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Assessment of 1-Triacontanol treatment of sweet corn (*Zea mays* L. convar. *saccharata*) aimed at the improvement of salt tolerance based on a pot experiment

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Abstract: In our research, the salt stress of two sweet corn hybrids (Tyson and Sweetstar) was induced by irrigation with saline water in a pot experiment. We studied the possibility of the decrease of the negative effects of salt stress by applying *1-Triacontanol* (TRIA) in various (3, 6, and 9 μM) doses. Plant height, raw biomass, root biomass, parameters characterizing the photosynthetic activity (SPAD, NDVI, chlorophyll content), and proline content were determined in order to quantify the effect of the TRIA treatment on salt tolerance. We concluded that triacontanol treatments had a positive effect on the studied parameters of both hybrids.

Keywords: salt stress, Sweetstar, photosynthetic activity, chlorophyll content

1. **Introduction**

The European Union and the USA are the most significant sweet corn growers worldwide. Preceding France, Hungary is the leading sweet corn producer in the European Union [1]. In Europe, the area utilized with sweet corn is the largest in Hungary, which provides 6 percent of the world's production.

Hungary is a net exporter regarding the most important vegetable products (green pepper, watermelon, green peas, sweet corn). The export of sweet corn products is 22 times higher than the import [2]. In the case of this vegetable, profitability has increased by 64% in the past four years, so farmers producing sweet corn should reckon with a strong market position [3]. The agroecological (climatic and soil) conditions of Hungary are favourable for sweet corn production, which is the most successful vegetable crop in Hungary.

As the water demand of sweet corn is high, safe production can only be ensured with irrigation. Increasing the proportion of irrigated areas is particularly important in horticultural crop production [4], making cultivation safer with higher yields. Nevertheless, irrigation could be a limiting factor in agriculture, as the salinity of irrigation water (and in soils) affects at least 800 million hectares throughout the world, which is more than 6% in terms of the total land area [5, 6].

Unfortunately, the salinity of waters used for irrigation is also high in some regions of Hungary [7], which causes difficulties in vegetable production, as a number of literature data have proven that most vegetable species are sensitive to high salinity [8]. It is an existing problem especially in the region of Great Cumania, as the water used for irrigation is saline in several cases. In their research, Zsembeli et al. [9] proved that in Karcag the water coming from the underground is not suitable for irrigation. According to García et al. [10], half of all irrigated soils are affected by soil salinity in that region.

Salinity is a major environmental challenge in agricultural production, as the salts transported to and accumulated in the root zone could affect crop yield and quality negatively as they are generating an osmotic pressure [11, 12].

The growing population of the world poses a major challenge to agricultural production. Based on several research studies, the population of the world will grow significantly over the next 50 years, reaching more than 9-10 billion people. The growing population requires more intensive food production, so it is necessary to increase the amount of corn products as well [13].

As the population grows very rapidly, land and water resources are used in a volume that can lead to the exploitation of fertile agricultural land and freshwater resources. As sweet corn is a high-value vegetable, it is definitely recommended in the long term to expand the cultivation areas, which calls for the inclusion of less favourable but still high-quality land and water resources in production [14].

Under abiotic stresses, exogenously applied plant hormones, such as *Triacontanol* (TRIA), which is a long-chain primary alcohol, can increase the growth, photosynthetic pigments, and yields of several crop species (rice, wheat, maize, tomato) [15, 16].

Numerous researchers have reported that TRIA has a positive effect on several plant physiological processes such as carbon dioxide and nitrogen fixation, protein synthesis, uptake of water and nutrients; furthermore, it can increase free amino acid content and enzyme activities in maize (*Zea mays* L.) [17, 18].

The aim of our experiment was to investigate and quantify the effect of three different doses of TRIA, applied as a foliar fertilizer, on the salt tolerance of two sweet corn hybrids. In our research, salt stress was induced by irrigation with saline water in a pot experiment, as we examined the possibility of utilizing agricultural areas that are suitable for sweet corn production yet characterized with unfavourable agro-ecological conditions (saline irrigation water, risk of secondary salinization). Given that the population demand for food resources is estimated to increase [19], the examination of the possibilities of growing crops in less favourable areas is becoming more and more important in order to promote sustainable agriculture.

2. Material and methods

In our study, the farming practice of Great Cumania was represented, which is a typical region with salt-affected soils in Hungary. We induced salt stress by using irrigation water characterized by high (> 500 mg L^{-1}) salt content. The aim of the experiment was to increase the salt tolerance of sweet corn and to quantify the difference in salt tolerance between the two hybrids under study.

The pot experiment

In our pot experiment (*Fig. 1*), we tested two sweet corn hybrids, namely Sweetstar and Tyson (Syngenta®), which are proven to be salt-tolerant on the basis of the results of a preliminary experiment.

Figure 1. Sweet corn hybrids in the pot experiment

The experiment was set up on 17/09/2020 in plastic pots filled with 5 kg of calcareous chernozem soil. The pots were placed in a heated greenhouse, where we could maintain air temperature values (22 ± 7ºC) optimal for the development of sweet corn. The main properties of the soil filled into the pots are summarized in *Table 1*.

Table 1. Some parameters of the 0–20 cm layer of the soil used in the pot experiment

Para-meter	**$PH_{(KCl)}$**	**Electronic conductivity (EC)**	**Humus content**	**NO_3-N**	**P_2O_5**	**K_2O**	**Na**	**$CaCO_3$**
Unit		mS cm^{-1}	%	mg kg^{-1}	mg kg^{-1}	mg kg^{-1}	mg kg^{-1}	mg kg^{-1}
	7.8	0.44	1.2	18.4	722	334	58	2.49

The soil can be characterized by weakly alkaline pH and 0.44 mS cm^{-1}, which means low salinity. The soil used in the experiment is poor in humus (1.2%). The soil of the pots is very well supplied with phosphorus and potassium and has a medium sodium content. In terms of calcium carbonate content, this calcareous chernozem soil can be considered weakly calcareous.

Induction of salt stress

Besides the beneficial effects of irrigation, we must also pay attention to its unfavourable effects reducing soil fertility and yields. If the irrigation water applied is inadequate, improper use can result in secondary salinization. The process of salinization is a change in the adsorption conditions in which the amount and proportion of sodium ions among the cations bound on the surface of the soil colloids increase, resulting in unfavourable soil physical properties. Secondary salinization occurs when the negative effects are caused by the high salinity of the irrigation water. As the total dissolved salt content of the water used for irrigation is also very high in Karcag and in its surrounding area, we used irrigation water originating from the drinking water network of Karcag to induce salt stress in our experiment. The main parameters of the irrigation water applied are summarized in *Table 2*.

Table 2. Some parameters of the irrigation water in Karcag

pH	EC	Dry matter content	Ca^{2+}	Mg^{2+}	Na^{+}	K^{+}	CO_3^{2-}	HCO_3^{-}	Cl^{-}	SO_4^{2-}	NO_2^{-} $+NO_3^{-}$
	mS cm^{-1}	g l^{-1}	mg l^{-1}	mg l^{-1}	mg l^{-1}	mg l^{-1}	mg l^{-1}	mg l^{-1}	mg l^{-1}	mg l^{-1}	mg l^{-1}
7.5	1.4	0.8	4.2	2.5	9.0	0.1	0	800	88.8	0.00	5.1

As according to the Hungarian standards water is not suitable for irrigation (total soluble salt content is above 500 mg L^{-1}), its use carries the risk of secondary salinization and can cause salt stress in cultivated plants.

Stressing the plants with salty water was initiated in the same time as sowing and was continued until the end of the experiment (the beginning of male flowering). The irrigation protocol, the electrical conductivity and salt concentration of the irrigation water, and the salt loads by irrigation are summarized in *Table 3*.

Table 3. The irrigation protocol

Date of irrigation	**Dosage of irrigation**	**EC**	**Salt concentration of the irrigation water**	**Salt input**
	L per pot	*mS cm^{-1}*	*mg L^{-1}*	*mg per pot*
09/09/2020	0.5	1.41	902.4	451.2
14/09/2020	0.5	1.41	902.4	451.2
18/09/2020	1	1.41	902.4	902.4
21/09/2020	0.3	1.41	902.4	270.7
06/10/2020	0.2	1.41	902.4	180.5
09/10/2020	0.3	1.41	902.4	270.7
16/10/2020	0.3	1.6	1024	307.2
27/10/2020	0.3	1.6	1024	307.2
30/10/2020	0.3	1.6	1024	307.2
04/11/2020	0.3	1.6	1024	307.2
09/11/2020	0.3	1.6	1024	307.2
Total	4.3			4,063

In total, 4.3 litres of saline water containing 4,063 mg of salts was irrigated on each pot in order to satisfy the water needs adapted to the growth of the plants until the beginning of flowering.

The indicator crops were fertilized four times with Ferticare IV (YARA) water-soluble fertilizer and two times with Mono Zn (Natur Agro) foliar fertilizer.

Triacontanol treatments

The plants exposed to salt stress were treated with foliar fertilizer made from TRIA. It is known to affect metabolism and regulate a number of physiological and biochemical processes.

During our study, we treated the plants with three different doses: 3, 6, and 9 micromolar (µM) of TRIA. In each case, these solutions were prepared from 1 litre of deionized water and the amount of TRIA (molar mass: 438.8 g MOL^{-1}) corresponding to the doses used, to which 1 ml of TWEEN 20 (E432) adhesive was added in order to make the applied solution adhere to the foliage as much as possible.

As control, only deionized water was sprayed on the plants. TRIA foliar treatment was performed a total of 7 times, each time 2 ml being applied onto the foliage of each plant.

As per to the main goal of the experiment, we wanted to investigate the possibility of using TRIA treatment to reduce the negative plant physiological effects on sweet corn suffering from salt stress.

Examined parameters

The indicator crops were harvested at the time of flowering. Because of the different lengths of the vegetation period of the hybrids, Sweetstar was harvested at 75 days of age and Tyson at 87 days of age. During the research, SPAD (Minolta SPAD 502, Japan), NDVI values (Trimble® GreenSeeker® crop sensor), and the total chlorophyll content (mg per 100 g fresh weight) of the plants were measured based on the "AOAC 942.04 (1995)" method, these values characterizing the photosynthetic activity of the plants. Plant height (cm), the raw biomass of the young plant (g), root weight (g), and number of leaves (per plant) were also examined to monitor the growth and development of the plants. Furthermore, proline content (100 µM per 100 g of fresh weight) as a plant stress hormone was also determined based on the method of Bates et al. [20].

Statistical data analysis

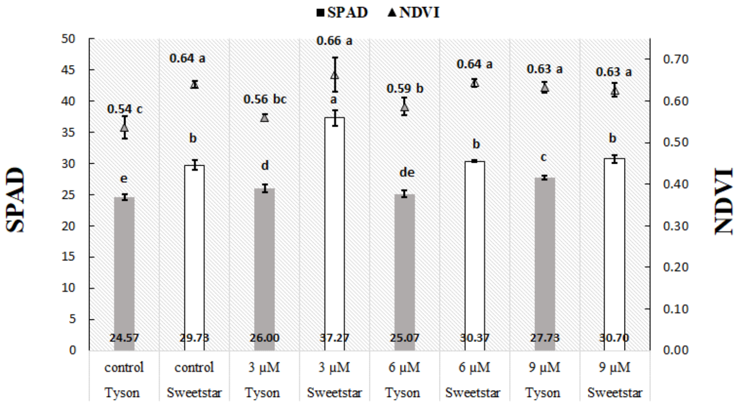
The statistical reliability of our results was tested with the SPSS 25.0 software package. One-way analysis of variance ($p < 0.05$) was used, as we were primarily interested in the effects of the different doses of the foliar fertilizer. Duncan's test ($p < 0.05$) was used to determine the statistical reliability of the differences between the means. One treatment was performed in 5 replicates.

3. Results and discussion

Effect of the treatments on the parameters characterizing the photosynthetic activity of sweet corn

We considered important to examine the effect of the treatments on the photosynthetic activity of sweet corn, as this parameter can be easily modified by environmental and technological conditions as well as by various stress factors.

Figure 2 shows the effect of the treatments on the SPAD and NDVI values of plants. The SPAD value gives us information about the relative chlorophyll content of the leaves while the NDVI about the relative chlorophyll content of the foliage.



Note: Means signed with the same letter are not significantly different at the 5% significance level.

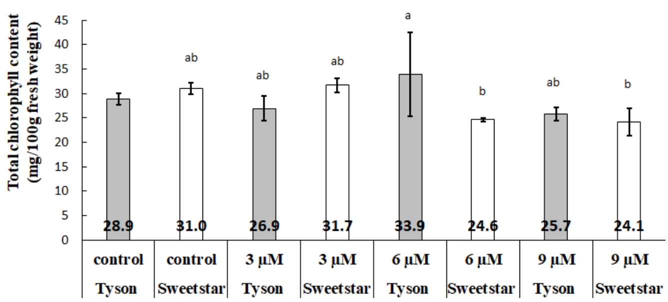
Figure 2. Effect of the treatments on the SPAD and NDVI values of sweet corn

Based on our results, it can be established that in the case of the hybrid Tyson, the different doses of TRIA foliar treatment slightly improved the SPAD and NDVI values compared to the control of the same hybrid, and these differences were statistically proven. The best results were measured in the case of the dose of the 9 µM treatment (SPAD: 27.7 NDVI: 0.63).

For the hybrid Sweetstar, the different doses of TRIA treatments improved the SPAD values in all cases compared to the control, while the NDVI values were only improved by the 3 µM TRIA treatment. The 3 micromolar treatment was proven to be the best for both parameters (SPAD: 37.2, NDVI: 0.66) for that hybrid.

The SPAD and NDVI values measured for Sweetstar were significantly more favourable than those for Tyson.

Chlorophylls are photosynthetic pigments that fundamentally determine the photosynthetic activity, and therefore the effect of the treatments on the total chlorophyll content of the plants was also examined. The relevant results are shown in *Figure 3*.



Note: Means signed with the same letter are not significantly different at the 5% significance level.

Figure 3. Effect of the treatments on the total chlorophyll content of sweet corn

In the case of the hybrid Tyson, the dose of 6 µM of the TRIA treatment (33.9 mg per 100 g of fresh weight) while in the case of hybrid Sweetstar the effects of the 3 µM dose (31.7 mg per 100 g of fresh weight) were the highest in terms of the total chlorophyll content of the plant leaves. Examining the total chlorophyll content of Sweetstar, we found that the use of TRIA treatment in doses higher than 3 µM had a negative effect on this parameter. However, these results could not be verified at a significance level of 5%.

Effect of the treatments on some morphological parameters of sweet corn

Since abiotic stress factors also affect the vegetative plant development, we considered to determine the effect of the treatments on the parameters of plant height, total aboveground (raw) biomass, root mass, and total number of leaves as important in our experiment. The results of these measurements are summarized in *Table 4*.

Table 4. The effect of different doses of TRIA treatments on some morphological parameters of sweet corn

Hybrid	Treatment	Plant height	Total aboveground biomass	Root mass	Total number of leaves
		cm	g per plant	g per plant	leaves per plant
Tyson	control	56.0cd	43.5c	1.6d	11.0ab
Sweetstar	control	76.2b	56.8ab	3.1bc	10.5b
Tyson	3 µM	57.3c	51.4b	1.8d	10.7b
Sweetstar	3 µM	86.0a	61.8a	4.0a	9.7c
Tyson	6 µM	62.0c	51.6b	1.8d	11.0ab
Sweetstar	6 µM	48.1c	32.6d	3.5ab	9.7c
Tyson	9 µM	64.3c	52.9b	1.8d	11.7a
Sweetstar	9 µM	71.3c	38.2cd	2.9c	9.7c

Note: Means followed by the same letter within the column are not significantly different at the 5% significance level.

Based on the morphological parameters, it can be concluded that for Tyson the TRIA treatments resulted in greater plant height and total aboveground (raw) biomass in all cases compared to the untreated individuals. The highest plant height and total aboveground biomass values of Tyson were measured for the 9 µM TRIA treatment (64.3 cm and 52.9 g) – the differences compared to the control were statistically proven.

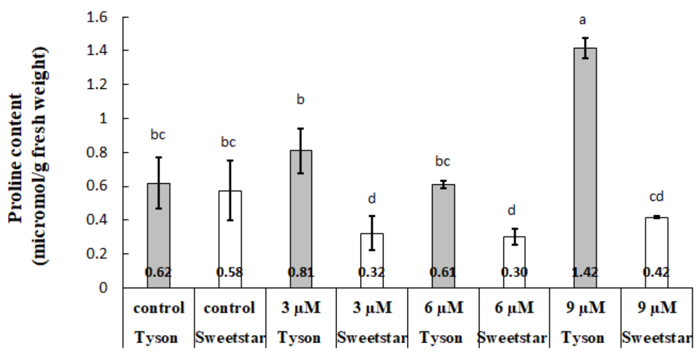
In the case of Sweetstar, both plant height and total aboveground biomass were improved by the 3 µM treatment compared to the control (86.0 cm and 61.8 g); however, the higher doses negatively affected these parameters, just like in the case of the total chlorophyll content.

Based on the examination of the root mass of the sweet corn hybrids we used, it can be established that in the case of Tyson, the different doses of TRIA improved the root weight equally (1.8 g), while in the case of Sweetstar the TRIA treatment of 3 µM dose was the most favourable, resulting in a 4.0 g increase, which was statistically significant.

The salt stress induced in our study did not cause such a stress effect on sweet corn that would have seriously affected the leaf number of the plants and thus the size of the assimilation surface. In the case of Tyson, the 9 µM TRIA treatment, which had a positive effect on several parameters, minimally increased the average number of leaves per stem (11.7). However, for Sweetstar, the TRIA treatments had no positive effect on the average leaf number.

Effect of the treatments on the proline content of the two sweet corn hybrids

Against salinity and osmotic stress, plants accumulate proline in their organisms in order to deal with environmental stresses [21]. Numerous researchers have reported that the accumulation of proline in sweet corn is a natural response to salt stress [22]. Based on Celik and Atak [23], proline accumulation can be a stress effect (a response to stress factors) or can be the cause of stress tolerance (a kind of protector agent).



Note: Means signed with the same letter are not significantly different at the 5% significance level.

Figure 4. Effect of the treatments on the proline content of sweet corn

Based on the results gained from the measurement of proline content, it can be established that regardless of the treatment, the hybrid Sweetstar produced less proline than the hybrid Tyson, suggesting that Sweetstar can be considered more salt tolerant than Tyson. This fact was also proven by the research of Karimi et al. [24], as they found that the amount of the accumulated proline was significantly higher in the more salt-sensitive maize genotypes than in the more salt-tolerant ones.

As Sweetstar is proven to be more salt tolerant, it accumulated significantly less proline than Tyson. Since the lowest proline (0.32 and 0.30 µM per 100 g of fresh weight) was measured in Sweetstar after the 3 and 6 micromolar treatments, we can conclude that the less proline accumulates in a more salt-tolerant plant, the less stress the latter experiences under the given environmental conditions.

In the case of Tyson, which was proven to be more sensitive to salt stress, proline accumulation increased as a result of the TRIA treatments. Proline production was the highest as a result of the 9 µM dose treatment (1.42 µM per 100 g of fresh weight), which was found to be favourable for other parameters too, which indicates that the plants tried to protect themselves against the negative effects of salt stress.

4. Conclusions

Based on our results, we found that the hybrid Sweetstar was more salt tolerant than the hybrid Tyson in all tested parameters. In the case of Sweetstar, the 3 µM TRIA treatment improved the parameters characterizing the photosynthetic activity of the plant, as well as plant height, raw biomass, root weight, and proline content. However, the higher doses negatively affected several parameters of Sweetstar (total chlorophyll content, plant height, raw biomass, leaf number). For the less salt-tolerant Tyson, higher doses of TRIA treatments were proved to be more effective for most of the parameters.

We concluded that TRIA as a foliar fertilizer is suitable for improving the salt tolerance of sweet corn exposed to salt stress. Based on the examined parameters, it can be stated that different doses of TRIA can be recommended for treating the hybrids with different degrees of salt tolerance. We suggest a lower dose (3µM) of TRIA application for tolerant hybrids and a higher dose (9µM) for salt-sensitive ones.

Acknowledgement

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Farmers' use of sustainable production practices for yellow pepper crop in the Nsukka agricultural zone, Enugu State, Nigeria

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Abstract: This study investigated farmers' use of sustainable production practices for yellow pepper crop in the Nsukka agricultural zone, Enugu State, Nigeria. Multi-stage sampling procedure was used in selecting 135 farmers and interview schedule using structured questionnaires was employed for data collection. Data generated were analysed with SPSS software using frequency and percentage, mean scores, standard deviation, factor analysis, and logistic regression. Findings revealed that the majority (91.1%) of the yellow pepper farmers had moderate knowledge on sustainable production practices and that some of the practices used were mass selection (97.0%), use of poultry manure (95.60%), use of improved varieties of yellow pepper (94.0%), crop rotation practices (91.10%), and minimum use of agrochemicals (78.50). Among the factors considered to be potential determinants of farmers' use of sustainable production practices, only sex was statistically significant, at 5% level. Also, findings indicated that technical, institutional, and economic constraints affected farmers' use of sustainable production practices. Furthermore, results indicated that sustainable production practices could be enhanced through the use of improved seeds (85.5%), access to credit facilities (77.8%), access to extension services (71.8%), and training of farmers (68.9%). The study therefore recommends that government and private sectors should provide services that will facilitate the development of sustainable production practices of Nsukka yellow pepper crop, as this will have a long-term effect on productivity and the production environment.

Keywords: pepper, sustainable, manure, crop, Nigeria

Introduction

Pepper is a major spicy crop grown in Nigeria. The bulk production of the crop occurs in the dryer Savannah areas of Nigeria where irrigation is often practised. In the south-east zone, pepper is widely cultivated, but the production is mostly carried out in rain-fed systems. This crop used to be termed “women’s crop” because it was mostly grown by women [21, 20], but today most men are into the production of pepper due to its lucrative nature. Pepper crop offers income security to many farmers and provides employment to about 70% of the population (including local farm labourers) in the region [21].

Pepper is an important food crop with essential nutrients. It provides minerals, pro-vitamin A, vitamins C and E, carotenoids, phenolic compounds, and metabolites [15, 19]. Also, it provides flavour, colour, and pungency to human food. Apart from using pepper for (domestic) culinary purposes [4], it is also used for the seasoning of some processed foods, in hot sauces and in pickling [22]. Additionally, pepper is used in medicine as anti-oxidant in reducing degenerative diseases, asthma, cough, sore, and toothache [26]. It is also used in plant-based insecticides and in the cosmetics industry for fragrance ingredients, as well as for hair and skin conditioning [11].

There are different varieties of pepper in Nigeria. However, the common species that has offered an important source of livelihood to many farmer families in the Nsukka agricultural zone, owing to its acceptability and potential, is the Nsukka yellow pepper. The Nsukka yellow pepper is indigenous to Nsukka, widely grown in Enugu State, and rarely grown elsewhere in the country, probably because of its propensity to lose pungency, aroma, and colour [28]. This species of pepper has a unique aroma, and the fruits are considerably large, green at the early stage and yellowish at maturity stage [10]. The distinctive aroma of the pepper enhances its acceptability in the market by attracting more customers than other pepper types in the rural and urban markets within the zone. Similarly, [17] asserted that the distinctive aroma of the crop makes it very much cherished by women and hotel managers for cooking and other uses. The yellow pepper fruit also exhibits traits such as pendant, conical, pointed end, and slight fruit corrugation at the immature and mature stages [8]. It bears an adequate number of fruits at a given time [2], with about 1-3 fruits occurring in the axils of one leaf [9]. These desirable characteristics of the Nsukka yellow pepper have drawn the attention of researchers to take measures to preserve them, and one of the ways is the adoption of sustainable production practices.

Sustainable agricultural production practices are management procedures that work with natural processes to conserve all resources, minimize waste and environmental impact, prevent problems, and promote agro-ecosystem resilience, self-regulation, evolution, and sustained production for the nourishment and satisfaction of everyone [16]. It is the production practices and farming systems that maintain the ability of agriculture to produce agricultural commodities and products, maintain a decent standard of living for the farmers yet jeopardize neither the ability of future generations of farmers to produce and maintain a decent standard of living nor the quality of the environment for both present and future generations [12]. By implication, agricultural practices that diminish long-term prospects for food production, regardless of their short-term benefits, are not considered sustainable [3]. A holistic discussion on sustainability is often done under the three dimensions of social, environmental, and economic sustainability [25]. The three pillars of sustainability are intertwined and sometimes inseparable. For instance, when environmental sustainability is compromised, other aspects will be affected. According to [24], changes in land and overuse of land as a result of increased cropping cause loss of biodiversity (environmental problem) and, by extension, reduce food production as well as affect income generation from the farm. On the contrary, the use of crop rotation, increased crop diversity, use of cover crops, no-till and reduced-till systems, integrated pest management (IPM), sustainable agro-forestry practices, and precision farming, among others, facilitate ecosystem protection, increase farm productivity, reduce poverty, and advance food security [23]. Based on these premises, this study sought to gain an insight into farmers' use of sustainable production practices on yellow pepper crop in the Nsukka agricultural zone, Enugu State, Nigeria.

So far, many researchers ([21], [28], [17], [10], [6], [29], [5], [22], [2], [8], and [9], among others) have conducted studies on the Nsukka yellow pepper but none have focused on sustainability. This study was carried out to fill this gap by providing information on sustainability practices for the crop. Specifically, it looked into farmers' knowledge on sustainable production practices, the sustainable production practices used, factors that influence farmers' use of sustainable production practices, constraints to the use of sustainable production practices, and it determined the measures to be taken to enhance the use of sustainable production practices for the Nsukka yellow pepper.

Methodology

Study area

The study was conducted in the Nsukka agricultural zone, Enugu State, Nigeria. The zone comprises three areas as follows: Nsukka, Igbo-Etiti, and Uzo-Uwani, is situated on a gentle slope with hills and valleys, and is located between longitude

$7^{0}20^{1}$E and $7^{0}29^{1}$E and latitude $6^{0}54^{1}$N and $7^{0}00^{1}$N. The predominant crops produced in the area include yellow pepper, cassava, maize, cocoyam, yam, rice, cucumber, oil palm, and vegetables. The area has been recognized for growing an indigenous pepper popularly known as the Nsukka yellow pepper.

Population and sampling procedure

The population for the study comprised all yellow pepper farmers in Nsukka. Multi-stage sampling production was used to select respondents for the study. At the first stage, all blocks (Nsukka, Uzo-Uwani, and Igbo Etiti) in the Nsukka agricultural zone were used for the study. At the second stage, 3 cells were randomly selected from each of the blocks in the zone, giving a total of 9 cells. At the third stage, 15 yellow pepper farmers were randomly selected from each of the 9 cells based on their involvement in the Nsukka yellow pepper production, giving a total of 135 respondents that were used for the study.

Method of data collection and measurement of variables

Data for the study were collected using structured interview schedule administered by the researcher and the assistants. The knowledge levels of farmers on sustainable production practices was elicited by providing 18 positive and negative statements/ items on sustainable production practices and having the farmers respond to each of the questions by ticking either Yes or No. One mark was assigned to each correct answer and zero to a wrong answer. Each respondent had a composite score. The negative questions were reversed, and respondents were categorized into four groups based on their knowledge level, namely: No knowledge (for respondents with 0 scores); Low knowledge (for respondents with 1–6 scores); Moderate knowledge (for respondents with 7–12 scores), and High knowledge (for respondents with 13–18 scores). To ascertain the sustainable production practices that farmers are using, a list of sustainable production practices (such as use of conservative tillage, use of mass selection, minimal use of agrochemicals, use of crop rotation to control built-up of pests and diseases, use of cover crops, and integrated pest management among others) was provided, and respondents indicated either “used” (1) or “not used” (0). A composite score for the use of sustainable practices was obtained for each respondent. The scores were categorized into three levels (low use, moderate use, and high use) and subjected to further analysis. Also, to ascertain the constraints to the use of sustainable production practices by Nsukka yellow pepper farmers, a list of constraints was provided and farmers rated them on a four-point Likert-type scale as follows: to a great extent (3), to some extent (2), to a small extent (1), and to no extent (0). The values were added and divided by 4 to give a mean score of 1.5. Any variable with a mean value equal to or greater than 1.5 was regarded as a constraint to the use of sustainable production practices in yellow pepper production, while variables with mean scores lower than 1.5 were considered otherwise. The obtained data were subjected to further analysis, and variables with a value of 0.4 or higher were regarded as constraints (technical, institutional, or economic). Furthermore, information on measures to enhance the use of sustainable production practices was gathered. To achieve this, respondents were asked to suggest measures that could be used to enhance the sustainability of yellow pepper production, and their responses were collated. To determine the factors that influence farmers' use of sustainable production practices for yellow pepper crop, the model is represented as thus:

$$Logit\ (P\ (Y \leq j)) = \beta_{j0} + \beta_{j1}X_1 + \beta_{j2}X_2 + \cdots + \beta_{jn}X_n \ for\ j=1,\ldots,j-1 \quad (1)$$

$$Logit\ (P\ (Y \leq j)) = \beta_{j0} + \beta_1 X_1 + \beta_2 X_2 + \cdots + \beta_n X_n \quad (2)$$

where: Y = ordinal outcome variable (level of use of the sustainable production practices)

P = predictors

J = ordered categories for the dependent variable (low, moderate, and high)

β_0 = intercept

$\beta_{1-}\ \beta_n$ = parameter estimates

X_1-X_n = independent variables

X_1 = age (years)

X_2 = sex (male = 1; female = 0)

X_3 = marital status (married =1; not married = 0)

X_4 = household size (continuous)

X_5 = member of organization (member = 1, not member = 0)

X_6 = year of farming experience (continuous)

X_7 = farm size (continuous)

X_8 = education status (formal education = 1; no formal education = 0)

Data analysis

Data generated were analysed with SPSS software using frequency and percentage, mean scores, standard deviation, ordinal logistic regression, and factor analysis.

Results and discussion

Yellow pepper farmers' knowledge level of sustainable production practices

Figure 1 revealed that the majority (91.1%) of the yellow pepper farmers had a moderate level, 8.2% had a high level, and 0.7% had a low level of knowledge on sustainability production practices. This implies that respondents lack adequate access to timely information on innovations and sustainability practices involved in yellow pepper production. It could also mean that there is a lack of training on sustainable farming. The poor information dissemination and trainings on sustainable practices might be due to inadequate extension service delivery to farmers. Extension service is entrusted with supplying reliable and sustainable agricultural information to farmers in order to improve their production practices and productivity. A few farmers that have a high level of knowledge about sustainable production practices may be cosmopolitans who sought information about pepper production and its sustainable practices elsewhere. This finding agrees with [27], who found that pepper farmers in Malaysia were knowledgeable about sustainable agricultural practices.

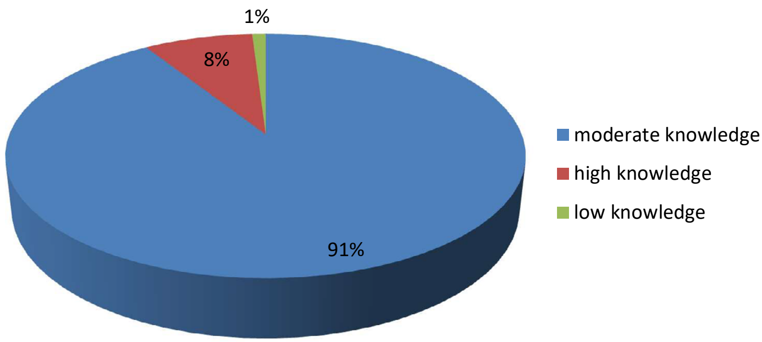

Figure 1. Yellow pepper farmers' knowledge level of sustainable production practices

Sustainable production practices for the Nsukka yellow pepper crop

The results in *Table 1* indicate that 99.3% of the respondents harvest their pepper at optimum time, 98.5% use mass selection (selecting and preserving seeds of the best yellow pepper plants for the next planting season), 97.8% use poultry manure, 95.6% use improved varieties of yellow pepper, 94.8% use appropriate plant spacing, 92.6% use crop rotation practices, and 80% make minimum use of agrochemicals, among others. Results imply that farmers use one or more sustainable production practices for the Nsukka yellow pepper crop. Mass selection may have been used by the majority of the yellow pepper farmers because it has the best seed properties for planting. It could be that the use of the mass selection method is easier for them than the use of the pedigree selection method and pure-line method, which few people practise. [1] noted that mass selection is very important in the domestication of major crop species. The practice of mass selection will provide farmers the opportunity to select the Nsukka yellow pepper with differing phenotypes and to develop a new cultivar by improving the average performance of the crop species. Similarly, the practice of optimum harvesting time in yellow pepper crops could be a strategy to get seed traits or fruit quality and reduce postharvest losses or pest infestation. Also, the high usage of poultry manure and other organic manures in yellow pepper production could be because of the awareness that organic manure improves plants' biodiversity and environment. This may be the case because of poultry dung and other organic manures are available and relatively cheaper than inorganic fertilizers. [10] noted that yellow pepper fruits grown with 10 t/ha poultry manure had the highest percentage of fat, crude fibre, moisture content, alkaloid, flavonoid, tannin, and volatile oil. Also, [18] reiterated that organic fertilizers are vital for the healthy development of pepper and vegetable blooms and fruits because they provide rapid growth with superior quality to all species. Furthermore, the minimum use of agrochemicals and crop rotation are good sustainability practices. While crop rotation aims to reduce the amount of the pest population present on the production site and allows the land to regain fertility, the minimum use of agrochemicals, such as fertilizers, herbicides, and pesticides, among others, is good for maintaining soil structure and reducing harm to crops and animals. This finding is consistent with [30], who found that crop rotation, compost manure, the planting of tolerant crop varieties, and cover cropping were some of the sustainable agricultural practices adopted by farmers in the Ohaukwu Local Government Area of Ebonyi State.

Table 1. Use of sustainable production practices by Nsukka yellow pepper farmers

Variables	**Frequency**	**Percentage**
Optimum harvest time	134	99.30
Use of mass selection of seeds	133	98.50
Use of poultry manure	132	97.80
Use of improved varieties of yellow pepper	129	95.60
Appropriate plant spacing	128	94.80
Crop rotation practices	125	92.60
Seed and seedling selection practices	114	84.40
Minimum use of agrochemicals	108	80.00
Minimized bush burning practice	100	74.10
Pure-line selection	83	61.50
Use of recommended doses of fertilizers	63	46.70
Use of compost manure	48	35.60
Use of lime/ash in pH control	47	34.80
Inter-plant with cover crops	39	28.90

Variables	Frequency	Percentage
Treatment of organic manure before use	32	23.70
Use of mulching	23	17.00
Treatment of yellow pepper seeds before nursery	23	17.00
Integrated pest management	17	12.60
Pedigree selection of seeds	6	4.40
Biological weed control practices	5	3.70
Conservative tillage practices	4	3.00

Source: field survey, 2020

Factors that influence the level of use of sustainable practice for yellow pepper crop

Table 2 shows the ordinal logistic regression analysis carried out to identify the main factors influencing farmers' use of sustainable practices for yellow pepper crop. Several factors were considered as potential determinants in this regard. However, results revealed that only sex was statistically significant (coefficient = -1.632; $p < 0.05$) at 5% significance level. The value is negative, and it implies that a unit increase in the number of male or female farmers will decrease the level of use of sustainable practice for yellow pepper crop, given that all other variables in the model are held constant. The result is unusual because, ideally, a unit increase in the number of farmers should lead to increase in the use of sustainable practices on a specific farm. Nevertheless, the reason might not be far from the fact that the use of any agricultural practice is dependent on an individual's willingness in addition to the wherewithal to use it. This finding corroborates the result from [30], who revealed that sex was significantly correlated with farmers' adoption of sustainable agricultural practices in Ebonyi State. On the other hand, [7] found that farmers' age, farming experience, and education status were factors that affected citrus farmers' adoption of sustainable agriculture practices in the Northern Ghor of Jordan valley. Similarly, [17] found that education level, income from agriculture, farmer cooperative and credit were determinant factors in the adoption of most of the agricultural practices that were considered.

Table 2. Factors that influence the level of use of sustainable practice for yellow pepper crop

Variables	Coefficient	Std. error	Wald	P-value
Age	0.052	0.031	2.749	0.097
Sex	**-1.632****	0.775	4.430	**0.035**
Marital status	-0.472	1.028	0.210	0.646
Household size	0.023	0.127	0.033	0.856

Variables	Coefficient	Std. error	Wald	P-value
Member of organization	-1.066	0.967	1.215	0.270
Year of farming experience	0.064	0.058	1.224	0.269
Farm size	0.021	0.025	0.689	0.407
Education status	1.759	1.215	2.095	0.148

Notes: test of parallel lines: 0.064; goodness-of-fit test of overall model: Pearson (0.069), deviance (1.00); model fitting information: chi-square = 8.860; Nagelkerke's R = 0.115.

Constraints to the use of sustainable production practices on the Nsukka yellow pepper crop

Table 3 revealed the major constraints to farmers' use of sustainable production practices on the Nsukka yellow pepper crop. The Varimax Rotation results on constraints were classified into three factors based on the variable loading. Technical constraint (Factor 1) is made up of variables relating to insufficient knowledge in production, processing, and sustainable practices of the Nsukka yellow pepper crop. These variables include among others: lack of technical know-how on yellow pepper production (M = 0.817), lack of capability in soil fertility management (M = 0.811), lack of access to training on sustainable practices (M = 0.735). The training of farmers in sustainable practices is sacrosanct because these farmers depend on the indigenous knowledge and information from fellow farmers to grow yellow pepper. The training will build their capacity on both sustainable production and processing of the yellow pepper crop. Adequate training of the rural farmers is the sure way to achieving sustainable agriculture and increasing productivity, while poor access to training limits productivity. This is in agreement with the findings by [30], who revealed that poor knowledge of sustainable practices and low literacy limit farmers' use of sustainable agricultural practices. [14] also found that the major constraint to sustainable high-yielding wheat production in Tajikistan was lack of knowledge among farmers (lack of knowledge about the variety grown, lack of crop rotation, and poor crop performance).

In a similar way, the institutional constraint (Factor 2) comprises inadequate extension services (M = 0.879), lack of access to information on yellow pepper innovations (M = 0.819), and poor access to finance/credit facilities (M = 0.489), among others. Lack of extension services has been a reoccurring problem for the development of agriculture in developing countries. Extension workers are meant to disseminate and/or train farmers on agricultural innovations, practices, or techniques. However, due to poor extension service delivery in most farming communities, farmers depend on their own knowledge and the information they receive from neighbours. This has not only hindered the acceptance of innovation or practices (such as sustainability), but it has also deprived farmers of important information and skills in agriculture. This is in agreement with [30], who found that inadequate extension contact, climate factors, and poor extension in promoting sustainable agriculture reduce farmers' opportunities to use sustainable agricultural practices. Similarly, lack of access to credit facilities also constitutes a huge hindrance to the development of agriculture. Farmers who have access to credit facilities can procure farm production inputs, adopt innovations or practices, and pay for labour. On the other hand, farmers without enough capital and opportunities for credit facility will find it difficult to accept new practices. This finding is consistent with [31], who found that the major constraints faced by farmers in sustainable agricultural production were financial problems, labour scarcity, and irrigation water shortage.

Furthermore, economic constraints include high perishability of yellow pepper (M = 0.753), low return on investment in yellow pepper (M = 0.706), cross-pollination with other cultivars (M = 0.633), and unavailability of improved varieties of yellow pepper (M = 0.532). Most farmers lost their produce during and after harvest because of the perishable nature of the yellow pepper crop. This reduces farmers' income generation from the produce. Similarly, the low return on the sales of yellow pepper crop, especially during glut, discourages farmers from expanding production and embarking on sustainable practices. Again, the absence of improved varieties of yellow pepper leads to farmers growing the same species that does not give the best yield and are not resistant to pests, diseases, and harsh environmental conditions. This finding is consistent with [30], who found that unavailability of inputs and poor incentives were some of the constraints to the adoption of sustainable agricultural practices. Similarly, [14] found that not using certified seed and resistant varieties in production and the lack of suitable management systems, in particular for weeds (co-production of wheat and weeds and no weed management), hinders sustainable high-yielding wheat production.

Table 3. Constraints to the use of sustainable production practices for the Nsukka yellow pepper crop

Constraints	**Technical constraints (Factor 1)**	**Institutional constraints (Factor 2)**	**Economic constraints (Factor 3)**
Lack of technical know-how on yellow pepper production	**0.817**	0.076	0.198
Lack of capability regarding soil fertility management	**0.811**	0.224	0.098
Lack of access to training on sustainable practices	**0.735**	0.183	0.173
Lack of processing capabilities	**0.684**	0.326	-0.039
Herdsmen attacking the farm	**0.571**	-0.038	-0.291
Inadequate extension services	0.014	**0.879**	-0.071

Constraints	Technical constraints (Factor 1)	Institutional constraints (Factor 2)	Economic constraints (Factor 3)
Lack of access to information on yellow pepper innovations	0.039	**0.819**	-0.035
Pest and disease insurgence	0.162	**0.567**	0.206
Poor access to finance/credit facilities	0.323	**0.489**	0.016
High cost of adopting sustainable practices	0.170	**0.409**	0.235
High perishability of yellow pepper	-0.297	0.119	**0.753**
Low return on investment in yellow pepper	-0.062	0.111	**0.706**
Cross-pollination with other cultivars	0.210	-0.010	**0.633**
Unavailability of an improved variety of yellow pepper	0.313	0.000	0.532

Source: field survey, 2020

Notes: extraction method: Principal Component Analysis; rotation method: Varimax with Kaiser Normalization.

Measures to enhance the use of sustainable production practices by the Nsukka yellow pepper farmers

Results in *Table 4* indicated measures to enhance the sustainable production of the Nsukka yellow pepper, and these include: provision of improved seeds (85.2%), access to credit (77.8%), security against herdsmen (77.0%), pest-/disease-resistant variety (76.3%), access to extension services (71.8%), training on the use of sustainable practices (68.9%), etc. Provision of an improved cultivar of the Nsukka yellow pepper is necessary to ensure the increased productivity and sustainability of the crop. The use of mass selection, pedigree selection, pure-line selection methods, or other crop breeding techniques may improve yellow pepper production cultivars. Similarly, access to credit facilities is essential for the expansion of farms, the procurement of farm inputs, and the adoption of new and better farming practices. Farmers who have access to credit facilities are more likely to engage in sustainable farming practices in yellow pepper crop. Again, the provision of extension services to yellow pepper farmers is imperative. Extension workers will not only create awareness but also enlighten yellow pepper farmers on the need to embark on sustainable production practices. Furthermore, extension workers will also improve farmers' knowledge through provision of trainings on sustainable production practices. This finding supports [14], who revealed that education, use of certified seeds of suitable wheat varieties, and appropriate crop management practices were important measures for increasing wheat yield and improving sustainability.

Table 4. Measures to enhance the use of sustainable production practices by Nsukka yellow pepper farmers

Measures	Frequency	Percentage
Provision of improved seeds	115	85.2
Access to credit facilities	105	77.8
Security against herdsmen	104	77.0
Pest-/disease-resistant variety	103	76.3
Access to extension services	97	71.8
Training on the use of sustainable practices	93	68.9
Access to export markets	84	62.2
Access to bio-fertilizers	73	54.1
Construction of irrigation dams	64	47.4
Access to tractors	53	39.3
Access to organic manure	17	12.6
Cheap labour	9	6.7

Source: field survey, 2020

Conclusions and recommendations

Based on this study, Nsukka yellow pepper farmers have a moderate knowledge on sustainable production practices, especially on the use of crop rotation, poultry manure, selection of good seeds, optimum harvesting time, minimal use of agrochemicals, and reduced bush burning. This level of knowledge on sustainable production practices is good for their production activities but needs improvement. Again, famers' sex influences the use of sustainable production practices on yellow pepper crop. Furthermore, farmers' use of sustainable production practices was affected by technical, institutional, and economic constraints. Thus, the government, non-governmental organizations, and private sectors should provide yellow pepper farmers with adequate trainings on sustainable production practices, as this would improve their indigenous knowledge about such practices. Also, the government, donor agencies, and private sectors should assist the Nsukka yellow pepper farmers with incentives and credit facilities to boast their adoption and use of sustainable production practices. Furthermore, the government should also enact policies regarding the adoption and use of sustainable production practices in crop production to boost agricultural productivity and conserve the environment.

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Conflict of interest

Authors have no conflict of interest.

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Methylene blue and Congo red removal by activated carbons: A current literature

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Abstract: Dye is a major source of water pollution. This mini-review highlights the origin of dye, its removal strategies, and the recent literature of methylene blue and Congo red adsorption by activated carbons. Adsorption is a unique process that relies upon the physicochemical properties of the activated carbon and the inherent characteristics of dye. Also, factors affecting adsorption, such as contact time, temperature, and concentration, were discussed to gain insight into dye removal mechanisms.

Keywords: activated carbon, adsorption, dye, methylene blue, Congo red, wastewater treatment

1. Introduction

Dyes are a common organic contaminant in many areas across the world. A British scientist named William Henry Perkin discovered the world's first synthetic purple dye in 1856. It was originally known as Tyrian purple, but after being commercialized in 1859, it was renamed mauve or aniline purple [1]. Dyes are commonly used in the textile and food industries for the purpose of colouring products. Many commercial dyes are manufactured each year, resulting in approximately seventy thousand tonnes of dye pollution. Dyes in water are less biodegradable due to their chemically stable aromatic molecular structures [2].

Due to the excessive discharge of dyes into environment, the textile industry is one of the major contributors to dye pollution. The amount of waste dye in the textile industry is 3,000 ADMI, which exceeds the department of environment's allowable limit of standard A (100 ADMI) and standard B (200 ADMI) in Malaysia [3]. Dye wastewater is discharged into a river or waterway, contaminating the aquatic environment [4]. Dyes have certain adverse effects on the environment and humans. For example, dyes have a significant effect on bacteria, and hence affect their growth. Dyes, considered carcinogenic, can also cause serious diseases in humans. Also, dyes can affect the aquatic living organism by causing chemical and biological activities in rivers, as well as consuming oxygen that is dissolved in the water [5].

Dyes are coloured, ionizing, and aromatic organic substances that have a specific affinity for the substrate to which they are applied in an aqueous solution. It colourizes a substrate inasmuch as the colouring remains resistant to washing, heat, light, and other conditions. However, not all dyes are coloured substances, as there are whiteners or optical brighteners that are also referred to as "dye". Because they contain carbon, most dyes are organic. In the visible area, dyes strongly adsorb light, and the physical and chemical interaction between the functional groups of the dyes and those on the support allows a strong bond between the dye and the support. In other words, physical forces, such as hydrogen bonding (van der Waals), and ionic forces enable dyes to be physically linked to the fibre, and in certain situations dyes are chemically bound to the substrate via covalent links [6].

2. Classification of dyes

Dyes can be grouped in several ways at the international level to ensure an easier import and export trading and dye manufacturing. Different dyes can be classified based on their chemical composition, application, colour, dyeing support, and commercial name. Chemical composition and application are the most used classification criteria. In the dyeing industry, dyes are classified mostly based on application rather than on chemical composition. The chemical composition of a dye indicates how many chromophores it has, but it does not reveal the dye's structural characteristics [7]. *Table 1* summarizes the classification and application of dyes.

Concerning the dyes' class, colour, and overall composition as found in the Colour Index of the Society of Dyers and Colourists, they are basically separated into three broad categories, which are as follows: dyes containing anionic groups, dyes containing cationic groups, and special colourant class. Numerous compounds from various dye classes, such as acid, direct, and reactive dyes, contain carboxylic acid or sodium salts of sulfonic acid groups [8]. Although these anionic dyes have different structural characteristics and substrate affinity, they all have the same property of providing water solubility and ionization to the dyestuff.

Table 1. Classification and application of dyes [7]

Dyes	Application
Acid dyes	Coloured anions applied to silk and nylon at low pH.
Basic dyes	Coloured cations used on polyacrylonitrile textiles with acid side-chains; mainly used on proteinaceous fibres and tannic acid-treated cotton.
Natural dyes	Dyes made or extracted from plants or animals.
Direct dyes	Acid dyes with big molecules that can directly bond to cellulose fibres.
Disperse dyes	Insoluble coloured substances in fine suspension which infiltrate hydrophobic materials.
Reactive dyes	Coloured substances that have side-chains which react with the substrate to form covalent bonds; used to dye cellulose and other materials.
Azoic dyes	An insoluble azo dye formed within the substrate through the reaction of diazonium salt with naphtol.
Solvent dyes	Coloured substances that are only soluble in hydrophobic solvents and used to colour liquids and polymers.

Basic dyes are also known as cationic dyes, and these coloured cationic salts belong to the family of amine derivatives. The basic groups in the structure of basic dyes enable the migration and the reaction of basic dye cations with the acidic or negatively charged fibre surface, resulting in an ionic linkage formation. These cationic dyes contain amine side-chains, which are protonated in acidic solutions, or quaternary nitrogen atoms, which are cationic in neutral or high pH environments. A non-conjugated chain of carbon atoms may be bound to the charged group [9]. Basic dyes have the advantage of producing bright colours and intensities, as well as being extremely quick on acrylic fibres. These cationic dyes have a high tinctorial value and are soluble in mild acidic conditions. Basic dyeable acrylic, cationic dyeable polyester, wool, silk, and nylon are among the fabrics to which basic dyes are applied. Basic dyes have also been found to be extremely strong and effective colorants for ready-made textiles such as acrylic fibres [10].

An example of a special colourant class is disperse dyes. The small polar dye molecules used in the dyeing of hydrophobic synthetic fibres, such as nylon, acetate, triacetate, polyester, and other manufactured fibres, are known as disperse dyes. Disperse dyes, unlike other cationic or anionic colourants, contain anthraquinone or azo groups but do not contain either positively or negatively charged groups. Disperse dyes have good light and wash fastness, as well as provide powerful bright colours although have a low water solubility [11].

2.1 Methylene Blue

Heinrich Caro, a German scientist, synthesised methylene blue dye in 1876 by oxidizing dimethyl-4-phenylenediamine in the presence of sodium thiosulphate [12]. Methylene blue is also known as methylthionine chloride, having the chemical formula of $C_{16}H_{18}ClN_3S$. Some of its characteristics are: it is a dark green powder, has a deep blue colour when diluted in water, and is odourless. In addition, it has distinct physicochemical characteristics that are dependent on the polar co-solvent in solution. Methylene blue, like most organic dyes, does not obey Beer's Law, which is related to the light absorption properties of the substance through which light passes. This is linked to the reversible formation of dye aggregates, which is one of the characteristics of organic dyes [13].

The medical sector and the textile industry are the two main applications of methylene blue. In the medical sector, Paul Ehrlich was the first to employ this dye to treat malaria in 1891 [14]. Even though its mechanism in antimalarial activity is undetermined, its use in the treatment of malaria has proven to be quite effective [15]. It is also widely used to treat methemoglobinemia, Alzheimer's disease, and as a tracer in the mapping of sentinel lymph nodes during gynaecologic laparoscopy [16].

Next, methylene blue is widely used in the textile industry for colouring products, as well as in the cosmetic, pharmaceutical, and paper industries. It is a cationic dye that is widely used in the textile industry to dye wools, cotton, and silk, as well as for hair colouring and paper coating. The increasing use of dyes in the industries results in wastewater containing excessive amounts of dye, which is then discharged into the environment. Textile industrial wastewater is one of the most environmentally hazardous wastewaters [17]. Therefore, more studies should be focused on its removal from wastewater to avoid the harmful effect on the health of living creatures.

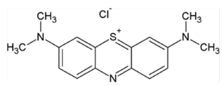
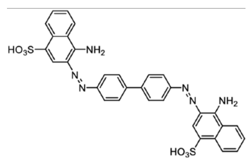
2.2 Congo red

Congo red was reportedly created in 1884 by Paul Bottiger, a German scientist who was looking for a pH indicator [18]. It is a synthetic compound with a variety of formal chemical names, but each molecule is made up of two molecules of naphthionic acid (1-naphthylamine-4-sulfonic acid or 4-aminonaphthalene-1-sulfonic acid) linked by azo groups to one molecule of benzidine (diaminophenyl or 4-(4-aminophenyl) aniline). Horobin and Kiernan (2002) gave Congo red the Colour Index number 22120 and the name Direct Red 28. The molecular formula is $C_{32}H_{22}N_6Na_2O_6S_2$. At a pH range below 3, it appears as a blue-violet colour, while it changes into red colour when the pH is above 5, and it is also odourless [19]. Due to its high solubility in water, Congo red can be easily removed via adsorption using carbonaceous adsorbent or activated carbon.

Congo red is mostly used in two main sectors, the medical and the textile. Its main application in the medical sector is in the diagnosis of amyloid. Bennhold described the staining of amyloid with Congo red in a short technical paper published in 1922, and this is now one of its most common applications. Bennhold was not, however, the first to use this dye as a microscopy staining. In 1886, Griesbach investigated numerous azo dyes, but he did not mention amyloid. Bennhold is credited with being the first to report its use in amyloid detection. This was identified in a study of Congo red intravenous injections, which had previously been used to measure blood volume [20].

Congo red is widely used in a variety of industries, including textile, printing, leather, paper, pulp, and cosmetics, and a large amount of its effluent is released into the aquatic system, particularly during dyeing processes. As a result, the overuse of the dye pollutes the environment and has become a major challenge for environmental protection organizations. Benzene and naphthalene rings in Congo red cannot be degraded using traditional methods. Due to the presence of an aromatic structure, it has a high optical and physiochemical stability [21]. Therefore, more studies should be focused on the removal of methylene blue and Congo red from wastewater. *Table 2* summarizes the characteristics of these two dyes.

Table 2. Characteristics of methylene blue and Congo red

Dyes	**IUPAC name**	**Commercial name**	**λ_{max} (nm)**	**Molecular structure**	**Reference**
Methylene Blue	[7-(dimethylamino)-phenothiazin-3-ylidene] dimethylazanium chloride	– Basic blue 9 – Methylthioninium chloride – Solvent Blue 8 – Swiss blue	650		[22]
Congo Red	disodium-4-amino-3-[4-[4-(1-amino-4-sulfonato-naphthalen-2-yl)-diazenylphenyl]-phenyl]-diazenyl-naphthalene-1-sulfonate	– Direct Red 28 – Direct Red K – Haemonorm – Solucongo	497		[23]

3. Dye removal technologies – An overview

Dyes are difficult to remove from wastewater because of their high toxicity and tinctorial value, which makes them visible even at low concentrations. Despite this, a variety of wastewater decolourization techniques have been developed, which can be classified into three categories: physical or physicochemical techniques, chemical techniques, and biological techniques [24]. Each dye removal technology has different applications, advantages, and drawbacks. In the following section, these dye removal techniques are further discussed.

3.1 Physical and physicochemical treatment

Dyes are physically removed from the wastewater in the course of the physical or physicochemical treatment. Physical, chemical, or biological unit processes are used in physicochemical wastewater treatment; in most cases, the physical process is in the first stage, followed by a sequence of single-unit processes. This means that the physical treatment removes dyes using specific equipment, without the use of any chemicals or reactions, whereas physicochemical techniques combine physical and chemical processes with the use of substances, such as coagulant and flocculant, to improve dye removal efficiency. Physical dye removal techniques include adsorption, ion exchange, and membrane filtration, which includes reverse osmosis, nanofiltration, microfiltration, and ultrafiltration, while physicochemical treatments include precipitation, coagulation, and flocculation [25].

Coagulation and flocculation are two physicochemical techniques that have been used in industrial wastewater treatment and decolourization over the years. Since it removes colloidal particles of turbidity, colour, and bacteria, this traditional method belongs to the initial treatment in an overall wastewater treatment scheme. The coagulation process does not remove all types of dyes [26]. Cationic dyes, for example, do not coagulate at all, whereas acid, direct, and reactive dyes coagulate in most cases, but their flocs are of poor quality and cannot settle without the addition of flocculant. Only disperse dyes coagulate well and sink rapidly.

Membrane filtration is a physical treatment that removes the dyes from industrial effluent in a continuous process. The micropores and selective permeability of the membrane are used to filter and separate residual dyes and pollutants from the treated water. In membrane separation processes, such as microfiltration, ultrafiltration, nanofiltration, and reverse osmosis, the membrane pressure is also a critical factor. This technique is highly effective, simple to adopt, and resistant to temperature and microbial attack, but its drawbacks are membrane fouling and clogging, as well as a high cost [27].

Adsorption is one of the most effective methods for eliminating dyes and other stable pollutants among all treatment methods. Furthermore, this physical treatment is cost-effective, simple to operate, and produces a high-quality product. In the absence of any reaction, the adsorption process involves the spontaneous accumulation of molecules of a gas, liquid, or vapour at the contacting surface. Physicochemical parameters, such as adsorbent surface area, dye adsorbent interaction, particle size, pH, temperature, and contact time, influence adsorption [28]. Adsorption efficiency is also influenced by the properties of the wastewater and the type of carbon used. Activated carbon, peat, coal, wood chips, silica gel, and other materials can be used for adsorption.

Ion exchange technique, on the other hand, cannot accommodate a wide range of dyes, and thus it is rarely used in the decolourization of dye effluent. In this physical treatment method, dye effluent passes through ion exchange resins to allow anions or cations to exchange until the exchange sites are saturated. To avoid adsorbent loss, the spent adsorbent can be regenerated. Nonetheless, because organic solvents are expensive and dispersed dyes are resistant to ion exchange, it is a high-cost treatment [29].

3.2 Chemical treatment

Chemical treatment employs a variety of techniques to remove dyes from textile wastewater, including ozonolysis, chemical oxidation, and reduction. When the specific chromophore of colour is decreased and the dye molecules are broken down into small fragments, decolourization by chemical techniques occurs. Chemical treatment methods such as oxidative treatments are commonly used in industries due to their ease of use. Despite this, most dyes are stable, and mild oxidation conditions are insufficient to remove dyes from wastewater. To improve dye removal in oxidative treatments, strong oxidizing agents, such as chlorine, ozone, Fenton's reagent, sodium hypochlorite, or other oxidizing alternatives, are used [30].

Chlorine is a good dye-oxidizing agent, and recent research has shown that it can be used for dye removal. Chlorine gas or sodium hypochlorite (NaOCl) can be used in the chlorination process. Chlorination can rapidly remove acid and reactive dyes, while direct and disperse dyes are resistant to chlorine treatment, even at high chlorine concentrations. Although chlorination is an effective and low-cost method of removing dyes, it can react with nitrogen-containing components, resulting in the formation of undesirable compounds [3]. The public has expressed a strong opposition to chlorination because it produces harmful chlorinated chemicals.

Using Fenton's reagent as an oxidant is another chemical oxidative technique. The Fenton reaction is widely used in the preliminary or primary treatment phase to manage sewage that is resistant to biological treatment and dangerous to all living beings. The hydroxyl radical is a powerful oxidizing agent that completely converts organic materials, including dyes, to water, carbon dioxide, and inorganic substances. Although this technique has a high efficiency in dye and COD removal, it still has several significant drawbacks, including the large amount of sludge produced, pH manipulation, which necessitates a significant amount of acid and alkali, the need to reduce excess iron concentration, and unfitness for final effluent emission [31].

Due to the characteristic of high instability, ozone is a stronger oxidizing agent than chlorine. Oxidation with ozone can break down detergents, chlorinated hydrocarbons, insecticides, aromatic substances, and phenols. Ozone oxidation is only effective for specific types of dyes: for example, a significant percentage of reactive dyes can be removed via ozonation, whereas the removal of azoic and basic dyes in wastewater is only moderately effective. Despite this, ozonation fails to separate dispersed dyes from the textile effluent. In other words, ozonation works best with molecules that are double-bonded [32].

Irradiation, in addition to oxidative methods, is a conventional chemical treatment that uses electron beams or gamma rays to destroy organic contaminants and harmful microorganisms in wastewater. When there is enough dissolved oxygen present, radiation can efficiently rupture organic pollutants. Due to the extremely rapid exhaustion of dissolved oxygen, a consistent and sufficient resource is required, which has an impact on operating costs. Although irradiation is a simple and effective technique, its use on a broad scale is limited due to maintenance and cost considerations [33].

3.3 Biological treatment

Dyes are removed from wastewater via adsorption on activated sludge or the biological digestion of dye molecules in biological treatment. However, because dye components are not a source of food for microorganisms, the competence of biological agents in the dye removal of dye wastewater is ambiguous and inconsistent. Bacterial breakdown of organic substances can take place in either aerobic or anaerobic environments. In other words, based on the oxygen requirement, biological treatment methods can be divided into aerobic and anaerobic treatment [34]. Nonetheless, dissolved oxygen concentrations in water may decrease because of rapid development with increased water pollution.

4. Adsorption of dyes

Heinrich Gustav Johannes Kayser, a German scientist, coined the term "adsorption" in 1881. Adsorption varies from absorption in that the former is the attraction between a substance and the outer surface of a medium termed adsorbent, whereas the latter is when a substance diffuses into a medium to produce a solution [35]. Because of capillary pressure, absorption is the fundamental driving force for transferring bulk fluid into a substance. Adsorption occurs when the atoms, ions, or molecules of the adsorbate bind to the surface of the adsorbent.

Adsorption is described as the spontaneous accumulation of gas, vapour, or liquid molecules at the contacting surface without any reaction. Adsorption is a phase of equilibrium in which the adsorbent meets the bulk stage and the interfacial layer, and it can occur in a variety of systems, including liquid–gas, liquid–liquid, solid–liquid, and solid–gas. In the evolution of industrial-scale adsorption processes, solid–liquid and solid–gas interfaces are equally the most common ones. Adsorption is defined as the penetration of the adsorbate molecules into the mass solid stage, whereas adsorbate is defined as the material in the adsorbed state [36].

Many researchers are interested in the development over several years of novel categories of solid adsorbents such as activated carbon fibres, carbon molecular sieves, microporous glasses, and nanoporous substances. Some adsorbents are used extensively as catalyst supports, catalysts, or desiccants, while others are used for liquid purification, gas separation or storage, pollution management, and respiratory protection. They are also interested in converting easily accessible and low-cost biomass into adsorbents, such as banana stems, mangosteen peels, oil palm, tea waste, and palm shell [37]. In terms of industry, industrial adsorbents can be divided into two categories: carbon adsorbents and mineral adsorbents.

4.1 Adsorption phenomena

There are multiple applications linked to the adsorption mechanism, including gas phase, liquid phase, and environmental applications. Gas phase applications include moisture removal in gases and fluids with activated alumina and nitrogen generation from air with carbon molecular sieves, while liquid phase applications include water treatment and protein uptake at the solid–liquid interface. Adsorption heat pumps and adsorption gadgets in spacecraft environmental control are examples of environmental applications. The adsorption process is used on a large scale in the industry for many applications: for instance, the separation and purification of gas and liquid blends, air, isomers, and bulk chemicals, water treatment, dehumidification of liquids and gases, chemical recovery from industrial gases, and removal of impurities in liquid and gas media [9].

Certain adsorbates in gas and liquid mixture are applicable in adsorption-related applications. The pore size and arrangement of solid adsorbents should be large enough to ensure rapid diffusion for adsorption-related operations. Generally, the van der Waals forces between the adsorbate and the adsorbent have a significant impact on selective adsorption [38]. Often, the higher the BET area (or pore volume), the greater the removal capacity because of the more adsorbate–adsorbent interaction probabilities. Also, the existence of surface functionalities in the adsorbent helps in the adsorption of certain target molecules/compounds. Nonetheless, adsorption is a unique process that relies upon not only the physicochemical properties of the adsorbent (e.g. specific surface, pore size, surface chemistry, etc.) alone but also on the inherent characteristics of the adsorbate (e.g. molecular size, polarity, charge density, steric hindrance, etc.) and the environment or operating conditions (e.g. temperature, concentration, retention time, co-existing substances, etc.) that bring about the affinity of adsorbate–adsorbent interactions and mechanisms, such as π-π, hydrogen bonding, pore filling, electrostatic, ion-exchange, etc.

The application of adsorption mechanisms to the removal of impurities encounters an advanced breakthrough when the artificial zeolite was formulated, and operation cycles are designed to recover product and regenerate used adsorbents. Every system including an adsorption process performs two critical actions: the separation of one or more substances with higher affinity and the regeneration of spent adsorbents from the adsorbent bed. These two procedures can be carried out in a continuous sequence or in a parallel multi-bed setup, with no adsorbents being wasted. The newly regenerated adsorbents are reused in cycles, and the adsorbate is recovered at the same time [39].

4.2 Methylene blue and Congo red adsorption

Methylene blue is a cationic dye that is poisonous and carcinogenic if accidentally ingested, whereas Congo red is an anionic dye that can have a serious impact on aquatic living organisms if discharged into the aquatic environment from industry effluent [40]. Therefore, it is important to separate these dyes from the industry effluent and the water bodies. Surface area, initial concentration, contact time, pH, and adsorption temperature are some parameters that influence the adsorption capacity of dyes. The adsorption of methylene blue and Congo red by various activated carbons is illustrated in *Table 3*. The number of active sites, porosity, and pore volume of the adsorbent facilitate the removal of dyes through several synergistic interactions. Meanwhile, an increase in temperature reduces solution viscosity and gives dye molecules more kinetic energy, increasing their mobility and diffusion onto the surface of the adsorbent and the internal pores, resulting in enhanced dye uptake [41].

Likewise, when the initial concentration of the dye increases, the quantity of the molecules absorbed per unit mass of the adsorbent also increases. For instance, the adsorption capacity of methylene blue by rubber-sludge-based activated carbon increases from 224 to 458 mg/g when the initial concentration increased from 250 to 500 mg/L. Similarly, the capacity of Congo red removal using sargassum-based activated carbon increases from 125.66 to 234.00 mg/g when the initial concentration increased from 100 to 200 mg/L. The concentration gradient offers a driving force to facilitate dye diffusion from bulk solution onto the adsorbent. In the case of activated carbon, the contact time is adjusted depending on the initial concentration, as a longer contact time to attain equilibrium is required for a higher initial concentration [42].

Table 3. Adsorption of methylene blue and Congo red by various adsorbents.

Adsorbent	Dye	BET area (m2/g)	Concentration (mg/L)	Contact time (T, °C), [pH]	Capacity (mg/g)	Removal (%)	Reference
Banana-stem-based activated carbon	Methylene blue	837	100	90 min, [7]	101	99.8	[43]
Mangosteen-peel-based activated carbon	Methylene blue	1,832	500	5 h, (25°C), [7]	871	–	[44]
Oil-palm-based activated carbon	Methylene blue	553	50	2 h, [3]	24	48	[45]
Rubber-sludge-based activated carbon	Methylene blue	-	250 500	2 h, (30°C), [5]	224 458	94.8	[46]
Tea-waste-based activated carbon	Methylene blue	851	500	-	357	71.4	[47]
Palm-shell-based activated carbon	Methylene blue	1,038	55	50 min, [10]	51.50	91.8	[48]
Kenaf-based activated carbon	Congo red	843	25	2 h, (27°C), [4]	19.7	78.9	[49]
Myrtus-communis-based activated carbon	Congo red	104	30	-	19.2	64.1	[50]
Orange-peel-based activated carbon	Congo red	1,169	750	2 h, (35°C), [4]	667	88.9	[51]
Casuarina-empty-fruit-based activated carbon	Congo red	1,072	300	144 h, (25°C)	232	77.3	[52]
Sargassum-fusiforme-based activated carbon	Congo red	1,329	100 200	3 h, (50°C), [7]	126 234	94.7	[53]
Walnut-shell-based activated carbon	Congo red	800	200	9 h, (25°C)	154	77	[54]

5. Conclusions

Dyes are toxic and recalcitrant to biodegradation. Methylene blue and Congo red are among the widely used dyes in industries. Therefore, there is an urgency to treat effluent laden with dyes prior to be discharged to the environment. In this mini-review, we highlighted recent works on methylene blue and Congo red adsorption by activated carbons. Synergistic interactions in terms of the physicochemical properties of the adsorbent, the intrinsic characteristics of dye, and the adsorption environment are expected to result in favourable adsorbate–adsorbent interactions in wastewater treatment.

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Growing media, water stress and re-watering effects on the growth and dry matter production of cocoa seedlings

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Abstract: Cocoa (*Theobroma cacao*) seedlings are very sensitive to water stress during the nursery stage and early field establishment. Sawdust, carbonated rice husk, and compost in the following ratios (i) 60: 40: 0 – M1, (ii) 60: 30: 10 – M2, (iii) 60: 20: 20 – M3, (iv) 60: 10: 30 – M4, (v) 60: 0: 40 – M5, and (vi) topsoil – M6 as control were used to investigate the effect of growing media, water stress, and re-watering on the growth, dry matter production, and partitioning of cocoa seedlings. Each combination was subjected to water stress 6 weeks after sowing for a period of 4 weeks, after which they were rewatered. Plant height, stem girth, leaf number and area, and seedling biomass were significantly higher in soilless potting media as compared to topsoil. M4 and M5 significantly recorded the highest total dry weight, plant height, stem girth, leaf number, leaf area, and root volume before imposition of water stress. Following imposition of water stress, cocoa seedlings grown in M4 and M5 showed a recovery that was superior to the other growing media, indicating that higher proportions of compost together with sawdust and carbonated rice husk provide an alternative for growing cocoa seedlings.

Keywords: cocoa, water stress, re-watering, growing media, carbonated rice husk

1. Introduction

Cacao is an important cash crop in Ghana; it accounts for a significant part of Ghana's GDP and foreign earnings [1]. Cocoa industry in Ghana provides livelihood for about 4 million farmers and their households [2].

Cocoa production per unit area in Ghana has been on decline since 2010 [3]. Efforts to improve cocoa production in Ghana and to achieve the 1 million metric tonnes produced in 2010 have been targeted, increasing yield through rehabilitation, soil fertility management, and the use of improved varieties, with little attention to the production of quality seedlings and subsequent survival and establishment in the field. Sustainable cocoa production is limited by seedling mortalities at the nursery stage and on the field due to high temperature, uneven distribution of rainfall, and drought [4; 5; 6; 7].

Climate change is likely to intensify the current stress factors, such as increasing temperature, uncertain rainfall patterns, and moisture deficits, on cocoa seedling growth and development in the nursery and field in the coming years. One strategy to overcome the climate change effect could be the development of growing media that increases the resilience of cocoa seedlings against unfavourable weather conditions.

Nursery growing media affects the quality of cocoa seedlings [8], their establishment in the field and eventual development [9]. The traditional nursery growing medium is topsoil obtained from farmlands. The continuous use of topsoil as growing medium causes land degradation and is therefore not environmentally sound [10]. The compactness of topsoil also restricts seedling root growth and therefore affects overall seedling growth and establishment in the field. In recent times, there have been renewed efforts to search for environmentally sound options for raising cocoa seedlings at the nursery. [11] reported that a good growing medium enhances the growth of a healthy fibrous root system, and it can be used to support and supply nutrients in order to achieve a healthy plant growth.

Recent studies have paid attention to the use of soilless growing media that are organic-based, less expensive, and suitable for plant growth [12]. The ability of an organic substrate in retaining moisture for plants will help overcome the issue of crops being stressed during uncertain weather conditions. [13] noted that the application of organic amendments increased water stress resistance in corn varieties. Compost and biochar (carbonated rice husk) are among the best organic substrates that could enhance the water- and nutrient-holding capacity and availability of growing media with a view to improving plant resilience against water stress.

Compost has been demonstrated to be efficient in improving the resilience, yield, and tolerance of plants to harsh conditions [14]. Applying compost could improve water-holding capacity, soil organic matter content, and mineral nutrition [15; 16]. Compost also has other beneficial effects on soil structure and decreases the soil pH [17]. Previous research has demonstrated that adding compost to soil can increase its ability to withstand environmental pressures by increasing, among other things, microbial activity [18]. Microorganisms are crucial for a stable, secure, and sustainable agricultural and biomass production because they considerably contribute to the compost's absorption of nutrients through biochemical transformations [19].

It has been proven that biochar can be added to soil in order to improve soil fertility and reduce water stress [20]. Biochar supports crop production under water stress conditions by improving moisture retention [21; 22].

Applying biochar and compost together resulted in a synergistic effect on water-holding capacity and soil nutrients [23]. Furthermore, the combined application of biochar reduces the application of chemical fertilizers and improves soil structure [24; 25].

The objective of the present study, therefore, was to evaluate the combined effect of carbonated rice husk (biochar) and compost on growth performance, dry matter production and partitioning, susceptibility to water stress, and response to the re-watering of cocoa seedlings in the nursery.

2. Materials and methods

Experimental site

The study was conducted in pots (polybags) at a nursery of the University of Ghana's Forest and Horticultural Crops Research Centre (FOHCREC), Kade. Kade, the capital of Kwaebibirem District, is in the eastern part of Ghana and falls within the semi-deciduous forest zone. It is 114 m above sea level on latitude 6˚09' and 6˚06'N and longitude 0˚55' and 0˚49'W. The average temperature and relative humidity in the area is 28^{0}C and 78% respectively.

Experimental materials

The treatments consisted of five different combinations of growing media (M) obtained by mixing sawdust (S), carbonated rice husk (CRH), biochar and compost (C) at different percentage ratios: M1: S + CRH + C (60:40:0), M2: S + CRH + C (60:30:10), M3: S + CRH + C (60:20:20), M4: S + CRH + C (60:10:30), M5: S + CRH + C (60:0:40), and M6: Topsoil. Topsoil (M6) was included as control. The topsoil from the Kokofu series (Lixisols) was sampled from a field at FOHCREC at a depth of 0–20 cm. The carbonated rice husk (biochar) and the compost were locally prepared. The size of the polybag used was 20 x 15 cm and was perforated at the base for aeration and drainage. The various combinations of the soilless growing media were thoroughly mixed and filled into the polybags as per the required ratio v/v. The quantity of M1, M2, M3, M4, M5, and M6 used to fill each polybag weighed 297.1, 280.6, 355.5, 381.7, 401.4, and 1,210.0 g respectively. Each treatment consisted of 60 plants and was arranged in a completely randomized block design with three replications. The polybags were placed on a wooden platform to prevent direct contact with the soil. The setup was watered and stabilized for 24 hours before sowing the cocoa seeds. Seeds were sown at one seed per pot (polybag). Thirty days after planting, each plant was given 150 ml solution of 10 g NPK fertilizer per litre of water twice a week. Watering was maintained at 80% field capacity for the first six weeks. The hybrid cocoa seeds were obtained from the Research Centre's cocoa plantation established with the help of Ghana Cocoa Board.

Six weeks after planting, 20 plants of each treatment were sent to a protected place, where water stress was imposed on them for 4 weeks. To minimize evaporation, the surfaces of the growing media were covered with aluminium foil sheets. Twenty plants of each treatment continued to receive normal watering. Drought plants were re-watered to 80% field capacity for a period for 8 weeks after the imposition of water stress. Twelve plants of each treatment were used for the determination of biomass production and partitioning.

Growing media analysis

Physical characteristics

Bulk density was estimated using the core method based on the below formula:

$$\text{Bulk density} = \frac{\text{Weight of oven dry core soil}}{\text{volume of the sample } \left(\text{g cm}^{-3}\right)} \tag{1}$$

Total soil porosity was estimated from bulk density and particle density using the below formula:

$$\text{Total porosity} = \frac{\text{Bulk density}}{\text{Particle density}} \tag{2}$$

Water-holding capacity was determined as the volume of water retained by a medium after drainage.

Chemical characteristics

Total nitrogen was determined with a macro-Kjeldahl apparatus. The phosphorus content was determined using molybdenum blue colorimetric method, while potassium content was determined by a flame photometer. Calcium and Mg were determined as described by [26]. The NO_3 and NH_4 were determined based on the procedure described by [27]. The pH of the media was determined in a 1:5 suspension with a pH meter. Electrical conductivity (EC) of the 1:5 extract was determined with an EC meter. Total organic matter (OM) content was determined by the ignition method at 550^0C. Organic carbon analyser was used to measure the organic carbon (OC) content.

Plant growth analysis

The measurement of parameters started with seedling emergence one week after planting. Germination was periodically recorded every week from the date of sowing and continued until the germination ceased. Germination indices were measured as follows:

$$\text{Rate of Germination} = \frac{1}{\text{Germination Time}} \tag{3}$$

$$\%\ \text{emergence} = \frac{\text{Total Number of Seeds Geminated}}{\text{Total Number of Seeds Sown}} \times 100\ \% \tag{4}$$

$$\text{Germination Index} = \frac{\text{Number of Geminating Seeds}}{\text{Days of First Count}} + \frac{\text{Number of Germinating Seeds}}{\text{Days of Final or Last Count}} \tag{5}$$

Data on seedling growth parameters and dry mater production were measured before and after imposition of the drought stress and at the end of the re-watering period. Data were collected on seedling growth parameters such as plant height, stem diameter (girth), leaf number, total leaf area, leaf chlorophyll content, leaf weight, stem weight, root weight, root length, and root volume. Dry matter yield and distribution (leaves, stem, roots, and shoot-to-root ratio) were measured. The cocoa seedlings' response to water stress and re-watering were evaluated.

Statistical analysis

Data were statistically treated using analysis of variance (ANOVA) in GenStat software version 12. Duncan Multiple Range Test (DMRT) was used to separate significant means at 5% probability ($P < 0.05$).

3. Results

Growing media – Physical and chemical properties

The water-holding capacity (WHC) of the soilless growing media (M1 to M5) was significantly higher ($p < 0.05$) than that of the topsoil (*Table 1*). No significant differences were recorded among the various soilless growing media in terms of air porosity. However, the topsoil had a higher porosity than the soilless media. The bulk density of the topsoil (1.14 g/cm^3) was significantly ($p < 0.05$) higher than that of the soilless media (M1 to M5). The lowest bulk density value among the growing media was recorded in M1 (0.33 g/cm3) (*Table 1*).

Table 1. Physical properties of the growing media

Codes	Growing media	Water-holding capacity (%)	Bulk density (g/cm³)	Porosity (%)
M1	S + CRH + C (60:40:0)	73.5[ab]	0.33[d]	54[b]
M2	S + CRH + C (60:30:10)	75.3[a]	0.53[c]	55[b]
M3	S + CRH + C (60:20:20)	75.8[a]	0.62[b]	56[b]
M4	S + CRH + C (60:10:30)	70.3[b]	0.67[b]	57[b]
M5	S + CRH + C (60:0:40)	70.8[b]	0.73[a]	57[b]
M6	Topsoil	40.0[c]	1.14[e]	61[a]

Note: Values with the same letters in the same column are not significantly different ($p < 0.05$) by DMRT.

The pH of the topsoil was more acidic (5.7) than that of the soilless growing media (M1–M5), while M1 was the most basic (7.8) growing medium (*Table 2*). Soilless growing media containing compost had a lower pH than those without compost (M1). Increasing the compost content enhanced the EC of the growing media from 591 dS/cm (M1) to 772 dS/cm (M5). M6 showed a significantly lower EC (300 dS/cm) value than the rest of the growing media. The C/N ratio of the soilless growing media (M1–M5) increased with increasing the amount of CRH (*Table 2*). The topsoil had significantly lower C/N ratio (3.7) (*Table 2*).

Table 2. Chemical properties of the growing media

Chemical properties	Growing media					
	M1	M2	M3	M4	M5	M6
pH	7.8[a]	6.8[b]	6.6[b]	6.6[b]	6.1[b]	5.5[c]
EC (dS/cm)	460[c]	591[ab]	676[b]	688[b]	772[a]	300[d]
C/N	81.1[a]	28.9[b]	26.4[b]	24.0[b]	16.9[b]	3.7[c]
N (%)	0.21[c]	0.57[b]	0.61[b]	0.66[b]	0.82[a]	0.26[c]
OC (%)	17.03[a]	16.48[ab]	16.11[ab]	15.0[ab]	13.82[b]	0.96[c]
S (%)	0.03[b]	0.02[bc]	0.09[a]	0.08[a]	0.07[a]	0.01[c]
OM (%)	2.8[d]	6.3[b]	10.5[ab]	11.5[ab]	13.8[a]	5.2[c]
NH_4 (mg/kg)	309.6[c]	360.2[b]	367.2[b]	367.0[b]	403.2[a]	144.3[d]
NO3 (mg/kg)	209.2[d]	243.2[c]	367.2[b]	381.6[b]	408.8[a]	108.0[e]
P (%)	0.11[c]	0.14b[c]	0.14[bc]	0.17[b]	0.25[ab]	0.42[a]
K (%)	2.2[b]	2.6[ab]	2.7[a]	2.7[a]	2.7[a]	0.8[c]
Ca ($cmol_{(+)}$/kg)	3.4[b]	4.5[ab]	4.8[ab]	5.4[a]	5.6[a]	2.3[c]
Mg($cmol_{(+)}$/kg)	3.7[a]	3.0[ab]	2.7[b]	2.6[b]	2.9[ab]	1.3[c]

Note: Values with the same letters in the same row are not significantly different ($p < 0.05$) by DMRT.

Germination indices

In terms of percentage emergence, the soilless growing media did not differ significantly ($p < 0.05$) (M1–M5). However, the topsoil (M6) recorded a significantly ($p < 0.05$) lower percentage emergence (78%) compared to the soilless growing media (*Table 3*) and a significantly ($p < 0.05$) lower (0.08) germination index (GI) than the other growing media. Among the soilless growing media, the lowest GI was recorded in M1 (0.11). The number of days from sowing to 50% seedling emergence was significantly higher (20) for M6 than for the other growing media (*Table 3*).

Table 3. Effect of growing media on the germination indices of cocoa seeds

Growing media	Rate of germination (day^{-1})	Germination index	Days to 50% emergence	Emergence (%)
M1	0.049^{ab}	0.11^{ab}	9.0^{b}	100^{a}
M2	0.052^{a}	0.13^{a}	8.0^{b}	100^{a}
M3	0.053^{a}	0.13^{a}	8.0^{b}	100^{a}
M4	0.053^{a}	0.13^{a}	8.0^{b}	100^{a}
M5	0.050^{a}	0.12^{a}	8.0^{b}	100^{a}
M6	0.030^{c}	0.08^{c}	20.0^{a}	80^{b}

Note: Values with the same letters in the same column are not significantly different ($p < 0.05$) by DMRT.

Growth, biomass production, and partitioning

M5 produced a significantly ($p < 0.05$) greater plant height (23.4 cm), higher number of leaves (9.9) and leaf area (245.9 cm^2) among the growing media six weeks after planting (*Table 4a*). M6 produced the lowest plant height (17.0 cm), stem girth (0.1 cm), and root volume (0.6 cm^3), but it recorded the highest chlorophyll content (14.6) among the growing media. There was no difference in the stem girth among the soilless growing media.

Table 4a. Effects of growing media on the growth of cocoa seedlings before water stress, six (6) weeks after sowing

Growing media	Plant height (cm)	Stem girth (cm)	Number of leaves	Leaf area (cm^2)	Root length (cm)	Root volume (cm^3)	Chlorophyll content
M1	20.0^{c}	0.2^{a}	5.7^{d}	147.2^{d}	13.4^{c}	1.2^{b}	7.1^{d}
M2	20.9^{c}	0.2^{a}	7.0^{c}	174.0^{c}	15.0^{b}	1.7^{ab}	8.1^{cd}
M3	21.7^{b}	0.2^{a}	7.0^{c}	181.2^{c}	15.2^{b}	1.9^{a}	8.2^{cd}

Growing media	Plant height (cm)	Stem girth (cm)	Number of leaves	Leaf area (cm²)	Root length (cm)	Root volume (cm³)	Chlorophyll content
M4	22.1^{b}	0.2^{a}	8.5^{b}	194.1^{b}	16.6^{a}	2.2^{a}	9.9^{c}
M5	23.4^{a}	0.2^{a}	9.9^{a}	245.9^{a}	16.9^{a}	2.4^{a}	10.5^{b}
M6	17.0^{d}	0.1^{b}	7.3^{c}	153.7^{dc}	13.6^{c}	0.6^{c}	14.6^{a}

Note: Values with the same letters in the same column are not significantly different ($p < 0.05$) by DMRT.

M5 produced the highest leaf and stem and root dry weight, resulting in the highest total dry weight (1.99 g) (*Table 4b*). M6, on the other hand, produced the least leaves, stem and root dry weight and hence the lowest total dry weight (1.37 g). M6, however, recorded a significantly higher shoot-to-root ratio (8.6).

Table 4b. Effects of growing media on total biomass production and partitioning of cocoa seedlings before water stress treatment, six (6) weeks after sowing

Growing media	Leaf dry weight (g)	Stem dry weight (g)	Root dry weight (g)	Total dry weight (g)	Shoot/root ratio
M1	0.77^{c}	0.55^{b}	0.34^{a}	1.66^{b}	3.9^{c}
M2	0.82^{b}	0.53^{b}	0.34^{a}	1.69^{b}	4.3^{bc}
M3	0.84^{b}	0.54^{b}	0.33^{a}	1.71^{b}	4.0^{bc}
M4	0.85^{b}	0.56^{b}	0.35^{a}	1.76^{b}	5.0^{b}
M5	0.99^{a}	0.64^{a}	0.36^{a}	1.99^{a}	4.5^{bc}
M6	0.73^{c}	0.48^{c}	0.14^{b}	1.37^{c}	8.6^{a}

Note: Values with the same letters in the same column are not significantly different ($p < 0.05$) by DMRT

At the end of the water stress period, M5 produced a significantly higher plant height (24.2 cm), number of leaves (10.9), root volume (3.0), and chlorophyll content (26.8) (*Table 5*). M6, on the other hand, produced a significantly lower plant height, stem girth, number of leaves, root volume, and chlorophyll content. Among the soilless media, chlorophyll content increased with increasing the amount of compost. None of the soilless growing media produced a significant difference ($p < 0.05$) in stem girth. In well-watered plants, M4 produced the greatest plant height (30.2 cm), the highest number of leaves (16.9), root length (31.9), and root volume (5.9 cm^3) (*Table 5*). However, M5 recorded the highest chlorophyll content (33.4). M6 recorded the lowest plant height (20.8 cm), stem girth (0.2 cm), number of leaves (7.7), root length (18.5 cm), and root volume (1.8 cm^3). There was no difference in stem girth among the soilless growing media.

Table 5. Effects of growing media on the growth of water-stressed and continuously watered cocoa seedlings ten (10) weeks after sowing

Growing media	Plant height (cm)	Stem girth (cm)	Number of leaves	Root length (cm)	Root volume (cm³)	Chlorophyll content
Water-stressed						
M1	20.1[c]	0.2[a]	7.0[c]	15.4[c]	1.6[b]	17.8[d]
M2	22.3[bc]	0.2[a]	9.5[b]	18.0[b]	2.2a[b]	18.8[d]
M3	22.7[bc]	0.2[a]	9.5[b]	18.7[b]	2.3[ab]	21.0[c]
M4	23.9[a]	0.2[a]	9.8[b]	19.9[a]	2.8[a]	24.5[b]
M5	24.2[a]	0.2[a]	10.9[a]	18.9[b]	2.7[a]	26.8[a]
M6	17.9[d]	0.1[b]	7.3[d]	16.0[d]	0.8[c]	4.2[e]
Continuously watered						
M1	22.7[c]	0.3[a]	9.0[d]	21.4[d]	3.6[c]	27.5[c]
M2	26.0[bc]	0.3[a]	12.0[c]	26.5[c]	4.5[b]	30.4[bc]
M3	26.5[bc]	0.3[a]	13.5[c]	26.9[c]	4.7[b]	30.6[bc]
M4	30.2[a]	0.3[a]	16.9[a]	31.9[a]	5.9[a]	32.6[b]
M5	28.1[b]	0.3[a]	15.7[b]	29.4[b]	5.4[a]	33.4[a]
M6	20.8[d]	0.2[b]	8.7[d]	18.5[e]	1.8[d]	30.1[bc]

Note: Values with the same letters in the same column are not significantly different ($p < 0.05$) by DMRT.

At the end of the water stress period, total biomass production was reduced by 43, 52, 51, 49, 29, and 42% in M1, M2, M3, M4, M5, and M6 respectively (*Table 6*). Biomass production was less affected in M5 than the other growing media. Plant total dry weight differed significantly between the soilless growing media (M1–M5) and the topsoil (M1) under drought stress. M5 produced the highest leaf, stem, and root dry weight and hence the highest total dry weight (3.4 g) (*Table 6*). M6, on the other hand, produced the least leaves, stem and root dry weight and hence the lowest total dry weight (1.8 g). M6, however recorded a significantly higher shoot-to-root ratio (6.5).

Table 6. Effects of growing media on biomass production and partitioning of water-stressed and continuously watered cocoa seedlings ten (10) weeks after sowing

Growing media	Leaf dry weight (g)	Stem dry weight (g)	Root dry weight (g)	Total dry weight (g)	Shoot/root ratio
Water-stressed					
M1	1.0b[c]	0.7[b]	0.3[bc]	2.0[bc]	5.7[b]
M2	1.0b[c]	0.8[b]	0.4[b]	2.2[bc]	4.5[c]

M3	1.2[b]	0.8[b]	0.4[b]	2.4[b]	5.0[bc]
M4	1.7[ab]	0.8[b]	0.5[b]	3.0[b]	5.0[b]
M5	2.0[a]	0.9[a]	0.5[a]	3.4[a]	5.8[b]
M6	0.8[c]	0.5[c]	0.2[c]	1.5[c]	6.5[a]
Continuously watered					
M1	1.9[c]	1.0[b]	0.6[b]	3.5[c]	4.8[b]
M2	2.8[b]	1.1[ab]	0.7[b]	4.6[b]	5.6[ab]
M3	2.9[b]	1.3[ab]	0.7[b]	4.9[b]	6.0[a]
M4	3.6[a]	1.4[a]	0.9[a]	5.9[a]	5.4[ab]
M5	2.8[b]	1.3[ab]	0.7[b]	4.8[b]	5.9[a]
M6	1.4[c]	0.8[c]	0.4[c]	2.6[d]	5.5[ab]

Note: Values with the same letters in the same column are not significantly different ($p < 0.05$) by DMRT.

Under well-watered conditions, M4 produced the highest leaf (3.6 g), stem (1.4 g), root (0.9 g,) and hence total (5.9 g) dry weights (*Table 6*), whilst M6 recorded the lowest leaf (1.4 g), root (0.4 g), and total (2.6 g) dry weights ten weeks after planting. The highest and the lowest shoot-to-root ratio were obtained in M3 (6.0) and M1 (4.8) respectively. Among the soilless growing media, M1 produced the lowest dry biomass for all parameters measured.

At the end of the re-watering period, none of the cocoa plants in M6 recovered. The imposition of water stress resulted in the death of all cocoa plants in M6 (*Table 7*). M5 produced the greatest plant height (59.1 cm), the highest number of leaves (20.1), root length (29.9), and chlorophyll content (39.3) at the end of the re-watering period. Among the soilless growing media, M1 recorded the lowest values for all the growth parameters measured. The highest root volume (19.2 cm^3) was obtained in M4.

Table 7. Effects of growing media and re-watering on the growth of water-stressed and continuously watered cocoa seedlings, eighteen (18) weeks after planting

Growing media	**Height (cm)**	**Girth (cm)**	**Number of leaves**	**Root length (cm)**	**Root volume (cm^3)**	**Chlorophyll content**
Water-stressed						
M1	25.6[d]	0.6[b]	7.3[d]	24.2[c]	14.3[d]	25.7[d]
M2	51.2[c]	1.0[a]	10.2[c]	28.0[b]	16.1[c]	35.1[c]
M3	52.7[bc]	1.0[a]	18.3[b]	29.3[a]	18.3[b]	36.7[b]
M4	56.3[b]	1.0[a]	19.4[a]	29.5[a]	19.5[a]	38.9[ab]
M5	59.1[a]	1.0[a]	20.1[a]	29.7[a]	18.8[ab]	39.3[a]
M6	0.0[e]	0.0[c]	0.0[e]	0.0[d]	0.0[e]	0.0[e]

Growing media	Height (cm)	Girth (cm)	Number of leaves	Root length (cm)	Root volume (cm^3)	Chlorophyll content
Continuously watered						
M1	34.2^{d}	0.8^{b}	24.2^{c}	24.3^{c}	15.7^{d}	24.3^{d}
M2	47.2^{c}	1.1^{a}	30.3^{b}	28.0^{b}	19.0^{c}	34.3^{c}
M3	49.2^{b}	1.2^{a}	31.8^{ab}	29.3^{a}	22.7^{b}	36.5^{b}
M4	49.7^{b}	1.2^{a}	32.4^{a}	29.0^{a}	25.7^{a}	37.2^{a}
M5	56.7^{a}	1.2^{a}	33.0^{a}	29.8^{a}	25.9^{a}	37.9^{a}
M6	39.1^{e}	0.8^{b}	20.7^{d}	19.5^{d}	10.4^{e}	25.7^{e}

Note: Values with the same letters in the same column are not significantly different ($p < 0.05$) by DMRT.

In the continuously watered plants, M5 produced the greatest plant height (56.7 cm), the highest number of leaves (33.0), root length (31.9 cm), root volume (25.9 cm^3), and chlorophyll content (37.9) (*Table 8*). However, M1 recorded the lowest plant height (34.2 cm) and chlorophyll content (24.3). M6 recorded the lowest number of leaves (20.4), root length (19.5 cm), and root volume (10.4 cm^3). The lowest stem girth (0.8 cm) was obtained by M1 and M6.

At the end of the re-watering period, M5 recorded the highest leaf dry weight (11.6 g), stem dry weight (10.6 g), total dry weight (28.0 g), and shoot-to-root ratio. Among the soilless growing media, M1 obtained the lowest biomass production for all parameters measured (*Table 8*).

M5 obtained the highest leaf dry weight (13.0 g), stem dry weight (9.0 g), root dry weight (5.7 g), and hence the highest total dry biomass (27.9 g). The highest shoot-to-root ratio was observed in M3. Total biomass production increased with increasing the amount of compost. M6 recorded the lowest biomass production (*Table 8*).

Table 8. Effects of growing media and re-watering on biomass production and partitioning of water-stressed and continuously watered cocoa seedlings, eighteen (18) weeks after planting

Growing media	Leaf dry weight (g)	Stem dry weight (g)	Root dry weight (g)	Total dry weight (g)	Shoot/root ratio
Water-stressed					
M1	2.5^{e}	2.1^{d}	1.8^{c}	6.4^{e}	2.5^{c}
M2	5.0^{d}	4.3^{c}	3.6^{b}	12.9^{d}	2.6^{c}
M3	9.5^{c}	9.2^{b}	4.3^{ab}	23.3^{c}	3.4^{b}
M4	10.5^{b}	9.8^{b}	4.9^{a}	24.0^{b}	4.1^{ab}
M5	11.6^{a}	10.6^{a}	4.8^{a}	28.0^{a}	4.8a

Growing media	Leaf dry weight (g)	Stem dry weight (g)	Root dry weight (g)	Total dry weight (g)	Shoot/root ratio
M6	0.0[f]	0.0[e]	0.0[d]	0.00[f]	0.0[d]
Continuously watered					
M1	6.5[e]	4.6[d]	2.7[c]	13.8[e]	4.1[a]
M2	8.2[d]	6.1[c]	4.1[b]	18.4[d]	3.5[b]
M3	9.9[c]	7.9[b]	4.1[b]	21.9[c]	4.3[a]
M4	11.2[b]	7.9[b]	4.7[b]	23.3[b]	4.0[a]
M5	13.0[a]	9.0[a]	5.9[a]	27.9[a]	3.7[ab]
M6	3.0[f]	3.0[e]	2.7[c]	8.7[f]	2.2[c]

Note: Values with the same letters in the same column are not significantly different ($p < 0.05$) by DMRT.

4. Discussion

Effective and affordable measures that enhance crop productivity are required for the sustainable management of water stress. The integrated use of biochar and compost in plant growth media is considered an efficient and affordable measure as it is environmentally sound. Several studies have demonstrated their effectiveness in enhancing crop growth and output under water-stressed conditions.

Physico-chemical properties of the growing media used for the study

The growing media were analysed for certain physico-chemical characteristics. Data reported showed a considerable variability in most of the physico-chemical properties, especially between the soilless growing media and the topsoil. The soilless growing media with compost included had an improved physico-chemical status for better plant growth and development due to increased aeration, water-holding capacity, and nutrient content. Increasing the amount of compost resulted in increased nutrient content. The increased C/N ratio with increasing the amount of CRH is likely due to greater organic C content. Among the soilless growing media, pH increased with increasing the amount of CRH. Organic matter content and the macronutrients N, P, K, Ca, and S increased with increasing the amount of compost.

The influence of growing media on germination indices of cocoa seeds

The soilless growing media used in this study had a higher rate of seedling emergence (number of seedlings appearing every 5 days), and it took fewer days to reach 50% emergence. This might be due to their higher WHC and low BD as compared to the topsoil medium. The high WHC of the soilless growing media is likely to have reduced the imbibition period and allowed seedlings to emerge at a faster rate, reducing the number of days to reaching 50% emergence compared to the low WHC of the topsoil. High bulk density affects seedling emergence, as indicated by [28]. It was noticed at the nursery that the shoot had emerged, which suggests that the high BD of the topsoil may have inhibited the emergence of the cotyledons. However, it took longer for the cotyledons to emerge, while some never did, and they eventually died. The high BD may have also prevented seedling emergence and caused some seeds to suffocate and perish by producing soil compaction after watering. This finding is consistent with [29], who claimed that soil compaction occurs when plants are cultivated in soil-filled containers due to the frequent watering requirements of the plants. Similar findings of low seedling emergence of African breadfruit in 100% soil media as compared to medium comprised of 1:2:3 Rice hull: PM: River sand were also reported by [30].

Effects of growing media and water stress on the growth and biomass of cocoa seedlings

Drought stress severely affects plant biomass allocation, photosynthetic rate, and growth [31]. Crop growth and development are limited under water stress conditions. The results showed that growing media and water stress significantly affected cocoa seedling growth, biomass production, and partitioning.

During drought imposition, water availability for the cocoa plants decreased in all the growing media. The soilless growing media with an improved physico-chemical status were less affected. The results of the study showed that the plant growth medium containing compost significantly performed better for plant growth parameters compared to the topsoil. The study indicated that shoot growth and root production increased with increasing the amount of compost for the soilless growing media. The positive effect of compost on plant growth may be a result of increased nutrient availability [32; 33], which in the present study was reiterated by increased N, P, K, Ca, S, and also organic matter content, which could increase water availability. The higher potassium concentration in the media may have contributed to the conservation of water during drought, as it is widely known that potassium enhances plant water status, the turgor pressure of cells, and stomatal regulation [34]. Again, the application of compost in soil is reported to increase the crop's tolerance to abiotic stresses by improving microbial activity [18]. Generally, seedling growth responses to water stress indicated that media M4 and M5 were the best. Seedlings grown in these media had delayed the appearance of water stress symptoms, indicating a better water economy. After imposition of water stress, seedlings grown in these media remained turgid for a longer period than those grown in other growing media, especially in the topsoil. Biomass production was less affected in the medium (M5) containing the highest amount of compost.

The effect of water stress in M6 was more prominent. In general, the topsoil produced seedlings with poorer growth. The imposition of water stress resulted in the death of all the plants in M6. This might be due to the poor root development observed for M6, which might be the result of high bulk density, which, in turn, discourages root development and hence plant growth and development.

Effects of growing media and re-watering on water-stressed cocoa plants

Cocoa plants in soilless growing media with compost recovered rapidly after re-watering as a result of a larger root system, which improves the crop's ability to utilize more water [33]. This study showed that compost-formulated soilless media increased seedling growth and accelerated recovery after drought stress compared to the topsoil. This is a result of the larger root system, which could improve the crop's ability to take up water during re-watering. CRH had less effect on cocoa plants' water stress recovery than compost, but it may help to maintain a more stable growing-media water content than topsoil due to its higher water-holding capacity in the short term.

This study has proved that increasing the amount of compost incorporation increased cocoa seedling growth and accelerated the recovery of plants after drought stress. Overall, all the soilless growing media with compost (M2–M5) were able to recover after one week of re-watering, whilst it took two weeks for M1 without compost. All the plants in the topsoil (M6) were unable to recover and subsequently died. The quicker recovery of the plants with increasing the amount of compost (M2–M5) could be attributed to the larger root system, as indicated by higher root biomass production, which could lead to increased water and nutrient absorption by the plants. The enhanced recovery could also be attributed to increased microbial activities [35].

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Evaluation of genetic variation among maize inbred lines for salinity stress at seedling stage through salt-stress-responsive traits

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Abstract: Saline conditions affect plant development and significantly reduce its yield. Maize (*Zea mays*) is the one of main cash crops in Pakistan, and unfavourable saline conditions are among the core reasons for its reduced productivity, especially in arid and semi-arid regions. The identification of potential genotypes is essential for genetic modifications. By considering this situation, the current experiment was conducted to evaluate the inbred maize lines under different salinity levels. We evaluated ten maize inbred maize lines at seedling stage under three salinity levels (0 mM, 75 mM, and 125 mM NaCl). The highly significant ($p \leq 0.001$) differences in inbred lines, salinity levels, and in their interaction were revealed by analysis of variance results for most of the traits. The results indicated that inbred lines D-135 and NCIL-20-4 performed better under saline conditions. Our results showed that salinity severely affects seedling growth. Accordingly, a significant decline was observed in root length, shoot length, root weight, and shoot weight, and these traits offered the maximum values for heritability and genetic advance. From the correlation and path coefficient analysis, it has been concluded that root length, shoot length, fresh root weight, and root density are the traits that can be beneficial for the identification of better germplasms under saline conditions and that are helpful for improving tolerance against saline conditions.

Keywords: *Zea mays*, heritability, genetic advance, path coefficient analysis

1. Introduction

Soil salinization is a severe threat to crop productivity [1], and it is expected to be exacerbated in the near future as the consequences of global climatic changes [2]. Salinity affects almost 50% of the world's irrigated land and one-fifth of cultivable land [3], while in Pakistan almost 14 million acres of arable land are affected by salinity [4]. The situation is particularly adverse in arid and semi-arid areas owing to low precipitation and high transpiration, as well as inadequate water and soil management techniques, which disrupt the salt balance in the soil, worsening its impact on plant growth [5]. Maize is a highly valuable agricultural crop that is used for food, animal feed, and bioenergy raw materials all over the world [6], and its production rate has a direct impact on food security [7]. Maize is a salt-sensitive crop [8], and under 1.7 dS m^{-1} of soil electrical conductivity (EC_e) threshold it shows moderate sensitivity, while every 1 dS m^{-1} increase in EC_e results in 12% loss in grain production [9]. Thus, the breeding of salt-tolerant cultivars is one viable approach to rise to this challenge and boost the productivity of crops in salt-affected soils [10]. By 2050, almost half of the arable land is predicted to be damaged by salinity if no major steps will be taken [11].

Soil salinity grossly affects plant growth and development [12]. Plants respond to salinity stress from the molecular to morphological levels [13, 14]. Plant cells respond to salt stress by undergoing major changes – e.g. salt stress induces ion stress that causes ionic imbalance, results in ionic toxicity and osmotic stress, and subsequently produces reactive oxygen species (ROS) in plant cell [15]. Salt stress affects photosynthetic and transpiration rate by decreasing chlorophyll content and stomatal conductance [16]. Soil water potential and leaf water potential decrease due to soil salinity that subsequently disturbs plant–water relations and leads to osmotic stress [17]. High soil salinity caused a reduction in the stem and root length, biomass, and yield [18]. However, different crops respond differently to soil salinity because this is dependent upon their resistance to salinity stress. For example, beans (*Vicia faba*) and rice (*Oryza sativa*) are referred to as salt-sensitive, while barley (*Hordeum vulgare*) and Upland cotton (*Gossypium hirsutum*) are more resilient to salinity stress [19]. Overall, modifications in numerous morpho-anatomical alternations in roots and leaves enable the plant to adjust during salt stress [20].

Salinity is a genetically complicated abiotic stress that is influenced by several physiological and biochemical processes, subsequently affecting yield production [21]. Plant breeders have devised a number of techniques for combating salinity. One of the most important steps is to investigate the genetic variability of the available germplasms in order to find a tolerant genotype that is capable of maintaining a fair production in salt-affected soil [22]. Genetic diversity for salt tolerance has been documented in maize [23]. However, the identification of tolerant and promising genotypes requires a detailed understanding of genetic correlations among many features [24]. For example, correlation is the tool that is used to calculate the link between the traits, and it directly observes the phenotypic and genotypic correlation, which expresses the relationship between traits at the gene level [25, 26]. The path coefficient analysis is also utilized to determine the direct and indirect effects of traits. This method of trait selection is based on the traits' direct and indirect substantial effects on the dependent trait and is useful for determining attributes for selection criteria [27, 28, 29].

In maize, the progress in the determination of genetic architecture of salt tolerance and detecting the underlying genes and genetic factors has slowed down [30]. Therefore, the current study was designed to 1) assess and choose the best-performing maize inbred lines under saline conditions and 2) perform correlation and path coefficient analysis to identify the most contributing traits towards salt tolerance and suggest their role in selection procedure.

2. Material and methods

2.1. Plant material and experimental details

The experiment was conducted in the autumn season of 2018 in the greenhouse of the Department of Plant Breeding and Genetics, University of Agriculture Faisalabad, where the temperature was maintained at 28–30°C. Ten inbred lines used for this evaluation – namely WM-13RA, B-34, W-82-3, A-545, D-135, D-103, NCIL-20-4, OH-54-3A, W-187R, and A-638 (represented as V1, V2, V3, V4, V5, V6, V7, V8, V9, V10 in the figures respectively) – were selected based on the characterization/information provided by the contributor. The inbred lines were sown in polythene bags filled with sand by following completely randomized design having three replications under factorial arrangement. Three salinity levels were applied: control (0 mM), 75 mM, and 125 mM NaCl. The salinity levels were applied on the first day of experiment, and then the normal water was applied throughout the experiment. After 24 days of sowing, chlorophyll content and leaf temperature were examined with the help of chlorophyll content meter CCM200 plus and infrared thermometer, and then seedlings were uprooted and washed. Roots and shoots were detached, and their lengths were measured (cm) using a one-meter ruler. Root density was recorded in grams per millilitre (g/ml) by dipping the roots in a 100 mm test tube. The fresh weight of roots and shoots was measured using a digital balance (g). Then the roots and shoots were oven-dried at 70°C for 72 hrs, and the root dry weight (g) and shoot dry weight (g) were measured. Root/shoot length ratio, fresh root/shoot ratio, and dry root/shoot ratio were also computed.

2.2. Statistical analysis

Analysis of variance of the data for the observed traits was conducted using IBM SPSS statistics 22. The means were compared by employing Tukey's HSD test ($p \leq 0.05$). The variability analysis was performed using R software [31]. Calculations were performed according to Singh and Chaudhary [32].

Heritability was calculated by the following equation:

$$h^2 = \sigma^2_g / \sigma^2_p,$$

where σ^2_g = genotypic variance and σ^2_p = phenotypic variance.

Genetic advance was computed by the following equation:

$$GA = \sigma_p \times h^2 \times I,$$

where σ_p = the phenotypic standard deviation, h^2 = estimate of broad sense heritability, and i = standardized selection differential.

The genetic and phenotypic correlation coefficient ($r_{g,}$ r_p) was calculated by performing the correlation analysis proposed by Kown and Torrie [33].

$r_g = COV_G(X_1\ X_2)/\surd\ V_G(X_1).\ V_G(X_2)$

$COV_G(X_1\ X_2)$ = genetic covariance between traits X_1 and X_2

$V_G(X_1)$ = genetic variance for trait X_1

$V_G(X_2)$ = genetic variance for trait X_2

$r_p = COV_p(X_1\ X_2)/\surd\ V_p(X_1).\ V_G(X_2)$

$COV_p(X_1\ X_2)$ = phenotypic covariance among traits X_1 and X_2

$V_p(X_1)$ = phenotypic variance for trait X_1

$V_p(X_2)$ = phenotypic variance for trait X_2

The path coefficient analysis was used to split the correlation coefficient into direct and indirect constituents. This method was described by Dewey and Lu [34]. The root/shoot length ratio was considered the dependent variable and checked the direct and indirect effect of other traits upon the dependent variable.

3. Results

3.1. Phenotypic variations of maize inbred lines at seedling stage

The two-way analysis of variance results are presented in *Table 1*. The analysis of variance revealed highly significant variations among the inbred lines for all observed traits. The interaction between inbred lines and salinity levels also revealed highly significant differences for most of the observed traits. Based on the data reported for twelve traits at seedling stage (*Figure 1*), inbred lines V5 (D-135) and V7 (NCIL-

20-4) performed relatively better as compared to other inbred lines under saline conditions. Although the other inbred lines yielded higher values under normal conditions, such as V10 (A-638), they were more susceptible to salinity and had a higher reduction rate (*Table 2*). All observed traits significantly decreased under saline stress, except for leaf temperature, which produced increased values (ranging from 7.03% to 27.95%); thus, the ratio of different traits presented fluctuations in values from decreased to increased *(Figure 1*, *Table 2*). Data regarding the percentage (%) variation of traits under different irrigation conditions as compared to normal conditions are presented in *Table 2*. The chlorophyll content decreased (ranging from 6.74% to 34.48%) in response to salinity stress in all inbred lines. The lowest reduction rate of root length was observed in V5 under both salinity levels (75 mM, 125 mM): 1.04% and 35.86% respectively, while the lowest reduction rate of shoot length was observed in V7, which was 29.64% and 48.83% for both salinity levels [*Figure 1(d)*, *Table 2*]. For fresh and dry root weights, V7 and V5, respectively, performed comparatively better. The reduction rate of fresh root weight was ranging from 8.22% to 22.35%, while the reduction rate for dry root weight was between 18.38% and 56.19% for these two inbred lines under both salinity levels [*Figure 1(e)*, *(h)*, *Table 2*]. The lowest reduction rate in root density was observed in the V7 and V5 inbred lines (ranging from 11.72% to 40.83%). Regarding fresh and dry shoot weights, fresh and dry root/shoot ratios, and root/shoot length ratios, inbred lines presented significant variations among themselves under all applied conditions [*Figure 1(f)*, *(i)*, *(g)*, *(j)*, *(l)*]. The lowest variations in the root/shoot length ratio were observed for V7 and V5, which may indicate their higher survival rate (*Table 2*).

Table 1. Statistical summary of maize traits at seedling stage under different saline conditions

Traits	**Inbred lines (IL)**	**Salinity levels (S)**	**IL × S**
Chlorophyll content	***	***	*
Leaf temperature	***	***	NS
Root length	***	***	***
Shoot length	***	***	**
Fresh root weight	***	***	***
Fresh shoot weight	***	***	**
Fresh root/shoot ratio	***	***	*
Dry root weight	***	***	***
Dry shoot weight	***	***	***
Dry root/shoot ratio	***	***	***
Root density	***	***	***
Root/shoot length ratio	***	***	***

Notes: NS = Non-significant; * = Significant at $p \leq 0.05$; ** = Significant at $p \leq 0.01$; *** = Significant at $p \leq 0.001$

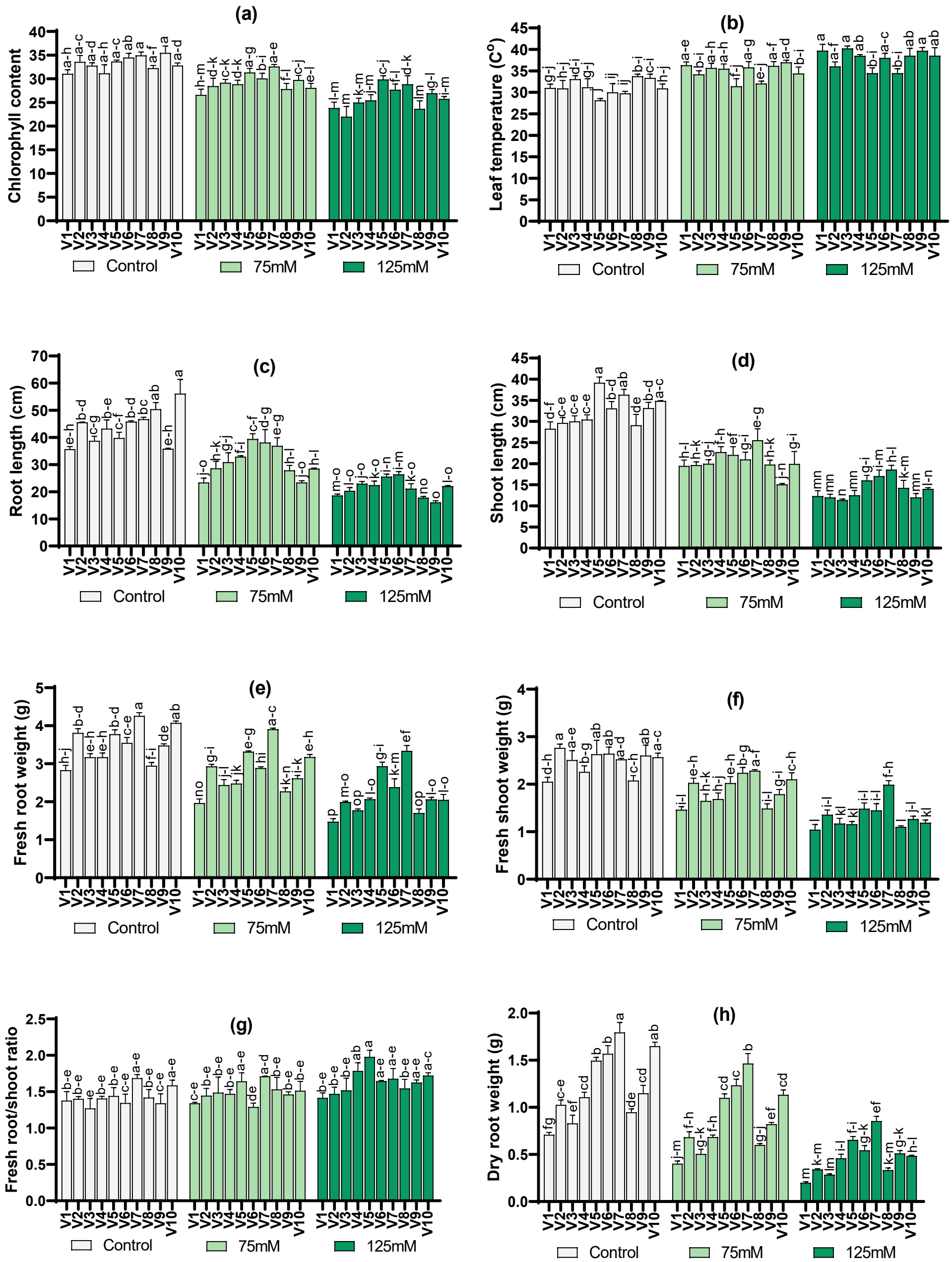

Figure 1a. Comparative morphological characteristics of maize inbred lines at seedling stage under different saline conditions: (a) Chlorophyll content, (b) Leaf temperature, (c) Root length, (d) Shoot length, (e) Fresh root weight, (f) Fresh shoot weight, (g) Fresh root/shoot ratio

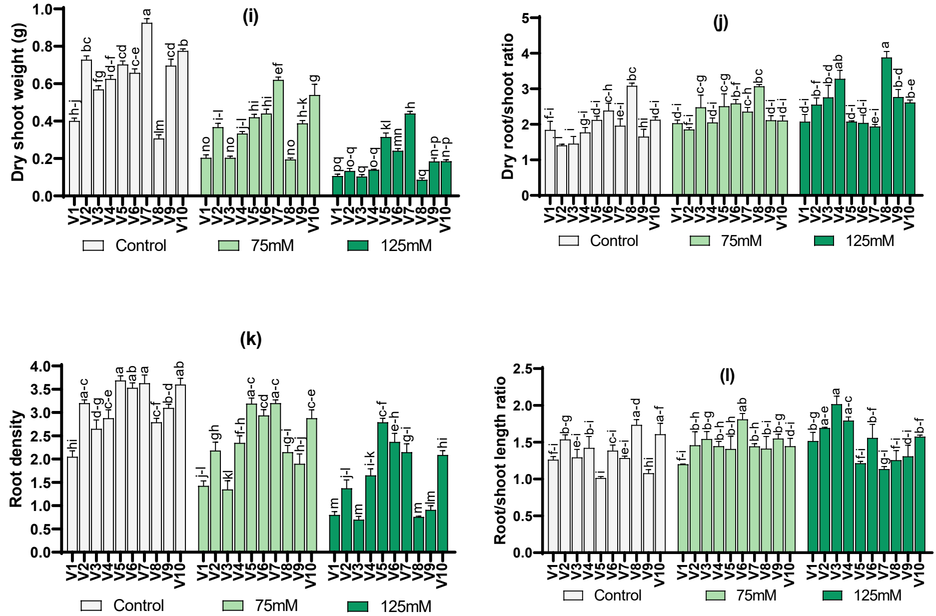

Figure 1b. (h) Dry root weight, (i) Dry shoot weight, (j) Dry root/shoot ratio, (k) Root density, (l) Root/shoot length ratio

Table 2. Percentage (%) variation of the different traits of maize inbred lines as compared to control at seedling stage under different saline conditions

Traits	Levels	Inbred Lines									
		V1	**V2**	**V3**	**V4**	**V5**	**V6**	**V7**	**V8**	**V9**	**V10**
Chlorophyll Content	75 mM	-14.34	-15.25	-10.95	-7.44	-6.86	-12.79	-6.74	-13.8	-16.07	-14.41
	125mM	-23.25	-34.48	-23.75	-18.35	-11.27	-19.68	-18.47	-26.73	-23.99	-21.49
Leaf Temperature	75mM	17.02	10.59	7.85	13.78	11.68	19.32	7.64	7.03	10.89	11.17
	125mM	27.95	16.48	21.55	23.4	22.41	26.73	15.81	14.1	18.89	24.6
Root Length	75mM	-34.5	-37.17	-20.36	-23.92	-1.04	-16.72	-21.02	-44.69	-34.59	-49.15
	125mM	-47.78	-55.37	-40.81	-48.08	-35.86	-42.33	-54.75	-64.67	-54.99	-60.66
Shoot Length	75mM	-31.15	-33.64	-33.46	-25.21	-43.61	-36.42	-29.64	-31.98	-54.46	-39.95
	125mM	-56.35	-59.52	-62.08	-58.87	-46.3	-48.36	-48.83	-51.01	-63.82	-59.82
Fresh Root Weight	75mM	-30.57	-23.2	-23.15	-21.89	-12.17	-18.59	-8.22	-22.88	-24.86	-22.02
	125mM	-47.88	-47.71	-44.09	-34.8	-22.35	-32.82	-21.6	-42.2	-40.66	-49.69
Fresh Shoot Weight	75mM	-28.75	-26.58	-34.26	-25.22	-23.15	-15.31	-9.5	-28.37	-31.29	-18.09
	125mM	-49.17	-50.81	-53.19	-48.67	-43.64	-45.18	-21.09	-46.88	-51.25	-53.7

Traits	Levels	Inbred Lines									
		V1	**V2**	**V3**	**V4**	**V5**	**V6**	**V7**	**V8**	**V9**	**V10**
Fresh Root/ Shoot Ratio	75mM	-2.77	3.12	17.13	4.55	14.05	-3.99	1.41	7.84	8.99	-4.61
	125mM	2.6	4.87	19.51	27.13	37.42	22.3	-0.49	8.68	21.28	8.51
Dry Root Weight	75mM	-43.23	-33.45	-39.1	-38.18	-26.39	-21.34	-18.38	-36.66	-28.55	-31.21
	125mM	-72.01	-66.7	-65.6	-58.48	-56.19	-65.29	-52.37	-64.66	-55.54	-70.61
Dry Shoot Weight	75mM	-49.27	-49.48	-64.13	-46.6	-40.16	-33.08	-32.96	-36.42	-44.21	-30.37
	125mM	-73.55	-81.58	-81.74	-77.63	-55.15	-63.23	-52.4	-71.87	-73.47	-76.08
Dry Root Shoot Ratio	75mM	10.06	31.67	69.84	15.75	18.16	8.47	20.19	-0.41	27.83	-0.92
	125mM	12.6	81.47	89.07	85.2	-2.33	-14.49	-1.18	25.81	67.22	22.92
Root Density	75mM	-30.49	-31.72	-48.96	-18.43	-13.69	-16.74	-11.72	-22.94	-38.61	-20.03
	125mM	-60.98	-57.03	-73.53	-42.61	-24.39	-32.77	-40.83	-72.76	-70.52	-42.01
Root Shoot Length Ratio	75mM	-4.96	-5.18	19.37	1.65	43.9	30.56	12.35	-18.58	43.55	-15.16
	125mM	19.99	10.15	55.87	26.04	19.57	12.46	-11.7	-27.61	24.59	-2.07

Note: Negative (-) values indicate the reduction, and positive (+) values show the increment of the specific traits as compared to the normal.

3.2. Genetic components of the traits of various maize seedling stages

Genotypic variance was slightly lower than phenotypic variance for all the observed traits of maize inbred lines under normal and salinity stress conditions (*Table 3*). The highest values of genetic and phenotypic variance were observed for root length and shoot length traits under all applied conditions, while the other traits yielded lower values, except for chlorophyll content and leaf temperature, which presented slightly higher values under 125 mM salinity level. Other traits displayed marginal changes compared to the normal.

The genotypic coefficient of variance (GCV) was slightly lower than the phenotypic coefficient of variance (PCV). Dry shoot weight, root density, dry root weight, fresh root weight, and shoot length revealed higher values of GCV and PCV under salinity stress. The environmental coefficient of variations (ECV) was higher for shoot length, followed by root density under stressful conditions. These results suggested that shoot length, dry shoot weight, fresh and dry root weight, and root density might be helpful for the selection of germplasm as indicator traits for salinity stress tolerance.

Dry shoot weight, dry root weight, and root density presented the highest values of heritability. The genetic advance was higher for root length and shoot length and moderate for chlorophyll content, leaf temperature, and root density; thus, other traits presented lower values of genetic advance under different salinity levels (*Table 3*).

Table 3. Genetic components for various seedling traits of maize inbred lines under normal and different saline conditions

Traits	Levels	σ^2_g	σ^2_p	GCV	PCV	ECV	h^2	GA
Chlorophyll Content	Control	1.691	2.247	3.913	4.510	2.242	0.753	1.5799
	75 mM	2.640	3.111	5.547	6.021	2.342	0.849	2.0955
	125 mM	5.043	5.823	8.677	9.324	3.413	0.866	2.9258
Leaf Temperature	Control	2.285	3.092	4.839	5.629	2.876	0.739	1.8191
	75 mM	2.722	3.412	4.731	5.297	2.382	0.798	2.0632
	125 mM	3.674	4.457	5.066	5.579	2.338	0.824	2.4364
Root Length	Control	39.763	42.408	14.388	14.858	3.711	0.938	8.5483
	75 mM	29.940	33.046	17.633	18.525	5.679	0.906	7.2916
	125 mM	10.151	10.669	14.906	15.282	3.369	0.951	4.3507
Shoot Length	Control	11.533	12.597	10.476	10.948	3.181	0.916	4.5494
	75 mM	10.064	11.350	15.003	15.932	5.362	0.887	4.1823
	125 mM	10.162	10.787	21.943	22.607	5.440	0.942	4.3318
Fresh Root Weight	Control	0.224	0.229	13.484	13.635	2.020	0.978	0.6556
	75 mM	0.321	0.325	20.224	20.337	2.147	0.989	0.7889
	125 mM	0.317	0.324	25.808	26.079	3.753	0.979	0.7798
Fresh Shoot Weight	Control	0.047	0.061	8.838	9.991	4.660	0.782	0.2698
	75 mM	0.084	0.090	15.423	16.010	4.298	0.928	0.3908
	125 mM	0.072	0.076	20.264	20.839	4.858	0.946	0.3661
Fresh Root/Shoot Ratio	Control	0.011	0.015	7.365	8.593	4.426	0.735	0.1263
	75 mM	0.012	0.016	7.241	8.371	4.200	0.748	0.1307
	125 mM	0.023	0.028	9.152	10.177	4.452	0.809	0.1890
Dry Root Weight	Control	0.139	0.141	30.359	30.582	3.692	0.985	0.5175
	75 mM	0.122	0.123	40.442	40.682	4.414	0.988	0.4860
	125 mM	0.037	0.037	41.181	41.472	4.899	0.986	0.2668
Dry Shoot Weight	Control	0.032	0.032	27.908	28.000	2.259	0.993	0.2487
	75 mM	0.020	0.021	38.564	38.790	4.176	0.988	0.1991
	125 mM	0.013	0.013	58.136	58.316	4.578	0.994	0.1574
Dry Root/Shoot Ratio	Control	0.228	0.243	24.060	24.795	5.992	0.942	0.6493
	75 mM	0.112	0.129	14.423	15.474	5.606	0.869	0.4367
	125 mM	0.366	0.388	23.216	23.917	5.750	0.942	0.8217
Root Density	Control	0.272	0.280	16.754	16.995	2.851	0.972	0.7197
	75 mM	0.455	0.464	28.606	28.900	4.108	0.980	0.9348
	125 mM	0.574	0.581	48.518	48.828	5.493	0.987	1.0539
Root/Shoot Length Ratio	Control	0.047	0.050	15.940	16.461	4.111	0.938	0.2947
	75 mM	0.017	0.024	8.960	10.512	5.497	0.727	0.1573
	125 mM	0.071	0.077	17.635	18.309	4.921	0.928	0.3598

Notes: σ^2_g = Genotypic variance, σ^2_p = Phenotypic Variance, GCV = Genotypic coefficient of variation, PCV = Phenotypic coefficient of variation, ECV = Environmental coefficient of variation, h^2 = Heritability, GA = Genetic advance.

3.3. Genotypic and phenotypic correlation among seedling stage traits

Significant positive genotypic correlation was observed between chlorophyll content and shoot length, fresh root weight, fresh shoot weight, dry root weight, and root density, while highly positive phenotypic correlation was demonstrated for these traits (*Table 4*). Fresh root weight indicated a significant positive correlation with fresh shoot weight, dry root and shoot weight, root density, and shoot length at both the genotypic and phenotypic level. Thus, fresh root weight showed significant negative correlation with leaf temperature for both the genotype and phenotype. Dry root weight and dry shoot weight exhibited significant positive correlation with chlorophyll content, fresh root weight, and fresh shoot weight at both the genotypic and phenotypic level (*Table 4*), while these traits showed significant negative correlation with leaf temperature under all applied conditions. Root density indicated significant positive correlation with all observed traits, except leaf temperature, which showed significant negative correlation (*Table 4*).

3.4. Path coefficient analysis for seedling stage traits

This analysis considered the root/shoot length ratio as dependent trait. The data were divided into direct and indirect effects on the root/shoot length ratio and are presented in *Table 5*. Root/shoot length ratio was dependent upon a variety of connected traits. The highest direct positive effect was presented by the fresh root weight and root length under stressful conditions. Results indicated that the direct effect of the fresh root weight was negative (-0.59311) under normal conditions, but, interestingly, it increased manifold under salinity stress conditions: 0.9279 for 75 mM and 1.94424 for 125 mM salinity stress (*Table 5*). The maximum negative direct effect was presented by fresh shoot weight and dry root and shoot weight under salinity stress conditions. Chlorophyll content and root density indicated the highest positive indirect effect on the dependent trait under salinity stress, via shoot length. Fresh root and shoot weight, dry root and shoot weight, and root density indicated the highest negative effect on the dependent trait under all applied conditions, via shoot length.

Table 4. Genotypic (above diagonal) and phenotypic (below diagonal) correlation coefficient for various seedling traits of maize under control and different saline conditions

Traits	Levels	***Chlorophyll content***	***Leaf temperature***	***Root length***	***Shoot length***	***Fresh root weight***	***Fresh shoot weight***	***Fresh root/shoot ratio***	***Dry root weight***	***Dry shoot weight***	***Dry root/ shoot ratio***	***Root density***	***Root/shoot length ratio***
CC	Control		-0.0260	0.0680	0.7018*	0.7132*	0.8493*	0.2852	0.6701*	0.7340*	-0.0952	0.7631*	-0.3837
	75 mM		-0.7257*	0.8564*	0.7008*	0.9142*	0.7555*	0.9216*	0.8276*	0.6932*	0.2451	0.7504*	0.5711
	125 mM		-0.5164*	0.5703*	0.9022*	0.8881*	0.6960*	0.9519*	0.8924*	0.8649	-0.5261*	0.7570*	-0.5535
LT	Control	-0.1453		-0.1473	-0.8569*	-0.5881*	-0.5456	-0.3840	-0.7080*	-0.6093*	0.0834	-0.6197*	0.3492
	75 mM	-0.6737**		-0.8681*	-0.9869*	-0.9431*	-0.7857*	-0.8932*	-0.6928*	-0.7029*	0.0702	-0.8469*	-0.0639
	125 mM	-0.4559*		-0.4289*	-0.8302*	-0.9435*	-0.9340*	-0.5316	-0.8006*	-0.8459*	0.4797*	-0.8076*	0.5367*
RL	Control	-0.0192	-0.0654		0.1773*	0.4800*	0.1244	0.7281*	0.5252*	0.2049	0.5358*	0.5222*	0.7819*
	75 mM	0.7471**	-0.6908**		0.8797*	0.6982*	0.6834*	0.4877	0.7378*	0.5079*	0.4519*	0.7846*	0.5106*
	125 mM	0.4956*	-0.3663		0.5746*	0.4652*	0.3451	0.6024*	0.3346	0.3797	-0.4704*	0.7845*	0.2247
SL	Control	0.5771**	-0.6713**	0.1438		0.8123*	0.6737*	0.6468*	0.8928*	0.7234*	0.1264	0.8948*	-0.4861*
	75 mM	0.5852**	-0.8714**	0.8331**		0.6761*	0.5334*	0.7227*	0.6026*	0.4888	0.2423	0.7355*	0.0283
	125 mM	0.8001**	-0.7820**	0.5636**		0.8543*	0.7357*	0.7513*	0.8054*	0.8499*	-0.5175*	0.8629*	-0.6589*
FRWT	Control	0.6409	-0.5632**	0.4478*	0.7563**		0.8692*	0.7700	0.8696*	0.9327*	-0.1641	0.8868*	-0.0825
	75 mM	0.8154**	-0.8342**	0.6487**	0.6282**		0.9311*	0.7309	0.9147*	0.9321*	-0.0636	0.8615*	0.2777
	125 mM	0.8040**	-0.8381**	0.4471*	0.8383**		0.9568*	0.7218	0.9846*	0.9805*	-0.5366	0.7936*	-0.5632
FSWT	Control	0.7374**	-0.3762	0.0853	0.5482*	0.7487**		0.3437	0.6097	0.8783*	-0.4796	0.8380*	-0.3220
	75 mM	0.6962**	-0.6284**	0.6479**	0.4544*	0.8987**		0.3864	0.9474*	0.9350*	-0.1251	0.8860*	0.4910
	125 mM	0.6244**	-0.7978	0.3115	0.6995**	0.9359**		0.4874	0.9291*	0.9631*	-0.5736	0.6191*	-0.5307
FRSRT	Control	0.1729	-0.4151	0.6152**	0.5207*	0.6974**	0.0492		0.8127	0.6274	0.2640	0.6043	0.2644
	75 mM	0.6220**	-0.7491**	0.3650	0.6024**	0.6626**	0.2731		0.5099	0.5634	0.1300	0.5425	-0.2114
	125 mM	0.7786**	-0.5078*	0.5399*	0.6870**	0.6548**	0.3501		0.7435	0.6121	-0.1871	0.8931*	-0.3298
DRWT	Control	0.5838**	-0.6181**	0.5095*	0.8433**	0.8578**	0.5339*	0.7023**		0.7868*	0.2044	0.9467*	-0.1004
	75 mM	0.7777**	-0.6317	0.6795**	0.5421*	0.9033**	0.9119**	0.4338		0.9342*	0.1015	0.9327*	0.4674
	125 mM	0.8288**	-0.7200**	0.3398	0.7758**	0.9628**	0.8818**	0.6818**		0.9496*	-0.4440	0.7704*	-0.6392
DSWT	Control	0.6335**	-0.5285*	0.1968	0.6927**	0.9208**	0.7668**	0.5419*	0.7720**		-0.4202	0.7814*	-0.3030
	75 mM	0.6674**	-0.6230**	0.4817*	0.4421	0.9149**	0.9074**	0.4424	0.9293**		-0.2193	0.8475*	0.2066
	125 mM	0.8022**	-0.7801	0.3671	0.8260**	0.9677**	0.9381**	0.5473*	0.9406**		-0.6617	0.7526*	-0.6412
DRSRT	Control	-0.0764	0.0495	0.5071*	0.0932	-0.1484	-0.4139	0.2451	0.2219	-0.4232		0.1661	0.4567
	75 mM	0.1806	-0.0042	0.3338	0.1882	-0.0367	-0.1226	0.2079	0.1145	-0.2189		0.1923	0.4959
	125 mM	0.5005*	0.4291	-0.4266	-0.4892*	-0.5260*	-0.5715**	-0.1336	-0.4159	-0.6525**		-0.5492*	0.1986
RD	Control	0.6679**	-0.5648**	0.4901*	0.8410**	0.8799**	0.7361**	0.5311*	0.9188**	0.7709**	0.1502		-0.0726
	75 mM	0.6654**	-0.7310**	0.7537**	0.7034**	0.8419**	0.8226**	0.4702*	0.9117**	0.8291**	0.1656		0.3228
	125 mM	0.7208**	-0.7234**	0.7461**	0.8297**	0.7844**	0.6117**	0.7808**	0.7524**	0.7450**	-0.5411*		-0.3482
RSLRT	Control	-0.3811	0.3537	0.7898**	-0.4834*	-0.0951	0.2824	0.2102	-0.0911	-0.2977	0.4416	-0.0833	
	75 mM	0.4691*	0.0886	0.5109*	-0.0483	0.2191	0.4691*	-0.2295	0.3975	0.2002	0.3141	0.2831	
	125 mM	-0.4955*	0.5482*	0.2260	-0.6664**	-0.5070*	-0.5070*	-0.3191	-0.6014**	-0.6206**	0.1912	-0.3377	

* = Significant, ** = Highly significant

Table 5. Direct (bold) and indirect effect of various seedling traits of maize inbred lines at root/shoot length ratio under normal and different saline conditions

Traits	Levels	Chlorophyll content	Leaf temperature	Root length	Shoot length	Fresh root weight	Fresh shoot weight	Fresh root/ shoot ratio	Dry root weight	Dry shoot weight	Dry root/ shoot ratio	Root density
CC	**Control**	**-0.04766**	0.00112	0.08374	-0.07835	-0.42302	-0.04245	-0.03318	-0.80854	0.99616	-0.05063	0.01916
	75 mM	**0.11195**	-0.04683	1.17629	-1.07834	0.84829	-0.10181	-0.17882	0.56739	-0.83052	-0.01971	0.12320
	125 mM	**0.18169**	0.05971	0.53629	-1.49832	1.72664	-0.82738	-0.32664	-0.94434	0.71682	-0.15250	-0.02541
LT	**Control**	0.00124	**-0.04306**	-0.18151	0.09567	0.34880	0.02727	0.04466	0.85416	-0.82686	0.04436	-0.01556
	75 mM	-0.08124	**0.06453**	-1.19231	1.51856	-0.87510	0.10588	0.17331	-0.47498	0.84213	-0.00564	-0.13903
	125 mM	-0.09382	**-0.11562**	-0.40334	1.37875	-1.83431	1.11038	0.18242	0.84718	-0.70105	0.13905	0.02711
RL	**Control**	-0.00324	0.00634	**1.23234**	-0.01979	-0.28467	-0.00622	-0.08469	-0.63424	0.27808	0.28484	0.01311
	75 mM	0.09588	-0.05601	**1.37348**	-1.35369	0.64788	-0.09210	-0.09463	0.50581	-0.60846	-0.03634	0.12881
	125 mM	0.10362	0.04960	**0.94032**	-0.95429	0.90450	-0.41023	-0.20670	-0.35407	0.31466	-0.13636	-0.02633
SL	**Control**	-0.03345	0.03689	0.21846	**-0.11164**	-0.48177	-0.03367	-0.07523	-1.07716	0.98178	0.06723	0.02247
	75 mM	0.07845	-0.06368	1.20827	**-1.53879**	0.62732	-0.07188	-0.14023	0.41313	-0.58554	-0.01949	0.12075
	125 mM	0.16391	0.09599	0.54031	**-1.66080**	1.66094	-0.87460	-0.25783	-0.85223	0.70437	-0.15002	-0.02896
FRWT	**Control**	-0.03399	0.02532	0.59148	-0.09068	**-0.59311**	-0.04344	-0.08956	-1.04924	1.26574	-0.08725	0.02227
	75 mM	0.10234	-0.06085	0.95899	-1.04033	**0.92790**	-0.12547	-0.14181	0.62709	-1.11666	0.00512	0.14144
	125 mM	0.16135	0.10909	0.43745	-1.41880	**1.94424**	-1.13744	-0.24771	-1.04188	0.81266	-0.15553	-0.02664
FSWT	**Control**	-0.04048	0.02349	0.15333	-0.07521	-0.51552	**-0.04998**	-0.03998	-0.73563	1.19195	-0.25497	0.02104
	75 mM	0.08458	-0.05070	0.93867	-0.82081	0.86397	**-0.13476**	-0.07498	0.64957	-1.12009	0.01006	0.14546
	125 mM	0.12645	0.10800	0.32448	-1.22183	1.86021	**-1.18882**	-0.16725	-0.98311	0.79822	-0.16626	-0.02078

Traits	**Levels**	Chloro-phyll content	Leaf tempera-ture	Root length	Shoot length	Fresh root weight	Fresh shoot weight	Fresh root/ shoot ratio	Dry root weight	Dry shoot weight	Dry root/ shoot ratio	Root density
FRSRT	**Control**	-0.01359	0.01653	0.89729	-0.07221	-0.45671	-0.01718	**-0.11631**	-0.98053	0.85152	0.14038	0.01517
	75 mM	0.10317	-0.05763	0.66983	-1.11207	0.67816	-0.05207	**-0.19403**	0.34959	-0.67494	-0.01046	0.08907
	125 mM	0.17294	0.06146	0.56641	-1.24782	1.40345	-0.57941	**-0.34316**	-0.78674	0.50731	-0.05423	-0.02998
DRWT	**Control**	-0.03194	0.03048	0.64782	-0.09967	-0.51580	-0.03047	-0.09453	**-1.20651**	1.06776	0.10868	0.02377
	75 mM	0.09265	-0.04470	1.01332	-0.92725	0.84871	-0.12768	-0.09894	**0.68560**	-1.11923	-0.00817	0.15312
	125 mM	0.16215	0.09257	0.31464	-1.33761	1.91436	-1.10452	-0.25514	**-1.05815**	0.78705	-0.12870	-0.02586
DSWT	**Control**	-0.03498	0.02623	0.25252	-0.08076	-0.55318	-0.04390	-0.07298	-0.94927	**1.35711**	-0.22337	0.01962
	75 mM	0.07761	-0.04536	0.69758	-0.75210	0.86488	-0.12599	-0.10932	0.64051	**-1.19801**	0.01764	0.13914
	125 mM	0.15714	0.09780	0.35700	-1.41145	1.90637	-1.14494	-0.21005	-1.00484	**0.82881**	-0.19181	-0.02526
DRSRT	**Control**	0.00454	-0.00359	0.66025	-0.01412	0.09734	0.02397	-0.03071	-0.24664	-0.57019	**0.53165**	0.00417
	75mM	0.02744	0.00453	0.62068	-0.37285	-0.05902	0.01686	-0.02523	0.06961	0.26277	**-0.08043**	0.03157
	125mM	-0.09559	-0.05547	-0.44236	0.85953	-1.04322	0.68187	0.06419	0.46983	-0.54844	**0.28986**	0.01843
RD	**Control**	-0.03637	0.02668	0.64357	-0.09990	-0.52598	-0.04188	-0.07029	-1.14223	1.06043	0.08829	**0.02511**
	75 mM	0.08401	-0.05464	1.07758	-1.13177	0.79938	-0.11939	-0.10527	0.63944	-1.01529	-0.01546	**0.16418**
	125 mM	0.13754	0.09338	0.73767	-1.43304	1.54301	-0.73603	-0.30648	-0.81519	0.62375	-0.15920	-0.03356

4. Discussion

Salinity affects plant development and is considered as one of the main abiotic stresses damaging crop production [35]. Salinity becomes the mainstream concern for plant scientists in the short as well as long term. Although the breeding techniques have made great progress in the past 15 years, conventional breeding is still the main method to improve the stability and production of crops under stressful conditions – in this method, screening of the potential germplasm is the most fundamental and critical point [36]. The genetic variability for salinity tolerance exists in maize crops due to its highly polymorphic nature [37]. Thus, considering certain agronomical characteristics, screening for salt tolerance in early growth stages is often considered valuable [38, 39]. Here we have screened the maize inbred lines to confirm the most promising salt-tolerant maize germplasm and provide more solid evidence for potentially effective salt-tolerant indicator traits. Our results offer a deeper understanding of maize responses towards salinity stress, and we have revealed the traits that make for salt-tolerant indicators in maize.

Maize is more sensitive for salinity at the vegetative growth stages [40, 41, 42]. In the current study, several morphophysiological traits were assessed in ten maize inbred lines at the seedling stage to investigate their relative tolerance ability to salt stress. The highly significant variations among the genotypes at the control and salt stress levels for almost all traits (*tables 1–2*, *Figure 1*) indicated the genetic difference between the maize genotypes used for salt tolerance. All observed traits, such as root and shoot lengths, fresh root and shoot weights, dry root and shoot weights, root density, and chlorophyll content, exhibited significant decline due to salt stress, whereas leaf temperature showed a rising trend in values. The results show that shoot length is reduced in all genotypes under all salt treatments. The better shoot length value under salt treatments indicated that the specific maize genotypes showed tolerance to salt stress and managed to adjust plant growth and development under stressful environment [43, 44, 45].

In the current investigation, maize inbred line V7 and V5 showed higher shoot length under salt stress as compared with other inbred lines, and therefore these may be used for enhancing the grain productivity of maize in salt-affected soils [46, 47]. Salt stress represses leaf initiation and expansion, including internode growth, which eventually leads to reduction in shoot growth [48, 49, 50]. This repression is mainly due to a reduction in cell elongation [51]. Maize is a salt-sensitive crop, and its shoot growth is highly inhibited at the seedling stage under salt stress [52, 53]. The fresh and dry weight of root and shoot as well as their length as the measure for maintaining growth during salinity stress were considered the driving traits for most variations among the genotypes. These traits have been acknowledged as good predictors of salinity tolerance [54, 55]. Giaveno et al. [56]

proposed that seedling weight should be used to screen for salt-tolerant maize germplasms under salinity stress.

The decrease in chlorophyll content was detected in all maize genotypes under salinity stress, and this decrease is a frequently reported phenomenon, which is used as a vulnerability indicator of cellular metabolic state in several findings [57]. Many plant species reported the decline in chlorophyll content under salinity stress due to membrane deterioration [58, 59]. The reduction of chlorophyll content decreases the photosystem II efficiency and net photosynthetic rate [60]. Salt-induced photosynthesis reductions are associated with both non-stomatal and stomatal limitations and their combination in maize [61]. However, the germplasm with the lowest reduction rate in chlorophyll content exhibited salt-tolerant behaviour [46]. Leaf temperature increment under salinity stress in all the inbred lines was observed to indicate the lower availability of water in leaves because saline conditions hindered plants' water-absorbing capacity [62].

The study of genetic variability and genetic advance offers useful information regarding the extent of variability in available germplasm sources [26, 63]. Based on genetic advance and heritability, selection can be useful [64]. Genetic advances have shown that the additive type of gene action can be used to improve specific traits by fixing them for the next generation [65]. Heritability enabled the entire variation because of genetic variability, and it can play a crucial role in determining the selection criteria [47]. However, heritability alone is not sufficient to determine the selection because high heritability may not always be linked with high genetic advance [66]; thus, heritability and genetic advance may prove to be the most useful for an effective and reliable selection. These selected traits may respond to phenotypic assortment and could be enhanced via heterosis breeding [67]. The highest heritability values were recorded for dry shoot weight, dry root weight, root density, and root length, while genetic advance was higher for root length and shoot length. These results indicated that the salt-tolerant behaviour of maize genotypes depend upon those traits [68], and, consequently, these traits may be used for enhancing the grain productivity of maize in salt-affected soils [46, 69]. Higher heritability in maize seedlings for shoot and root traits under salinity stress was also reported by Tanzeel-ur-Rehman et al. [70], who suggest that root and shoot traits could be taken into consideration for salt tolerance breeding in maize.

For the improvement of traits, the fundamental feature is the assessment of trait variation. It is imperative to describe the relative amount of trait variation components for the development of plant type under any stress condition with further selection parameters by using effective breeding methods [71, 72, 73]. The correlation of seedling traits indicated that the shoot and root length and their weights may be utilized for the selection of better-yielding maize genotypes under salt stress conditions. The higher shoot and root length caused to increase photosynthetic rate in leaves and led to the accumulation of organic matters in the seedling body, due to which the growth and development of maize seedlings improved even under salt stress conditions [69, 74, 75, 76].

For plant breeders, the path coefficient analysis is an effective tool to evaluate the direct and indirect effects of various traits (independent traits) on one dependent trait [64], and it enables a stronger focus on the selection procedure [26, 77]. The traits with the most positive direct effect could be favourable for selection in the breeding cycle [78]. In the current study, the highest direct positive effect was presented by fresh root weight and root length under stressful conditions, while the maximum negative direct effect was exhibited by fresh shoot and dry root and shoot weight under salinity stress conditions, indicating their importance in the selection procedure under stressful conditions [79]. Thus, these traits can be used as reliable screening criteria for the evaluation of salt-tolerant maize genotypes.

5. Conclusions

Comparatively, all inbred lines behaved differently under salinity conditions. Root fresh and dry weights, shoot fresh and dry weights, root and shoot lengths, and root density indicated the highest heritability, which suggested their vital role as selection criteria. Furthermore, root and shoot length and chlorophyll content presented higher genetic advance. The significant correlation among root and shoot lengths and their fresh and dry weights expressed that these traits are essential for assortment against salinity stress conditions. Overall, two inbred lines, namely D-135 and NCIL-20-4, performed comparatively better than other inbred lines. Furthermore, future transcriptomic studies of these tolerant inbred lines could provide an extra glimpse into the gene regulatory components of salt tolerance and investigate their possible use in maize breeding programmes aimed at salinity tolerance.

Authors' contributions

Farrah Zaidi and Ali Shahzad performed the experiment, measurements, and analyses; Muhammad Ahsan supervised the study; Farrah Zaidi, Ali Shahzad, Hameed Gul, and Shareef Gul wrote the manuscript draft; Muhammad Ahsan and Muhammad Shahzad reviewed and edited the manuscript. All authors have read and agreed to the published version of the manuscript.

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Declarations

Conflict of interest
Authors declare that they have no conflict of interest.

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Comparative examination between traditional and worldwide-known red wine grapes and vines based on their qualitative and quantitative characteristics

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Abstract: The tradition of drinking wine has a very old history. Romania has a very good geographical location and a favourable climate for grapes; it is ideal for growing vines and producing quality wines. In our research, we analysed quantitative and qualitative parameters of four varieties of grapes in the Miniş-Măderat Vineyard. The objective was to look for differences between traditional varieties (Cadarcă/Kadarka and Fetască Neagră/Fekete Leányka) and world varieties (Cabernet Sauvignon and Merlot). From the data, it could be concluded that the worldwide-known varieties yielded better results, but for a new plantation in the Miniş-Măderat Vineyard, we recommend the use of traditional varieties, as they have a better history in the area.

Keywords: vine, productivity, must sugar, must acidity, alcohol

1. Introduction

Wine is a beverage that plays a very important role in the present as well as in the past. However, we should not forget that wine could not exist without viticulture [1]. According to some studies, the grape was the first horticultural crop [2, 3]. The harvest of grapes can be used in the form of fruit, must, and wine; however, the most important one is winemaking.

Since ancient times, red wines have been more important than white wines. If we look back at any ancient myth, red wine is always mentioned, for example: “God turned water into red wine.” Although white and rosé wines are catching up more and more nowadays, a true wine lover still swears by red wine.

Among the crops processed worldwide, including all foods and agricultural products, wine is one of the most important products in the world [4]. Grapes can be cultivated on five continents, between the parallels of 30° and 50°, on both the Northern and Southern hemisphere [5].

The conditions for wine production in Romania are favourable, as the conditions for growing grapes are given, both in terms of climate and soil properties [6, 7]. Two of the most important elements of viticulture are soil and land exposure [8] in addition to the climate factors, for example, temperature and precipitation [9]. Besides this, grapes that come from vines are the raw material for winemaking; from grapes, we can obtain jams, raisins, compotes, juice, concentrated must, etc. [10].

Romania can be divided into eight grape-growing regions, within which there are 37 wine regions [11]. On the territory that our country occupies now, wild vine (*Vitis vinifera* ssp. Sylvestris) dates back to the 7000s BC. From this wild vine derived the varieties that are now cultivated such as Fetească Neagră/Fekete Leányka [12, 13].

Based on data from 2017, the world population's wine consumption is 243 million hL/year, while in Romania this is 4.1 million hL/year. So, it could be said that the greatest significance of growing grapes is the wine culture tour. At the same time, grapes are a landscape element, as well as an aesthetic and mood-influencing factor due to changes in the terrain and cellars. As a culture linked to a place, the wine made from it withstands the climate, soil, location of the area, and even the difference between vintages, the characteristics of that particular year [14]. In addition to all this, grape and winemaking provide a livelihood for countless people, as well as a supplement to earnings. All of the above are true for the world and for Romania.

The world's total vineyard area was 7.5 million ha in 2004 [15]. According to a 2017 survey, wine grapes are grown on approximately 191,000 hectares in Romania, which accounts for 6% of the wine grapes grown in EU countries [16, 17]. The largest grape-growing country in the EU is Spain (941,000 ha), followed by France (803,000 ha), Italy (610,000 ha), and Portugal (199,000 ha). Approximately 77.2 million tons of grapes are produced across the world, while approximately 828 thousand tons are produced in Romania.

In the present experiment, we compared the quantitative and qualitative parameters of Cadarcă/Kadarka, Fetească Neagră/Fekete Léányka (traditional, local varieties) and Cabernet Sauvignon, Merlot (worldwide varieties). The aim was to determine which variety is more appropriate to plant in the newly planted vineyards in terms of climate and natural conditions.

2. Materials and methods

The site of the experiment was provided by the Balla Géza winery located in Păuliş (Ópálos), 26 km from Arad, and the Miniş (Ménes) Viticulture and Winery

Research Institute. The Miniş (Ménes) wine region is located in the western part of Romania, near the city of Arad. The region is known as a good grape-growing region.

Considering the area of the wine region, we encountered a very diverse soil composition. Among the bedrocks, there could be found: granite, crystal slate, mica slate, sandstone, limestone, etc. 70% of the soils are slightly acidic (5.57–6.45 pH), while the remaining 30% are neutral, slightly alkaline (7.05–7.10 pH) [18]. Examining the humus content of the soil, it could be observed that the area has 32% optimal, 40% medium, and 28% low humus, the P content is between 111 and 155 ppm, and the K content is between 67 and 145 ppm.

Based on the metrological measurements of the Miniş (Ménes) research station, it could be mentioned that comparing the 60-year data: the average temperature is 11.2°C, the average amount of precipitation is 626.6 mm, the vegetation period of the grapes is 212.8 days on average, the number of hours of sunshine in these days is 1,955 hours, and the prevailing south and southwest wind has an average speed of 10-12 km/h. The region has an eastern sub-Mediterranean climate, so it is suitable for growing quality grapes.

Observing the topography, it could be determined that the highest area used for grape growing around Păuliş (Ópálos) is located at 150 m above sea level. However, most of the vineyards in the wine region are located at an altitude of 180–300 metres above sea level [19]. The wine region has an excellent geographical location, perfect weather and soil properties for red wines, and a suitable microclimate because of the River Mureş.

The winery has a vineyard of 120 hectares, of which 80% are blue grapes and 20% white grapes. Some of the more important grape varieties appearing in the plantation are: Fetească Neagră (Fekete Leányka), Cadarcă (Kadarka), Burgund Mare (Kékfrankos), Cabernet Sauvignon, Merlot, Cabernet Franc, Fetească Regală (Királyleányka), Mustoasă de Măderat (Mustos Fehér), Riesling, Furmint, and Sauvignon Blanc. In addition to the plantation, the winery has a fully equipped processing plant, where all the necessary equipment for red, white, and rosé wine technology can be found. The winery's storage capacity is around 10,000 hL.

The experiment started in mid-August 2017 by marking the selected vines, and then with the start of the harvest we continued with the examination of the parameters of the grapes.

During our experiment, we made measurements with these four grape varieties: Cadarcă (Kadarka), Fetească Neagră (Fekete Leányka), Cabernet Sauvignon, and Merlot. To examine vine productivity, we also examined the amount harvested from 30 vines for each variety. 3,600 kg of grapes were used to determine the amount of grape must.

3. Examination of grape parameters

Vine productivity

In order to determine vine productivity, we selected 30 vines from each variety, which were marked with a red ribbon, collected the harvest from these vines every year, measured them, and then calculated the average productivity. We removed the grape bunches from the stems with pruning shears and collected them in plastic boxes.

Yield per hectare

All trailers arriving at the winery were weighed using a bridge scale, and we calculated the average yield per hectare from the quantities received from the following vineyards:

Cadarcă/Kadarka – Hotarul Kövi (Kövi dűlő) (6.7 ha),

Fetească Neagră/Fekete Leányka – Hotarul Ghioroc (Gyoroki dűlő) (5.5 ha),

Cabernet Sauvignon – Hotarul Tei (Hársfa dűlő) (10.2 ha),

Merlot – Hotarul Groapa vulpii (Rókagödör dűlő) (4 ha).

Grape must quantity: L/10 kg grapes.

For the experiment, we selected ten, 10-kg samples from the received grape varieties in three repetitions and then separated the amount of juice from the solid part by manual pressing.

Grape must sugar content

The sugar content of the must was measured with a refractometer.

Grape must acidity

To determine the acid content, we titrated with a strong alkaline-measuring solution using a bromothymol blue indicator until the equivalence point (pH 7) was reached.

3. Results and discussions

Vine productivity of the tested grape varieties

From the data, it could be concluded that Merlot reached better results in all three years, the highest vine productivity in the case of Merlot being in the year 2019 (1.97 kg/vine). The lowest vine productivity in 2017 could be observed in the case of Cadarcă/Kadarka, where the average vine productivity was only 1.52 kg/vine (*Fig. 1*).

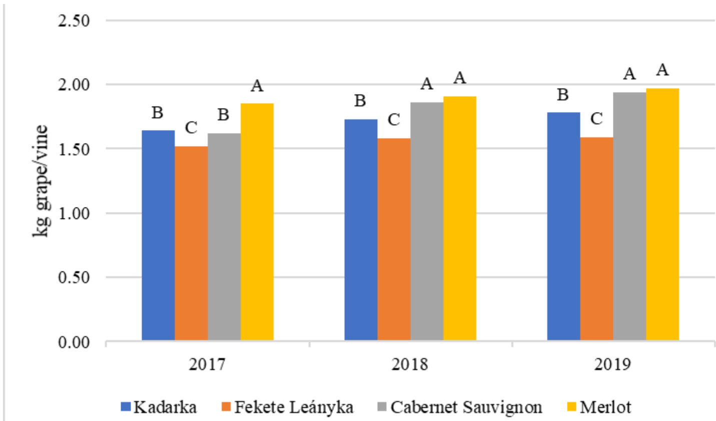

Figure 1. Vine productivity

The hectare-based yield of the examined grapes

It is important to mention that in viticulture technology yield is regulated since the quality of the grapes is prioritized, not the quantity. Under our experimental conditions, the highest yield was determined for the Merlot variety in 2019 with a value of 8.82 t/ha and the lowest for Fetească Neagră/Fekete Léányka in 2017 with a value of 6.81 t/ha (*Fig. 2*).

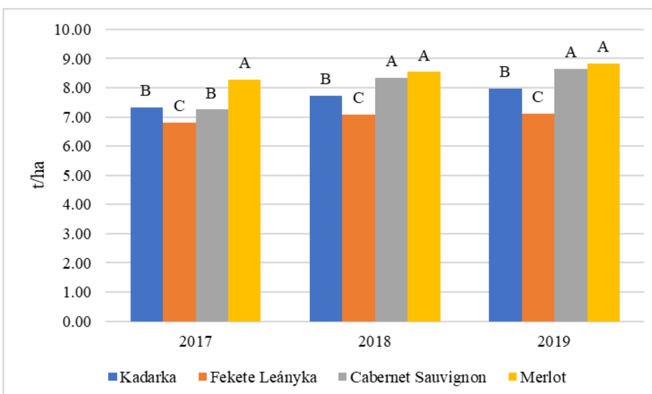

Figure 2. Hectare-based yield

The amount of must obtained from the tested blue grape varieties

In 2017, Fetească Neagră/Fekete Léányka and Merlot yielded equal results: 6.72 L/10 kg of grapes – in the case of Cadarcă/Kadarka, 6.70 L/10 kg of must was obtained from the grapes, and the lowest amount of must was extracted from Cabernet Sauvignon in 2017: 6.54 L/10 kg.

Regarding 2018, the highest amount of must was obtained for Merlot (6.94 L/10 kg of grapes) and Cadarcă/Kadarka (6.93 L/10 kg of grapes), followed by Fetească Neagră/Fekete Léányka with 6.87 L/10 kg of grapes and then by Cabernet Sauvignon with an amount of 6.67 L/10 kg of grapes.

In the last year of the experiment (2019), Cadarcă/Kadarka provided the largest amount of grape must, 7.14 L/10 kg of grapes, while Cabernet Sauvignon provided the lowest amount, of only 6.78 L/10 kg of grapes (*Fig. 3*).

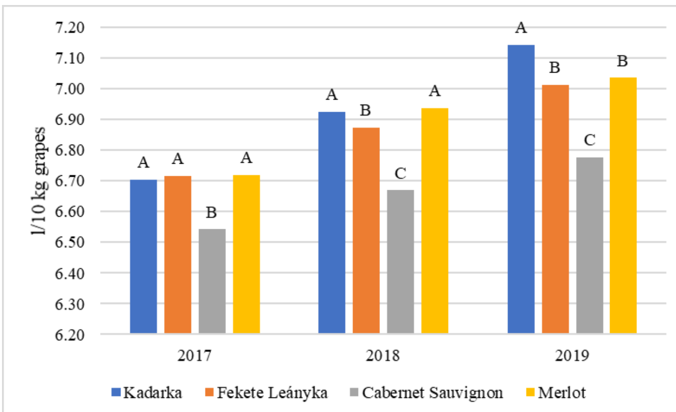

Figure 3. Must quantity

The sugar content of the grape must obtained from the blue grape varieties examined in the experiment

In 2017, Cabernet Sauvignon recorded the highest sugar content at 237.32 g/L, while Cadarcă/Kadarka the lowest at 221.68 g/L, and Fetească Neagră/Fekete Leányka and Merlot produced almost the same amount. Considering 2018, Fetească Neagră/Fekete Leányka and Cabernet Sauvignon obtained similar sugar content (Fetească Neagră/Fekete Leányka 233.92 g/L, Cabernet Sauvignon 232.90 g/L), and the must derivate from Cadarcă/Kadarka had the lowest sugar content: 218.62 g/L. Regarding 2019, the must extracted from all four grape varieties had a slightly lower sugar content compared to previous years: Fetească Neagră/Fekete Leányka 228.48 g/L, Cabernet Sauvignon 223.89 g/L, Merlot 218.45 g/L, and, finally, Cadarcă/Kadarka with 214.88 g/L (*Fig. 4*).

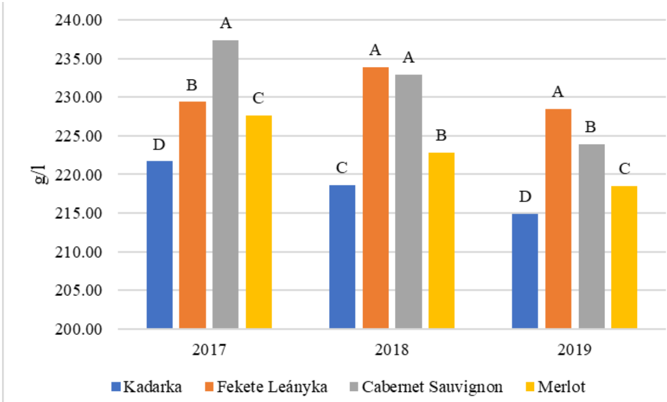

Figure 4. Must sugar content

The acidity of the must extracted from the blue grape varieties examined in the experiment

Under our experimental conditions, we have noticed that in 2017 the highest acid content was determined for Fetească Neagră/Fekete Leányka, 7.25 g/L, and the lowest one for Cadarcă/Kadarka, 6.22 g/L. Cabernet Sauvignon slightly exceeded the acidity of Cadarcă/Kadarka, the result of the measurements being 6.25 g/L, while Merlot showed a value of 6.59 g/L. In 2018, Cabernet Sauvignon produced the highest result with 7.67 g/L, Fetească Neagră/Fekete Leányka had 6.98 g/L, Cadarcă/Kadarka had 6.81 g/L, while Merlot yielded the lowest results, 6.77 g/L. Regarding 2019, the acidity content was as follows: Cabernet Sauvignon 7.37 g/L, Fetească Neagră/Fekete Leányka 7.13 g/L, Merlot 7.11 g/L, and Cadarcă/Kadarka 6.55g/L. The tests proved that the ranking was not the same every year.

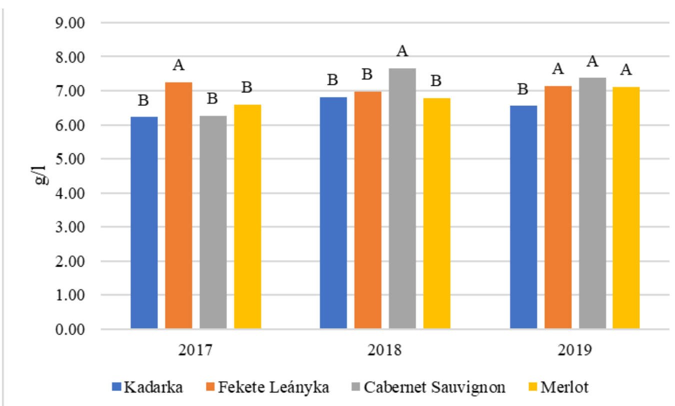

Figure 5. Must acidity

The expected alcohol content of wines made from blue grape varieties tested in the experiment

In this study, the expected alcohol content was the highest for Cabernet Sauvignon, 14.02%, and the lowest for the Cadarcă/Kadarka variety, 13.06%, in the year 2017. As for alcohol content in 2018, the highest score was recorded for the Fetească Neagră/Fekete Leányka (13.80%) and Cabernet Sauvignon (13.75%) varieties, followed by Merlot (13.14%), and the lowest alcohol content was observed for Cadarcă/Kadarka (12.91%). In 2019, our data showed that the highest alcohol content for the Fetească Neagră/Fekete Leányka variety was 13.49% and the lowest for the Cadarcă/Kadarka variety was 12.69% (*Fig. 6*).

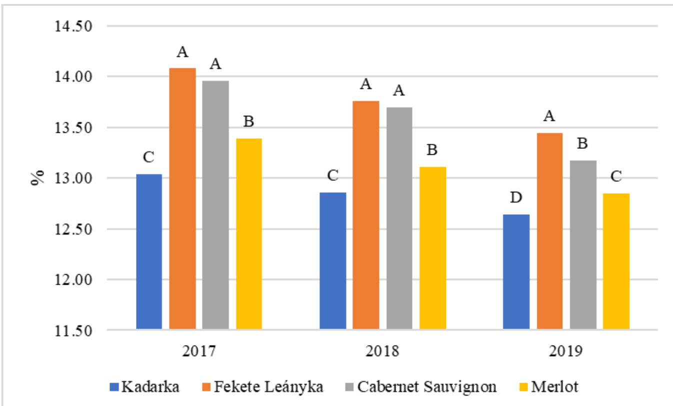

Figure 6. Expected alcohol content

4. Conclusions

From the present study, it could be concluded that in terms of average yield (t/ha), Merlot (2019, 8.82 t/ha) and Cabernet Sauvignon (2019, 8.66 t/ha), whereas in terms of vine productivity Merlot (2019, 1.97 kg/vine) and Cabernet Sauvignon (2019, 1.94 kg/stock) recorded the highest parameters. In the case of must quality parameters, Cabernet Sauvignon had the highest sugar content in 2017 at 237.32 g/L, and in terms of acidity Cabernet Sauvignon (7.64 g/L) yielded the highest result in 2018.

Based on the tests, it is clear that both worldwide-known and traditional varieties are capable of producing good-quality grapes, which is the basis of good-quality wines. In the case of planting new blue grape varieties, we could recommend opting for the traditional Cadarcă/Kadarka and Fetească Neagră/Fekete Leányka, as they are decisive in the history and uniqueness of the wine region.

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Examination of the most important red wine grape varieties of the Miniş (Ménes) wine region based on their quantitative and qualitative parameters

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Abstract: Winemaking has a very old history, which goes back thousands of years in the past. Numerous countries and geographical regions are known and acknowledged for their winemaking history. In Romania, there are countless winemaking regions that produce high-quality red and white wines. In the present experiment, we measured the quantitative and qualitative parameters of two red wines manufactured from worldwide-known varieties (Cabernet Sauvignon and Merlot) and two red wines produced from traditional grape varieties (Cadarcă/Kadarka and Fetească Neagră/Fekete Leányka). The data were collected in three different years to see if the traditional varieties could compete with the worldwide-known varieties. In terms of wine quality parameters, looking at total acidity, the Cabernet Sauvignon in 2018 while in terms of alcohol content the Fetească Neagră/Fekete Leányka in 2017 showed the highest values. From the sensory examination, it could be concluded that Fetească Neagră/Fekete Leányka yielded the best results in 2017, in 2018 the Cadarcă/Kadarka, and in 2019 the Merlot variety.

Keywords: winemaking, anthocyanin, acidity, sugar, alcohol, traditional varieties

1. Introduction

Wine is a worldwide-known beverage with a tradition of thousands of years. It had always played an important role for humanity. Numerous research studies demonstrate its beneficial effect on health due to its phenolic compound [1, 2]. Also, a beneficial property is that it can improve the human body's antioxidant defence status, and it can even lower oxidative stress [3, 4, 5].

Nowadays, due to advanced winemaking technologies and the use of specific vineyards, the quality of wine has improved significantly [6, 7, 8]. Wine quality mostly depends on its chemical composition, and with red wine technology, quality is strongly related to the amount and type of polyphenolic compounds [9]. The polyphenolic compound plays an important role in improving wine colour intensity and stability, the structure and the mouthfeel of the wine, as well as their ageing potential [1]. Furthermore, the polyphenolic compound in wine could be greatly beneficial to human health [10]. It has been demonstrated in several studies that the phenolic compound has a major effect on the human body [1], and it is proved that it helps in reducing all-cause mortality risk [11], prevents cardiovascular diseases [12], diabetes [13], and improves cognitive functions [14]. The determination of grape varieties and their composition is also an important step [15, 16].

Romania has a suitable climate for grape growing, and viticulture is a traditional occupation [17, 18]. This is the reason why Romania is one of the main wine-producing countries of the world. It has 192,000 ha of vine plantation with a production of about 5 million hL/year [19]. Besides the well-known international varieties, we can find autochthonous grape varieties in each Romanian wine region such as Fetească Neagră/Fekete Leányka or Cadarcă/Kadarka [20].

2. Materials and methods

The experiment was carried out in Păuliş (Ópálos), which is 26 km distance from Arad, at the Balla Géza Winery and at the Miniş (Ménes) Viticulture and Winery Research Institute.

The Miniş (Ménes) wine region is in the western part of Romania, near Arad, and it is known as a good wine-producing region. It has a very good geographical location with suitable soil properties and weather for grape growing and wine production, especially for red wines. This favourable microclimate is provided by the River Mureş.

In October 2017, we examined the parameters of the already matured, stable wines made from four grape varieties: Cadarcă/Kadarka, Fetească Neagră/Fekete Leányka, Cabernet Sauvignon, and Merlot. We have used 24 L of wine to determine the parameters. All four wines were made with the same red wine technology.

Determination of total acid content

To determine the acidity, we titrated the wine samples with a strong alkaline-measuring solution. We used bromothymol blue until we reached the pH level of 7.

Determination of anthocyanin content

The measurement was carried out using a spectrophotometer, examining the colour absorption capacity of anthocyanins. The colour absorption capacity is directly proportional to the anthocyanin content. For the experiment, we used a Helios alpha spectrophotometer, a glass cuvette, and a pipette. The measurements were made at a wavelength of 520 nm.

Alcohol and sugar-free extract

To determine the alcohol content and the extract content, we used the Alex 500 alcohol and extract meter. We obtained the sugar-free extract content by subtracting the amount of sugar over 1 g from the total extract content.

Sensory examination

For the sensorial examination, the following scoring system was used:
Colour and clarity: 0–15 points;
Fragrance quality and intensity: 0–20 points;
Flavour and varietal character intensity: 0–40 points;
Total impression: 0–25 points;
The maximum possible score was 100 points.
At the sensory examination, four participants were present: Géza Balla, Gergő Nyilas, Gyula Besenyei, Iuon Nicolae. Scoring was based on a 100-point system.

3. Results and discussion

The total acid content of the wines produced from red wine grapes examined in the experiment

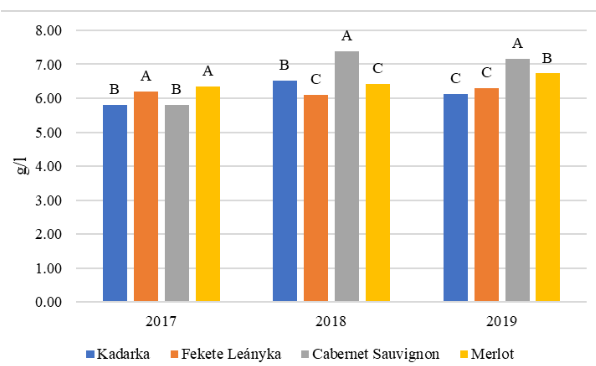

Figure 1. Total acidity content

From the titration of a sample taken from a 10,000-litre tank after malic acid decomposition for each variety, assessments were made.

We could clearly observe that in 2017 Merlot (6.34 g/L) had the highest total acid content, followed by Fetească Neagră/Fekete Leányka with 6.21 g/L total acid content and then Cadarcă/Kadarka and Cabernet Sauvignon with 5.81 g/L. In 2018, Cabernet Sauvignon showed the highest value with 7.39 g/L, while the lowest was measured in the sample from Fetească Neagră/Fekete Leányka, 6.10 g/L. In 2019, Cabernet Sauvignon had the highest total acid content of 7.16g/L, while on the other hand the lowest was measured for Cadarcă/Kadarka: only 6.12g/L (*Fig. 1*).

The anthocyanin content of the wines produced from red wine grape varieties examined in the experiment

Based on the data, it can be determined that Cabernet Sauvignon had the highest (955 mg/L) anthocyanin content in 2017 and Cadarcă/Kadarka the lowest in 2019: only 427 mg/L (*Fig. 2*).

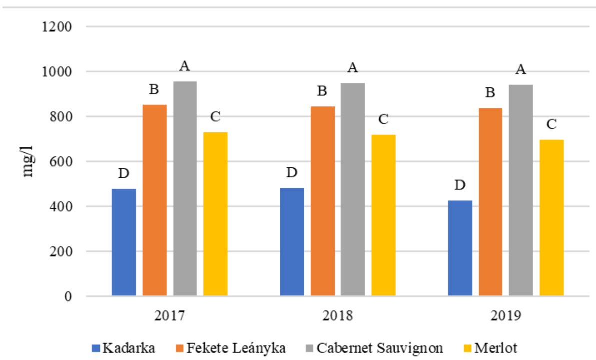

Figure 2. Anthocyanin content

The alcohol content of the wines produced from the red wine grape varieties examined in the experiment

Under our experimental conditions, we found that (*Fig. 3*) in 2017 Fetească Neagră/Fekete Leányka and Cabernet Sauvignon had similarly high alcohol content (Fetească Neagră/Fekete Leányka – 14.05%, Cabernet Sauvignon – 14.01%), Merlot a reduced alcohol content (13.33%), and Cadarcă/Kadarka approximately 13%. The 2018 wines had a slightly lower alcohol content due to less sugar; accordingly: the highest values were for Cabernet Sauvignon, 13.76%; Fetească Neagră/Fekete Leányka showed almost the same value, 13.64%, followed by Merlot with 13.10%

and Cadarcă/Kadarka with 12.71%. In 2019, the alcohol content of Fetească Neagră/Fekete Leányka was measured the highest, with 13.46%, followed by Cabernet Sauvignon with 13.22%, Merlot with 12.78%, and Cadarcă/Kadarka with 12.55%.

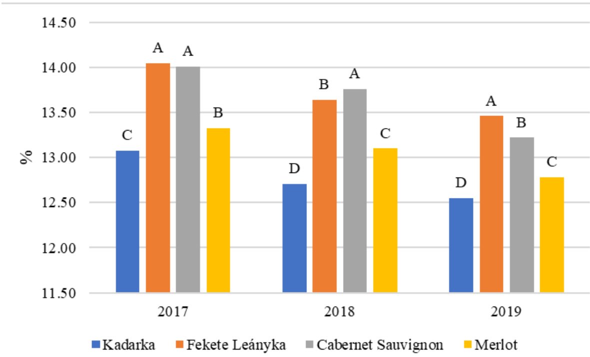

Figure 3. Alcohol content

The sugar-free extract content of the wines produced from red wine grape varieties tested in the experiment

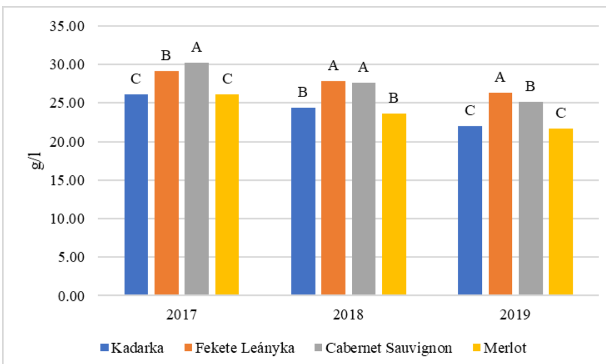

Figure 4. Sugar-free extract content

Considering the sugar-free extract content, in 2017, Cabernet Sauvignon had the highest sugar-free extract, 30.25 g/L, followed by Fetească Neagră/Fekete Leányka with 29.13 g/L, then Cadarcă/Kadarka with 26.15 g/L, and, finally, Merlot with 26.12 g/L. In 2018, the sugar-free extract values determined from Fetească Neagră/Fekete Leányka and Cabernet Sauvignon are almost similar (Fetească Neagră/Fekete Leányka: 27.82 g/L, Cabernet Sauvignon: 27.60 g/L), while Cadarcă/Kadarka and Merlot also showed lower values (Cadarcă/Kadarka: 24.42 g/L, Merlot: 23.65 g/L). In 2019, the sugar-free extract content of Fetească Neagră/Fekete Leányka was recorded the highest at 26.38 g/L, the sample from Cabernet Sauvignon was

25.14 g/L, the sample from Cadarcă/Kadarka was 22 g/L, while the sample from Merlot was 21.71 g/L (*Fig. 4*).

The average total scores of the sensory tests of the wines produced from red wine grape varieties examined in the experiment

In the sensory examination, the colour, purity, aroma quality and intensity, flavour and varietal character intensity, and the overall impression were scored (*Fig. 5*). The sensory test showed that Fetească Neagră/Fekete Leányka produced in 2017 scored the most points (90), followed by Cabernet Sauvignon and Merlot with 88 points, and Cadarcă/Kadarka the least with 87 points. For the wines produced in 2018, the participants' favourite wine was Cadarcă/Kadarka with 89 points, then Fetească Neagră/Fekete Leányka with 85 points, Merlot with 84 points, and the minimum points were reached by the Cabernet Sauvignon wine: only 82 points. In the year 2019, Merlot recorded the highest score with 89 points, followed by Cabernet Sauvignon with 88 points, Cadarcă/Kadarka with 87 points, and, finally, Fetească Neagră/Fekete Leányka wine only with 86 points.

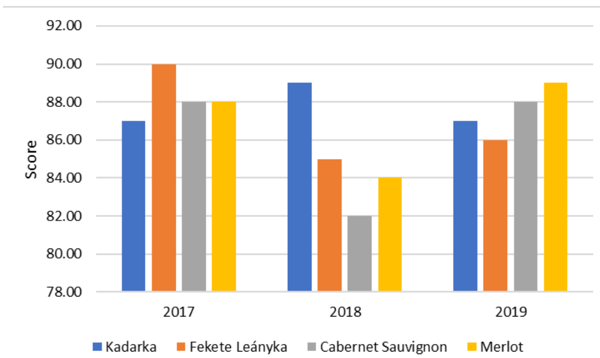

Figure 5. Sensory test score

4. Conclusions

From our data, it can be concluded that in terms of wine quality parameters, regarding the total acidity, in 2018 Cabernet Sauvignon (7.39 g/l) reached the highest content. Considering the alcohol content in 2017, Fetească Neagră/Fekete Leányka (14.05 %) showed the highest values.

During the sensory examination, Fetească Neagră/Fekete Leányka achieved the best results in 2017 (90 points), in 2018 Cadarcă/Kadarka (89 points), and in 2019 Merlot (89 points) were the most appreciated by the participants.

Based on the experiment, it can be stated that both traditional and worldwide-known varieties are suitable for making high-quality wines in the Romanian regions. Although the varieties selected for this work are not similar in different ways, the experiment presented here proves that traditionalism can and should be continued, as it is possible to create "gems" that will not be forgotten decades or even millennia later.

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Predicting the expected impact of climate change on the reproductive success of roe deer and wild boar

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Abstract: We have identified weather factors that may influence the reproductive characteristics of roe deer and wild boar, and thus the harvest rates in the future. By exploring the weather and other factors affecting reproductive success, considering the likely scenarios of climate change, we hope to predict future changes in reproductive capacity and, in the light of these, estimate the necessary changes in harvest rates to maintain reasonable numbers of animals.

Keywords: climate scenarios, wildlife reproduction, population control, corpora lutea, foetuses

1. Introduction

In Hungary, annual temperatures have risen by 1.2°C–1.8°C over the past 30 years, and the frequency of extreme droughts has increased [1, 2]. Climate change is particularly threatening the xeric limits of temperate continental forests. Predictions of expected changes in major site factors predict dramatic future droughts, and, consequently, a significant shift in forest climate classes is expected, especially at low elevations [3]. Population sizes of the big game species with the greatest impact on forest habitats have shown a significant increase in recent decades, not only in Hungary but also across Europe [4, 5]. This is mainly due to underharvest, but habitat factors that facilitate reproduction are also important in this respect. Most notably, climate warming facilitates reproductive survival. These changes inevitably have and will continue to have an impact on the reproductive capacity of native big game species and the tree species composition of forests, and through this on the future extent of forest damage [6].

The aim of this research is to identify the weather and reproductive characteristics and their interrelationships that may influence the population dynamics of roe deer and wild boar and, through this, the harvest rates in the future. By exploring the weather and other factors affecting reproductive performance, considering the likely scenarios of climate change, we hope to predict future changes in reproductive performance and, in the light of these, estimate the necessary changes in harvest rates to maintain reasonable population levels.

2. Materials and methods

The study was carried out in Zala County, Hungary (*Figure 1*). First, we analysed the population dynamics of the two big game species of Zala County, using the 28-year data set of the National Game Management Database (OVA).

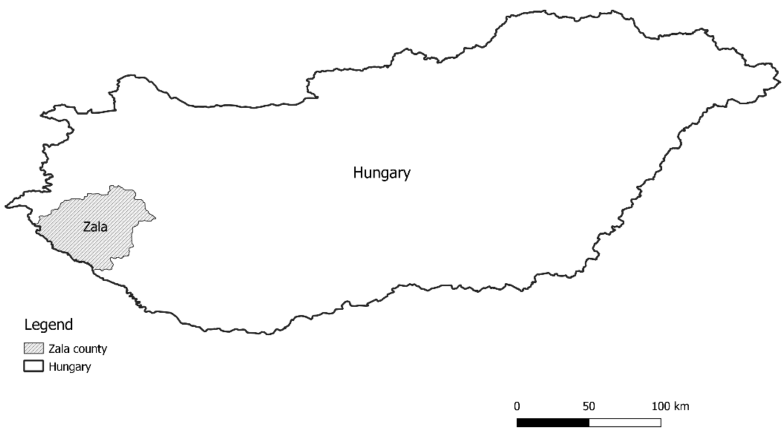


Figure 1. Location of the study area: Zala County, Hungary

Subsequently, we explored the factors influencing population dynamics using our own historical data and international literature. Our previous reproductive biology studies [7]–[9], which determined the reproductive characteristics of big game species, were extended over time and to species that had not been studied before. For this purpose, we determined the number of embryos (foetuses) of females hunted during the hunting season. After removing the reproductive organs, we examined the ovary, counted the number of corpora lutea, dissected the uterus, counted the number of embryos, and recorded their sex according to the methodology used in former wildlife reproduction studies [9–11].

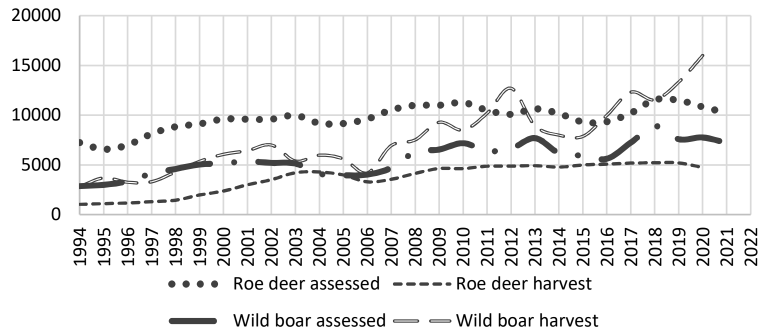
In the phase of early gestation, we counted only the number of corpora lutea, as it is not possible to determine the characteristics of the embryos accurately due to their undeveloped nature. The number of corpora lutea is a good approximation of the number of offspring that will be born later. For the big game species studied (roe deer and wild boar), maternal body condition was determined by kidney fat index analysis [12]. The age estimation was first performed based on tooth wear, followed by counting the cementum layers of the M1 tooth for a more accurate estimation of age [13–15]. We investigated whether the age and body condition of females influenced the presence, number, and sex of the foetus and then examined the variation of body condition with age.

In addition to recording individual-specific features, we continuously estimated the population's reproductive success. The number of breeding females and their respective reproductive success was counted in the observed big game groups; so, the offspring per female was continuously monitored. In the case of big game species, the results were processed to look for correlations between meteorological characteristics (amount of precipitation and monthly mean temperature) and the rate of pregnancy and the number of embryos. The effect of weather data on the survival of new-borns (fawns, piglets) was investigated. We examined the period of the year when the reproduction of each big game species was most exposed to risk of mortality. We looked for general trends in mortality throughout the year and modelled the effect of weather. We studied the relationship between weather characteristics and the reproductive success.

The differences between the number of corpora lutea and the number of foetuses were tested using Wilcoxon's test. To test the correlations and determine the strength of relationships between different sets of data, Spearman's rank correlations were calculated. Statistical analyses were carried out using STATISTICA version 13.5.0.17 (TIBCO 2022) and Microsoft Excel.

3. Results and discussions

For both big game species, estimation and shooting numbers increased steadily over the studied period with small or major peaks and troughs (*Source:* OVA [17] *Figure 2*). The increase was driven by more favourable conditions for reproductive survival, but the main reason for this phenomenon across Europe is that the densities of these species exceeded the level where the regulating effect of hunting (and, where relevant, predator) can operate [5, 9, 16]. The main cause of this relies on stagnant or declining hunting pressure [5]. Estimation and harvest data for big game species follow a similar trend, which is not possible, as increased shooting should lead to a decline in the following year's population. For this reason and because of high harvest numbers relative to estimates, population extinctions should occur in the longer term, so we concluded that the estimate numbers are highly unreliable. This is because in the absence of adequate estimation methods, hunters give their estimation numbers by guesswork ("guesstimation"), relying heavily on shooting data and/or considering their management objectives. Accordingly, during population trend analyses, only the shooting data were taken into account, and it was found that the population of both species clearly increased during our study period, with an explosive increase in the case of wild boar.



Source: OVA [17]

Figure 2. Trends in big game estimation and harvest data in Zala County, Hungary

In order to model future changes in the required level of harvest, we need to look at species-specific factors influencing population trends, such as birth and mortality characteristics, and the weather effects on these.

In the analysis of the reproductive biology of roe deer, the pregnancy rate was 95.6% for all individuals examined [9]. We examined the number of corpora lutea per doe for pregnant individuals, as this allows us to determine the reproductive rate per doe with a high degree of certainty in the early stages of pregnancy. The difference between the number of corpora lutea and the number of foetuses was not significant ($T = 42.5$; $p = 0.107$, $n = 93$), but we found a strong, positive, and significant correlation between these features ($r = 0.717$; $p < 0.05$, $n = 93$). The number of corpora lutea per doe in the studied period was 1.6 ± 0.7. The effect of the meteorological data for May–July was analysed, but the evolution of the number of corpora lutea was not influenced by the monthly mean temperature ($r = -0.43$; $p = 0.247$, $n = 9$) or by the amount of precipitation ($r = 0.159$; $p = 0.683$, $n = 9$). No effect of these weather factors could be detected for pregnancy rate (temperature: $r = -0.282$; $p > 0.05$, $n = 9$; rainfall: $r = 0.463$; $p > 0.05$, $n = 9$).

In addition to abiotic factors, the effects of body weight, body condition, and age on reproduction were analysed. We found no statistically significant difference in the number of corpora lutea per doe for age ($r = 0.15$, $p = 0.699$, $n = 9$). There was no effect of increasing body condition on the change in the proportion of non-pregnant does (r = -0.142, p > 0.056, n = 9).

Furthermore, we examined the effect of autumn and winter condition on the development of the number of corpora lutea and foetuses of the current and the following year. Only autumn body condition had significant effect on the number of foetuses in the following year. A strong positive correlation was found between dressed body mass and the number of corpora lutea (r = 0.933, p = 0.000, n = 9), where the number of corpora lutea increased with increasing body weight. When pregnant does were examined, 44% of cases had 1 foetus, 52% had 2 foetuses, and 4% had 3 foetuses.

Once the survival rate of fawns was established, we examined the factors that might have influenced it. In the case of postnatal mortality (identified based on June monitoring data), we examined temperature and precipitation conditions in May. In contrast to temperature, which showed no detectable relationship (r = -0.36, p = 0.329, n = 9), rainfall showed a positive correlation with fawn survival (r = 0.685; p = 0.04, n = 9) (*Figure 3*).

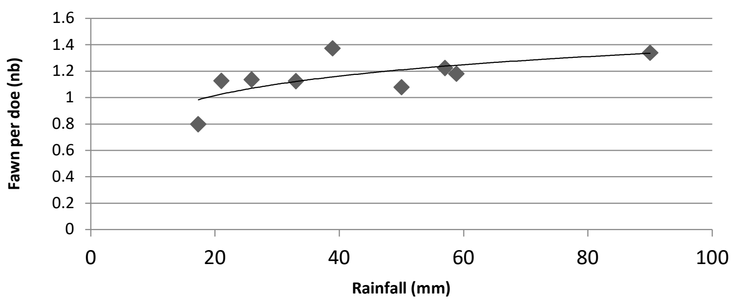


Figure 3. Evolution of fawn survival in relation with precipitation in May

For the summer survival of fawns (based on September monitoring data), we examined mean monthly temperature (r = 0.188, p = 0.627, n = 9) and precipitation (r = -0.056; p = 0.886, n = 8) for July–August but found no statistically significant relationships. For winter survival (based on April monitoring data), no statistically confirmed effect of temperature was found (r = -0.583; p = 0.129, n = 8). The intrauterine sex ratio was 1:0.8, skewed in favour of the males. The prenatal (intrauterine) mortality rate was 5.1% during the study period. Neonatal mortality was 23.3%, summer mortality was 2%, winter mortality of surviving individuals was 24%, and, overall, 49.3% of the born fawns died by the end of winter.

The birth rate in the wild boar study was 6.7 ± 2.1 (n = 51) [8]. No significant differences were found between the pregnancy rates of the study years (p > 0.05). The foetal sex ratio was 1:1.2 (♂:♀), the standard deviation of the number of female and male foetuses did not differ among study years (F = 1.6063), and the t-test showed no statistically significant difference from 1:1 ($t = -1.464$, $p > 0.05$; $n = 49$). The number of foetuses (FN) increased with age ($FN = 0.5134*age + 4.7579$; $p < 0.01$; $n = 36$), and age explained 25% of the variance in the number of foetuses ($r = 0.5$). A correlation was found between the number of foetuses and the number of corpora lutea (CLN) ($FN = 0.7374*CLN + 0.7316$; $F = 62.56$; $p = 0.000$; $n = 51$). The number of corpora lutea explained 55% of the variance in the number of foetuses ($r = 0.74$). In late gestation, the number of foetuses vs. corpora lutea ratio was 0.83 ± 0.15. No correlation was found between pregnancy rate and sows' height at withers, chest girth, or neck circumference. However, a strong correlation was found between sows' body weight (BW) and the number of foetuses ($FN = 3.7457*Ln(BW) - 10.075$; $F = 20.14$; $r = 0.5648$; $p = 0.000$; $n = 41$) (*Figure 4*).

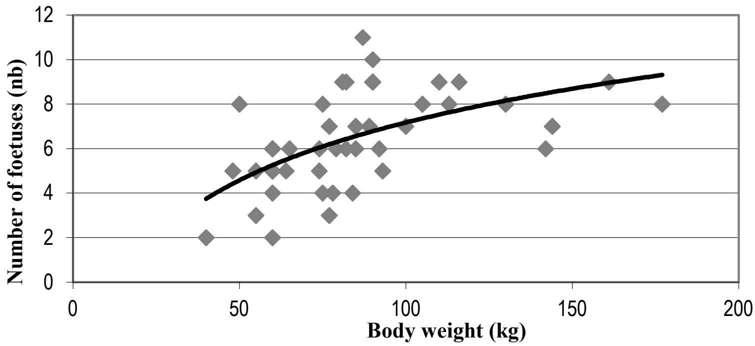


Figure 4. Changes in the number of foetuses as a function of sow's body weight

Our estimates showed relatively high mortality rates in early life stages. The estimated new-born mortality rate was 55.1% (3.75 piglets/sow) in the first year of the study and 60.9% (4.04 piglets/sow) in the second year. Summer mortality was 6.2% (0.19 piglets/sow) in the first year and 9.3% (0.24 piglets/sow) in the second year. As a consequence of mortality, the average number of piglets after early life was 3.05 ± 1.64 ($n = 124$) in the first year and 2.59 ± 1.33 ($n = 63$) in the second year, the difference not being significant ($p > 0.05$). There was no statistically proven difference ($p > 0.05$) between the recruitment up to the end of September in the two studied years (2.86 ± 1.54; $n = 83$ and 2.35 ± 1.41; $n = 63$). No statistical correlation was found for weather effects; however, based on a European survey with a larger sample size, it was clear that some extreme weather effects (long and cold winter, high snow cover, wet spring) have a strong influence on the recruitment [5].

4. Conclusions

Based on the results, we found that the future population number of the species under study will be influenced primarily by harvest rate. At the same time, changes in climatic conditions may affect the body condition of individuals and the survival rate of offspring, which will influence harvest rate. An increase in the frequency of extreme weather events in a given year may have an impact on the longer-term dynamics.

Based on our study results and literature data processing, the body condition of the wild boar and roe deer populations is expected to increase over the coming period. In the case of roe deer, this is due to an increase in average winter temperature as an influencing factor on snow cover [16]. In the case of wild boar, mean annual temperature is also an influencing factor. For this species, not only a change in body condition is predicted [18], but an increase in the survival of juveniles is even more plausible [19]. The study of the reproductive performance of the roe deer showed that neonatal mortality (currently 23.3%) will decrease with the decrease in May precipitation [9]. These changes and processes and their magnitude should be considered when planning future harvest rate.

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