health problem. Probably, the occupational exposure is the most common source of exposure that results in unintentional acute intoxication. The most important route to occupational route is dermal which occurs by contamination of clothing, damaged skin, and sweat. All of these factors are common in agricultural work that forces absorption through the skin. The use of personal protective equipment can be useful for exposed workers. In occupational occasions, poisoning with organophosphates, and carbamates are rather frequent. They can easily penetrate through the skin, the respiratory tract or through ingestion. The clinical

manifestations of poisoning by these pesticides reflect the organs where the acetylcholine is the transmitter of nerve impulses. The exposed workers have the clinical sings and symptoms such as: miosis, diarrhea, urination, excitation, lacrimation, bronchorrhea, pulmonary oedema. Usually, the cause of death in acute poisoning is respiratory failure. Chronic health effects in exposed workers are: carcinogenicity, neurobehavioral effects, teratogenicity and skin effects. Organochlorine insecticides have acute and subacute clinical forms in occupational poisoning. These pesticides produces a picture of generalized CNS excitability and dysfunction. The symptoms and sings of poisoning are: dizziness, headache, confusion, weakness, convulsions and coma. The most mentioned chronic effect of chronic poisoning by organochlorine insecticides is cancer, especially breast cancer, and prostate cancer (O' Malley, 2014).

Pesticides can contaminate soil, water, air, and plants. United Nations Environment Programme (UNEP) estimated that pesticides intoxicate at least 3 million agriculture workers in less-developed countries and at the minimum 300,000 workers in the United State. The Academy of Sciences mentioned that pesticide residues in food cause approximately 4.000-20.000 cancer cases annually in the United States (Miller & Spoolman,2016).

The Occupational Safety and Health Administration (OSHA) regulates and enforces worker protection standards to ensure that employers provide a safe working environment for employees and ensure that workers receive personal protection equipment when handling pesticides. According to World Health Organization, each year, about 3,000,000 cases of pesticide poisoning and 220.000 deaths are reported in developing countries (Lah, 2011).

Exposure of the general population to pesticides occurs through eating food and drinking water contaminated with pesticides. Non-occupational exposure is from residues in food, air and drinking water, and generally involves low doses and it is chronic. The risk to human health from many studies is mentioned as a result of pesticide use in or around the home. Individuals can be exposed during the preparation and application of pesticides, whereas delayed exposure can occur through inhalation of residual air concentrations or exposure to residues found on surfaces, clothing, bedding, dust, discarded pesticide containers. Accidental poisoning with pesticides in the home is a possible from pesticide use around the house or garden (Davis et al.,1992).

The significant amount of pesticide residues can be found in daily food, including: water, fruits, juices, fish, meat. Additionally, it is mentioned that washing and peeling cannot remove completely the residues of pesticides (Nicolopoulou-Stamati et al., 2016). According to WHO more than 4 million people are poisoned by pesticides each year in the world, of these, at least a million people are hospitalized (Raven, 2011).

Organochlorines pesticides are substances that are classified as persistent organic pollutants because of their long-term environmental persistence. Due to their high persistence, low aqueous solubility, low polarity and lipid solubility, organochlorine pesticides are more dangerous to humans, plants, animals and the

atmosphere. They are stable chemical substances that can attach to soil and air, and have been identified as chronic exposure agents for animals and humans. The example of a major organochlorine pesticide with structure chemical name and toxicity is Dichlorodiphenyltrichloroethane (DDT) (Kedari, 2020).

In 1940s DDT was used as the first modern synthetic insecticide to control insect in agriculture, housing, institutes and to combat insect-borne human diseases (EPA, 2012). According to many studies the widespread use of DDT was signed as a very toxic substance to humans, and environment. Investigations showed that DDT was a reproductive toxic with increasing evidence of its adverse effects (Longnecker et al, 2005). Also the results of the other studies showed that high level of DDT was detected in food of animal origin, vegetable, fruit, soil and water (Dogheim et al, 1996). DDT was also signed as one of 12 restricted persistent organic pollutants in the world from the international agreement Stockholm Convention 2001 (WHO, 2011).

In one study by USA researchers in cooperation with Canadian provinces, conducted in Canadian provinces, pesticides were commonly found in water consumed by populations. Groundwater was found to have residues of 39 pesticides and their degradation products. Residues of pesticides that are "severely restricted" because of their serious effects on human health were also found in significant quantities in the water sources. Residues enter the water supply as they are leached from the soil into groundwater after home, lawn, roadway, and agriculture spraying (Zahm,1998).

The pesticides can enter the body through skin, eyes, digestive system, and respiratory system. Pesticides can be toxic to humans and animals. The important source of exposure to pesticides is diet. Variations in dietary more concentrated with certain foods high in pesticides may also have outcomes such as neurological, behavioral, endocrinological, and oncological. Anyone who uses pesticides or is present when pesticides are used is at risk for dangerous exposure. The findings suggest that the consumption of eggs and meat is also a significant source of exposure to the majority of organochlorine chemicals studied (Mc Connell et al, 1993).

In our work, we also investigated key legal international and domestic regulations about the use and transport of pesticide. Also, we mentioned some results that indicate the hazards of the pesticides related to health of general population, as well as environmental pollution. Some results are related to professional exposure to pesticide, and professional diseases due to pesticides.

Some of the most important documents are:

- *Rotterdam Convention*. It entered into force on 23 February 2004. Its aims are to: (1) promote shared responsibility and cooperation in the international trade of certain hazardous chemicals in order to protect human health and the environment; and (2) contribute to the environmentally sound use of certain hazardous chemicals information exchange about their characteristics, establishment of a national decision-making process on their import and export and dissemination

of these decisions to the Parties of the Convention (Rotterdam Convention, 2003).

- *Stockholm Convention on Persistent Organic Pollutants* (EPA, 2021).
- *Regulation (EC) N. 396/2005* of the European Parliament and of the Council of 23 February 2005on maximum residue levels of pesticides in or on food and feed of plant and animal origin.
  - ✓ Key points: High and consistent level of consumer protection across EU
  - ✓ Community harmonised MRLs
  - ✓ Consumer risk assessment reviewed by EFSA (*Regulation (EC) N. 396/2005* of the European Parliament, 2005).
- Directive 2009/128/EC of the European Parliament and of the Council on the sustainable use of pesticides, (the "Directive"), was adopted on 21 October 2009 as part of the 2006 Thematic Strategy on the sustainable use of pesticides. The Directive provides for a range of actions to achieve a sustainable use of pesticides in the European Union by reducing the risks and impacts of pesticide use on human health and the environment and promoting the use of Integrated Pest Management (IPM) and of alternative approaches or techniques, such as non-chemical alternatives to pesticides (Directive 2009/128/EC, 2009).
- Regulation (EU) No 649/2012 of the European Parliament and of the Council of 4 July 2012 concerning the export and import of hazardous chemicals (recast) Text with EEA relevance (Regulation EU № 649, 2012).

In the Republic of Srpska, pesticide poisoning refers to jobs and workplaces where exposure to pesticides exists, and where there is evidence of the intensity and duration of the exposure. The clinical picture of poisoning with specific damage to two organs or organ systems is taken into account as an occupational disease. In that case, occupational pesticide poisoning is on the List of occupational diseases of the Republic of Srpska, and the insured person has certain rights to free treatment, the possibility of professional rehabilitation, as well as the possibility of early retirement (List of occupational diseases, 2018).

With the aim to support the elimination and reduction of the release of persistent organic pollutants (POPs) into the environment, the United Nations member states signed the Stockholm Convention in 2001. Bosnia and Herzegovina (B&H) ratified this Convention in 2010, thus, the country should meet its requirements, which include avoiding the use of hazardous POPs by shifting to safer alternatives and removing old supplies and equipment containing these substances. According to Food Safety Agency of Bosnia and Herzegovina, there was research about the pesticide residues measured in the food products analysed by the national competent authorities. Pesticide residues resulting from the use of plant protection products on crops that are used for food or feed production may pose a risk factor for public health (EFSA, 2021). The chemical monitoring data collected and

published by EFSA include the analytical results provided by EU Member States, Iceland, Norway and three pre-accession countries: Bosnia-Herzegovina, Montenegro and North Macedonia (Food Safety Agency of Bosnia and Herzegovina, 2021),

In Bosnia and Herzegovina, a comprehensive legal framework was adopted, which defines the rules for the approval of active substances used in plant protection products, the use of plant protection products, and prescribes the maximum permitted amounts of pesticide residues in and on food. In order to ensure a high level of consumer protection, the permitted limits, the so-called "maximum permitted amount" or "MRL" (Ordinance on maximum levels of pesticide residues in and on food and feed of plant and animal origin, 2017) which is harmonized with Regulation (EC) 396/2005 as well as Law on Phytopharmaceutical Products of BiH. Pesticide residue in foods can be present in many products and can lead to many health damages. In B&H was conducted study that investigated pesticide residue in foods, and the result was that about 27% of total samples contained pesticide residues above the quantification level (Tomović et al., 2021). These data may be a signal to relevant institutions in B&H to promote better legal regulation in the field of protection from pesticides.

CONCLUSION

Pesticides have very important role for global food security, but the importance of that contribution can cause harm to human health and environment. It is known that the use of pesticides causes long-term severe negative effects on human health and the environment. Many studies have been concerned the problems with use of pesticides. Many pesticides are banned or restricted due their negative impacts, as outlined in Regulation (EC) No 1107/2009 and Regulation (EC) No 396/2005 in EU, but some pesticide companies headquartered within the EU export large amounts of pesticides banned for use to developing countries where regulations regarding their use are less strict. This problem is concerned by the some regulation bodies of UN and European Commission. The most of pesticides are hazardous if misused, and have the potential to seriously damage human health as well as environment. Pesticides are the significant factor of professional diseases due to their production or use. The protection of the people and environment from pesticides must be the priority of all international institutions that are included in this problem in terms of pesticide risk reduction, the use of high-risk pesticides, as well as the promotion of integrated pest management.

In our research the most of the selected documents shows that production, use and exposure to pesticides leads to the occurrence of occupational diseases, accidental poisoning of the population, as well as environmental pollution. The analysis suggests that permanent control of the production and use of pesticides through legislation is needed, in order to achieve the best possible protection against these agents.

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SEED SIZE AND ALLOMETRIC RELATIONSHIPS AMONG LOCAL MAIZE VARIETIES IN GALICIA (NW SPAIN)

Óscar GARCÍA TUESTA[1]*, Jessica GROBA[1], Laura PIÑA[1], Seyla GIL[1], Emilio ANDÚJAR[1], Carmela DARRIBA[1], Pablo GONZÁLEZ NOVOA[1], Yaiza SAN MIGUEL[1], Francisco MORA[1,2], Javier MONTALVO[1,3]

[1]The Matrix Foundation, Research and Sustainable Development, Spain
[2]Department of Statistics and Operations Research & [3]Department of Ecology and Animal Biology, University of Vigo, Spain
*Corresponding author: oscargarcia793@gmail.com

ABSTRACT

The AGRIECO research project promotes organic farming with local varieties of maize (*Zea mays* L.). Conservation of agrobiodiversity is important because local varieties show different traits that are valuable for adaptation to climate change. Seed size is a relevant trait for germination and seedling growth, fundamental processes for successful crop production. We analyse the differences in grains of 20 maize local varieties from Galicia (NW Spain), from a seed bank (Phytogenetic Resources Center, CRF-CSIC-INIA) and from local farmers. Multivariate characterization of grain samples from these varieties showed relatively high phenotypic variability in average grain size and other grain physical traits. A measure of grain size, Thousand Kernel Weight (TKW) showed a range of 2.4 times among varieties, from 203 to 497 g, with statistically significant differences between some of them. In addition, TKW showed a 2-fold variation range in the coefficients of variation (CV) among varieties. The average and CV of TKW showed a relatively high negative intervarietal correlation. In addition, intervarietal and intravarietal allometric relationships between grain weight and volume were identified. The results demonstrate evidence of phenotypic differences in reproductive attributes among maize local varieties. They underline the feasibility and importance of a characterization of the components of biodiversity in order to promote their value and agricultural use.

Keywords: *agrobiodiversity, allometry, grain size, Galicia, sustainability.*

INTRODUCTION

Production of maize (*Zea mays* L. subsp. *mays*) is one of the most important worldwide among cereal crops. It is grown for human and animal food, and this crop is also a valuable renewable natural resource for many industrial processing facilities (Zhang et al., 2021; Anuada et al., 2022). Maize was domesticated from a wild grass in southwest Mexico, being a good example of how human selective

breeding and seed saving allow for adaptation to agronomic environments (Stitzer & Ross-Ibarra, 2018). Maize moved to Europe, Africa and Asia, and thousands of traditional open-pollinated varieties were developed although nowadays are rapidly being replaced by modern more productive commercial hybrid varieties. Maize is one of the most variable and adaptable species of the Poaceae family. Adaptation in maize means good performance with respect to yield and other agronomic traits in a given environment (Brown et al., 1985; Stitzer & Ross-Ibarra, 2018). Maize crops present high diversity in grain size, form and biochemical composition due to genetic and environmental factors, including the grain location in the cob (Pérez de la Cerda et al., 2007). Maize grain size is a relevant trait for germination, seedling growth and yield (Kara, 2011; Akinyosoye et al., 2014). In the National Inventory of Phytogenetic Resources of Spain there are 2,459 traditional maize varieties, 30% of which come from the region of Galicia, located in the NW of the Iberian Peninsula (CRF-CSIC-INIA, 2020). The coexistence of different local maize varieties within the same region reflects their agronomic adaptation to different environments and uses (Magdaleno-Hernández et al., 2020). Local maize varieties are more tolerant to drought periods, show greater resistance to pests and diseases, or exhibit advantages for traditional processing methods and hence they are generally considered tastier. Risk of maize crop failure in hybrid varieties is higher than in local open-pollinated varieties if conditions are substandard (Andersen et al., 2022). Seed Banks are important facilities for phytogenetic resource conservation of crop varieties, as well as assuring food security. It is paramount that the genetic and phenotypic characteristics of maize varieties are known by the farmers for their conservation and use (Fuentes López, 2008). Global warming is a significant threat to crop yields, causing declines in production, even in the most productive areas. Due to the susceptibility to extreme heat of maize crops, switching varieties is a farmer option of adaptation to climate change (Roberts & Schlenker, 2011). Food needs are expected to double by 2050, and agrobiodiversity helps meeting this demand in a socially and ecologically sustainable way (Kremen and Miles, 2012). Organic farming is associated with greater biodiversity (species richness), probably due to the semi-natural characteristics of this type of production (Wickramasinghe et al., 2003) and the reduced use of chemical fertilizers and pesticides (Paoletti et al., 1992; Hole et al., 2005). Organic farming increases biodiversity by 30% on average compared to conventional farming in temperate climates of the northern hemisphere (Tuck et al., 2014). Besides, organic farming is valuable because more biodiversity implies more resilience to climate change (Arnés et al., 2013). The aim of this paper is to characterize a set of local maize varieties from different localities of Galicia region based on physical variables of grain. This work results from AGRIECO research project, which promotes agrobiodiversity conservation and organic farming using local maize varieties in Northern Spain.

MATERIALS AND METHODS

Grains of 20 local maize varieties from different locations of Galicia region (NW Spain) were characterised in the Applied Ecology Laboratory of the University of Vigo in late spring 2022. Fifteen varieties samples were supplied by a Seed Bank (Phytogenetic Resources Center, CRF-CSIC-INIA) and the rest by local farmers (Fig. 1). The following methodology was used to estimate eight grain physical variables of each maize variety. First, kernels (grains) that were broken, had insect holes or signs of a fungal infection were removed from the samples. Secondly, length (L), width (W) and thickness (T) were measured in a sample of 20 grains of each variety using a digital calliper reading to 0.01 mm. The geometric mean diameter (D_g), surface area (A), volume (V) and sphericity index (Φ) of grain samples were calculated using equations (Karababa & Coskuner, 2007). Thirdly, individual kernel fresh weight was measured in a sample of 100 grains using an analytical balance. Thousand Kernel Weight (TKW) was used for description of grain weight (Wu et al., 2018; Paulsen et al., 2019).

The mean value and the coefficient of variation (CV) of the grain physical variables of each variety were calculated; CV expresses the intravarietal variability. Tukey's test was used for pairwise comparisons of TKW means among varieties.

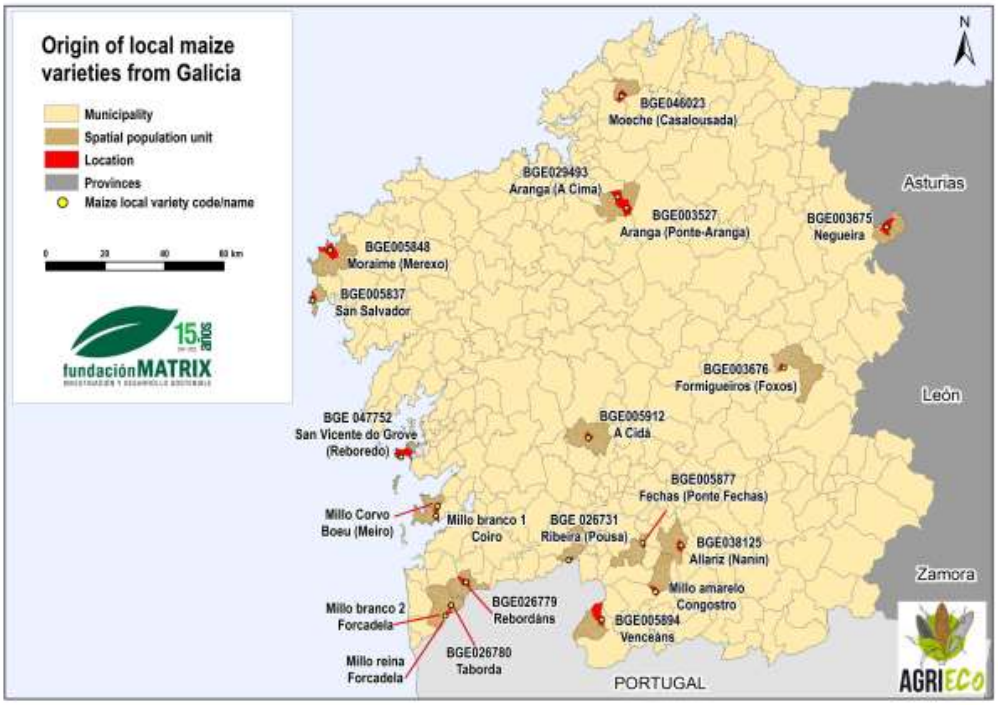

Figure 1. Provenance of 20 local maize varieties of Galicia region (NW of Spain) supplied by CRF-CSIC-INIA (codes are indicated) and local farmers (local name in Galician).

The allometric relationship between TKW and V was examined by a potential regression model ($TKW = aV^b$) for each variety and for all varieties at the same time. Tukey's test was also used for pairwise comparisons of scaling exponent (b) among varieties. To examine the variability of the local maize varieties according to the mean values of the set of grain physical variables, the scaling exponent, as well as the CV of the physical variables, a Principal Component Analysis (PCA) was used, after data standardisation. An agglomerative hierarchical clustering of varieties was also performed using the same variables, based on the Euclidean distance and Ward's method. Software R (version 4.2.0 Patched) was used for statistical analyses.

RESULTS AND DISCUSSION

Grain weight spectrum of local maize varieties

The grain weight spectrum showed a wide variation range of more than 2.4 times among the 20 maize varieties (Fig. 2). There were statistically significant differences in TKW means between many of them. For example, Millo Reina, with a TKW = 497 g outperforms 19 varieties, and this variety along with five others outperform the TKW of the remaining 14 varieties. On the opposite side, grain weight of the variety BGE003527 was the lowest, TKW = 203 g, significantly less than all the others. The phenotypic differences among varieties in this reproductive trait suggest a wide spectrum of adaptation to local environment.

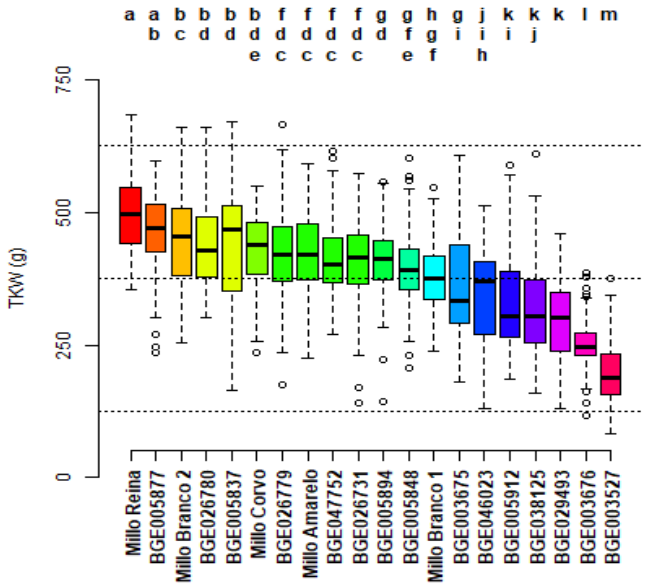

Figure 2. Box-plot of TKW of local maize varieties. Different letters or groups of letters denote statistically significant differences between pairs of varieties ($p<0.05$).

Allometry in maize grain size

Figure 3 plots TKW versus grain volume for 20 maize varieties (20 grains of each variety). An intervarietal negative allometry between both variables was identified. For the whole set of varieties, grain weight scales roughly as the square root power of grain volume rather than isometrically ($TKW = 18.5V^{0.549}$; $p<0.01$; $R^2 = 0.44$; $N = 400$). An explanation could be that grain bulk density decreases among varieties with increasing grain volume (Niklas, 1994).

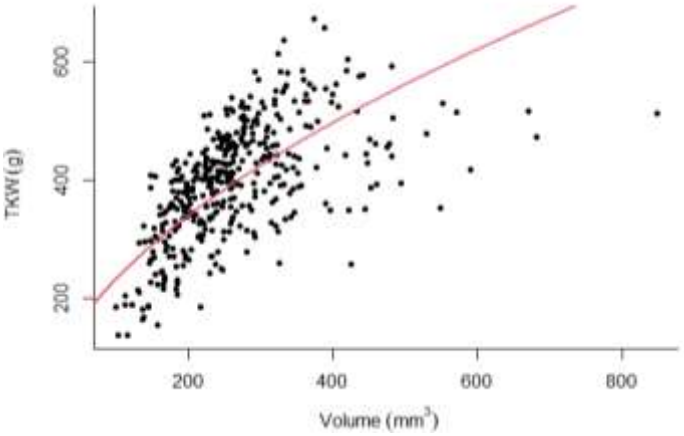

Figure 3. Allometric relationship between TKW and volume (V) of grains of 20 local maize varieties. The line expresses the regression fit to an allometric equation ($TKW = aV^b$).

The allometric relationship was statistically significant for every variety considered separately (intravarietal allometry; $p<0.01$, except in the variety BGE004858, which is $p<0.05$; R^2 varied between 0.29 and 0.90; $N = 20$). This size-dependent relationship showed that grain weight grows proportionally less than grain volume in all maize varieties, with a variation range among varieties in scaling exponent from 0.299 to 0.853. However, there were no statistically significant differences between varieties in scaling exponent.

Maize intervarietal and intravarietal allometry in grain weight versus grain volume suggest a relationship between ontogeny of seed size and changes in size among mature individual grains, i.e., between ontogenetic and static allometries (Pélabon et al., 2013). We interpret that pressure of selective breeding in different environments during the development of different maize varieties in Galicia was closely linked to environmental constraints of growth and development of grains and, therefore, their size and shape in mature stage.

Multivariate characterization of maize varieties

The result of the PCA (Fig. 4) shows that two components explain almost 80% of the variability among the 20 varieties based on 9 physical features of grains. Component 1 described the main trend of variation, which explains 57.7% and

arrange the varieties according to numerous grain size traits, including two perpendicular grain dimensions. This trend segregates at the negative end the varieties with lower values of D_g, S, V, L, W and TKW (e.g., the variety BGE003527, with L = 7.71 mm and TKW = 203 g), and at the positive end the varieties with the highest values of these physical variables (e.g., Millo Corvo, with L = 15.67 and TKW = 430 g).

This trend coincides with a climatic geographic gradient associated with continentality, with grain size being smaller in the varieties from inland Galicia, located at a distance of between 20 and 100 km from the coastline and at a higher altitude (200-800 m asl), and larger in the varieties of provenances near the sea (< 20 km) and lower altitude (< 200 m asl).

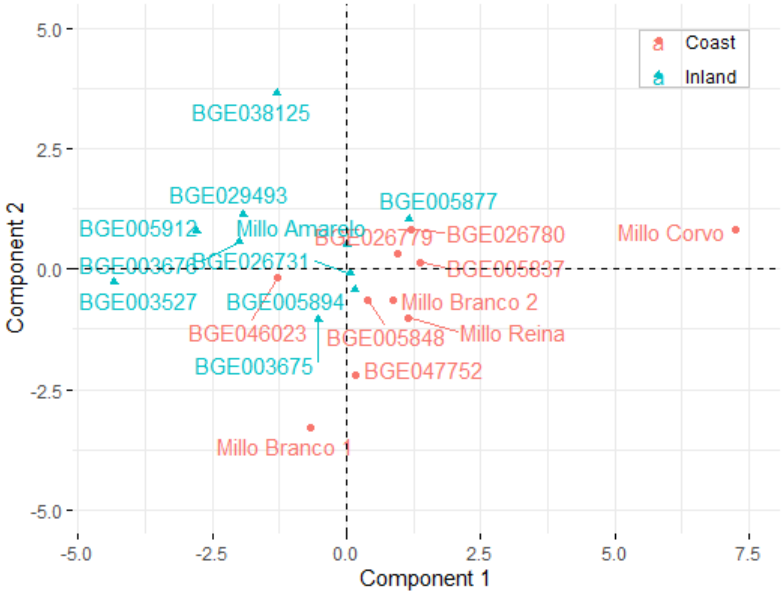

Figure 4. Distribution of local maize varieties in the ordination space defined by the two components of a PCA based on 9 physical traits. Code/name and origin of varieties are indicated (see Fig. 1).

The second trend (Component 2), which is independent of the previous one, explained 21.8% of the variability among maize varieties. It orders them according to two grain traits: thickness and sphericity. The negative end of this Component 2 segregated the varieties with less thickness and sphericity (e.g., Millo Branco 1, with a T = 5.16 mm and Φ = 66%); on the positive end were varieties with coarser, more spherical grains, e.g., the variety BGE038125, with a T = 6.81 mm and Φ = 103%). This secondary trend was not associated with a clear geographic variation pattern.

The scaling exponent did not contribute to explain the variability among varieties, which is consistent with described results on allometry of grain size.

The dendrogram of the classification (Fig. 5) allows us to identify three different groups of maize varieties, that roughly coincide with the groups defined by their origin (Fig. 4), designated as Group 'Inland-M' with 9 varieties of small grains, with an average volume of 228 mm^3 (average L = 9.34 mm); Group 'Coast-L' with 10 varieties of big grains, with an average volume of 276 mm^3 (average L = 10.70 mm), and Group 'Coast-XL', with a single variety from the coast (Millo Corvo) and more voluminous grains than the previous group, with V = 489 mm^3 (L = 15.68 mm).

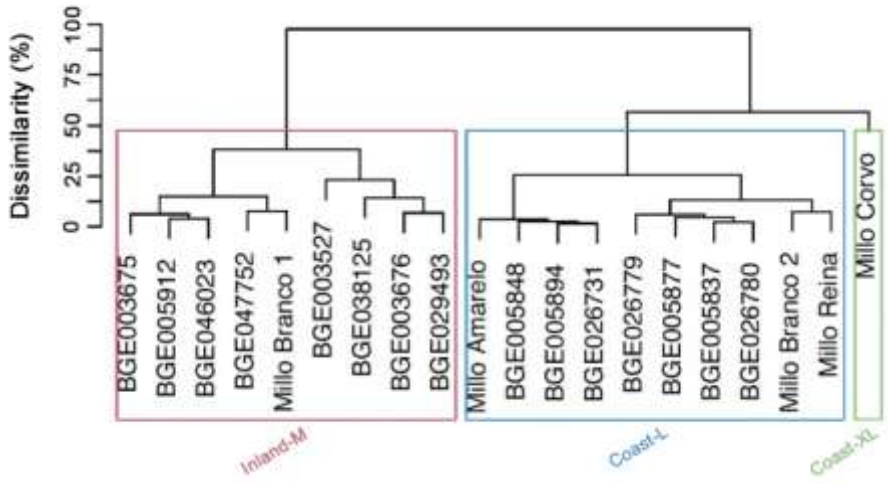

Figure 5. Hierarchical clustering dendrogram of local maize varieties using 9 physical variables of grains at once, which defines three groups: Inland-M (9 varieties), Coast-L (10 varieties) and Coast-XL (1 variety). Code/name of varieties are indicated (see Fig. 1).

The result of a PCA using eight additional traits that correspond to the coefficient of variation of physical variables, which express the intravarietal variability, revealed that varieties with a smaller grain size tended to present greater intravarietal heterogeneity in TKW, D_g, L, S and W, and those varieties with larger grain size showed greater intravarietal uniformity of their grain physical traits. The TKW variable can be highlighted and at the same time illustrate this fact. CV of TKW showed a 2-fold variation range among varieties. The average and CV of TKW showed a relatively high negative intervarietal correlation ($r = -0.66$; $p<0.01$). Phenotypic variance of maize grain traits is attributed to genotypic effects (Chen et al., 2016; Zhu et al., 2018).

Intravarietal differences in grain size dimensions could be related due to their position on the cob, since apical grains are smaller and rounder than others (Pérez

de la Cerda et al., 2007). However, differences in grain size can also be of genetic or environmental origin.

Maize grain size effect on germination is complex. In vitro germination is positively correlated with grain size (Akinyosoye, 2014). Smaller maize grains show faster germination than larger ones in drier soils (Iowa State University, 2020). This is consistent with their higher amylase activity, which is relevant for seed vigour and seedling emergence (Sulewska et al., 2014). The prevalence of small-sized grain in maize varieties from inland areas of Galicia could be interpreted as a selection pressure on this trait of less favourable environmental conditions than in coastal areas.

Within the same maize variety, a higher TKW is associated with lower yield in a temperate climate (Sulewska et al., 2014). Nevertheless, under other climates, lower TKW is associated with lower maize yield and water stress (Çakir, 2004; Kara, 2011; Alemayehu et al., 2017; Anuada et al., 2022). Soil moisture conservation promotes higher TKW (Magdaleno-Hernández et al., 2020). The length of the growing period is greater on the coast –and also at lower altitudes– than in inland locations; the thermal amplitude shows an opposite pattern. These different environmental conditions could allow larger plants with larger grain size on the coast than in inland areas. This suggests a selective value of TKW in the differentiation of local maize varieties across Galicia localities. This hypothesis is consistent with experimental evidence of the effect of growing period length on seed weight in some plants (e.g., Winn & Gross, 1993).

The absence of differences among varieties in the scaling exponent of the relationship between TKW and grain volume suggests an ontogenetic nature of this intervarietal negative allometry. This hypothesis means that grain size spectrum across maize varieties would express a specific adaptation effect (the outcome of environmental differences linked to its geographical provenance) compatible with an environmental modulation of gene expression (the outcome of an interaction between maize variety and its growing environment).

CONCLUSIONS

The study showed phenotypic differences among traditional maize varieties from Galicia in the physical characteristics of the grain. There was a wide variation range in grain weight spectrum, expressed as TKW, of up to 2.4-fold. A multivariate characterization of maize varieties based on grain physical traits allows to identify a variation trend associated to their origin, segregating a group of them from the inland of Galicia, and two groups of varieties from the coast land areas, with smaller and larger grains, respectively. This empirical evidence suggests that continentality determines differences in adaptation to the local environment among traditional maize varieties.

Greater length of growing period and thermal amplitude might be the underlying climatic factors associated to differences in grain size among Galician maize varieties. This hypothesis is also supported by the maize intervarietal and intravarietal allometry, which indicates that grain weight grows proportionally less

than grain volume. This fact suggests that development of traditional maize varieties in Galicia was clearly constrained by local environment conditions. Being maize grain size and yield two traits expected to be strongly correlated in Galicia, results highlight the relevance of increasing knowledge, conservation efforts and use of traditional maize varieties in this region to meet the needs of organic farming and challenges of climate change.

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PREDICTION OF MICROBIAL INACTIVATION IN UV LIGHT TREATMENT OF WHITE TEA USING MACHINE LEARNING AND NEURAL NETWORKS

Sibel UZUNER[1*],Büşra Zeynep GÜLER[2], Selen BİLGİÇ[2], Nurullah BULUT[2], Bahar ATMACA[3]

[1]Department of Food Engineering, Faculty of Engineering, Izmir Institute of Technology, İzmir, Turkey
[2]Department of Food Engineering, Faculty of Engineering, Bolu Abant Izzet Baysal University, Bolu, Turkey
[3]Centeral Research Lab. Application and Research Center, Mardin Artuklu University, Mardin, Turkey
*Corresponding author: sibeluzuner@iyte.edu.tr

ABSTRACT

The potential of ultra-violet (UV) light to replace the traditional brewing process to make cold tea in terms of inactivation of endogenous microflora has not been explored. Thus, the efficacy of emerging technologies such as UV-C by tea leaves/water ranging from 1 to 3 %, number of lamps ranging from 2 to 8, and number of cycles ranging from 4 to 8 were performed to determine the inactivation of total mesophilic aerobic bacteria (TMAB) and total mold and yeast (TMY) and changes in quality properties in cold drip white tea. The UV-light process was effective to reduce both TMAB and TMY. Increased number of cycles provided a significant amount of inactivation on both TMAB and TMY. The reduction of initial number of TMY was determined as 3.40±0.03 log cfu/mL with the number of lamps of 5, the number of cycle of 4, and tea leaves/water ratio of 1%, whereas TMAB were found as 3.12±0.08 log cfu/ with the number of lamps of 2, the number of cycles of 6 and tea leaves/water ratio of 1%. The resulting datasets were used to predict the inactivation of TMAB and TMY in cold drip white tea using gradient boosting regression tree (GBRT), random forest regression (RFR), and artificial neuron network (ANN) models. The ANN model provided the lowest RMSE and highest R^2 value for predicted inactivation of TMAB. TMY has not been predicted using either machine or neural networks. UV treatment possess a viable alternative for microbial inactivation without adverse effect on the quality properties of cold drip white tea.

Keywords: *Ultraviolet light, Cold drip white tea, Total mesophilic aerobic bacteria, Machine learning.*

INTRODUCTION

Tea, served as hot or ice-cold, is a trendy popular beverage worldwide. Depending on the variations in harvesting, processing, and associated degree of oxidation of fresh tea leaves; white, green, oolong, and black teas are produced from the leaves and buds of the *Camellia sinensis* (L.) (family *Theaceae*) (Unachukwu *et al*., 2010). Among those, white tea, due to being very rare and produced in minimal quantities because the leaves are collected only at dawn during a few days in the spring when the buds are still closed, is a very precious type of tea, and thus, it has been receiving increasing attention in the United States and Europe, recently (Obanda *et al.*, 2004). Ultra-violet (UV) light processing, is currently used to pasteurize food as an alternative non-thermal processing technology. UV-light can eliminate the microbial flora without the sensory quality of the food (Falguera et al., 2011). It is used for various food products to inactivate bacteria, molds, yeast, and protozoa (Guerrero-Beltran and Barbosa-Canovas, 2005)

Machine learning and neural networks can correlate large and complex datasets in solving many complex (non-linear) problems (Torrecilla *et al*., 2004). They have been used as a modeling tool in several food processing applications such as quality control, microbiology inactivation etc. (Goni *et al*., 2008; Yin and Ding, 2009).

To the best of our knowledge, the potential of UV-light to replace the traditional process to make cold tea in terms of the prediction of inactivation of yeast and bacteria using machine learning and neural networks has not been explored. Thus, the objectives of the study were to (i) treat white tea by UV-light as a function of the number of lamps, the number of cycles, and tea leaves/water ratio, and (ii) inactivate total aerobic mesophilic bacteria (TAMB) and total mold and yeast (TMY), (iii) evaluate and compare the several popular machine learning algorithms and neural networks for predicting inactivation of TMAB and TMY in white tea.

MATERIAL AND METHODS

Tea samples. Dry white tea leaves were acquired from the General Directorate of Tea Enterprises (Çay-Kur, Rize Turkey), grounded through one mm sieve, and stored in plastic bags at room temperature (22±2 ^{o}C) until processed.

Cold Drip Tea Preparation. The slower extraction process of cold brewed tea was prepared by using drip cold brew tower. This tower has a ceramic filter. Vessel was loaded by ice and water mixture. Tea leaves put into filter and adding 1L of ice-water mixture at room temperature to 10-30 g of tea leaves. Water dripped over the leaves to brew at room temperature (20-25 ^{o}C) for 8h. Water dripped at 30 drips per minute (1 every 2 seconds).

Ultra-Violet (UV) Light Processing. The UV light system was constructed in the Food Engineering Dept. at Bolu Abant İzzet Baysal University, Bolu, Turkey. The UV light system was consisted of an annular tube made from quartz glass and 12 (254 nm) UV lamps. UV lamps were placed around the outer cylinder of the quartz

tube at an equivalent distance. 12 lamps are controlled independently on the panel and the desired lamp is turned on and off alone or together with other lamps. The diameter of the area created by UV lamps was 15 cm. The UV system consists of 12 UV lamps, each with 65 W power measuring 740 mm in size, which can see the products equally. Samples for microbial analysis were taken by using glass sample taps after each cycle.

Microbial inactivation. Inactivation of total mesophilic aerobic bacteria (TMAB) and total mold and yeast (TMY) were performed with the appropriate dilutions prepared by 0.1 % (w/v) peptone (Fluka, Seelze, Germany). TMAB samples were surface plated on plate count agar (PCA, Fluka, Seelze, Germany) and TMY samples on potato dextrose agar (PDA, Fluka, Seelze, Germany) acidified with 10 % (w/v) tartaric acid (Sigma Chemical Co., Stockholm, Sweden). PCA plates were incubated at 35±2 °C for 24-48 h, whereas PDA plates were incubated at 22±2 °C for 3-5 days, respectively. Results were reported as log cfu/mL.

Construction of machine learning algorithms and ANN. Two supervised machine learning (ML) algorithms including gradient boosting regression tree model and random forest regression and ANN model were evaluated to predict the inactivation of TMAB and TMY in cold drip white tea by controlling input variables such as number of lamps, number of cycle, and tea leaves/water ratio. Parameters for the gradient boosting regression tree model (GBRT-M) algorithm are the number of trees, the number of splits in the trees, the learning rate and minimum number of observations in nodes of trees. The random forest regression model (RFR-M) creates predictions by generating many decision trees and combining their predictions in a weighted average giving the final prediction. ANN models with one and two hidden layers and two activation functions such as hyperbolic tangent (TanH) and Gaussian were investigated. For all machine learning and ANN models, the dataset was randomly split into training (65% of the initial dataset), validating (25% of the initial dataset) and testing (10% of the initial dataset) sets. An optimal hyperparameter for each model was selected based on a trained model with a small RMSE value. All the models were performed by JMP Pro software package. The performance of each model was evaluated on the test data sets with RMSE (root mean square error) and the coefficient of determination (R^2) to measure the difference between the observed and predicted values of the selected model.

Data analyses. Box-Behnken experimental design was applied with total of 15 runs using explanatory variables of number of lamps, number of cycle, and tea leaves/water ratio (Table 1). Each run was conducted in triplicate.

Table 1. Sample codes, variables and their levels for cold drip white tea.

Sample code	Tea leaves/water ratio (%)	Number of lamps	Number of cycle
UV1	2	5	6
UV2	1	8	6
UV3	1	5	4
UV4	1	5	8
UV5	1	2	6
UV6	2	8	4
UV7	3	5	4
UV8	3	5	8
UV9	2	8	8
UV10	3	8	6
UV11	3	2	6
UV12	2	2	4
UV13	2	2	8

RESULTS AND DISCUSSION

Control cold drip white tea samples had the initial TAMB and TMY count of 2.54±0.18 and 2.77±0.86 log cfu/mL, respectively. UV process with different number of lamps, number of cycles, and tea leaves/water ratio was effective to reduce both TAMB and TMY (Fig.1). Generally, the higher number of lamps, number of cycles, and tea leaves/water ratio were more effective for microbial inactivation (Fig. 1).

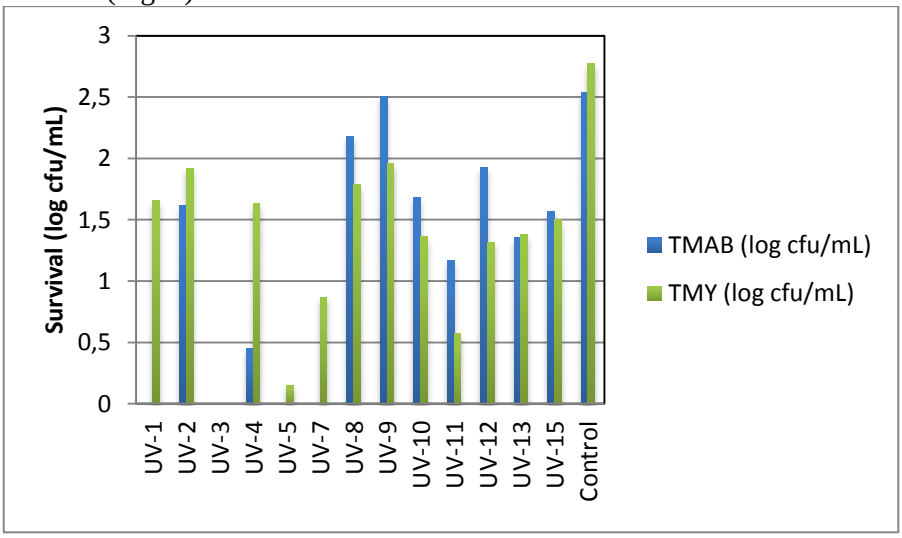

Figure 1. Inactivation of total mesophilic aerobic bacteria and total mold and yeast in cold drip white tea processed by UV

Microbial behavior was predicted for microbial inactivation using machine learning and ANN. The relationships between independent variables and explanatory variables can be determined empirically from the data processing approach using machine learning and ANN instead of a statistical approach. Different numbers of the hidden layers (one and two), number of neurons, and activation functions of the hidden layer (Gaussian and Tan H) were tested for ANN models of TMAB and TMY inactivation values. The number of hidden neurons is one of the crucial parameters of ANN. Thus, the number of neurons in the hidden layer was determined by the trial-and-error method. Several ANN models with different network topologies were trained, tested, and validated to select the best network topology. The R^2 and RMSE from training, validating, and testing data for different ANN topologies were summarized in Table 2.

Table 2. Characteristics of ANN for TMAB inactivation

Index	**No. of hidden layer**	**ANN network model**	**Training set**		**Validating set**		**Testing set**		**Activation function in hidden layer**
			RMSE	**R^2**	**RMSE**	**R^2**	**RMSE**	**R^2**	
TMAB inactivation	1	3-3-1	0.89	0.49	0.88	0.62	0.87	0.56	Tan H
	1	**3-4-1**	**0.002**	**0.99**	**0.002**	**0.99**	**0.003**	**0.99**	**Tan H**
	1	3-5-1	0.96	0.59	0.75	0.74	0.96	0.52	Tan H
	2	3-2-3-1	0.80	0.68	0.73	0.66	0.91	0.63	Tan H
	2	3-2-4-1	0.53	0.48	0.85	0.75	1.04	0.68	Tan H
	2	3-3-3-1	0.80	0.61	0.74	0.78	0.06	0.79	Tan H

The 3-4-1 topology (Fig.2) was the best with minimum RMSE and maximum R^2 values for TMAB inactivation (Table 2). The best fit ANN model with one hidden layer was tested for TMAB inactivation. ANN model for TMAB inactivation was used as the first hidden layer by TanH as an activation function. The highest R^2 of 99.9% for TMAB belonged to the best-fit ANN models based on the TMAB inactivation testing data (Table 2). RFR-M, GBRT-M, and ANN-M were evaluated for TMAB and TMY inactivation. The ANN model provided the lowest RMSE and highest R^2 value for the predicted inactivation of TMAB (Table 2). TMY was not predicted using either machine or neural networks.

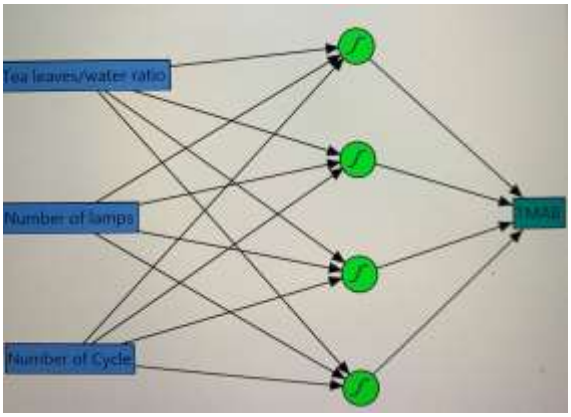

Figure 2.Architecture of ANN model used in the present study.

TMAB inactivation for all test data was predicted and plotted by ANN model against the observed values (Table 2). The models revealed R^2 values of 0.99, 0.99, and 0.99 for training, validation, and testing data of ANN-M with the RMSE values of 0.002, 0.002, and 0.003 (Table 2). ANN does not require a standard experimental design to build the model, and it is flexible and permits to addition of new experimental data to build more trustable models. This may be why ANN models can handle nonlinear responses better than the others (Chau *et al*., 2018).

CONCLUSIONS

Two machine learning algorithms (GBRT and RFR) were performed to predict the inactivation of TMAB and TMY. ANN-M predicted better inactivation of TMAB than the machine learning algorithms. However, TMY was not predicted using either machine or neural networks.

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APPLICATION OF THE INTERNET OF THINGS IN PRECISION BEEKEEPING IN LATVIA

Aleksejs ZACEPINS[1]*, Vitalijs KOMASILOVS[1], Jans JELINSKIS[2], Niks OZOLS[3], Armands KVIESIS[1]

[1]Department of Computer Systems, Faculty of Information Technologies, Latvia University of Life Sciences and Technologies, Jelgava, Latvia
[2]"1NCE Latvia" SIA, Riga, Latvia
[3]Institute of Plant Protection Research 'Agrihorts', Latvia University of Life Sciences and Technologies, Jelgava, Latvia
*Corresponding author: aleksejs.zacepins@llu.lv

ABSTRACT

Beekeeping is one of the traditional branches of agriculture and honeybees are very valuable economic insects, as they are the main pollinators in the world. Precision beekeeping is a sub-branch of the precision agriculture, which combines information and communication technologies with beekeeping and is aimed at managing an apiary more effectively to minimise the bee colony losses. Real-time, remote monitoring of the colonies applying information and communication technologies (ICT) can help the beekeepers to detect abnormalities and identify different states of the colony. One of the trending information technologies is the Internet of Things (IoT), which helps to link remotely located objects with the web services and data platforms. This research presents the IoT approach in precision beekeeping for the remote bee colony real-time monitoring system using the IoT SIM card for the data transfer. The approach is tested in a real apiary located in Platone, Latvia. Five systems were installed and used for bee colony temperature and weight monitoring. For the data management MQTT protocol and interactive visualization web application Grafana were used. As an IoT SIM card provider company 1NCE was selected, which provided SIM cards for the evaluation purposes. In addition, system power consumption and data volumes were evaluated within this research. It was evaluated that the system can operate up to 40 days before the need for the battery change or charge. IoT SIM cards worked properly. Using this approach beekeeper was able to remotely monitor the weight gain of the colonies and decide when to move the colonies to a new location.

Keywords: *Precision beekeeping, smart apiary, IoT, IoT sim card, HIVEOPOLIS*.

INTRODUCTION

Nowadays Cloud Computing and the Internet of Things (IoT) are the two trending points in the Internet field (Zacepins et al., 2017). IoT approach and methods can be applied in many branches and domains of human life including but not limited

to medicine (Lu et al., 2021), transportation (Zantalis et al., 2019), agriculture (Farooq et al., 2019), etc. The IoT can be defined as a network of Internet enabled objects linked with web services that interact with these objects (Zhao et al., 2010). This concept can be applied also to the beekeeping sector, which allows to convert beehives as a physical thing into a data generating device that is connected to the Internet. With the increase of the number of such active devices connected to the Internet around the globe, the possibility to identify different bee colony states and predict upcoming potential issues is becoming a solvable task. Beekeeping is an important productive branch of agriculture dedicated to the breeding and maintenance of bees; in addition, bees play a fundamental environmental role (Patel et al., 2021). Bees are in charge of producing special and healthy food and products' such as wax, royal jelly, bee venom, honey, pollen and propolis (Souza Cunha et al., 2020; Flores et al., 2021). Currently, bee colonies are faced with various challenges such as climate change, pesticides, and land use changes (LeBuhn and Vargas Luna, 2021) and the IoT approach can help in minimising these impacts. IoT technology became relevant to the end users and massive practical implementations as the decline of sensor size, cost and energy consumption, allows the manufacturing of extremely small and inexpensive low-end devices and microchips. IoT can be applied in a beekeeping sector too for remote and real-time monitoring of honeybee colonies, extending the precision beekeeping (PB) approach. PB is defined as an apiary management strategy based on monitoring of individual bee colonies to minimize the resource consumption and maximize the productivity of bees (Zacepins et al., 2021; Zacepins et al., 2015). Remote colony monitoring and decision making minimizes the consumption of resources and stress in the colony (Kviesis et al. 2020). It is important to emphasize that processing the collected data, transforming it into information, and extracting information from the data are the essential operations within data science (Aydin and Aydin, 2022). There are some examples of the IoT application and implementation in the beekeeping (Debauche et al., 2018; Dineva and Atanasova, 2017; Kridi et al., 2016; Zabasta et al., 2019; Cejrowski et al., 2020). The aim of this paper is to describe a developed IoT system for honey bee colony temperature and weight monitoring, using the IoT SIM cards for data transmission. An IoT SIM card is a variation of traditional SIM cards used in personal mobile devices that have additional features designed specifically for IoT devices. These features include things like being more durable, secure, and flexible.

Today in the market there are plenty of options for the IoT SIM cards (https://www.emnify.com, https://1ot.com, https://1nce.com, https://www.hologram.io, https://www.simoniot.com, and others). For the end-user, the most important questions are price and data volume as well as if additional features are available, like remote control of activating/deactivating the card, transparent information about data usage, etc. Prices can differ based on a geographical region. Some companies have flexible data plans based on a consumed data volume and additional fee for the device itself, some companies have fixed prices with limited data amount and defined lifespan. Authors selected

the IoT SIM card from 1NCE (https://1nce.com) as they have an office in author's local country Latvia and agreed to provide several SIM cards for the practical experiments and evaluation. 1NCE is the global Tier-1 IoT carrier specialized in providing managed connectivity services for low bandwidth IoT applications.

IoT SIM cards work by establishing a connection to the host network and transferring data between the device and the IoT data platform. IoT SIMs and traditional SIMs perform the same primary function, which is keeping internet-enabled devices connected to the Internet, and technically use of traditional SIM cards is possible for this purpose. But the advantage of the IoT SIM providers have extra management features available for the end-users. On the top of the SIM there is a powerful and flexible BSS system (Business Support System) which allows to manage even very large numbers of cellular connections via 1NCE Portal or via API integration. It also has powerful data transformation tools to minimize payload from IoT devises and ensure minimal data consumption. Also, such SIM is able to connect multiple operators in each country – depending on availability with the global coverage.

MATERIAL AND METHODS

This section describes the approach, used monitoring devices and data transfer procedure methods used within this study.

Location description

This research and measurements were carried out in Platone, Latvia, GPS coordinates: 56°33'32.8"N 23°41'04.1"E, during the honeybee foraging period of 2022, starting from 19.05.2022. Apiary is surrounded by the winter oil seed rape (*Brassica napus L.*) fields. This is a 60–130 cm high annual herbaceous plant belonging to the family of cruciferous plants (*Brassicaceae*).

Apiary description

Five honey bee (*Apis mellifera*) colonies were placed for remote monitoring (Fig. 1). Colonies were placed in polyfoam hives with one section dimensions of 170 mm (height), 550 mm (width) and 450 mm (depth) for brood and for honey. All hives were put in the same location in an open environment with a distance of at least 1 m between hives in one row. Hives were with different number of frames starting from 10 and up to 40 frames. Weight and temperature (inside the hive and outside) of the colonies were continuously measured with the time interval of 30 minutes between two measurements by the automated bee colony scales, and two digital temperature sensors.

Figure 1. Apiary with five honeybee colonies

Monitoring device

All five colonies were equipped with bee colony monitoring systems based on the ESP8266 microchip inspired by the monitoring system developed within the SAMS project (Wakjira et al., 2021) and described in detail in another author publication (Zacepins et al., 2020). For weight monitoring, a single-point load cell Bosche H30A is used. For the bee colony temperature monitoring DS18S20 1-Wire® sensors were used. The accuracy and precision of the single-point load cell were empirically evaluated by (Kviesis et al., 2020). The precision of the scale measurement system (single-point load cell H30A together with the 24-bit HX711 A/D converter) was observed to be around 10g.

One temperature sensor (Dallas DS18S20) per colony was installed inside the hive above the hive body (brood frames) as suggested by Stalidzans and Berzonis (2013).

The monitoring system was powered by a Sony Li-ion 18650 3.7 V 3120 mAh battery. Additional GSM/GPRS module SIM800L was attached to the monitoring device for the IoT SIM card, which required additional power supply. For this purpose an extra Sony Li-ion 18650 3.7 V 3120 mAh battery was used per monitoring system. Data about the bee colony and battery charging status were collected every 30 minutes and sent to the remote data platform.

Data transfer approach

Schematic overview of the data transfer procedure is demonstrated in the figure below.

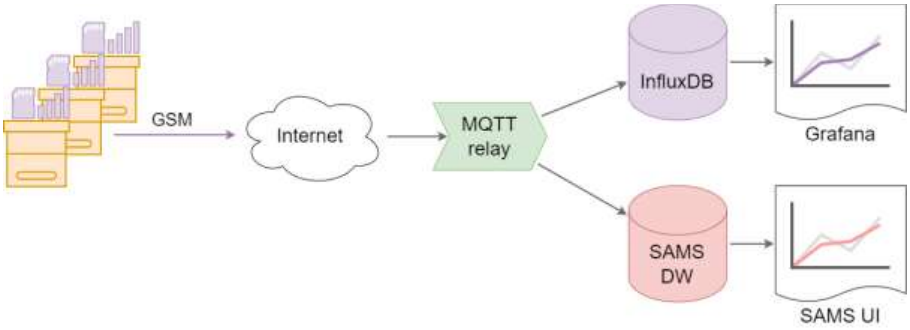

Figure 2. Architecture of the data transfer approach.

Five bee colony monitoring nodes are transferring data using the MQTT protocol. The MQTT broker coordinated the incoming and outgoing messages. Within this data transfer procedure, authors developed an "MQTT relay" component, which subscribes for specific data-in MQTT topics and carries out data storing routines upon the incoming messages. Two data platforms are used for data persistence: InfluxDB instance hosted on-premises and SAMS DW (Zacepins et al., 2020). Internet connectivity is ensured using IoT SIM cards provided by the 1NCE company. For the data observation multi-platform open source analytic and interactive visualization web application Grafana (https://grafana.com) is used to present data stored in InfluxDB, and SAMS UI for the SAMS DW stored data. The screenshots below (Fig. 3 and Fig. 4) demonstrate how the summary of one bee colony monitoring was shown to the beekeeper in real-time in both applications.

Figure 3. Summarized view in the SAMS UI.

Figure 4. Detailed view in the Grafana UI.

RESULTS AND DISCUSSION

Data packet size

As the IoT SIM cards have limited data volumes, optimisation of the data packets sent to/from the data platform is crucial in the development process.

Data packet consists of several parts: bee colony measurement data itself and technical data defined by network protocols (such as handshake and security certificate exchange). The total overhead to establish a new TLS session is around 6500 bytes on average (http://netsekure.org/2010/03/tls-overhead). Different technical messages sent during the TLS session are: ClientHello, ServerHello, Certificate, ClientKeyExchange, ChangeCipherSpec, Finished. In the authors' case, the total overhead is equal to 6567 bytes. It should be mentioned that measurements data package itself is only 47 bytes, which is less than 1% of the whole data usage, and if data packets can be optimized by the developers, technical data defined by TCP, TLS and MQTT protocols cannot be affected.

There is an option to use data communication without TLS, then the total overhead is 668 bytes. But it is not recommended as in this case sensitive information from the monitoring nodes, like passwords, are transferred in plain text. One can use plain UDP packet with only reading data – plain binary. This can be sent to AWS via 1NCE Connectivity Suite and the SIM card can be used as a secure Authorization tool. In this case, plain UDP packet almost without any overhead after being received on AWS account with the serverless Lamda function can be securely transferred to any backend – with all the security protocols. This will ensure minimal payload data consumption from the cellular modem via mobile network. For better data usage tracking, 1NCE provides a web interface where users can observe and monitor data usage for all IoT SIM cards and individually for each SIM card.

Evaluation of the system battery life for the continuous monitoring

The bee colony monitoring system was powered by two Sony Li-ion 18650 3.7 V 3120 mAh batteries (one for the monitoring node, one for the SIM module). By summarizing the battery discharge dynamics, it was concluded that the monitoring system's daily battery drain is around 8.0 – 18.5 mV, where battery discharge for the SIM module is higher than for the monitoring module. Difference in discharge rate can be explained by the power consumption of the SIM module (peak current draw can reach ~2A during transmission burst mode and up to ~400mA during data mode (GPRS) (Shanghai SIMCom Wireless Solutions Ltd., 2015)) and the time needed for the module to connect to the cell tower, enable data mode and perform the data transfer procedure. It was evaluated that the system can operate up to 40 days before the need for the battery change or charge to avoid over-discharge and potential damage to the battery.

Costs estimation

Beekeepers are not willing to invest much in the digital solutions, thus the economic aspect of the system is very important and system costs should be as minimal as possible. The list of used system hardware components with approximate unit price are summarized in Table 1.

Table 1. The list of used system hardware components with unit price.

Nr.	Name of the component	Cost (in EUR)
1	BOSCHE Wagetechnik Single point load cell H30A (200kg)	50.00
2	Platform for load cell	50.00
3	ESP8266 microchip including adapter plate	13.00
4	Temperature sensor DS18B20 (x2)	8.00
5	A/D converter OKYSTAR HX711	6.00
6	Additional components (PCB, wires, resistors, capacitors, connectors, etc.)	10.00
7	Rechargeable battery Sony Li-ion 18650 3.7 V 3120 mAh (x2)	18.00
8	SIM800L module	5.00
Monitoring node overall costs		160.00

The calculated costs for one monitoring system are 160.00 EUR (based on local prices in Latvia). System installation, maintenance, data storage, IoT sim card with

appropriate data plan and usage of the web system is not considered in these calculations.

Analysis of bee colony data

Focus of this study was not on a comprehensive data analysis and the bee colony state and health recognition, but more on the approach of how to collect and transfer bee colony data to the endpoint. But for demonstration purposes authors would like to provide some charts visualizing bee colony temperature and weight dynamics of the monitored colonies indicating useful scenarios for the beekeeper. Data was collected from 19.05.2022 and the process is still ongoing.

By continuous weight monitoring it is possible to identify daily patterns of the bee activity during sunny days. Continuous monitoring of the honey bee colony weight allows beekeepers to identify daily patterns of their activity during sunny days. Based on the weight data, honey bee day can be split into 3 periods: nectar processing by reducing the water content during the night time; flying out for the foraging when ambient temperature reaches 8 ℃ and it is sunny; coming back with collected nectar (see Fig. 5).

Figure 5. Daily routine of one honey bee colony during the sunny days.

By continuous temperature monitoring, conclusions can be made about the status of brood rearing in the colony during the summer period. To ensure the optimal development of brood, a honeybee colony needs to maintain its temperature within a certain range of values (thermoregulation), regardless of environmental changes in biotic and abiotic factors (Godeau et al., 2022).

Figure 6 below shows the temperature in colonies and indicates that optimal development of the brood is ensured, when temperature is between 34 ℃ and 36 ℃.

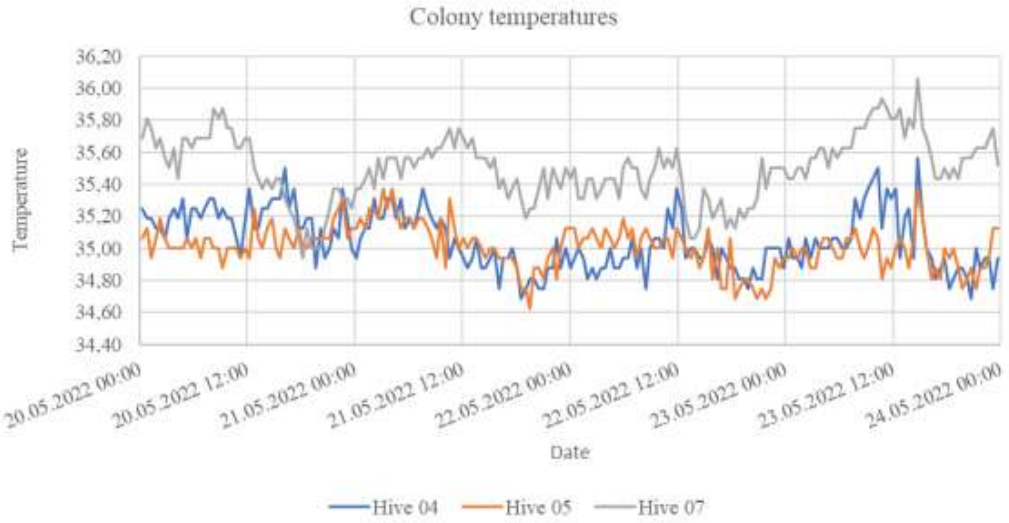

Figure 6. Temperature dynamics in some honeybee colonies.

CONCLUSIONS

Latest trends indicate an increasing correlation between the use of modern information and communication technologies with the successful development of beekeeping practice. New technologies enable beekeepers to make their business more efficient by reducing costs, minimising the number of on-site colony inspections. IoT solutions help to build-up the interaction between beekeepers and bee colonies. The ability to transfer data from a practically unlimited number of sensors over a long distance and the ability to analyse all the data in the cloud, makes the Internet of Things an indispensable technology to digitize beekeeping.

By using individual GSM/GPRS module per measurement node, the colony monitoring in the apiary becomes more distributed, decentralized and is not dependent on one central element (gateway, WiFi router) that allows data transmission to the cloud, therefore limiting the potential data loss of all connected nodes in case the central unit fails. One of the potential future research direction within this topic is the optimization of payload data from the cellular modem.

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DNA MARKERS AS A MEANS OF ASSESSING THE GENETIC DIVERSITY AND IDENTIFICATION OF GRASSES

Iryna KONDRATSKAYA*, Andrei YUKHIMUK, Olga CHIZHIK, Vladimir RESHETNIKOV

The State Scientific Institution «Central Botanical Garden of the National Academy of Sciences of Belarus» Minsk , Belarus
*Corresponding author: ikondratskaya@mail.ru

ABSTRACT

The effectiveness of breeding is largely depends on the presence of the gene variability in the initial breeding material. The genetic variability assessment helps to evaluate the source material during a fundamentally new forms with economically valuable traits creating. In this regard, the assessment of the genetic polymorphism of the cereal grasses genotypes which are of interest both as a source of breeding material and as a variety candidate was carried out by DNA markers. Seventy-three loci were identified for the Festulolium and Lolium L. genotypes using the SCoT marker system, 69 of markers were polymorphic. This marker system revealed a high level of polymorphism in the studied genotypes of Festulolium and Lolium L. – 94.52%. For the genotypes of intergeneric hybrids of the Agropyron L. genus and their parent forms 90 loci were identified – 46 SCoT markers and 44 SRAP markers. From the total pool of markers 73 markers were polymorphic. On average, the level of polymorphism was 81.1%. For the genotypes of the Alopecurus L. genus interspecific hybrids and their parent forms 157 loci were identified – 52 for RAPD-PCR and 105 – for ISSR-PCR. Of the total pool of markers, 104 were polymorphic According to the results of DNA genotyping the genetic passports of cereal grasses were compiled.

Keywords*: cereal grasses, genetic passport, genotype, SCoT markers, ISSR, RAPD, SRAP.*

INTRODUCTION

Scientific and practical achievements in the fields of genetics and breeding lead to the reduction of the erms for hybrids and varieties creation. So, today one can see a significant increasing in both the rate of new varieties creation and their number. At the same time the modern breeding is characterized by a tendency to reduce the genetic distances between newly created varieties. This is due to the fact that when a new varieties creating, the same genotypes with economically valuable properties are often involved in crossing. The genetic diversity of varieties reduction demands to identify a large number of varieties of the closely related origin (Kilchevski,

2014). At the present time such assessment can be carried out using DNA marker systems.

To solve the problem of pure undegraded DNA extraction from plant objects is very important to define the first stage of molecular genetic research. Different plant species, plants and organs at different stages of development, even different organs of the same species at the same stage of plant development, contain different amounts and classes of secondary metabolites and spare substances (Raybushkina, 2019). The component composition has a significant impact on the quality and quantity of extracted DNA. For DNA isolation it is better to use young plant organs and leaves, since they contain a smaller amount of spare substances and secondary metabolites. However, young leaves and plant organs are not always available. The methods of DNA isolation have to be modified to obtain the satisfactory results for the adult plant organs, herbarium material, etc. To isolate DNA both dry and sprouted seeds can be used or lyophilically dried or fixed in silica gel leaf tissue. To carry out DNA labeling and to create the DNA collection of perennial grasses we carried out the research work to optimize the method of DNA isolation, which provides a high yield of high-quality DNA preparations.

The DNA extraction procedure was specific for cereal grasses which are characterized by the high protein content and a high concentration of polysaccharides in leaves.

MATERIAL AND METHODS

Object of investigations: cereal grasses – the representatives of Festulolium, Lolium, Agropyron and Alopecurus families. To isolate DNA, we used: 1) dried seeds; 2) turgid seeds (12 hours of soaking); 3) fresh leaf material; 4) leaf tissue fixed at -200 C and -800 C; 5) leaf tissue dried from 370°C to 420°; 6) dried plant tissue with silica gel until complete dehydration. Preparations of high-quality DNA were obtained using a modified STAB method from silica-dehydrated leaf tissue of cereal grasses. (Dempster E.L.,1999). Total DNA labeling was carried out using PCR technique. To label the hybrid forms and varieties of perennial grasses included in the studies, the multilocus primers were selected: ISSR (inter simple sequence repeat); RAPD (randon amplification of polymorphic DNA); SCoT (start codon targeted); SRAP (Sequence-related amplified polymorphism) and microsatellite primer SSR (Simple Sequence Repeat).

RESULTS AND DISCUSSION

After optimizing of the DNA isolation process we obtained the high-quality DNA preparations of cereal grasses. Preparations with a DNA concentration of at least 50 ng/ml and ratios A260/A280 $\geq$ 1.7 and A260/A230 $\geq$ 1.5 were considered qualitative. Data on the quality preparations of total DNA of the *Lolium* L. genus and their hybrid forms are represented in the table 1.

Table 1. Qualitative and quantitative parameters of total DNA preparations

№	Name	A260/280	A260/230	c (DNA), ng/μL
1	Lolium perenne L. cv. Guslayr	1,8	2,1	602
2	Lolium multiflorum Lam. cv. Matador	1,8	2,1	906
3	Lolium perenne × multiflorum hybrid 22–7	1,8	2,1	931
4	Lolium perenne × multiflorum hybrid 22–7–21	1,8	2,1	700
5	Lolium perenne × multiflorum hybrid 22–7–23	1,9	2,3	817

The obtained DNA preparations of hybrid forms and varieties of perennial grasses have been included in the DNA collection of perennial grasses and placed for guaranteed long-term storage at a temperature of -80 ° C in the DNA BANK of the Department of Biochemistry and Biotechnology of plants of the State Scientific Institution "Central Botanical Garden of the National Academy of Sciences of Belarus"(Table2).

Table 2. Passport of the basic DNA collection on the example of representatives of the genus Lolium L, and hybrid forms. Curator of the DNA collection – a scientific researcher Yukhimuk A.N.

№	**Name**	**Source of DNA**	**Selection method**	**Quality indicators (A260/A280)/ (A260/A230)**	**Concentration**	**Volume**
1	Lolium perenne L. Cv Guslayr	leaf tissue	Demster, 1999	1,8/2,1	602	>100
2	Lolium multiflorum Lam. cv. Matador	leaf tissue	Demster, 1999	1,8/2,1	906	>100
3	Lolium perenne × multiflorum Bybor 22–7	leaf tissue	Demster, 1999	1,8/2,1	931	>100
4	Lolium perenne × multiflorum, hybrid 22–7–211	leaf tissue	Demster, 1999	1,8/2,1	700	>100
5	Lolium perenne × multiflorum, hybrid 22–7–25	leaf tissue	Demster, 1999	1,9/2,3	817	>100
					ng/μL	μL

Currently, the Department of Biochemistry and Biotechnology of Wildebeest Plants of the Central Botanical Garden of the National Academy of Sciences of Belarus has formed a collection of DNA varieties of representatives of genera:
- Lolium perenne L, Lolium multiflorum Lam.,
- Festuca arundinacea SCHREB.; - Agropyron cristatum L.;
- Festulolium; - Alopecurus pratensis L., Alopecurus arundinaceus Poir. and their:

- Lolium perenne L X Lolium multiflorum Lam.;
- Alopecurus pratensis L.X Alopecurus arundinaceus Poir.;
- Lolium perenne L, X Agropyron cristatum L.;
- Lolium perenne L, X Festuca arundinacea SCHREB.

39 samples in a total.

The obtained high-quality total DNA preparations of varieties and hybrid forms of perennial grasses were used for DNA labeling using marker systems.

Based on molecular markers, we have developed a system of DNA certification of varieties and hybrids of perennial grasses. For molecular genetic certification of varieties and hybrids of perennial grasses, based on the literature data (Paäakinskiene,2000, Arghavani,2010) a pool of primers was selected. All primers were tested for highly polymorphic, reproducible markers obtaining.

DNA labeling of all hybrid forms and varieties of perennial grasses using the marker systems, listed above, made it possible to differentiate all the genotypes studied, develop and compile unique profiles for each of them, calculate the genetic distances of kinship/remoteness. Based on the obtained DNA spectra the genetic passports were compiled for the studied samples (Table 3).

Table 3. Molecular genetic passports on the example of an interspecific hybrid of the genus *Lolium* L. and parental forms, compiled on the basis of the results of multilocus labeling of total DNA

Lolium multiflorum LAM. , Matador

Праймер	Маркер, bp
SCoT	
SCoT–01	1656, 1271, 828, 781, 715, 658, 607, 538, 427, 357, 248
SCoT–06	1003, 675, 604, 527, 382, 310, 269, 221, 176
SCoT–13	849, 690, 610, 475, 408, 356, 280, 202
SCoT–21	879, 693, 669, 481
SCoT–32	1093, 884, 534, 453, 386, 317, 264, 226
SRAP	
SRAP–Em06/Me02	970, 579, 449, 376, 298, 223
SRAP–Em06/Me09	1074, 907, 847, 686, 489, 453, 369, 213
SRAP–Em12/Me09	990, 896, 776, 533, 429, 349, 327
SRAP–Em13/Me05	1573, 1429, 1251, 544, 423, 365

Lolium perenne L., Guslyar

Праймер	Маркер, bp
SCoT	
SCoT–01	1656, 1271, 973, 828, 715, 607, 559, 510, 427, 357, 248, 201
SCoT–06	1003, 701, 527, 421, 221
SCoT–13	729, 690, 610, 488, 449, 408, 356, 280, 231, 202
SCoT–21	879, 693, 669, 550, 481, 304
SCoT–32	1525, 1093, 884, 613, 490, 352, 317, 264, 226
SRAP	
SRAP–06/Me02	579, 472, 376, 343, 258, 223
SRAP–06/Me09	1074, 847, 686, 630, 489, 453, 413, 249, 213
SRAP–12/Me09	896, 776, 459, 349
SRAP–13/Me05	1573, 887, 513, 423, 342, 288

Lolium L., hybrid 22-7, Vybor

Праймер	Маркер, bp
SCoT	
SCoT–01	1656, 1094, 866, 715, 658, 538, 486, 427, 357, 275, 201
SCoT–06	1119, 1003, 845, 742, 476, 310, 269, 221
SCoT–13	1002, 690, 610, 449, 408, 356, 280, 231, 202
SCoT–21	879, 812, 693, 669, 481, 432, 227
SCoT–32	1093, 884, 613, 490, 453, 431, 386, 317, 264
SRAP	
SRAP–m06/Me02	970, 895, 579, 472, 404, 376, 223
SRAP–m06/Me09	1074, 974, 907, 847, 630, 489, 453, 413, 369, 213
SRAP–m12/Me09	925, 842, 776, 459, 385, 349
SRAP–m13/Me05	1573, 730, 513, 423

CONCLUSIONS

The DNA passport presence for new highly competitive varieties of cereal grasses creation will allow:

- to check the compliance of new varieties with the criteria of the OOS test;
- to evaluate the genetic novelty of varieties, lines and hybrids;
- to assess the compliance of seed batches with the standard;
- to confirm the conditioning of seeds purchased abroad;
- eliminate the possibility of falsification of varieties and related economic losses.
- to improve the system of patenting new varieties, as well as to solve controversial issues of conformity and authorship of the variety according to the characteristics of the allel state of loci.

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EFFECT OF SEASON IN ROOTING STEM-TIP CUTTINGS OF MEDITERRANEAN SAGES (*Salvia* spp.) NATIVE TO GREECE

Aikaterini N. MARTINI*, Konstantinos BERTSOUKLIS, Georgia VLACHOU, Maria PAPAFOTIOU

Laboratory of Floriculture and Landscape Architecture, Department of Crop Science, School of Plant Science, Agricultural University of Athens, Iera Odos 75, 11855 Athens, Greece
*Corresponding author: martini_agr@yahoo.com

ABSTRACT

Mediterranean sages (*Salvia* spp. family Lamiaceae) are readily propagated by stem cuttings. However, their rooting efficiency may be affected by season because of differences in mother plant physiology and climatic conditions. In the present study, stem-tip cuttings of *Salvia fruticosa, S. officinalis*, *S. pomifera* ssp. *pomifera*, *S. ringens* and *S. tomentosa*, 8-12 cm long, were collected from greenhouse grown mother plants at the end of November 2020, February, May and August 2021, indicative of four seasons. The aim was to define the most appropriate period for effective rooting of cuttings for each species that could enhance their potential use in floriculture industry through the regeneration of selected genotypes. Cuttings were treated with dusting powder for soft wood cuttings Rhizopon (0.5% w/w IBA) and were placed for rooting on peat-perlite substrate 1:1 (v/v) in a mist for 2 weeks. Then, they remained on the greenhouse bench in a semi-shaded bench for another 4 weeks. Cuttings of all species rooted more efficiently during the period from autumn to spring, excepting *S. fruticosa*, whose cuttings rooted at the lowest percentage in spring, probably because of insufficient lignification, while they presented high rooting percentage in summer similar to that of autumn and winter. Therefore, propagation of studied *Salvia* spp. by stem-tip cuttings was feasible throughout the year, although rooting percentages were reduced during spring (for *S. fruticosa*) and summer (for all species, excepting *S. fruticosa*). By choosing the appropriate season maximum rooting percentages of cuttings can be achieved.

Keywords: *Salvia fruticosa, Salvia officinalis, Salvia pomifera ssp. pomifera, Salvia ringens, Salvia tomentosa, clonal propagation*.

INTRODUCTION

Mediterranean sages (*Salvia* spp. family Lamiaceae) are ideal for xeriscaping, due to their reduced water and cultivation requirements, as well as their high ornamental value and bee friendliness. *Salvia fruticosa* is up to 1.20 m, with white felted stems, leaves often with 1-2 pairs of small lobes below the main one, flowers with high colour variability, pink, lilac, or sometimes white, in March-June. The

leaves are used for flavoring and for an herbal tea (Blamey and Grey-Wilson, 1988). *Salvia officinalis* is a strongly aromatic, rather grayish shrub up to 60 cm, with branches spreading to erect, becoming woody below, leaves oblong to elliptical, rough greenish above but white felted beneath, margin finely toothed, flowers violet, blue, pink or white in May – July (Tutin *et al.*, 1972; Blamey & Grey-Wilson, 1993). It is cultivated worldwide with many varieties as pharmaceutical and ornamental. *Salvia pomifera* ssp. *pomifera* is up to 1.00 m high, with strongly aromatic grey-green oval leaves and pink and violet flowers of intense color, with calyx often reddish-purple (Tutin *et al.*, 1972; Blamey & Grey-Wilson, 1988; Thanos and Doussi, 1995). *Salvia ringens* is up to 0.30 m high (up to 60 with the inflorescences), woody at base, with leaves pinnatisect or pinnate with 3-6 pairs of small lateral segments, appressed-hairy and flowers dark violet-blue or blue, (Tutin *et al.*, 1972). *Salvia tomentosa* is up to 0.80 m high, similar to *S. officinalis*, but has leaves with a rounded or heart-shaped base and flowers usually light violet or pink with reddish-brown calyces (Blamey & Grey-Wilson, 1993).

The asexual propagation by stem cuttings is a simple and easily applied method of plant propagation, which is preferred in case of medicinal and aromatic herbs rather than propagation by seeds (Nicola *et al.*, 2005). Mediterranean *Salvia* species are readily propagated by stem cuttings, as shown by several reports on rooting cuttings of *S. officinalis* (Nicola *et al.*, 2003; Nicola *et al.*, 2005; Kaçar *et al.*, 2009; Paradiković *et al*., 2013; Gudeva *et al*., 2017) and *S. fruticosa* (Sağlam *et al.*, 2014). Besides, rooting of spring cuttings of all *Salvia* species of the present work, collected from native plants, has already been studied testing various treatments for rooting induction and obtaining the mother plants for this study (Martini *et al.*, 2020). However, their rooting efficiency may be affected by season because of differences in mother plants physiology and climatic conditions. So, experimentation for each specific plant is necessary in order to determine the appropriate rooting hormone treatment, since it is well established that exogenous application of auxin accelerates the rates of rooting, increases final rooting percentage and the number of produced roots in leafy cuttings (De Klerk *et al.*, 1999).

The aim of this study was to define for each *Salvia* species the most appropriate period for effective rooting of cuttings collected from greenhouse grown mother plants, which could enhance their potential use in floriculture industry through the regeneration of selected genotypes.

MATERIALS AND METHODS

Stem-tip cuttings of *Salvia fruticosa, S. officinalis, S. pomifera* ssp. *pomifera, S. ringens* and *S. tomentosa*, 8-12 cm long, were collected from mother plants maintained in a greenhouse at the Agricultural University of Athens (37°58’53.94” N, 23°42’25.01” E), at the end of November 2020, February, May and August 2021, indicative of four seasons. After each collection of cuttings, mother plants were fertilized monthly with 2 g/L water soluble fertilizer (20-20-20

plus, HUMOFERT, Metamorfosi, Greece). In each pot, 100 ml of fertilizer was applied. Almost three months later, the following collection was done from the same mother plants. Cuttings were treated with dusting powder for soft wood cuttings Rhizopon (0.5% w/w IBA), which was one of the most effective treatments for all species in a previous study with spring cuttings (Martini *et al.*, 2020). Then, they were placed for rooting on peat-perlite substrate 1:1 (v/v) in a mist for 2 weeks, followed by transfer on the greenhouse bench in a semi-shaded location for another 4 weeks.

At the end of the experiment, rooting percentage (%) of cuttings was recorded. Cutting quality was also evaluated per species considering seasonally cutting final growth and foliage healthiness. The completely randomized design and three repetitions of ten cutting per treatment were used, the significance of the results was tested by one- or two-way analysis of variance (ANOVA) and treatment means were compared by Student's *t* test at $p \leq 0.05$.

RESULTS AND DISCUSSION

The two-way ANOVA revealed significant interaction of the two main factors of the experiment (*Salvia* species and season of cuttings collection), so rooting percentages were analysed by one-way ANOVA per *Salvia* species. Season of cutting collection had a significant effect on the rooting percentage of all species, excepting *S. pomifera* ssp. *pomifera* (Figure 1).

Cuttings of *S. fruticosa* rooted at higher percentage (>80%) during the summer to winter period compared to spring (Figure 1a), probably because spring cuttings, produced after the winter collection, were not sufficiently lignified and elongated, as shown in Figure 2a. In summer and autumn, not only high rooting percentages were recorded but also quality of rooted cuttings was more satisfactory (Figure 1a, Figure 2a).

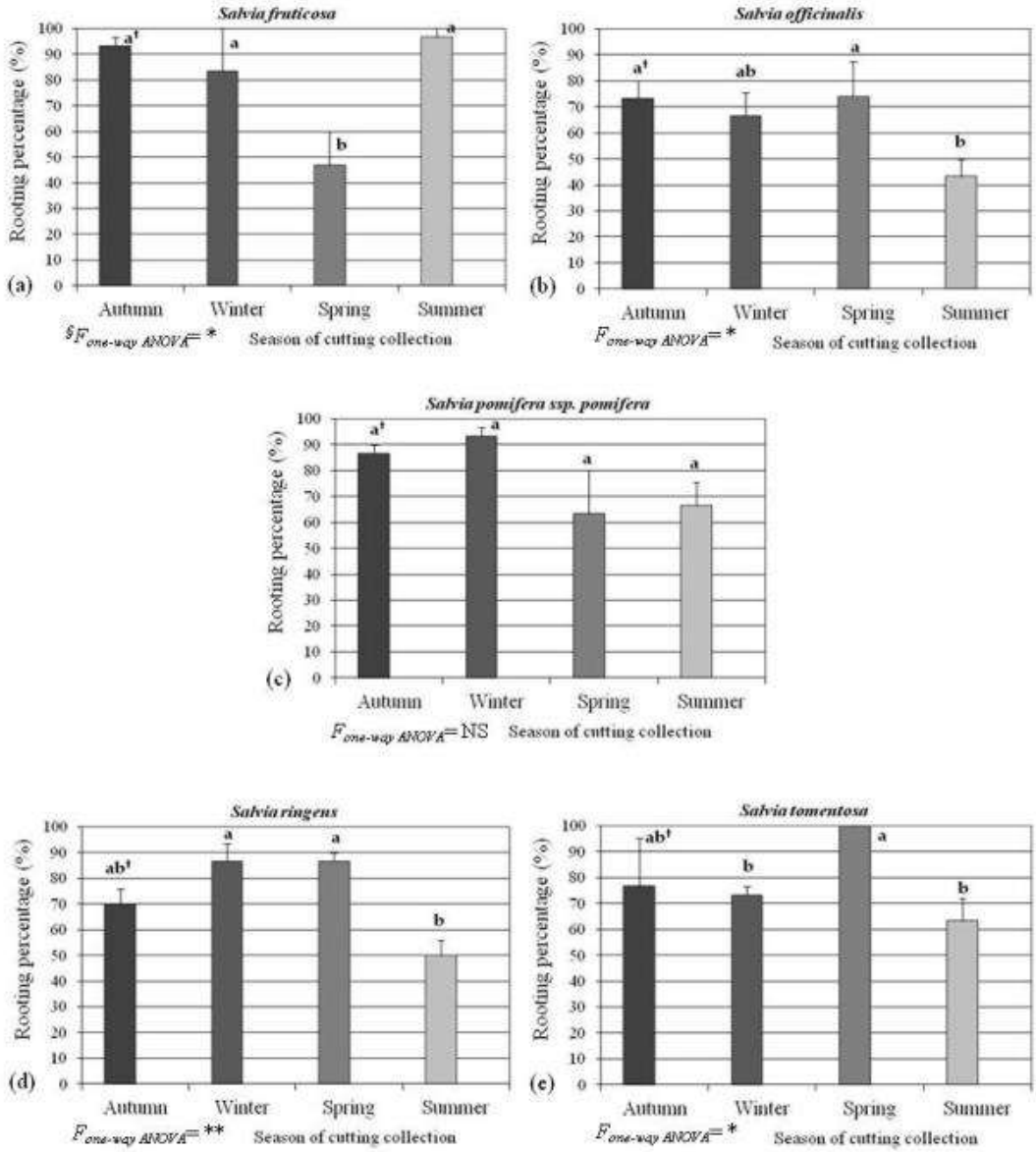

Figure 1. Effect of season in rooting percentage (%) of stem-tip cuttings of Mediterranean sages, i.e., *Salvia fruticosa* (a), *S. officinalis* (b), *S. pomifera* ssp. *pomifera* (c), *S. ringens* (d) and *S. tomentosa* (e), collected from greenhouse mother plants during November 2020 to August 2021 and treated with dusting powder Rhizopon (0.5% w/w IBA).

† Mean values per species (n = 3 replications of 9-10 cuttings each) followed by the same lowercase letter did not differ significantly at $p \leq 0.05$ using Student's t-test.

§ NS or * or **, non-significant at $p \leq 0.05$ or significant at $p \leq 0.05$ or $p \leq 0.01$, respectively.

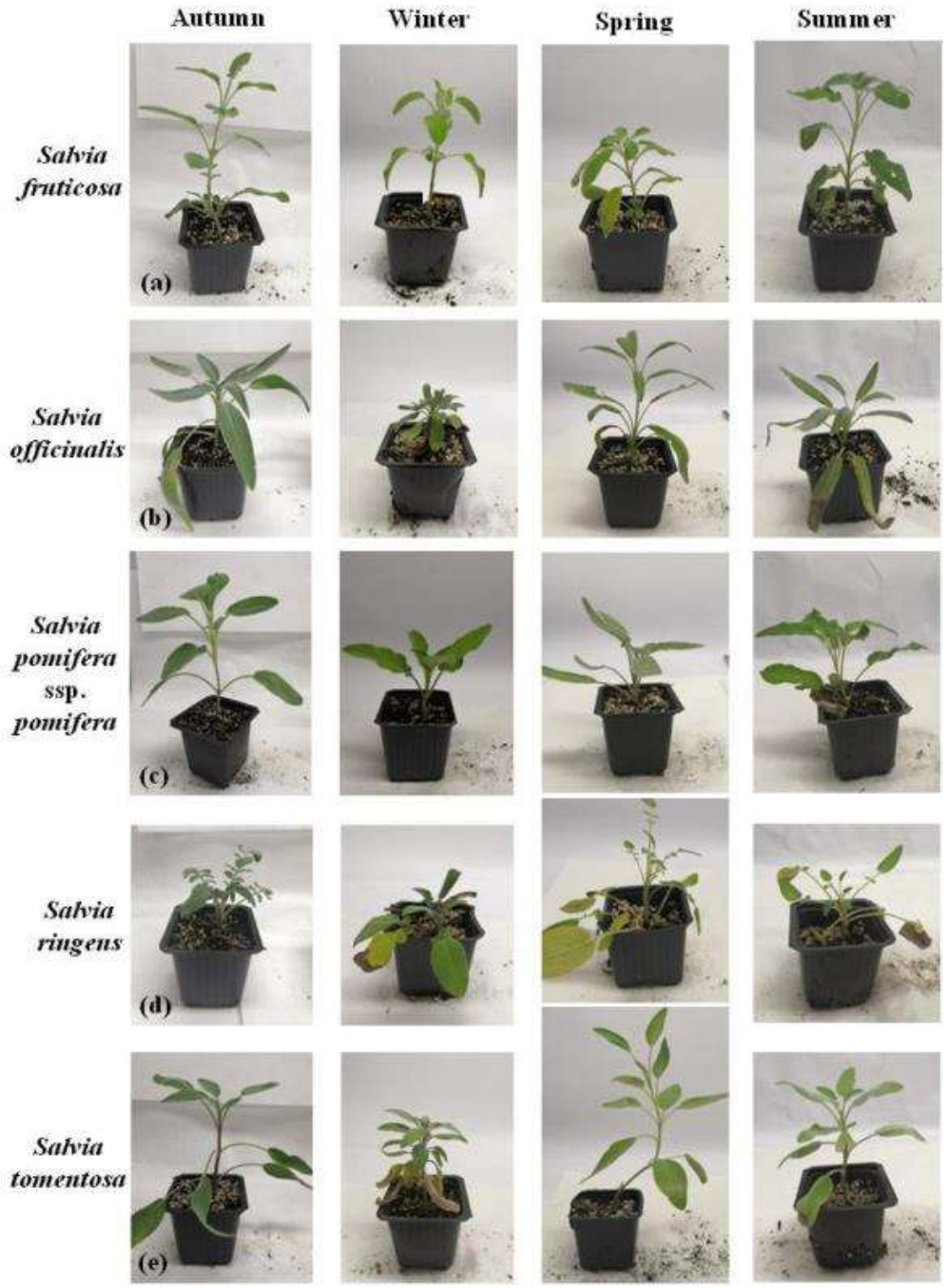

Figure 2. Rooted cuttings of the species *Salvia fruticosa* (a), *S. officinalis* (b), *S. pomifera* ssp. *pomifera* (c), *S. ringens* (d) and *S. tomentosa* (e), collected from greenhouse grown mother plants in marked season, six weeks after their treatment

with dusting powder Rhizopon (0.5% w/w IBA) and their placement for rooting on peat-perlite substrate 1:1 (v/v).

In *S. officinalis,* higher rooting percentages (about 70%) were recorded in autumn to spring period compared to summer (Figure 1b), in verification of previous reports, in which higher than 70% rooting percentages were achieved either using Rhizopon (Paradiković *et al.*, 2013) or after immersion in 1000 mg L^{-1} IBA for 5 sec (Kaçar *et al.*, 2009). Rooting percentages were increased compared to our preliminary study (Martini *et al.*, 2020), in which spring cuttings collected from native mother plants were used and those of *S. officinalis* rooted at the lowest percentages (35%) of all species, probably because of insufficient lignification and the presence of blooming. Quality of rooted cuttings was better in autumn and spring (Figure 2b).

There were no statistically significant differences between the seasons in rooting percentages of *S. pomifera* ssp. *pomifera* cuttings (63-93%), although higher percentages were observed in autumn to winter period (Figure 1c) and quality of rooted cuttings was better in autumn (Figure 2c). Spring cuttings were less lignified and elongated, as in the case of *S. fruticosa* (Figure 2a and c).

Cuttings of *S. ringens* rooted more sufficiently (87%) during winter to spring period compared to summer (Figure 1d), while quality of rooted cuttings, excepting autumn cuttings, seemed to be poorer than those of the other species (Figure 2). However, rooted cuttings of all studied species grew satisfactorily after transplantation in plastic pots with various substrates of peat-perlite (1:1, 2:1 or 3:1, v/v) and monthly fertilization with 2 g L^{-1} water soluble fertilizer 20-20-20 (Tassoula *et al.*, 2021).

Cuttings of *S. tomentosa* rooted at the highest percentage (100%) in spring, followed by autumn (Figure 1e). Quality of rooted cuttings was also optimum in spring, while it was satisfactory in autumn and summer too (Figure 2 e).

In previous studies on *S. officinalis* and *S. fruticosa*, hormones enhanced rooting of cuttings (Nicola *et al.*, 2003) and had a positive effect on root system and plant development (Nicola *et al.*, 2005; Paradiković *et al.*, 2013; Sağlam *et al.*, 2014). Besides, spring cuttings of all the *Salvia* species investigated in the present work rooted more efficiently after treatment with dusting powder Rhizopon or immersion in a 2000 or 3000 mg L^{-1} IBA solution compared to immersion in a solution of 500 or 1000 mg L^{-1} IBA or the control (Martini *et al.*, 2020).

CONCLUSIONS

Propagation of studied *Salvia* spp. by stem-tip cuttings was feasible throughout the year, although rooting percentages were reduced during spring (for *S. fruticosa*) and summer (for all species, excepting *S. fruticosa*). Quality of rooted cuttings in terms of cutting final growth and foliage healthiness was better in autumn for all species, while other seasons were also satisfactory, depending on the species. By choosing the appropriate season for cutting collection maximum rooting percentages and better quality of cuttings can be achieved.

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MORPHOLOGICAL ANALYSIS IN NEW INTERSPECIFIC HYBRIDS OF *SALVIA* SPP. ORIGINATED FROM *S. FRUTICOSA, S. OFFICINALIS, S. POMIFERA* SSP. *POMIFERA* AND *S. RINGENS*

Konstantinos BERTSOUKLIS*, Aikaterini N. MARTINI, Sofia-Nikoleta ROUSSAKI, Ilias MELEAS, Maria PAPAFOTIOU

Laboratory of Floriculture and Landscape Architecture, Department of Crop Science, School of Plant Science, Agricultural University of Athens, Iera Odos 75, 11855 Athens, Greece
*Corresponding author: kber@aua.gr

ABSTRACT

The introduction of new hybrids and clones suitable for xeriscaping is a challenge for modern floriculture. The Mediterranean sages *Salvia ringens* and *S. pomifera* ssp. *pomifera* are unexploited versus *S. officinalis* and *S. fruticosa* that are widely used in floriculture and medicinal industry. The present study was conducted to test quantitative and qualitative morphological traits to find suitable descriptors for the discrimination of new *Salvia* hybrids. Morphological characteristics were used to study new hybrids, i.e., OR (*S. officinalis* × *S. ringens*), FR (*S. fruticosa* × *S. ringens*), PR (*S. pomifera* ssp. *pomifera* × *S. ringens*). A total of eight quantitative (leaf and flower/inflorescence morphometrics) and 14 qualitative characters (characters of vegetation, flowers and fragrance) were selected and used based on descriptors for other plant species. One way ANOVA was used for determination of the differences between the mean values of leaf, stem and flower traits and a dendrogram was generated based on the genetic distance matrix. *S. ringens* inherited its segmented leaves and their light aroma to all its hybrids. PR and FR hybrids formed leaves and inflorescences with intermediate length between their parents, while the inflorescence length of OR had no difference with *S. officinalis*. The present study suggests morphological characteristics to differentiate the new hybrids from their parents in order to enhance their introduction to the floricultural industry.

Keywords: *Dendrogram, descriptors, flower morphometrics, Mediterranean sage, leaf morphometrics, qualitative and quantitative characters.*

INTRODUCTION

The Greek flora is a pool of genetic material and *Salvia* species native in Greece have a high potential value for floriculture industry. *Salvia* includes 2,100 scientific plant names; 1,042 are accepted species names being one the largest genera of flowering plants, (WFO, 2022). The genus has three distinct regions of diversity,

i.e., Central and South America, Eastern Asia and Central Asia and the Mediterranean (Walker *et al.,* 2004). 30 taxa (species and subspecies) of the genus *Salvia* can be found in Greece, the *Salvia fruticosa* Mill. having the widest distribution, as in all the Mediterranean basin (Karousou *et al.,* 2000). *S. officinalis* is one of the most widely used species in traditional medicine (Llurba-Montesino and Schmidt, 2018) and with *S. fruticosa* one of the most researched European species (Karalija *et al,* 2022). *S. officinalis* is a perennial subshrub, native to the coastal regions of the southern Europe with a habitat reaching south into northwest Greece (di Pietro, 2011). *S. pomifera* spp. *pomifera* occurs in dry, rocky places in Crete and Peloponnese being unexploited (Strid, 2016). *S. ringens,* is a hardy herbaceous perennial herb, up to 30 cm (60 cm with inflorescences) that inhabits dry stony and grass-covered places of South and Eastern parts of Balkan Peninsula, being drought tolerant and long lived (Hedge, 1972). The floriculture industry is looking for introducing new native plant, species, hybrids or clones for cultivation. Therefore, artificial hybrids between *Salvia* species found in Greece could be introduced for exploitation providing new ornamental plants for use either as pot or landscape plants. Taking into account that few instances of natural hybridization has been documanted between native Salvia species (Celep *et al.,* 2020) it would be a challenge to point out the morphological characteristics of new, artificial hybrids. Morphological analysis has proved an effective tool for both characterizing and distinguishing hybrids and studying their relationships (Bertsouklis and Papafotiou, 2016; Bertsouklis *et al,* 2021). Morphological traits are widely used for diversity studies (Lopes *et al.,* 2012) and assessing variability in plant species (Khurshid *et al.,* 2004); leaf size and flower characters have been used as descriptors of a number of *Salvia* species (Celep *et al.,* 2011; Leontaritou *et al*., 2020; Bertsouklis *et al.,* 2021). The objective of the present study was to test quantitative and qualitative morphological traits aiming to find a set of suitable descriptors for exploring the phylogenetic relations between new artificial hybrids and their parental species aiming to enhance their introduction to the floricultural industry.

MATERIALS AND METHODS

Three Salvia species S. fruticosa (F), S. officinalis (O), S. ringens (R), one subspecies, S. pomifera ssp pomifera (P), and three new hybrids S. fruticosa × S. ringens (FR), S. officinalis × S. ringens (OR), S. pomifera ssp pomifera × S. ringens (PR), were sampled for identification in 2022, at Agricultural University of Athens (37°58′58.051′′N, 23°42′17.499′′E). The hybrids were products of the SALVIA-BREED-GR research project. The pollen parent of all hybrids was S. ringens. Three-year-old plants derived from stem cuttings of plants grown in a glasshouse. A total of eight quantitative (leaf and flower/inflorescence morphometrics) and 14 qualitative characters (characters of vegetation, flowers and fragrance) were selected and used based on descriptors for other plant species (Table 1, Figure 1). Some of these descriptors had been used in a previous study of morphometrics of S. fruticosa in Greece (Bertsouklis et al., 2021). One-way ANOVA was used for determination of the differences between the mean values of

leaf and flower traits and a dendrogram was generated based on the genetic distance matrix. Cluster analysis was conducted on the taxonomic distance matrix with the Unweighted Pair Group Method based on Arithmetic Average (UPGMA) and the dendrograms were generated based on Euclidean distance-squared of morphological character analysis (Greenacre and Underhill, 1982). Principal coordinate analysis (PCA) was used in order to verify cluster analysis and to assist in visualizing the data, and statistical analysis of morphological markers was conducted by the software NTSYS-pc version 2.11f (Rohlf, 1992).

RESULTS AND DISCUSSION

As regards the quantitative characteristics, analysis revealed that were differences in all morphological traits (Table 2). R had the longest and widest leaves, as well as the longest inflorescences, flowers and internodes (Table 2). As regards the quantitative characteristics of the hybrids, FR had intermediate leaf length, width, length/width, inflorescence length, flower and calyx length compared to its parents having the smallest internode length of all species and hybrids analyzed; OR and PR had intermediate leaf length, width, inflorescence length, and internode compared to their parents (Table 2).

Table 1. Morphological traits were measured in three *Salvia* species (F, O, R), one subspecies (P) and three interspecific hybrids (FR, OR, PR) and used as descriptors. There is a scoring code for each one depending on its status among different individuals tested.

Code	Descriptor	Score code - descriptor state					
1	Leaf pubescent	1: Low	2: Medium	3: High			
2	Leaf texture	1: Leathery-elastic	2: Membranaceous-smooth	3: Membranaceous-tough			
3	Leaf shape	1: Elliptical to lanceolate	2: Elliptical				
4	Leaf colour of upper side	1: Light green	2: Green				
5	Leaf simple	1: Yes	2: No				
6	Leaf lobes	1: Yes	2: No				
7	Existence of dark/linear zone on stems	1: Yes	2: No				
8	Colour of petals	1: Pink	2: Light pink	3: Light pink-purple	3: Light purple	5: Purple	6: Dark purple
9	Inflorescence density	1: Low	2: Medium	3: High			
10	Existence of dark/linear zone on calyx	1: Yes	2: No				
11	Intensity of dark/linear zone on calyx	1: Low	2: Medium	3: High			

12	Calyx pubescent	1: Yes	2: Medium	3: High
13	Colour of calyx	1: Light green	2: Green	3: Dark green
14	Strength of leaf and flower fragrance	1: Low	2: Medium	3: High

Cluster analysis separated the species and their hybrids according to their morphological characteristics in two main branches (Figure 2). R and PR hybrid were found to be distinct from the other two species and their hybrids and R inherited its segmented leaves and light aroma of the leaves to all its hybrids. OR and PR were closer to F than R (Figure 2). PCA analysis confirmed cluster analysis and five components have been arranged in decline order according to their importance, explaining the 93.48% of the total variability among the different individuals. All descriptors grouped in the same principal component have strong correlation and each component is strongly correlated with a group of the used descriptors so it could be estimated their contribution to variability (Table 3, Figure 3). Morphological traits have been proved useful to study the variability of *S. fruticosa* in a previous work (Bertsouklis *et al*., 2021) The present study provides morphological traits to differentiate new interspecific hybrids originated from *S. fruticosa, S. officinalis, S. pomifera* spp. *pomifera* and *S. ringens* serving the aim to distinguishing plants with special ornamental characteristics, which could be the initial plant material for breeding programs and clonal propagation of plants to be introduced to the floricultural industry. The applied morphological characteristics could be a basis for the development of a complete list of discriminating characteristics for new *Salvia* hybrids.

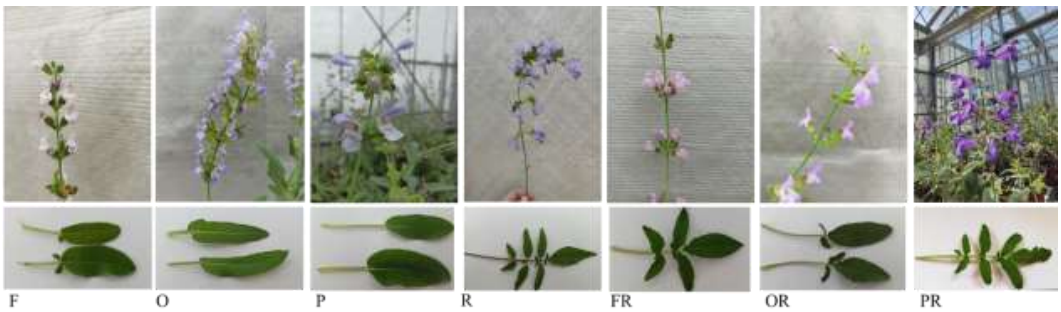

Figure 1. Leaves and inflorescences of *Salvia* spp and interspecific hybrids used in analysis

Table 2. Leaf, stem and inflorescence traits of three *Salvia* spp (F, O, R), one subspecies (P) and three interspecific hybrids (FR, OR, PR)

	Leaf				**Inflorescence**			**Stem**
Code	**Length (cm)**	**Width (cm)**	**Length/ Width**	**Thickness (mm)**	**Length (cm)**	**Flower length (cm)**	**Calyx Length (cm)**	**Internode length (cm)**
F	4.4 d	2.2 e	2.0 b	0.8 a	20.5 d	1.8 e	0.8 f	1.6 ab
O	4.4 d	1.4 f	3.2 a	0.7 b	32.3 c	2.3 d	1.2 d	1.0 d
P	4.4 d	2.4 e	1.8 bc	0.7 b	24.8 d	3.7 b	1.6 a	1.2 c
R	11.0 a	9.5 a	1.2 d	0.8 a	74.5 a	4.0 a	1.3 c	1.7 a
FR	5.2 c	3.5 d	1.5 c	0.8 a	45.0 b	2.2 d	1.0 e	0.9 f
OR	6.4 b	4.2 c	1.5 c	0.7 b	34.5 c	2.8 c	1.1 d	1.3 c
PR	6.8 b	6.2 b	1.1 d	0.8 a	47.8 b	3.7 b	1.5 a	1.5 b
F	***	***	***	***	***	***	***	***

Mean separation in columns by Student's t test at $P \leq 0.05$, ***significant at $P \leq 0.001$. Values followed by different lowercase letter within each trait are significantly different

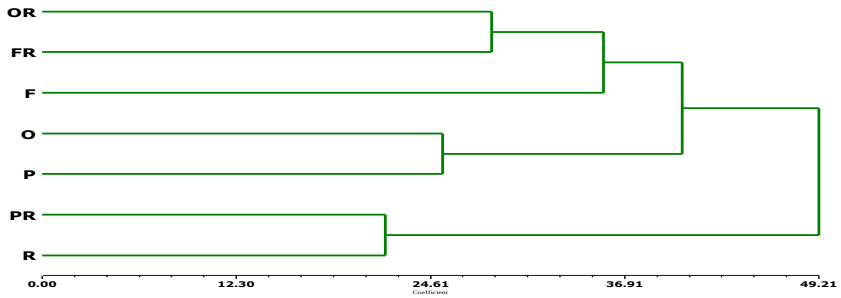

Figure 2. UPGMA dendrogram of three new interspecific hybrids (FR, OR, PR) originated from three *Salvia* spp (F, O, R) and one subspecies (P) based on Euclidean distance-squared of morphological character analysis

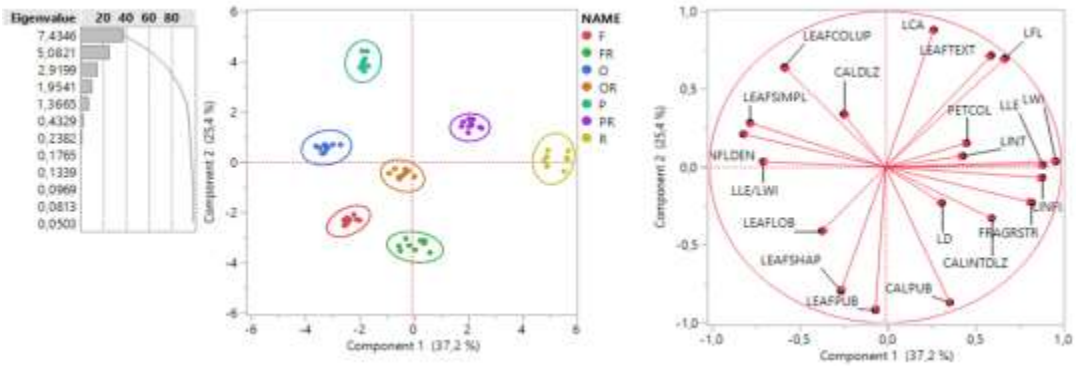

Figure 3. Evaluation of the descriptors and their contribution to the variability of the species and hybrids studied

Table 3. Results of principal components calculation

Principal Components				
1	2	3	4	5
% Contribution of variability				
37.17	25.41	14.59	9.77	6.8
Related descriptors				
LLE	CALPUB	CALDLZ	LLE/LWI	LD
LWI	LEAFPUB	LINT	PETCOL	INFLDEN
LINFL	LCA	LEAFLOB	CALINTTDLZ	LEAFSHAPE
FRAGRSTR	LFL		LEAFSIMPLE	
	LEAFTEXT		LEAFCOLUP	

Leaf pubescent (LEAFPUB), Leaf texture (LEAFTEXT), Leaf shape (LEAFSHAPE), Leaf colour of upper side (LEAFCOLUP), Colour of petals (PETCOL), Inflorescence density (INFLDEN), Existence of dark/linear zone on calyx (CALINTTDLZ), Intensity of dark/linear zone on calyx (CALDLZ), Calyx pubescent (CALPUB), Strength of leaf/flower fragrance (FRAGRSTR), Leaf Length (LLE), Leaf Width (LWI), Leaf Width/Leaf Length (LLE/LWI), Leaf Thickness (LD), Inflorescence Length (LINFL), Flower length (LFL), Calyx Length (LCA), Internode length (LINT), Leaf simple (LEAFSIMPLE), Leaf lobes (LEAFLOB)

CONCLUSIONS

Evaluating data of the present study and taking in account that the commercial production and use in the landscape of *Salvia* spp plants will be affected by the changing climate, the production of new hybrids could be a key point to face the problem. The present study revealed morphological characteristics to differentiate the new hybrids from their parents so that they could facilitate their clonal propagation and exploitation for ornamental and pharmaceutical use.

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EFFECTS OF SUBSTRATES ON GROWTH, YIELD AND TUBER QUALITY OF SWEET POTATO CULTIVATED USING SOILLESS CULTURE SYSTEM

Yaseer Suhaimi MOHD*, Izyani RASHIP, Noor Ismawaty NORDIN, Mohd Effendi MOHAMED NOR, Siti Nurzahidah ZAINAL ABIDIN, Zakry Al-Asyraf ABDUL LATIFF, Muhammad Faidhi TOWHID, Noor Safuraa SAMSUDIN

Industrial Crop Research Centre, Malaysian Agricultural Research and Development Institute (MARDI), Malaysia
*Corresponding author: ysuhaimi@mardi.gov.my

ABSTRACT

The soilless culture system effectively promotes plant growth by facilitating water and nutrient uptake by plant roots. To increase sweet potato (*Ipomoea batatas*) production, we developed containerized planting in which tuberous roots were grown in solid substrates in the polybags supplied with a nutrient solution through an irrigation system. Five combinations of growth substrates were evaluated: 100% coir dust; 100% burnt paddy husks; 70% coir dust + 30% burnt paddy husks; 30% coir dust + 70% burnt paddy husks; and 50% coir dust + 50% burnt paddy husks. The sweet potato plants were harvested 90 days after planting. Plants grown in 100% coir dust gave the best yield compared to the other treatments. They produced the highest tuber yield (2788 g) compared to the plants grown in 100% burnt paddy husk which produced the lowest tuber yield (1174 g). Tubers obtained from 100% coir dust showed the highest Total Soluble Solid (TSS) value (12.1° Brix) and moisture content (76.73%) compared to other treatments. These results showed that sweet potato cultivated in coir dust substrates increased the tubers yield by 2.3 times compared to those grown in burnt paddy husks. Studies revealed that planting sweet potato in 100% or high coir dust substrates increased the plant growth, tuber yield and enhanced tuber quality compared to substrates containing high burnt paddy husk.

Keywords: *Sweet potato, soilless culture system, coir dust, burnt paddy husk, tuber.*

INTRODUCTION

Sweet potato (*Ipomoea batatas* L. Lam.) belongs to the family Convolvulaceae that produces nutritious tuberous roots. The main problem in cultivating sweet potato in Malaysia is that the current average tuber yield grown using the conventional method is still low at 9 mt/ha compared to 20-30 mt/ha targeted. Self Sufficiency

Rate (SSR), which has not yet reached 100% for sweet potatoes (75.1%), has forced Malaysia to import with an Import Dependence Rate (IDR) for sweet potatoes reaching 26.3% (Mohd Yusrizal, 2021). Vast open market opportunities and high domestic demand for this commodity require a more sustainable and high yielding system of cultivation. There is potential to increase the growth and yield of sweet potato using soilless system based on significant increase in yields of ginger, chilies, rockmelons, tomatoes, and other leafy and fruity vegetables grown on various substrates (Yaseer Suhaimi *et al*., 2011). Many types of substrates such as rockwool, perlite, vermiculite and peat can be used to grow many kinds of crops in soilless culture system (Böhme 1995; *Komada et al*., 1997). Soilless substrates such as rockwool, perlite and vermiculite are expensive because they have to be imported. Hence, alternative substrates that are cheaper and locally available such as coir dust and burnt paddy husks should be used as alternative media. One of the most important factors influencing plant fertility in soilless culture system, besides water and nutrient content, is oxygen availability or level of aeration in the substrates (Wall and Heiskenen, 2003; Humara *et al*., 2002). Substrates need to provide adequate storage of water and nutrients for the plant, while maintaining good aeration. There are number of crops had been tested in coir dust and burnt paddy husk as substrates. For instance, Raviv *et al*. (2001) revealed rose plants grown using coir dust as substrates gave 19% higher number of flowers. Previous studies found faster root development of tomatoes and consequently better yield (Islam *et al*., 2002). Ginger able to grow and produce rhizomes when cultivated using burnt paddy husk (Suhaimi *et al*., 2012). Therefore, cultivating sweet potato using soilless culture system could be an alternative method to plant sweet potato that can give a higher yield than the conventional method. Thus, this study was conducted to determine the effects of soilless substrates such as coir dust and burnt paddy husks on the growth and yield of sweet potato. The main objective was to determine the optimum growth substrate for sweet potato cultivation using soilless culture system.

MATERIALS AND METHODS

Sweet potato var. Lembayung was used in this study. Stem cuttings were used as plant material for the cultivation of sweet potato. 30 cm long stem cuttings with 5-7 leaves were taken from a 2.5-month-old sweet potato plant. The stem cuttings were treated with Benomyl and copper solution before planting. Cuttings were planted 5 cm deep horizontally in substrates. A side-netted rain shelter of 30 m long x 10 m wide x 4.5 m high located in MARDI Serdang, Selangor, Malaysia was used in the study. The treatments were arranged in a randomised complete block design (RCBD) with five levels of treatment,three replicates and 30 plants per treatment. The treatments were as follows: T1 = 100% coir dust; T2 = 70% coir dust and 30% burnt paddy husks; T3 = 50% coir dust and 50% burnt paddy husks; T4 = 30% coir dust and 70% burnt paddy husks; and T5 = 100% burnt paddy husks. Each mixture was thoroughly mixed in a 10-liter bucket before filling into 60 cm x 60 cm black polythene bags. Planting materials were planted into the media according to the

treatments. Each polyethylene bag was placed randomly on four irrigation lines under the side-netted rain shelter and individually irrigated with a nutrient solution via a dripper on the surface of the substrates.

The fertiliser was formulated by MARDI based on the needs of the plant tubers. The irrigation solutions were prepared in a 1,500-litre tank. Stock A and stock B were added into the tank at a 1:1 ratio until the needed electricity conductivity (EC) was achieved. The EC of the fertigation solution was between 1800 μS/cm and 2600 μS/cm. The irrigation duration was 3 minutes and an identical amount of fertiliser solution was applied to all polyethylene bags. The irrigation scheduling was automatically implemented by a digital timer, three times per day in the first three weeks (07:00 h, 10:00 h and 17:00 h) and six times per day after four weeks (07:00 h, 08:00 h, 10:00 h, 11:00 h, 12:00 h and 17:00 h) until the end of cultivation periods. The daily irrigation volumes per plant were 675 ml in the first three weeks and 1,350 ml after four weeks until the end of the cultivation periods. If necessary, routine horticultural practices for pest, disease, and weed control were performed using biopesticides. The growth of the sweet potato plants was measured by measuring the main stem length, stem diameter, number of lateral shoots, vegetative biomass and SPAD value. The sweet potato plants were randomly selected and the tubers were harvested after three months of planting to determine the yield and growth of tubers. The weight was measured immediately after harvest to prevent desiccation and water loss from the tubers. The container moisture capacity (CMC) is the amount of water present after the substrates have been saturated and allowed to drain. The CMC of the five different substrates mixtures was taken at two different time intervals and calculated using the formula: (saturated mass – dry mass)/dry volume. The measurement was taken by weighing the container at one hour and five hours after watering one month after planting. Air-filled porosity (AFP) or air capacity can be defined as the proportion of the volume that contains air after it has been saturated with water and allowed to drain. Data obtained were subjected to statistical analysis using analysis of variance (ANOVA) procedures to test the significant effect of all the variables investigated using SAS version 9.1. Means were separated using the Duncan Multiple Range Test (DMRT) as the significance test at $p \leq 0.05$.

RESULTS AND DISCUSSION

There were significant differences in the AFP and CMC values between each treatment (Table 1). The 100% coir dust treatment had the highest porosity after one hour and five hours of irrigation. T5 or 100% burnt paddy husks (initial: 6.6% / final: 8.7%) had the lowest initial and final porosity at both times after irrigation. The AFP value from 100% coir dust and mixture with higher coir dust increased compared to 100% burnt paddy husks and combination with higher burnt paddy husk. Mixtures with high content of burnt paddy husks had lower AFP values due to their compaction and high-water retention properties. The CMC values decreased five hours after irrigation (Table 1). The highest initial and final CMC values were obtained from 100% burnt paddy husks. The differences in CMC

values between 100% burnt paddy husks and 100% coir dust were 27.5% and 29.7%, respectively, both times after irrigation. The availability of air, air retention and moisture in the substrate is an essential factor affecting the success of growing plants in containers (Aendekerk, 1994). The water content of substrates and AFP gives the estimation of oxygen availability or level of aeration in the substrates (Wall and Heiskenen 2003).

Table 1. Physical properties of five growing substrates at two different times after irrigation

Treatment	Air-filled porosity (%)		Container moisture capacity (%)	
	1 h	5 h	1 h	5 h
T1	10.4 [a*]	14.5 [a]	40.5 [e]	35.0 [e]
T2	9.0 [b]	10.9 [b]	49.7 [d]	46.0 [d]
T3	8.4 [c]	10.0 [c]	53.9 [c]	51.0 [c]
T4	7.4 [d]	9.0 [d]	65.0 [b]	60.0 [b]
T5	6.6 [e]	8.7 [e]	68.0 [a]	64.7 [a]

*Mean values in the same column followed by the same letter are not significantly different at $p < 0.05$

There were significant differences in plant growth between treatments (Table 2). The longest main stem was produced by plants cultivated in 100% coir dust with an average length of 398 cm and the shortest were those grown in 100% burnt paddy husks (average length 236 cm). Treatment containing 100% coir dust produced plant with the thickest stem diameter, the highest number of lateral shoots, vegetative biomass and SPAD value compared to other substrates. The highest vegetative biomass was recorded from plants cultivated in 100% coir dust with an average weight of 3850 g. The lowest biomass was obtained from plants grown in 100% burnt paddy husks. According to Humara *et al*. (2002), the high water content in the growing substrates can reduce both AFP and aeration, which can lead to logging and hypoxia which are detrimental to most plant species. This explains the lower plant growth in the 100% burnt paddy husks compared to other substrates mixtures. In extreme waterlogging conditions, sweet potato plants fail to develop storage roots (Sakamoto *et al*., 2018).

Table 2. Effects of substrates on plant growth after three months of cultivation

Treatment	Main stem length (cm)	Stem diameter (cm)	Number of lateral shoots	Vegetative biomass (g)	SPAD value
T1	398 [a*]	2.08 [a]	13.30 [a]	3850 [a]	51.45 [a]
T2	385 [b]	1.69 [b]	9.13 [b]	3270 [b]	49.9 [b]
T3	262 [c]	1.29 [c]	7.97 [c]	2700 [c]	49.6 [b]
T4	263 [c]	1.24 [c]	7.98 [c]	2450 [d]	48.9 [c]
T5	236 [d]	1.01 [d]	8.10 [c]	1860 [e]	47.6 [c]

*Mean values in the same column followed by the same letter are not significantly different at $p < 0.05$

There were significant differences in tuber yield between treatments after 90 days of cultivation (Table 3). The highest average fresh tuber yield was obtained from plants cultivated in 100% coir dust. These results showed that sweet potato cultivated in a higher amount of coir dust substrates increased the tubers yield by 2.3 times compared to those grown in substrates containing a higher amount of burnt paddy husks. Plant cultivated in a higher amount of coir dust substrates gave more tubers than those grown in substrates containing a higher amount of burnt paddy husks. Tuber yield per plant is positively correlated to the number of tubers as the higher the tuber yield per plant gave rise to high tubers number per plant. The average weight per tuber obtained from 100% coir dust also was higher compared to other treatments. These results were similar to the study done by Hayden *et al*. (2004) found that the growth of rhizomes is dependent on the type of medium. Substrates containing more than 50% of coir dust showed 0.72 harvest index indicating biomass partitioning of storage roots and shoots accordingly (Hermans *et al*., 2006). Substrates that ensure proper root aeration such as coir dust were suitable for the soilless culture cultivation of sweet potato (Kitaya *et al*., 2008).

Table 3. Effects of substrates on tuber yield after three months of cultivation

Treatment	Tuber yield per plant (g)	Number of tubers per plant	Average weight per tuber	Harvest index
T1	2788 [a*]	7.1 [a]	374.39 [a]	0.72 a
T2	2350 [b]	6.06 [b]	368.17 [b]	0.72 a
T3	1858 [c]	5.10 [c]	349.30 [c]	0.69 b
T4	1528 [d]	5.09 [c]	287.26 [d]	0.62 c
T5	1174 [e]	4.9 [d]	275.89 [e]	0.63 c

*Mean values in the same column followed by the same letter are not significantly different at $p < 0.05$

The selection of substrates is critically important to obtain high sweet potato tuber yield in the soilless culture system. High oxygen availability in the coir dust substrates supports the underground tuber requirement for high oxygen for growth. It is crucial for crops grown in containers to consider the tendency of most root systems to grow gravitropically to form a dense layer at the bottom of the containers (Raviv *et al*., 2011). Coir dust has a strong capillarity that provides more uniform moisture conditions for roots. These conditions can increase aeration in the base mix and reduce surface drying by lifting the moisture higher up in the polyethylene bags. This redistribution of moisture is perhaps one of the reasons for plants grown in pure coir dust to have higher tuber yield. Aeration in the growing substrates is positively related to AFP and negatively to water content (Raviv and Lieth, 2008).

Table 4. Effects of substrates on tuber quality after three months of cultivation

Treatment	Total Soluble Solid (Brix value)	Tuber dry matter content (%) (1 g)	Tuber flesh pH	Tuber diameter (mm)			Tuber length (cm)
				Upper part	Middle part	Lower part	
T1	12.1 [a*]	76.73 [a]	5.9 [b]	32.14 [a]	36.67 [a]	29.34 [c]	18.50 [a]
T2	12 [a]	74.11 [b]	5.84 [b]	27.44 [c]	37.82 [a]	31.10 [a]	18.80 [a]
T3	11.7 [b]	63.43 [d]	6.08 [a]	30.07 [b]	37.10 [a]	27.61 [d]	15.92 [b]
T4	10.3 [d]	67.09 [c]	5.85 [b]	23.93 [e]	28.15 [c]	19.41 [e]	14.79 [c]
T5	11.2 [c]	68.06 [c]	5.89 [b]	25.01 [d]	32.91 [b]	30.27 [b]	14.70 [c]

*Mean values in the same column followed by the same letter are not significantly different at $p < 0.05$

There were significant substrate effects on tuber quality between treatments (Table 4). Tubers obtained from a high content of coir dust gave higher Total Soluble Solid (TSS) or Brix value, tuber dry matter content and tuber length compared to tuber grown in high burnt paddy husk substrates. Most of the dry matter in sweet potatoes consists of carbohydrates, primarily starch and sugars and to a lesser extent pectins, cellulose and hemicellulose (Truong *et al*., 2018). Tuber flesh pH in all treatments ranging between 5.85 – 6.08, indicated that the flesh is slightly acidic. The shape of the tuber is tapered at the top and bottom with the middle part is expanded. All treatments produced similar tuber shapes with T2 giving the longest tuber length. However, there were no significant differences between T1 and T2, with substrates containing high coir dust giving high tuber length compared to substrates containing high burnt paddy husk. Good quality sweet potatoes should be smooth and firm, with uniform shape and size (Cantwell and Suslow, 2002). Planting sweet potato in high coir dust substrates increased the tuber quality compared to substrates containing high burnt paddy husk.

CONCLUSION

Data revealed that planting sweet potato in high coir dust substrates increased the plant growth, tuber yield and enhanced tuber quality compared to substrates containing high burnt paddy husk. It can be concluded that 100% coir dust is the best substrate for growing sweet potato in a soilless culture system. However, studies on burnt paddy husks in combination with other agricultural wastes like sago waste, industrial by-products like polystyrene beads, or any other cheaper substrates such as coarse sand should be continued to increase the use of burnt paddy husks as a growing substrate for growing sweet potato or any suitable crop in soilless culture production system.

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- 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.

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