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In vitro responses of two barley varieties to *Moringa* extract and growth regulators under drought stress

SAJA JAWAD SHNAISHEL BADAY[1,♥], MAYADA TARIQ AL-JUBORI[2], MAHA IBRAHIM SALIH[3]
[1]Department of Desertification Combat, College of Agricultural Engineering Sciences, University of Baghdad. Sector 755, st 4, Baghdad 10063, Iraq. Tel.: +964-77-1380-2191, ♥email: saja.jawad@coadec.uobaghdad.edu.iq
[2]Department of Horticulture and Landscape Engineering, College of Agricultural Engineering Sciences, University of Baghdad. Sector 543, st 22, Baghdad 10922, Iraq
[3]Institute of Genetic Engineering and Biotechnology, University of Baghdad. Sector 423, st 15, Baghdad 8929, Iraq

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Abstract. *Baday SJS, Al-Jubori MT, Salih MI. 2025. In vitro responses of two barley varieties to* Moringa *extract and growth regulators under drought stress. Asian J Agric 9: 358-367.* The world has recently turned to find alternatives to plant growth regulators, especially materials used in plant tissue culture, as they are the main pillar in establishing tissue cultures and participate in all stages of agriculture. Plant extracts from (seeds or leaves) are most important that can encourage growth, as they contain many active compounds that may compensate for hormones and increase the plant's resistance to drought. *Moringa* extract contains phenols, ascorbic acid, in addition to growth-promoting hormones (auxins, gibberellins and cytokinins), and abscisic acid, and the activities of the antioxidant enzymes (catalase, peroxidase, and superoxide dismutase). In the research, barley embryos were grown in the Murashige and Skoog (MS) culture media containing of *Moringa* leaf extract with (0, 1.5, 3 and 4.5 mL L^{-1}), versus kinetin (Kin) treatments at 0, 1, 2 and 3 mg L^{-1} and indole acetic acid (IAA) at 0.5 mg L^{-1} then, plantlets that achieved highest average number of shoots, total chlorophyll and dry weight were transferred to the culture media containing Poly Ethelene Glycol (PEG) at 0, 2, 4 and 6 g L^{-1} and the work was performed from a fully Complete Randomized Design (CRD), the sample size on which the analysis was conducted was 160 samples resulting from the use of two varieties of barley, 4 concentrations of *Moringa* extract, 4 concentrations of Kin, and 5 replications. The results estimated that the highest average number of the vegetative shoots formed was 16.8 shoots $plant^{-1}$, the total amount of chlorophyll was 323.4 mg g^{-1}, and the dry weight of vegetative shoots was 88.9 mg in concentration 3 of *Moringa* and Kin extract in the Buhuth244 variety. The average plant relative growth rate was 49.2 mg day^{-1}, and the average plant growth rate was 56.1 mg day^{-1} in Buhuth244, and 3 mL L^{-1} of *Moringa* extract and 4 of PEG. The examination, diagnosis, and estimation of the active compounds in the *Moringa* leaf methanolic extract was identified by Gas Chromatography with Mass Spectrometer unit (GC-MS). These extracts can serve as useful antioxidant materials. This study has concluded that *Moringa* extract can be used as an alternative to synthetic plant hormones due to its effect on improving vegetative growth characteristics.

Keywords: Drought, Kin, PEG, plant tissue culture

INTRODUCTION

Barley, or *Hordeum vulgare* L., enhances a chromosome count of (2n = 28). It has proudly belonged to the diverse Poaceae family (Attia et al. 2023). This noteworthy cereal plant displays grains that are not just staples but are packed with protein, carbohydrates, calcium, phosphorus, and vitamin B. It's cultivated in an increase of environments and stands tall as the world's fourth-largest cereal crop, following in the footsteps of wheat, rice, and corn (Geng et al. 2021). Historically, barley played an important role, decorating our tables in the form of traditional breads, hearty soups, and even healthcare products. Nowadays, its primary role has shifted where it serves mainly as animal feed and is a cornerstone of the industry of alcoholic drinks, particularly in making rich, flavorful beers (Lukinac and Jukić 2022). Yet, the ghost of climate change appears large, troubling agricultural landscapes and intensifying the pressures on farming rules.

As these urban areas of cultivation wrestle with the ongoing impacts of the change of climate, all the time, abiotic stresses such as fluctuation of light, drought, and extreme temperatures always threaten to impose limits on agricultural productivity (Ashraf and Foolad 2007). Alarmingly, the above challenges could result in a confusing reduction in crop yields, potentially slicing product by as much as 50% (Vinocur and Altman 2005).

In the intricate world of plant biology, researchers (Qin et al. 2011; Vianna and Sentelhas 2014; Voss-Fels and Snowdon 2016) have concentrated themselves in the attracting world of how plants react to drought and stress. At the microscopic level, these green wonders undergo amazing changes transformations that involved alterations in pigment levels, especially chlorophyll, as ensured by Cha-Um et al. (2012) and Banerjee and Roychoudhury (2019). Plants also searched for these challenges with noteworthy skill, making critical osmotic modifications an essential survival tactic that de Melo-Abreu and Ribeiro (2010) and Patade et al. (2011) have discussed.

Moreover, in the face of stress, they don't pause to quickly close their stomata, a fascinating response analyzed as discussed by Van Heerden et al. (2004), straight with the insights shared by de Almeida Silva et al. (2013) and Zhao et al. (2013). These insights offer just a glimpse into the intricate dance between plants and the often harsh environments they face. Yuniati et al. (2022) reported that *Moringa* extract contains important organic compounds, minerals and hormones. The results showed El-Lethy et al. (2024) that the addition of *Moringa* leaf extract significantly improved callus formation and shoot regeneration in wheat, as effect was attributed to the presence of natural cytokinin (zeatin), known for its role in stimulating cell division and regulating the balance between auxin and cytokinin in cultured tissues. Bakry et al. (2021) show that a 20% *Moringa* leaf extract are effective than 10 and 30%, it caused the highest increases in studied growth, biochemical, and parameters of yield in the two tested flax varieties.

Yap et al. (2021) proved that the increase in contents of silybin was escort by the ascending order of the chalcone synthase 1 and 3 genes, that have been involved in the silybin synthesis when add extract of *Moringa oleifera* leaf work in the enhancement of growth, yield, and content of silybin while relieving salt-induction reverse effects on the *Silybum marianum* growth. Plant growth regulators play a crucial role in plant tissue culture, as they enhance cell division and elongation, thereby improving the properties of vegetative growth (Nasrallah et al. 2015). This, in turn, affects yield, and all stages of tissue culture depend on them (Jha et al. 2020). Plant regulators are characterized by their high prices, as they are all imported and not manufactured locally. This necessitates the need to find cheaper alternatives that are locally available (Toma 2022) and to enhance the characteristics of vegetative growth and drought resistance (Baday and Hamza 2024).

The hormonal modulation for stress response hypothesis suggests the phytohormones within *Moringa* extract, particularly cytokinins, can fine-tune the plant's physiological response to water scarcity. This may involve optimizing stomatal regulation (controlling water vapor release) and delaying leaf senescence (aging), allowing the plant to sustain photosynthetic activity for longer periods under challenging conditions. In essence, *Moringa* extract is hypothesized to be a holistic biostimulant, arming plants with a powerful blend of growth promoters, vital nutrients, and stress-adaptive compounds that enable them to thrive even when faced with limited water resources. The research aims to enhance vegetative growth characteristics and increase drought tolerance by supplementing the plant hormones Kin and IAA with *Moringa* leaf extract.

MATERIALS AND METHODS

An experiment was performed in the laboratory of plant tissue culture at the College of Agricultural Engineering Sciences, Baghdad University, Baghdad, Iraq. The variables were conducted 160 samples resulting from the use of two varieties of barley, 4 concentrations of *Moringa* extract, 4 concentrations of Kin, and 5 replications.

Varieties

Seeds Buhuth244 a registered and approved variety in 2010 (Database of Agricultural Varieties and Hybrids 2022), and Iba99, a registered and approved variety in 1999 (Database of Agricultural Varieties and Hybrids 2022), were sterilized using mercuric chloride at 1 g L^{-1} for 10 minutes, as described by Sawant et al. (2021).

Preparation of media

MS media (Murashige and Skoog 1962) was used as a culture media at 121°C for 15 minutes.

Preparation for *Moringa* extract

The dried *Moringa* leaves were ground, 100 g of powder was weighed, and 1000 mL of methanol was added to it. It was left to soak for 2 hours to allow the active ingredients to be extracted. The mixture was filtered, and the filtrate was then collected and concentrated using rotary evaporator, where it was left until the volume reached 100 mL. The extract was filtered using Whatman No. 1 filter paper, the pH of nutrient medium was adjusted to be 5.8 before adding the extract and sterilizing the entire medium. The extract was sterilized by filtration through 0.22 μM membrane filters after heat sterilization of the basic medium to preserve heat-sensitive compounds. Barley embryos were planted in the media containing the methanolic extract of *Moringa* leaves at 0, 1.5, 3, and 4.5 mL L^{-1} (El-Lethy et al. 2024), and plant growth regulators Kin at 0, 1, 2 and 3 mg L^{-1} and IAA at 0.5 mg L^{-1} (Al-Jubori and Al-Amery 2022).

Measurement of chlorophyll

Then the plantlets that achieved the highest average shoots number and the total chlorophyll, which were measured according to (Dere et al. 1998) using 15 mL of acetone with a quarter gram of the paper sample, the filtrate was separated from the precipitate through filter paper, one mL of the filtrate was taken. The volume was completed to 15 mL by adding acetone. Then the reading was taken with a spectrophotometer at wavelengths of 642.5 and 660 nm based on the following equation: T.ch1 = {(7.12 *A 660) + (16.8 * A 642.5)} * {(v / w)} * 100}, and the dry weight, which was measured according to (Ikram et al. 2014) the growths were extracted from the bottles in which they were planted, placed on filter paper. The remnants of the culture medium were removed using a surgical blade.

Measurement of dry weight

The dry weight of the growths was calculated, and then the growths were dried using an electric oven at 70°C, until the weight was constant.

PEG treatment

Which was 3 mL L^{-1} of *Moringa* leaf extract, and 3 mg L^{-1} of Kin for the nutrient media containing PEG at 0, 2, 4, and 6 g L^{-1} and the cultures were incubated at of 25 ± 2°C, and at light intensity of 1000 lux for 16 hours of lighting and 8 hours of darkness (Al-Jubori and Al-Shamari 2025). Observations were taken after four weeks of cultivation, and then the stages of including rooting and acclimatization, were carried out using the plant tissue culture method (Baday 2020).

Measurement of PRGR and PGR

The physiological characteristics were measured as the average relative growth as described by Farshadfar et al. (2014), using the plant relative growth rate (PRGR) formula: PRGR = [(W2-W1)]/W1, where W1 and W2 represent the initial weight of the plant before and after four weeks, respectively. The average plant growth rate (PGR) (mm/day) of cultured embryos on MS media was measured at 7, 14, 21, and 28 days after transferring the plant to the media.

GC-MS analysis

The active compounds in *Moringa* leaf extract were separated, characterized and quantified using GC-MS before being added to the nutrient media. Preparation of the Moringa leaf methanolic extract and analysis by GC-MS were carried out according to Al-Taweel et al. (2019) and Bhalla et al. (2021). The analysis was performed using a GC-MS-TQ8050 NX gas chromatograph mass spectrometer from Shimadzu.

Data analysis

Data analysis was performed using a factorial experiment design with a Complete Randomized Design (CRD) and five replications for each treatment, concentration, and variety across all experiments. The averages were analyzed using Least Significant Difference (LSD) test at 0.05 probability level, as implemented in the Genestat 2007 program (Lamidi et al. 2024).

RESULTS AND DISCUSSION

Effect of *Moringa* extract and Kin on the number of vegetative shoots

Tables 1-2 and Figure 1 shows the two barley varieties, where Buhuth244 exhibited the highest average number of shoots of 9.9 shoot plant^{-1}, while Iba99 exhibited a lower average number of shoots of 6.5 shoot plant^{-1}. The *Moringa* leaf extract shows that 3 mL L^{-1} indicated the highest average number of shoots plant^{-1}, up to 11.9 shoots plant^{-1}, because *Moringa* extract contains many active compounds, as shown in Table 9 and Figure 4. In contrast, the comparison treatment showed a lower average number of vegetative shoots, up to 5.6 shoots plant^{-1}. The 3 mg L^{-1} of Kin was the dominant treatment because it yielded the highest number of vegetative shoots, approximately 12.3 shoots plant^{-1}, and the lowest average number of vegetative shoots in comparison to the other treatments, which was 7.4 shoots plant^{-1}. The interaction between the varieties and the concentrations of *Moringa* leaf extract was noted where Buhuth244 at the of 3 mL L^{-1} gave the highest number of vegetative shoots of 12.8 shoot plant^{-1}, but the lowest number of vegetative shoots was 3.2 shoot plant^{-1} was obtained with Iba99 at 0 mL L^{-1}. For the interaction between the varieties and Kin concentrations, Buhuth244 at 3 mg L^{-1} showed a high number of vegetative shoots at 13.5 shoots plant^{-1}, and Iba99 at 0 mg L^{-1} yielded a lower number of vegetative shoots at 5.7 shoots plant^{-1}. The interaction between *Moringa* leaf extract concentrations and Kin concentrations, the highest number of vegetative shoots was recorded at 3 mL L^{-1} of *Moringa* leaf extract and the 3 mg L^{-1} of Kin, which measured to 15.3 shoot plant^{-1}, but Moringa leaf extract and Kin obtained the lowest number of vegetative shoots, which was 3.9 shoot plant^{-1}. From the data, a note that the presence of high differences between the varieties, *Moringa* leaf extract and Kin, as Buhuth244 performed at the 3 mL L^{-1} of *Moringa* extract and the of 3 mg L^{-1} of Kin, and gave highest the number of vegetative shoots, which reached to 16.8 shoot plant^{-1}, the reason for the superiority of the *Moringa* extract concentrations in both fresh and dry weights by comparing to the comparison treatment may be due to the fact that this extract contains active substances and compounds such as vitamins and antioxidants that improve plant growth (Al-Jubori et al. 2023), while the Iba99 showed the lowest average number of vegetative shoots at the 0 mL L^{-1} of *Moringa* leaf extract and Kin, it was reached to 3.0 shoot plant^{-1} as shown in Figures 3.A and 3.B.

Table 1. The effect of *Moringa* leaves extract and Kin on the number of vegetative shoots plant^{-1}

Varieties	Kin conc.	*Moringa* extract conc.				Varieties average × Kin conc.
		0	1.5	3	4.5	
Iba99	0	3.0	4.6	8.0	7.0	5.7
	1	3.8	5.4	9.6	8.6	6.9
	2	4.4	7.6	10.0	9.8	8.0
	3	5.4	8.6	11.8	10.4	9.1
Buhuth244	0	4.8	5.8	9.6	8.9	7.3
	1	6.8	8.6	13.0	10.4	9.7
	2	7.4	9.4	14.8	13.6	11.3
	3	9.0	12.6	16.8	15.4	13.5
LSD0.05				13.8		8.9
Varieties		*Moringa* extract × Varieties				Varieties average
Iba99		3.2	5.1	9.1	8.5	6.5
Buhuth244		7.3	8.1	12.8	11.5	9.9
LSD0.05				8.9		3.1
Kin Conc.		*Moringa* extract × Kin				Kin average
0		3.9	5.8	10.3	9.7	7.4
1		5.8	7.0	11.8	10.6	8.8
2		6.9	8.0	13.4	12.2	10.1
3		7.2	11.6	15.3	14.9	12.3
LSD0.05				11.4		4.2
Average of *Moringa* extract		5.6	7.7	11.9	10.8	10.0
LSD0.05				4.2		

Effect of *Moringa* extract and Kin on the total chlorophyll content

Tables 3 and 4 indicates that there are clear differences between the two barley varieties, as the Buhuth244 achieved the highest total chlorophyll content of 255.1 mg g^{-1}, while the Iba99 achieved a lower total chlorophyll content of 181.0 mg g^{-1}. The *Moringa* leaf extract indicated that the 3 mL L^{-1} concentration was clearly high and exhibited the highest total chlorophyll amount, reaching 262.2 mg g^{-1}. At the same time, the comparison treatment recorded a lower average total chlorophyll amount, reaching 198.2 mg g^{-1}. The 3 mg L^{-1} Kin treatment was the most dominant, as it showed the highest amount of chlorophyll, at 263.8 mg g^{-1}, while the lowest amount of chlorophyll in comparison to the other treatments was 242.3 mg g^{-1}. The interaction between the varieties and *Moringa* leaf extract was significant where Buhuth244 at 3 mL L^{-1} yielded a high chlorophyll amount of 266.8 mg g^{-1}, and the lowest chlorophyll amount of 157.4 mg g^{-1} was achieved with Iba99 at 0 mL L^{-1}. While the interaction between the varieties and Kin, the Buhuth244 at the 3 mg L^{-1} showed a high chlorophyll amount of 290.9 mg g^{-1}, and the Iba99 at the 0 mg L^{-1} gave a lower chlorophyll amount of 159.3 mg g^{-1}. The interaction between *Moringa* leaf extract and Kin yielded the highest chlorophyll amount at 3 mL L^{-1} of *Moringa* leaf extract and 3 mg L^{-1} of Kin, which was 312.6 mg g^{-1}. However, the combination of *Moringa* leaf extract and Kin exhibited the lowest chlorophyll amount, at 195.2 mg g^{-1}. From the data, the presence of significant differences between the varieties, *Moringa* leaf extract and Kin, as the Buhuth244 outperformed at the 3 mL L^{-1} of *Moringa* leaf extract and the 3 mg L^{-1} of Kin, and gave highest total chlorophyll amount 323.4 mg g^{-1}, while the Iba99 recorded the lowest total chlorophyll amount at the 0 mL L^{-1} of *Moringa* leaf extract and Kin. That reached 157.2 mg g^{-1}.

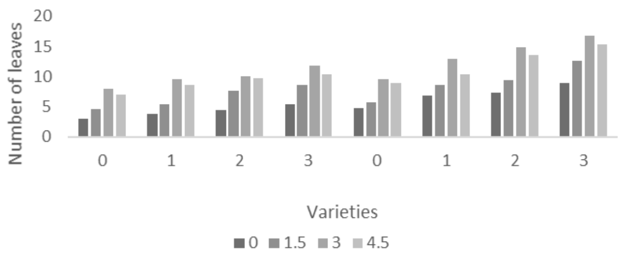

Figure 1. The effect of *Moringa* leaves extract and Kin on the number of vegetative shoots $plant^{-1}$

Table 2. Analysis of variance

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Varieties	1	612.3	612.3	806.3	<. 001
Moringa. con.	3	181.7	60.6	79.7	<. 001
Kin. con.	3	1015.2	338.4	445.6	<. 001
Varieties. *Moringa*. Con.	3	60.8	20.3	26.8	< .001
Varieties. Kin con.	3	63.9	21.3	28.1	<. 001
Moringa. con. Kin con.	9	50.9	5.7	7.5	< .001
Varieties *Moringa* con. Kin con.	9	10.7	1.2	1.6	0.134
Residual	128	97.2	0.76		
Total	159	2092.7			
Stratum standard errors and coefficients of variation					
d.f.	s.e.	cv%			
128	0.87	11.0			

Table 3. Effect of *Moringa* extract and Kin on the total chlorophyll content mg g^{-1}.

Varieties	Kin conc.	*Moringa* extract concentrations				Varieties average × Kin conc.
		0	1.5	3	4.5	
Iba99	0	157.2	159.8	161.4	158.6	159.3
	1	157.4	166.8	187.2	182.0	173.4
	2	158.2	168.4	199.4	189.2	178.8
	3	158.8	196.4	301.8	211.6	217.2
Buhuth244	0	233.2	235.8	238.6	236.4	236.0
	1	239.4	245.6	286.6	255.2	256.7
	2	241.0	262.4	298.6	286.2	272.1
	3	244.2	287.4	323.4	308.6	290.9
$LSD_{0.05}$		166.2				131.6
Varieties		*Moringa* extract × Varieties				Varieties average
Iba99		157.4	172.4	212.5	181.6	181.0
Buhuth244		235.7	256.3	266.8	261.6	255.1
$LSD_{0.05}$		131.6				56.6
Kin conc.		*Moringa* extract × Kin				Kin average
0		195.2	196.8	289.6	287.5	242.3
1		197.9	203.2	292.9	291.6	246.4
2		199.1	215.4	299	295.7	252.3
3		200.1	241.9	312.6	300.6	263.8
$LSD_{0.05}$		142.7				63.4
Average of *Moringa* extract		198.2	214.9	262.2	246.2	230.4
$LSD_{0.05}$		63.4				

Table 4. Analysis of variance

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Varieties	1	266700.0	266700.0	375900.0	<.001
Moringa. con.	3	62350.0	20780.0	29295.8	<.001
Kin. con.	3	59630.0	19880.0	28019.2	<.001
Varieties. *Moringa*. Con.	3	2494.0	831.3	1171.9	<.001
Varieties. Kin con.	3	1395.0	465.1	655.6	<.001
Moringa. con. Kin con.	9	35260.0	3917.0	5522.2	<.001
Varieties *Moringa* con. Kin con.	9	12520.0	1391.0	1960.9	<.001
Residual	128	90.8	0.71		
Total	159	440400.0			
Stratum standard errors and coefficients of variation					
d.f.	s.e.	cv%			
128	0.84	8.2			

Effect of *Moringa* extract and Kin on the dry weight of shoots

Tables 5 and 6 indicates the barley, as the Buhuth244 achieved the highest average dry weight of shoots, reaching 74.4 mg, while the Iba99 achieved a lower average dry weight of shoots, reaching 53.5 mg. *Moringa* leaf extract showed that the 3 mL L^{-1} was significant and recorded the highest dry weight of shoots, 70.0 mg, while the comparison treatment recorded the lowest dry weight of shoots 58.7 mg. Using Kin at 3 mg L^{-1} predominated, because it gave the highest dry weight of shoots, compared to 73.1 mg, and recorded the least normal dry weight of shoots within the comparison treatment which was 59.2 mg. The interaction between the varieties and *Moringa* leaf extract was critical for Buhuth244, at a concentration of 3 mL L^{-1}, the highest dry weight of the shoots was 79.8 mg, while the lowest dry weight of the shoots was 46.3 mg, which was recorded with Iba99 at the same concentration. The interaction between the varieties and Kin, the Buhuth244 as observed at the 3 mg L^{-1} exhibited the highest dry weight of the shoots at 80.4 mg, while the Iba99 at the 0 mg L^{-1} of Kin gave the lowest dry weight of the shoots at 47.2 mg. At the same time, the interaction between *Moringa* leaf extract and Kin, the highest dry weight of shoots was achieved at 3 mL L^{-1} of *Moringa* leaf extract and 3 mg L^{-1} of Kin, which was 89.7 mg, while at 0 mL L^{-1} of *Moringa* extract and Kin, the lowest dry weight of shoots was 58.1 mg. The data, revealed that there are significant differences between the varieties and *Moringa* leaf extract and Kin, where Buhuth244 exceed at 3 mL L^{-1} of *Moringa* leaf extract and 3 mg L^{-1} of Kin, and gave the highest dry weight of shoots, which was 88.9 mg, while the Iba99 recorded at a concentration of 0 mL L^{-1} of *Moringa* leaf extract and Kin, lowest dry weight of shoots, which was 45.8 mg.

Effect of PEG in plantlets that achieved the highest number of shoots, total chlorophyll and dry weight treated with *Moringa* extract and Kin on plant relative growth rate

Table 7 shows significant differences between the two grain varieties, where Buhuth244 exceeded at 3 mL L^{-1} of *Moringa* extract and was dominated with plant relative growth rate and gave of 40.2 mg day^{-1} compared to Iba99, at 3 mg L^{-1} of Kin, which gave a lower plant relative growth rate of 32.8 mg day^{-1} (Baday 2020). The effect of PEG on the relative plant growth rate is presented in Table 7, which reveals significant differences caused by PEG. The highest plant relative growth rate of 43.1 mg day^{-1} was achieved at 43.1 mg day^{-1} was achieved at a PEG concentration of 1 to 4 g L^{-1}. In comparison plant relative growth rate lowered to 26.6 mg day^{-1} when the PEG increased to 6 g L^{-1} this is because of the decreasing of plant relative growth rate may be attributed to the lowering in the efficiency of photosynthesis due to drought due to the addition of PEG, that causes lowering in photosynthesis necessary for carbohydrates and proteins building (Khierallah and Jawad 2017; Yavas et al. 2024). Additionally, Table 7 shows the interaction between the varieties and PEG refers to significant differences where the lowest plant relative growth rate was achieved in Iba99 at 3 mg L^{-1} of Kin, was 24.4 mg day^{-1} at 6 g L^{-1} of PEG, but the highest plant relative growth rate was appeared with Buhuth244 at 3 mL L^{-1} of *Moringa* extract was 49.2 mg day^{-1} at 4 g L^{-1} of PEG, which may be attributed to the nature of these cells to the stress surrounding the living body, and the use of plasticity process that DNA has in the cells of plant to form genetic structures that are more resistant to stress conditions (Zhao 2020) as shown in Figures 3.C and 3.D.

Effect of PEG on plantlets that achieved the highest number of shoots, total chlorophyll and dry weight treated with *Moringa* extract and Kin on plant growth rate

Table 8 and Figure 2 shows significant differences between the barley, as the Buhuth244 with a 3 mL L^{-1} of *Moringa* extract was significantly superior in plant growth rate and yielded of 46.6 mg day^{-1} compared to the Iba99 with a 3 mg L^{-1} of Kin, which gave a lower plant growth rate of 36.3 mg day^{-1}, this may be attributed to the adaptive nature of these cells to the stress conditions they are exposed to outside the living body, and the exploitation of the plasticity process that DNA possesses in plant cells to create genetic structures that are more tolerant to stress conditions. As for the effect of PEG on plant growth rate, the results showed significant differences between PEG treatments, with the highest plant growth rate of 47.9 mg day^{-1} achieved at a PEG concentration of 1 to 4 g L^{-1}. In contrast, the plant growth rate decreased to 30.7 mg day^{-1} when the PEG concentration was increased to 6 g L^{-1}. That the interaction between the varieties and PEG indicates that the lowest plant growth rate was recorded in Iba99 at a 3 mg L^{-1} concentration of Kin, reaching 26.4 mg day^{-1} at a 6 g L^{-1} concentration of PEG (Hadi et al. 2019). The decrease in the plant growth rate at high of PEG cell division and cytoplasmic volume decrease, loss of cell turgor, and nutritional imbalance due to a decrease in water and nutrient absorption, an increase in electrical potential, and a decrease in cellular water content with increasing stress, or perhaps the increase in stress causes physical damage to the carbon metabolism mechanism as a result of exposure to continuous stress, or perhaps it is attributed to the fact that the cytoplasmic content of the plant cell becomes denser (Ikram et al. 2014), and its volume decreases when the cell is deficient in water, the decrease in dry weight may be attributed to the decreased efficiency of photosynthesis due to drought stress resulting from the addition of PEG, which causes a decrease in photosynthetic products necessary for the construction of carbohydrates and proteins (Hamza and Ali 2017; Hu et al. 2023), while the highest plant growth rate was achieved in the Buhuth244 at 3 mL L^{-1} of *Moringa* extract, reaching 56.1 mg day^{-1} at 4 g L^{-1} PEG, this result reflects the effectiveness of Moringa leaf extract in improving the tolerance of varieties to drought stress as shown in Figures 3.E and 3.F. The decrease in the average fresh weight of calli at high concentrations of mannitol may be attributed to decreased cell division, reduced cytoplasmic volume, loss of cell turgor, and nutritional imbalance due to decreased water absorption and nutrients, an increase in electrical potential, and a decrease in the cellular water content with increasing stress, or perhaps the increase in stress causes physical damage to the carbon metabolism mechanism as a result of exposure to continuous stress, or perhaps it is.

Table 5. Effect of *Moringa* extract and Kin on the dry weight of shoots mg

Varieties	Kin conc.	***Moringa* extract concentrations**				Varieties average × Kin conc.
		0	**1.5**	**3**	**4.5**	
Iba99	0	45.8	46.3	49.3	47.5	47.2
	1	46.4	50.3	56.8	54.3	52
	2	46.8	50.4	57.9	55.5	52.7
	3	47.2	52.4	60.6	59.4	54.9
Buhuth244	0	70.3	70.7	72	71.4	71.1
	1	70.8	70.9	84.5	72.6	74.7
	2	71.3	72.8	86.9	76.2	76.8
	3	72.5	77.7	88.9	82.4	80.4
$LSD_{0.05}$		43.2				33.4
Varieties		*Moringa* extract × Varieties				Varieties average
Iba99		46.3	49.8	63.6	54.2	53.5
Buhuth244		70.6	73	79.8	74.1	74.4
$LSD_{0.05}$		33.4				20.3
Kin conc.		*Moringa* extract × Kin				Kin average
0		58.1	58.6	60.6	59.4	59.2
1		58.4	60.6	66.6	63.4	62.3
2		58.6	61.6	75.9	73.8	67.5
3		58.8	65.1	89.7	78.9	73.1
$LSD_{0.05}$		36.2				24.5
Average of *Moringa* extract		58.7	61.4	70.9	65.93	
$LSD_{0.05}$		24.5				

Table 6. Analysis of variance

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Varieties	1	20983.3	20983.3	34837.9	<.001
Moringa. con.	3	4136.3	1378.8	2289.1	<.001
Kin. con.	3	4431.8	1477.3	2452.6	<.001
Varieties. *Moringa*. Con.	3	37.8	12.6	20.9	<.001
Varieties. Kin con.	3	730.2	243.4	404.1	<.001
Moringa. con. Kin con.	9	3122.7	346.9	576.1	<.001
Varieties *Moringa* con. Kin con.	9	1845.6	205.1	340.5	<.001
Residual	128	77.1	0.6		
Total	159	35364.8			
Stratum standard errors and coefficients of variation					
d.f.	s.e.	cv%			
128	0.78	1.2			

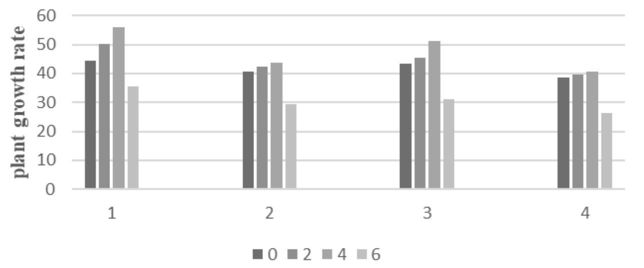

Figure 2. Effect of PEG in plantlets that achieved the highest number of shoots, total chlorophyll and dry weight treated with *Moringa* extract and Kin on plant growth rate mg day^{-1}

Table 7. Effect of PEG in plantlets that achieved the highest number of shoots, total chlorophyll and dry weight treated with *Moringa* extract and Kin on plant relative growth rate mg day^{-1}

Varieties PEG Conc.	1	2	3	4	Average
0.0	39.5	36.2	37.4	33.7	36.7
2.0	42.3	38.2	40.6	35.6	39.2
4.0	49.2	39.5	46.3	37.5	43.1
6.0	29.9	25.9	26.2	24.4	26.6
LSD0.05		25.3			17.1
Average	40.2	35.0	37.6	32.8	
LSD0.05		8.5			
Stratum standard errors and coefficients of variation					
d.f.		s.e.	cv%		
64		1.04	13.2		

Note: 1. Buhuth244 treated 3 mL L^{-1} of *Moringa* extract, 2. Buhuth244 treated 3 mg L^{-1} of Kin, 3. Iba99 treated 3 mL L^{-1} of *Moringa* extract, 4. Iba99 treated 3 mg L^{-1} of Kin

Table 8. Effect of PEG in plantlets that achieved the highest number of shoots, total chlorophyll and dry weight treated with *Moringa* extract and Kin on plant growth rate mg day^{-1}

Varieties PEG Conc.	1	2	3	4	Average
0.0	44.4	40.5	43.4	38.7	41.8
2.0	50.2	42.3	45.6	39.6	44.4
4.0	56.1	43.6	51.3	40.5	47.9
6.0	35.7	29.3	31.2	26.4	30.7
LSD0.05		30.2			17.8
Average	46.6	38.9	42.9	36.3	
LSD0.05		11.3			
Stratum standard errors and coefficients of variation					
d.f.		s.e.	cv%		
64		0.94	12.1		

Note: 1. Buhuth244 treated 3 mL L^{-1} of *Moringa* extract, 2. Buhuth244 treated 3 mg L^{-1} of Kin, 3. Iba99 treated 3 mL L^{-1} of *Moringa* extract, 4. Iba99 treated 3 mg L^{-1} of Kin

Moringa oleifera extract is a valuable natural extract used to enhance the vegetative growth of plants. Its composition is rich in many bioactive materials. These materials enhance physiological processes that lead to useful growth of leaves, stems and the vegetative parts. *Moringa* extract contains natural plant hormones, such as cytokinins, which stimulate cell division and differentiation, leading to an increase in leaf size. They also contribute to delaying the aging of leaves, thereby preserving their photosynthetic activity. *Moringa oleifera* extract contains auxins, which play a vital role in cell elongation for the growth of stems and roots. *Moringa oleifera* extract can serve to increase the plant's total biomass. Gibberellins can elongate stems and expand the area of plant leaves, so increasing the photosynthesis efficiency. *Moringa* extract contains two powerful antioxidants like carotenoids and flavonoids. These compounds help the plant resist environmental stresses (such as drought, heat, and salinity) by reducing the damaging effects of free radicals. When a plant is less stressed, it can direct more of its energy toward vegetative growth. This means the plant becomes more efficient at converting light energy into chemical energy, which supports stronger and faster vegetative growth.

Moringa leaf extracts promote the accumulation of compatible solutes, or osmolytes, such as proline, soluble sugars, and glycine betaine. These osmolytes help maintain cell turgor and water potential under osmotic stress, preventing water loss and protecting cellular structures, particularly membranes and proteins. This allows the plant to continue essential metabolic processes despite reduced water availability.

Moringa leaf extracts are rich in natural plant growth regulators, particularly cytokinins (e.g., zeatin), auxins, gibberellins, and salicylic acid. Under drought, the balance of these hormones is disrupted. *Moringa* leaf extracts application can re-establish a favorable hormonal balance, promoting root growth (for better water uptake), delaying senescence, enhancing photosynthetic efficiency, and improving stomatal regulation. For instance, increased cytokinin levels can delay leaf senescence and maintain photosynthetic activity under stress.

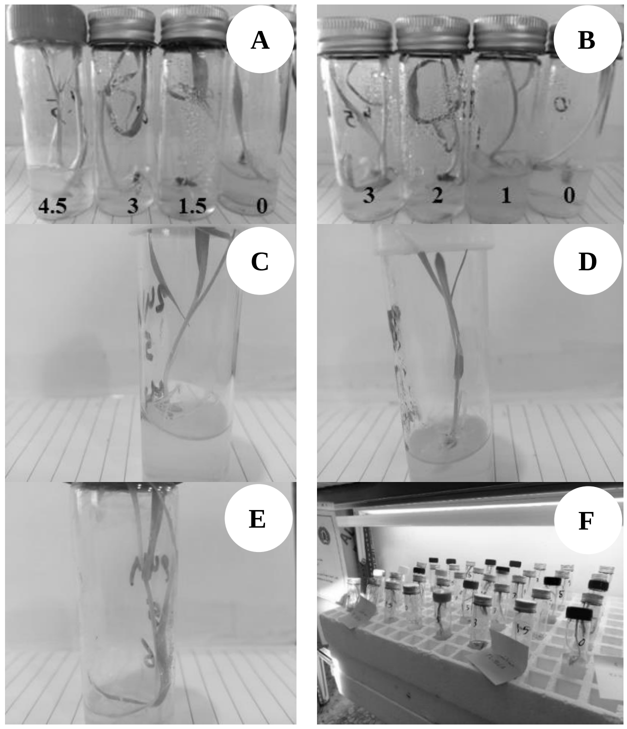

Figure 3. A. Buhuth244 is treated with *Moringa* leaf extract at concentrations of 0, 1.5, 3, and 4.5 mL L^{-1}, from right to left, as shown in. B. Buhuth244 is treated with Kin at 0, 1, 2, and 3 mg L^{-1} from right to left, as shown in. C. Buhuth244 resulting from a 4.5 mg L^{-1} of Kin treated with 4 g L^{-1} PEG. D, E. Buhuth244 and Iba99 respectively resulting from 3 mL L^{-1} of *Moringa* extract treated with 4 g L^{-1} of PEG, as shown in F. Tissue culture

Enhance water use efficiency by improving osmoregulation and root development, *Moringa* leaf extracts allow plants to extract and utilize limited water resources more effectively. This reduces the water footprint of crops, crucial in water-stressed ecosystems. Boost nutrient cycling while direct studies on *Moringa* leaf extracts long-term impact on soil microbial communities are ongoing. Its organic nature and nutrient content suggest it could contribute to healthier soil microbiomes, fostering natural nutrient cycling and reducing the need for synthetic fertilizers that can lead to runoff and water pollution.

Increasing biomass production improved growth under stress means higher biomass accumulation. This can lead to greater carbon sequestration in agricultural lands and potentially richer organic matter in the soil, benefiting overall ecosystem health. Support biodiversity (indirectly) by enhancing crop resilience and yield, Moringa leaf extracts can reduce pressure to expand agriculture into pristine natural habitats, thereby helping conserve biodiversity. Furthermore, as a natural product, it avoids the negative impacts of chemical pesticides and fertilizers on non-target organisms. Numerous studies worldwide, across diverse crops (e.g., wheat, maize, mung bean, chili pepper, lettuce, tomato), have consistently reported improved germination, seedling vigor, plant height, leaf area, chlorophyll content, biomass, and ultimately, yield under both normal and abiotic stress (drought, salinity, heat) conditions. This global consistency strengthens the validity of the hypotheses regarding phytohormone, nutrient, and antioxidant roles.

GC-MS analysis

Retention Time (RT) and compound identification

The RT values span a range from 1.80 to 49.57. This wide range suggests the presence of compounds with varying volatilities and interactions with the chromatographic column. Compounds with lower RT (e.g., 1.80 for 1,3-Dihydroxy acetone (dimer)) elute earlier, indicating they are more volatile or have less affinity for the column. Compounds with higher RT (e.g., 49.57 for Ethene, ethoxy-) elute later, suggesting they are less volatile or have a stronger affinity for the column. The ID column provides the chemical identity of each peak, which is essential for understanding the sample's composition.

Peak area % and relative abundance

The peak area % values indicate the relative amount of each compound in the sample. A higher Peak Area % suggests a relatively higher concentration of that compound. For instance, the compound with the highest peak area % is Pyran-4-one-2,3-dihydro-3,5-dihydroxy-6-methyl, with 8.56%. Compounds with low peak area % are present in smaller amounts.

Molecular weight

The molecular weight values significantly increase, ranging from 72 g/mol (Ethene, ethoxy-) to 343 g/mol (Tetra acetyl-d-xylonic nitrile). This variation indicates the presence of compounds with different sizes and complexities. There's no immediately obvious correlation between molecular weight and RT, although some general trends might emerge with further analysis in specific chemical contexts.

Observations and potential insights

Diverse compound mixture: The table shows a complex mixture of various organic compounds, including acids, amines, esters, cyclic compounds, and sugars.

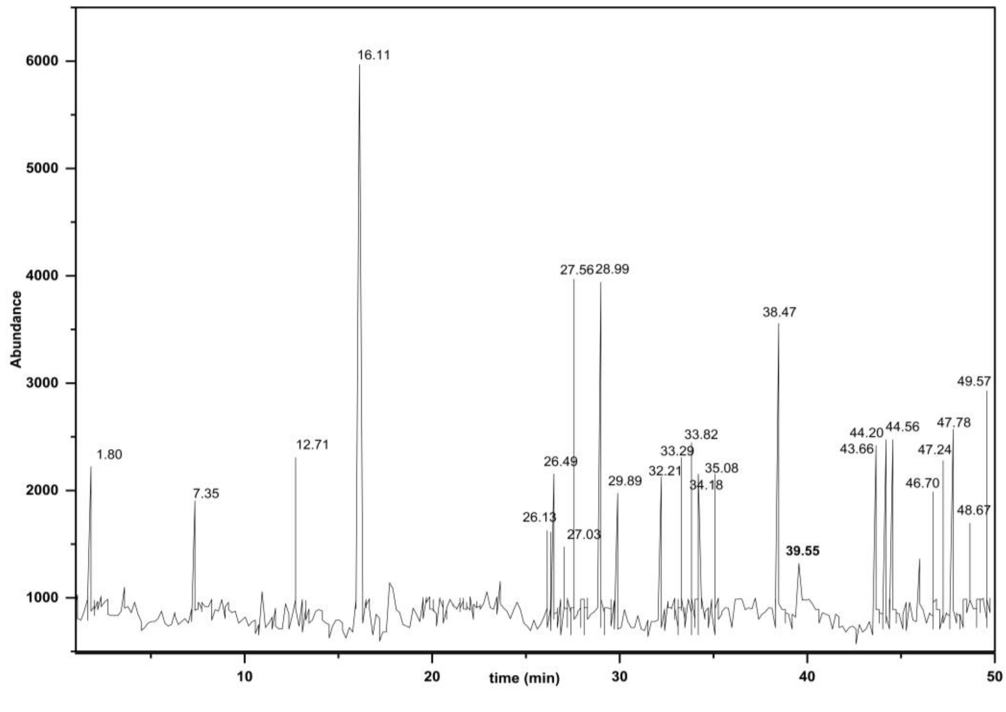

Figure 4. GC-MS of Moringa extract

Table 9. GC-MS chromatogram of compounds characterized in the *Moringa* leaves extract using methanolic extraction

Sr. No.	RT	Peak area %	ID	Molecular weight (g/mol)
1	1.80	3.55	1,3-Dihydroxy acetone (dimer)	180
2	7.35	3.25	Acetic acid (dimer)	120
3	12.71	2.24	2,6-Diamino pyrimidinone	126
4	16.11	8.56	2,3-Dihydro-3,5-dihydroxy-6-methyl 4-pyranone	144
5	26.13	1.12	Hexa-2-yne acid	112
6	26.49	2.14	(S)-2-Hydroxy-2-methyl-butane dioic acid	148
7	27.03	0.92	3,30 -Imino bispropyl amine	131
8	27.56	1.07	1-Amine hexane	101
9	28.99	5.16	4,5-Dimethyl-1,3-Dioxolan-2-one	116
10	29.89	1.30	2-Butenethioic acid, 3-(ethyl thio)-, S-(1-methyl ethyl) ester	204
11	32.21	2.02	Propane amide-N,N-dimethyl-	101
12	33.29	1.07	2-Isopropoxyethyl propionate	160
13	33.82	1.06	D-Mannoheptulose	210
14	34.18	1.02	Azetidin-2-one 3,3-dimethyl-4-(1-aminoethyl)	142
15	35.08	1.04	Carbonic acid, butyl 2-pentyl ester	188
16	38.47	4.21	Tetra acetyl-d-xylonic nitrile	343
17	39.55	3.44	Alpha-D-Glucose	180
18	43.66	1.25	1H-Cyclopenta[c]furan-3(3aH)-one,6,6a-dihydro-1-(1,3-dioxolan-2-yl)-, (3aR,1-trans,6a-cis)-	196
19	44.20	1.52	3-[1-(4-Cyano-1,2,3,4-tetrahydro naphthyl)] propane nitrile	210
20	44.56	1.40	Quinolinium, 1-ethyl-, iodide	285
21	46.70	1.04	N-Isopropyl-3-phenyl propane amide	191
22	47.24	1.03	Propane amide	73
23	47.78	1.28	1,2-Ethane diamine, N-(2-amino ethyl)-	103
24	48.67	1.01	1,4-Benzene diol, 2-methyl-	124
25	49.57	1.03	Ethene, ethoxy-	72

In conclusion, the results of this study demonstrated that the use of *Moringa* leaf extract in barley culture media resulted in a significant improvement in shoot and root multiplication rates compared to synthetic growth regulators. This effect is attributed to the extract's high content of natural cytokinins, particularly zeatin, as well as the presence of phenolic compounds and antioxidants in the extract. These components not only improve hormonal balance in plant tissue cells but also play a crucial role in mitigating oxidative stress caused by laboratory culture conditions. This dual action of *Moringa* leaf extract provides reassurance about its potential benefits. These results are consistent with numerous scientific studies that have confirmed the effectiveness of natural extracts, particularly *Moringa*, as growth promoters and alternatives to synthetic growth regulators in plant tissue culture of cereal crops. Based on the above, this study provides scientific support for expanding the use of *Moringa* leaf extract in micropropagation programs and breeding crops resistant to harsh environmental conditions. It recommends conducting future studies on different crops in more detail to analyze the active components of *Moringa* leaf extract and evaluate their molecular effects on cell differentiation pathways as well as the expression associated with plant organ formation. Developing tailored biostimulant formulations derived from plant extracts (e.g., seaweed, *Moringa*, watermelon rind hydrogels), These formulations can be optimized for specific barley growth stages and drought scenarios, offering targeted and sustainable plant manipulation without synthetic inputs.

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