Acclimatization of tissue-grown Grand Nain banana seedlings using Zeolite rocks

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Abstract:

Bananas are the fourth most economically valuable fruit crop worldwide. As bananas lack economically viable seeds, they are mostly propagated using vegetative means and tissue culture techniques. Tissue-cultured plantlets experience significant difficulties during the acclimatization stage, limiting their establishment rate. The purpose of this study is to enhance the acclimatization of Grand Nain tissue cultured plantlets by determining the most effective growing medium. Seven growing media were examined in the study: 100% zeolite; zeolite, peat moss (2:1); zeolite, peat moss (1:2); zeolite, peat moss, sand (1:1:1); zeolite, peat moss, sand (2:1:1); peat moss, sand (1:2); and zeolite, peat moss, sand (1:2:1). It was found that zeolite, peat moss (2:1) medium was the most effective in supporting vegetative growth, providing maximum shoot length (23.66 cm) during the final acclimatization stage. At the same time, 100% zeolite proved to be the least effective for seedling growth. The maximal leaf number (6.66) was observed on zeolite, peat moss, sand (1:2:1) medium. From the results, it appeared that addition of peat moss into growing medium increased water retention capacity and promoted plant growth and acclimatization. Moreover, the chemical composition of growing medium considerably affected the endogenous hormones levels in leaves. In conclusion, the experiment has shown that the mixture of peat moss and zeolite is ideal for growing tissue-cultured Grand Nain banana plantlets.

Keywords: Micro propagation, Peat moss, *Musa acuminata*, Zeolite, Cytokinin, Auxin

Introduction

Bananas are a crucial global crop, ranking fourth in worldwide and a key herbaceous fruit crop economically. The banana is the monocot herbaceous plants belonging to the genus Musa (2). Identifying the varieties is difficult because the majority stem from or were crossbred with two wild species, *Musa acuminata* and *Musa balbisiana*. The type (Grand Nain) is one of the important types that may be called the Cavendish banana or the big dwarf (23). Bananas are propagated by vegetative propagation because they do not produce seeds. Plant tissue culture is among the most efficient techniques of plant propagation due to production of a lot of

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employed to fight off fungal infections (16). It has been found in previous researches that a significant number of tissue cultured banana plantlets die during the process of acclimatization, whereas those plantlets which survive are characterized by weak growth and poor establishment (13). Tissue-cultured banana plantlets are relatively fragile at an early age and are very sensitive to the changes in their environmental conditions (3). Once these plantlets are transferred to the nursery, they face a lot of difficulties such as poor rooting (6), increased susceptibility to microbes, and maintaining hormonal balance (10). Thus, tissue-cultured banana plantlets are highly susceptible to environmental stresses during the acclimatization period. Since they face more fluctuation in terms of temperature, humidity, and light intensity as compared to the protected in vitro condition, so if acclimatization is not done properly then plantlets lose water immediately, wilt, and eventually die. Thus, the medium selected for acclimatization is vital, significantly impacting cost reduction and success enhancement (20).

The present study focused on the significance of using an appropriate growth medium for the acclimatization process after tissue culture of banana plantlets, which will aid them in adapting to the external environment prior to transplanting to the nursery or field. This is because it is important for the healthy physiological growth and survival of the plantlets. The purpose of this study is to determine the most appropriate growth medium for the acclimatization of tissue culture banana plantlets.

Materials and methods

Growth condition

The experiment took place during the summer of 2016 in the Tissue Culture Laboratory of the Basra Agriculture, Directorate The experiment was conducted according to a Completely Randomized Design (CRD), with each treatment replicated three times and the seedlings that have the same genetic material like that of the parent plant (4). Plant tissue culture is the cultivation and multiplication of plant cells, tissues, and organs using specialized growth media under sterile, controlled conditions. To ensure the success of micropropagation or tissue culture, it's essential to maximize plant survival during transport and keep costs low (15). Acclimatization, or hardening, is another very important phase in plant tissue culture technology. This is the process through which the plantlets that have been cultured in vitro undergo adaptation to environmental conditions after being moved out of the culture (19). There are a number of physiological and anatomical changes that take place during this phase to ensure the survival and growth of the plantlets in greenhouse or open field environment. Nonetheless, acclimatization takes time and is an expensive phase, which contributes significantly to the cost of tissue culture production (11). In order to increase the viability and successful adaptation of banana tissue cultured plantlets, especially at the stage of acclimatization, it is vital to ensure proper conditions for growing that will allow them to adapt to the external environment. At the acclimatization stage, banana plantlets are moved from a sterile in vitro environment, which provides nutrients, water, and favorable growth conditions to greenhouse or field conditions, where they need to perform photosynthesis and independently take up water and nutrients from the soil by means of roots (18; 8). For this experiment, seven types of growing media have been selected to analyze the effect of growing media on the growth and acclimatization of banana plantlets. One of the components of the growing media was zeolite, which is a hydrated alumino-silicate mineral with high porosity (21; 14). In agriculture, it is used to stabilize soil ammonia and boost its nitrogen content. It functions as a fertilizer, effectively managing soil moisture by absorbing and releasing water, which helps maintain humidity. Additionally, this substance is

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3. Zeolite-peatmoss in a ratio of 1:2

4. Zeolite-peatmoss-sand in a ratio of 1:1:1

5. Zeolite-peatmoss-sand in a ratio of 2:1:1

6. Peatmoss-sand in a ratio of 1:2

7. Zeolite-peatmoss-sand in a ratio of 1:2:1

The mixture was sterilized by autoclaving at a temperature of 121° for 20 minutes

Plant transfer and measurements

For acclimatization, plants grown in solid media were transferred after removing the suspended media, rinsing them with sterile distilled water, and soaking them in a fungicide for 15 minutes. The vegetative and root group fleet and the number of leaves on each plant were then transferred to the agricultural medium prepared for acclimatization as mentioned previously, with three replicates for each treatment. They were watered with distilled water containing half the strength (MS), i.e. 2.2 g/liter, and the plant was monitored and the data was read (the length of the vegetative group and the number of leaves) every two weeks. The internal acclimatization of the plant was carried out inside a special cabin where the temperature was 28°C and the lighting was a long day with 14 hours of light and 10 hours of darkness, as the acclimatization continued for 8 weeks. Endogenous hormone extraction and determination of auxin and cytokinin content was done according to method of (1).

Results:

Shoot Length

As evident from Table 1 and Figure 1, the growing medium had an effect on the acclimatization and vegetative growth of the tissue cultured banana plantlets. In comparison to other tested mediums, the zeolite and peat moss at a ratio of 1:2 yielded maximum plant height. The 100% zeolite growing medium showed minimum vegetative growth in comparison to other treatments randomly assigned to the experimental units.

. The tissue-cultured plantlets of the banana variety Grand Nain served as the source of the experimental material. Preparation of banana explants involved stripping off the older leaves to obtain apical buds. Banana explants were subjected to surface sterilization using 15% chlorine solution for a short duration and then washed several times with sterile distilled water.

Sterilized apical buds were inoculated into a Murashige and Skoog (MS) nutritional medium containing sucrose, 6-benzylaminopurine (BAP), sodium phosphate ($NaH_2 PO_4 \cdot 2H_2 O$), adenine sulfate, and agar to induce shoot regeneration (5; 7). Regenerated shoots were further sub-cultured on a shoot multiplication medium that had the same composition, except for the increased concentration of BAP. The process of shoot multiplication involved five subcultures within which the leaves were trimmed off at each subculture to stimulate the formation of new shoots.

Following the shoot multiplication, the shoots were subcultured to an MS medium that contained kinetin instead of BAP and left unpruned for a month. Finally, plantlets were subcultured to an MS rooting medium that had α-naphthaleneacetic acid (NAA) added to stimulate root formation (9; 17).

Agricultural media

After developing healthy shoots and roots, the banana plantlets obtained through tissue culture were subjected to acclimatization. Seven growing media with varying ratios of ingredients were prepared to determine their suitability for plantlet acclimatization. There were seven treatments in the experiment, each replicated thrice, as shown below:

1. Zeolite 100%

2. Zeolite-peatmoss in a ratio of 2:1

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weeks, the plantlets continued to grow vegetative after being removed from the in vitro growing medium. However, the growth continued in all medium except the 100% zeolite. growing medium. A brief fall in the growth rate of the plants was observed during the initial stage of the acclimatization process in most of the growing mediums. After two

Table 1: Effect of substrate composition on shoot length (cm) of micropropagated Grand Nain banana plantlets during acclimatization.

Treatment	Substrate Composition	30 May	14 June	29 June	13 July
A	Zeolite 100%	16.17 ± 0.44 b	15.00 ± 0.29 c	13.83 ± 0.60 e	13.67 ± 0.33 f
B	Zeolite/Peat (1:2)	15.17 ± 0.60 c	13.83 ± 0.44 d	16.00 ± 0.76 d	17.67 ± 0.67 d
C	Zeolite/Peat (2:1)	18.33 ± 0.73 a	18.83 ± 0.93 a	21.33 ± 0.88 a	23.66 ± 0.84 a
D	Zeolite/Peat/Sand (1:1:1)	17.33 ± 0.84 ab	13.00 ± 0.29 e	14.17 ± 0.17 e	15.33 ± 0.33 e
E	Zeolite/Peat/Sand (1:1:2)	18.67 ± 1.20 a	12.50 ± 1.26 f	16.75 ± 1.65 c	19.75 ± 1.06 c
F	Peat/Sand (2:1)	18.33 ± 0.73 a	16.67 ± 0.88 b	19.50 ± 0.76 b	21.17 ± 0.83 b
G	Zeolite/Peat/Sand (1:2:1)	13.83 ± 1.69 d	14.00 ± 0.58 d	15.50 ± 0.87 d	16.50 ± 1.04 d
LSD (0.05)		1.24	0.58	0.71	0.88

*Values represent mean ± standard error (n=3). Means within a column followed by different superscript differ significantly according to LSD test at $P \leq 0.05$.

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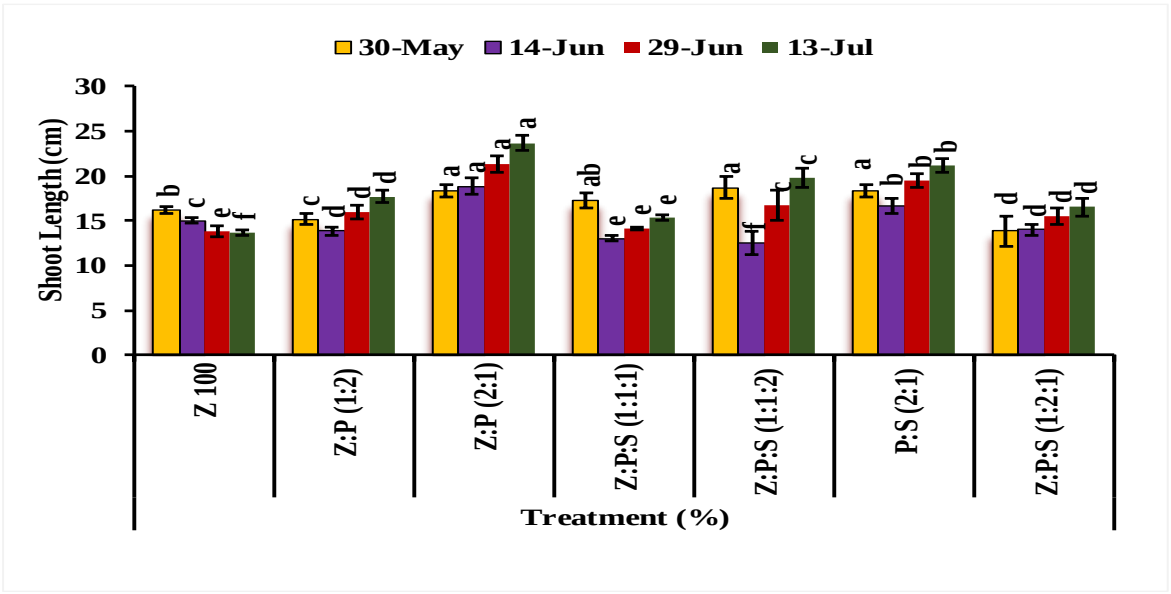


Fig. 1. Effect of growing media on shoot length of tissue-cultured banana plantlets during acclimatization stage (Zeolite (Z), Zeolite-peatmoss (Z:P), Zeolite-peatmoss-sand (Z:P:S).

changes in the growth media composition. Treatment zeolite, peat moss, sand (1:2:1), emerged with the highest mean cytokinin content at 3.645, closely followed by Treatment zeolite, peat moss (1:2) at 3.604, with no statistically significant difference detected between them according to the LSD test (Table 3). A contrasting pattern was observed for auxin, where Treatment zeolite, peat moss, sand (1:1:1) yielded a distinctly superior mean concentration of 28.147. On the lower end of the spectrum, Treatment zeolite, peat moss, sand (1:1:2) recorded a markedly lower cytokinin mean of 1.794, placing it in a statistically distinct and inferior category compared to all other formulations tested. The calculated LSD values of 1.192 for cytokinin and 3.465 for auxin provided the statistical threshold confirming the significance of these observed differences among the treatment groups.

Number of Leaves

A clear effect of the growth medium on acclimatization of banana plants, which contains zeolite/peatmoss at a ratio of 1/2, gave the highest average number of leaves while the medium which contains zeolite at a ratio of 100%, gave the lowest number of leaves (Table and Figure 2). It was observed that the plants in most growth media faced deterioration of vegetative growth two weeks after transferring them from the solid nutrient medium to the growth medium prepared for acclimatization. Also, vegetative growth recovered in all acclimatization media except for media (Z, Z:P, Z:P:S), especially during the last acclimatization period.

Auxin and sytokinin

The experimental data revealed a distinct response pattern in plant hormone levels to

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Table 2: Effect of substrate composition on leaf number of micropropagated Grand Nain banana plantlets during acclimatization.

Treatment	Substrate Composition	30 May	14 June	29 June	13 July
A	Zeolite 100%	4.67 ± 0.33 c	3.67 ± 0.33 f	5.00 ± 0.00 c	4.33 ± 0.33 e
B	Zeolite/Peat (1:2)	5.33 ± 0.67 b	4.67 ± 0.67 e	5.33 ± 0.33 c	5.00 ± 0.00 d
C	Zeolite/Peat (2:1)	5.67 ± 0.67 b	5.33 ± 0.33 d	6.67 ± 0.33 a	6.00 ± 0.00 bc
D	Zeolite/Peat/Sand (1:1:1)	6.00 ± 0.58 a	5.00 ± 0.58 d	5.33 ± 0.33 c	5.00 ± 0.00 d
E	Zeolite/Peat/Sand (1:1:2)	6.33 ± 0.33 a	4.00 ± 0.58 f	4.50 ± 0.29 d	5.00 ± 0.58 d
F	Peat/Sand (2:1)	4.33 ± 0.33 c	5.67 ± 0.33 c	6.67 ± 0.33 a	6.00 ± 0.00 bc
G	Zeolite/Peat/Sand (1:2:1)	5.33 ± 0.67 b	6.33 ± 0.33 a	6.00 ± 0.00 b	6.67 ± 0.33 a
LSD *(0.05)*		0.55	0.40	0.49	0.55

*Values represent mean ± standard error (n=3). Means within a column followed by different superscript differ significantly according to LSD test at $P \leq 0.05$.

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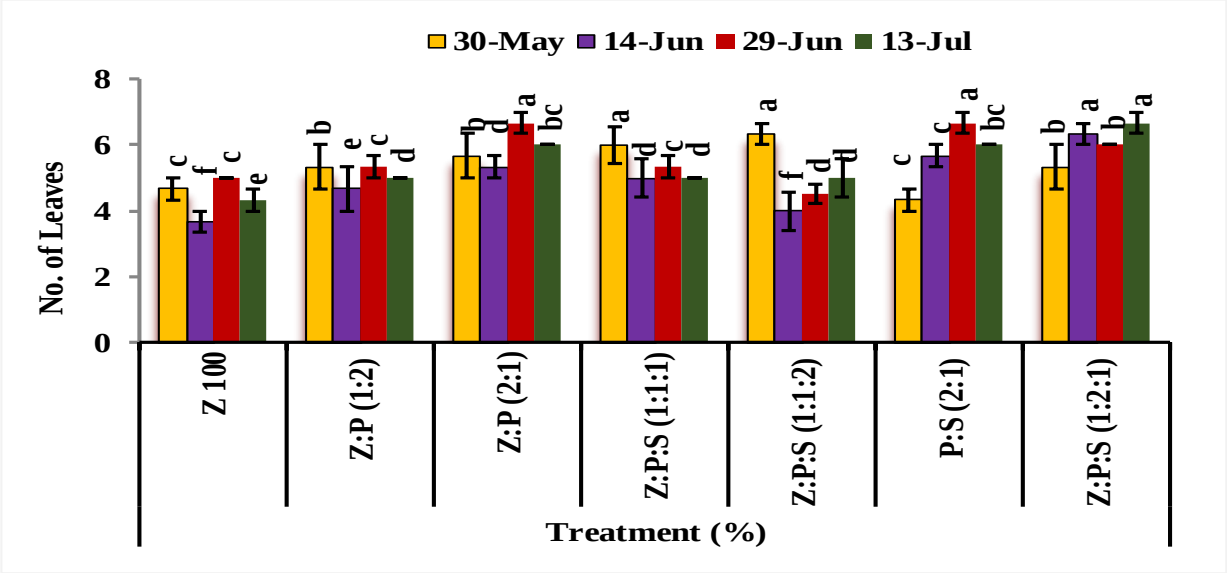


Fig. 2. Effect of growing media on the number of leaves of tissue-cultured banana plantlets during acclimatization stage (Zeolite (Z), Zeolite-peatmoss (Z:P), Zeolite-peatmoss-sand (Z:P:S).

Table 3. Effect of substrate composition on endogenous hormone concentrations (µg g^{-1} fresh weight) in leaf plantlets during acclimatization stage.

Treatment	Substrate Composition	Auxin (IAA)	Cytokinin (Zeatin-type)
A	Zeolite 100%	25.577 ± 0.64 c	3.544 ± 0.35 a
B	Zeolite/Peat (1:2)	26.773 ± 0.46 b	3.604 ± 0.30 a
C	Zeolite/Peat (2:1)	25.367 ± 0.28 c	3.439 ± 0.19 a
D	Zeolite/Peat/Sand (1:1:1)	28.147 ± 0.63 a	3.480 ± 0.23 a
E	Zeolite/Peat/Sand (1:1:2)	25.497 ± 0.54 c	3.508 ± 0.19 a
F	Peat/Sand (2:1)	26.920 ± 0.71 b	3.645 ± 0.17 a
G	Zeolite/Peat/Sand (1:2:1)	26.610 ± 0.61 b	1.794 ± 0.21 b
LSD (0.05)		3.465	1.192

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*Values represent mean ± standard error (n=3). Means within a column followed by different superscript differ significantly according to LSD test at $P \leq 0.05$.

zeolite-peatmoss-sand (1:1:2) media represented another group. Finally, the third group included the following growing media: zeolite-peat moss-sand (1:1:1), peat moss-sand (2:1), 100% zeolite, and zeolite-peat moss (1:2). The 100% zeolite and zeolite-peat moss (1:2) media demonstrated the closest relation because of their minimal clustering distance. It can be said that the composition of the growing medium played an essential role in the growth pattern of tissue-cultured banana plantlets in the process of acclimatization.

Dendrogram

The dendrogram showed (Figure 3) the hierarchical classification of the seven growing media on the basis of the growth behavior of tissue cultured banana plantlets in the process of acclimatization. It is clearly seen that the growing media could be divided into three main groups, depending on their similarities. Thus, the growing medium, which consisted of zeolite-peat moss-sand (1:2:1), represented a separate group and it demonstrated the different growth response compared to other media. Peat-Sand (2:1) and

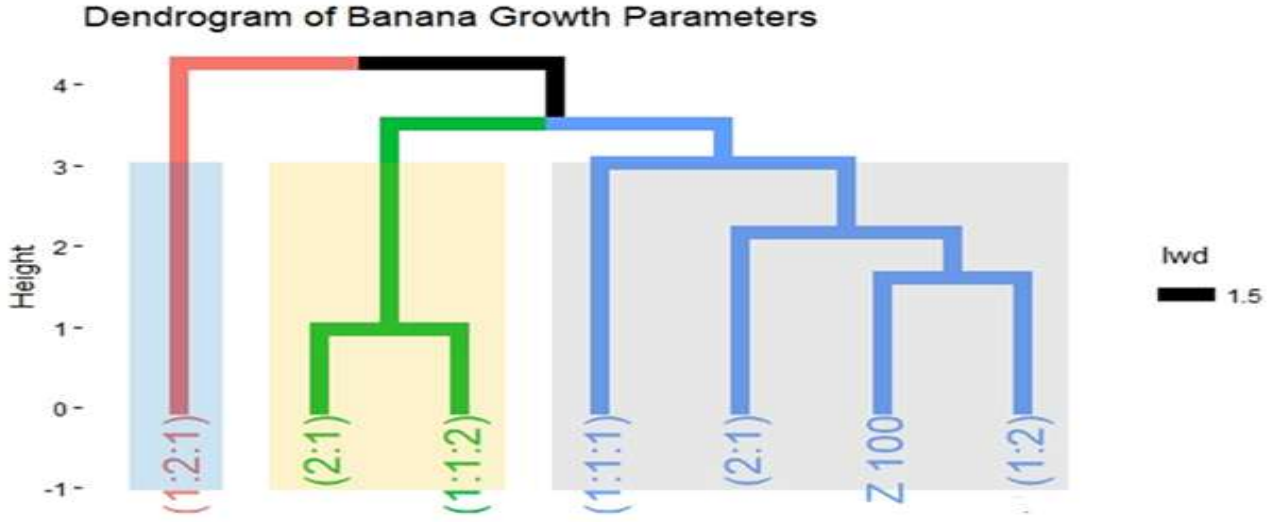


Figure 3:- Dendrogram showing the similarity among various growing media based on the growth attributes of tissue-cultured banana plantlets during acclimatization stage. Zeolite-Peatmoss-Sand (1:2:1); Peat-Sand (2:1); Zeolite-Peat-Sand (1:1:2); Zeolite-Peatmoss-Sand (1:1:1); Zeolite-Peat (2:1); Zeolite (100%); Zeolite-Peat (1:2).

and the corresponding growth characteristics of tissue-cultured banana plantlets during acclimatization are depicted in figure 4. Two principal components accounted for 69.5% of total variability, where PC1 (Dim1) contributed 43.9%, while PC2 (Dim2) contributed 25.6% of variability.

Principal Component Analysis

The results of the principal component analysis (PCA) for different growing media

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other treatments, demonstrating the unique effect on the growth of tissue-cultured banana plantlets compared to other growing media. Zeolite–peat moss (1:2) treatment showed that auxin concentration increased during acclimatization in this treatment.

In general, PCA proved that the type of the growing medium influenced the growth performance and endogenous hormones of tissue-cultured banana plantlets during the acclimatization period.

In the context of the PCA, the growing media could be differentiated depending on their influence on the plant growth. The media consisting of zeolite–peat moss–sand (1:1:2) and zeolite–peat moss (2:1) were close to vectors for shoot length, leaf number, and cytokinin, indicating that the growth of plants was associated with such kind of medium. In turn, the 100% zeolite treatment was situated far from vectors, which means that it had less impact on plant growth. Zeolite–peat moss–sand (1:2:1) treatment was separated from

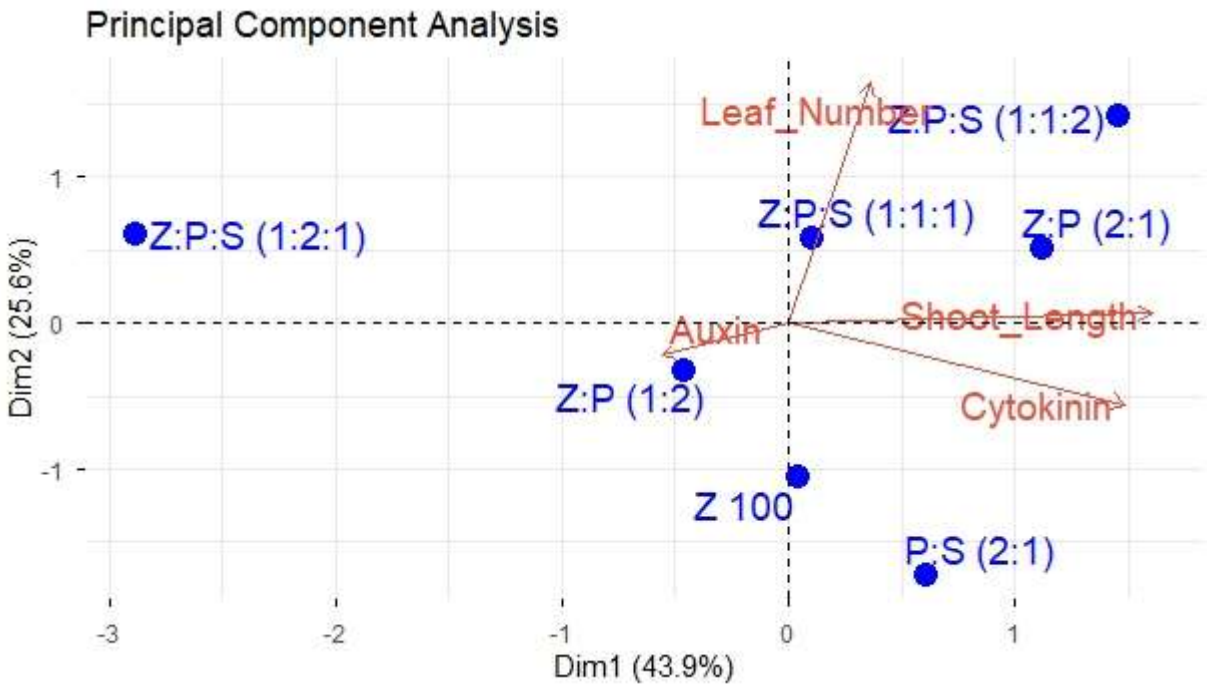


Figure 4:-Principal component analysis showing the relationship among various growing media and growth attributes of tissue-cultured banana plantlets during acclimatization stage.

was a very weak positive relationship between shoot length and leaf number (r = 0.21), implying that an increase in shoot length causes leaf number to slightly increase. However, there was a weak negative relationship between shoot length and auxin (r = –0.20). On the other hand, there was almost no relationship between cytokinin and either leaf number (r = –0.04) or auxin (r = –0.06).

Correlation Matrix

The relationship between shoot length, cytokinin, leaf number, and auxin in acclimatized banana tissue-cultured plantlets is represented in figure 5. There was a positive relationship between shoot length and cytokinin (r = 0.69), which shows that an increase in cytokinin is associated with an increase in shoot length. In addition, there

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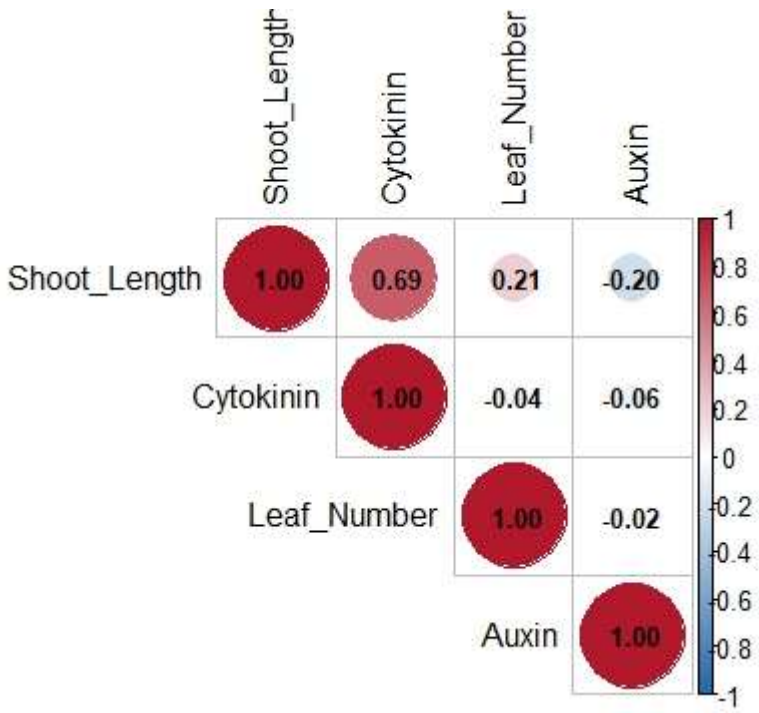


Figure 5:- Correlation matrix illustrating the association between shoot length, cytokinin, leaf number, and auxin in banana tissue culture plantlets at the acclimatization phase.

Clustered Heatmap

The clustered heatmap of the growth characteristics and endogenous hormones evaluated in the banana plantlets tissue culture grown in various media under the process of acclimatization is represented in figure 6. It is evident from the heatmap that dark red color showed higher values for that trait. Dark blue showed lower values for that trait.The treatments that were clustered together in the dendrogram were those having comparable growth and hormonal response.From the heatmap, it is evident that different growing media led to different responses in the growth of banana plantlets.

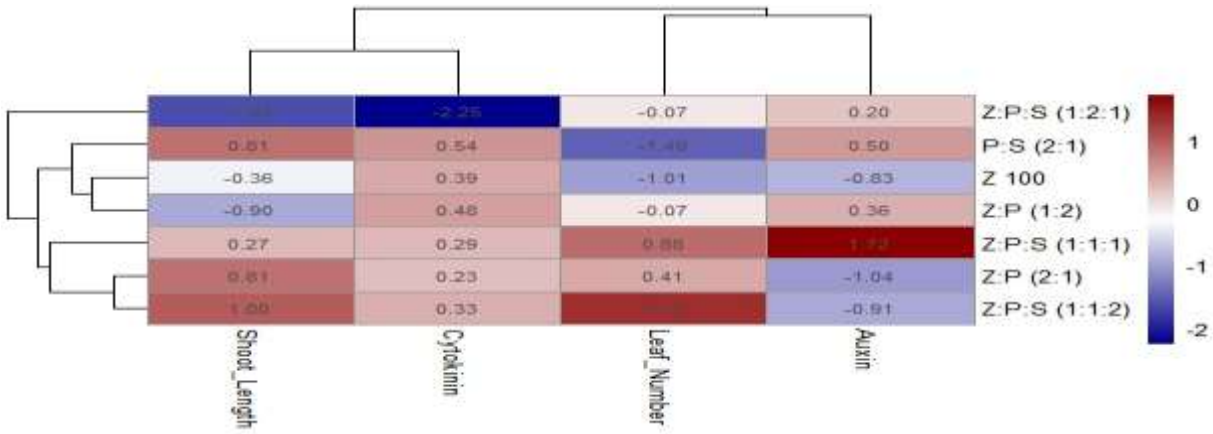


Figure 6:- Clustered Heat Map representing the impact of varying growing media on shoot length, leaf numbers, cytokinin and auxin in tissue culture derived banana plantlets at acclimatization stage.

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Chord Diagram

Each colored region within the circle is an individual trait. The ribbon between any two regions is a symbol for the relationship between the two traits. Thick ribbons imply a strong relationship between them.Thinner ribbons imply weak relationship between them. It is clear from diagram that shoot length is strongly correlated with cytokinin. Leaf number has a moderately strong relationship with shoot length. Auxin is weakly correlated with the other traits.

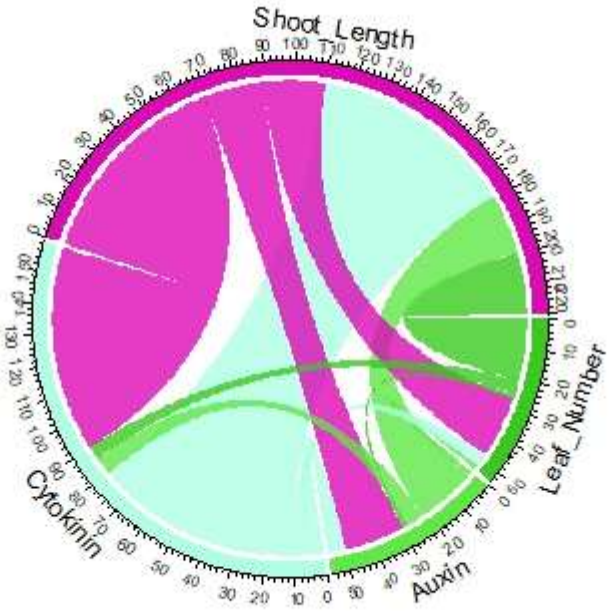


Figure7:- A chord diagram showing the relationship among various growth attributes.

notable enhancement in endogenous hormone balance for banana seedlings during the critical acclimatization phase. Treatment comprising zeolite/peat moss in a 1:2 ratio, increased cytokinin, content, which reflects this combination of treatment in providing a good environment to cell division and new bud formation during dual mechanism by improved bioavailability of potassium which in basis of cytokinin synthesis (22), and increased water and nutrient retention capacity because of the high porosity of zeolite, thus reducing water stress, a major inhibitor of hormone biosynthesis (5). A mix of zeolite, peat moss, and sand in equal parts, showed the greatest amount of auxin, a key hormone for root development. This suggested that this

Discussion

The results demonstrated that mixing peat moss in double proportion with zeolites or sand or both has a positive effect on the acclimatization of tissue-produced banana plants in terms of the length of the vegetative group and the number of leaves. The reason is that this medium retains moisture, preventing it from drying out, and also allows adequate air circulation for the plant, thus avoiding over-watering (22; 12). The preliminary study suggested a 1/2 ratio of peat moss to zeolite for improved acclimatization and plant biomass. The findings showed that combining zeolite and peat moss in particular proportion led to a

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conditions for plant growth and development. This means that a growth medium containing zeolite and peat moss, either alone or in combination with sand, should be used to improve the acclimatization of tissue-cultured banana plantlets.

Author contributions

The concept was developed by Ahmed Z Resan and Aqeel H Abdulwahid, who also helped in writing the manuscript. Asmahan S Abdulkareem and Khudhair M Al-Kanany handled draft preparation, review, and editing. Ahmed Z Resan, Aqeel H Abdulwahid, Asmahan S Abdulkareem, and Khudhair M Al-Kanany edited the draft. All the authors equally contributed in the manuscript.

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Conflict of Interest

The authors declare that there is no conflict of interest.

Novelty Statement

An ideal medium comprising of zeolite and peat moss is helpful in improving the acclimatization and vegetative growth as well as the endogenous hormone responses of the tissue culture raised Grand Nain red banana.

References

1- Abdulwahid, A . H. , 2011 . A Genetic Fingerprinting Study of Two Male Date Palm Cultivars (Phoenix dactylifera L.) and the Effect of Their Pollen on the Physical and Chemical Characteristics of 'Al-Halawi' Date Fruits. PhD theses, College of Agriculture, University of Basrah, Iraq: 202 p.

2- Ahmed, R. T., and O.S. Ibrahim, 2024. Modeling and analyzing banana prices in the city of Mosul using the ARFIMA model: Predictive market study**.** *Iraqi J. Stat. Sci.,* 21(2): 95-111

combination, particularly the sand contribution to aeration, creates favorable conditions for root growth. Retains moisture and nutrients (as a result of zeolite and peat moss), fostering tryptophan-driven auxin production and establishing optimal redox conditions for enzymes such as tryptophan aminotransferase (23).

These results exhibited that the physical and chemical properties of the culture medium through porous and improved materials such as zeolite is an effective strategy for manipulating the plant internal physiology, particularly the delicate hormonal balance between cytokinins and auxins (CK/Auxin ratio), which directly controls the transition from in vitro dependence to autotrophicity during acclimatization (17). Therefore, this study provides evidence that improving the rhizosphere environment via engineered culture media acts as a vital link to enhance the efficiency of tissue culture propagation in high value crops such as bananas.

Conclusion

The findings indicated that the growing medium composition plays a major role in the successful acclimatization of tissue-cultured Grand Nain banana plantlets. Out of the various growing media, the medium that contained zeolite and peat moss in the ratio 1:2 recorded the best performance regarding vegetative growth by showing maximum plant height, whereas the medium containing zeolite, peat moss, and sand in the ratio 1:2:1 showed maximum leaf production. On the other hand, 100% zeolite was the poorest performing medium, recording poor growth of plants.

In addition, the growth media affected the endogenous hormones of the plantlets where the growth of shoots was positively correlated with the levels of cytokinin hormone. Overall, growth media consisting of peat moss and zeolite showed better water retention capability and thus creating favorable

under salt stress conditions *in vitro*. *Euphrates J. Agric. Sci., 16*(4): 69–75.

10- Eatshan, K. M., M.J,.H. Al-shareefi, and D.H. Jawad, 2025. *Impact of the growth regulator B.A in salt stress tolerance of Swingle Citrumelo Citrus rootstock shoots in vitro*. *Euphrates J. Agric. Sci.,* 17(1): 893–901.

11- Elyazid, D.M.A., Salama, A.M., Zanaty, A.F.M.E. and Abdalla, N., 2021. In vitro propagation and acclimatization of banana plants: antioxidant enzymes, chemical assessments and genetic stability of regenerates as a response to copper sulphate. Plants, 10(9):1853. *https://doi.org/10.3390/plants10091853*.

12- Fikri, E., R. Athaya Mutiara Farid, Y. Ardiani Septiati, N. Djuhriah, N. Yety Hanurawaty and A. Sharaf Eldin Khair, 2022. Korespondensi: Effect of Zeolite and Activated Carbon Thickness Variation as Adsorbent Media in Reducing Phenol and Manganese Levels in Wastewater of Non-Destructive Testing Unit. *J. Ecol. Eng., (JEE), 23*(8): 40-48. https://doi.org/10.12911/22998993/150653

13- Hussein, S. S., A.A. Matrood and M.H. Abass, 2023. Isolation and identification of fungi associated with tissue cultures of banana tree (*Musa acuminata*) and the synergistic inhibitory effect of Beltanol and copper sulfate. *Iraqi J. Agric. Res., 27*(1): 1–19. https://doi.org/10.33899/ijar.2023.184567.

14- Ibrahim, H. A., I.N. Khelkal and Y.M. Muhsin, 2024. Improvement of Bacteriocin Production by Natural Zeolite and Detection of Antibacterial Activity of Leuconostoc carnosum Purified Carnosin. Basrah J. Agric. Sci.. 37(2): 37–53. https://doi.org/10.37077/25200860.2024.37.2.4.

15- Mahendra, R., Chauhan, N., Sharma, J.B., Rana, K. and Bakshi, M., 2020. Ex-vitro establishment of tissue cultured plants in fruit crops-A review. *Int J Curr Microbiol* https://doi.org/10.33899/iqjoss.2024.185244

3- Al-Ameri, A., and M.M.M. Almasoody, 2025. *The effect of plant growth regulators on in vitro production of Yellow Royal Dutch Iris bulbs*. *Euphrates J. Agric. Sci.,* 17(2): 132–139.

4- Al-Asadi, A. Z. ., A.H. Abdulwahid and A.M. Al-Mayahi, 2019. The Effect of Thidiazuron on Callus and in vitro Shoots Development of Date Palm (*Phoenix dactylifera* L.) cv. Barhee. *Basrah J. Agric. Sci.,32*: 258–265. https://doi.org/10.21276/basjas

5- Al-kanany, K. M., A.H. Abdulwahid, and M.A. Ibrahim, 2023. Physical characteristics of in vitro date palm cultures under the influence of polyethylene glycol induced water stress and brassinosteroid treatment. *Dysona, Appl. sci.4*(1): 21-27. https://doi.org/10.30493/DAS.2022.368280.1067

6- Al-Mousawi, Z. J., and L.K. Al-Amery, 2025. *Effect of ultraviolet ray and Moringa leaf extract on growth and accumulation of digoxin in Digitalis lanata in vitro*. *Euphrates J. Agric. Sci.,* 17(1): 268–279.

7- Althuluth, A. H.,A.A. Suhaim, and M.S. Auda, 2024. Detection of bioactive compounds produced by in Vitro culture of Jojoba plants (Simmondsia chinesi (Link) Schn.) Using GCMS and FTIR. *Basrah J. Agric. Sci., 37*(2), 54-66. https://doi.org/10.37077/25200860.2024.37.2.5

8- Aswandi, A., A. ., B.L. Syaefullah, D. Iyai, and M.J. Wajo, 2022. Utilization of carbohydrate potential in various kinds of banana cob flour banana in kacang goats. *Iraqi J. Agric. Sci.*,53(4): 732-742. https://doi.org/10.36103/ijas.v53i4.1583

9- Atiyah, F. S., M.J.H. Al-shareefi, and B.M.H.A. Altaei, 2024. Effect of growth regulators on vegetative parameters of (*Catharanthus roseus* L.) shoots grown

Appl Sci., 9(11): pp.3321-3329. https://doi.org/10.20546/ijcmas.2020.911.397

16- Nassif, N. A and A.S. Nasser, 2024 . Effect of natural zeolites on blood parameters of common carp (*Cyprinus carpio*) fed on aflatoxin-contaminated diet. *Iraqi J. Agric. Res.*, *28*(2): 36–44. https://doi.org/10.33899/ijar.2024.185245.

17- Omari, E. N., M. Mucheru-Muna, B.K. Mburu, and A.A. Odawa, 2024. Assessing the advantages of tissue culture bananas technology production of banana farmers in Kisii County, Kenya. *Environ. Chall.*, *14*: 100843.https://doi.org/10.1016/j.envc.2024.100843.

18- Othman, S.H., N.A. Abdullah, N. Nordin, N.N.A.K. Shah, M.Z.M. Nor, and K.F.M. Yunos, 2021. Shelf life extension of Saba banana: Effect of preparation, vacuum packaging, and storage temperature. *Food Packag. Shelf Life.*,*28*, p.100667. https://doi.org/10.1016/j.fpsl.2021.100667

19- Sharma, N., N.Kumar, J. James, S. Kalia, and S. Joshi, 2023. Strategies for successful acclimatization and hardening of in vitro regenerated plants: Challenges and innovations in micropropagation techniques. *Plant Sci. Today* ., 10(sp2), 90–97.https://doi.org/10.14719/pst.2376

20- Sharma, P., M. Mishra, S. Rajan, T. Damodaran and M. Trivedi, 2025. An innovative hardening technology for micro propagated banana plantlets for medium size tissue culture industries. *J. Appl. Hortic.*,*27*(1), pp.143-148. https://doi.org/10.37855/jah.2025.v27i01.27

21- Tzeng, J.H., Weng, C.H., Huang, J.W., Shiesh, C.C., Lin, Y.H. and Lin, Y.T., 2019. Application of palladium- modified zeolite for prolonging post- harvest shelf life of banana. *J Sci Food Agric.*, 99(7):3467-3474. https://doi.org/10.1002/jsfa.9565.

22- Yin, C. Y., M.A.M. Akhir, and M.N.A. Uda , 2020. Effect of potassium permanganate and zeolite on shelf life and quality of Musa acuminata. In *IOP Conference Series. Mater. Sci. Eng.*, (Vol. 864, No. 1). IOP Publishing.https://doi.org/10.1088/1757-899X/864/1/012141.

23- Zhang, J., Bei, S., Li, B., Zhang, J., Christie, P. and Li, X., 2019. Organic fertilizer, but not heavy liming, enhances banana biomass, increases soil organic carbon and modifies soil microbiota. *Appl. Soil Ecol.*,*136*, pp:67-79. https://doi.org/10.1016/j.apsoil.2018.12.017

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