Chapter 8

Conclusion

For over five millennia, roses have captivated humans not only as ornamental plants but also for their medicinal properties and culinary uses. Celebrated as symbols of beauty and grace, they have inspired a wide array of artistic expressions. Economically, roses hold significant value as one of the top five ornamental plants globally. In many developed nations, the revenue generated from ornamental plant production surpasses that of vegetables and fruits. This economic importance makes roses a compelling subject for research and even as a model plant. What makes roses particularly suitable for this role is their unique combination of traits not found in any other woody species. These includes:

- A relatively small genome size.
- Vast biodiversity, encompassing a wide range of species and cultivated varieties with diverse morphological and physiological traits.
- Absence of a juvenility period, with recurrent flowering genotypes having a generation time of approximately one year.
- Ease of vegetative propagation for most genotypes.
- Simple production of segregating progeny at various ploidy levels, even between different species.
- Availability of multiple protocols for regeneration and transformation.
- Close phylogenetic ties to other rosaceous crops, many of which have more comprehensive genomic data available.

Moreover, roses exhibit distinctive morphological, physiological, and genetic traits that are not present in other model species. These include the production and release of a wide array of volatiles in petals, significant morphological diversity with various flower structures, growth forms, and prickle types, as well as a unique form of meiosis not observed in other taxa. Additionally, as long-lived woody perennials, roses provide opportunities to explore intriguing questions related to the generation and preservation of genetic diversity in natural populations, particularly in both diploid and polyploid species.

Our laboratory has successfully developed protocols for the micropropagation of rose plant species. However, the current use of agar-gelled semi-solid media in these protocols has significantly contributed to the high production costs of tissue-cultured plants. This has highlighted the need to establish more cost-effective methodologies. As a result, this study was undertaken to evaluate the feasibility of using a liquid culture system, eliminating the need for agar in the micropropagation of Rose plant species. The research also explored the effects of various factors such as support structures, temporary immersion, different types of vessels, stopper variations, and CO_2 enrichment when using a liquid medium. The study included an assessment of morpho-physiological and biochemical parameters in shoot cultures grown in liquid medium under different culture vessel conditions. This analysis aimed to provide insights into the degree of variation induced in the shoot cultures by the altered *in vitro* environment.

The increased proliferation rates associated with liquid culture systems are well-recognized when compared to agar-gelled solid media. In this study, the liquid medium notably stimulated the *in vitro* growth and shoot multiplication of roses. Significant improvements were observed in shoot growth, multiplication rate, and elongation when utilizing the liquid medium. Furthermore, the liquid medium led to a considerable increase in both the number and surface area of leaves.

During our investigation into selecting suitable supports for the liquid culture medium, we identified glass marbles as the optimal choice. The glass marbles used in the medium, produced locally, exhibited key characteristics such as inertness, autoclavability, and reusability, making them an ideal support option.

Temporary immersion or cultivation in a liquid medium was utilized for growing roses, which effectively promoted shoot elongation and multiplication. Moreover, a noticeable increase in leaf area was observed under these specific culture conditions.

CO_2 enrichment, whether applied in semi-solid or liquid medium, significantly enhanced *in vitro* shoot growth and proliferation of roses. The lack of essential carbon sources, such as CO_2 and sucrose, led to a gradual decline in the cultures, ultimately resulting in their failure in both semi-solid medium (SFSM) and liquid medium

(SFLM) due to nutrient deprivation. Notably, substantial improvements in *in vitro* growth and multiplication were observed when sucrose-free cultures were grown in a controlled environment with enriched CO_2, allowing the shoots to achieve full photoautotrophic growth. Additionally, a synergistic effect between CO_2 enrichment and sucrose in the medium was noted, promoting optimal *in vitro* plant growth. Furthermore, the liquid medium demonstrated superior overall growth compared to the semi-solid medium under CO_2-enriched conditions.

In the current study, the growth of rose cultures was significantly affected by the type of culture vessels used. The best shoot multiplication occurred in 250 ml flasks and Magenta™ boxes (square-shaped vessels), indicating their effectiveness in providing optimal growth conditions.

The choice of gelling agent in the media composition has a significant impact on the *in vitro* growth of roses cultivated on semi-solid medium. Notably, agar shows superior performance in promoting shoot proliferation, as well as increasing both dry weight and fresh weight.

Polyamines (PAs) positively impact rose micropropagules during multiplication by enhancing growth and biochemical parameters at moderate concentrations. This level of PAs increased shoot proliferation, biomass, carbohydrates, proteins, and antioxidant enzyme activity while reducing phenolic content, supporting plant growth and stress resilience. Higher concentrations reduced growth, indicating an optimal PA range for rose tissue cultures. PAs act as growth promoters and oxidative stress modulators by boosting POD and SOD enzyme activities.

In this study, efforts were made to improve the rooting capability of roses by using a liquid medium. Upon transferring the shoots propagated in the liquid medium to the rooting medium, successful root formation was achieved. Among the various plant systems evaluated, the best rooting response characterized by factors such as the number of roots, average root and shoot length, average number of leaves, and rooting percentage was observed in the agar-free liquid medium. Notably, glass marbles proved to be the most effective support matrix for *in vitro* rooting across all rose plants examined.

The use of a liquid culture system enabled the production of a significant number of healthy plants, improved plant traits, and a higher survival rate during the *in vitro* hardening process in rose plant.

In this study, we examined how *in vitro* conditions influence leaf surface structures using scanning electron microscopy and light microscopy. We compared leaf surface features, including stomatal frequency, stomatal size, epicuticular wax deposition, and stomatal function, between plants cultivated *in vitro* and those grown in the field. Notable differences were observed in these characteristics across leaves from various micropropagation stages and field-grown plants.

Histological analyses were carried out on the stems, leaves, and roots of Rose grown in semi-solid and liquid media to identify any structural differences between these conditions. Cross-sections of the aerial stem and leaves showed consistent anatomical features across both media for all three plant species, indicating a minimal impact of the liquid medium on hyperhydricity. However, transverse sections of the submerged roots revealed hyperhydric tissue development in the cortical area.

Variations in water loss percentages were observed throughout different stages of the Rose micropropagation process. Leaves from *in vitro* plants displayed higher water loss compared to those from *in vitro*-hardened plants, likely due to reduced wax deposition, increased stomatal density, and inconsistent stomatal function. The culture medium type did not significantly affect the water loss across any plant species.

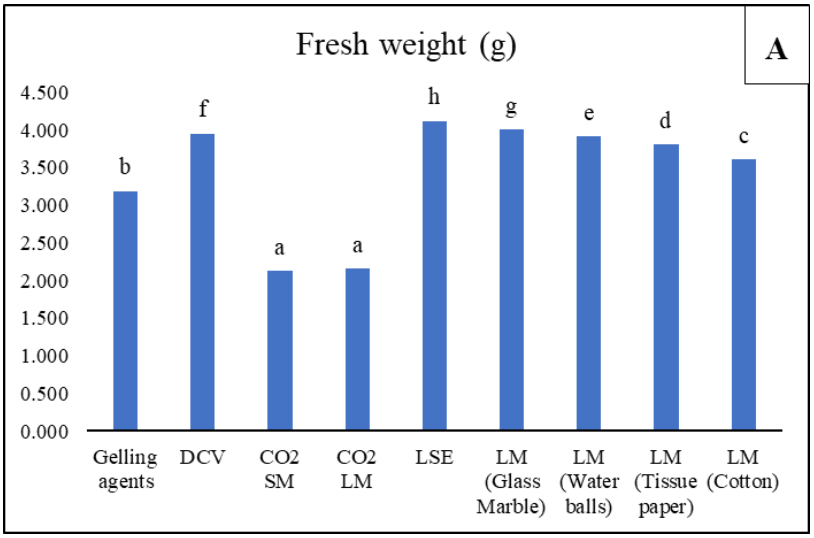
In the *in vitro* multiplication of Rose, fresh and dry weight accumulation was examined across different culture setups, with a liquid medium supported by glass marbles yielding the highest weights in shoot clumps. Factors like temporary immersion, carbon dioxide enrichment with sucrose, and the use of larger culture vessels further enhanced weight accumulation. A strong correlation was found between multiplication rate and biomass, showing that a higher proliferation rate led to increased shoot clump mass. Additionally, moisture content was consistently higher in shoot cultures grown in a liquid medium across all conditions.

The enzymatic activity of carbonic anhydrase (CA) was evaluated in Rose cultures grown *in vitro* compared to those in a natural field environment. Notably, CA activity was highest in the *in vitro* proliferating cultures grown in a liquid medium.

This research on Rose identified key changes in biochemical parameters, including metabolite and enzyme levels, at different stages of *in vitro* cultivation. Notable differences emerged between cultures grown on agar-gelled and semi-solid media compared to those in a liquid medium, particularly in carbohydrate accumulation, which varied by growth stage and medium type. Peroxidase (POD) activity decreased as plants progressed from multiplication to hardening, with liquid medium cultivation not leading to oxidative stress but displaying diverse response patterns, although total chlorophyll content was substantially higher in liquid medium cultures, indicating improved photosynthetic efficiency under these conditions.

The liquid medium proved to be beneficial for the proliferation of Rose, as conditions within the culture vessel, alongside the liquid medium, supported optimal growth. Morpho-physiological and biochemical assessments showed that the liquid medium did not induce significant stress, with observed morphological traits closely mirroring those seen in agar-gelled semi-solid media. The effectiveness of the liquid medium in large-scale Rose cultivation offers promising potential for developing a more cost-effective production system.

The genetic fidelity of Rose was evaluated using Random Amplified Polymorphic DNA (RAPD) analysis. After optimizing the polymerase chain reaction (PCR) conditions, genomic DNA was amplified using the RAPD method. A total of 53 randomly selected decamer primers were screened, leading to the identification of 24 primers that produced clear and easily scorable amplification products. Each primer yielded a unique set of amplification results. The plantlets analysed across different culture passages showed consistent RAPD profiles compared to the maternal plant, with no significant genetic variations observed under any of the growth conditions tested. While subtle differences in band intensity were noted, the number and size of the bands remained stable across all samples, indicating no significant variations in the profiles. These findings suggest that the accession examined maintains genetic stability across various cultured environments. In this investigation, the cultivation of plant tissue cultures under different growth conditions did not significantly affect the genetic integrity of the micro-clones. The Random Amplified Polymorphic DNA (RAPD) profiles remained unchanged across the various culture conditions.



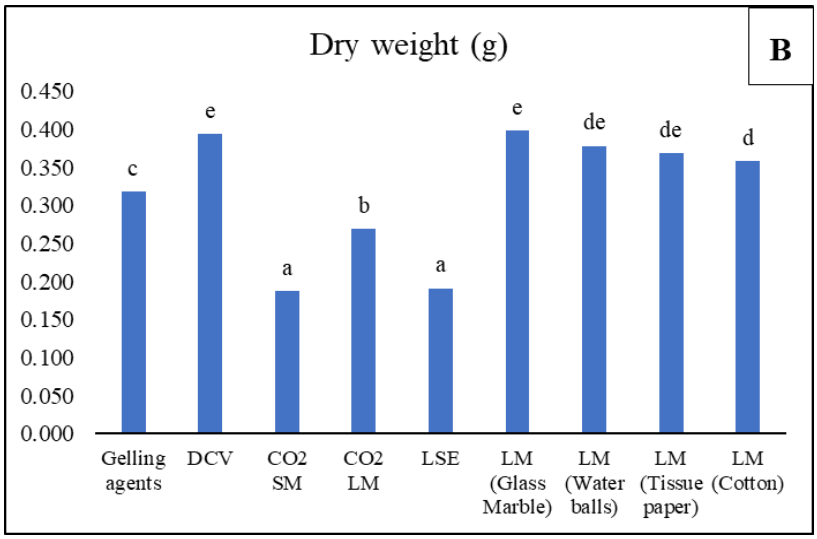


Figure 8.1(a) Summary of comparative analysis of different morphological and biochemical parameters: Effect of different growth condition on Rose during *in vitro* growth. In figure (A & B) comparison of each treatment effect on plant morphological parameters.

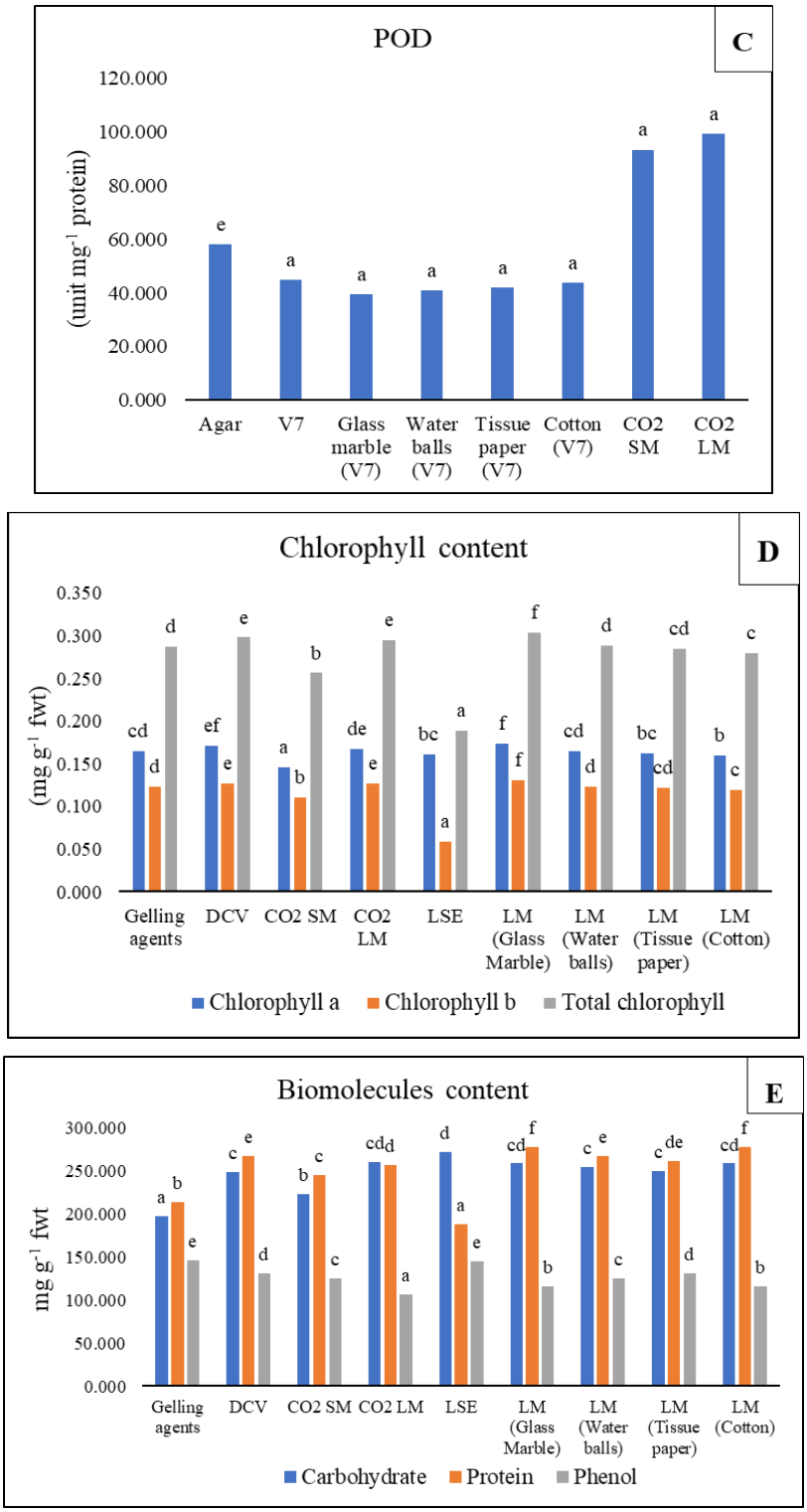


Figure 8.1(b) Summary of comparative analysis of different morphological and biochemical parameters: Effect of different growth condition on Rose during *in vitro* growth. In figure (C, D, & E) comparison of each treatment effect on plant biochemical parameters