Chapter 8

Summary

Musa Acuminata, commonly known as banana, stands as a significant botanical entity with multifaceted relevance. Indigenous to diverse regions, bananas play a pivotal role in agriculture, nutrition, and economic sustenance. In many states region in, India, offers a unique context for understanding the dynamics of banana cultivation and its impact on the local ecosystem. This tropical plant, renowned for its versatility and nutritional value, contributes substantially to the agricultural landscape. However, the implications of banana cultivation extend beyond mere agricultural considerations, intertwining with ecological and socio-economic dimensions. In the context of the prevailing agricultural practices and the demand for bananas, it becomes imperative to explore the intricate balance between cultivation practices and environmental sustainability in many regions. To meet the growing market demand for fruits, the imperative for large-scale production of crucial plant species has emerged. Our laboratory has successfully devised laboratory protocols for the micropropagation of *Musa acuminata* plant species. Notably, the existing protocols employed agar-gelled semi-solid media, contributing significantly to the overall production cost of tissue culture plants. Hence, there arose a necessity to formulate economically efficient methodologies. Consequently, this investigation was initiated to assess the appropriateness of a liquid culture system, obviating the requirement for agar in the micropropagation of *Musa* plant species. The study also delved into the impact of various variables, including support structures, temporary immersion, vessel types, stopper varieties, and CO_2 enrichment, in conjunction with the implementation of a liquid medium. The investigation encompassed the evaluation of morpho-physiological and biochemical parameters in shoot cultures cultivated in liquid medium, subjected to diverse culture vessel environments. This scrutiny aimed to contribute insights into the extent of deviations induced in the shoot cultures by the modified *in vitro* environment.

The heightened proliferation rates inherent in the liquid culture system are widely acknowledged in comparison to agar-gelled solid medium. In the current study, the liquid medium exerted a stimulating influence on the *in vitro* growth and shoot multiplication of *Musa acuminata*. Significantly favorable enhancements were

noted in the *in vitro* shoot growth, multiplication rate, and elongation when subjected to the liquid medium. Additionally, this aqueous medium demonstrated a consequential augmentation in both the quantity and surface area of leaves.

In the course of our examination regarding the selection of supports for liquid culture medium within the framework of our investigations, it was determined that glass bead support emerged as the optimal choice. The indigenous production of glass beads employed in the medium demonstrated characteristics of inertness, autoclavability, and reusability.

Temporary immersion or culture in a liquid medium was employed for the cultivation of *Musa acuminata*, facilitating elongation and multiplication of shoots. Additionally, a discernible augmentation in leaf area was observed under these specific culture conditions.

CO_2 enrichment, whether in semi-solid or liquid medium, exhibited a significant enhancement in *in vitro* shoot growth and proliferation of *Musa acuminata*. The absence of essential carbon sources, namely CO_2 and sucrose, led to a gradual decline in cultures, ultimately resulting in their demise in both Semi-Solid Medium (SFSM) and Semi-Liquid Medium (SFLM) due to nutrient deprivation. Remarkable enhancements in *in vitro* growth and multiplication were evident when sucrose-free cultures were cultivated in a controlled environment enriched with CO_2. Under these conditions, shoots demonstrated full photoautotrophic growth. Notably, a synergistic effect between CO_2 enrichment and sucrose in the medium was observed, fostering optimal *in vitro* plant growth. Furthermore, it was observed that the liquid medium outperformed the semi-solid medium in overall growth under CO_2-enriched conditions.

The growth of *Musa acuminata* cultures in the current study was markedly influenced by the type of culture vessels employed. Optimal shoot multiplication was observed in 250 ml flasks and Magenta™ boxes (square-shaped vessels), highlighting their efficacy for promoting superior growth conditions.

The selection of a gelling agent in the media composition significantly influences the *in vitro* growth of *Musa acuminata* cultivated on semi-solid medium. Notably, Phyta gel demonstrates superior growth performance in terms of shoot proliferation, dry weight, and fresh weight.

In the current investigation, endeavors were made to enhance the rooting capability of *Musa acuminata* by employing a liquid medium. Upon transferring the shoots propagated in the liquid medium to the rooting medium, root formation was successfully achieved. Among the various plant systems studied, optimal rooting response, characterized by parameters such as the number of roots, average root and shoot length, average number of leaves, and percentage of rooting, was discerned in the liquid medium devoid of agar. Notably, the utilization of glass beads emerged as the most efficacious support matrix for *in vitro* rooting across all examined species of *Musa acuminata.*

The implementation of a liquid culture system facilitated the attainment of a substantial quantity of proficient plants, enhanced plant characteristics, and an elevated survival percentage during the *in vitro* hardening process in *Musa acuminata*.

In the current investigation, an exploration of the impact of *in vitro* conditions on the leaf surface structures was conducted, employing scanning electron microscopy and light microscopy. Comparative analyses were performed on the leaf surface characteristics of plants undergoing micropropagation *in vitro* and those growing in a field setting. A diverse array of distinctions in stomatal frequency, stomatal size, epicuticular wax formation, and functional attributes of stomata were discerned across leaves at various stages of micropropagation and those from field-grown plants. Generally, *in vitro* leaves exhibited predominantly non-functional stomata and lacked epicuticular wax. The composition of the culture medium exerted a discernible influence on the leaf surface structures, with more pronounced changes observed in terms of stomatal size and frequency. Leaves cultivated on a liquid medium displayed larger stomata. The extent of malformation was heightened in leaves from liquid medium cultures across all three plant species, particularly in the case of leaves in the root differentiation stage (LM). Notably, structural alterations crucial for the appropriate acclimatization of micro propagated plants initiated at the root differentiation stage, with LR leaves displaying more pronounced changes. This suggests that plants cultivated in a liquid medium may require less time for the hardening and acclimatization process, potentially resulting in a higher survival rate upon transplantation.

Histological examinations were conducted on the stem, leaves, and roots of *Musa acuminata* cultivated in semi-solid and liquid mediums. The objective was to

discern any structural variations between the two growth conditions. Examination of cross sections of the aerial stem and leaves demonstrated that the anatomical characteristics remained largely consistent across both media types for all three plant species. This suggests minimal influence of the liquid medium in inducing hyperhydricity. Nevertheless, transverse sections of the submerged roots revealed the existence of hyperhydric tissues in the cortical region.

In this investigation, chlorophyll fluorescence parameters, including F0, Fm, Fv/Fm, ϕPS2, ETR, qP, and qN, were evaluated in *Musa acuminata* under cultivation in semi-solid and liquid mediums. The primary aim was to discern potential structural variations arising from the two distinct growth conditions. Analysis of cross-sectional anatomy of the aerial stem and leaves indicated a predominantly consistent anatomical profile across both media types for all three plant species. This observation implies minimal impact of the liquid medium on inducing hyperhydricity. However, examination of transverse sections of submerged roots revealed the presence of hyperhydric tissues within the cortical region. These assessments were conducted at various growth phases and on diverse media types. The investigations conducted suggest that the cultivated plants experienced a temporary stress within the *in vitro* environment, potentially attributed to low light intensity and photomixotrophic conditions. This stress-induced situation resulted in alterations to the ultrastructure of the chlorophyll molecule. Notably, the adverse conditions did not cause significant harm to the reaction centers. Although a considerable percentage of open photochemical centers remained accessible for photochemical reactions, it is plausible that the insufficient presence or impaired functionality of photosynthetic enzymes hindered their effective utilization. Assessment of these conditions was carried out at various growth phases and on diverse media formulations. The potential explanation for the diminished electron transport rate may underlie the observed decrease in the photosynthetic rate. These fluctuations began to reverse during the rooting phase. A juxtaposition of cultures cultivated in semi-solid and liquid culture systems demonstrated that the liquid medium did not elicit significant hyperhydricity symptoms, as evidenced by nearly identical fluorescence parameter values to their semi-solid counterparts. In certain instances, the physiological well-being of the plants thrived in the liquid medium compared to the semi-solid medium.

Consequently, the implementation of a liquid culture system proves efficacious in the micropropagation of these three pivotal medicinal plant species.

The evaluation of the photosynthetic capacity in *Musa acuminata* involved the introduction of CO_2 enrichment to the cultures, and this was measured through chlorophyll fluorescence assessments. In the case of *Musa acuminata* plant systems cultivated on a sucrose-supplemented medium, it was observed that CO_2 enrichment did not exert a significant impact on parameters such as Fv/Fm and ΦPS2, along with other fluorescence indicators in shoot cultures. Interestingly, the highest photochemical yield was documented in the *in vitro* multiplying cultures derived from SCSM and SCLM, cultivated under CO2-free conditions. Remarkably, under these conditions, the absence of carbon dioxide led to the maximum photochemical efficiency. Conversely, as the concentration of carbon dioxide increased, there was a subsequent decline in photochemical efficiency. This decline was attributed to the downregulation of photosynthesis, induced by the presence of sucrose in the growth medium.

In contrast, heightened carbon dioxide (CO_2) levels in the absence of sucrose within the growth medium significantly augmented the chlorophyll fluorescence parameters in *Musa acuminata*. The photoautotrophic cultivation of plantlets on saccharide-free medium facilitated the maturation of a fully operational photosynthetic apparatus.

Discrepancies in the percentage of water loss were documented across various phases of the micropropagation process of *Musa acuminata*. The *in vitro* leaves of plant exhibited elevated percentage water loss compared to *in vitro* hardened plants, attributable to limited wax deposition, heightened stomatal density, and the heterogeneous functionality of stomata. It was observed that the type of culture medium employed did not exert a statistically significant impact on the overall water loss in any of the plant species.

During the *in vitro* multiplication of *Musa acuminata*, the accrual of fresh and dry weights was documented in diverse culture environments, particularly on a liquid medium supported by glass beads. This investigation revealed that the glass bead-supported liquid medium consistently exhibited the highest content of fresh and dry weights in the shoot clumps in plant. Additionally, various factors, including temporary immersion, carbon dioxide enrichment with sucrose, and employing larger

culture vessels, were found to enhance the accumulation of fresh and dry weights in *Musa acuminata*. A significant correlation was observed between the proliferation rate and biomass accumulation, indicating that a higher rate of multiplication corresponded to an increased fresh mass of the shoot clump. It was consistently noted that the percentage of moisture content in shoot cultures was elevated when cultivated in a liquid medium across all experimental conditions.

The enzymatic activity of carbonic anhydrase (CA) was assessed in *in vitro* proliferating cultures of *Musa acuminata* and with their counterparts cultivated in the natural field environment. Remarkably, the CA activity exhibited its maximum in the *in vitro* proliferating cultures when cultivated in a liquid medium.

In the course of current research focused on *Musa acuminata*, alterations in biochemical parameters pertaining to metabolites and enzymes were noted across various stages of *in vitro* cultivation. Substantial variations in biochemical profiles were documented between cultures cultivated on agar-gelled and semi-solid medium in comparison to those on liquid medium. The accumulation of carbohydrates exhibited variability corresponding to both the growth stage and the nature of the culture medium. The Superoxide Dismutase (SOD) enzymatic activity in *Musa acuminata* exhibited a discernible decrease as the plants transitioned from the multiplication stage to the hardening stage. While the utilization of a liquid medium did not induce oxidative stress, the observed responses displayed a diverse range of characteristics. A study into proline accumulation unveiled an augmentation in its synthesis in *Musa acuminata* throughout various growth stages under the influence of osmotic stress induced by the liquid medium. Contrary to expectations, there was no significant augmentation in proline accumulation within liquid proliferating cultures of *Musa acuminata*. Conversely, the total chlorophyll content, as assessed in cultures cultivated in liquid medium, exhibited a notable increase when compare with those cultivated on agar medium. These findings suggest an enhanced photosynthetic proficiency in cultures subjected to liquid medium conditions.

Therefore, the aqueous medium demonstrated a stimulatory effect on the proliferation of *Musa acuminata*. Concurrently, various alterations in the conditions within the culture vessel were conducive to growth in conjunction with the liquid medium. The morpho-physiological and biochemical analyses indicated that the aqueous medium did not induce significant stress signals, and the morpho-

physiological aberrations observed were nearly analogous to those observed in semi-solid medium gelled with agar. The appropriateness of a liquid medium for the expansive-scale cultivation of *Musa* plant species undoubtedly paves the way for novel prospects in establishing an economically efficient production framework.

Genetic fidelity assessment of *Musa acuminata* was conducted through Random Amplified Polymorphic DNA (RAPD) analysis. Following the optimization of polymerase chain reaction (PCR) conditions, genomic DNA underwent PCR amplification employing RAPD methodology. A comprehensive screening of 53 randomly selected decamer primers was conducted, resulting in the identification of 22 primers that generated distinct and readily scorable amplification products. It is noteworthy that each primer exhibited a distinctive set of amplification products. The plantlets examined across various culture passages exhibited congruent RAPD (Random Amplified Polymorphic DNA) profiles in comparison to the maternal plant. No discernible genetic variations were noted under any growth condition investigated. Despite detecting subtle differences in RAPD profiles concerning band intensity, the quantity and size of bands remained consistent across all samples. Noteworthy is the absence of any significant variations in the profiles. The findings from this investigation suggest that the examined accession exhibits genetic stability under diverse growth conditions in cultured environments.

RAPD analysis was carried out to evaluate genetic fidelity of *Musa acuminata* grow in different growth condition. The selected primers yielded a total of 54 scorable bands with an average of 6 bands per primer. Number of bands for each primer varied from 6 to 10 (Table 6.3). The size of the bands produced by these primers ranged from 100 bp in OP-06 to 1200 bp in OP09. RAPD profiles indicted uniformity among all the micropropagules, obtained from different culture passages, as observed in the form of monomorphic banding patterns and were similar to the mother plant. All the primers produced monomorphic bands across all the micropropagules analyzed irrespective of the culture indicating that no genetic variation had occurred in any different growth condition during Micropropagation. Minor variability in terms of band intensity was observed in the RAPD profiles.

In the current investigation, the cultivation of plant tissue cultures under diverse growth conditions did not exert discernible impacts on the genetic integrity of

micro-clones. No alterations were discerned in the Random Amplified Polymorphic DNA (RAPD) profiles obtained across various culture.

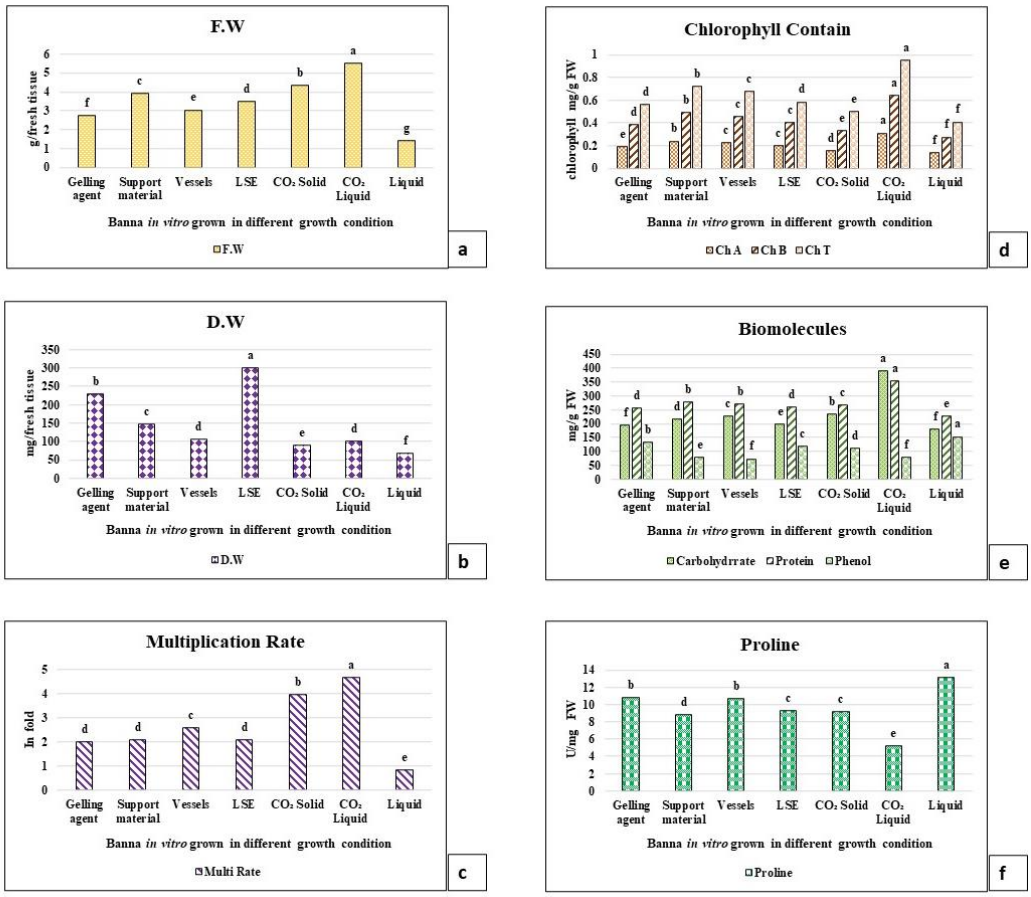

Figure: 8.1. Summary of comparative analysis of different morphological and biochemical parameters: Effect of different growth condition on *Musa acuminata* during *in vitro* growth. In figure (a, b, c) comparison of each treatment effect on plant morphological parameters. In figure (d, e, f) comparison of each treatment effect on plant biochemical parameters.