



Available Online at EScience Press

## Plant Protection

ISSN: 2617-1287 (Online), 2617-1279 (Print)  
http://esciencepress.net/journals/PP

### Research Article

## OPTIMIZING CULTURING VESSELS FOR DISEASE-FREE SHOOT PROLIFERATION IN OLIVE (*OLEA EUROPAEA* L.) CULTIVARS

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### ARTICLE INFO

#### Article history

Received: 28<sup>th</sup> December, 2024

Revised: 17<sup>th</sup> March, 2025

Accepted: 23<sup>rd</sup> March, 2025

#### Keywords

*Olea europaea* L.

Single node

Culturing vessels

Shoot proliferation

Tissue culture

### ABSTRACT

Researchers worldwide are striving to develop rapid, simple, disease-free and cost-effective methods for plant multiplication to produce healthy, high-quality plants. To achieve these objectives, a study was conducted at the Tissue Culture Laboratory, Agriculture Research Institute Tarnab, Peshawar, to investigate the influence of different culturing vessels on pathogen-free shoot proliferation in olive (*Olea europaea* L.) cultivars. *In vitro*-proliferated shoots were cultured as single nodes under laboratory conditions on Rugini Olive Medium supplemented with zeatin (3 mg/L) using three different types of culturing vessels viz. test tubes with caps, test tubes with cotton plugs, and jars with lids. The choice of culturing vessel significantly affected the growth of both *in vitro*-proliferated olive cultivars, with the best results observed in jars with lids and test tubes with caps compared to test tubes with cotton plugs. The shortest time to shoot induction, highest shoot induction percentage, and enhanced plantlet fresh and dry weight, number of shoots per explant, number of leaves per shoot, shoot length, leaf fresh and dry weight, and percent leaf water content were recorded when cv. Gemlik was cultured in jars with closed lids, followed by test tubes with caps, and test tubes with cotton plugs. It is, therefore, recommended that cv. Gemlik be cultured in jars with lids for efficient micropropagation and growth.

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### INTRODUCTION

Olive (*Olea europaea* L.), a member of the Oleaceae family, is a highly significant species native to the eastern Mediterranean basin, widely recognized for its

nutritional and medicinal value. It is one of the oldest cultivated trees, with a history deeply intertwined with human civilization. Olive fruits are rich in essential nutrients, comprising approximately 50% water, 22%

oil, 5.8% cellulose, 1.6% protein, 19.1% carbohydrates, 1.5% inorganic substances, and 1-3% phenolic compounds. They also contain organic acids, pectin, and pigments, with organic acids serving as metabolic intermediates in various biochemical pathways within the plant (Millan-Linares et al., 2021).

Olive oil, extracted primarily from the fruit, is rich in oleic acid and also contains smaller quantities of other fatty acids, including stearic, palmitic, linoleic, palmitoleic, and  $\alpha$ -linolenic acids, all of which contribute to its nutritional and health benefits (Paiva-Martins and Kiritsakis, 2017).

The olive tree is a perennial, evergreen, and long-lived species that thrives in Mediterranean climates characterized by dry, warm, and subtropical conditions. It is particularly well adapted to challenging environmental factors such as shallow soils and extreme temperatures (Fraga et al., 2020). Its resilience to drought and ability to produce fruit under harsh conditions make it a valuable crop in arid and semi-arid regions. Traditionally, olives have been propagated vegetatively and begin to bear fruit after a few years.

The Mediterranean basin remains the dominant region for olive cultivation, accounting for approximately 95% of the world's olive groves, with Spain, Italy, and Greece being the leading producers of both table olives and olive oil (Bassi et al., 2024).

In recent years, olives have gained increasing significance due to their high oil content and the health benefits of olive oil, particularly in preventing cardiovascular diseases. As a result, demand for olive cultivation has risen in regions like Pakistan, which has made substantial investments to enhance olive productivity and oil production (Alam et al., 2021).

Olive trees can be propagated through various methods, including seed propagation, hardwood cuttings, air layering, and grafting. Although seeds are viable, they often fail to maintain the genetic consistency of the parent tree, limiting their effectiveness. Hardwood cuttings and air layering involve vegetative propagation, where cuttings or wounded branches develop roots under controlled conditions, ensuring genetic uniformity. Grafting is also widely used to propagate specific olive varieties with desirable traits (Rehman et al., 2013).

Micropropagation has emerged as an efficient technique for the rapid and sustainable mass production of olive plants. This method involves growing plant tissues, cells, or organs *in vitro* under sterile conditions, enabling large-

scale propagation while maintaining genetic uniformity (De Filippis, 2013). It has revolutionized olive cultivation by facilitating the rapid multiplication of healthy, disease-free plants. However, the success of micropropagation depends on several factors, including the choice of culturing vessels, which significantly influence the growth, development, and overall performance of olive explants during *in vitro* culture (Lambardi et al., 2023).

Recent studies indicate that the type and design of culturing vessels in micropropagation protocols significantly influence key factors such as nutrient uptake, light exposure, and gas exchange, all of which are essential for optimal olive tissue growth (Cardoso et al., 2018). The impact of different culturing vessels on shoot proliferation in olive cultivars has become an area of growing interest. Given the increasing demand for efficient and cost-effective olive propagation methods, it is crucial to explore how various culturing vessels enhance or hinder shoot proliferation, ultimately affecting the success of micropropagation protocols. The present research aims to evaluate the effects of different culturing vessels on the shoot proliferation of various olive cultivars, providing insights into optimizing micropropagation techniques for large-scale olive production in a disease-free environment. Specifically, it seeks to identify the most suitable culturing vessel and the best-performing olive cultivar for micropropagation while also examining the interaction between culturing vessels and olive cultivars. The findings will contribute to the development of more efficient and sustainable micropropagation systems, enhancing the scalability of pathogen-free olive cultivation.

## MATERIALS AND METHODS

The research was conducted in the Plant Tissue Culture Laboratory, Agriculture Research Institute, Tarnab, Peshawar, in March 2023. The experiment followed a completely randomized design with two factors, and treatments were replicated three times. The study aimed to evaluate the effects of three different culturing vessels, jars with lids (J), test tubes with cotton plugs (T. cot), and test tubes with caps (T. cap), on the shoot proliferation of two olive cultivars, Gemlik and Coratina in a disease free environment, and to identify the most suitable vessel for large-scale olive micropropagation. Single-node explants from *in vitro* proliferated shoots of the two olive cultivars were cultured under laboratory conditions on Rugini Olive Medium (OM) supplemented with zeatin (3 mg/L) using the three different culturing vessels.

### Explant sources

Single nodes from *in vitro*-proliferated shoots of olive cultivars Coratina and Gemlik were used as explants for this experiment, conducted in the tissue culture lab at the Agriculture Research Institute, Tarnab, Peshawar.

### Growth media, culturing vessel preparation, and incubation

Olive Rugini medium (OM) was used for shoot proliferation. A 1-liter OM solution was prepared by supplementing it with 3 mg/L zeatin and 30 g/L sucrose. The pH was adjusted to 5.7-5.8 before gelling with 7.5 g/L Agar.

The selected culturing vessels were first washed with dishwashing detergent and then rinsed thoroughly with distilled water. They were dried in an oven before being filled with the prepared OM media. Sterilization was carried out in an autoclave at 15 PSI and 121°C for 25 min, ensuring complete sterilization of both the media and vessels.

In addition to media sterilization, the *in vitro*-proliferated shoots used as explants were surface-sterilized with 70% ethanol for 1-2 min to eliminate contaminants. Ethanol effectively denatures proteins on microbial surfaces, creating a sterile environment without damaging the explant tissue. These sterilization conditions were carefully optimized to minimize contamination risks and enhance shoot regeneration.

Under sterile conditions in a laminar flow hood, explants were handled using sterilized forceps, blades, and Petri dishes, which were flamed with a lamp after ethanol spraying. Single-node explants were cut with sterile blades and transferred onto the prepared growth media. The cultures were then placed in a growth room under controlled conditions. They were exposed to 50-70  $\mu\text{mol}/\text{m}^2/\text{s}$  of cool white fluorescent light with a 16-h light/8-h dark photoperiod to promote shoot proliferation. The incubation temperature was maintained at 24-26°C to support optimal metabolic activity and growth (Kyte et al., 2013).

### Attributes studied

Explants cultured on OM media in vessels were regularly

observed in the growth room until shoot formation was detected. The number of days from the initial culturing date to shoot induction was recorded for all treatments in each replication, and the average was then calculated. The percentage of shoot induction and the proportion of shoot-induced plants were recorded for each replication, and the percentage for each treatment was determined using the following formula:

$$\text{Shoot Induction (\%)} = \frac{\text{Shoot Induced Explants}}{\text{Total Cultured Plants}} \times 100$$

For measuring plantlet fresh weight, plantlets were removed from the vessels using forceps after 60 days of culturing in the first experiment and 30 days in the second experiment. They were then washed with distilled water to remove any residual media. Excess water was absorbed using filter paper before weighing the fresh plantlets on a digital balance. The fresh weight of the plantlets was recorded immediately after harvesting.

After measuring the fresh weight, the plantlets were dried in an oven at 70°C for 24 h, above ambient temperature, and their dry weight was recorded using a digital balance.

The number of shoots per explant was recorded by counting the total number of shoots in each treatment and replication, followed by calculating the average. Similarly, the total number of leaves was counted on each shoot in each treatment and replication, and the average was determined. Shoot length was measured using a ruler, and mean values were calculated for statistical analysis.

For measuring the fresh weight of leaves, harvested leaves were detached from the media using forceps after 30 days. The leaves were washed with distilled water to remove any media residues, and excess water was absorbed using filter paper. The fresh weight of the leaves was then measured using a digital balance.

After determining the fresh weight, the leaves were dried in an oven at 70°C for 24 hours, and their dry weight was recorded using a digital balance. Leaf relative water content was calculated using the following formula:

$$\text{Leaf Water Content (\%)} = \frac{\text{Fresh leaf weight} - \text{Dry leaf weight}}{\text{Fresh leaf weight}} \times 100$$

### Statistical analysis

The experiment was conducted using a completely randomized design. All data were analyzed using a two-way analysis of variance (ANOVA) in Statistix 8.1 software. Significant differences were determined using

the least significant difference (LSD) test at a significance level of  $P \leq 0.01$ .

### RESULTS

The mean data for various growth parameters, including

days to shoot induction, shoot induction percentage, plantlet fresh and dry weight, number of shoots per explant, and leaf water content percentage, are provided in Table 1, and the interactions are shown in Figures 1, 2, 3, and 4.

The earliest shoot induction (5.55 days) was observed in explants cultured on OM medium in jars with lids, while the longest induction time (10.48 days) was recorded in test tubes with cotton plugs. Regarding

cultivars, the earliest shoot induction (6.90 days) was observed in the Gemlik cultivar, compared to Coratina (7.88 days). The highest shoot induction percentage (87.68%) was recorded in jars with lids, whereas the lowest percentage (54.93%) was observed in test tubes with cotton plugs. Among the cultivars, Gemlik exhibited the highest shoot induction percentage (78.18%), while Coratina had the lowest (73.29%) (Table 1).

Table 1. Effect of different culturing vessels on growth parameters of olive cultivars.

| Culturing Vessels (V) | Days to shoot induction | % Shoot induction percentage | Plantlet fresh weight (mg) | Plantlet dry weight (mg) | Number of shoots explant <sup>-1</sup> | % Leaf water content |
|-----------------------|-------------------------|------------------------------|----------------------------|--------------------------|----------------------------------------|----------------------|
| Jars with lids        | 5.55B                   | 87.68A                       | 198.10A                    | 47.41A                   | 1.55A                                  | 76.31A               |
| Test tubes with       | 10.48A                  | 54.93C                       | 61.17B                     | 23.85C                   | 1.10B                                  | 65.78C               |
| Test tubes with caps  | 6.15B                   | 84.58B                       | 187.20A                    | 34.33B                   | 1.44AB                                 | 72.71B               |
| LSD                   | 0.72                    | 2.49                         | 30.17                      | 8.81                     | 0.36                                   | 2.02                 |
| Olive Cultivars (C)   |                         |                              |                            |                          |                                        |                      |
| Coratina              | 7.88A                   | 73.29B                       | 130.62B                    | 29.66B                   | 1.19B                                  | 69.99B               |
| Gemlik                | 6.9B                    | 78.18A                       | 167.02A                    | 40.93A                   | 1.54A                                  | 73.21A               |
| LSD                   | 0.58                    | 2.03                         | 24.63                      | 7.19                     | 0.29                                   | 2.48                 |
| Interaction V x C     | NS                      | NS                           | NS                         | NS                       | NS                                     | NS                   |

The maximum plantlet fresh weight (198.10 mg) was recorded in jars with lids, while the minimum fresh weight (61.17 mg) was observed in test tubes with cotton plugs. Plantlet fresh weight was also significantly influenced by cultivar, with Gemlik showing a higher fresh weight (167.02 mg) than Coratina (130.62 mg). The maximum plantlet dry weight (47.41 mg) was recorded in jars with lids, whereas the minimum dry weight (23.85 mg) was observed in test tubes with cotton plugs. Among the cultivars, the maximum plantlet dry weight (40.93 mg) was recorded in the Gemlik cultivar, while the minimum was recorded in Coratina (29.66 mg).

Additionally, the highest number of shoots per explant was observed in jars with lids (1.55), while the lowest was recorded in test tubes with cotton plugs (1.10). Similarly, Gemlik produced the highest number of shoots (1.54), whereas Coratina produced fewer shoots (1.19) (Table 1).

Significant variations in shoot length were observed due to the interaction between olive cultivars and culturing vessels. The longest shoot length (3.6 cm) was recorded in the Gemlik cultivar grown in jars with lids, while the shortest (0.87 cm) was observed in the Coratina cultivar grown in test tubes with cotton plugs (Figure 1).

The interaction between cultivar and culturing vessel significantly affected the number of leaves per shoot. The highest number of leaves per shoot (13.66) was observed in Gemlik cultured in jars, while the lowest (3.26) was recorded in Coratina cultured in test tubes with cotton plugs (Figure 2).

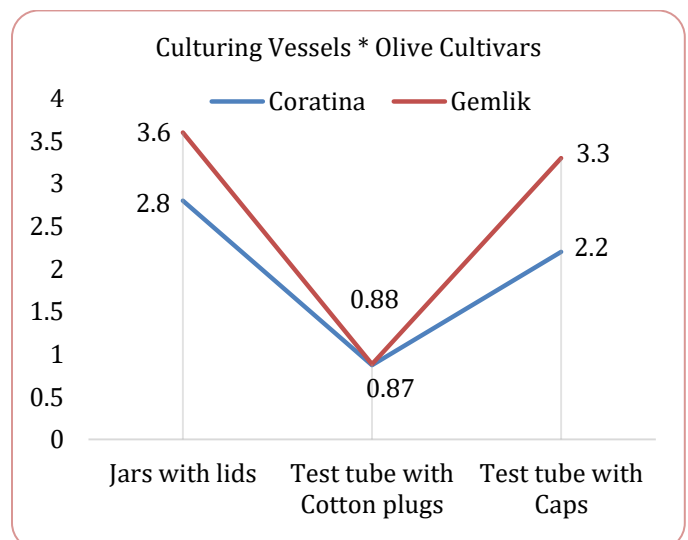


Figure 1. Effect of different culturing vessels and olive cultivars on shoot length.

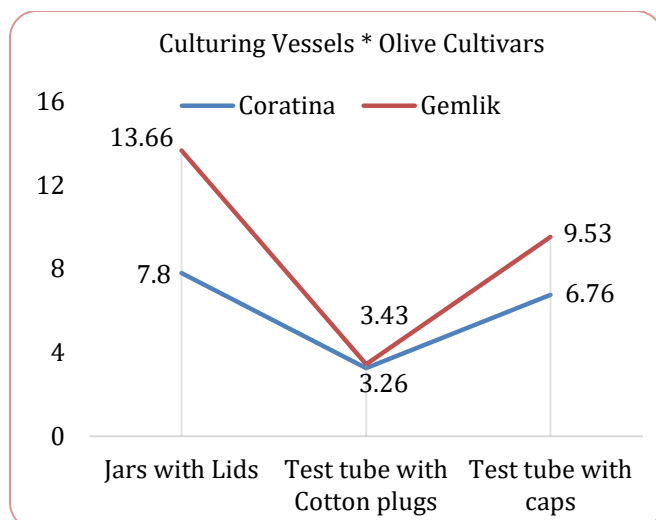


Figure 2. Effect of different culturing vessels and olive cultivars on the number of leaves per shoot.

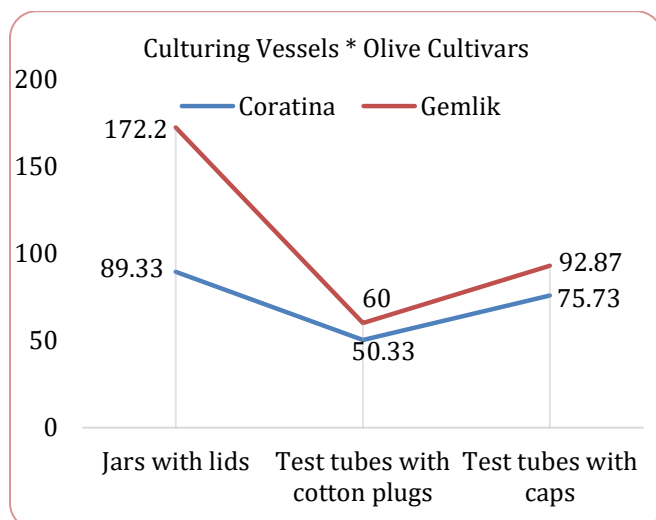


Figure 3. Effect of different culturing vessels and olive cultivars on leaf fresh weight.

Leaf fresh and dry weights were significantly influenced by different culturing vessels and cultivars, with both factors interacting significantly to affect these parameters. The maximum leaf fresh weight (172.2 mg) was observed in Gemlik cultured in jars, while the minimum (50.33 mg) was recorded in Coratina cultured in test tubes with cotton plugs (Figure 3). Similarly, the maximum leaf dry weight (45 mg) was observed in Gemlik cultured in jars, while the minimum (12.36 mg) was recorded in Coratina cultured in test tubes with cotton plugs (Figure 4).

The leaf water content also significantly influenced both the culturing vessels and the Olive cultivars. The

maximum leaf water content (76.31%) was recorded in jars with lids while the minimum leaf water content (65.78%) was observed in plants cultured in test tubes with cotton plugs. Similarly, in case of cultivars the maximum leaf water content (73.21%) was recorded in Gemlik cultivar while the minimum leaf water content (69.99%) was observed in Coratina (Table 1).

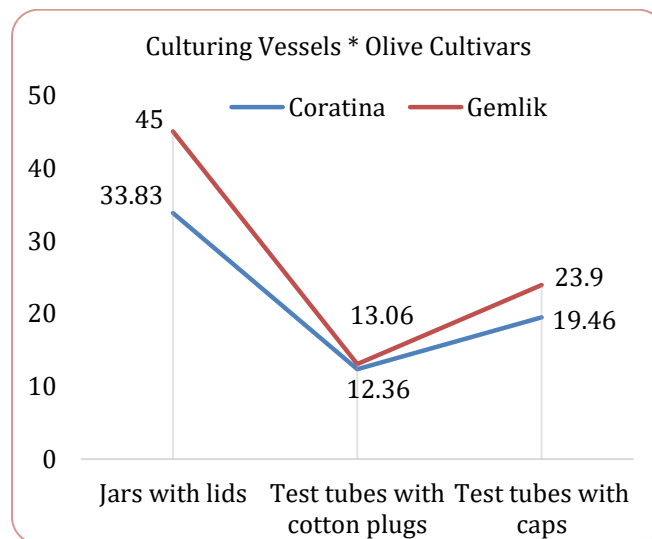


Figure 4. Effect of different culturing vessels and olive cultivars on leaf dry weight.

## DISCUSSION

The successful micropropagation of olive cultivars is a complex process influenced by multiple environmental and physiological factors. The substantial variation in shoot induction times across different culturing vessels highlights the critical role of environmental factors such as humidity, light, and gas exchange in regulating plant development. Jars with closed lids create an optimal microenvironment for explant growth, primarily by increasing humidity, which reduces water loss and maintains favorable turgor pressure within the explants (Das et al., 2021). This high-humidity environment minimizes stress, thereby enhancing the physiological processes associated with shoot initiation.

One of the key factors influencing shoot induction is the hormonal balance within plant tissues. Higher moisture levels facilitate the accumulation of cytokinins, a group of hormones known to promote shoot initiation (Sosnowski et al., 2023). In sealed jars, where evaporation was minimized, cytokinin concentrations likely remain at optimal levels, reducing delays in shoot emergence.

Moreover, the sealed environment helps maintain temperature stability, which is crucial for biochemical reactions and metabolic activity. Temperature fluctuations can hinder enzyme activity, disrupt hormonal signaling, and negatively impact growth (Awasthi et al., 2015). By providing a stable temperature, jars help regulate enzymatic processes involved in growth and differentiation, thereby accelerating shoot induction.

Among the tested cultivars, the Gemlik cultivar exhibited the shortest shoot induction time and the highest shoot induction percentage (Figure 5). This superior performance is likely due to its genetic predisposition, possibly including a more efficient cytokinin synthesis pathway, which enables a stronger response to the cytokinin-rich medium in the jars (Rodrigues et al., 2024).

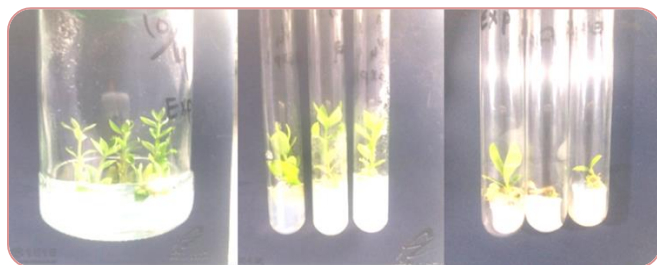


Figure 5. Cultivar Gemlik cultured in jars with lids, test tubes with caps, and test tubes with cotton plugs.

Genetic differences also influence the ability of a cultivar to regulate various physiological processes, including nutrient uptake, water-use efficiency, and metabolic flexibility. For instance, the greater fresh and dry weights observed in Gemlik could be attributed to its superior nutrient utilization efficiency (El-Mahdy et al., 2018). Olive cultivars with higher genetic adaptability often exhibit better growth under controlled tissue culture conditions, as their metabolic pathways are optimized for nutrient assimilation and biomass production (Martins et al., 2024). Thus, the enhanced performance of Gemlik is likely due not only to the culturing vessel but also to its genetic ability to thrive in a nutrient-rich, high-humidity environment.

On the other hand, the lower shoot induction percentage and slower shoot emergence in the Coratina cultivar may be linked to its genetic limitations in hormone regulation and nutrient assimilation (Figure 6). This cultivar might be less responsive to the cytokinin levels provided in the culture medium or may have a reduced capacity to efficiently balance its metabolic pathways under tissue culture conditions.

The culturing vessel also plays a crucial role in gas exchange, which is a key factor in explant growth (Vahdati et al., 2017). The enclosed environment of jars allows for controlled gas exchange, ensuring sufficient oxygen levels for cellular respiration while maintaining low ethylene concentrations, a plant hormone associated with stress (Florez et al., 2016). Oxygen availability is essential for cellular growth, as it supports oxidative phosphorylation in mitochondria, providing the energy required for various developmental processes (Wilson, 2017). In contrast, test tubes with cotton plugs may restrict gas exchange, potentially limiting oxygen availability and impeding cellular respiration, thereby slowing shoot induction and overall plant growth.

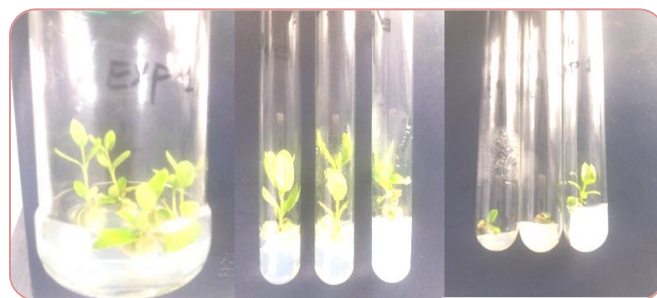


Figure 6. Cultivar Coratina cultured in jars with lids, test tubes with caps, and test tubes with cotton plugs.

Additionally, light exposure within jars is more consistent and controllable compared to test tubes with cotton plugs. Light intensity and duration directly influence photosynthetic activity, which affects carbohydrate production, an essential energy source for shoot growth and differentiation. The ability of jars to provide a stable light environment likely enhances the photosynthetic capacity of plantlets, leading to increased biomass accumulation and more vigorous growth (Hashim et al., 2021).

The significantly higher leaf water content observed in explants cultured in jars can be attributed to the high-humidity environment provided by the closed vessel system. Water retention in plant tissues is crucial for maintaining cellular turgor pressure, which is essential for cell expansion and growth (Feng et al., 2016). The sealed jar minimizes water loss through transpiration, thereby preserving water within the plant tissues, particularly in the leaves. This optimal water status helps sustain cellular functions and supports the development of healthy, turgid leaves, which are vital for efficient photosynthesis and overall plant health (Abd El-Mageed et al., 2017).

The enhanced leaf water content also highlights physiological differences between cultivars. The Gemlik cultivar, which exhibits higher leaf water content, may possess superior water-use efficiency mechanisms, such as more effective stomatal regulation or improved water uptake and retention within leaf tissues. These traits are particularly beneficial in micropropagation, as they help maintain physiological balance and reduce water stress, ultimately supporting better growth and development in a controlled culture system (Khan et al., 2018).

The success of micropropagation in olive cultivars is not solely determined by the culturing vessel or environmental conditions but is also significantly influenced by the intrinsic genetic traits of a cultivar. The Gemlik cultivar, which demonstrates superior performance in shoot induction, leaf development, and biomass accumulation, appears to be genetically predisposed to thrive in controlled culture conditions. This advantage is likely due to enhanced physiological processes such as nutrient uptake, hormonal regulation, and water retention. These findings emphasize the importance of considering both environmental factors (such as culturing vessels and nutrient media) and genetic characteristics when optimizing micropropagation protocols.

#### Correlation and principal component analysis of olive cultivars cultured in various vessels

Principal component analysis (PCA) was performed using the mean values of several key growth parameters in olive plants, including days to shoot induction (DSI), shoot induction percentage (SI%), fresh plantlet weight (FPW), dry plantlet weight (DPW), number of shoots per explant (NSE), shoot length (SL), number of leaves per shoot (NLS), fresh leaf weight (FLW), dry leaf weight (DLW), and leaf water content percentage (LWC%).

The PCA results, as depicted in the scree plot, indicated that the highest contribution to the total variance was from the first principal component (PC1), which explained 83.8% of the variance. The second principal component (PC2) contributed 7.5%, together accounting for 91.3% of the total variance. This suggests that the majority of the variation in the dataset is captured by these two components, providing a simplified yet effective representation of the data.

The analysis further illustrated the relationships among the traits, revealing a strong correlation among most of the measured variables. Notably, all traits except DSI exhibited a highly positive correlation along PC1. In contrast, DSI was negatively correlated with FPW, as

shown in Figure 7. This negative correlation suggests that plants with shorter shoot induction periods tend to have lower fresh plantlet weights, indicating a potential trade-off between rapid shoot formation and overall biomass accumulation. Eigenvalues for the growth-related parameters, presented in Figure 8, support these findings and highlight the primary contributors to the observed variance.

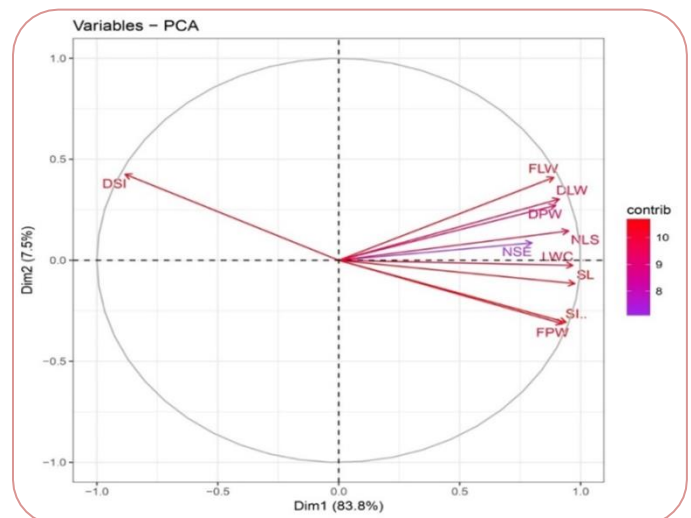


Figure 7. Principal component analysis of different attributes of olive cultivars cultured in different culturing vessels.

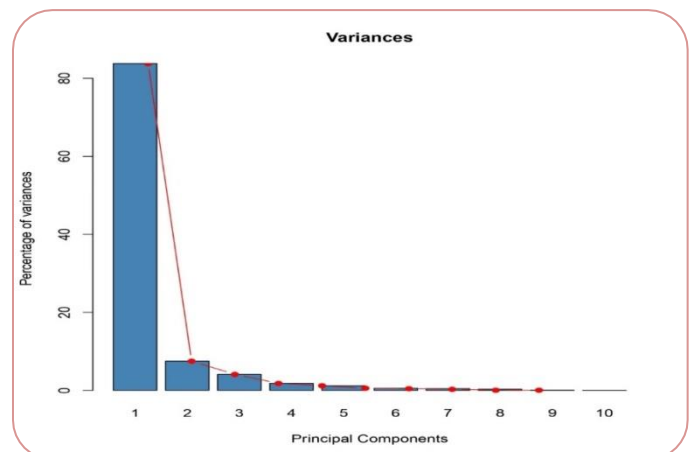


Figure 8. Eigenvalues for growth related parameters of olive cultivars cultured in different culturing vessels.

Correlation analysis (Figure 9) further clarified the relationships among the traits. DSI exhibited a negative correlation with all other measured parameters, indicating that faster shoot induction is associated with lower values in these traits. In contrast, SI% showed strong positive correlations with several traits, including FPW (0.96), DPW (0.76), NSE (0.67), SL (0.95), NLS

(0.84), FLW (0.70), DLW (0.79), and LWC% (0.90). This suggests that a higher shoot induction percentage is linked to improved growth outcomes across various parameters.

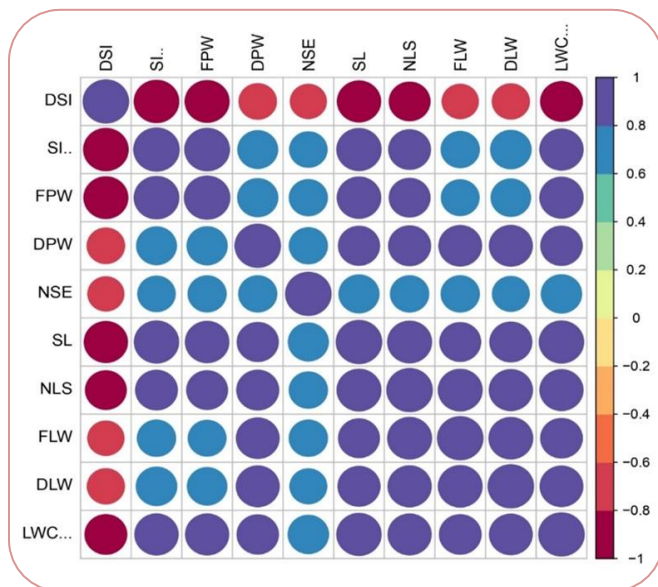


Figure 9. Correlation of different attributes of olive cultivars cultured in different culturing vessels.

FPW exhibited a strong positive correlation with most other traits, with correlation coefficients ranging from 0.77 to 0.88, highlighting its strong association with overall growth characteristics. Similarly, DPW showed a positive correlation with NSE (0.77), SL (0.81), NLS (0.81), FLW (0.85), DLW (0.89), and LWC% (0.83), indicating that plants with higher dry weight tend to have more shoots, longer shoots, and a greater number of leaves, as well as higher leaf weights and water content.

NSE was positively correlated with SL (0.77), NLS (0.70), FLW (0.70), DLW (0.65), and LWC% (0.70), suggesting that explants with more shoots also tend to develop longer shoots, produce more leaves, and exhibit higher leaf biomass. The correlation between SL and NLS was particularly strong (0.93), as were the correlations between SL and FLW (0.81), SL and DLW (0.85), and SL and LWC% (0.95). These results indicate that longer shoots are strongly associated with increased leaf production, leaf biomass, and water content.

NLS also displayed high positive correlations with FLW (0.93), DLW (0.90), and LWC% (0.89), further reinforcing the interdependence between leaf characteristics and overall plant growth. Moreover, FLW was positively correlated with DLW (0.92) and LWC%

(0.84), while DLW showed a strong positive correlation with LWC% (0.88), emphasizing the close relationship between leaf biomass and water content.

In conclusion, PCA analysis revealed strong interrelationships among various growth traits in olive plants, with SI% and SL emerging as key indicators of overall plant development. The negative correlation between DSI and other traits suggests a potential trade-off between rapid shoot induction and overall plant growth. The predominantly positive correlations among growth parameters indicate that improvements in one aspect, such as shoot length or leaf weight, are often accompanied by enhancements in other traits, providing valuable understandings for optimizing olive plant development.

## CONCLUSION

The superior performance of CV. Gemlik in shoot induction, plantlet fresh and dry weight (mg), number of shoots per explant, fresh and dry leaf weight (mg), number of leaves per shoot, shoot length (cm), leaf moisture content (%), and days to shoot induction can be attributed to enhanced physiological processes. These processes include improved nutrient uptake, hormonal regulation, and an optimized environment with controlled humidity, temperature, gas exchange, water retention, and leaf development in closed-lid jars. Together, these factors contributed to the successful micropropagation of the Gemlik cultivar. It is recommended that CV. Gemlik be cultured in closed-lid jars for efficient, disease-free micropropagation and growth.

## AUTHORS' CONTRIBUTIONS

MA conceived the Idea; GG supervised the experiment; ZUH performed the experiment and collected the data; HA, SJ and MSK prepared the manuscript and analyzed the data statistically and graphically; AAA and BR provided technical assistance during the experiment; HH and SAJ proofread the manuscript.

## CONFLICT OF INTEREST

The authors declare no conflict of interest.

## REFERENCES

- Abd El-Mageed, T.A., Semida, W.M., Rady, M.M., 2017. Moringa leaf extract as biostimulant improves water use efficiency, physio-biochemical attributes of squash plants under deficit irrigation. *Agricultural Water Management* 193, 46-54.
- Alam, R., Sajid, M., Rahman, K.U., 2021. Assessment of

- phenological, carpometric and yield allied attributes of olive cultivars harvested at different maturity stages. *Journal of Pure and Applied Agriculture* 6(4), 44-53.
- Awasthi, R., Bhandari, K., Nayyar, H., 2015. Temperature stress and redox homeostasis in agricultural crops. *Frontiers in Environmental Science* 3, 11.
- Bassi, D., Cirilli, M., Rossini, L., 2024. Most important fruit crops in Mediterranean Basin (Mb).
- Cardoso, J.C., Sheng, L.T.G., Teixeira da Silva, J.A., 2018. Micropropagation in the twenty-first century. *Plant Cell Culture Protocols*, 17-46.
- Das, A., Basu, P.S., Kumar, M., Ansari, J., Shukla, A., Thakur, S., Singh, P., Datta, S., Chaturvedi, S.K., Sheshshayee, M.S., Bansal, K.C., 2021. Transgenic chickpea (*Cicer arietinum* L.) harbouring AtDREB1a are physiologically better adapted to water deficit. *BMC Plant Biology* 21, 1-17.
- De Filippis, L.F., 2013. Crop improvement through tissue culture. *Improving Crops in the Era of Climate Change* 1, 289-346.
- El-Mahdy, M.T., Youssef, M., Eissa, M.A., 2018. Impact of *in vitro* cold stress on two banana genotypes based on physio-biochemical evaluation. *South African Journal of Botany* 119, 219-225.
- Feng, W., Lindner, H., Robbins, N.E., Dinnyen, J.R., 2016. Growing out of stress: the role of cell- and organ-scale growth control in plant water-stress responses. *The Plant Cell* 28(8), 1769-1782.
- Florez, S.L., Curtis, M.S., Shaw, S.E., Hamaker, N.K., Larsen, J.S., Curtis, W.R., 2016. A temporary immersion plant propagation bioreactor with decoupled gas and liquid flows for enhanced control of gas phase. *Biotechnology Progress* 32(2), 337-345.
- Fraga, H., Moriondo, M., Leolini, L., Santos, J.A., 2020. Mediterranean olive orchards under climate change: A review of future impacts and adaptation strategies. *Agronomy* 11(1), 56.
- Hashim, M., Ahmad, B., Drouet, S., Hano, C., Abbasi, B.H., Anjum, S., 2021. Comparative effects of different light sources on the production of key secondary metabolites in plants *in vitro* cultures. *Plants* 10(8), 1521.
- Khan, A., Pan, X., Najeeb, U., Tan, D.K.Y., Fahad, S., Zahoor, R., Luo, H., 2018. Coping with drought: stress and adaptive mechanisms, and management through cultural and molecular alternatives in cotton as vital constituents for plant stress resilience and fitness. *Biological Research* 51.
- Kyte, L., Kleyn, J., Scoggins, H., Bridgen, M., 2013. *Plants from test tubes: Introduction to Micropropagation*. 4<sup>th</sup> Edition. Timber Press, ISBN-10: 1604692065, ISBN-13: 978-1604692068.
- Lambardi, M., Fabbri, A., Micheli, M., Vitale, A., Baldoni, L., Caruso, T., Famiani, F., 2023. Olive propagation and nursery. *The Olive: Botany, Production and Uses*, 228-256.
- Martins, S., Pereira, S., Dinis, L.T., Brito, C., 2024. Enhancing olive cultivation resilience: Sustainable long-term and short-term adaptation strategies to alleviate climate change impacts. *Horticulturae* 10(10), 1066.
- Millan-Linares, M.C., Montserrat-de, L.S., Martin, M.E., 2021. Pectins and olive pectins: Biotechnology and human health. *Biology* 10(9), 860.
- Paiva-Martins, F., Kiritsakis, A., 2017. Olive fruit and olive oil composition and their functional compounds. In: *Olives and Olive Oil as Functional Foods: Bioactive Chemistry and Processing*, 81-115.
- Rehman, M., Awan, A.A., Khan, O., Haq, I., 2013. Response of olive cultivars to air-layering at various timings. *Pakistan Journal of Agricultural Sciences* 50(4), 555-558.
- Rodrigues, L., Estêvão, C., Garcia, R., Rato, A.E., Campos, C., Marques, M., Pires, R., Peixe, A., Cardoso, H., 2024. Metabolomics connecting genomes and phenomes in plant molecular breeding-A special focus on Mediterranean agronomically important tree species. *Plant Molecular Breeding in the Genomics Era: Concepts and Tools*, 283-319.
- Sosnowski, J., Truba, M., Vasileva, V., 2023. The impact of auxin and cytokinin on the growth and development of selected crops. *Agriculture* 13(3), 724.
- Vahdati, K., Asayesh, Z.M., Aliniaiefard, S., Leslie, C., 2017. Improvement of ex vitro desiccation through elevation of CO<sub>2</sub> concentration in the atmosphere of culture vessels during in vitro growth. *HortScience* 52(7), 1006-1012.
- Wilson, D.F., 2017. Oxidative phosphorylation: regulation and role in cellular and tissue metabolism. *The Journal of Physiology* 595(23), 7023-7038.