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ASSESSMENT OF THE SUSTAINABILITY OF SELECTED NEGLECTED AND UNDERUTILISED CROP SPECIES IN NIGER

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ABSTRACT

There is a gap in the assessment of the sustainability of neglected and underutilized crop species (NUS), especially in developing countries. Therefore, this work assesses the sustainability of six NUS in Niger: sweet potato, cassava, roselle, moringa, okra and Bambara groundnut/voandzou. The assessment was carried out using a framework developed within the project SUSTLIVES (Sustaining and improving local crop patrimony in Burkina Faso and Niger for better lives and ecosystems) that contains 27 indicators related to eight themes covering the three sustainability dimensions (viz. environmental, social, economic). For each NUS, a reference major crop was selected. Data collection was carried out in 2023 through a literature review and, where necessary, interviews with informants and experts. A NUS is considered sustainable if it has a sustainability score equal to or greater than 5 (cf. score of the reference crop), on a 0–10 scale. Results indicate that only okra, moringa and roselle have scores above 5 and are, therefore, sustainable; while the score for sweet potato is close to 5, the cases of cassava and voandzou are more critical. In general, it is at the economic dimension where the lowest scores were recorded. This study provides useful indications on the weaknesses and strengths of the NUS, which should be taken into consideration to improve their performance and sustainability and, therefore, their competitiveness compared to major crops. Particular attention should be paid to social and, especially, economic aspects to ensure the promotion of the concerned NUS in Niger and Sahel.

Keywords: *NUS, neglected and underutilised species, sustainability assessment, Sahel, SUSTLIVES.*

INTRODUCTION

Niger is a Sahelian country that covers an area of 1,267,000 km², two-thirds of which is located in the desert zone (Ministère de l'Agriculture – Niger, 2015). The population is estimated at 25,130,810 inhabitants, the majority of which is rural, i.e. 84% (INS-Niger, 2020). The main activities of the population are agriculture and

livestock breeding. Indeed, the primary sector has a significant socio-economic impact in Niger. Data from the World Bank reveals that agriculture, forestry, and fishing contributed 42.0% of the gross domestic product (GDP) in 2022 (World Bank, 2024a). Meanwhile, employment in agriculture accounted for 71% of total employment in 2021 (World Bank, 2024b). Despite this, food insecurity and malnutrition remain significant challenges (FAO et al., 2023; République du Niger – Haute Autorite à la Sécurité Alimentaire, 2011). In the period 2020 – 2022, the overall prevalence of undernourishment in the Nigerien population remained high at 16.1%. The situation is even more worrying when considering the prevalence of moderate or severe food insecurity which was at 71.4% during the same period (FAO et al., 2023). Moreover, evidence suggests that Sub-Saharan Africa will face the most significant impacts of climate change (Baarsch et al., 2020; Bakshi et al., 2019; Hassan, 2010; Lokonon et al., 2019). Agriculture, which primarily relies on rainfall, is highly vulnerable to climate variability (El Bilali, 2021; Sultan & Gaetani, 2016). The challenges mentioned above indicate the urgent need for the transition towards a sustainable and resilient agri-food system (El Bilali et al., 2023b).
NUS, or neglected and underutilized species, are highly regarded as important assets in the transition towards sustainable and resilient agri-food systems (El Bilali et al., 2023a). Reports indicate that the promotion of underutilized and neglected crop species significantly contributes to the conservation of agrobiodiversity, as well as food and nutrition security, climate change adaptation and mitigation, environmental integrity, human health, and the sustainability and resilience of rural livelihoods (El Bilali et al., 2023c; Mabhaudhi et al., 2019). However, it is still difficult to assess the sustainability potential of NUS due to the lack of a specific framework. According to El Bilali et al. (2022), there is a dearth of quality scholarly documents that deal with the assessment of the sustainability of NUS. One exception is the framework to assess the environmental, social and economic sustainability of NUS developed within the project SUSTLIVES (*SUSTaining and improving local crop patrimony in Burkina Faso and Niger for better LIVes and EcoSystems*) (SUSTLIVES, 2023). In this context, the objective of this study is to apply the framework to assess the sustainability of six NUS selected within SUSTLIVES project in Niger viz. sweet potato, cassava, roselle, moringa, okra and Bamabara groungnut/voandzou.

MATERIAL AND METHODS

The assessment of sustainability was carried out using a framework developed within the project SUSTLIVES (El Bilali et al., 2023d; El Bilali et al., 2022). The framework used contains 27 indicators (Table 1) divided between different themes covering the three dimensions of sustainability viz. environmental, social and economic.

Table 1. SUSTLIVES matrix for the assessment of the sustainability of NUS.

Dimension	Theme	Indicator
Environmental (Env)	Env1. Environmental integrity	Env1.1 Nitrogen requirement
		Env1.2 Phosphorus requirement
		Env1.3 Pesticide requirement
		Env1.4 Water demand
		Env1.5 Crop evapotranspiration
		Env1.6 Genetic diversity
		Env1.7 Nitrogen fixation
	Env2. Agronomic performance and productivity	Env2.1 Yield
		Env2.2 Length of the growing season
		Env2.3 Growing degree days
		Env2.4 Level of tolerance to salinity
		Env2.5 Level of tolerance to high temperatures
		Env2.6 Level of tolerance/resistance to pests and diseases
		Env2.7 Seed availability
		Env2.8 Seed suitability
		Env2.9 Seed quality
Social (S)	S1. Cultural significance and relevance	S1.1 Number of documented uses
	S2. Nutritional quality and diversity	S2.1 Content of bioactive and health-promoting compounds
		S2.2 Protein content
		S2.3 Duration of fresh produce conservation
	S3. Employment	S3.1 Labour requirement
	S4. Equity and fair accessibility	S4.1 Seed access
Economic (Econ)	Econ1. Competitiveness	Econ1.1 Price
		Econ1.2 Market demand
		Econ1.3 Production cost
	Econ2. Profitability	Econ2.1 Gross profit margin
		Econ2.2 Income

In line with Capone et al. (2016) and El Bilali et al. (2020), the developed framework uses equal weighting for indicators within themes as well as for themes within each sustainability dimension. Actually, the scores of the indicators (not absolute values) are aggregated to obtain an overall score on the performance/sustainability of a NUS (El Bilali et al., 2023d). For each NUS, a reference crop was selected: potato for sweet potato and cassava, cabbage for roselle and moringa, pepper for okra, and cowpea for voandzou. The reference crop was chosen from the main crops in each country within the group of the selected NUS (viz. roots/tubers, vegetables, legumes) and taking into account the product uses. The determination of the reference crop allows calculating the benchmark and developing the scoring scale for each indicator. Indeed, a scoring system was proposed for each indicator; from 0 (unsustainable) to 10 (very sustainable) with 5 corresponding to the sustainability benchmark value (cf. value of the reference crop). A NUS is considered sustainable if it has a sustainability score equal to or greater than 5, on a 0–10 scale (El Bilali et al., 2023d).

Data collection was performed in 2023 through a review of the relevant literature and semi-structured interviews with experts in Niger. The literature review consisted of gathering quantitative and qualitative information. It took place on the internet as well as at research institutes (e.g. National Agricultural Research Institute of Niger – INRAN, International Crops Research Institute for the Semi-Arid Tropics – ICRISAT, Regional Agro-Hydro-Meteorological Center – Agrhymet), the University Abdou Mumouni, the National Network of Chambers of Agriculture (RECA) and the Ministry of Agriculture and Livestock. Individual interviews were conducted at INRAN (Pr. Haougui Adamou and Dr. Bori Haoua), the General Directorate of Agriculture (Bassirou Boubacar), the Federation of Horticulture Cooperatives in Niger – FCMN (Moudi Kabirou), the non-governmental organization Agri Focus (Tassiou Amani Mourtala). The technician Moustapha Amadou from ICRISAT was approached via email. Other individual interviews were conducted with producers at the Chamber of Commerce of the Dosso region, during NUS fairs in certain regions of Niger, and in some markets in Niamey (e.g. Katako, Harobanda, Banifondou 1, Bobiel). In addition, individual interviews on the prices of NUS products were conducted with a few traders. The data collected were processed by Excel 2016. Some difficulties of several types were encountered. For the documentary research, it was the difficulty of accessing or non-availability of data on the NUS in question. For the interviews, the main difficulty concerns the unavailability of interviewees.

RESULTS AND DISCUSSION

The results indicate that the six NUS considered have different performances not only in terms of overall sustainability but also concerning each sustainability dimension (Table 2). As for the environmental dimension, only okra (6.41) and sweet potato (5.87) are sustainable, while moringa (4.99), cassava (4.56), voandzou (4.30) and roselle (4.24) are not. Regarding the social dimension, only moringa (5.67), roselle (5.66) and okra (5.66) are sustainable while the other NUS (viz. sweet potato, 4.75; cassava, 4.62; voandzou, 4.62) are not. Concerning the economic dimension, NUS that result sustainable are okra (8.75), roselle (7.50) and moringa (6.75), the other NUS (viz. sweet potato, 3.83; cassava, 2; voandzou, 1.25) resulting unsustainable. In general, it is at the level of the economic dimension that there are the lowest scores (especially for cassava and voandzou); this has also implications in terms of overall sustainability. Indeed, only okra (6.94), moringa (5.80) and roselle

(5.80) have scores above 5 and are, therefore, sustainable; while the score for sweet potato (4.81) is close to 5, so to the sustainability threshold, the cases of cassava (3.72) and voandzou (3.39) are more critical.

Table 2. Scores of environmental, social and economic sustainability of the six NUS.

NUS	Dimension	Theme	Theme score	Dimension score	Global score
Sweet potato	Environmental (Env)	Env1	7.5	5.87	4.81
		Env2	4.25		
	Social (S)	S1	5	4.75	
		S2	7		
		S3	5		
		S4	2		
	Economic (Econ)	Econ1	7.67	3.83	
		Econ2	0		
Cassava	Environmental (Env)	Env1	5	4.56	3.72
		Env2	4.12		
	Social (S)	S1	5	4.62	
		S2	6.5		
		S3	5		
		S4	2		
	Economic (Econ)	Econ1	4	2	
		Econ2	0		
Voandzou	Environmental (Env)	Env1	4.75	4.30	3.39
		Env2	3.87		
	Social (S)	S1	5	4.62	
		S2	2.5		
		S3	5		
		S4	6		
	Economic (Econ)	Econ1	2.50	1.25	
		Econ2	0		
Moringa	Environmental (Env)	Env1	5.33	4.99	5.80
		Env2	4.66		
	Social (S)	S1	10	5.67	
		S2	ND		
		S3	5		
		S4	2		
	Economic (Econ)	Econ1	7.5	6.75	
		Econ2	6		
Roselle	Environmental (Env)	Env1	5.83	4.24	5.80
		Env2	2.66		
	Social (S)	S1	10	5.66	

NUS	**Dimension**	**Theme**	**Theme score**	**Dimension score**	**Global score**
		S2	ND		
		S3	5		
		S4	2		
	Economic (Econ)	Econ1	5	7.50	
		Econ2	10		
Okra	Environmental (Env)	Env1	5.83	6.41	6.94
		Env2	7		
	Social (S)	S1	10	5.66	
		S2	ND		
		S3	5		
		S4	2		
	Economic (Econ)	Econ1	7.50	8.75	
		Econ2	10		

Legend: Env1. Environmental integrity; Env2. Agronomic performance and productivity; S1. Cultural significance and relevance; S2. Nutritional quality and diversity; S3. Employment; S4. Equity and fair accessibility; Econ1. Competitiveness; Econ2. Profitability; ND: No Data.

The results indicate that the score of sustainability changes from one NUS to another. This depends on the intrinsic characteristics of each NUS but also on its relative performance with respect to the reference crop since the scoring system considers the performance of the reference crop as a benchmark. The particularity and strength of the SUSTLIVES framework are that it focuses on the sustainability of each crop, which means that it can be used to distinguish between the performances of different NUS not only in the same context but even on the same farm. This makes it more appropriate than other approaches and frameworks for the assessment of sustainability that have been used in sub-Saharan African countries such as the Democratic Republic of the Congo – DRC (Ndjadi et al., 2021) and Benin (Ahouangninou, 2013). Indeed, Ndjadi et al. (2021) and Ahouangninou (2013) assess the sustainability of horticulture farms in eastern DRC and southern Benin, respectively, but do not provide any information about the sustainability of the different crops, which makes such an analysis of low importance for value chain actors (e.g. farmers that want to know which crops to grow).

In a transition framework for NUS, El Bilali et al. (2024) argue that the success of the transition, so the possibility of a NUS becoming a major crop and replacing the reference one, depends, among others, on the characteristics of the niche NUS (cf. strengths and weaknesses). The authors stipulate that "*Different features of the niche NUS determine not only their own potential but also their potential to compete with major commercial crops. These relate to the intrinsic strengths and weaknesses of NUS*" (p. 19). The results obtained suggest that okra, roselle and moringa have comparative advantages with respect to their reference crops (cf. okra vs. pepper, roselle vs. cabbage, moringa vs. cabbage), which means that okra can substitute pepper and roselle and moringa can substitute cabbage. Meanwhile, sweet potato, cassava and voandzou are not competitive with respect to their reference, major crops (viz. sweet potato vs. potato, cassava vs. potato, voandzou vs. cowpea). In other words, sweet potato and cassava can hardly substitute potato in Niger, and the same applies to voandzou with cowpea. The analysis also allows to compare NUS when they have the same reference crops (cf. potato for sweet potato and cassava, cabbage for roselle and moringa). Indeed, the results suggest that the performance of sweet potato is better than cassava in the Nigerien context; which might imply that sweet potato has a better chance than cassava to replace potato. Meanwhile, moringa and roselle have the same sustainability scores and the same performance so it is difficult to state which one of them is more competitive with respect to cabbage.

The study results have different implications. On the one hand, the study provides useful indications of the weaknesses and strengths of each of the six NUS which should be taken into account to improve their performance and sustainability and, therefore, their competitiveness in relation to dominant, reference crops. In this regard, it seems that particular attention must be paid to the social and, above all, economic aspects to ensure the promotion of the NUS in question in Niger. On the other hand, the study shows that, among the six considered NUS, the most promising ones in the context of Niger seem to be okra, moringa and roselle. Therefore, the various stakeholders concerned can focus, in the short and medium term, on the promotion of these three species.

CONCLUSIONS

Niger faces major environmental, social and economic challenges such as biodiversity erosion, climate change, land degradation and desertification, water pollution and food insecurity. Therefore, the integration and promotion of NUS should be reconsidered to foster the transition towards a sustainable and resilient agri-food system in Niger. Nevertheless, the assessment of the sustainability of NUS has not yet been the subject of specific studies in Niger. To the best of our knowledge, this is the first assessment of the sustainability of the selected NUS (viz. sweet potato, cassava, roselle, moringa, okra and voandzou) in Niger. In general, it is at the level of the economic dimension that there are the lowest scores; this has also implications in terms of overall sustainability. Indeed, only okra, moringa and roselle are sustainable; while the score for sweet potato is close to the sustainability threshold, the cases of cassava and voandzou are more critical. This study provides valuable insights into how to improve the performance and sustainability of NUS and, consequently, their competitiveness compared to major crops. It suggests that particular attention should be paid to economic aspects to ensure an effective promotion of the concerned NUS in Niger. The sustainability assessment framework developed within SUSTLIVES project is valid and enables the objective evaluation of the environmental, social, and economic sustainability of each NUS. It also enables the comparison of each NUS's sustainability to that of the main reference crop. Therefore, the framework can be used for the prioritization of NUS based on their potential impact.

The study has some limitations and highlights some research gaps that should be addressed in the future. Despite all, it was not possible to assess the sustainability of certain indicators due to a lack of data. Indeed, there are no specific data on nitrogen fixation and the level of tolerance to salinity (cf. environmental dimension) and, sometimes, the content of bioactive and health-promoting compounds, and the shelf-life of fresh products (cf. social dimension). Therefore, it is necessary to carry out further research on NUS in Niger. Scientific research is crucial to fill existing knowledge gaps in order to have a solid basis for the development of these crops in Niger, Sahel and beyond.

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REFERENCES

Ahouangninou, C. (2013). Durabilité de la production maraîchère au sud-Bénin : un essai de l'approche écosystémique. Thèse de doctorat, Université d'Abomey-Calavi. http://agritrop.cirad.fr/572410/1/document_572410.pdf

Baarsch, F., Granadillos, J. R., Hare, W., Knaus, M., Krapp, M., Schaeffer, M. and Lotze-Campen, H. (2020). The impact of climate change on incomes and convergence in Africa. World Development, 126, 104699. https://doi.org/10.1016/j.worlddev.2019.104699

Bakshi, B., Nawrotzki, R. J., Donato, J. R. and Lelis, L. S. (2019). Exploring the link between climate variability and mortality in Sub-Saharan Africa. International Journal of Environment and Sustainable Development, 18(2), 206. https://doi.org/10.1504/IJESD.2019.099518

Capone, R., El Bilali, H. and Bottalico, F. (2016). Assessing the Sustainability of Typical Agro-Food Products: Insights from Apulia Region, Italy. New Medit, 15(1), 28–35.

El Bilali, H. (2021). Climate change and agriculture in Burkina Faso. Journal of Aridland Agriculture, 22–47. https://doi.org/10.25081/jaa.2021.v7.6596

El Bilali, H., Calabrese, G., Iannetta, M., Stefanova, M., Paoletti, F., Ladisa, G., Bottalico, F. and Capone, R. (2020). Environmental sustainability of typical agro-food products: a scientifically sound and user friendly approach. New Medit, 19(2). https://doi.org/10.30682/nm2002e

El Bilali, H., Cardone, G., De Falcis, E., Naino Jika, A., Rokka, S., Diawara, A. B., Nouhou, B. and Ghione, A. (2023). Neglected and underutilised species (NUS): An analysis of strengths, weaknesses, opportunities and threats (SWOT). AGROFOR International Journal, 8(1), 9–29. http://agrofor.ues.rs.ba/data/20230410-03_El_Bilali_et_al.pdf

El Bilali, H., Cardone, G., Rokka, S., De Falcis, E., Naino Jika, A., Diawara, A. B. and Nouhou, B. (2024). Transition framework for neglected and underutilized crop species. AGROFOR International Journal, 9(1), 16–26.

El Bilali, H., Cardone, G., Rokka, S., De Falcis, E., Naino Jika, A., Diawara, A. B., Nouhou, B. and Ghione, A. (2023a). Orphan crops and sustainability transitions in agri-food systems: Towards a multidimensional and multilevel transition framework. International Conference on Life Sciences (ICLISC-23), 42–57.

El Bilali, H., Cardone, G., Rokka, S., De Falcis, E., Naino Jika, A. K., Diawara, A. B., Nouhou, B., Diadie, H., Tarri Aliou, M., Kiebre, Z. and Kabre, J. (2023). Assessment of the sustainability of neglected and underutilised crop species: SUSTLIVES approach. AGROFOR International Journal, 8(2), 12–23.

El Bilali, H., Cardone, G., Rokka, S., De Falcis, E., Naino Jika, A. K., Diawara, A. B., Nouhou, B. and Ghione, A. (2023b). Sustainability transitions in West African agriculture and food systems. Journal of Survey in Fisheries Sciences, 10(4S), 1847–1865. http://sifisheriessciences.com/journal/index.php/journal/article/view/1410/1443

El Bilali, H., Cardone, G., Rokka, S., Naino Jika, A. K., De Falcis, E., Diawara, A. B., Nouhou, B. and Ghione, A. (2022). Sustainability of neglected and underutilised species (NUS): Towards an assessment matrix for crop species. AGROFOR International Journal, 7(3), 17–30.

FAO, IFAD, UNICEF, WFP and WHO (2023). The State of Food Security and Nutrition in the World 2023. FAO. https://doi.org/10.4060/cc3017en

Hassan, R. M. (2010). The double challenge of adapting to climate change while accelerating development in sub-Saharan Africa. Environment and Development Economics, 15(6), 661–685. https://doi.org/10.1017/S1355770X10000306

INS-Niger (2020). Projections démographiques 2012 - 2024. https://www.stat-niger.org/?s=d%C3%A9mographiques+

Lokonon, B. O. K., Egbendewe, A. Y. G., Coulibaly, N. and Atewamba, C. (2019). The Potential Impact of Climate Change on Agriculture in West Africa: A Bio-Economic Modeling Approach. Climate Change Economics, 10(04), 1950015. https://doi.org/10.1142/S2010007819500155

Mabhaudhi, T., Chimonyo, V. G. P., Hlahla, S., Massawe, F., Mayes, S., Nhamo, L. and Modi, A. T. (2019). Prospects of orphan crops in climate change. Planta, 250(3), 695–708. https://doi.org/10.1007/s00425-019-03129-y

Ministère de l’Agriculture – Niger (2015). Stratégie de la Petite Irrigation au Niger (SPIN). https://reca-niger.org/IMG/pdf/SPIN_FINALE_Niger.pdf

Ndjadi, S. S., Ahoton, L. E., Kizungu, R. V., Saidou, A., Mugumaarhahama, Y., Mushagalusa, A. C., Safina, F. B. and Mushagalusa, G. N. (2021). Évaluation de la durabilité des exploitations maraîchères au Sud-Kivu (Est de la République Démocratique du Congo). Cahiers Agricultures, 30, 15. https://doi.org/10.1051/cagri/2020050

République du Niger – Haute Autorite à la Sécurité Alimentaire (2011). Conférence Internationale sur la Sécurité Alimentaire et Nutritionnelle au Niger (CISAN) - Termes de Référence du Symposium.

https://www.fao.org/fileadmin/templates/ess/ess_test_folder/Workshops_Events/ICFNS_mar2011/tdr_symp.pdf

Sultan, B. and Gaetani, M. (2016). Agriculture in West Africa in the Twenty-First Century: Climate Change and Impacts Scenarios, and Potential for Adaptation. Frontiers in Plant Science, 7, e1262. https://doi.org/10.3389/fpls.2016.01262

SUSTLIVES (2023). The project - About. https://www.sustlives.eu/en/a-propos

World Bank (2024a). The World Bank Data – Agriculture, forestry, and fishing, value added (% of GDP). https://data.worldbank.org/indicator/NV.AGR.TOTL.ZS

World Bank (2024b). The World Bank Data – Employment in agriculture (% of total employment). https://data.worldbank.org/indicator/SL.AGR.EMPL.ZS

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MOLECULAR AND MORPHOLOGICAL CHARACTERIZATION OF WHEAT GENOTYPES UNDER DROUGHT CONDITION IN NIGERIAN SUDAN SAVANNAH

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ABSTRACT

Drought stress is one of the most important abiotic constrains affecting cereal crop in the world that causes serious yield loses and threat to sustainability and food security especially in wheat thereby causing the insufficient supply of food. Therefore, understanding the genetic characterization of drought stress response is very important. This study was designed to reveal the morphological and molecular characterization of wheat genotypes using simple sequence repeat (SSR) markers and to estimate the genetic diversity and relationships among the cultivars subjected to drought conditions. A total of 15 wheat genotypes were evaluated for quantitative morphological traits such as plant height, number of seeds per spike, 1000 seed weight, spike length, flag leaf length, grain weight and proline content. Molecular characterization was done using a set of simple sequence repeat (SSR) markers. A total of 12 SSR markers were used to analyze the varieties and the genetic diversity and relationship among them. The results showed that, the drought-stressed plants had lower plant height, number of seeds per spike, 1000 seed weight, spike length, flag leaf length and grain weight than the non-stressed plants, while proline content was found to be higher in stressed plants than the non-stressed. Molecular analyses indicated significant variation among the genotypes with the mean PIC value of 0.64, mean heritability ratio of 0.67 and mean allele number of 8.9. This study also indicates the significance of SSRs as a useful tool in marker-assisted breeding about drought tolerance and for developing strategies for improving drought tolerance in cereals.

Keywords: *Wheat genotypes, Drought stress, Genetic diversity, Proline concentration, SSR markers.*

INTRODUCTION

Wheat (***Triticum aestivum*** L.) is one of the most widely grown staple diet cereal crops, contributing to the global food supply and economic security. It is one of the earliest domesticated plants and is widely accepted to originate from the levant region of the Near East and Ethiopian Highlands and provides 21% of the food calories and 20% of the protein for more than 4.5 billion people across the globe (Liu *et al.*, 2013). Cereals provide more protein to people than meat, fish, milk, and egg combined in most developing regions, except for Latin America, making them an essential source of protein for over half the world's population (FAO, 2020). Among the cereals, wheat serves significantly as a source of carbohydrate, protein, vitamins, and mineral elements. Moreover, it also is used as raw materials in industries for the preparation of alcoholic beverages, starch, and straw, or as animal feed, due to its high level of protein, carotenoid, and fiber, feeding more than 35% of the world (Colasuonno *et al.,* 2017). It is an exceptional cereal plant among recently domesticated species as it spread across all parts of the world except Antarctica after originating in the Fertile Crescent (Rasheed & Xia, 2019). It is popularly known as 'Stuff of life or king of the cereals due to its nature to occupy large acreage of land, its ranking position in the international food grain trade, and high productivity (Sharma *et al.*, 2018).

Nigeria's wheat production currently stands at about 37,200 tonnes from about 51,000 hectares, according to the Food and Agricultural Organization (FAO, 2020). Yield is an important complex trait strongly influenced by environmental conditions. Breeders are challenged to enhance the current level of wheat production due to the rapid increase in population, climate change, and environmental stresses that arose as the main threat to staple crop production. Therefore, increasing yield potential is important in solving the wheat food shortfall and meeting the rising demand for wheat grain by the ever-increasing world population (EL Sabagh *et al.*, 2021), especially in Africa. In West Africa, Nigeria is a major wheat-growing region and mostly grown under irrigation in the northern parts of the country between latitudes 100 and 140 N and altitudes 240-306m above mean sea level from November to March during the cold harmattan period, which provides the considerable low temperature for production (Olugbemi, 1990). Drought stress, insufficient water supply during the growing season, and other factors in most arid and semi-arid areas severely reduce yield. The reproductive stage of wheat is adversely affected by the depletion of the residual soil moisture, while the vegetative growth is heavily influenced by soil moisture retention owing to changes in the direction of the prevailing winds of the region; hence the stress of drought prevalence reduces wheat productivity more severely than any other environmental stress (Karim *et al*., 2000; Raza *et al*., 2019).

Drought stress (DS) is critical abiotic stress that adversely affects wheat plant growth and development. It has a negligible effect on the growth rate and shortens the linear growth phase (LGP). LGP is directly associated with final grain mass. Climate change produces challenging conditions for producing the required quantity of crops that will meet fulfil the demand for the population (Kumar *et al*., 2020). Under extreme conditions, drought stress may severely disrupt several metabolic processes, resulting in reduced photosynthesis, delayed cell enlargement and division, and finally passed on the cells (Kramer, 1983; Chowdhury *et al*., 2021). The plant reproductive stage is heavily affected by DS due to the effect on plant metabolic processes compared with the vegetative growth phase, and the effect usually happens during flowering or anthesis, which reduces reproductive development, photosynthesis and grain yield (Karim *et al*., 2000; Araus *et al*., 2002). The study was designed to evaluate the morphological characteristics of different wheat cultivars grown during dry and rainy seasons, estimate the genetic diversity and relationships among the cultivars subjected to drought conditions using molecular markers.

MATERIAL AND METHODS

Plant Material and Study Site

The field and laboratory research were conducted at the Institute of Agricultural Research (IAR) Farm Samaru Zaria and at the African Centre of Excellence, Centre for Biotechnology Research and training Laboratory Ahmadu Bello University Zaria. The study site was located in Nigeria's Sudan Savannah ecological zone between latitudes 11^{o} 5' to 7.9476' N and longitudes 7^{o} 43' to 11.8020' E, with temperatures ranging from 12^{o}C - 32^{o}C Nigerian Meteorological Agency (NIMET). The area was characterized by two seasons: the rainy season with a long day length usually begins from May and ends in September, with heavy rainfalls in July and August. A short-day length that begins in October and ends in April to May characterizes the dry season. The mean annual rainfall was 756 mm, while the minimum and maximum relative humidity was 33.1 and 56.0%, respectively.

Treatments and Experimental Design

The experiments consist of fifteen different wheat varieties collected from Lake Chad Research Institute, Institute of Agricultural Research Ahmadu Bello University Zaria and Kadawa research Farm Kano. The cultivars include Lacri-4 (V1), Lacri-6 (V2), Norman (V3), Atila-50 (V4), Seri -3 (V5), Teves (V6), Rabih - 10 (V7), Hubara-1 (V8), Rabih-3 (V9), Cham-8 (V10), Cham-6 (V11), Atila1 (V12), Tesfa (V13), Bacarona-T8 (V14) and Rena-28 (V15). They are characterized by high yielding, with medium tillering ability, tolerant to stem borer, early to medium maturing habit with plant height ranging from 70-90cm. The experiment was laid out in a Randomized Complete Block Design (RCBD) and replicated three times. Thirty seeds were sown in each row, with each plot comprising of 6m row spaced at 30cm apart. The cultivars were also screened for drought tolerance by irrigating up to the booting stage, and then the water was withdrawn for 3 weeks and resumed later. The cultivars that exhibited drought tolerance and those susceptible to drought were identified. All other agronomic practices were carried out on time to achieve a good crop stand. At maturity, the following parameters were observed and recorded, including Plant height (PH), number of seeds per spike (NS), 1000 seed weight (TSW), spike length (SL), flag leaf length (FL), grain weight (GW).

DNA Extraction

Genomic DNA was isolated from 21-day-old green leaves according to the manufacturer's protocol. Quick-DNA Plant/Seed mini prep Kit obtained from Zymo Research Corp with minor modification was purchase from Inqaba Biotech west Africa Ltd with a catalog No. D6020. The procedures are described as follows: 150g of finely cut leaves sample were added to Zymo Research (ZR) BashingBead™ lysis tube (2.0 mm) and 750 µl BashingBead™ Buffer was added to the tube and caped tightly. The sample were then secure in a bead beater fitted with a 2 ml tube holder assembled and process at maximum speed for 3 minutes using high-speed cell disrupters (FastPrep® -24). The ZR BashingBead™ lysis tube was centrifuge at 10000 x g for 1 minute using microcentrifuge. 400 µl supernatant was transferred to Zymo spin III-F filter in to collection tube and centrifuged at 8000 x g for 1 minute and the Zymo-Spin™ III-F Filter was discarded. 1200 µl of genomic lysis buffer were added to the filtrate in the collection tube from above and mixed well. 800 µl of the mixture from above step was transferred to Zymo-Spin™ IICR column in a collection tube and was centrifuged at 10000 x g for 1 minute. The flow through from the collection tube was discarded and the above step was repeated. Subsequently, 200µl of DNA pre wash buffer was added to the Zymo-Spin™ IICR column in a new collection tube and centrifuged at 10000 x g for 1 minute. Later 500 µl g-DNA wash buffer was added to the Zymo-Spin™ IICR column and centrifuged at 10000 x g for 1 minute. Zymo-Spin™ IICR column was transferred to a clean 1.5 microcentrifuge tube and 100 micro liter DNA elution buffer was added directly to the column matrix and was centrifuged at 10000 x g for 30 seconds to elude the DNA. Zymo-Spin™ III HCR filter was placed on a clean collection tube and 600 µl prep solution was added and centrifuged at 8000 x g for 3 minutes. The eluded DNA was transferred to a prepared Zymo-Spin™ III HRC spin filter in a clean 1.5 ml microcentrifuge tube and centrifuged at exactly 16000 x g for 3 minutes. The filtrate DNA is ready for PCR and other downstream application. The genomic DNA concentration and purity was measured using nano drop spectrophotometer.

PCR Cocktail

The cocktail was performed by preparing 25 µl of nuclease free water, 2* master mix with standard buffer, 0.5 µl forward primer, 0.5 µl reverse primer and 1 µl template DNA. The reaction mix was preheated at 94°C for 30 seconds followed by 30 cycles of 20 seconds of denaturation at 94°C, 30 seconds at 48-54°C based on the primer annealing temperature and elongation at 68 °C for 1 min, after the last cycle a final step was maintained at 68 °C for 5 min to allow complete extension of all amplified fragments followed by holding at 4°C until electrophoresis.

GEL Electrophoresis

2 % agarose gel was prepared by dissolving 2g of agarose in 100 ml 1X TAE buffer and heated in a microwave oven for 2 min and 1 microliter of ethidium bromide solution was added and then poured into a casting plate with a comb placed and was allowed to solidified. The sample was loaded into the wells and a larder of 100 bp was used. The gel was illuminated by UV trans- illuminator and photographed for assessing the DNA profiles.

Molecular Screening

The markers were scored for the presence (1) or absence (0) of amplified bands. Comparison of genotypes and examination of genetic relationships between genotypes were done by the help of Numerical Taxonomy and Multivariate Analysis System software (NTSYSpc, version 2.1) (Rohlf, 1998). To be able to obtain a dendrogram of wheat genotypes, the DendroUPGMA (D-UPGMA) program (http://genomes.urv.cat/UPGMA) was used. The genetic similarity index of wheat genotypes was calculated according to Jaccard (1908). SSR and ISSR marker polymorphism rates were determined using Polymorphism Information Content (PIC) values, which were calculated based on the following formula: $PIC = 1 - \sum Pij^2$, where Pi is the frequency of the *ith* allele (Anderson *et al.*, 1993). The heterozygosity (He) was calculated according to Liu and Wu (1998).

Determination of Proline Concentration

The analysis of proline concentration (PRC) in the leaves was measured by using acid ninhydrin reagent following the method described by Bates *et al.* [1973] with some modifications. The amount of PRC in response to water stress was performed at the flowering stage after the water stress was induced using the leaf sample. Fresh plant material (0.1g) was homogenized with 10 ml of 3% aqueous sulfosalicylic acid, and the homogenate was filtered using Whatman No. 2 filter paper. 1 ml of filtrate was then used for the determination of PRC by adding 1ml of acid ninhydrin and 1 ml of glacial acetic acid in a test tube. The mixture was shaken by hand and incubate to react for 1 h in a water bath at 100°C. After that it was then transferred to ice bath allowed to be cooled at room temperature until producing reddish color. 2 ml of Toluene were added to the reaction mixture, mixed vigorously in a test tube, and stirred for 15–20 seconds until separate layers were formed. The chromophore or upper toluene layer containing the color complex due to proline ninhydrin reaction was separated from the aqueous phase to another test tube and was warmed at room temperature, and absorbance was read at 520 nm by UV spectrophotometer. PRC was determined from the standard curve constructed from the known concentration of PRO and was expressed in μmol of proline per gram fresh weight of the leaf and calculated on a fresh weight basis as indicated in the following equation

$$\text{Proline concentration} = \frac{(\mu g\ \text{proline}^{-ml}) \times (ml\ \text{toluene}/115.5\mu g^{-\mu mol})}{(g\ \text{sample}/5)}$$

Data Collection and Statistical Analysis

The recorded data during the experiments includes, Plant height (cm), flag leaf length (cm), spike length (cm), number of seed per spike, grain weight (g), thousand seed weight (g), and proline content. The data collected on the quantitative traits was analyzed statistically using the analysis of variance (ANOVA) and coefficient of variation following the procedure of Panse and Sukhatme [1962]. The significance among treatment means were compared by employing Duncun's Multiple Range Test (DMRT) at p ≤ 5% level of probability.

RESULTS AND DISCUSSION

Drought stress (DS) is a major contributing factor among other environmental stresses that causes significant yield losses by decreasing crop growth and productivity (Pour-Aboughadareh *et al.,* 2019) and has adverse effects on physiological and agronomic characters in wheat (Qaseem *et al.*, 2019). The mean performance of different wheat genotypes investigated for water stress tolerance on all measured variables shows a significant variation. The great variability can play a vital role in grain yield improvement of wheat in different breeding programs. The mean data response of all measured variables in Table 1 and Table 2 of wheat genotypes under irrigated and drought conditions indicated a significant decline in yield and related traits. This is in line with the work of Pour-Aboughadareh *et al.* (2020), who reported a decrease in yield and other traits due to drought stress. The response of all measured variables studied under irrigated and drought stress conditions is presented in Table 1 and Table 2.

Table 1. Mean performance of morphological traits of different wheat genotypes under irrigated conditions

Genotype	PH[a] (cm)	FL[b] (cm^2)	SL[c] (cm)	NS[d]	1000SW[e] (g)	SW[f] (g)	Proline content (µmol/g)
V1	77.17b	10.47c	9.52de	49.71f	36.51cd	303.00a	0.20a
V2	73.17c	11.10b	10.25d	62.00bcd	38.52b	280.12b	0.25a
V3	69.3bc	9.00f	9.32e	52.10ef	39.32a	227.90e	0.15b
V4	65.5g	7.52j	8.21g	63.81bc	40.21a	224.01e	0.18b
V5	62.2de	12.80a	11.10a	70.52b	38.72b	257.30c	0.13b
V6	77.3a	10.35c	9.70d	64.32bc	34.51c	301.12a	0.18b
V7	58.83ef	9.17f	8.32g	54.03def	41.70a	194.90f	0.17b
V8	65.83cd	10.47c	9.33e	65.51bc	34.02de	240.41cd	0.20a
V9	68.00c	8.80g	8.38fg	52.70ef	40.22a	279.90b	0.18b
V10	85.67a	8.20h	9.22e	64.70bc	34.80c	279.71b	0.24a
V11	77.33b	10.07d	11.21b	65.80bc	34.22d	238.42d	0.20a
V12	72.33c	9.50e	10.72bc	64.34bc	37.00b	257.02c	0.16b
V13	61.00def	9.50e	8.71ef	59.73cde	34.00d	270.00b	0.23a
V14	86.00a	13.03a	11.90a	80.73a	33.80d	189.91f	0.15b
V15	67.50c	11.00b	10.61c	66.52bc	36.72b	240.21cd	0.18b

Note: [a]PH = Plant height, [b]FL = Flag leaf length, [c]SL = Spike length, [d]NS = Number of seeds per spikes, [e]1000 SW = Thousand seed weight, [f]SW = Seed weight per plot

The mean variation of plant height (PH) ranges from 53.33 to 86.00 (cm) for both irrigated and drought condition. The genotype *V14* has the highest PH mean of 86.00 cm and 75.32 cm and *V7* with the lowest mean of 58.83 and 53.33 (cm) in both irrigated drought conditions respectively. The mean PH of the wheat genotypes has significant decrease of 6.8% under water stress compare with irrigated condition (Table 1 and Table 2) which could be attributed to the environmental factors (Abdulkerim *et al.*, 2015), and genetic makeup of the varieties (Shahzad *et al.,* 2007). Tefera *et al*. (2021) reported a significant reduction of 26% in PH under water stress compared with irrigated condition. Similarly, PH reduction in PH of 14% decrease due to drought was also identified by Bayoumi *et al.* (2008). Reduction in PH due drought stress was reported by Gupta *et al.* (2001). In addition, the highest mean performance in number of seeds per spikes (NS) was recorded in genotypes *V14* (80.73) and *V2* (63.50), with the lowest mean values of 49.71 in *V1* and 32.67 in *V4* for both irrigated and drought stress conditions respectively. The NS is economically important and has a direct measure on yield. this finding is in line with Kumar *et al.* (2020), who reported a significant decrease in NS under drought condition.

Table 2. Means values of morphological traits of different wheat genotypes under drought conditions

Genotype	PH[a] (cm)	FL[b] (cm^2)	SL[c](cm)	NS[d]	1000SW[e] (g)	SW[f] (g)	Proline content (µmol/g)
V1	61.71de	10.30de	8.54e	47.01e	33.51d	217.11d	0.51a
V2	70.53b	9.71ef	9.42cde	63.50a	33.00de	206.20e	0.32c
V3	65.67g	8.74g	8.05h	42.50efg	34.17cd	116.97h	0.37b
V4	58.33ef	8.72g	8.50d	32.67g	35.17bc	179.21f	0.40a
V5	56.17f	10.17ef	9.65bc	40.67efg	34.67bc	253.63a	0.36b
V6	60.83def	8.07g	8.37cd	50.67bcd	34.33cd	246.63a	0.49a
V7	53.33f	7.00h	8.58d	39.33fg	37.06a	184.73f	0.27c
V8	60.80de	9.91ef	8.37f	37.17gh	33.33de	232.50c	0.39b
V9	55.54ef	9.02g	8.40ef	40.17efg	35.17bc	239.50bc	0.45a
V10	65.52bcd	8.60g	8.78d	43.83defg	33.17e	218.07d	0.47a
V11	74.34a	11.71bc	8.88d	39.83fg	32f	231.60cd	0.43b
V12	71.33a	11.44c	8.70d	44.67cdef	35.33bc	255.20a	0.29c
V13	59.71def	9.02g	8.42ef	39.83fg	33.50de	161.80f	0.40b
V14	75.32a	12.02a	11.10a	57.17ab	31.00f	152.23fg	0.53a
V15	63.72cd	10.71d	8.32g	51.50bc	35.33bc	142.70g	0.25c

Note: [a]PH = Plant height, [b]FL = Flag leaf length, [c]SL = Spike length, [d]NS = Number of seeds per spikes, [e]1000SW = Thousand seed weight, [f]SW = Seed weight per plot

The spike length (SL), flag leaf length (FL), seed weight per plot (SW) and thousand seed weight (TSW) are directly contributed to yield components. The SL of different wheat genotype was found to be shorter in drought condition for most genotypes compared with irrigated condition (Table 2). The highest mean was recorded from the genotype V14 with 11.9 cm and 11.10 cm for both irrigated and drought conditions, while the lowest mean was recorded in V4 (8.21 cm) and *V3* (8.05cm) for both conditions respectively (Table 1). The difference in SL may be due to environment effect and genetic make-up of varieties (Shahzad *et al.*, 2007). Similarly, the highest and lowest mean value for FL was recorded in *V14* (13.03 cm^2) and *V4* (7.52 cm^2) in irrigated condition (Table 1), whereas the genotypes *V14* has the highest value of 12.02 cm^2 and *V7* with the lowest value 7.00 cm^2 (Table 2).
Accumulation of proline content (PC) under DS is one of the most common features in plants (Buttar *et al.*, 2005). Generally, genotypes are selected as drought-tolerant, having higher PC in DS than in normal conditions and the PC of all genotypes also increased under drought stress. The PC of all genotypes increases under drought condition when compared with irrigated condition. The highest mean performance was recorded in genotype *V14* with 0.53µmol/g and the lowest on *V15* 0.25µmol/g under drought condition. Whereas, the genotype *V2* has the highest mean value of 0.25µmol/g and V5 has the lowest value of 0.13 µmol/g (Table 1; Table 2). Increased PC accumulation acts as an osmotic for lessening of osmotic potential and increases water availability for many of fundamental biochemical pathways ongoing in plants and hence induces drought resistance (Ramond & Smirnoff, 2002). Liang *et al.* (2008), reported a significant increase of PC in wheat genotypes under stress condition and the accumulation of proline under DS, serve as a sensor of drought injury along with its prime role in stress tolerance mechanisms. High proline levels allow plants to attain low water potential, and thus imparts tolerance against moisture deficiency by increasing the biosynthesis of intermediate enzymes (Mwadzingeni *et al.*, 2016).
A total of 15 SSR primers were tested and twelve were polymorphic and used in this study (Table 3; Table 4; Figure 1).

Table 3. SSR primers, number of alleles, heterozygosity ratio and PIC values

Primer name	Allele number	He[a]	PIC[b]	Band size (bp)
Xwmc 695	11	0.48	0.46	201–273
Xgwm 350	10	0.61	0.56	145 - 197
Xwmc 233	10	0.68	0.64	218 - 276
Xwmc 182	10	0.58	0.56	100 - 159
Xwmc 9	10	0.58	0.56	155 -221
Xgwm 332	10	0.58	0.56	147 - 721
Xgwm 260	5	0.91	0.89	165 - 181
Xwmc 603	6	0.86	0.84	206 - 240
Xwmc 17	10	0.58	0.56	125 - 192
Xbarc 121	8	0.74	0.72	100 - 120

Xgwm 573	9	0.66	0.64	179 - 191
Xgwm 130	8	0.74	0.72	114 - 137
Total	**107**	**8.00**	**7.70**	-
Average	**8.9**	**0.67**	**0.64**	-

Note: [a]He, Heterozygosity; [c]PIC, polymorphism information contents

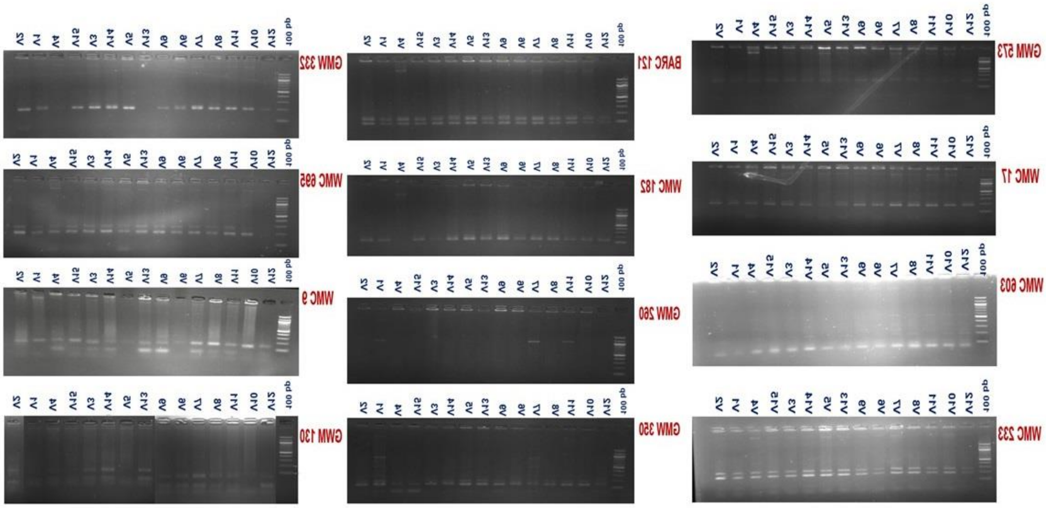

Figure 1. Gel image for molecular characterization of 15 wheat genotypes with 12 SSR primers (L:100bp)

Likewise, for the seed weight per plot (SW), the highest mean of 303.00 g and 255.20 g was observed in genotypes V1 and V12, and lowest mean values of 189.91 g and 116.97 g was recorded in genotypes V14 and V3 for both irrigated and drought conditions respectively. Under irrigation condition, wheat genotypes performed differently for 1000 seed weight (TSW). The highest TSW was recognized in genotypes V9 with 40.22 g and V14 with the lowest values of 33.80 g. whereas the genotypes V7 has the highest mean value of 37.06 g and V14 with the lowest value of 31.00 g under drought condition. Generally, under drought condition, TSW has direct effect on grain yield, leading to significant decrease in grain yield Dadbakhsh et al. (2011). The cumulative influence of environmental and genetic variables is responsible for the variations among the genotypes for all analyzed characters. Kumar et al. (2020) reported that severe water stress has a great influence in yield component. Similarly, drought stress has caused a reduction in all the yield contributing characters (Kilic & Yagbasanlar 2010). Blum and Pnuel (1990) reported that yield and yield contributing traits of wheat were rastically decreased under least annual precipitation.

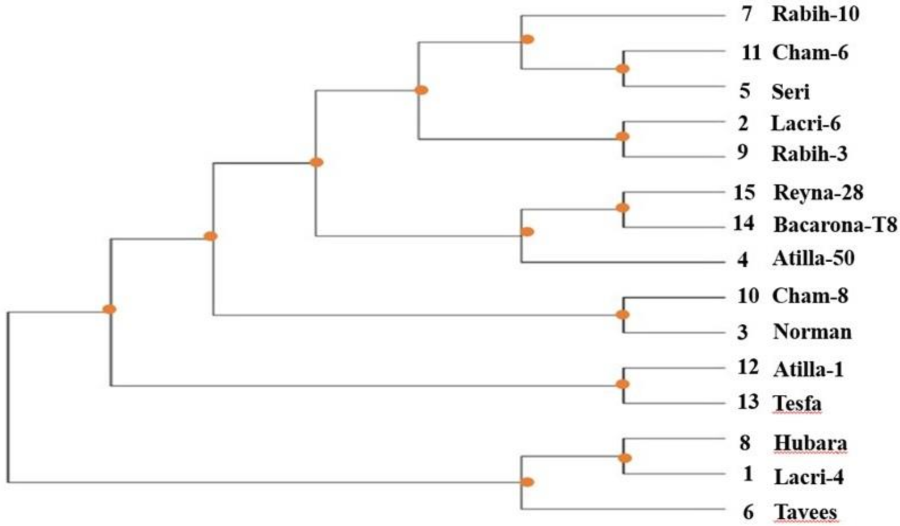

Figure 2. Dendrogram showing phylogenic relationship in a cluster for the fifteen wheat varieties

Table 4. Presence (1) versus absence (0) of PCR-amplified fragments from fifteen genotypes using twelve SSR primers

Pimer Name	V1	V2	V3	V4	V5	V6	V7	V8	V9	V10	V11	V12	V13	V14	V15
XWMC695-3A	0	1	1	1	1	0	1	0	1	1	1	0	1	1	1
XGWM350-4A	0	1	0	1	1	0	1	1	1	1	1	0	0	1	1
XWMC233-5D	0	1	0	1	1	0	1	1	1	0	1	1	0	1	0
XWMC182-6B	1	1	0	1	1	1	0	1	1	1	0	1	0	0	1
XWMC9-7A	0	1	1	1	1	0	1	0	0	0	1	1	1	1	1
XGWM332-7A	0	1	1	0	1	1	1	0	1	0	1	1	0	1	1
XGWM260-7A	0	0	1	0	1	0	0	0	1	0	1	0	0	1	0
XWMC603-7A	1	1	0	1	1	0	0	0	0	1	1	0	0	0	0
XWMC17-7A	1	1	0	0	1	1	1	1	0	1	0	1	1	0	1
XBARC121-7A	0	0	1	0	1	0	1	0	1	1	1	0	1	1	0
XGWM573-7B	1	1	0	1	1	0	1	0	1	0	1	0	1	0	1
XGWM130-7D	0	0	1	0	1	1	1	1	0	1	1	0	0	0	1

The Polymorphic information content (PIC) was estimated to determine the genetic diversity and the level of gene variation in plants (Ateş Sönmezoğlu & Terzi, 2018; Al-Tamimi & Al-Janabi, 2019). The locus of gene is considered high diversity if the PIC value is > 0.5, while if the value is < 0.25, it is considered to be low diversity (Nagy *et al*., 2012; Ramadugu *et al*., 2015). In the present study the mean PIC values for the SSR markers ranged from 0.46 to 0.89 with the mean recorded as 0.64. The highest PIC value was observed in *Xgwm260* marker with 0.89 and the lowest was determined in *Xwmc695* with value of 0.46**.** Similarly, there was no much significant different between the heterozygosity ratio (He) and the PIC values. The highest He was reported to be 0.91 in *Xgwm260* primer and the lowest in *Xwmc695* primer with the value of 0.48 (Table 3). The results indicates that out of the twelve SSR marker used in this study nine of them were found to be highly informative due to significant genetic variation, therefore they can be utilized to assist selection of drought stress tolerance in breeding programs. Previous studies suggest that genetic diversity in wheat genotypes should be reflected by PIC values as well as number of alleles per locus due to significant variation (Mkhabela *et al.,* 2020). These results are in accordance with Ateş Sönmezoğlu and Terzi (2018) who characterized different wheat varieties using SSR, single nucleotide polymorphisms (SNP) and randomly amplified fragment polymorphisms (RAPD) markers to screen for drought tolerance genotypes. They reported the reliability and significance of SSR markers in genetic characterization of drought tolerance in bread wheat. Other studies were conducted using 27 bread wheat genotypes to evaluate their response to drought stress through six SSR and eight ISSR markers which they reported significant genetic diversity among the genotypes which could be utilized for marker assisted selection and other breeding programs (Ates-Sonmezoglu *et al*., 2022). Yadav *et al.*, (2018) reported significant variation using 15 ISSR markers for six drought tolerance and six drought sensitive wheat varieties in which 14 markers gave reproductive bands.

Table 5. Genetic similarity values among 15 wheat genotypes

Genotypes	**V1**	**V2**	**V3**	**V4**	**V5**	**V6**	**V7**	**V8**	**V9**	**V10**	**V11**	**V12**	**V13**	**V14**
V2	0.71													
V3	0.57	0.72												
V4	0.62	0.78	0.78											
V5	0.76	0.85	0.76	0.79										
V6	**0.52**	0.61	0.58	0.69	0.67									
V7	0.71	0.76	0.69	0.72	0.71	0.67								
V8	0.56	0.66	0.59	0.65	0.72	0.56	0.79	.						
V9	0.68	0.85	0.71	0.81	0.69	0.66	0.84	0.64						
V10	0.62	0.77	0.69	0.75	0.86	0.67	0.81	0.62	0.81					
V11	0.82	0.89	0.68	0.77	**0.92**	0.64	0.89	0.65	0.86	0.79				
V12	0.54	0.58	0.60	0.62	0.71	0.56	0.69	0.61	0.76	0.68	0.68			
V13	0.55	0.57	0.59	0.62	0.72	0.57	0.70	0.61	0.76	0.68	0.68	0.57		
V14	0.62	0.73	0.63	0.69	0.80	0.76	0.81	0.65	0.85	0.76	0.72	0.62	0.67	
V15	0.68	0.76	0.63	0.70	0.85	0.77	0.86	0.66	0.86	0.76	0.73	0.65	0.69	0.77

A dendrogram was constructed among the genotypes based on the genetic similarity coefficient value using the SSR information (Figure 2). The varieties V5 and V11 gave the highest genetic similarity value (0.92). The closest genotypes were found in V5 and V11 with genetic similarity of 0. 92 (Table 5). In addition, 12 out of the 15 bands of V5 cultivar had similar amplified bands with V11 (Table 4). This implies that cluster analysis can be a useful tool for identifying drought tolerance genotypes. However, V1 and V6 varieties had the least genetic similarity value of 0.52 (Table 5) indicating that V6 is the most divergent genotype among the genotypes as it is located in separate cluster. This work is similar to the findings of (Mansour et al.,

2020) and Tahir (2008). Similarly, Tungalag *et al*. (2018), reported that ISSRs markers could be used to determine genetic relationships by using 17 ISSR markers to define variants in six Mongolian local wheat varieties.

CONCLUSION

The current study was designed to evaluate the morphological characteristics of different wheat cultivars grown during dry and rainy seasons and estimate the genetic diversity and relationships among the cultivars subjected to drought conditions using molecular markers. Identifying variations between phenotype and genotype of targeted germplasm will effectively use genetic resources. The results reveal a significant variation among the genotype with PIC values ranged from 0.46 to 0.89 with the mean value recorded as 0.64, which allowed the identification and selection of drought related genotypes. Those genotypes will be very useful genetic resources for various breeding programs especially for characterizing genotypes for drought tolerance for molecular breeding.

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REFERENCES

Abdulkerim, J., Tana, T., & Eticha, F. (2015). Response of bread wheat (Triticum aestivum L.) varieties to seeding rates at Kulumsa, South eastern Ethiopia. *Asian Journal of Plant Sciences*, *14*(2), 50–58. https://doi.org/10.3923/AJPS.2015.50.58

Al-Tamimi, A. J. T., & Al-Janabi, A. S. (2019). Genetic diversity among bread wheat genotypes using rapd and ssr markers. *Sabrao Journal of Breeding and Genetics*, *51*(3), 325–399.

Araus, J. L., Slafer, G. A., Reynolds, M. P., & Royo, C. (2002). Plant Breeding and Drought in C3 Cereals: What Should We Breed For? *Annals of Botany*, *89*(7), 925–940. https://doi.org/10.1093/AOB/MCF049

Ates-Sonmezoglu, Ö., Çevik, E., & Terzi-Aksoy, B. (2022). Assessment of some bread wheat (Triticum aestivum L.) genotypes for drought tolerance using SSR and ISSR markers. *Biotech Studies*, *31*(2), 45–52. https://doi.org/10.38042/biotechstudies.1159128

Ateş Sönmezoğlu, Ö., & Terzi, B. (2018). Characterization of some bread wheat genotypes using molecular markers for drought tolerance. *Physiology and Molecular Biology of Plants*, *24*(1), 159–166. https://doi.org/10.1007/s12298-017-0492-1

Chowdhury, M. K., Hasan, M. A., Bahadur, M. M., Islam, M. R., Iqbal, M. A. H. A., Javed, T., Raza, A., Shabbir, R., Sorour, S., Elsanafawy, N. E. M., Anwar, S., Alamri, S., Sabagh, A. EL, & Islam, M. S. (2021). Evaluation of Drought Tolerance of Some Wheat (Triticum. *Agronomy*, *11*(9), 1–20.

Colasuonno, P., Lozito, M. L., Marcotuli, I., Nigro, D., Giancaspro, A., Mangini, G., De Vita, P., Mastrangelo, A. M., Pecchioni, N., Houston, K., Simeone, R., Gadaleta, A., & Blanco, A. (2017). The carotenoid biosynthetic and catabolic genes in wheat and their association with yellow pigments. *BMC Genomics*, *18*(1), 122. https://doi.org/10.1186/s12864-016-3395-6

Dadbakhsh, A., Yazdansepas, A., & Ahmadizadeh, M. (2011). Study drought stress on yield of wheat (Triticum aestivum L.) genotypes by drought tolerance indices. *Advances in Environmental Biology*, *5*(7), 1804–1810.

EL Sabagh, A., Islam, M. S., Skalicky, M., Ali Raza, M., Singh, K., Anwar Hossain, M., Hossain, A., Mahboob, W., Iqbal, M. A., Ratnasekera, D., Singhal, R. K., Ahmed, S., Kumari, A., Wasaya, A., Sytar, O., Brestic, M., ÇIG, F., Erman, M., Habib Ur Rahman, M., … Arshad, A. (2021). Salinity Stress in Wheat (Triticum aestivum L.) in the Changing Climate: Adaptation and Management Strategies. In *Frontiers in Agronomy* (Vol. 3, p. 661932). Frontiers Media S.A. https://doi.org/10.3389/fagro.2021.661932

FAO. (2020). *China | Global Partnership Initiative for Plant Breeding Capacity Building | Food and Agriculture Organization of the United Nations*. http://www.fao.org/in-action/plant-breeding/our-partners/asia/china/en/

Gupta, N. K., Gupta, S., & Kumar, A. (2001). Effect of Water Stress on Physiological Attributes and their Relationship with Growth and Yield of Wheat Cultivars at Different Stages. *Journal of Agronomy and Crop Science*, *186*(1), 55–62. https://doi.org/10.1046/J.1439-037X.2001.00457.X

Kumar, L., Singh, M., & Arora, V. (2020). Influence of Drought Stress on Morphological Characters in Indian Wheat Cultivars. *International Journal of Current Microbiology and Applied Sciences*, *9*(10), 816–832. https://doi.org/10.20546/ijcmas.2020.910.098

Liu, S., Sehgal, S. K., Li, J., Lin, M., Trick, H. N., Yu, J., Gill, B. S., & Bai, G. (2013). Cloning and characterization of a critical regulator for preharvest sprouting in wheat. *Genetics*, *195*(1), 263–273. https://doi.org/10.1534/genetics.113.152330

Mansour, H. A., El Sayed Mohamed, S., & Lightfoot, D. A. (2020). Molecular studies for drought tolerance in some Egyptian wheat genotypes under different irrigation systems. *Open Agriculture*, *5*(1), 280–290. https://doi.org/10.1515/opag-2020-0030

Mkhabela, S. S., Shimelis, H., & Mashilo, J. (2020). Genetic differentiation of selected drought and heat tolerant wheat genotypes using simple sequence repeat markers and agronomic traits. *South African Journal of Plant and Soil*, *37*(3), 211–219. https://doi.org/10.1080/02571862.2020.1718787

Mwadzingeni, L., Shimelis, H., Tesfay, S., & Tsilo, T. J. (2016). Screening of bread wheat genotypes for drought tolerance using phenotypic and proline analyses. *Frontiers in Plant Science*, *7*(AUG2016), 182193. https://doi.org/10.3389/FPLS.2016.01276/BIBTEX

Nagy, S., Poczai, P., Cernák, I., Gorji, A. M., Hegedűs, G., & Taller, J. (2012). PICcalc: An online program to calculate polymorphic information content for molecular genetic studies. *Biochemical Genetics*, *50*(9–10), 670–672. https://doi.org/10.1007/s10528-012-9509-1

Pour-Aboughadareh, A., Mohammadi, R., Etminan, A., Shooshtari, L., Maleki-Tabrizi, N., & Poczai, P. (2020). Effects of Drought Stress on Some Agronomic and Morpho-Physiological Traits in Durum Wheat Genotypes. *Sustainability*, *12*(14), 5610. https://doi.org/10.3390/SU12145610

Pour-Aboughadareh, A., Omidi, M., Naghavi, M. R., Etminan, A., Mehrabi, A. A., Poczai, P., & Bayat, H. (2019). Effect of Water Deficit Stress on Seedling Biomass and Physio-Chemical Characteristics in Different Species of Wheat Possessing the D Genome. *Agronomy*, *9*(9). https://doi.org/10.3390/AGRONOMY9090522

Qaseem, M. F., Qureshi, R., Shaheen, H., Pakistan, R., & Rawalpindi, P. (2019). Effects of Pre-Anthesis Drought, Heat and Their Combination on the Growth, Yield and Physiology of diverse Wheat (Triticum aestivum L.) Genotypes Varying in Sensitivity to Heat and drought stress. *Scientific Reports*, *9*(1), 1–12. https://doi.org/10.1038/s41598-019-43477-z

Ramadugu, C., Keremane, M. L., Hu, X., Karp, D., Federici, C. T., Kahn, T., Roose, M. L., & Lee, R. F. (2015). Genetic analysis of citron (Citrus medica L.) using simple sequence repeats and single nucleotide polymorphisms. *Scientia Horticulturae*, *195*, 124–137. https://doi.org/10.1016/j.scienta.2015.09.004

Rasheed, A., & Xia, X. (2019). From markers to genome-based breeding in wheat. In *Theoretical and Applied Genetics* (Vol. 132, Issue 3, pp. 767–784). Springer Verlag. https://doi.org/10.1007/s00122-019-03286-4

Raza, A., Razzaq, A., Mehmood, S. S., Zou, X., Zhang, X., Lv, Y., & Xu, J. (2019). Impact of Climate Change on Crops Adaptation and Strategies to Tackle Its Outcome: A Review. *Plants 2019, Vol. 8, Page 34*, *8*(2), 34. https://doi.org/10.3390/PLANTS8020034

Sharma, B. R., Mohan, G., Manchanda, S., & Amarasinghe, U. A. (2018). *Water Productivity Mapping of Major Indian Crops*. *October*.

Tefera, A., Kebede, M., Tadesse, K., & Getahun, T. (2021). Morphological, Physiological, and Biochemical Characterization of Drought-Tolerant Wheat (Triticum spp.) Varieties. *International Journal of Agronomy*, *2021*. https://doi.org/10.1155/2021/8811749

Yadav, V., Kumar, P., & Goyal, M. (2018). Evaluation of genetic diversity in drought tolerant and sensitive varieties of wheat using ISSR markers. *Electronic Journal of Plant Breeding*, *9*(1), 146–153. https://doi.org/10.5958/0975-928X.2018.00017.0

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OPTIMIZING HYDROPONIC FORAGE PRODUCTION: THE IMPACT OF WATER QUALITY ON WHEAT GROWTH AND YIELD

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ABSTRACT

This study investigates the impact of water quality on the productivity of hydroponic wheat forage. Two water sources were compared: water from a local lake and water from a fish farming center. After a six-week acclimation period, repeated experiments were conducted for 17 days each to evaluate the growth and productivity of hydroponic wheat. The results show that lake water, despite its higher mineral content, led to faster growth and greater final height of wheat plants compared to fish farming water. Dry matter yields were also significantly higher with lake water (36.6 tons/ha) compared to fish farming water (22.5 tons/ha). Analyses revealed significant differences in ash content, indicating a higher mineral accumulation in forage grown with lake water. These findings suggest that the source and quality of water play a crucial role in the productivity of hydroponic forage, offering a viable and sustainable solution for small-scale farmers, particularly in regions where traditional forage production is challenging. This technology promises to enhance the sustainability of future livestock farming systems.

Keywords: water quality, growth, productivity, wheat.

INTRODUCTION

Currently, the hydroponic forage production system is an alternative technology used to produce animal feed without using soil as a planting medium. Instead, it uses water with or without a mix of dissolved minerals (Ceci et al., 2023; Harerimana et al., 2023). The hydroponic forage production technique converts energy-rich food sources like barley or wheat grains into green fodder. This method requires less soil, water, and labor, and it enables year-round production regardless of climatic conditions. Additionally, the fresh forage produced in hydroponic systems is known for its high digestibility (Harerimana et al., 2023).

Hydroponic cultivation has been integrated into aquaculture systems to produce a valuable byproduct by recovering accumulated nutrients in the water and producing green forage (Snow et al., 2008). In these integrated systems, nutrient-rich effluents from the aquaculture facility provide moisture and nutrients for plant production (Ghaly et al., 2005).

MATERIALS AND METHODS

The Hydroponic System: This study was conducted from January to April 2023, including a 6-week acclimatization period to determine the optimal water quantity for wheat germination. After the acclimatization period, the experiments were conducted and repeated three times, each lasting 17 days. The experiments took place at the Faculty of Istom located in western France.

The wheat species cultivated was Rgt Sacramento, provided by a local farmers' cooperative.

A hydroponic system was designed and installed at Istom's experimental unit. The hydroponic unit featured a greenhouse structure with a three-tier system and manual spray irrigation to control the water amount used in each tray. Trays with an area of 600 cm^2 each and a reservoir height of 2 cm were used.

During the experimental phase, each treatment involved 3 trays per repetition, with a wheat density of 200 g/m^2. Water spraying of 200 ml per tray was performed twice daily at 8 am and 6 pm, five times a week. Each day of the experiment, the germination height was measured during the morning spray, and mold or abnormal growth was monitored. One tray from the first repetition was excluded due to abnormal germination delay.

Temperature was controlled and maintained between 19°C-20°C. No artificial lighting was used, relying on a natural light cycle of approximately 12 hours of light and 12 hours of darkness.

Once a repetition was completed (17 days), the forage was collected from the six trays, and the fresh yields of each tray were weighed. The forage was then dried in an oven to determine the dry matter content of each tray and stored until all three repetitions were completed to conduct chemical analyses simultaneously.

Treatments: Two types of water were used in these experiments: the first was sourced from a local lake near Istom's experimental unit. This water was collected once during the acclimatization period and again before starting the actual experiments. It was stored in closed, opaque containers and used daily for spraying at 200 ml per tray.

The second type of water was obtained from a fish farming center. This water was filtered and treated by the center to remove waste and food residues.

Chemical Analysis: Immediately after each series of experiments, the fresh forage was collected, and the productivity of each tray in each treatment was weighed. Each sample was then placed in an oven to obtain the dry matter within 72 hours. These samples were stored in bags until the chemical analyses were performed. The forage samples were analyzed using proximate analysis according to AOAC (AOAC, 2004).

Water quality analyses were performed before each repetition using specialized test strips for these types of analyses.
The Anova test using the R system was employed to compare means, with a significance level of 5% adopted for all results.

RESULTS AND DISCUSSION

The results of the water quality analysis reveal a difference in the quality of water collected from the local lake and the fish farming center (Table 1). Contrary to expectations, the fish farming water was found to be purer than the local lake water. Total water hardness is an indicator of water mineralization by divalent alkaline earth cations, which can precipitate as calcareous deposits. It is determined solely by calcium and magnesium ions. Water is classified according to its hardness as follows:Very soft: hardness less than 7; Soft: 7 < hardness < 15 ; Moderately hard: 15 < hardness < 30 ; Hard: 30-40 ; Very hard: more than 40

Table1. Average values of water parameters measured before each repetition

Water parameters	Lac Water	Fish Farming Water
Total water hardness (mg/L)	25±3[a]	0[b]
Carbonate Hardness KH (mg/L)	80±9[a]	60±3[b]
Nitrate(NO3-)(mg/L)	25±4[a]	10±0.5[b]
Chlore (mg/L)	0	0
pH	8±0.3	6,8±0.1

The means in each row that are followed by different letters are significantly different with a 5% probability.

As shown by the analysis results in the table above, the lake water is considered moderately hard and falls within the upper range (25 mg/L), while the fish farming water, after filtration, is classified as very soft, containing no traces of magnesium or calcium.
The carbonate hardness (KH) of the water corresponds to the amount of carbonates present in the medium, indicating its capacity to stabilize the pH. Our results show that the carbonate hardness in lake water is 33% higher than in fish farming water (80±9 vs. 60±3, $P<0.05$).
Water analysis reveals that both types of water contain nitrates (NO3-), with a significant difference between the two samples. Nitrate (NO3-) is an ion produced during the nitrogen cycle in water, often responsible for pollution. Its natural concentration in waters, in the absence of fertilization, ranges from 5 to 15 mg/L. The nitrate concentration in lake water is two and a half times higher than in fish farming water, 25±4 vs. 10±0.5 mg/L for lake and fish farming water, respectively ($P<0.05$).

Effect of Water Quality on Hydroponic Barley Growth

Figure 1 shows the daily growth of hydroponic barley sprayed with two types of water, from the first day until harvest after 17 days.

From the first to the twelfth day, no difference was observed in the growth of barley between the two treatments. However, starting from day 13, the growth rate began to differ depending on the water used. Barley grown with lake water outperformed barley grown with fish farming water. The three-leaf stage began on day 14 for the lake water treatment and on day 15 for the fish farming water treatment. From day 15 to day 17, the growth rate differences between the two treatments became significant.

The average height of the three trays of barley on day 15 was 123 mm for fish farming water versus 135 mm for lake water. By the final day (day 17), the barley height reached 165 mm for lake water and 141 mm for fish farming water.

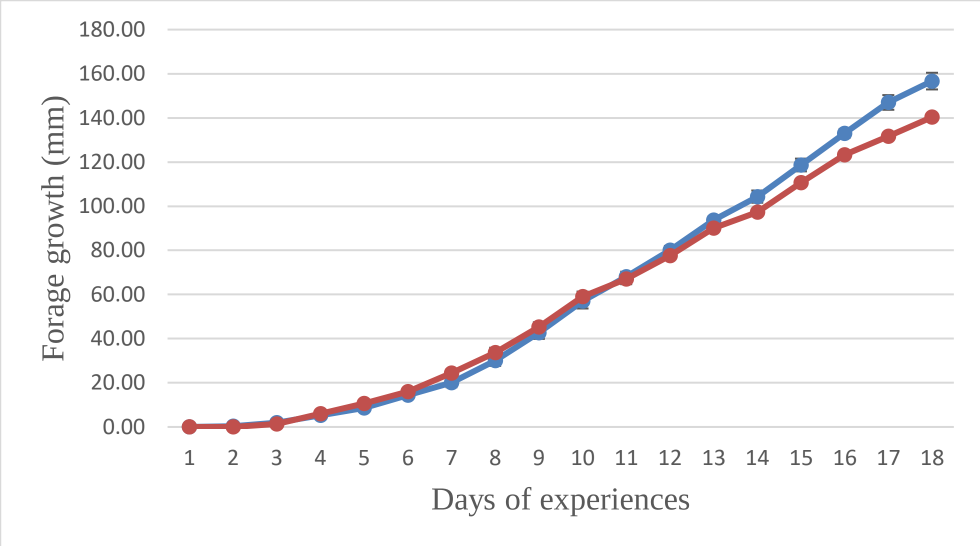

Figure1: shows the average daily growth of barley depending on the type of treatment, with SD for each day

The * shows a significant difference at level 5%

Fresh and Dry Matter Yields: The results shown in Table 2 present the average yield of fresh forage collected immediately after each experiment, as well as the average dry matter yield according to the type of treatment. These results indicate that the type of water impacts the amount of forage produced. Although the fresh forage yield produced with lake water was higher than that produced with fish farming water (100.16 T/Ha vs. 98.5 T/Ha for lake water and fish farming hydroponic forage, respectively), this difference is not significant (P=0.6).

Table2: The means of forage yield in fresh and dry materials by tray ± SD

Hydroponic fodder yield	Wheat fish farming water	Wheat lake water
Average fresh material per tray (g)	591±10[a]	601±9[a]
Average dry matter per tray (g)	135±14[a]	220±23[b]

The means in each row that are followed by different letters are significantly different with a 5% probability.

In contrast, the dry matter from fresh forage dried in an oven at 70°C for 72 hours showed a significant difference. As shown in Table 2, the dry matter yield from forage treated with lake water reached 36.6 tonnes per hectare, while the dry matter yield from fish farming water was 22.5 tonnes per hectare.

Ash analysis revealed significant differences between the forage samples based on the type of water used. Our results indicate that the ash content in lake water forage samples was 34.7% DM (347±78 g/kg DM) compared to only 27.38% DM (273±42 g/kg DM) in fish farming water samples.

The analyses of organic matter (carbohydrates, lipids, nitrogenous materials) are still ongoing

The aim of this experiment was to understand the effect of the water source on the productivity of hydroponic forage. Initially, it was hypothesized that fish farming water, rich in waste and nitrogenous substances, would accelerate the growth process and increase productivity (Snow et al., 2008; Ceci et al., 2023). Some cereals (wheat, barley, and oats) grown hydroponically have significantly reduced the pollutant load in wastewater and aquaculture water. However, during the two-week acclimatization experiments, it was found that the fish farming water was treated, filtered, and very low in organic matter. Consequently, we compared the effects of fish farming water with water sourced from a local lake.

Within 24 hours of starting the experiment, the seeds in all trays began to absorb water and swell. After 2 days, the radicle and plumule emerged from the seed coat and were visible in most seeds. During the germination period, the crops in all trays grew rapidly and uniformly regardless of the water type used, appearing healthy with a green color, and no signs of mold or rot. Until the thirteenth day, no growth differences were observed between the two treatments. The three-leaf stage began on day 14 for the lake water treatment and on day 15 for the fish farming water treatment. From day 15 to day 17, the growth rate differences between the two treatments became significant.

The average height of the barley trays on day 15 was 123 mm for fish farming water versus 135 mm for lake water. On the final day (D17), the barley height reached 165 mm for lake water and 141 mm for fish farming water.

Several studies have demonstrated the ability of cereals grown hydroponically to reduce mineral content (Clarkson and Lane et al., 1991) and nutrient content (Kamal and Ghaly, 2002) in wastewater. According to numerous references, the mineral and waste content of the water affects the growth rate of hydroponic forage crops. According to Snow et al. (2008), growth rates and forage height vary depending on the species, with wheat reaching 19 cm versus 25 cm for barley at 21 days of hydroponic culture under the same treatment. Al-Karaki and Al-Hashimi 2012 found that plant height varies within the species, with heights of 18.7, 20.3, and 22.7 cm for three wheat varieties (ACSAD 176, Rum, and Local, respectively) during a 10-day hydroponic production cycle. However, Clarkson and Lane et al. (1991)

achieved 20 cm in 10 days using aquaculture water from Cyprinus carpio and Oncorhynchus mykiss, and up to 25 cm with Tilapia aquaculture water (Kamal and Ghaly, 2002). These references suggest that factors leading to differences in growth rates and plant heights are varied and complex, potentially due to differences in plant species or even plant variety. It appears that water content and source play a significant role in growth rates. This hypothesis is supported by the significant increase in ash content (minerals) in the forage extract from lake water (34.7% vs. 27.3%), indicating mineral accumulation in the resulting feed.

The hydroponic system reduces nitrite presence in water using cereals. Ghaly et al. (2005) reported a decrease in NO_3^- from 76.7% to 75.1% using oats and from 68.8% to 64.8% using barley. In our study, the higher nitrate content in lake water compared to aquaculture water could have contributed to the rapid forage growth.

Fresh forage yields were 100.16 T/Ha vs. 98.5 T/Ha for lake water and fish farming hydroponic forage, respectively, with dry matter yields of 36.6 tonnes per hectare for lake water and 22.5 tonnes per hectare for fish farming water. Al-Karaki and Al-Hashimi (2012) found yields of 113 tonnes per hectare with 22.9 tonnes/ha of dry matter. However, other studies have reported lower yields, with Snow et al. (2000) obtaining 64.59 tonnes/ha of fresh matter and Saidi and Abo Omar (2015) reporting 37.03 tonnes/ha. Forage productivity of hydroponic wheat varies across studies and also for other cereal species. Barley forage yields, according to Al-Karaki and Al-Momani (2011), were 222, 236, and 281 tonnes/ha for three barley varieties: ACSAD176, Rum, and a local cultivar, respectively, with dry matter yields of 27.1, 28.6, and 33.7 tonnes/ha.

Seed density, light intensity, and materials used for root support also influence these yields. Pettersen (1987) reported yields ranging from 1 to 65 tonnes/ha depending on light intensity and root support materials used.

CONCLUSION

Hydroponic forage production systems generate large quantities of nutritious and palatable green feed for livestock. Hydroponic germination of wheat using locally available materials has the potential to enhance the technical and economic viability of this technology among small-scale farmers. The quality and source of water play a crucial role in the productivity and nutrient content of these feeds. Regardless of the water source, hydroponic forage productivity exceeds that of traditional forage, making this method promising for areas where conventional forage production is challenging. Utilizing this technology can help sustain livestock farming in the future and create a more sustainable farming system.

REFERENCES

Al-Karaki G N and Al-Hashimi M. 2012. Green fodder production and water use efficiency of some forage crops under hydroponic conditions. International Scholarly Research Notices. 9246–72. doi:10.5402/2012/924672

Al-Karaki G. N. and N. Al-Momani, 2011. Evaluation of some barley cultivars for green fodder production and water use efficiency under hydroponic conditions, Jordan Journal of Agricultural Sciences. Volume 7-3.

AOAC. 2004. Official Methods of Analysis. Association of Official Analytical Chemists, Gaithersburg, MD.

Ceci L, Cavalera MA, Serrapica F, Di Francia A, Masucci F and Carelli G, 2023. Use of reclaimed urban wastewater for the production of hydroponic barley forage: water

characteristics, feed quality and effects on health status and production of lactating cows. Front. Vet. Sci. 10:1274466. doi: 10.3389/fvets.2023.1274466.

Clarkson, R. and S.D. Lane, 1991. Use of a small scale nutrient-film hydroponic technique to reduce mineral accumulation in aquarium water. Aquacult. Fish. Manage., 22: 37-45.

Harerimana T, Hatungimana E, d'Andre Hirwa C, Niyireba R, 2023. Effect of hydroponic wheat fodder supplementation on dry matter intake and growth performances of piglets, Scientific African, Volume 19, 2023, https://doi.org/10.1016/j.sciaf.2022.e01507

Ghaly, A.E., M. Kamal and N.S. Mahmoud, 2005. Phytoremediation of aquaculture wastewater for water recycling and production of fish feed. Environ. Int., 31: 1-13.

Kamal, M. and A.E. Ghaly, 2002. Effectiveness of phytoremediation for treatment and recycling of aquaculture wastewater. ASAE Annual International Meeting, Chicago, Illinois. 28-31

Saidi, A.M.A.; Jamal, A.O. The biological and economical feasibility of feeding barley green fodder to lactating awassi ewes. J. Anim. Sci. 2015, 5, 99–105. DOI: 10.4236/ojas.2015.52012

Snow, A.M., Ghal, A. E., & Snow, A. (2008). A comparative assessment of hydroponicallygrown cereal crops for the purification of aquaculture wastewater and the production offish feed. American Journal of Agricultural and Biological Sciences, 3, 364-378. DOI:10.3844/ajabssp.2008.364.378

Pettersen, B.W., 1987. Ability of lettuce, rye grass and barley to reduce the nutrient salt content of wastewater from fish farming. Water Sci. Technol., 19: 129-138

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CLIMATE RESILIENTE AGRICULTURE IN THE SEMI-ARID REGION OF CEARÁ STATE, BRAZIL

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ABSTRACT

This study aims to evaluate climate resilient agriculture (CRA) in the semi-arid zone cultivated as rainfed food crops in Ceará State of Brazil. The research specific objectives were: a) to classify the distribution of rainfall in Ceará from 1901 to 2020 into three periods: drought, normal and rainy; b) to examine the ooccurrence years of these periods between 1945-2020; c) to evaluate the behavior of rice, bean, cassava and corn production in the state in each of these periods; d) to estimate the instabilities of rainfall, as well as those associated with the defining variables of the production of the studied crops; e) to verify whether there is resilience in the production of these crops in response to the occurrence of droughts, as defined in this study. The rainfall data used were taken from NOAA (National Oceanic & Atmospheric Administration), 2022. Information on harvested areas, yields and production values per hectare of the crops studied was available from the Brazilian Institute of Geography and Statistics (IBGE) and covered the period 1945- 2020. Instabilities were estimated using the coefficients of variation (CV) of the variables. Factor analysis was used to construct the synergy index (SIN) to assess the interface between the variables used to measure crop production. The evidence found proved that the studied rainfall series were unstable and that it was possible to classify the state rainfall into drought, normal and rainy periods as suposed. In addition, the existence of self-resilience in the production of the crops studied was demonstrated.

Keywords: *Rainfed food crops; Drought, Rainfall instability; Synergy; Self-resilience.*

INTRODUCTION

The semi-arid is a type of climate characterized by the prevalence of high temperatures, low relative humidity and with unstable rainfall regimes both, from a temporal and spatial point of view (Marengo et al., 2017; Salviano; Praxedes and Lemos, 2020).

In general rainfed agricultural activities, which depend exclusively on rainfall precipitation, prevail in the Brazilian semi-arid region. These are crops of great importance as promoters of occupation, monetary income, and food production for the families of farmers who engage in these activities. The use of technologies that neutralize, or at least mitigate the impacts of rainfall irregularities, which are very common in the Brazilian semi-arid region, isn't accessed by the majority of farmers (Carvalho, 2010; Gurjão, 2020). These facts make the agriculture practiced under this climatic regime, in the semi-arid region, difficult to conduct and is an activity very subject to risks: economic, social and environmental (Fischer; Shah and Vanvelthuizen, 2002; Rosenzweig and Hillel, 2005; Obermaier, 2011; Beyer et al., 2016; Pereira, 2018; Lemos et al., 2020).

Soil and water management, in areas where rainfed agriculture prevails, are the main constraints in maintaining production, productivity, and income of these crops. Thus, the water scarcity that is caused by the systematic occurrence of droughts plays a crucial role, given that it induces vulnerabilities that rebound in persistent poverty in places that coexist with this difficulty, in addition to impacting on the degradation of natural resources and the environment, thus eroding productivity and weakening coping strategies and their resilience (Berkes, 2007; Devendra, 2016; Deschênes and Greenstone, 2007; Duque, 1973; Fraiture et al., 2009; Mallari, 2016; Mohinder Singh et al., 2018; Sathyan et al., 2018).

On the other hand, it is known that farmers in general, and those who cultivate rainfed crops in particular, develop strategies of adaptation and coexistence with the rainfall uncertainties that are their rule since they entered the activities, either through the knowledge acquired from their parents and relatives, or by their own experiences. This learning leads them to devise survival strategies in the most critical periods. A behavior that can be identified as self-resilience, because it is independent of the action of external agents, or of public policies. They function as a self-defense mechanism for farmers who cultivate rainfed crops (Hanjra and Qureshi, 2010; Machado, 2018).

In 2010 the FAO presented the concept of Climate-Smart Agriculture (CSA) at The Hague Conference on Agriculture, Food Security and Climate Change. This concept, according to the document produced at that event, would contribute to the achievement of sustainable development goals. The concept would reflect the synergy between three of the dimensions of sustainable development: economic, social and environment, seeking to find food security under the challenge of climate change (FAO, 2013; Rao, 2016).

According to FAO (2013), the CSA concept is anchored on three pillars: 1- increasing productivity growth and income in the agricultural sector; 2- adapting and building resilience to climate variations; and 3- reducing and/or removing greenhouse gas emissions, when possible.

According to Maheswari (2015), Climate Resilient Agriculture (CRA) means the incorporation of adaptation, mitigation, and other practices in agriculture that are able to increase the productive system in responding to different climate instabilities, by resisting damage, recovering quickly and ensuring sustainable production. In short, it is the ability of a system to return to its position prior to the occurrence of the stress to which it was subjected. CRA involves increment in management of natural resources, such as land, water, soil, besides genetic resources always seeking

better practices. In this way, the concept of Climate Resilient Agriculture (CRA) can be conceived as a step prior to CSA (Hanjra and Qureshi, 2010; IIED, 2015; Maheswari, 2015; Rai et al., 2015; Mendelsohn, 2009; Rao and Gopinath, 2021).
In the Brazilian semi-arid region in general, and in Ceará State in particular, which has 175 of its 184 municipalities recognized by the Federal Government as being included in this region, it can be said that there are some practices exercised by the farmers themselves that can be characterized as CRA. These are experiences and adaptations, most of the times spontaneous, without the intervention of public authorities, which lead them, for example, to select seeds and seedlings of the crops that show the best results in times of good rainy seasons and even in those periods when there are rain difficulties (Obermaier, 2011; Hanjra and Qureshi, 2010; WMO, 2012). Map 1 shows the position of Ceará State in Brazil.
Based on these fundamentals, this research has the following objectives: a) Based on the historical series of average rainfall for the State of Ceará between 1901 and 2021, to classify the rainfall distribution into three periods: drought, normal and rainy; b) as the information regarding crop production extends from 1945 to 2020, to evaluate the years of occurrence of the drought, normal and rainy periods, defined in objective “a” in this time span; c) to evaluate how the production of rice, beans, cassava and corn in Ceará State behave in each of these periods in the years 1945 to 2020; d) To assess the total instability/stability of rainfall, as well as those associated with the defining variables of rice, beans, cassava and corn production between 1945 and 2020; e) To assess evidence of the existence of resilience in the production of these crops in rainy years or years of normal rainfall following years of drought, as defined in this study.

MATERIAL AND METHODS

Database description

The study uses rainfall series provided by two sources, covering the period from 1901 to 2020 (120 years). The sources of information were: Meteorology and Water Resources Foundation of Ceará (Funceme, 2020) and National Oceanic and Atmospheric Agency (NOAA, 2022).
The data referring to harvested areas, productivity, prices of rice, beans, cassava and corn production were collected from 1945 to 1973 from the IBGE Statistical Yearbooks. From 1974 to 2020 were taken from the documents Municipal Agricultural Productions (PAM) in the reference years. The values were updated for 2020 using the General Price Index - Internal Availability (GPI-IA) from Getúlio Vargas Foundation - GVF.
The selected rainfed food crops were: rice, beans, cassava and corn, because they are cultivated by the majority of farmers in the State of Ceará (IBGE). From these crops the following variables were analyzed: A_{it} = Harvested area in hectares (ha) with crop "i" (i = 1, 2, 3, 4) in year t (t = 1945,..., 2020) in Ceará; R_{it} = land productivity or, simply, productivity ($kg.ha^{-1}$) in the production of crop "i" in year "t"; P_{it}= average price ($USD.kg^{-1}$), in 2020 values, of crop "i" in year "t".

The choice of variables is justified for the following reasons. Besides being the ones that define the production of the crops, they have the following characteristics. Farmers have some power of definition about the areas that they will plant (areas that they have) and the technologies that they will use for the cultivation of crops, which will influence the productivities. However, the areas that will be harvested, as well as the yields, depend on exogenous factors, being the rainfall instability the most relevant in semi-arid region. Thus, these variables are random for the farmers. Prices, on the other hand, are set in markets over which farmers have no control. Thus, the crop prices are also random variables for them.

Classification of rainy years in Ceará State from 1901 to 2020

For the classification of rainfall periods for the State of Ceará, are used historical series of rainfall for the State between the years 1901 and 2020. The average (AV) and the standard deviation (SD) were calculated for these years of observations. Thus the following periods are defined, according to the annual rainfall intensity: Years of rainfall scarcity, called "Drought". Years with excessive rainfall, called "Rainy". Periods when the rainfall levels are in an intermediate position between "Drought" and "Rainy", called "Normal". The definitions of these three periods are shown in Table 1.

Table 1. Rainfall periods definition in Ceará State from 1901 to 2020

Periods	**Ranges of Variation in milimeter (mm)**
Drought	Rainfall < (AV– ½ SD)
Normal	(AV – ½SD) ≤ Rainfall ≤ (AV + ½ SD)
Rainy	Rainfall > (AV + ½ SD)

Source: NOAA (2022) and Funceme (2020).Note: SD = standard deviation.

Once the years that make up each period are defined, the test is performed to verify if the mean of these periods are statistically different. To perform the test we use dummies variables (D_1 and D_2) defined as follows: D_1=1 in normal years. D_1=0 in drought and rainy periods; D_2= 1 in rainy years. D_2= 0 in drought and normal periods; D_1=D_2=0 in drought periods Equation (1) is used to perform the test:

$$C_t = \beta_0 + \beta_1 D_1 + \beta_2 D_2 + \eta_t \quad (1)$$

In Equation (1), the linear coefficient β_0, being statistically different from zero, with $D_1 = D_2 = 0$, will be the average rainfall for the drought years; the coefficient β_1 being statistically different from zero, with D_1= 1 and D_2 = 0, means that the average rainfall of the normality period is different from the other periods. The coefficient β2 being statistically different from zero, with D_1 = 0 and D_2= 1, means that the average rainfall of the rainy period differs from the other periods.

The random term η_t, by hypothesis, meets the assumptions of the classical linear model, of being white noise. Thus the parameters of Equation (1) can be estimated by the ordinary least squares method–OLS (GREENE, 2012; WOOLDRIDGE, 2013).

Assessment of stability/instability in the variables

To gauge the stability/unstability associated with rainfall, it was used the coefficient of variation (CV). The CV is widely used as a measure of variation by the researchers in the disciplines like finance, climatology, engineering, risk sensitivity; variability in agricultural experiments. An advantage associated with the CV is that it allows the comparison between variables of different nature and measurement (Brooks, 2003; Garcia, 1989; Hamer et al., 1995; Nairy and Rao, 2003; Santos and Dias, 2021).

The smaller the CV is, the more homogeneous, or more stable will be the distribution of the variable observations around its mean Santos and Dias (2021). To use the CV as a measure of the degree of stability/instability of a distribution, some knowledge about the definition of its critical values may be useful. Gomes (1989) established limits for classifying CVs in agricultural experimentation. These are the references used in this study (Gomes, 1989): Low ($CV < 10$); Medium ($10 \leq CV < 20$); High ($20 \leq CV < 30$); e Very High ($CV \geq 30$).

Synergies among the defining variables of rainfed production

The variables assumed to define the production of rice, bean, cassava and corn were aggregated: harvested areas, productivities and prices. As these are variables measured in different units and supposed to be correlated, the aggregation was made through the synergy index (SIN) built in the research. To build this index it was used the factor analysis methodology (FA) thru principal components decomposition.

Brief summary of the AF procedure as it applies to the study

Factor analysis is a method designed to investigate whether a number of variables Y_1, Y_2, ...,Yn, can be summarized into a smaller number ($k < n$) of unobservable factors: F_1, F_2, ..., F_k. The technical assumption of AF is anchored in the linear correlation between the variables that are used. In order to adequately perform the AF you need to follow these steps: perform the Bartlett's test of sphericity to confirm that the correlation matrix is not an identity; ensure that the Kaiser-Meyer-Olkin (KMO) statistic has a value equal or greater than 0.500; evaluate the percentage of explanation of the accumulated variance by the estimated factors (Chan, 2017; Fávero and Belfiore, 2017; Hahn and Foster, 2009; Hassan et al., 2012; Guillaumont and Simonet, 2011; Mcclave et al., 2005).

Being generated more than one factor, the FA method provides the possibility to rotate these factors. In this study it was adopted the Varimax method, which will produce linearly independent factors. This property is used to build the synergy index (SIN). Once the extraction has been done and the number of factors has been determined, the FA possibility to estimate the coefficients associated with the factorial scores (Fávero and Belfiore, 2017; Hahn and Foster, 2009; Hassan et al., 2012; Xu et al., 2017).

With these procedures the "n" original variables generate "k" ($k < n$) unobserved variables that have zero mean and unit variance. These are the factor scores (FS) used in the construction of the synergy index (SIN) in a positive scale. The FS has zero mean, so they values gravitate around it are positives and negatives. To make all of them positives, without affecting the hierarchy in which they were generated, the variable (Y_{kt}) is created, which will have values ranging between zero and one. To do this we estimate the maximum FS value (FS_{max}) and its minimum value (FS_{min}) and use the transformation showed in Equation (2):

$$Y_{kt} = (FS_{kt} – FS_{min}) / (FS_{max} – FS_{min}) \quad (2)$$

Generated in this way, because the factors are orthogonals (caused by the varimax rotation), it is possibly to estimate the synergy index (SIN) by the arithmetic average of the Ykt.

$$SIN_t = \Sigma Y_{kt}/k \quad (3)$$

The synergy index will vary between zero and one ($0 \leq SIN_t \leq 1$). The closer to one (1) the SIN value is in a given year, the better the joint behavior of the original twelve (12) variables in that year will have been.

Measuring the resilience in the production of the studied crops

To assess whether there was resilience associated with the synergy between harvested areas, productivity and prices of rice, beans, cassava and corn in Ceará State from 1945 to 2020, as summarized in the SIN, is assumed the following procedure. It was computed in sequence, the rainfall years in the drought, normal and rainy periods. When sequences of more than one year occur in the drought periods, the SIN averages of these sequences of years are calculated. On the other hand, when sequences of normal and rainy periods occur, the averages of these periods are calculated in aggregated way. These are the non-drought periods. Therefore, it is assumed that resilience would be the ability to recover from the non-drought periods that followed drought periods.

This SIN sequence is then assembled into pairs identified as “after” and “before” drought periods. The test performed compares the SIN averages in the two groups. The null hypothesis is: the difference of SIN average after drought (μ_1) and its average before drought (μ_2) is equal to zero as shown in Equation (4):

$$H_0: (\mu_1 - \mu_2) = 0 \quad (4)$$

If the null hypothesis is accepted, it can be ascertained that there is resilience in the variables grouped. The used test is Student's “t” test with (n-1) degrees of freedom, where "n" is the number of pairs to be tested (Xu et al., 2017; Monteiro and Lemos (2019).

RESULTS AND DISCUSSION

The estimated average rainfall for the period from 1901 to 2020 was 799.5 mm, with a standard deviation of 268.3 mm and CV=33.6%. Based on these values we stipulated the upper limit for the drought period (668.50mm) and the lower limit for the rainy period of 933.7 mm. The normal years were situated between these two extremes.

It can be seen in Table 3 that the observed rainfall averages for the defined periods between the years 1901 and 2020 were statistically different. The limits established for the periods were applied for rainfall occurring between 1945 and 2020. The statistical test done for this period also showed that the averages are different. Thus, the following hierarchy can be constructed for the rainfall precipitation occurring in Ceará between the years 1901-2020 and between the years 1945-2020 as follows: rainy period > normal period > drought period (Table 2).

Table 2. Test to evaluate difference between the averages of the three rainfall periods created for Ceará

Variables	Results from 1901 to 2020			Results from 1945 to 2020		
	Coefficients	t statistic	Sign.	Coefficients	t statistic	Sign.
Constant	524.685	24.830	0.000	552.042	23.994	0.000
D_1	255.148	8.979	0.000	245.546	6.987	0.000
D_2	599.670	19.650	0.000	552.521	14.894	0.000
Adjusted R^2: 0.768				Adjusted R^2: 0.753		

Source: NOAA (2022) and Funceme (2020).

Table 3 shows the averages and the estimated CVs for the drought, normal, and rainy periods between the years 1901-2020 and 1945 - 2020.

Table 3. Classification by periods of Ceará State rainfall

Periods	Years of occurrence (1901 to 2020)				Years of occurrence (1945 to 2020)			
	Total	(%)	Average (mm)	CV (%)	Total	(%)	Average (mm)	CV (%)
Drought	38	31.7	524.7	20.6	28	36.8	552.0	19.1
Normal	47	39.2	779.8	10.5	28	36.8	803.4	11.4
Rainy	35	29.2	1124.4	17.0	20	26.3	1104.6	18.4
Total	**120**	**100.0**	**799.5**	**33.6**	**76**	**100.0**	**777.8**	**33.3**

Source: NOAA (2022) and Funceme (2020).

It is observed that between 1901-2020 the estimated average rainfall for Ceará was 799.5 mm with CV = 33.6%, and between 1945-2020 the average was 777.8 mm with CV=33.3%, showing the very high instability of rainfall in both analyzed series (Table 3).

Table 4 shows the means and the CV estimated for the variables used in the study. Through the evidence shown in Table 5 we can infer that the rainfall instability observed in the period investigated, was transmitted practically to all variables that define the production of rice, beans, cassava and corn in Ceará. In relation to the periods in which the annual rainfall of the state of Ceará was classified, the greatest instabilities observed in the variables that define the production of the products studied are in the drougt periods, excepting to cassava harvested lands (Table 4).

Table 4. Averages and variation coefficients (CV) of selected variables, Ceará State (from 1945 to 2020)

Variables	Drought		Normal		Rainy	
	Average	CV (%)	Average	CV (%)	Average	CV (%)
Rice harvested land (ha)	34677.68	50.28	43226.69	46.45	46990.72	28.32
Rice productivity (kg.ha^{-1})	1771.50	41.21	1973.64	30.95	2203.32	23.26
Rice price (USD.kg^{-1})	806.96	43.34	870.49	20.84	1144.89	37.12
Bean harvested land (ha)	336095.71	43.62	385732.90	44.95	462814.5	28.38
Bean productivity (kg.ha^{-1})	260.48	45.49	431.63	28.05	331.80	31.59
Bean price (USD.kg^{-1})	263.63	48.41	298.07	39.33	305.34	35.57
Cassava harvested land (ha)	87500.61	41.01	88143.69	46.95	97308.41	21.57
Cassava productivity ((kg.ha^{-1})	8843.20	35.93	11571.48	27.12	10242.52	27.36
Cassava price value(USD.kg^{-1})	736.84	50.87	811.38	43.05	921.73	33.40
Corn harvested land (ha)	368959.16	45.04	451570.83	37.92	525755.14	23.33
Corn productivity (kg.ha^{-1})	445.08	49.88	833.51	22.20	698.53	32.70
Corn price (USD.kg^{-1})	145.06	57.91	232.51	29.78	216.53	30.24
Years of occurrence	28		28		20	

Source: NOAA (2022), Funceme (2020) and IBGE (2020).

Results associated with the FA to build the synergy indexes (SIN)

The results found for the factor analysis used to estimate the synergy index (SIN) are shown in Table 5. The twelve original variables are reduced to three orthogonal factors. It is observed that the adjustments were all satisfactory, from a statistical point of view, considering that the Bartlet test evidences that the correlation matrix is not identity. The KMO statistic = 0.620 and the total explained variance (77.8%) by the three orthogonal factors, into which the twelve (12) variables studied were summarized, complement the information of the robustness of the adjustment (Table 5).

Table 5. Results from factor analysis to create the sinergy index (SIN)

Variables	Rotated Component Matrix			Component Score Coefficient Matrix		
	F_1	F_2	F_3	F_1	F_2	F_3
Rice harvested land (ha.year^{-1})	0.131	0.380	0.670	0.042	0.151	0.240
Rice productivity (kg.ha^{-1})	-0.054	0.723	-0.341	0.051	0.245	-0.112
Rice price value (USD.kg^{-1})	0.034	-0.035	0.676	-0.019	-0.002	0.241
Bean harvested land (ha.year^{-1})	-0.184	0.935	0.202	0.005	0.316	0.091
Bean productivity (kg.ha^{-1})	0.958	-0.083	-0.010	0.289	0.042	-0.043
Bean price value (USD.kg^{-1})	0.433	-0.422	0.674	0.075	-0.108	0.220
Cassava harvested land (ha.year^{-1})	-0.280	0.293	0.764	-0.095	0.091	0.289
Cassava productivity (kg.ha^{-1})	0.843	-0.296	0.109	0.233	-0.039	0.002
Cassava price value (USD.kg^{-1})	0.081	-0.255	0.742	-0.024	-0.075	0.261
Corn harvested land (ha.year^{-1})	-0.056	0.943	0.087	0.050	0.327	0.044

Corn productivity ($kg.ha^{-1}$)	0.879	0.325	-0.219	0.304	0.177	-0.110
Corn price value ($USD.kg^{-1}$)	0.867	-0.245	0.340	0.235	-0.017	0.085
Explained variances	29.001	25.165	23.627			
Cumalitive explained variances						
Approx. Chi-Square: 917.835						
Degree of freedom: 66						
Sig.: 0.000						
Bartlett's Test of Sphericity Kaiser-Meyer-Olkin (KMO)/Measure of Sampling Adequacy (MSA): 0.620						

Source: IBGE (2020). Note: Rotation converged in 5 iterations.

Contrast test to evaluate resilience in the rainfed Ceará production

The estimated average of the SIN for the drought periods was 0.572 while its value for the non drought periods was 0,796.

In Table 6 was showed the averages SIN contrasts measuring the differences of SIN average before non droughts periods (0.797) and SIN after drought periods (0.795).The estimated P value for performed "Student" "t" test, used to evaluate the existing statistical significance in the contrasts, showed that there was no significant difference between them. These results mean that the averages aggregated SIN, before drought and after drought periods, are statistically equals. Otherwise they permit to conclude that there were no reasons to reject the hypotheses that exist resilience in production of rice, bean, cassava and corn in Ceará State in the period of 1945-2020.

Table 6. Contrast test to evaluate difference of SIN before and after scarcity periods in Ceará State from 1945 to 2020

Contrasts	**Differences between ranked pairs with 95% fiducial probability**					
	Average difference	**Average before drought periods**	**Average after drought periods**	**t Statistic**	**Degrees of freedom**	**Sig. 2-tailed**
Before – After	0,001	0.797	0.795	0.029	17	0.977

Source: IBGE (2020).

CONCLUSIONS

The research succeeded in elaborating the proposed classification of the climate years in Ceará State between 1945 and 2020, years in which data about the agricultural production of the state is available. There were created 3 periods from the annual rain precipitation for that period: Drought, Rainy and Normal. It was made based on an available longer series from 1901 to 2020.

In general, the rainfall observed in the Ceará State is very unstable, but is more unstable in the drought years, results that are transmitted mostly to the variables used in the research for the construction of the synergy index (SIN), to evaluate the rice, bean, cassava and corn productions in the State between 1945 and 2020.

The general conclusion of the search is that, despite the difficulties faced by farmers, mostly family farmers, producers of rice, beans, cassava and corn, some of the most important rainfed crops in the Ceará State, these producers managed to develop adaptive capacities by themselves to the rainfall instabilities observed in the last 76 years and exhibited evidences of resilience in the production of these crops, after the occurrence of droughts, which were manifested in 31 of the 76 studied years.

REFERENCES

Alemaw, B. & Simalenga, T. (2015). Climate Change Impacts And Adaptation In Rainfed Farming Systems: A Modeling Framework For Scaling-Out Climate Smart Agriculture In Sub-Saharan Africa. American Journal Of Climate Change, 4, 313-329.

Altieri, M.A. & Nicholls, C. I. (2017). The Adaptation And Mitigation Potential Of Traditional Agriculture In A Changing Climate. Climatic Change, 140, 33- 45.

Berkes, F. (2007). Understanding Uncertainty And Reducing Vulnerability: Lessons From Resilience Thinking. Natural Hazards, 41, 283-295.

Beyer, M., Wallner, M., Bahlmann, L., Thiemig, V., Dietrich, J. & Billib, M. (2016). Rainfall Characteristics And Their Implications For Rain-Fed Agriculture: A Case Study In The Upper Zambezi River Basin. Hydrological Sciences Journal. 61(2), 321-343.

Brito, S. S. B., Cunha, A. P. M., Cunningham, C. C., Alvalá, R. C., Marengo, J. A. & Carvalho, M. A. (2018). Frequency, Duration And Severity Of Drought In The Semi- Arid Northeast Brazil Region. International Journal Of Climatology, 38(2), 517-529.

Brooks, N. (2003). Vulnerability, Risk And Adaptation: A Conceptual Framework. Tyndall Centre For Climate Change Research.

Carvalho, O. (2010). Semi-Arid Northeast: Transformations Of Potentialities Into Economic Possibilities. In: CHAMBER OF DEPUTIES. Semi-Arid. Brasília (DF).143-176.(In Portuguese).

Ceará Meteorology And Water Resources Foundation (Funceme). (2020). Ceará Hydrological Portal. (In Portuguese).

Chan, L. L. (2017). Validity And Reliability Of The Instrument Using Exploratory Factor Analysis And Cronbach's Alpha. International Journal Of Academic Research In Business And Social Sciences,7(10), 400-410.

Deschênes, O. & Greenstone, M. (2007). The Economic Impacts Of Climate Change: Evidence From Agricultural Output And Random Fluctuations In Weather. American Economic Review, 97(1), 354-385.

Devendra, C. (2016). Rainfed Agriculture: Its Importance And Potential In Global Food Security. Utar Agriculture Science Journal, 2(2), 4-17.

Fávero, L. P. & Belfiore, P. (2017). Handbook Of Data Analysis. 1st. Ed. Rio De Janeiro: Elsevier. (In Portuguese).

Fischer, G., Shah, M. & Vanvelthuizen, H. (2002). Climate Change And Agricultural Vulnerability. Johannesburg: International Institute For Applied Systems Analysis To World Summit On Sustainable Development, Special Report.

Food And Agriculture Organization Of The United Nations (FAO). (2013). Climate-Smart Agriculture Sourcebook. 570.

Fraiture, C., Karlberg, L. & Androckström, J. (2009). Can Rainfed Agriculture Feed The World? An Assessment Of Potentials And Risk. In: Wani, S.P.; Rockström, J. & Oweis, T. Rainfed Agriculture: Unlocking The Potential. London, UK. 124-132.

Garcia, C.H. (1989). Tables For Classifying The Coefficient Of Variation. Piracicaba: IPEF, (Technical Circular, 171), 12. (In Portuguese).

Gomes, F.P. (1985). Course In Experimental Statistics. 13th.Ed. São Paulo: ESALQ/USP, 467. (In Portuguese).

Greene, W. H. (2012). Econometric Analysis.71. Ed. New York: Stern School Of Business, New York University.

Guillaumont, P. & Simonet, C. (2011). Designing An Index Of Structural Vulnerability To Climate Change. FERDI-Fondation Pour Les Etudes Etrecherches Sur Le Developpement International, France, 42.

Gurjão, N.O. (2020). Advances In Environmental Degradation In The Northeast Region Of Brazil. 2020. 105 F. Dissertation (Master In Rural Economics) - Center For Agricultural Sciences, Federal University Of Ceará, Fortaleza, Ceará. (In Portuguese).

Hahn, M., Riederer, A. & Foster, S. (2009). The Livelihood Vulnerability Index: A Pragmatic Approach To Assessing Risks From Climate Variability And Change - A Case Study In Mozambique. Global Environmental Change, 19(1), 74-88.

Hamer, A.J. Et Al; (1995). A New Method Of Comparative Bone Strength Measurement, Journal Of Medical Engineering & Technology, 19(1), 1-5.

Hanjra, M.A. & Qureshi, M.E. (2010). Global Water Crisis And Future Food Security In An Era Of Climate Change. Food Policy, 35, 365–377.

Hassan, S., Ismail, N., Jaafar, W. Y. W.,Ghazali, K.,Budin, K., Gabda, D. & Samad, A. S.A. (2012). Using Factor Analysis On Survey Study Of Factors Affecting Students' Learning Styles. International Journal Of Applied Mathematics And Informatics. 6(1), 33-40. Available From: Https://Www.Naun.Org/Multimedia/Upress/Ami/17- 667.Pdf.

IBGE, Brazilian Institute Of Geography And Statistics. IBGE/SIDRA. (2019). Agricultural Census 2017: Final Results. Rio De Janeiro: IBGE. (In Portuguese)

IIED. (2015). Reviving Knowledge: India's Rainfed Farming, Variability And Diversity.

Jesus, E. T. De, Amorim, J. S., Junqueira, R., Viola, M. R., Mello, C. R. (2020). Meteorological And Hydrological Drought From 1987 To 2017 In The Doce River Watershed, Southeastern Brazil. Scientific/Technical Article. RBRH 25. (In Portuguese).

Lemos, J. J. S., Bezerra, F. N. R., Costa Filho, J. & Gurjão, N. O. (2020). Family Farming In Ceará: Evidence From The 2017 Agricultural Census. Rev. Econ. NE, Fortaleza, 51(Special Supplement), 93-112. (In Portuguese)

Machado, L. M. (2018). Climate Change Resilient Development Of Family Farmers In The Brazilian Semi-Arid: Ananalysis Of Public Policies And Of The Coexisting With The Semi-Arid Paradigm. Phd Thesis - Graduate Program In Energy Planning, COPPE, Federal University Of Rio De Janeiro, Rio De Janeiro, RJ, Pp. 161.(In Portuguese).

Maheswari, M., Sarkar, B., Vanaja, M., Srinivasa Rao, M., Srinivasa Rao, Ch., Venkateswarlu, B. & Sikka, A.K. (2015). Climate Resilient Crop Varieties For Sustainable Food Production Under Aberrant Weather Conditions. Central Research Institute For Dryland Agriculture (ICAR), Hyderabad, 47.

Mallari, A. E. C. (2016). Climate Change Vulnerability Assessment In The Agriculture Sector: Typhoon Santi Experience. Procedia - Social And Behavioral Sciences, 216, 440-451.

Marengo, J. A., Alves, L., Avala, R., Brito, S. & Moraes, O. (2017). Climatic Characteristics Of The 2010-2016 Drought In The Semiarid Northeast Brazil Region. Anais Da 118 Academia Brasileira De Ciências. São Paulo.

Marengo, J. A., Cunha, A. P.,Alves, L. M.(2016).The 2012-15 Drought In The Semiarid Northeast Of Brazil In Historical Context. Climanalysis Journal, 3:49-54. (In Portuguese).

Mcclave, J.T., Benson, P.G.,Sincich, T. (2005). Statistics For Business And Economics. Uppersaddle River, New Jersey, Pearson Prentice Hall.

Mendelsohn, R. (2009). The Impact Of Climate Change On Agriculture In Developing Countries. Journal Of Natural Resources Policy Research. 1(1), 5-19.

Mishra, A. K. & Singh, V. P. (2010). A Review Of Drought Concepts. Journal Of Hydrology, Amsterdam, 391, 202-216. Monteiro, A. P. & Lemos, J. J. S. (2019). Inequalities In The Distribution Of Pronaf Resources Among Brazilian Regions. Revista De Política Agrícola, Year XXVIII, 1: 6-17. (In Portuguese).

Mohinder Singh, N. K. T., Naveen Kumar, K. R. D. & Dehinwal, A. K. (2018). Dry And Rainfed Agriculture-Characteristics And Issues To Enhance The Prosperity Of Indian Farming Community. BEPLS, 6(10).

Nairy, K. Subrahmanya & Rao, K. Aruna (2003). Tests Of Coefficients Of Variation Of Normal Population, Communications In Statistics - Simulation And Computation, 32(3), 641-661.

NOAA, National Oceanic And Atmospheric Agency. (2022). Global Historical-Monthly Climatology Network (GHCN-M).

Obermaier, M. (2011). Old And New Dilemmas In The Hinterlands: Climate Change, Vulnerability And Adaptation In The Brazilian Semi-Arid. Phd Thesis - UFRJ, COPPE, Energy Planning Program. (In Portuguese).

Pedro-Monzonís, M., Solera, A., Ferrer, J., Estrela, T. & Paredes-Arquiola, J. (2015). A Review Of Water Scarcity And Drought Indexes In Water Resources Planning And Management. Journal Of Hydrology (Amsterdam), 527, 482-493.

Rai, R. K., Bhatta, L. D., Acharya, U. & Bhatta, A. P. (2018). Assessing Climate-Resilient Agriculture For Smallholders. Environmental Development, 27, September, 26-33.

Rao, C. S., Gopinath, K. A.,Prasad, Y., Sikka, A. & Singh, R. (2016).Climate Resilient Rainfed Agriculture. Indian Farming Agriculture, 66(2), 02-06.

Rao, C.S. & Gopinath, K.A. (2021). Resilient Rainfed Technologies For Drough Mitigation And Sustaninable Food Security. Mausam. 67(1), 169-182.

Rey, D., Holman, I. P. & Knox, J. W. (2017). Developing Drought Resilience In Irrigated Agriculture In The Face Of Increasing Water Scarcity. Regional Environmental Change, 17, 1527-1540.

Rosenzweig, C. & Hillel, D. (2005). Climate Change, Agriculture And Sustainability. In: Lal, R., Uphoff, N., Stewart, B.A. & Hansen, D.O. (Eds.).Climate Change And Global Food Security. Taylor & Francis, London, UK. 243-268.

Sain, G., Loboguerrero, A.M., Corner-Dolloff, C., Lizarazo, M., Nowak, A., Martínez- Barón, D. & Andrieu, N. (2017). Costs And Benefits Of Climate-Smart Agriculture: The Case Of The Dry Corridor In Guatemala. Agric. Syst. 151, 163-173.

Santos, C. & Dias, C. Note On The Coefficient Of Variation Properties (2021). Brazilian Electronic Journal Of Mathematics, 2(4), 101-111.

Sathyan, A. R., Funk, C., Aenis, T. & Breuer, L. (2018). Climate Vulnerability In Rainfed Farming: Analysis From Indian Watersheds. Sustainability, 10(9), 33-57.

Woli, P., Jones, J. W., Ingram, K. T. & Fraisse, C. W. (2012). Agricultural Reference Index For Drought (ARID). Agronomy Journal, 104(2), 287-300.

World Meteorological Organization (WMO). (2012). Standardized Precipitation Index User Guide. (M. Svoboda, M. Hayes And D. Wood), (WMO-No. 1090), Geneva.

Xu, M., Fralick, D.; Zheng, J. Z., Wang, B., Tu, X. M. & Feng, C. (2017). The Differences And Similarities Between Two-Sample T-Test And Paired T-Test. Shanghai Arch Psychiatry, 29(3), 184-188.

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NITROGEN FERTILIZATION AND BIOSTIMULANTS EFFECT ON SAFFLOWER CROP

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ABSTRACT

Carthamus tinctorius species or safflower as it is referred to by its common name is a plant that grows and thrives in dry heat climates. Due to climate change and the medicinal properties of the plant, this study was prepared to draw conclusions on the response of the crop to nitrogen enrichment and to study the effects of the application of bio-stimulants on its seed yield in Thessaly, Greece. A split-plot experimental design was used with two different nitrogen fertilization levels being the major factor (N1: 0 and N2: 80 kg ha^{-1}) and four different bio-stimulants as the sub-factor (C: control, A: *Ascophyllum nodosum* algae extract, S: plant amino acids, and A+S: a combination of previous two). The data analysis found no statistically significant difference between the study factors or their interactions on seed yield. Nitrogen fertilization (N2) almost doubled the seed yield, reaching up to 1.3 t ha^{-1}, with a harvest index of 0.2. In the case of biostimulants, the S treatment resulted in a numerically higher yield (1.2 t ha^{-1}), while interactions (N2xS) recorded a seed yield of 1.5 t ha^{-1}. Finally, a statistically significant difference was found in biostimulants treatments when measuring seed oil content. In addition to providing the rural population with a crop that is resistant to climate change, the aforementioned results provide the foundation for future study into the yield of safflower in dry-thermal conditions that will generate positive results in the manufacturing of oil with medicinal characteristics.

Keywords: *Safflower, Carthamus tinctorius, N-fertilization, biostimulant, yield.*

INTRODUCTION

The plant species known as *Carthamus tinctorius*, or safflower as it is commonly called, is a dry heat-loving plant, which has contributed to its widespread distribution and cultivation over the planet. Today, regions of Asia, North-East Africa, Europe, America, and Australia cultivate spindles for commercial use (Ren et al., 2017). In 2018, the global production of safflower seed was 627,653 tons, with Kazakhstan being the primary producer, contributing 34% of the total production. The United

States and India combined accounted for the remaining 26% of the global production (Oleynikova et al., 2021).

In the data items of the "Production Yearbook" published in 1993, the United Nations Food and Agriculture Organization formally recognized safflower as a dual-purpose medical oil (Peng, 1999). Furthermore, safflower exhibits promising prospects for use in food, cosmetics, new oil, industrial resources, and natural pigment additives (Liu, 2017; Zhang et al., 2016).

The plant produces long, delicate, light green leaves with tiny teeth or smooth surfaces during the initial phases of vegetative growth. However, the leaves do not acquire any kind of spines during the early stages of rosette formation and germination. Once the central stem has grown to its full height, the leaves turn a dark green hue and become leathery. Bract leaves, which are leathery, spine-adorned, and significantly shorter than the plant's other leaves, sprout at the base of each inflorescence. The stem of the plant is made up of a primary stem on which lateral branches grow. Depending on the growing environment, the capability of each variety, and the cultivation practices, the stem length of the various plant kinds ranges from 30 to 150 cm. The plant produces spherical inflorescences on top of both the main stem and the secondary stems. Depending on the cultivating method and the variety, the inflorescences can have a diameter of 1.3–1.4 cm and can produce from 3 up to 50 inflorescences in total.

Safflower seeds are classified as achenes. The seeds are rarely mottled or gray; they are typically white in appearance. A thousand seeds have a weight of between 30 and 45 g and a dimension of between 6 and 10 mm. They are greasy and contain nutritious and high-quality oil (Popov A.M. and Kang D., 2011). For the majority of types, ripe seed contents include around 30% dry weight oil content, 5-8% moisture content, 14–15% protein content, 32-40% crude fiber content, and 2-7% ash (Bijanzadeh et al., 2022; Günç Ergönül and Aksoylu Özbek, 2020). Similar to sunflower and olive seeds, safflower has a 20%–40% seed oil content (Kumar et al., 2016).

In order to maximize plant output and improve nitrogen absorption, growers primarily apply safflower fertilization to irrigated crops (Santos et al., 2018). Nitrogen is the primary inorganic element for plant growth since it directly influences the growth of the plant and the increase in biomass. Additionally, fertilizer with nitrogen helps plants produce more by encouraging the development of more flower heads per plant. The crop's nutritional needs begin at the rosette stage when the plant begins to adsorb nitrogen from the soil on a small scale. A higher rate of nitrogen uptake by the plant is observed during the central stem elongation stage, however the highest uptake rate is observed at the full flowering stage. From the stage of full flowering onwards, the nitrogen needs gradually decrease at a constant rate and the greater percentage of the absorbed nitrogen is channeled into the seeds.

In many crops, the usage of biostimulants has increased recently. Biostimulants are compounds that promote growth and fortify a plant's resistance to both biotic and abiotic stresses, such as a shortage of nutrients and water. When applied in modest amounts, biostimulants also aid in the growth of the plant without acting as nutrients

for the same purpose. Three categories can be used to categorize biostimulants: preparations containing humic acids in the first, plant hormones in the second, and amino acids in the third (du Jardin, 2015).
Applying biostimulants foliarly can strengthen the plant's defenses, raise the plant's dry biomass content, and improve crop seed output (Davari et al., 2022). In the case of safflower, the use of a biostimulants can improve plant nutrition in non-irrigated soil with low organic matter content and increase seed yield. Biostimulants use can also strengthen a plant's defenses against drought conditions in the soil substrate by preventing the entry of pathogenic microorganisms, regulating the opening of the plant's leaf stomata, and assisting in the regulation of the plant's uptake of water from the soil, according to research evaluating various biostimulants in spindle culture (Janmohammadi and Sabaghnia, 2023).
This study investigated the effects of nitrogen fertilization and biostimulants on seed yield of a rainfed safflower cultivation in Thessaly.

MATERIALS AND METHODS

Experimental Field

In order to estimate safflower seed yield, a field experiment was conducted on the University of Thessaly farm in Velestino, Magnesia, at coordinates 39°23'59"N and 22°45'14"E.
Sowing was carried out on November 25, 2022. At sowing, basic fertilization was carried out, where 200 kg/ha of the 15-15-15 (+5SO3) fertilizer was used. The experimental design included 24 total sowing lines with 50 cm distance between lines, and the required amount of seeds was equal to 91.6 kg/ha.
A factorial split-plot design with three replicates (blocks) and 8 plots per replication was used to accomplish the study goal. The prime-factor was the different nitrogen fertilization levels (N1: 0, N2: 80 kg N ha^{-1}, using 34.5-0-0), while the sub-factor was the different biostimulants (B1: control, B2: algal extract, B3: plant amino acids and B4: combination of B1 & B2). A 12 m^2 area, with dimensions of 6 m for width and 2 m for length, made up each trial plot. On March 20, 2023, 1 L/ha of the B1 biostimulant, and on April 25, 2023, 2 L/ha of the B2 biostimulant were sprayed to the crop.
Specifically, two complete rows of plants (the inner rows) were harvested from each plot in the experimental plot on July 24, 2023. After that, the seeds were manually removed from the heads and their weight was recorded.

Soil Characteristics

The soil in the experimental field had organic matter levels of 1.86% at 30–60 cm and 2.91% at 0–30 cm, making it a very productive calcixerollic xerochrept soil (USDA, 1975).

Meteorological Data and Statistical Analysis

A meteorological station located on the University of Thessaly farm in Velestino, provides the meteorological data.

Finally, an analysis of variance (ANOVA) was conducted on the collected data within the sample durations using the statistical program GenStat (7th Edition) for all measured and derived variables. Steel and Torrie (1982) employed the $LSD_{0.05}$ test criteria to assess any disparities in the main and/or interaction effect means. Statistics allowed for a thorough analysis of the data, ensuring that any differences between the variables under investigation that were discovered were statistically significant and not merely accidental.

RESULTS AND DISCUSSION

Climatic Data

The study region has a Mediterranean climate with distinct seasonal variations. Winters are cold and humid, while summers are hot and dry.

In contrast, the lowest average ten-day temperature value was recorded during the first decade of February, with an average temperature value of 3.3°C. The highest average ten-day temperature value was recorded during the third decade of July at 29.6°C. The greatest recorded 10-day rainfall value, 45.17 mm, occurred in the first decade of April, while the lowest recorded 10-day rainfall values, zero, occurred in the first, second, and third decade of July. Figure 1 shows that 366.2 mm of rain fell between the day of sowing and harvest.

In summary, the research area experiences chilly, humid winters and hot, dry summers due to its Mediterranean climate. Temperatures and precipitation data collected throughout harvest seasons show different weather patterns all year round. Increased precipitation in other seasons counteracts the scarcity of the summer, making the environment more conducive to cultivation.

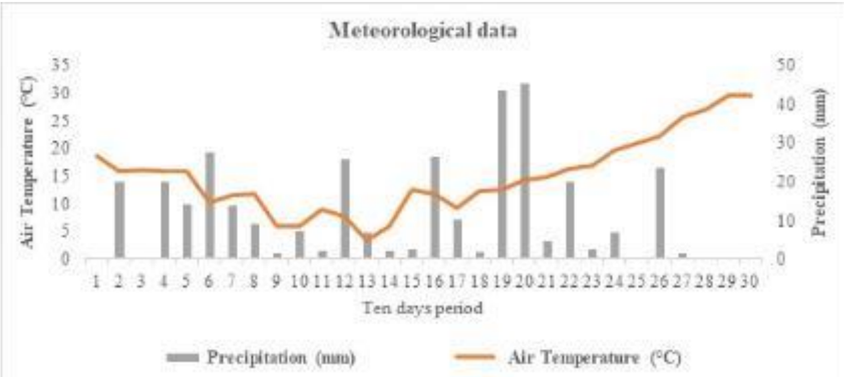

Figure 1. Temperature and precipitation (10-days mean values) occurring in studied site during the growing period of safflower (October 2022 - July 2023).

The data analysis conducted on the study factors and their interactions yielded a statistically insignificant difference in seed yield. Notably, nitrogen fertilization (N2) had a profound impact on seed yield, almost doubling it to reach a maximum of 1.3 tons per hectare, with a corresponding harvest index of 0.2. In contrast, the results of biostimulants treatments showed that the S treatment numerically outperformed the other treatments, yielding a seed output of 1.2 tons per hectare. Furthermore, the interaction between nitrogen fertilization and biostimulants (N2xS) resulted in an impressive seed yield of 1.5 tons per hectare. Interestingly, the analysis also revealed a statistically significant difference in seed oil content among the biostimulants treatments. This finding contradicts existing research that has demonstrated that nitrogen fertilizer can have a significant impact on plant biomass and seed production (Jaffar and Al-Refai, 2021; Mohamed et al., 2012). The discrepancy between the current study's findings and previous research may be attributed to differences in experimental design, environmental conditions, or genetic variability among plant species.

Table 1. Safflower seed yield (kg/ha) and harvest index.

	Seed Yield (kg/ha)	**Harvest Index**
Fertilization		
0N	651	0,15
8N	1258	0,20
$LSD_{.05}$	**ns**	**ns**
Biostimulants		
Control	906	0,18
A	761	0,15
S	1217	0,21
A+S	933	0,18
$LSD_{.05}$	**ns**	**ns**
Interaction		
0N*Control	563	0,16
0N*A	416	0,10
0N*S	942	0,20
0N*(A+S)	682	0,16
8N*Control	1249	0,20
8N*A	1106	0,20
8N*S	1492	0,22
8N*(A+S)	1185	0,20
$LSD_{.05}$	**ns**	**ns**
CV (%)	43,1	24,3

CONCLUSIONS

In summary, a Mediterranean climate characterizes the study region, with hot, dry summers and chilly, damp winters. Based on the data analysis, it was shown that nitrogen fertilization significantly increased seed output. Treatments with biostimulants also showed encouraging results, but the combination of biostimulants and nitrogen fertilization produced the maximum seed yield. Furthermore, the study's conclusions ran counter to earlier studies on the effects of nitrogen fertilizer on plant biomass and seed yield.

Based on the facts above, it can be concluded that safflower cultivation is not only climate change resistant but may also prove to be beneficial cultivation in the future. in the process of producing oil with therapeutic qualities, a fact that will become clear from more studies.

REFERENCES

Bijanzadeh E., Moosavi S.M., Bahadori F. (2022). Quantifying water stress of safflower (*Carthamus tinctorius* L.) cultivars by crop water stress index under different irrigation regimes. Heliyon, Vol. 8(3), 09010. https:// doi. org/ 10. 1016/j.

Davari K., Rokhzadi A., Mohammadi K., Pasari B. (2022). Paclobutrazol and Amino Acid-Based Biostimulant as Beneficial Compounds in Alleviating the Drought Stress Effects on Safflower (*Carthamus tinctorius* L.). Journal of Soil Science and Plant Nutrition. pp. 674-690.

du Jardin P. (2015). Plant biostimulants: Definition, concept, main categories and regulation, Scientia Horticulturae, Vol. 196, pp. 3-14.

Ergönül P.G., Özbek Z.A. (2020). Chapter 29 - Cold pressed safflower (*Carthamus tinctorius* L.) seed oil. Cold Pressed Oils Green Technology, Bioactive Compounds, Functionality, and Applications, pp. 323-333.

Jaffar R.A.A., and Al-Refai S.I. (2021). Effect distribution of plants and combination of fertilization (N.P.K) on some of growth, yield and its componant of safflower. (*Carthamus tintorius* L.). IOP Conf. Ser.: Earth Environ. Sci.735 012026. 10.1088/1755-1315/735/1/012026

Janmohammadi M. and Sabaghnia N. (2023). Effects of foliar spray of nano-micronutrient and growth regulators on safflower (*Carthamus tinctorius* L.) performance. Plant Nano Biology. pp. 100045.

Kumar S., Ambreen H., Variath M.T., Rao A.R., Agarwal M., Kumar A., Goel S., Jagannath A. (2016). Utilization of molecular, phenotypic, and geographical diversity to develop compact composite core collection in the oilseed crop, safflower (*Carthamus tinctorius* L.) through maximization strategy. Front. Plant Sci., Vol. 7, pp. 1554. 10.3389/fpls.2016.01554

Liu Li (2017) Summary of the study on *Carthamus tinctorius* L. Guangzhou Chem. Ind., Vol. 45(12), pp. 23-25-68

Mohamed S.J., Jellings A.J., Fuller M.P. (2012). effect of nitrogen on safflower physiology and productivity. Afr. Crop Sci. J., pp. 225-237.

Oleynikova E.M., Koltsova O.M., Mateyeva S.Z., Mateyeva A.E., Mirsaidov M.M. (2021). *Carthamus tinctorius* L. development and productivity under the influence of ecological and climatic factors. IOP Conference Series: Earth and Environmental Science. pp. 1-2.

Peng J.C. (1999). Development and utilization of safflower resources. Mod. Chin. Med. Vol. 9, pp. 45-46. 10.13313/j.issn.1673-4890.1999.09.021

Popov A.M. and Kang D. (2011). Chapter 118. Analgesic and Other Medicinal Properties of Safflower (*Carthamus tinctorius* L.) Seeds. Nuts and Seeds in Health and Disease Prevention, pp. 995-1002.

Ren C.X., Wu Y.Y., Tang X.H., Hu J., Chen J., Wu Q.H., Pei J. (2017). Safflower's origin and changes of producing areas. China J. Chin. Mater. Med., Vol. 42(11), pp. 2219-2222. 10.19540/j.cnki.cjcmm.2017.0101

Santos R.F., Bassegio D., Sartori M.M.P., Zannoto M.D., de Almeida Silva M. (2018). Safflower (*Carthamus tinctorius* L.) yield as affected by nitrogen fertilization and different water regimes. Acta Agronómica, pp. 264-269.

Steel R.G.D., Torrie J.H. (1982). Principles and Procedures of Statistics. A Biometrical Approach, 2nd ed.; McGraw-Hill, Inc.: New York, NY, USA pp. 633.

USDA. Soil Survey Staff. Soil Taxonomy. Basic System of Soil Classification for Making and Interpreting Soil Surveys. In Agricultural Handbook; USDA: Washington, DC, USA, 1975; Volume 466, p. 754.

Zhang L.L., Tian K., Tang Z.H., Chen X.J., Bian Z.X., Wang Y.T., Lu J.J. (2016). Phytochemistry and pharmacology of *Carthamus tinctorius* L. Am. J. Chin. Med., Vol. 44(2), pp. 197-226. 10.1142/S0192415X16500130

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ORGANOLEPTIC AND QUALITY PROPERTIES OF TOMATO FRUITS UNDER TREATMENT WITH MICROALGAE EXTRACTS

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ABSTRACT

The quality parameters of tomatoes can vary depending on different factors, including cultivar, fruits maturity stage, fertilisation and growing conditions. The research aimed to the evaluation of the organoleptic and quality properties of tomato fruits under treatment with microalgae extracts. Tomatoes (cultivar 'Belle' F1, Enza Zaden) were grown in peat (producer Laflora LTd, pH_{KCl} 5.5) in the conditions of polycarbonate greenhouse. From seedlings planting till start of harvesting plants were sprayed weekly (for a total of five times) with the solution of ethanol extractions of different microalgae species: *Spirulina* sp., *Dunaliella* sp., C*hlorella* sp., two concentrations of the extracts were compared with sprays with corresponding ethanol solution as a control. In total, nine plants per treatment were used. Yield was harvested 13 times from 30/09 till 23/12 once per week at the stage of full ripen. Colour components of L*, a*, b*, organoleptic properties, taste index and maturity index were determined. No negative effects of any tested treatments on organoleptic and quality properties as well as fruits` shape, diameter, number of locules were determined for tomato yield. Different fruit parameters including shape, diameter, number of locules, taste index and maturity index were determined. No negative effects of any tested treatments on organoleptic and quality properties as well as of tomato fruits were observed. Maturity index was higher for control treatment with drinking water (17.60), but the highest taste index (0.57) was observed for variant with *Spirulina*, concentration 10% v/v. The effect of microalgae extract on the organoleptic indicators of the fruit was not detected, which can be evaluated positively, and this moment indicates the stability of the cultivar.

Keywords: *Microalgae, Biostimulants, Quality parameters.*

INTRODUCTION

Tomato (*Solanum lycopersicum* L.) is one of the most economically important and widely cultivated vegetables in the world, consumed fresh or processed (Duckena at al., 2003; Alsina et al., 2023). Tomatoes` quality parameters can vary depending on different factors, including cultivar, growing conditions, fertilisation as well as fruits maturity stage. Concentrations of bioactive compounds in tomato fruits as well as their organoleptic properties can be genetically and environmentally determined (Carli et al., 2011).

Fruit colour is one of the most important attributes of fruit quality, especially in the case of choosing fresh tomatoes by consumers (Radzevičius et al., 2014; Tadesse et al., 2015; Ilahy et al., 2016;). It is observed, that colour is the primary factor that consumer evaluate before any fresh produce purchase (Ilahy et al., 2016). The complexity of tomato colour is due to the presence of a diverse carotenoid pigment system with appearance conditioned by pigment types and concentrations (Pandurangaiah et al., 2020).

By Felföldi et al. (2022), for increasing the economic efficiency, producers pay more attention to productivity of tomatoes than quality parameters, including organoleptic properties of fruits, especially savour. By Duma et al. (2019), medium sized tomatoes are less tasty, despite the tomato colour. As well as in other research of Duma et al. (2022) was observed, that tomatoes harvested in autumn contain more biologically active substances than harvested in spring.

Biostimulants from seaweeds have positive effect on the growth, yield and yield`s quality as well as on the tolerance of plants to abiotic and biotic stresses (Arioli et al., 2015; Shukla et al., 2019). By Santos de Paula et al. (2021), for mini tomato plants, treatment with algae extracts significantly affected the yield, diameter, length, colour and Brix degree of the fruits. But fruit volume and weight as well as the number of cracked fruits did not change.

The research aimed to the evaluation of the organoleptic and quality properties of tomato fruits under treatment with microalgae extracts.

MATERIAL AND METHODS

In August 2023, tomato seedlings (cultivar 'Belle' F1, Enza Zaden) were planted in 25 L pots, filled with peat (producer Laflora LTd., pH_{KCl} 5.5) and grown in polycarbonate greenhouse. Plants were sprayed weekly from seedlings` till start of harvesting, for a total of five times, with the solution of ethanol extractions of different microalgae species: *Spirulina* sp., *Dunaliella* sp., C*hlorella* sp. Two concentrations of the extracts 10% and 20% v/v were compared with sprays with corresponding ethanol solution as a control 2% and 4% v/v as well as with control spray with drinking water. In total, nine plants per treatment were used.

Yield was harvested 13 times from 30/09 till 23/12 once per week at the stage of full ripen. During vegetation, plants were regularly watered and fertilized, phytosanitary measures were provided. Additional lighting was provided by high-pressure sodium lamps as well as automatic ventilation was carried out by necessity. Plants were pruned by traditional scheme.

Colour components of L*, a*, b*, organoleptic properties, taste index and maturity index as well as fruits` shape, diameter, number of locules were determined for tomato yield.

Colour analysis. Colour of samples were measured in CIE L*a*b* colour system using a colorimeter ColorTec PCM (Accuracy Micro sensors Inc., USA). Before the measurement, the colorimeter was calibrated using a white reference tile and a light trap (black tile). Ten random tomato fruit were measured per each sample. Three coordinates CIE L*a*b* represent: the lightness of the colour L* = 0 means black and L* = 100 indicates diffuse white; negative value of a* indicate green while positive values indicate red; negative values of b* indicate blue and positive values indicate yellow (Radzevičius et al., 2014; Tarhan et al., 2010).

Morphometric and organoleptic indices. For tomato fruits such morphometric indices as shape, diameter, number of locules were determined by methodology of ECPGR descriptor (codes 7.2.2.5, 7.2.2.10, 7.2.2.31) (Descriptors..., 1996). Organoleptic indices of samples were evaluated at the stage of full ripen by 10 people. For tomato fruits, appearance, shape, aroma, taste, aftertaste and firmness were analysed using the marks from 1 to 10 (1 – very weak; 3 – medium; 5 – very high) (Kalnina et al., 2016).

Statistical analysis. Analysis of variance was used for data statistical processing, whereas the significance of differences between mean values was evaluated with p-value.

RESULTS AND DISCUSSION

For all variants slightly flattened fruit shape was dominant (Figure 1). For samples, fruits` diameter at the stage of full ripen was not significantly different ($p<0.05$). Maximal diameter (9 cm) was observed for fruits under treatment with *Spirulina* sp. extract, 20% v/v, for control variant with ethanol, 2% v/v as well as for control variant with drinking water. Minimal diameter (7 cm) was characterised for fruits under both variants with *Dunaliella* sp. extract.

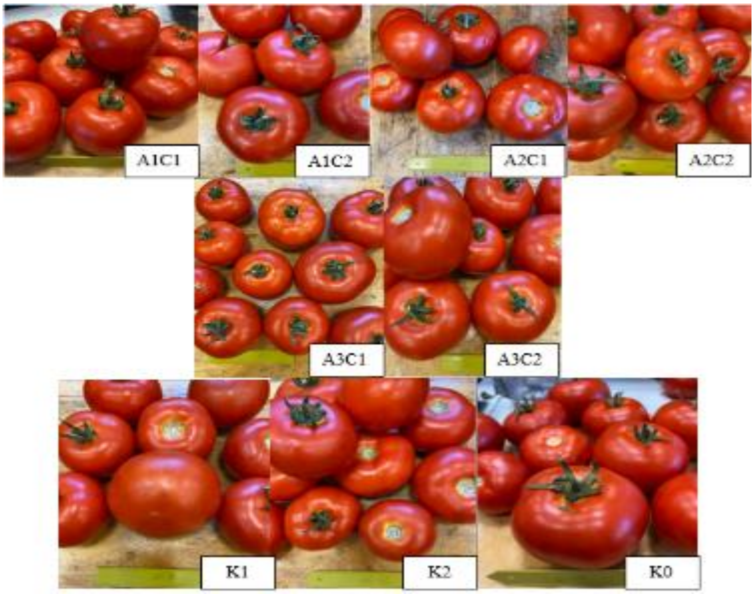

Figure 1. Appearance of tomato fruits according to variants:
A1C1 – *Spirulina* sp. extract, 10% v/v, A1C2 – *Spirulina* sp. extract, 20% v/v
A2C1 – *Dunaliella* sp. extract, 10% v/v, A2C2 – *Dunaliella* sp. extract, 20% v/v,
A3C1 – *Chlorella* sp. extract, 10% v/v, A3C12 – *Chlorella* sp. extract, 20% v/v,
K1 – control, ethanol, 2% v/v, K2 – control, ethanol, 4% v/v, K0 – drinking water.

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The two-colour space system known as L*a*b* is commonly used commercially for food colour measurement as it closely approximates the colour perception by humans. This system mathematically describes all perceivable colours in three dimensions and measures three values: the achromatic component L* (light vs. dark) and two colour descriptors a* (red vs. green) and b* (yellow vs. blue) values (Radzevičius et al., 2014). Differences between colour parameters presented in Table 1.

Table 1. Colour characteristics of the tomato fruits

Plant material	Colour parameter values		
	L*	a*	b*
Spirulina sp. extract, 10% v/v	33.05±2.21ab	33.77±0.94a	30.46±1.42a
Spirulina sp. extract, 20% v/v	31.09±2.03ab	34.60±1.53a	31.76±0.71a
Dunaliella sp. extract, 10% v/v	30.44±1.53ab	32.03±0.82a	26.25±1.63a
Dunaliella sp. extract, 20% v/v	31.47±.0.97ab	32.42±1.52a	27.28±0.82a
Chlorella sp. extract, 10% v/v	38.07±0.96c	30.94±1.84a	32.97±1.55a
Chlorella sp. extract, 20% v/v	33.19±0.78ab	32.81±0.92a	31.32±0.94a
Control, ethanol, 2% v/v	28.93±0.72a	31.15±1.53a	27.03±1.22a
Control, ethanol, 4% v/v	33.80±1.04bc	33.70±1.74a	31.24±1.11a
Drinking water	32.55±1.22ab	32.19±1.08a	28.37±1.96a

** Values represent the mean of ten replicates± standard deviation. Values, marked with the same letter, are not significantly different (p>0.05).*

The highest colour component L* value, related to the lightness, were found for *Chlorella* sp. extract, 10% v/v, but the lowest value – for control variant with ethanol, 2% v/v (indicates a darker colour intensity). By López Camelo and Gómez (2004) this characterises changes of colour from pink to full red. For variants, L* colour values were not significantly different ($p<0.05$). The highest values of colour components a* (indicates the redness) were obtained for samples under *Spirulina* sp. extract, 20%, but lowest – under *Chlorella* sp. extract, 10% v/v. In experiment, it was proved that the colour a* values were not significantly different ($p<0.05$), that means not change in the context of the redness.
For tomato samples under treatment with *Chlorella* sp. extract, 10% v/v the highest colour component`s b* value was determined, but the lowest was observed under *Dunaliella* sp. extract, 10% v/v. The difference between the lowest and highest colour b* value (related to yellowness) also was not significant ($p<0.05$). Results can be explored by the fact that carotenes reach their highest concentration before full ripening, where lycopene (red colour) and β-carotene (orange colour) achieve their peaks (López Camelo and Gómez, 2004). Obtained L*, a*, b* values are in accordance with data about tomatoes grown under different geographical areas (Ilahy et al., 2016).
Information about taste index and maturity index is presented in Figure 2. Per variants, both indices were significantly different ($p<0.05$).

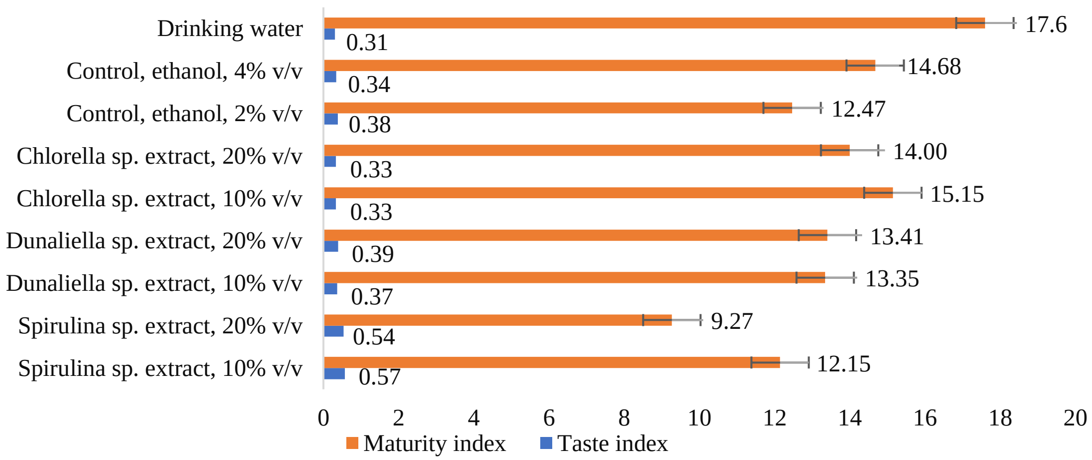

Figure 2. Taste index and maturity index for tomato fruits

In this research, maturity index was higher for control treatment with drinking water, but the highest taste index was observed for variant with *Spirulina*, concentration 10% v/v. Results were similar to data, described in research of Alsiņa et al. (2023), but observed average taste index was lower than described by Dūma et al. (2022).

During harvesting period, for fruits at the stage of full ripen, number of locules were not significantly different ($p>0.05$): five symmetric locules (Figure 3) were characterised for tomato fruits.

Figure 3. Tomato fruit in cross section, in average for all variants.

Figure 4 presents the results of organoleptic testing of tomato fruits. It is interesting, that colour was the least evaluated indice. Per variants, scores were not significantly different ($p>0.05$), it can be described with stability of the cultivar 'Belle' to different growing conditions, including application of extracts, explored in this research.

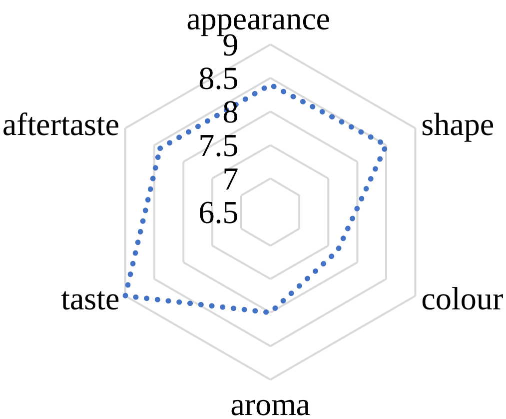

Figure 4. Organoleptic properties of tomato fruits, in average for all variants.

No negative effects of any tested treatments on quality properties of tomato fruits were observed.

CONCLUSIONS

Under treatments with ethanol, 2% v/v, tomato fruits were darker, but under *Chlorella* sp., 10% v/v tomatoes were lighter. The effect of microalgae extract on the organoleptic indicators of the fruit was not detected, which can be evaluated positively, and this moment indicates the stability of the cultivar. No negative effects of any tested treatments on tomato fruits` quality were observed.

ACKNOWLEDGMENTS

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REFERENCES

Alsina I., Dūma M., Dubova L., Alksnis R., Dučkena L., Erdberga I., Harbovska T., Avotiņš A. (2023). Determination of tomato quality with hyperspectral imaging. Agronomy Research, vol. 21(3), pp. 1035–1048.

Arioli T., Hepworth G., Farnsworth B. (2020). Effect of seaweed extract application on sugarcane production. Proceedings of the Australian Society of Sugar Cane Technology, vol. 42, pp. 393–396.

Carli, P., Barone, A., Fogliano, V., Frusciante, L., Ercolano, M. R. (2011). Dissection of genetic and environmental factors involved in tomato organoleptic quality. BMC Plant Biology, vol. 11. file:///C:/Users/Lietotajs/Downloads/1471-2229-11-58.pdf

Descriptors for tomato (Lycopersicon spp.) (1996). International Plant Genetic Resources Institute (IPGRI). 44 p.

Duckena L., Alksnis R., Erdberga I., Alsina I., Dubova L., Duma M. (2023). Non-destructive quality evaluation of 80 tomato varieties using Vis-NIR spectroscopy. Foods, vol. 12. https://doi.org/10.3390/foods12101990

Duma M., Alsina I., Dubova L., Augspole I., Erdberga I. (2019). Suggestions for consumers about suitability of differently coloured tomatoes in nutrition. FoodBalt 2019: 13th Baltic conference on food science and technology "Food. Nutrition. Well-Being": conference proceedings, Jelgava, May 2-3, 2019 / Latvia University of Life Sciences and Technologies. Faculty of Food Technology, pp. 261-264.

Dūma M., Alsiņa I., Dubova L., Gavare D., Erdberga I. (2022). Quality of different coloured tomatoes depending on the growing season. Proceedings of the Latvian Academy of Sciences. Section B, Vol. 76, No. 1 (736), pp. 89–95.

Felföldi Z., Ranga F., Roman I. A., Sestras A. F., Vodnar D. C., Prohens E., Sestras R. E. (2022). Analysis of physico-chemical and organoleptic fruit parameters relevant for tomato quality. Agronomy, Vol. 12, 1232.

Ilahy R., Siddiqui M.W., Tlili I., Piro G., Lenucci M.S., Hdider C. (2016). Functional quality and colour attributes of two high-lycopene tomato breeding lines grown under greenhouse conditions. Turkish Journal of Agriculture – Food Science and Technology, Vol. 4(5), pp. 365-373.

Kalnina I., Sterne D., Strautina S. (2016). Strawberry (Fragaria ananassa) cultivar ‘Rumba’ assessment under the northern climatic conditions. Acta Horticulturae, vol. 1139, pp. 259-264.

López Camelo A.F., Gómez P.A. (2004). Comparison of colour indexes for tomato ripening. Horticultura Brasileira, vol. 22(3), pp. 534-537.

Nielsen S. (2003) Food analysis (3rd ed.). New-York, Kluwer Academic/Plenum Publishers, 534 p.

Narvez B., Letard M., Graselly D., Jost M. (1999) Les criteres de qualite de la tomate. Infos-Ctifl, vol. 155, pp. 41-47.

Pandurangaiah S., Sadashiva A. T., Shivashankar K. S., Sudhakar Rao D. V. (2020). Carotenoid content in cherry tomatoes correlated to the color space values L*, a*, b*: a non-destructive method of estimation. Journal of Horticultural Sciences, vol. 15, No. 1, pp. 27-34.

Radzevičius A., Viškelis P., Viškelis J., Karklelienė R., Juškevičienė D. (2014). Tomato fruit color changes during ripening on vine. World Academy of Science, Engineering and Technology**.** International Journal of Bioengineering and Life Sciences**,** 8(2), pp. 112-114.

Santos de Paula B., Feltrim D., Hörz Engel D. C., Corte Baptistella J. L., Rodrigues M., Engel E., Mazzafera P. (2021). Algae-based biostimulants increase yield and quality of mini tomatoes under protected cultivation. JSFA Reports, https://onlinelibrary.wiley.com/doi/10.1002/jsf2.36

Shukla P, Mantin E, Adil M, Bajpai S, Critchley A, Prithiviraj B (2019) Ascophyllum nodosum-based biostimulants: sustainable applica-tions in agriculture for the stimulation of plant growth, stress toler-ance, and disease management. Frontiers in Plant Sciences, https://www.frontiersin.org/journals/plant-science/articles/10.3389/fpls.2019.00655/full

Tadesse T. N., Ibrahim A.M., Abtew W.G. (2015). Degradation and Formation of Fruit Color in Tomato (*Solanum lycopersicum* L.) in Response to Storage Temperature. American Journal of Food Technology, vol. 10(4), pp.147-157.

Tarhan S., Telci I., Tuncay M.T, Polatci H. (2010). Product quality and energy consumption when drying peppermint by rotary drum dryer. Industrial Crops and Products, vol. 32(3), pp. 420-427.

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DEVELOPMENT OF THE EMISSIONS TRADING SYSTEM OF THE EUROPEAN UNION

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ABSTRACT

The Emissions Trading System of the European Union (EU ETS) is a milestone of the EU's policy to combat climate change. It is established in 2005 and is its key instrument for reducing greenhouse gas emissions (GHG) cost-effectively. The EU ETS is the world's first major carbon market and remains the biggest one. It is established with Directive 2003/87/EC as a response to the commitments under the United Nations Framework Convention on Climate Change (UNFCCC) which is the first international agreement to recognize the detrimental effects of GHG emissions caused by human activity. Its objective is to stabilize GHG concentrations in the atmosphere to a point where they won't cause further deepening of climate change. The EU aims at achieving climate-neutrality and net-zero GHG emissions by 2050, but global temperature continues to rise, and so does the magnitude and frequency of natural disasters. Along with the mitigation of adverse climate effects, there are numerous advantages to lowering GHG emissions, such as improved human well-being and air quality, preserving biodiversity, and secure energy supply. Achieving zero-carbon emissions without sacrificing economic growth is essential to ensuring long-term sustainability. To meet the climate targets, additional reforms are required beyond the efforts accomplished so far. It is imperative to further decrease free emission allowances, improve their regulation, and encourage the shift to renewable energy sources. The main objective of the paper is to examine the development of the EU ETS in relation to the changes and updates of EU strategies and long-term environmental goals.

Keywords: *emissions trading system of the European Union, emissions trading, greenhouse gas emissions, climate change.*

INTRODUCTION

Europe has been warming twice as much as the global average since the 1980s, with deepening impacts on the social, economic and ecological systems. The late spring and summer heatwaves in 2022 led to more than 61 000 deaths in Europe and to numerous wildfires which affected more than 182 000 ha across the EU, 40 % above the 2003-2022 average. The heatwaves were followed by heavy precipitation and unprecedented floods with many fatalities (COM (2023) 653 final). The last four decades have been warmer than any previous decade, due to human activity.

Compared to the 1850-1900 period, the 2003-2012 was warmer with 0.78^{o} C and the 2011-2020 period – with 1.09^{o} C (UN, 2021). At the same time, according to the Emissions Database for Global Atmospheric Research (EDGAR) (EC, 2023), the global levels of GHG emissions, which are considered as the main cause of global warming and climate change, continue to rise. On the other hand, the EU has made progress in lowering the levels of GHG emissions, which is a step towards its goal of having a net-zero GHG emissions economy and becoming climate neutral by 2050, but achieving tangible and long-lasting global impact requires global commitment.

The United Nations Framework Convention on Climate Change (UNFCCC) (UN, 1992) is the first international document acknowledging the detrimental effects of GHG emissions caused by human activity, pointing out that since GHG concentrations in the atmosphere have been significantly rising. It could be expected that the Earth's surface and atmosphere will warm further on average, which would result in negative effects on both humankind and natural ecosystems. The UNFCCC claims that while historically and currently, the largest share of global emissions has originated in developed countries, emissions in developing countries are relatively low, but it is expected their share to increase to meet their development needs. Due to the global nature of climate change, all nations must work together and take part in an appropriate and effective international response, according to their unique social and economic circumstances, as well as their shared but distinct responsibilities.

Despite the efforts at international level, recent studies show that global emission levels continue to rise, which results in escalation in climate change (COM (2023) 653 final). So, in the early 2050s, worldwide net-zero CO_2 emissions must be attained if the goal is to keep temperature increases to 1.5^{o} C with little to no overshoot. By 2030, global GHG emissions must decrease by 43 % and by 84 % compared to 2019 levels.

More than three decades after the UNFCCC, the EU Climate Action Progress Report 2023 (COM (2023) 653 final) confirms that anthropogenic GHG emissions are causing global warming, which is escalating the frequency and intensity of climate and weather extremes. The effects of global warming will only get worse with each degree of warming, so stopping it and preparing for its effects requires immediate global climate action.

As climate change is already affecting every region across the globe, the year 2021 marks the beginning of the first global stocktake process, which will assess the collective progress towards the Paris Agreement goals (UN, 2021). As of 2021, the aim is for at least 20 % of key players across sectors to take a part in transforming the sector in line with the Climate Action Pathways by 2023. It is estimated that systemic change would be accelerated to a point where it can no longer be stopped by creating sufficient activity among a critical number of actors within a sector. By September 2021, actors across 18 designated sectors have risen to the challenge, and reached or surpassed the 20 % mark. The progress is monitored by the Global Climate Action Portal (GCAP), launched in 2014 and at the end of the first global stocktake period in 2023, the results lead to the conclusion that a greater ambition and accelerated action is needed to limit global warming to 1.5 °C by 2030 and build resilience (UN, 2023).

The main objective of the paper is to examine the development of the EU ETS in relation to the changes and updates of EU strategies and long-term environmental goals.

MATERIALS AND METHODS

This paper is based on a detailed review of reports, analysis and official documents as action plans, declarations, programs and data related to the measurement of GHG emissions levels, emission trading system, its implementation and the policies aimed at supporting the transition towards achieving climate-neutrality and an economy with net-zero GHG emissions by 2050.

Although the UNFCCC sets the target of reducing GHG emissions, the first steps and commitments towards the implementation of that target followed the adoption of the Kyoto Protocol in 1997 and its entry into force in 2005. In Article 3 a mandatory emission reduction objective of an average of 5% below 1990 levels over the first commitment period of 2008–2012 is established (UN, 1997). The Doha Amendment to the Kyoto Protocol (UN, 2012) marks the second commitment period, 2013–2020 with updated lists of GHGs to be reported on, new pledges, and updated versions of other items related to the initial commitment period. The next step in combating climate change and its negative effects is the Paris Agreement (UN, 2015). Its goal is to limit global average temperature increase to below 2°C above pre-industrial levels with the ambition to limit it to 1.5°C by the end of this century. To achieve this, GHG emissions must peak before 2025 and decline 43% by 2030.

RESULTS AND DISCUSSION

The scheme for GHG emission allowance trading is established with Directive 2003/87/EC (EU, 2003). According to Article 10, for the first three years period, starting on January 1, 2005, Member States should provide at least 95 % of the allowances free of charge. For the next five years period, starting on January 1, 2008, the free of charge allowances should be at least 90 %, as the percentage of free of charge allowances must decrease in each subsequent period. The decrease should be by 1.74 % for the 2013-2020 period, by 2.2 % for the period from 2021 to 2023, by 4.3% between 2024 and 2027 and by 4.4% from 2028. The Directive also states that agreements for the mutual recognition of allowances between the EU ETS and other GHG emissions trading schemes should be concluded with third countries that accepted the Kyoto Protocol.

In order to meet the commitments made under the European Climate Law, the European Green Deal, and the Paris Agreement to a greater extent, Directive 2003/87/EC have been amended several times, as the latest changes were adopted in Directives (EU) 2023/958 and 2023/959 in which some additional measures regarding stationary installations, aviation and maritime transport are included. Directive 2023/958 focuses on further emissions reduction from the aviation sector with the aim all aviation allowances to be traded by 2026 (EU, 2023), while Directive 2023/959 represents overall reform of the existing EU ETS with increased level of emission reduction of 62 % (previously 43 %), inclusion of new sectors and additional reduction of the annual cap on allowances (EU, 2023).

The changes in the GHG emissions levels are monitored by the European Commission, Joint Research Centre (JRC), the International Energy Agency (IEA), and the Emissions Database for Global Atmospheric Research (EDGAR, 2023) provides an independent, worldwide database for human-caused greenhouse gas emissions and air pollution (fig. 1).

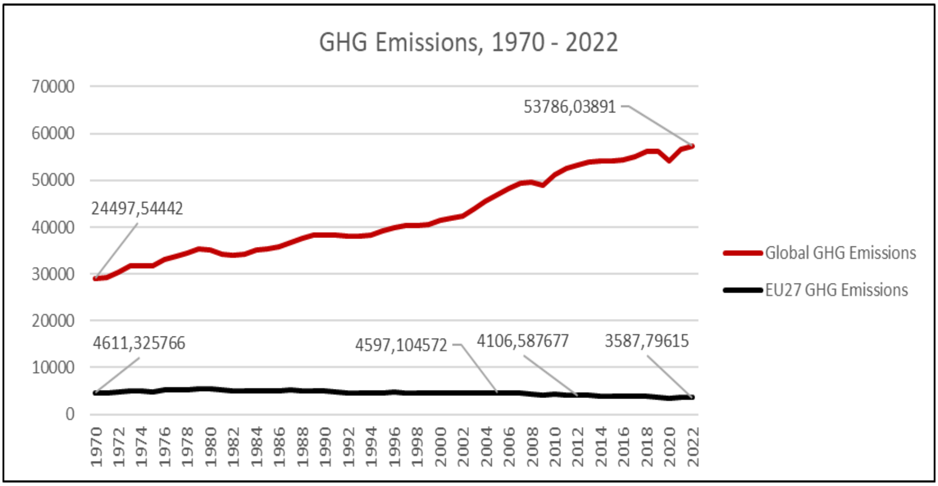

Figure 1. GHG emissions for the 1970-2022 period, Mt CO_2eq/year

Source: Emissions Database for Global Atmospheric Research, European Commission, 2023

As represented in fig. 1 above, global levels of GHG emissions continue to increase, while their levels in the EU are decreasing. After the launch of the EU ETS in 2005, there is a decrease of 490,5 Mt CO_2eq by 2012, which exceeds the commitment of 8 % emission reduction for the period. The trend continues and the decrease for the 2005-2022 period is 1 009,3 Mt CO2eq, or about 28 %. These results bring the EU closer to achieving the goal of a 55 % emissions reduction by 2030, but still, the EU is far from reaching the 2050 net-zero GHG emissions target and greater efforts and better international cooperation are needed. Still, this data validates the EU's leadership in climate action as the reduction of GHG emissions at EU level does not lead to negative impacts on the competitiveness and the performance of regulated actors.

The reduction of GHG emissions at EU level, while their global levels are constantly growing, calls for improved international cooperation. For achieving a global climate impact, more comprehensive change, better international cooperation and inclusion of more sectors and installations is needed.

According to Dechezleprêtre et. al. (Dechezleprêtre et. al., 2023), the EU ETS has a significant impact on carbon emissions and economic performance in Europe. It reduces carbon emissions by around 10 % and led to an increase in revenues and fixed assets for regulated firms. The EU ETS appears to have led to some reductions in carbon emissions without negative impacts on the economic performance of regulated firms and the competitiveness of European industry. But it has been found to have some carbon leakage (Wang et. al., 2024), primarily in the carbon content of imports to EU ETS participants. It also showed significant decreases in export values, carbon intensity, and carbon content for ETS countries. The total carbon embodied in trade has increased due to the ETS, as the carbon leakage offsets decreases in exported carbon content. At the same time, the ETS significantly inhibits carbon emission intensity (CEI) and restricts it through economic intensity promotion and green technology innovation (Liu et. al., 2024). However, the ETS policy produces different carbon reduction effects for different cities, with edge cities having a significant but negligible impact on CEI.

Exploring the impact of emission trading schemes, energy innovation, technology transfer, population growth, and inflation on economic performance, Zhang et. al. (Zhang et. al., 2024) come to the conclusion that carbon taxes have a positive impact to regulating businesses and providing high-quality resources. Renewable energy production reduces fossil fuel consumption, while technology transfer increases advanced technology use. High population growth and government efforts during inflation improve economic practices, leading to better performance.

On the other hand, a study by Adamolekun et. al. on the impact of membership in emission trading schemes (ETS) on corporate environmental practices show that firms that are members of ETS emit more carbon and have more environmental scandals, suggesting that they may join for greenwashing purposes. At the same time they are more effective in their carbon reduction efforts (Adamolekun et. al., 2024). However, membership could discourage a quick transition to sustainable operations and that firms that exit the scheme continue to emit more.

While the amount of free emission allowances has decreased, the price of traded allowances has increased by 45.5 € per ton for the 2005-2024 period (fig. 2).

As shown in fig. 2 below, since the establishment of the ETS in 2005, there have been two main deviations from the trend of gradual increase in price of emission allowances. The first one is in 2007, just before the start of the first commitment period 2008-2012, when the values were close to 0 € per ton. This suggests that very few traded allowances were available at the time, and sales did not occur prior to 2008 when free allowances were awarded. The first peak occurred in the middle of 2008, when prices were close to 30 € per ton. Prices stayed below this level almost until the end of 2020. After the economy started to recover from the COVID-19 pandemic, there was a second peak, and in February 2023 the price was above 106 € per ton, before dropping again to reach 70.25 € per ton in July 2024.

Figure 2: EU carbon permit prices, 2005 – 2024, € per ton

Source: https://tradingeconomics.com/commodity/carbon, *07. 2024*

The simultaneous increase in both price levels of GHG emission allowances and levels of GHG emissions could be indicative that the prices are not high enough to stimulate a faster transition to more sustainable and low-carbon production. On the other hand, the reduction of the total number of allowances also contributes to the price increase. However, more stringent measures in the allocation and reporting of emissions are needed, as well as joint efforts at both regional, national and global levels, to achieve climate targets.

CONCLUSION

Despite the increasingly alarming scientific data on climate change and its consequences, actions in this area continue to be insufficient to achieve real results. While emission levels are reduced within the EU, their global levels are continuously rising. Since the EU ETS's launch in 2005, there has been a decrease of about 28 % of GHG emissions levels in the EU by 2022. This brings EU closer to achieving its goal of a 55 % emissions reduction by 2030, but to meet the 2050 net-zero GHG emissions objective, EU will need to accelerate the transition of all sectors towards more sustainable production models and set more ambitious emission reduction targets.

On the other hand, the reduction of GHG emissions at EU level, while their global levels are constantly growing, indicates the need for improvement of international cooperation. As climate change is a global issue, achieving long-lasting global impact requires global commitment. In order resilience to be created and the global warming to be limited to 1.5 °C by 2030, more ambitious and rapid actions should be undertaken. In order for governments and other stakeholders to make the shift toward a net-zero resilient society, a collaborative regulatory framework is needed. Higher pricing of emission allowances could also contribute to encouraging the actors to shift towards more sustainable production processes.

REFERENCES

A. Dechezleprêtre, D. Nachtigall, F. Venmans, The joint impact of the European Union emissions trading system on carbon emissions and economic performance, Journal of Environmental Economics and Management, Volume 118, 2023, ISSN 0095-0696, available at: https://www.sciencedirect.com/science/article/pii/S0095069622001115

COM (2023) 653 final, EU Climate Action Progress Report 2023, Brussels, 24.10.2023, available at: https://eur-lex.europa.eu/legal-content/EN/TXT/?uri=CELEX%3A52023DC0653

Directive (EU) 2023/959 of the European Parliament and of the Council amending Directive 2003/87/EC and Decision (EU) 2015/1814, Official Journal of the European Union, L 130/134, 2023, available at: https://eur-lex.europa.eu/eli/dir/2023/959/oj

Directive (EU) 2023/958 of the European Parliament and of the Council amending Directive 2003/87/EC as regards aviation's contribution to the Union's economy-wide emission reduction target and the appropriate implementation of a global market-based measure, Official Journal of the European Union, L 130/115, 2023, available at: https://eur-lex.europa.eu/eli/dir/2023/958/oj

Directive 2003/87/EC of the European Parliament and of the Council for establishing a scheme for greenhouse gas emission allowance trading within the Community, Official Journal of the European Union, L 275/32, 2003, available at: https://eur-lex.europa.eu/legal-content/EN/TXT/?uri=celex%3A32003L0087

Doha Amendment to the Kyoto Protocol, UN, 2012, available at: https://unfccc.int/process/the-kyoto-protocol/the-doha-amendment

EDGAR (Emissions Database for Global Atmospheric Research) Community GHG Database, a collaboration between the European Commission, Joint Research Centre (JRC), the International Energy Agency (IEA), and comprising IEA-EDGAR CO_2, EDGAR CH_4, EDGAR N_2O, EDGAR F-GASES version 8.0, (2023) European Commission, JRC (Datasets), available at: https://edgar.jrc.ec.europa.eu/

G. Adamolekun, F. F. Adedoyin, A. Siganos, Firm-level pollution and membership of emission trading schemes, Journal of Environmental Management, Volume 351, 2024, ISSN 0301-4797, available at: https://www.sciencedirect.com/science/article/pii/S0301479723027585

Jiayu Liu, Feng Xu, Yan Lv, How an emission trading system affects carbon emissions? Evidence from the urban agglomeration in the Middle Reaches of the Yangtze River, China, Ecological Indicators Journal, Volume 160, 2024, ISSN 1470-160X; available at: https://www.sciencedirect.com/science/article/pii/S1470160X24003224

Kyoto Protocol to the United Nations Framework Convention on Climate Change, UN, 1997, available at: https://unfccc.int/documents/2409

M. Wang, T. Kuusi, Trade flows, carbon leakage, and the EU Emissions Trading System, Energy Economics Journal, Volume 134, 2024, ISSN 0140-9883, available at: https://www.sciencedirect.com/science/article/pii/S0140988324002640

Paris Agreement, UN, 2015, available at: https://unfccc.int/process-and-meetings/the-paris-agreement

United Nations Framework Convention On Climate Change, UN, 1992, available at: https://unfccc.int/process-and-meetings/what-is-the-united-nations-framework-convention-on-climate-change

Yearbook Of Global Climate Action 2021, UNFCCC secretariat, UN, 2021, ISBN: 978-92-9219-199-3, available at: https://unfccc.int/

Yearbook Of Global Climate Action 2023, UNFCCC secretariat, UN, 2023, ISBN: 978-92-9219-204-4, available at: https://unfccc.int/

YunQian Zhang, Ching-Chi Hsu, What role do emission trading schemes, energy innovation, and technology transfer play in sustainable recovery? A perspective from BRICS economies, Geoscience Frontiers Journal, Volume 15, Issue 4, 2024, ISSN 1674-9871, available at: https://www.sciencedirect.com/science/article/pii/S1674987124000021

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EVALUATION OF TWO METHODS OF TREATING *CROCUS SATIVUS* PATHOGENS

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ABSTRACT
Crocus (*Crocus sativus* L.) is a very important plant species for Western Macedonia, but also for Greece.In recent years, the flowering of *Crocus sativus* L. has shown a declining trend due to various causes including fungal infections. So it is important to find methods to deal with *Crocus sativus* pathogens. The research has been conducted in the area of Kozani, a city where crocus has been cultivated for the last 300 years. The aim of this research was the evaluation of two methods concerning the respective response of the crop. For this purpose, a physical method (sundisinfection), a chemical one (with systemic fungicide) and the control were installed. In addition, two different sowing depths (15, 20 cm) were tested to find the most suitable one. The experimental design was RCB with four replications. Specifically, the flowers of the crocus were measured and the number of infected bulbs from each experimental plot as well.The results showed that the number of flowersobtained by the two treatments (sundisinfection, chemical method) and the control were very close. Regarding the infected bulbs the most effective method was the chemical one and the most suitable sowing depth was that of 20 cm, except the control. Higher stigma yield was obtained from the control and the sun disinfection.

Key words:*crocus,sun disinfection, yield, sowing depth.*

INTRODUCTION
Crocus (*Crocus sativus* L.) is a very important plant species for Western Macedonia, and for Greece as wellbecause it is of great economic interest due to its use as a spice (saffron) and for medicinal purposes (Vogdopoulos and Lazaridou 2022). Saffron is derived from the stigma of *Crocus sativus*, a sterile autotriploid herb belonging to the family *Iridaceae* (Shah and Tripathi 2009, Ambardar and Vakhlu 2013).
Globally, the area under saffron cultivation has shown significant decline due to several biotic and abiotic factors. Among the various biotic constraints, bulb rot has been recognized as a major limiting factor for successful cultivation of crocus in both traditional and non-traditional areas. The disease is also known as dry rot, brown rot, violet rot, death blight and yellows. Infected plants die early, resulting in the

reduction in size and number of daughter bulbs and flowers. This leads to the reduction in flowering period and subsequently to low yield and poor quality of saffron. Pathogenic fungi infect the bulbs by penetrating the protective sheaths, subsequently converting the white-colored bulb surface to yellow and ultimately to black, resulting in the rotting and death of the bulbs. Under moist conditions, the infection progresses very gradually and ultimately causes drying of the infected parts. In newly infested fields, the disease first occurs in small patches that gradually enlarge with each passing year until the whole field is infested. Unfortunately, the origin of the disease and the conditions that contribute to its development remain unknown. Several species of Penicillium have been isolated from infected crocus bulbs. *Rhizopus nigricans* has been isolated from injured bulbs of *Crocus sativus* L. (White et al. 2002). The genus *Fusarium* includes several species of fungi and spreads rapidly in soils and organic substrates. One of the pathogens of this genus is *Fusarium oxysporum* which is the most destructive disease ofcrocusand more than 100 plant species (Berrocal Lobo and Molina 2008) and has caused severe losses in Italy (Cappelli 1994). Of the pathogens isolated from saffron, *Fusarium* has been detected in many different saffron growing regions causing the highest bulb losses. Infected plants die early, resulting in reduced yield, quality, and flower and stigma production (Palmero et al. 2014). *Rhizoctonia* and *Sclerotium fungi* are soil basidiomycetes, which cause serious diseases in saffron. *Phytium* species are soil pathogens, which under conditions of increased moisture cause disease in many plants, including saffron plants (de Souza et al. 2003). Therefore it is important to find methods to deal with *Crocus sativus* pathogens.

Chemical soil disinfection, when it is applicable, it is often the only way out of dealing with a large number of soil pathogens. As a pre-emergence application, it drastically affects their survival soil pathogens, while disinfection with sublethal doses of disinfectants in combination with non-chemical methods such as sun disinfection are considered as biologically milder methods (Katan, 1981). Sun soil disinfection is a recent agricultural phytopathological soil disinfection technique. The method is based on the utilization of solar radiation by using transparent polyethylene plastics during the warm period of the year (Katan & DeVay 1991).Sun disinfection effectively reduces pathogen populations to a fairly large extent (30-50 cm). The presence of moisture is also considered an important factor for the successful application of sun disinfection. The actual sensitivity of the various microorganisms increases with the level of humidity.

This research was undertaken to study the effectiveness of two different methods treating the pathogens of crocus andhow two different sowing depths influence their effectiveness.

MATERIALS AND METHODS

The experiment was established in the area of Kozani in Greece, in a field thathas proven to be contaminated with pathogenic organisms. The treatments used included a physical method (sun disinfection), a chemical one (with systemic fungicide) and the control (no application). In addition, two different sowing depths (15, 20 cm)

were tested to find the most suitable one. The experimental design was RCB with four replications. Regarding the sun disinfection method, a special 3-layer impermeable sheet with a thickness of 30-35μm, Orgasum, was used, which significantly reduces chemical losses during soil solar disinfection. Its permeability to chemicals is 100-200 times less than that of a simple polyethylene sheet of the same thickness. The sun disinfection method was installed onthe 21st ofJuly, 2022 and completed on the 10th of September, 2022. Regarding the chemical method, the systemic fungicide Neotopsin 70 WG was used, which was sprayed at the depth where the crocus was sowed, with a special machine that has the ability to penetrate the soil and spray at the desired depth. No treatment was applied on the control
The sowing of saffron took place on the 17th of September,2022, one week after the application of sun disinfection. Thetraditional way of planting (saffron planter) was used.In order to evaluate the effectiveness of theplant protection methodsthe flowers of the crocus were measured. Additionallya sample of 25 bulbs was taken from each experimental plot separately, which was examined in the Laboratory, both for the isolation and identification of pathogens and for the effectiveness of theplant protection methods.

RESULTS AND DISCUSSION

A phytosanitary check was carried out onall the samples studied for the presence of both external and internal symptoms on the saffron bulbs. A small percentage of infestation was observed in all the samples tested, while in the majority of them the affected bulbs presented mainly external changes. In particular, in the treatment of sun disinfection at the depth of 15 cm and 20 cm, the presence of both external and internal necrotic spots was 12%, and 8% respectively.Regarding the chemical method, the percentage of infestation at the depth of 15 cm and 20 cm, the presence of both external and internal necrotic spotswas found to be 9% and 6% respectively. Concerning the control the external lesions as necrotic spots at the depth of 15 cm and 20 cmwas 12% and 8% respectively, while there was no lesions inside the bulb(Table 1).

Table 1. Infection frequency in collected bulbs of *Crocus sativus* L. in three different treatments (plant protection methods)

Treatments	No of samples	Internal symptoms %	External symptoms %
Control 15cm depth	93	0	12
Control 20 cm depth	93	0	8
Sun disinfection 15 cmdepth	104	12	12
Sun disinfection 20 cm depth	92	8	8
Fungicide 15cm depth	93	9	9
Fungicide20 cm depth	94	6	6

As can be seen in the table above, in the control at both sowing depths (15, 20 cm), there are only external infestations and no internal lesions were observed. On the other hand, in the two other treatments used (sun disinfection, and chemical application), external and internal lesions were observed. This is because thesetreatments (sundisinfection, and fungicide) require irrigation for their proper effectiveness, during their installation in the field. The moisture that was created may have activated the pathogenic organisms, resulting in the acceleration of the start of their biological cycle, while in the experimental plots of the control, there was no moisture at all and the pathogenic organisms were deactivated. Tjamos and Faridis (1980) and Tjamos (1984) reported that sun disinfection, alone or in combination even with reduced doses of methyl bromide, successfully treated the fungus *Pyrenochaeta lycopersici* in a greenhouse experiment and the fungus *Verticillium dahliae* *(*Tjamos and Paplomatas 1987). Tjamos and Paplomatas(1988) also reported the long-term action of sun disinfection. Therefore, in our researchthe effectiveness of sun disinfection can be proven in the coming years.

Regarding the sowing depth in all treatments including the control, reduced infestation was observed at the 20 cm sowing depth compared to the 15 cm. Comparing the two methods (sun disinfection, and fungicide), less damage was observed in the chemical method (9 and 6% internal and external symptoms instead of 12 and 8%). Regarding the infected bulbs the mosteffective method was the chemical one and the most suitable sowing depth was that of 20 cm.

The results showed that the number of flowers obtained by the two treatments (sun disinfection and control) were very close both in total and at each of the two sowing depths separately. Slightly fewer flowers were obtained by the chemical method in total (Table 2). It should be emphasized that in both sun disinfection and control, more flowers were obtained from the 20cm sowing depth, while in the chemical method more flowers were obtained from the 15 cm sowing depth. So the appropriate sowing depth is dependent on the method applied. Higher stigma yield was obtained from the control and the sun disinfection.

Table 2.Number of flowersin three different protectionmethodsand two different sowing depth

Treatments	Number of flowers Sowing depth 15 cm	Number of flowers Sowing depth 20 cm	Total Number of flowers
Control	1705	1948	3653
Chemical method	1876	1561	3437
Sun disinfection	1729	1918	3647

CONCLUSION

It was concluded that concerning the infected bulbs the most effective method was the chemical one, except the control, and the most suitable sowing depth was that of 20 cm. Regarding the yield it was concluded that the total number of flowers obtained by the two treatments and the control were very close.

Howeverthe results concernedone year experiments. So further research, for more than one year, is needed to confirm the results of the present study.

REFERENCES

Ambardar S., Vakhlu J. (2013).Plant growth promoting bacteria from *Crocus sativus* rhizosphere World J MicrobiolBiotechnol(12) pp. 2271-9.doi: 10.1007/s11274-013-1393-2.

Berrocal-Lobo M., Molina A. (2008). Arabidopsis defence response against *Fusariumoxysporum.* Trends in plant science 13, pp. 145-150.

Cappeli C. (1994). Occurence of *Fusarium oxysporum* f. Sp. Gladioli on saffron in Italy. Phytopathologia Mediterranea 33, pp. 93-94.

Katan J. 1981. Solar heating (solarization) of soil for control of soilborne pests. Annual Review of Phytopatholology, 19, pp. 211-236.

KatanJ.,DeVay J.E. (1991).Soil Solarization: historical perspectives, principles anduses. In: Katan J., DeVay JE., eds. Soil Solarization. CRC Press, Boca Raton, FL, pp. 23-38.

Palmero D., Rubio-Moraga A., Galvez-Patón L.,Nogueras J.,Abato C., Gómez-Gómez L., AhrazemO. (2014). Pathogenicity and genetic diversity of *Fusarium oxysporum* isolates from corms of *Crocus sativus* Industrial Crops and Products 61: pp. 186-192

Shah A.A., Tripathi, R.B. (2009). Economics analysis of saffron production in Kashmir valley. Agriculture Update, 4 (3/4), pp. 277-279.

de Souza JT, Arnould C., Deulvot C., Lemancean P., Gianinazzi-PearsonV., Raaijmakers JM. (2003). Effect of 2,4-diacetylphloroglucinol on Pythium. Cellularr responses and variation in sensitivity among propagules and species. Phytopathology 93, pp. 966-975.

TjamosEC. (1983). Control of *Pyrenochaeta lycopersici* by combined soil solarization and low dosage of methyl bromide. Acta Horticulturae 152, pp. 252-258

TjamosEC., Paplomatas EJ. (1987).Effect of soil solarizationon the survival of fungal antagonists of *Verticillium dahliae*. Plant Prot. Organ. Bull17, pp.645-653.

TjamosEC., Paplomatas EJ. (1988).Long-term effect of soil solarizationin controlling*Verticillium* wilt of globe artichokes in Greece. Plant Pathology37, pp. 507-515.

TjamosEC., Faridis A. (1980). Control of soil-borne pathogens by solar heating in plastic houses in Greece. In: Proceedings of the Fifth Congress of the Mediterranean Phytopathological Union. Patras, Greece, pp. 82-84.

Vogdopoulos K., Lazaridou T.B. (2022). Morphological Description of *Crocus sativus* in the area of Kozani, Greece Proceedings of the XIII International Scientific Agricultural Symposium "Agrosym 2022"pp. 245-249.

White JF., Belanger F., Meyer W., Sullion RF., Bischoff JF., Lewis EA., (2002). Clavicipitalean fungal epibionts and endophytes-development of symbiotic interactions with plants. Symbrosis 33, pp. 201-213.

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FOOD ALLERGIES IN DOGS AND CATS: A BRIEF REVIEW

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ABSTRACT

Food allergies (FA) in companion animals can affect pet health and cause concern for their caretakers. Pet owners maintain emotional bonds with their pets, therefore emphasizing the importance of well-being in their companions. However, successful diagnosis and treatment of FA remains inconsistent due to lack of consensus among scientifically based literature articles. Therefore, this work aims to improve general reader knowledge by reviewing various crucial aspects of FA, including terminology, clinical signs, risk factors, known allergens, treatment management, and prognosis. Use of proper terminology encourages accurate diagnoses by limiting confusion between various conditions, particularly given food intolerances (such as toxicity) can be misdiagnosed for immune-related reactions because clinical signs may appear similar at times. Symptoms of FA may be expressed as dermatological or gastrointestinal, ranging in afflicted area, severity and appearance. Diet composition, life stage, genetics, species (canine or feline), and the gut microbiome may contribute to FA prevalence. Food allergens provoke a physiological immune response, which can be activated acutely or chronically. Common pet diet ingredients associated with inducing FA symptoms include beef, dairy, chicken and fish. Although prognoses for FA are good when the offending dietary component is successfully removed from the pet food, care must be taken to address additional contributing factors, such as flea and mite prevention. Natural hyposensitization rarely occurs and future episodes of FA are possible after diet adaptation. Future work should seek to establish standardized diagnostic protocols for FA as well as to methodically investigate the links between risk factors and FA occurrence.

Keywords: *Food allergies, pet nutrition, hypersensitivity, atopic dermatitis.*

INTRODUCTION

The broad history of FA in pets lacks clear methods of reliable identification. This may be due to the vague nature of symptom appearance. A technical definition has been described as involving any immune-related response related to food intake (Verlinden et al., 2006). The intestinal mucosa provides a crucial function in defending the host system from pathogens. Normally, the immune system can identify and appropriately respond to antigens in the gastrointestinal (GI) tract by suppressing T-cell activation, which produces oral tolerance (no allergic reaction).

However, in the circumstance of a hypersensitive pet, the immune response is unable to properly distinguish between harmless and harmful compounds. This results in a targeted antigen-specific attack leading to the release of IgM, IgG, or IgE cells (Verlinden et al., 2006). Physiological immune response is further divided into four types (I, II, III, IV) with each type associated with response time, physiological effects, and severity. Often, symptoms of FA may be expressed via the GI and dermatological systems. However, studying FA in an applied situation (such as a case study) can be challenging as these symptoms may result from other conditions, such as lactose intolerance or toxicity (Jackson, 2023). Therefore, these knowledge gaps are currently being studied to improve the accurate identification and treatment of pet FA. The current prevalence in dogs and cats is unknown, however, estimated numbers suggest 0.2% of dogs and 0.1% of cats in the United States are affected (Banfield Pet Hospital, 2018). However, the same report states that environmental and flea allergies are on the rise. Dogs and cats with FA are, respectively, six and fifteen times more likely to clinically present with pyoderma (skin infections) than their non-allergic counterparts (Banfield Pet Hospital, 2018). According to American Veterinary Medical Association, it was estimated in 2020 that there were roughly 83.7 million dogs and 60 million cats in the United States (AVMA, 2021). Following these numbers, there is an estimated 167,400 dogs and 60,000 cats suffering from FA in the United States. These data may reflect underdiagnosis, with skin conditions in pets being otherwise stated as commonly caused by adverse food reactions (AFRs) (Biel et al., 2022). This work aims to enhance general reader knowledge by reviewing several important aspects of FA, including terminology, symptoms, risk factors, known allergens, and treatments.

TERMINOLOGY

Many terms and abbreviations are involved when encountering issues associated with FA in pets. As previously mentioned, AFRs cover both food hypersensitivities and food intolerances. Food intolerance reactions are collectively grouped as non-immunological responses, meaning there is no association with FA. Such responses include idiosyncrasies, toxicities, and metabolic effects (Verlinden et al., 2006). These are typically variable and dose-dependent reactions that may be hours or days after a dietary exposure (Craig et al., 2018). On the other hand, food hypersensitivities are considered to be immunologically related responses following food intake. This may be objectively verified by the presence of various immune defense cells in the plasma. However, there may be overlap between both groups (hypersensitivities and intolerances), which proves differentiation difficult at times (Verlinden et al., 2006). Other terms commonly utilized include descriptions of dermatological symptoms. These are cutaneous adverse food reactions (CAFRs) and atopic dermatitis (AD). CAFRs simply refer to acute dermatological problems associated with an ingested food (as opposed to an environmental or parasitic cause). Atopic dermatitis, also known as eczema, is a common skin issue associated with patches or “hot spots” of reddened, dry and inflamed epidermis that is typically chronic in nature. The term “pruritis” is usually a description of frequent scratching behavior, usually affecting specific body areas. Alopecia refers to lost hair and bare patches. Although many of these terms are related, it is crucial to remember the differences to assist in accurate information portrayal.

CLINICAL SIGNS

When comparing the expression of clinical signs of FA in dogs and cats, it must be remembered that there is a lack of evidence-based diagnostic procedures due to the elusive nature of dietary allergy symptoms. In both species, there is manifestation of both dermatological and GI symptoms. As previously reported, there is not consistent data for symptom occurrence rates, with researchers providing contrasting results, including the type and prevalence of GI symptoms observed (Mueller et al., 2018; Wernimont et al., 2020). Muller and coworkers (Muller et al., 1989) generously stated that FA occurs in dogs as approximately 10% of all allergic skin diseases. Food allergy cases in cats have been far less common in comparison.

Dermatological signs include evidence of pruritis (licking, scratching, alopecia, compulsive chewing), poor hair quality (i.e., brittleness), and AD. Skin symptom presentation often involves the face, feet, and genital regions. Additional symptoms in dogs include GI signs (such as frequent defecation with loose stool and increased flatulence), conjunctivitis, sneezing, and anaphylaxis (Mueller and Olivry, 2018). Cats often present in a slightly different fashion, expressing vomiting, diarrhea, respiratory signs, conjunctivitis, and hyperactive behavior (Mueller and Olivry, 2018). A bibliometric study reported that AD in cats is more frequently reported in females than males, with some study participants demonstrating severe dermatological compromise as characterized by subjective appearance based on lesion size, type, exudate and physiological location (Santoro et al., 2021). However, a limitation is the poor ability to discern the differences between specific causes amongst cases of feline atopic syndrome (FAS). A previous review suggests additional symptoms may also be exhibited, but lack statistical correlation (Verlinden et al., 2006). Effects of FA are generally considered non-seasonal. Symptom expression is assumed to be based on continuous consumption of the antigen-containing ingredient unless weather conditions were to influence the ingredient profile. Incidences of FA can occur at any age but may be prevented by early encouragement of oral tolerance immediately after the first 9 weeks of life as shown by the partial introduction of ovalbumin dissolved in cow milk (Zemann et al., 2003). The 28-day treatment enabled allergy-prone puppies to immunologically overcome and adapt to a new food without provoking a dietary allergic reaction as opposed to the control group (Zemann et al., 2003).

RISK FACTORS

Potential determinants for FA have been identified with life stage, breed, genetics, diet, gut microflora, and other elements (Jackson, 2023; Marsella, 2009; Olivry & Bexley, 2018; Tawfik et al., 2020). A study sought to find the prevalence of allergic skin reactions in various breeds and ages of dogs (Tawfik et al., 2020). In this study, twenty-nine dogs were utilized ranging from 1.5 months to 2.5 years old. The main methods of sample collection were via skin scraping, skin biopsies and histological examination while biopsies were attained from body pinnae, nasal plenum, footpads, and small lesions via surgical resection (4 and 6 mm insert size). Results showed 13.79% of the dogs had FA-associated dermatitis based on elevated infiltration of lymphocytes, macrophages and neutrophils noted in biopsy cores related to immune responses. Infectious skin issues were more common than miscellaneous dermatitis. Flea allergy dermatitis was most common for infectious, while primary contact dermatitis was most often exhibited in miscellaneously caused cases. Food allergies were the second most common cause in miscellaneous cases (Tawfik et al., 2020). Although breeds were recorded, they varied greatly. However, another study found German Shepherds, Irish Setters, and Shar-Peis to be more commonly affected by GI symptoms accompanying FA (Roudebush et al., 2000). Ingredients have been reported to influence food acceptance, pruritic behaviors, hair quality and fecal score in dogs expressing AFR (Weemhoff et al., 2021). Further factors include the storage conditions of dry kibble. *Tyrophagus* storage mites can be found in contaminated commercial dry foods and may cause a false positive when testing dogs for FA. A study found that these mites were more likely to achieve high numbers in commercial stored dry foods when initial mite numbers were high and the kibble was crushed (Olivry and Mueller, 2019). Another cofactor for high mite proliferation rates includes storage of foods in ideal mold-proliferation conditions such as higher humidity and increased ambient temperatures. While cereal-rich foods are more commonly reported to sustain mite reproduction, *Tyrophagus putrescentiae* reproduces quickly in fat- and protein-rich diets (Olivry and Mueller, 2019). Mite multiplication is mitigated when foods are purchased fresh, utilized in shorter time periods, and stored in low humidity in addition to cooler temperatures. Existing microbial presence and diversity may influence susceptibility to increased FA-induced inflammatory responses. It is suggested that when FA manifests as GI disturbance, such as enteritis and diarrhea, alterations occur to the resident microbiome, with the most literature studying the colonic region (Wernimont et al., 2020). Gut barrier functionality may be improved by specific postbiotics, such as 10-hydroxy-cis-12-octadecenoic acid released during microbial fatty acid metabolism (Yamada et al., 2018). Exposure to probiotics (*Lactobacillus rhamnosus*) in the first 6 months of life for puppies significantly decreased allergen-specific IgE levels to ragweed, timothy and Dermatophagoides farinae as well as prevented partial AD after comparison with baseline challenge data (Marsella, 2009). Few studies have been conducted in pets evaluating microbiome differences between allergic and non-allergic individuals. However, one study compared two groups of adult humans, a non-allergic group and a group allergic to peanuts and tree nuts, exhibited that those with an allergy expressed lower numbers of intestinal *Clostridiales*, *Prevotella*, and *Ruminococcaceae* as well as higher numbers of *Bacteroides* as discerned by fecal analysis (Hua et al., 2016). Identified microbiological differences may be a dysfunctional causative cofactor, a result of chronic allergen response, or likely, a complex mixture of both.

KNOWN FOOD ALLERGENS

The focus here will remain with commercial pet foods. Although it may not be possible to discuss every potential allergen, common ones involve glycoproteins (animal or plant). Typically insoluble during digestive absorption, usual food allergens are reviewed varying from 15 to 40 kDa in size (Jackson, 2023). Review of various antigen suspects commonly encountered in canine and feline diets summarize that there is more data provided for dogs, showing that beef and dairy present the most allergic reactions in hypersensitive dogs (Verlinden et al., 2006). The same work suggested wheat, chicken, egg, lamb, soy, pork and fish also provoked a smaller number of allergic cases. Cats exhibit lesser reactivity with antigenic challenges from beef, chicken and fish associated with most cases while fewer cats also negatively responding to grains and dairy (Mueller et al., 2016). A separate work recruited 179 atopic dogs, which included 27 dogs with cod allergy (Imanishi et al., 2020). All dogs were collected for serological immune responses to crude cod oil. Thirty-nine of the 179 (20%) atopic dogs possessed specific IgE for crude cod oil and 12 of the 27 (44%) allergic dogs had the same verified via ELISA analysis. The dogs which tested positive for specific IgE, 25%, 39%, and 50% of this population also tested positive for specific IgE to, respectively, parvalbumin, collagen, and tropomyosin (proteins found in crude cod oil). This suggests that it may be beneficial to isolate specific proteins in ingredients to provide an ideal fit for individual dietary needs of pets based on clinical diagnosis. A study performed by North Carolina State University retrieved sera from 40 dogs and 40 cats with undetectable, low, medium and high corn-specific IgE reactivity (Olivry and Bexley, 2018). Extracts (except the derived corn kernel extract) were subjected to phosphate-based buffer twice with centrifugation to remove allergens. Findings via ELISA analysis concluded no detectable IgE action in two cornstarch extracts as opposed to a heightened response against allergens found in corn flour. This further shows that differences in ingredient processing can alter allergic responses in pets (Olivry and Bexley, 2018).

TREATMENT, MANAGEMENT AND PROGNOSIS

Once one or more specific allergens have been identified, treatment involves diet manipulation to accommodate for allergens present. It has been found that extensive hydrolyzation of poultry-derived protein resulted in the absence of clinical symptoms in poultry-allergic dogs and cats (Olivry et al., 2017). However, partial hydrolysis did not achieve the same positive outcome. Protein hydrolysate diets, protein/ingredient replacement, homemade diets, and commercial hypoallergenic diets are available. Selection of a new diet relies on numerous factors, including severity of symptoms, budget, availability, preparation time, and more. Objective methods, such as individual IgE levels and scoring scales based on lesion appearance, for quantifying FA severity have proven to be variable and conflicting, with varying types of dermatitis being documented in multiple articles (Cucerzan et al., 2020; Santoro et al., 2021; Tawfik et al., 2020; Weemhoff et al., 2021). Cucerzan and coworkers found that 10 out of 10 food-allergic dogs showed significantly reduced clinical AD signs after consuming a hydrolyzed protein diet for 3 months (Cucerzan et al., 2020). In individual pets in which there are numerous allergies or a lack of antigen identification, it may be necessary to administer glucocorticoids (Santoro et al., 2021; Verlinden et al., 2006). As it relates to dermal conditions, prevention of fleas and other causes of pruritis should not be ignored. Although prognosis is typically good after the offending antigen has been identified and manipulated out of the diet, relapse is possible (Olivry and Mueller, 2020). Natural hyposensitization to the existing allergens is rare (Muller et al., 1989).

CONCLUSION

Pet FA prevalence appears to be under-represented in the literature. However, this could be confirmed with improved diagnostic methods and protocols. Understanding the risk factors, pathways, GI components, and symptoms are crucial to initiate administered aid. Methodology that differentiates food intolerance (non-immune reaction) from food hypersensitivity (immune overreaction) is needed. Overall, further investigation of FA in pets is encouraged for not only the health of the pet but also the owners who struggle to ensure the comfort of the animal.

REFERENCES

American Veterinary Medical Association (AVMA). 2021. Pet population still on the rise, with fewer pets per household. https://www.avma.org/javma-news/2021-12-01/pet-population-still-rise-fewer-pets-household (Accessed 27 April 2023).

Banfield Pet Hospital. 2018. 2018 Pet Health Report. https://www.banfield.com/en/about-banfield/newsroom/press-releases/2018/2018-state-of-pet-health-report-explores-pet-aller (Accessed 27 April 2023).

Biel, W., M. Natonek-Wiśniewska, J. Kępińska-Pacelik, K. Kazimierska, E. Czerniawska-Piątkowska, and P. Krzyścin. 2022. Detection of chicken DNA in commercial dog foods. BMC Vet. Res. 18:1–10. doi:10.1186/s12917-022-03200-z. Available from: https://doi.org/10.1186/s12917-022-03200-z.

Craig, J. M. 2019. Food intolerance in dogs and cats. J. Small. Anim. Pract. 60:77-85. doi:10.1111/jsap.12959.

Cucerzan, A., I. Kalman, Morar, A., L. T. Cziszter, and E. TÎrziu. 2020. Research on the effect of a hypoallergenic diet in dogs with atopic dermatitis. Lucr. Stiinţ., Ser. Med. Vet. 53(3):15-25. ISSN:2668-2435.

Hua, X., J. J. Goedert, Pu, A., G. Yu, and J. Shi. 2016. Allergy associations with the adult fecal microbiota: Analysis of the American gut project. EBioMedicine. 3:172-179. doi: 10.1016/j.ebiom.2015.11.038.

Imanishi, I., J. Uchiyama, K. Mizukami, J. Kamiie, K. Kurata, K. Iyori, M. Fujimura, K. Shimakura, K. Nishifuji, and M. Sakaguchi. 2020. IgE reactivity to fish allergens from Pacific cod (Gadus macrocephalus) in atopic dogs. BMC Vet. Res. 16:1–10. doi:10.1186/s12917-020-02559-1.

Jackson, H. 2023. Food allergy in dogs and cats; current perspectives on etiology, diagnosis, and management. J. Am. Vet. Med. Assoc. 261:S23-S29. doi.org/10.2460/javma.22.12.0548.

Marsella, R. 2009. Evaluation of Lactobacillus rhamnosus strain GG for the prevention of atopic dermatitis in dogs. Am. J. Vet. Res. 70:735-740. doi:10.2460/ajvr.70.6.735.

Mueller, R. S., and T. Olivry. 2018. Critically appraised topic on adverse food reactions of companion animals (6): prevalence of noncutaneous manifestations of adverse food reactions in dogs and cats. BMC Vet. Res. 14:341. doi:10.1186/s129170181656-0.

Muller, G.H., R. W. Kirk, and D. W. Scott. 1989. Food hypersensitivity. In: J. Dyson, editor, Small animal dermatology. W.B. Saunders Company, Philadelphia, PA. p. 470–474.

Olivry, T., and J. Bexley. 2018. Cornstarch is less allergenic than corn flour in dogs and cats previously sensitized to corn. BMC Vet. Res. 14:1–8. doi:10.1186/s12917-018-1538-5.

Olivry, T., J. Bexley, and I. Mougeot. 2017. Extensive protein hydrolyzation is indispensable to prevent IgE-mediated poultry allergen recognition in dogs and cats. BMC Vet. Res. 13:1–9. doi:10.1186/s12917-017-1183-4.

Olivry, T., and R. S. Mueller. 2019. Critically appraised topic on adverse food reactions of companion animals (7): Signalment and cutaneous manifestations of dogs and cats with adverse food reactions. BMC Vet. Res. 15:1–5. doi:10.1186/s12917-019-1880-2.

Olivry, T., and R. S. Mueller. 2020. Critically appraised topic on adverse food reactions of companion animals (9): Time to flare of cutaneous signs after a dietary challenge in dogs and cats with food allergies. BMC Vet. Res. 16:1–4. doi:10.1186/s12917-020-02379-3.

Roudebush, P., W. G. Guilford, and K. J. Shanley. 2000. Adverse reactions to food. In: M. S. Hand, C. D. Thatcher, R. L. Remillard, and P. Roudebush, P., editors, Small animal clinical nutrition. Mark Morris Institute, Topeka, KS. p. 431–453.

Santoro, D., Pucheu-Haston, C. M., C. Prost, R. S. Mueller and H. Jackson. 2021. Clinical signs and diagnosis of feline atopic syndrome: detailed guidelines for a correct diagnosis. Vet. Dermatol. 32(1):26-e6. doi:10.1111/vde.12935.

Tawfik, M., S. Oda, and A. Khafaga. 2020. Pathological Study of Skin Disorders in Dogs and Cats at Alexandria Governorate, Egypt. Alexandria J. Vet. Sci. 65:66. doi:10.5455/ajvs.93531.

Verlinden, A., M. Hesta, S. Millet, and G. P. J. Janssens. 2006. Food allergy in dogs and cats: A review. Crit. Rev. Food Sci. Nutr. 46:259–273. doi:10.1080/10408390591001117.

Weemhoff, J. L., J. M. MacLeay, J. Brejda, H. Schiefelbein, S. M. Wernimont, and K. L. Gross. 2021. Successful nutritional control of scratching and clinical signs associated with adverse food reaction: A randomized controlled COSCAD ’18 adherent clinical trial in dogs in the United States. J. Vet. Intern. Med. 35:1884–1892. doi:10.1111/jvim.16193.

Wernimont, S. M., J. Radosevich, M. I. Jackson, E. Ephraim, D. V. Badri, J. M. MacLeay, D. E. Jewell, and J. S. Suchodolski. 2020. The Effects of Nutrition on the Gastrointestinal Microbiome of Cats and Dogs: Impact on Health and Disease. Front. Microbiol. 11:1–24. doi:10.3389/fmicb.2020.01266.

Yamada, M., N. Takahashi, Y. Matsuda, K. Sato, M. Yokoji, B. Sulijaya, T. Maekawa, T. Ushiki, Y. Mikami, M. Hayatsu, Y. Mizutani, S. Kishino, J. Ogawa, M. Arita, K. Tabeta, T. Maeda, and K. Yamazaki. 2018. A bacterial metabolite ameliorates periodontal pathogen-induced gingival epithelial barrier disruption via GPR40 signaling. Sci. Rep. 8:1–12. doi:10.1038/s41598-018-27408-y.

Zemann, B., C. Schwaerzler, M. Griot-Wenk, M. Nefzger, P. Mayer, H. Schneider, A. De Weck, J. M. Carballido, and E. Liehl. 2003. Oral administration of specific antigens to allergy-prone infant dogs induces IL-10 and TGF-β expression and prevents allergy in adult life. J. Allergy Clin. Immunol. 111:1069–1075. doi:10.1067/mai.2003.1411.

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OPTIMIZATION OF NUTRIENT MEDIA FOR RAPID AND EFFICIENT MICROPROPAGATION OF *BACOPA MONNIERI* AND *CERATOPHYLLUM DEMERSUM*

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ABSTRACT

Bacopa monnieri L. Pennell, which has an important medicinal value and *Ceratophyllum demersum* L. having a great potential in cleaning water contaminated with heavy metals are also an indispensable plants in aquariums. Due to these features, the production of uniform and healthy plants in abundance in these species is important. On the other hand, it is possible to produce thousands of healthy plants with the same genetic structure in a short time with *in vitro* techniques. In this study, the effects of 12 different basic nutrient media, six different sucrose and seven different agar concentrations were investigated for the rapid and low-cost *in vitro* production of B. *monnieri* and C. *Demersum* species. When shoot formation and rooting rate were evaluated together, it was observed that Schenk ve Hildebrandt (SH) for *C. demersum* and Orchimax basic nutrient media for *B. monnieri* gave better rate of micropropagation than other media tested. In different sugar applications, the highest micropropagation results were obtained from the medium containing 20 g/L sucrose in *B. monnieri* and 50 g/L sucrose in *C. demersum*. In addition, *C. demersum* achieved the highest micropropagation in liquid medium without agar, and *B. monnieri* in medium containing 5 g/L agar. As a result of this study, micropropagation was achieved at high rates in both species without using agar, the most expensive compound of nutrient media, or by using it at low rates. Regenerated plants were transferred to aquariums and achieved 100% adaptation.

Keywords: *In vitro, SH medium, Orchimax medium, agar, sucrose.*

INTRODUCTION

Aquatic plants produce organic compounds such as carbohydrates, lipids, proteins and pigments through photosynthesis using carbon, nutrients and solar energy (Mansour et al., 2022). Therefore, they are the main producers of aquatic environment and maintain the balance between aquatic organism (Özcan et al., 2023). Aquatic plants, which are resistant to several pollutants and can grow in soils that are not suitable for the cultivation of terrestrial plants, have begun to gain great importance in areas such as biofuel, cosmetics, functional foods and pharmaceuticals

(El-Shenody et al. 2019; Mansour et al.2022). Moreover, some aquatic plants play an important role as biological indicators in detecting water pollution and can also be used to clean water and protect shorelines, ponds and lakes from erosion. (Öztürk et al., 2004; Doğan, et al., 2018). Many aquatic plant species are also used as aquarium plants, and in addition to providing a beautiful appearance to aquariums, they also make a significant contribution to the protection and feeding of fish and the improvement of water quality (Özcan et al., 2023).

Bacopa monnieri L. Pennell and *Ceratophyllum demersum* L. species, which have important place in the pharmaceutical industry and phytoremediation of water (Keskinkan et al., 2004; Vishnupriya and Padma, 2017; Abu, 2017), are also indispensable plants in aquariums. These species can be propagated by seeds or cuttings, but these methods are very slow and costly. In addition, genetic changes occur in seed propagation, and the risk of contamination with bacterial and fungal diseases is much higher in propagation with cuttings. On the other hand, it is possible to propagate millions of disease-free plants with the same genetic structure within a year with *in vitro* techniques. For fast and low-cost *in vitro* production, the nutrient media content used for each plant must be optimized. Therefore, various basic nutrient media, different sucrose and agar concentrations were tested for the micropropagation of *B. monnieri* and *C. Demersum* in the current study.

MATERIALS AND METHODS

This study was carried out in the laboratories of Fisheries and Aquaculture and Field Crops Departments, Faculty of Agriculture, Ankara University in 2020 and 2021. In the study, 12 different basic nutrient media developed by different researchers for different plant species were tested for micropropagation of *B. monnieri* and *C. Demersum* (Table 1). Among these media, MS and B_5 vitamins were added to the SH medium. Additionally, 0-60 g/l sucrose and 0-7 g/l agar were added to the best medium determined for each plant species to find the best sugar and agar concentrations. After adjusting the pH of the prepared media to 5.6, they were autoclaved at at 121 °C under 1.4 kg/cm^2 pressure for 20 minutes.

Apical and axillary buds taken from plantlets developed *in vitro* under sterile conditions were cultured on nutrient media in Duchafe Sterivent culture containers. Four buds were placed in each culture container and measurements were made 4 weeks after the start of culture. All experiments were set up in 4 replicates of 4 explants each. The data obtained were subjected to analysis of variance (ANOVA) and the significance between the means were determined with the Tukey test. After four weeks of culture initiation, the plantlets that developed and rooted in the tested nutrient media were transferred to aquariums with white light, where the temperature was 24 °C and the pH was 6.5-7.2.

RESULTS AND DISCUSSION

Effect of different nutrient media

In both species, shoot development started one week after culture initiation. Shoot formation and rooting results obtained from 12 different basic nutrient media used for micropropagation of *Bacopa monnieri* are given in Table 1. As seen in the Table, the highest number of shoots per explant was obtained from Anderson's Rhododendron nutrient medium with 6.31 (Fig. 1a), while the highest shoot length was recorded on MS medium with 5.94 cm. The lowest number of shoots per explant and shoot length were obtained from MS and MS No: 3B basic nutrient media with 1.44 and 1.12 cm, respectively. In this species, the highest number of roots (14.5 pieces) and root length (7.15 cm) per explant were found in Orchimax medium (Fig. 1b). When the shoot formation and rooting results were evaluated together, it was determined that Orchimax nutrient medium was more suitable for micropropagation of *B. monnieri*.

Among the 12 different nutrient media tested for *in vitro* micropropagation of *C. demersum*, the highest number of shoots per explant was obtained from Knudson C Orchid medium with 10.25 and the highest shoot length of 4.80 cm (Fig. 1c, Table 2). However, the rooting rate was quite low in this medium. The lowest shoot number and shoot length per explant were recorded in CHU (N6) nutrient medium, with 3.0 and 2.17 cm, respectively (Table 2). While the highest number of roots per explant was obtained from Orchimax (12.25) and SH (11.50) nutrient media, the highest root length was found in SH + MSvit medium, with 2.97 cm (Fig. 1d). When all the results were evaluated, it was determined that the SH medium was the most suitable basic nutrient medium for the micropropagation of *C. demersum* (Table 2).

Table 1. Effect of different basal nutrient media on *in vitro* micropropagation of *Bacopa monnieri* after 4 weeks of culture initiation

Nutrient media	Shoot Formation		Rooting	
	Number of shoots/explant	Shoot length (cm)	Number of roots/explant	Root length (cm)
Schenk and Hildebrandt (SH)	3.31 bc	2.20 de	5.50 de	2.99 de
SH+MSvit	2.31 cd	5.03 ab	4.50 e	4.20 c
Murashige and Skoog (MS)	1.44 d	**5.94 a**	6.00 cde	2.46 ef
Anderson's Rhod.	**6.31 a**	2.41 de	4.25 e	1.66 f
CHU(N_6)	2.62 bcd	2.79 cd	8.50 bc	3.10 de
McCown	2.56 bcd	3.18 cd	5.75 de	2.40 ef
Orchimax	4.00 b	2.58 cde	**14.50 a**	**7.15 a**
Lindemann Orchid	2.75 bcd	3.30 cd	6.50 cde	3.27 cde
Knudson C orchid	2.81 bcd	4.02 bc	6.75 cde	3.82 cd
MS No: 3B	5.75 a	1.12 e	9.75 b	3.01 de
MS No: 1B	3.12 bc	2.51 cde	8.00 bcd	3.07 de
SH+B_5 vitamins	3.75 bc	3.63 bcd	8.50 bc	**6.01 b**

The difference between the means shown in different letters in the same column is significant at the 0.05 level according to the Tukey test results. 30 g/l sucrose and 5 g/l agar were added to the nutrient media.

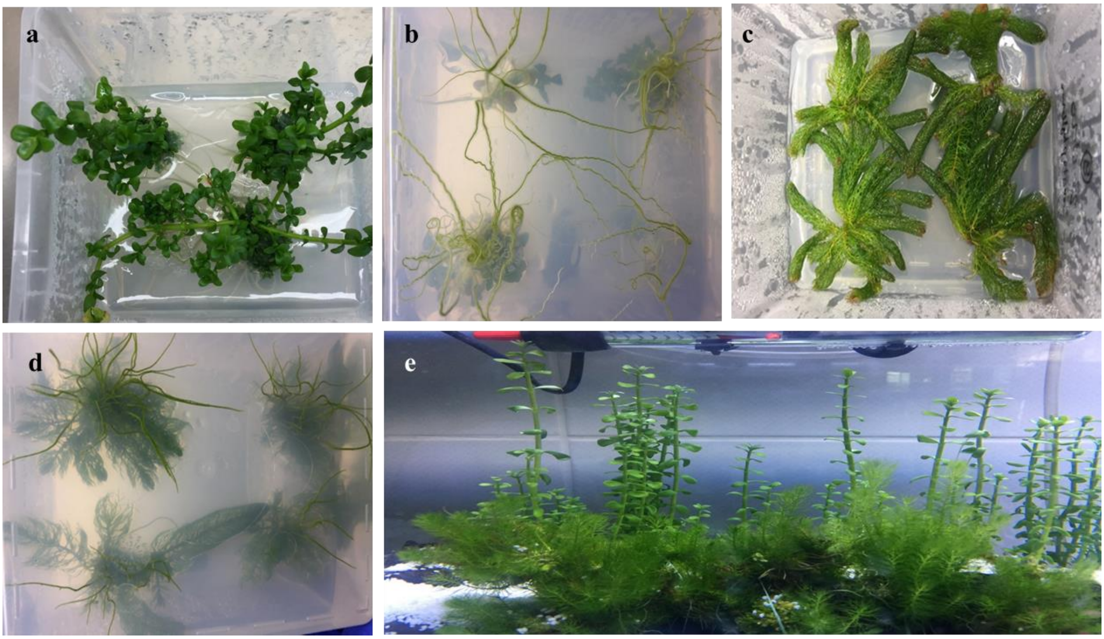

Figure 1. Micropropagation of *B. monnieri* and *C. demersum* 4 weeks after the culture initiation and growth in the aquarium. a) Shoot regeneration in *B. monnieri* on Anderson's Rhododendron medium containing 30 g/l sucrose and 5 g/l agar, b) Root formation in *B. monnieri* in Orchimax medium containing 30 g/l sucrose and 5 g/l agar, c) shoot regeneration in *C. demersum* on Knudson C orchid medium containing 30 g/l sucrose and 5 g/l agar, d) root formation in *C. demersum* in SH medium supplemented with 50 g/l sucrose and 5 g/l agar, e) growth of *B. monnieri* (in the background), and *C. demersum* in aquarium (in the foreground)

Table 2. Effect of different basal nutrient media on *in vitro* micropropagation of *Ceratophyllum demersum* after 4 weeks of culture initiation

Nutrient media	Shoot Formation		Rooting	
	Number of shoots/explant	Shoot length (cm)	Number of roots/explant	Root length (cm)
SH	8.75 b	3.92 cd	11.50 ab	2.12 b
SH+MSvit	7.25 c	3.67 cd	10.25 bc	**2.97 a**
MS	5.75 e	3.66 cd	5.50 e	1.92 bc
Anderson's Rhod.	7.25 c	4.12 bc	2.25 g	0.34 e

CHU(N_6)	3.00 f	2.17 f	3.50 fg	1.02 d
McCown	9.75 ab	4.62 ab	4.25 ef	0.62 de
Orchimax	6.25 cde	3.05 e	**12.25 a**	2.90 a
Lindemann Orchid	6.25 cde	3.07 e	9.00 c	3.08 a
Knudson C orchid	**10.25 a**	**4.80 a**	5.75 de	1.52 c
MS No: 3B	5.75 e	3.50 de	5.25 e	0.90 d
MS No: 1B	7.00 cd	3.85 cd	2.75 fg	0.37 e
SH+B_5 vit	6.00 de	3.57 de	7.25 d	1.87 bc

The difference between the means shown in different letters in the same column is significant at the 0.05 level according to the Tukey test results. 30 g/l sucrose and 5 g/l agar were added to the nutrient media.

Although their content depends on a number of variables such as genotype, explant and purpose of the study, basic nutrient media contain all macro and micro elements and vitamins necessary for plants (Saad and Elshahed 2012). By reducing or increasing these nutritional elements, it is possible to achieve success even in plants whose *in vitro* regeneration is very difficult (Saad and Elshahed 2012; Phillips and Garda 2019). In the present study, the effectiveness of 12 different basic nutrient media developed for different plant species was tested on two different aquarium plant species. Considering the shoot formation and rooting results, it was observed that the basic nutrient media SH for *C. demersum* and Orchimax for *B. monnieri* provided the highest rate of micropropagation. On the other hand, in previous studies with aquarium plants, only one or two basic nutrient media were used at the same time, and the most commonly used was the standard MS nutrient medium (Ceasar et al., 2010; Sharma et al., 2010; Koul et al. 2014; Barpete et al., 2015; Chaunhan and Shirkot 2020, Özcan et al., 2021). Apart from MS nutrient medium, B_5 (Koul et al. 2014) and SH media have also been used, although rarely, in different plant species (Özcan et al. 2023). However, in the present study, standard MS nutrient medium was not effective in both species. This result revealed the necessity of making adjustments in the nutrients contained in the developed basic nutrient media for high success in different plant species.

Effect of different sucrose concentrations

In optimizing the sucrose ratio for *B. monnieri*, Orchimax medium which gave the highest results in the determination of media was used. The effect of sucrose ratios on the number of shoots per explant was found to be statistically insignificant. While the highest number of shoots per explant was obtained from the addition of 20 g/l sucrose with 3.38, the highest shoot length was found in the sucrose-free medium with 6.87 cm (Table 3). While the effect of sucrose ratios on root length in *B. monnieri* was found to be statistically insignificant, the highest number of roots per explant was obtained from 30 g/l with 11.75, and the maximum root length was obtained from 60 g/l sucrose ratio with 2.74 cm (Table 3). Considering shoot formation and rooting together, it was observed that the majority of sucrose ratios were effective in *B. monnieri*. However, 20 g/l sucrose was used to optimize the agar ratio.

To determine the effect of different sugar ratios on *C. demersum*, SH medium which gave the highest micropropagation results in the nutrient media study was used. The highest number of shoots per explant (6.06) and the highest shoot length (4.93 cm) were accomplished from the SH medium containing 50 g/l sucrose (Table 4). Likewise, the highest number of roots per explant (11.12) and root length (3.45 cm) were also recorded in 50 g/l sucrose application. Additionally, high amounts of sucrose (50-60 g/l) caused reddening of shoot tips. According to these results, the most suitable amount of sucrose for *C. demersum* was 50 g/l (Table 4).

Table 3. Effect of different sucrose concentrations on *in vitro* micropropagation of *B. monnieri* after 4 weeks of culture initiation

Sucrose (g/l)	Shoot Formation		Rooting	
	Number of shoots/explant	Shoot length (cm)	Number of roots/explant	Root length (cm)
0	2.81nd	**6.87 a**	10.38 ab	2.32nd
10	2.25	6.56 a	9.62 ab	1.99
20	**3.38**	4.74 ab	8.75 ab	2.31
30	3.00	4.43 ab	**11.75 a**	2.45
40	1.81	5.56 ab	8.31 ab	2.40
50	2.69	2.50 b	5.06 b	2.22
60	2.81	3.11 b	5.75 b	**2.74**

The difference between the means shown in different letters in the same column is significant at the 0.05 level according to the Tukey test results. nd= not significant. Orchimax medium supplemented with 5 g/l agar was used.

Table 4. Effect of different sucrose concentrations on *in vitro* micropropagation of *C. demersum* after 4 weeks of culture initiation

Sucrose (g/l)	Shoot Formation		Rooting	
	Number of shoots/explant	Shoot length (cm)	Number of shoots/explant	Root length (cm)
0	2.31 b	2.10 cd	1.25 b	0.38 c
10	6.12 a	1.99 d	4.31 b	0.98 c
20	5.06 ab	2.42 bcd	3.25 b	2.61 b
30	5.625 ab	3.54 abcd	5.75 ab	2.36 b
40	6.44 a	4.10 ab	5.75 ab	2.93 ab
50	**6.06 a**	**4.93 a**	**11.12 a**	**3.45 a**

60	3.38 ab	3.85 abc	4.38 b	2.74 b

The difference between the means shown in different letters in the same column is significant at the 0.05 level according to the Tukey test results. SH medium supplemented with 5 g/l agar was used.

Although it varies depending on the plant species, the most commonly used sucrose ratio in *in vitro* culture is 30 g/L (Miroshnichenko et al. 2017, Doğan 2020b, Gao et al. 2020). On the other hand, in the present study, the highest micropropagation results were obtained when 20 g/L sucrose was added to the nutrient medium in *B. monnieri* and 50 g/L sucrose in *C. demersum*. Similar results were also reported in *B. monnieri* (Ranjan and Kumar 2018) with the application of 20 g/L sucrose and *R. fluitans* (Özcan et al. 2021) with the addition of 15 g/L sucrose to the nutrient medium.

Effect of Different Agar Concentrations

In the agar application, Orchimax medium containing 20 g/l sucrose were used for *B. monnieri*. The results of the different agar applications on shoot and root formation in *B. monnieri* are given in Table 5. While the highest number of shoots per explant (3.50) was obtained from the medium added with 5 g/l agar, the highest shoot length (6.89 cm) was obtained from the medium solidified with 7 g/l agar. The highest number of roots per explant (8.06) was observed in nutrient medium solidified with 5 g/l agar. The highest root length was found in agar-free medium with 7.26 cm (Table 5). When shoot formation and rooting are considered together, the highest micropropagation results in *B. monnieri* were obtained from the medium solidified with 5 g/l agar.

Table 5. Effect of different agar concentrations on *in vitro* micropropagation of *B. monnieri* after 4 weeks of culture initiation

Agar (g/l)	Shoot Formation		Rooting	
	Number of shoots/explant	Shoot length (cm)	Number of shoots/explant	Root length (cm)
0	2.50 b	3.37 c	4.81 c	**7.26 a**
1	2.31 bc	6.32 ab	4.69 c	4.67 b
2	2.56 ab	2.98 c	5.19 c	3.82 b
3	2.25 bc	3.85 bc	6.38 abc	3.52 b
4	2.81 ab	4.50 abc	7.87 ab	3.27 b
5	**3.50 a**	5.35 abc	**8.06 a**	3.01 b
6	1.37 cd	5.51 abc	6.06 bc	2.91 b
7	1.25 d	**6.89 a**	5.81 c	2.62 b

The difference between the means shown in different letters in the same column is significant at the 0.05 level according to the Tukey test results. Orchimax medium supplemented with 20 g/l sucrose was used.

In the agar experiment for *C. demersum*, SH medium supplemented with 50 g/l sucrose were used. The effect of different agar concentrations on the micropropagation of *C. demersum* is given in Table 6. The highest shoot formation and rooting were achieved from liquid medium without agar. In this medium, the number of shoots per explant was 10.69 and the shoot length was 6.26 cm (Table 6). The lowest shoot formation and rooting were recorded in SH medium solidified with 7 g/l agar. According to these results, the most suitable nutrient medium for *C. demersum* was liquid medium without agar.
C. demersum achieved the highest micropropagation in liquid medium without agar and *B. monnieri* in nutrient medium containing 5 g/L agar. The fact that plants have different *in vitro* responses to different agar ratios suggests that it may be related to their ability to survive under or above water. For example, the fact that *C. demersum* can live completely under water and without roots is an explanation for the fact that it shows its best development in *in vitro* liquid culture without the need for solid nutrient media. Karataş *et al*. (2014) in their study, they obtained the highest rate of *in vitro* micropropagation of *C. demersum* from liquid nutrient media, as in the present study. While *B. monnieri* can grow in water-rich and water-saturated soils (Visnoi et al. 2016), it can also be grown in field conditions (Rahe et al. 2020). This explains why *B. monnieri* requires a more solid medium than *C. demersum* in tissue culture.

Table 6. Effect of different agar concentrations on *in vitro* micropropagation of *C. demersum* after 4 weeks of culture initiation

Agar (g/l)	Shoot Formation		Rooting	
	Number of shoots/explant	Shoot length (cm)	Number of shoots/explant	Root length (cm)
0	**10.69 a**	**6.26 a**	**10.50 a**	**4.30 a**
1	7.19 b	5.84 a	8.00 ab	3.65 ab
2	6.31 b	5.39 a	8.81 ab	3.29 bc
3	6.00 bc	4.02 b	6.50 ab	2.80 bcd
4	6.56 b	4.06 b	9.50 ab	2.30 cd
5	4.88 bcd	3.90 b	6.75 ab	2.87 bcd
6	3.38 d	2.51 c	3.06 b	2.65 bcd
7	3.81 cd	2.00 c	4.12 ab	2.08 d

The difference between the means shown in different letters in the same column is significant at the 0.05 level according to the Tukey test results. SH medium supplemented with 50 g/l sucrose was used.

CONCLUSIONS

With present study, *Ceratophyllum demersum* and *Bacopa monnieri* species, which are important plants in aquariums and have medicinal value and water phytoremediation properties, were able to be propagated at a high rate within 4 weeks. Agar, the most expensive compound of nutrient media, was not required for the propagation of *C. demersum*. Moreover, in this species sucrose used in low amounts gave the highest results. *In vitro* propagated plants were acclimated to aquariums at a 100% rate. The methods developed for the plant species studied have low economic costs and can also be used for commercial production.

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CONFLICTS OF INTEREST

The authors declare no conflict of interest. The opinions and/or conclusions expressed are exclusively those of the authors.

REFERENCES

Abu T. (2017). A review: Aquatic macrophyte *Ceratophyllum demersum* L. (Ceratophyllaceae): Plant profile, phytochemistry and medicinal properties. International Journal of Science and Research, 6: 394-399.

Barpete, S., Özcan S. F., Aasim M., Özcan S. (2015). *In vitro* high frequency regeneration through apical shoot proliferation of *Hemianthus callitrichoides* Cuba'- a multipurpose ornamental aquatic plant. Turkish Journal of Biology, 39: 493-500.

Ceasar S.A., Maxwell S.L., Prasad K.B., Karthigan M., Ignacimuthu S. (2010). Highly efficient shoot regeneration of *Bacopa monnieri* (L.) using a two-stage culture procedure and assessment of genetic integrity of micropropagated plants by RAPD. Acta Physiol Plant, 32: 443-452.

Chauhan R., Shirkot P. (2020). Micropropagation of endangered medicinal plant *Bacopa monnieri* (L.) Pennell. Journal of Pharmacognosy and Phytochemistry, 9: 1614-1620.

Doğan M. (2020). The effects of different sucrose concentrations on the regeneration area of *Riccia fluitans* L., a medicinal aquatic plant. Journal of Engineering Technology and Applied Sciences, 5: 51-58.

Doğan M., Karatas M., Aasim M. (2018). "Cadmium and lead bioaccumulation potentials of anaquatic macrophyte *Ceratophyllum demersum* L.: A laboratory study. Ecotoxicol. Environ, 148: 431-440.

El-Shenody R.A., Ashour M., Ghobara M.M.E. (2019). Evaluating the chemical composition and antioxidant activity of three Egyptian seaweeds: *Dictyota dichotoma*, *Turbinaria decurrens*, and *Laurencia obtusa*. Braz. J. Food Technol., 22: 1-15.

Gao F., Peng C., Wang H., Tretyakova I.N., Nosov A.M., Shen H., Yang L. (2020). Key techniques for somatic embryogenesis and plant regeneration of *Pinus koraiensis*. Forests, 11: 11-15.

Karataş M., Aasim M., Doğan M. (2014). Multiple shoot regeneration of *Ceratophyllum demersum* L. on agar solidified and liquid mediums. Fresenius Environ. Bull., 23: 3-9.

Keskinkan O., Goksu M.Z.L., Basibuyuk M., Forster C.F. (2004). Heavy metal adsorption properties of a submerged aquatic plant (*Ceratophyllum demersum*). Bioresource technology, 92: 197-200.

Koul A., Sharma, A., Gupta, S. and Mallubhotla, S. 2014. Cost effective protocol for micropropagation of *Bacopa monnieri* using leaf explants. Int J Sci Res, 3(4), 210-212.

Mansour A.T., Ashour M., Alprol A.E., Alsaqufi A.S. (2022). Aquatic plants and aquatic animals in the context of sustainability: Cultivation techniques, integration, and blue revolution. Sustainability, 14/3257: 1-28.

Miroshnichenko D., Chaban I., Chernobrovkina M., Dolgov S. (2017). Protocol for efficient regulation of *in vitro* morphogenesis in einkorn (*Triticum monococcum* L.), a recalcitrant diploid wheat species. PLoS One, 12: 1-23, e0173533.

Özcan E., Atar H. H., Ali S. A., Aasim M. (2023). Artificial neural network and decision tree–based models for prediction and validation of *in vitro* organogenesis of two hydrophytes—*Hemianthus callitrichoides* and *Riccia fluitans*. In Vitro Cellular & Developmental Biology-Plant, 59:, 547-562.

Özcan E., Önlu S., Sezgin M.E., Barpete, S. (2021). The effect of improvised media and sugar concentration on *in vitro* shoot multiplication of *Riccia fluitans* L.: an amphibious liverwort. Fresenius Environmental Bulletin, 30: 1696-1702.

Öztürk M., Khawar K.M., Atar H.H., Sancak C., Özcan S. (2004). *In Vitro* Micropropagation of the aquarium plant *Ludwigia repens*. Asia Pacific Journal of Molecular Biology and Biotechnology, 12: 21-25.

Phillips G.C., Garda M. (2019). Plant tissue culture media and practices: An overview. In Vitro Cellular Developmental Biology-Plant, 55: 242-257.

Rahe M.A., Mollika S.R., Khan M.S., Banu T.A., Al Amin G.M., Habib, M.A. et al. (2020). *In vitro* Micropropagation of *Bacopa monnieri* (L.) Penn.-An Important Medicinal Plant. Plant Tissue Culture and Biotechnology, 30: 57-63.

Ranjan R Kumar S. (2018). A rapid *in vitro* propagation protocol of local germplasm of *Bacopa monnieri* (L.) induced through direct organogenesis from nodal explants. Journal of Pharmacognosy and Phytochemistry, 7: 2515-2518.

Saad A.I.M., Elshahed, A.M. (2012). Plant tissue culture media. In: Recent Advances in Plant *In Vitro* Culture. Leva, A. (ed). IntechOpen. https://www.intechopen.com/chapter/40181.

Sharma S., Kamal B., Rathi N., Chauhan S., Jadon V., Vats N. et al. (2010). *In vitro* rapid and mass multiplication of highly valuable medicinal plant *Bacopa monnieri* (L.) Wettst. African Journal of Biotechnology, 9: 8318-8322.

Vishnoi V.K., Singhal P., Goel K.K., Vishnoi-Singhal R. (2016). Review on *Bacopa monnieri*: A multipotential medicinal plant. International Journal of Engineering & Management Technology, 3: 168-174.

Vishnupriya P., Padma V.V. (2017). A review on the antioxidant and therapeutic potential of *Bacopa monnieri*. React Oxygen Spec., 3: 111-120.

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THE EFFICIENCY OF USING NON-TRADITIONAL FERTILIZERS FOR WINTER OILSEED RAPE CROPS

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ABSTRACT

Biogas plants and solid fuel boilers where wood ash and digestate are produced as by-products are widely used for energy and heat production. In agriculture, both wood ash and biomass digestate are used separately as liming and fertiliser materials, Field trials were conducted at the Study and Research Farm "Peterlauki" in sod calcareous soil. Winter oilseed rape sowings were established using different variants of fertilizer mix with cattle manure digestate (D) and wood ash (P) at different ratios: B1 – D; B2 – D + P 1:1; B3 – D + P 2:1; B4 – D + P 3:1; B5 – D + P 3:1 + N16P40K60 kg ha-^{1}; B6 – D + P 3: 1 + N 68.8 kg ha^{-1}; B7 – D + P 4: 1. The norms of the innovative mixed fertilizer from cattle manure digestate and wood ash were: A1 – 5 t ha^{-1}, A2 – 10 t ha^{-1}, and A3 – 20 t ha^{-1}. Unfertilized winter oilseed rape fields and cattle manure digestate (D) fertilizer rates were used as control options. Studies have shown that using wood ash and digestate mixtures, it is possible to obtain good winter rapeseed yields without the use of mineral fertilizers. The highest winter rapeseed yield in the particular experimental year was obtained from variants where fertilizer rates of 10 t ha^{-1} were used for fertilization.

Key words: *digestate, wood ash, mixtures, winter oilseed rape.*

INTRODUCTION

Winter oilseed rape (*Brassica napus* L.) is widely cultivated in Latvia, with increasing yields (from 2.5 t ha^{-1} in 2010 to 3.3 t ha^{-1} in 2020) and total size of the growing area (from 67.6 thous. ha in 2010 to 141.1 thous. ha in 2023). To grow winter oilseed rape, a particular attention should be paid to the agronomic and economically sound use of fertilizers, thus reducing the costs and potential risks of environmental pollution (Litke, Gaile, Ruža, 2019).

Currently in Latvia, biogas cogeneration plants and various solid fuel boilers are widely used for heat and energy production, with digestate and wood ash as by-products. So far, these materials have been used in agriculture only as liming materials and fertilizers (Patterson et al., 2004; Koszel et al., 2020); however, the mixtures of wood ash and digestate can provide a high quality fertilizer that can be used to fertilize a wide range of agricultural crops (such as winter oilseed rape),

providing increase in the productivity and quality of crops (Hejcman, Ondracek, Smrz, 2011; Koszel et al., 2020).
The aim of the research was to study the effects of the mixtures of digestate and wood ash on the yield and quality of winter oilseed rape.

MATERIAL AND METHODS

The field trials were established at the Study and Research Farm "Peterlauki" (56°53' N, 23°71' E) of the Latvia University of Life Sciences and Technologies in the autumn of 2020-2023. Soil characteristics: sod calcareous soil Luvisols (according to FAO classification); granulometric composition – heavy dusty sand clay. Soil agrochemical parameters: soil reaction pH_{KCl} 6.7; plant-available phosphorus (P_2O_5) content – 60 mg kg^{-1}; potassium (K_2O) content – 144 mg kg^{-1}; organic matter (OM) content – 2.6%.
Winter oilseed rape was sown using fertilizer mixtures from cattle manure digestate (D) (obtained from joint stock company "Ziedi JP") and wood ash (P) (obtained from company with limited liability "Gren Jelgava") at different ratios: B1 –D; B2 –D +P 1:1; B 3 –D +P 2:1; B 4 –D +P 3:1; B 5 –D +P 3:1 + N16P40K60 kg ha^{-1}; B 6 –D +P 3:1 + N 68.8 kg ha^{-1}; B 7 –D +P 4:1. Innovative results from the digestate and wood ash mixture application were observed at the rates of A1 – 5 t ha^{-1}, A2 – 10 t ha^{-1}, and A3 – 20 t ha^{-1}.
The chemical composition of the digestate and wood ash mixtures is presented in Table 1 by which it is possible to calculate the amount of nutrients applied with each fertilizer mixture and rate.

Table 1. Nutrient content of the digestate and wood ash mixtures

Nutrients	Content in dry matter, %				
	D	D+P 1:1	D+P 2:1	D+P 3:1	D+P 4:1
Nitrogen in a natural	0.29	0.27	0.30	0.51	0.34
sample (N)	1.20	0.43	0.40	0.76	0.37
Ammonium nitrogen	0.74	0.90	0.89	0.83	0.83
(N/NH4), g kg^{-1}	1.70	2.90	2.92	2.73	2.64
Phosphorus (P)	2.41	13.44	13.55	10.48	10.86
Potassium (K)	9.27	12.19	11.84	11.22	10.91
Calcium (Ca)					
pH					

D – cattle manure digestate; P – wood ash

Unfertilized rapeseed plots and different cattle manure digestate norms were used as control variants. The variants in two-factorial trials were randomly allocated in three repetitions. In total, 66 plots were established, where the area of each plot was 30 m^2.

Figure 1. Fild trials with winter oilseed rape crops

The pre-crop to winter oilseed rape was winter wheat (*Triticum aestivum* L.). To prepare the experimental plots, ploughing was carried out to a depth of 22 cm and the pre-prepared mixtures of wood ash, cattle manure digestate and B 5 –D + P 3:1 + N16P40K60 kg ha^{-1} were spread onto the plots, incorporating the mixture into the soil with the “ZIRKON 8” compactor. For sowing eding the winter oilseed rape, the variety ‘Visby’ was used at a seeding rate of 80 germinating seeds per m^2 at a depth of 1.5–2 cm. In the spring, when vegetation recovers, N 68.8 kg ha^{-1} ammonium nitrate was applied to the B6 variant plots. After threshing the winter rape, the yield of each plot was weighed and purified using a “PFEUFFER SLN3” sample cleaner. Afterwards, the moisture, and seed oil content (%) and volume mass (kg hL^{-1}) of samples were determined using a FOSS “Infratec NOVA” analyser. Basing on the results, the yield (t ha^{-1}) and the amount of oil (t ha^{-1}) obtained were calculated at standard moisture (8%) and 100% purity of samples. The mass of 1000 grain was determined using a standard method (LVS EN ISO 520). Data processing was performed using "Microsoft Excel" and "R-Studio" computer programs.

RESULTS AND DISCUSSION

The average winter oilseed rape yield of 1.97–2.48 t ha^{-1} in the experimental plots studied was low compared to varietal potential (Table 2). This was due to the dry and hot weather conditions during winter oil rapeseed emergence and the start of vegetation. According to the data in the literature, drought stress in oilseed rape reduces stem diameter and length, negatively affecting the seed yield (Sangtarash et al., 2009). In our study, the lowest winter rapeseed yield of 1.97 t ha^{-1} was directly obtained from control field plots (Table 2).

Table 2.The effect of different digestate and wood ash mixture rates on winter rape seed yield, t ha^{-1}

Fertilizer rate, t ha^{-1} (F_A)	Digestate and wood ash ratio in the mixture (F_B)							(FA) p=0.003 RS(LSD) $_{0.05}$ = 0.149
	D	D+P 1:1	D+P 2:1	D+P 3:1	D+P 3:1 + NPK	D+P 3:1 + N	D+P 4:1	
Control	1.97							**1.97**
5 t ha^{-1}	1.85	2.21	1.94	2.24	2.12	1.77	2.30	**2.05**
10 t ha^{-1}	2.76	2.47	2.45	2.24	2.53	2.06	2.66	**2.45**
20 t ha^{-1}	2.26	2.67	2.13	2.03	2.11	2.25	2.50	**2.28**
On average p=0.046 RS(LSD) $_{0.05}$ (B) = 0.230 RS(LSD) $_{0.05}$ (AB) = 0.393	**2.29**	**2.45**	**2.17**	**2.17**	**2.26**	**2.03**	**2.48**	×

D – *cattle manure digestate*; P – *wood ash*

Significantly higher (p<0.05) average seed yields of winter rape in the trial years were obtained when fertilizer norm rates of 10 un 20 t ha^{-1} were used. The lowest fertilizer norm rate of 5 t ha^{-1} in variants D, D + P 2:1, and D + P 3:1 + N gave lower yields than in the control variants; however, the differences were not significant (p>0.05). Significantly higher (p<0.05) average winter rapeseed yields were for variants D, D + P 1:1, D + P 3:1 + NPK, and D + P 4:1, of which the highest yields were obtained from the fertilizer variants D + P 1:1 and D + P 4:1 (Table 2). Studies have shown that the use of liquid digestate of at least 25.000 L ha^{-1} as a fertilizer can significantly (p<0.05) increase the yield of winter rapeseed, and by increasing the digestate rates, the yield also increases (Koszel et al., 2020).

In the present trial, compared to the control, the use of digestate alone increased the average winter rapeseed yields at a fertilizer rate of 10 t ha^{-1} in all mixed fertilizer treatments, which suggests that nutrient deficiency was not a yield-limiting factor.in the trial years. The observed conclusion is also supported by the fact that the average increase in seed yield applying the digestate-wood ash mixture supplemented with N fertilizer only slightly increased the yield, although there are findings in the literature that the increase in N fertilizer to 60 kg ha^{-1} has also resulted in a significant increase in winter rapeseed yield regardless of the trial year (Litke, Gaile, Ruža, 2019).

One of the main indicators of winter rapeseed quality is the oil content of rape seeds. The analysis of the results showed that a significantly higher (p=0.07) average oil content in winter rape seeds was obtained using the 5 t ha^{-1} digestate and wood ash mixture rate. Among the average values of all mixture fertilizer variants, a significantly higher (p=0.001) oil content was obtained in the variants D + P 1:1, D + P 2:1, D + P 3:1, and D + P 3:1 + NPK; whereas, a significantly lower (p=0.001) oil content was obtained in the variants where ammonium nitrate (Table 3) was additionally used for fertilization. Such trend has also been observed in other studies where the increase in N fertilizer rate significantly decreased the oil content of winter rape seeds (Farahbakhsh, Pakgohar, Karimi, 2006).

Table 3.The effect of different digestate and wood ash mixture rates on oil content of winter rape seeds, %

Fertilizer rate, t ha^{-1} (F_A)	Digestate and wood ash ratio in the mixture (F_B)							On average (F_A)
	D	D+P 1:1	D+P 2:1	D+P 3:1	D+P 3:1 + NPK	D+P 3:1 + N	D+P 4:1	p=0.007 LSD $_{0.05}$ = 0.295
Control	47.3							**47.3**
5 t ha^{-1} 10 t ha^{-1} 20 t ha^{-1}	47.70 47.23 47.43	48.03 47.50 47.73	48.06 47.70 47.76	48.06 47.63 47.93	48.03 47.57 47.87	46.27 45.87 46.37	47.27 47.40 48.06	**47.63** **47.27** **47.59**
On average LSD $_{0.05}$ (B) = 0.390 LSD $_{0.05}$ (AB) = 0.781	**47.45**	**47.75**	**47.84**	**47.87**	**47.82**	**46.17**	**47.58**	×

D – *cattle manure digestate;* P – *wood ash*

The research suggests that the usage of wood ash and digestate mixtures as a fertilizer had no significant effect (p=0.334) on the average oil content in winter rapeseed. But on the other hand, increasing the fertilizer rates to 10 and 20 t ha^{-1} resulted in higher (p=0.004) oil yields in winter rapeseed (Table 4).

Table 4. The influence of different digestate and wood ash mixture rates on winter rapeseed oil yield, t ha^{-1}

Fertilizer rate, t ha^{-1} (F_A)	Digestate and wood ash ratio in the mixture (F_B)							On average (F_A)
	D	D + P 1:1	D + P 2:1	D + P 3:1	D + P 3:1 + NPK	D + P 3:1 + N	D + P 4:1	p=0.004 LSD $_{0.05}$ = 0.128
Control	0.93							**0.93**
5 t ha^{-1} 10 t ha^{-1} 20 t ha^{-1}	0.88 1.30 1.07	1.06 1.17 1.28	0.93 1.17 1.02	1.07 1.07 0.97	1.02 1.21 1.01	0.80 0.94 1.04	1.11 1.26 1.18	**0.98** **1.16** **1.08**
On average p=0.334	**1.08**	**1.17**	**1.04**	**1.04**	**1.08**	**0.93**	**1.18**	×

D – *cattle manure digestate*; P – *wood ash*

Analysis of the changes in seed volume mass showed that increased wood ash and digestate fertilizer norms significantly reduced (p=0.001) winter rapeseed volume mass compared to the control. Comparing the mixture variants, a significantly higher (p=0.001) volume mass was observed when additional ammonium nitrate fertilizer was applied. In this particular variant, the bulk density reached 67.96 kg hL^{-1}, which is similar to the control variant (Table 5).

Table 5.The influence of digestate and wood ash mixture rates on the volume mass of winter rape seeds, kg hL^{-1}

Fertilizer rate, t ha^{-1} (F_A)	Digestate and wood ash ratio in the mixture (F_B)							On average (FA)
	D	D + P 1:1	D + P 2:1	D + P 3:1	D + P 3:1 + NPK	D + P 3:1 + N	D + P 4:1	p=0.001 LSD $_{0.05}$ =0.162
Control	**67.93**							
5 t ha^{-1}	67.10	67.33	67.27	67.20	67.20	68.03	67.33	**67.35**
10 t ha^{-1}	67.17	66.83	67.50	67.17	66.93	67.93	67.50	**67.29**
20 t ha^{-1}	67.13	67.13	66.97	67.23	67.20	67.93	67.23	**67.26**
On average p=0.001 LSD $_{0.05}$ (B)=0.210 LSD $_{0.05}$ (AB)=0.430	**67.13**	**67.10**	**67.25**	**67.20**	**67.11**	**67.96**	**67.35**	×

D – *cattle manure digestate*; P – *wood ash*

In the trial, the effect of wood ash and digestate mixtures on 1000 grain weight of winter rapeseed was evaluated. The average 1000 seed mass of the samples ranged between 4.37 and 4.79 grams. Slight differences between fertilizer treatments were observed, but they were not significant ($p=0.671$). Also, no significant differences ($p=0.095$) were found between winter rapeseed samples and the applied fertilizer mixture rates. The low 1000 grain weight values obtained in the trial could be explained by the dry conditions these seasons, which hindered plant development in the spring and caused heat stress in plants at the end of flowering, before full maturity of rapeseed.

CONCLUSIONS

Using wood ash and digestate mixtures as a fertilizer, good winter rapeseed yields can be obtained without using mineral fertilizers.

The highest winter rapeseed yields in the trial years were obtained in the variants treated with 10 t ha^{-1} fertilizer mixture rates.

The highest oil content of winter oilseed rape was obtained in the variants treated with 5 t ha^{-1} fertilizer mixture rates.

The volume mass of winter rapeseed in the variants studied was slightly higher than 67.0 kg hL^{-1}.

REFERENCES

Farahbakhsh H., Pakgohar N., Karimi A. (2006). Effects of nitrogen and sulphur fertilizer on yield, yield components and oil content of oilseed rape (*Brassica napus* L.). *Asian Journal of Plant Sciences*, Vol. 5, p. 112–115.

Hejcman M., Ondracek J., Smrz Z. (2011). Ancient waste pits with wood ash irreversibly increase crop production in Central Europe. *Plant and Soil*, Vol. 339(1), p. 341–350.

Koszel M., Parafiniuk S., Szparaga A., Bochniak A., Kocira S., Atanasov A., Kovalyshyn S. (2020). Impact of Digestate Application as a Fertilizer on the Yield and Quality of Winter Rape Seed. *Agronomy*, Vol. 10(6), p. 878–896.

Litke L., Gaile Z., Ruza A. (2019). Effects of nitrogen fertilizer and soil tillage on winter oilseed rape yield and quality. *Balanced agriculture*: Proceedings of the LLU LF, LAB and LLMZA scientific-practical conference (February 21, 2019), Jelgava: LLU, 44-49. (In latvian).

Patterson S. J., Acharya S. N., Bertschi A. B., Thomas J. (2004). Application of Wood Ash to Acidic Boralf Soils and its Effect on Oilseed Quality of Canola. *Agronomy Journal*, Vol. 96(5), p. 1344–1348.

Sangtarash M. H., Qadri M. M., Chinnapa C. C., Reid D. M. (2009). Differential sensitivity of canola (*Brassica napus*) seedlings to ultraviolet-B radiation, water stress and abscisic acid. *Environmental and Experimental Botany*, Vol. 66, p. 212 – 219.

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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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