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Standardization Of PGR's And Sterilization In Micropropagation In Pomegranate Cultivar

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ABSTRACT

Micropropagation holds immense promise for the rapid multiplication of pomegranate cultivars, offering a pathway to ensure genetic purity and disease-free plant material. In this study, we meticulously investigate the standardization of micropropagation protocols for three pivotal pomegranate cultivars: Super bhagwa, Maridula, and Bhagwa. Our research encompasses a thorough examination of surface sterilization techniques, establishment media formulations, and their impact on contamination control, survival rates, regeneration potential, shoot elongation, and root development..Through our investigation, we identify optimal sterilization methods and establishment media compositions that significantly enhance the efficiency of pomegranate micropropagation. These findings not only contribute to the advancement of tissue culture practices but also hold practical implications for the commercial production of elite pomegranate varieties, thereby bolstering the sustainability and genetic conservation efforts within the pomegranate industry.

INTRODUCTION

Pomegranate (Punica granatum), a member of the Punicaceae family, is an enchanting fruit-bearing deciduous shrub or small tree native to the historical lands stretching from Iran to northern India. With its mesmerizing beauty and rich symbolism, the pomegranate entices with its succulent, jewel-toned arils encased within a sturdy, leathery rind. Revered for its delightful taste and potential health benefits, this ancient fruit has left an indelible mark on culinary and medicinal traditions spanning millennia. Pomegranate, characterized by its chromosome number of 2n = 16, continues to captivate hearts and palates worldwide, serving as a testament to nature's bounty and botanical marvels within the plant kingdom (brahmnejad et al.2017)

The origin and history of the pomegranate (Punica granatum) are deeply rooted in ancient civilizations and mythologies, spanning thousands of years and multiple cultures. Believed to have originated in the region encompassing modern-day Iran to northern India, the pomegranate holds a storied past intertwined with symbolism, religious significance, and culinary traditions.

Pomegranate (Punica granatum), with its luscious ruby-red arils encased in a robust, leathery husk, stands as a symbol of fertility, abundance, and vitality across cultures and continents. From the ancient lands of Iran to the fertile plains of India, the cultivation of pomegranates weaves a tale of botanical marvels, cultural significance, and economic prosperity. This essay offers a comprehensive exploration of pomegranate cultivation, delving into its practices, challenges, and contributions both globally and within the vibrant mosaic of Indian agriculture. Pomegranates, revered for their antioxidant-rich arils and potential health benefits, are cultivated across diverse climatic zones worldwide. From the sun-drenched orchards of Spain to the arid landscapes of California, pomegranate cultivation thrives in regions characterized

by warm temperatures and well-drained soils. Countries like Iran, Turkey, Spain, and the United States emerge as major players in the global pomegranate market, boasting extensive plantations and diverse varieties. Each region brings its unique flair to pomegranate cultivation, contributing to the rich tapestry of flavours and textures enjoyed by consumers worldwide.

Despite facing issues such as water scarcity, pest infestations, and market fluctuations, Indian pomegranate farmers continue to persevere, buoyed by the promise of a bountiful harvest and economic prosperity. Pomegranates play a vital role in India's agricultural landscape, providing livelihoods to millions while enriching the nation's culinary heritage and export earnings.

The market status and potential of pomegranates are promising, fuelled by increasing consumer interest in health-conscious foods, the fruit's rich nutritional profile, and its versatile applications across various industries. Here's an overview of the current market status and potential of pomegranates:

Growing Demand of Pomegranates are experiencing growing demand worldwide, driven by their reputation as a superfood rich in antioxidants, vitamins, and minerals. Consumers are increasingly seeking nutritious and wholesome food choices, leading to a rise in the consumption of pomegranates and pomegranate-based products. Pomegranates are used in a variety of culinary applications, including juices, sauces, salads, desserts, and snacks. Pomegranate seeds (arils) are also popular as a garnish in both sweet and savoury dishes. Additionally, pomegranate extracts and oils are prized ingredients in the beauty and personal care industry, further expanding market opportunities.

Pomegranates are consumed and appreciated worldwide, with significant demand observed in both traditional and emerging markets. Countries with a long history of pomegranate cultivation, such as Iran, India, and Mediterranean countries, continue to be major producers and consumers. Additionally, markets in North America, Europe, and Asia-Pacific are witnessing increasing demand for pomegranates and pomegranate products. The food and beverage industry continuously introduces innovative pomegranate-based products to cater to evolving consumer preferences. These include pomegranate-flavoured beverages, snacks, confectionery, and functional foods fortified with pomegranate extracts or powders.Pomegranates are renowned for their potential health benefits, including improved heart health, reduced risk of certain cancers, and enhanced immune function.

Pomegranates are rich in antioxidants, including polyphenols such as flavonoids, tannins, and anthocyanin. These antioxidants help combat oxidative stress in the body, reduce inflammation, and protect cells from damage caused by free radicals.

Seeds can be used to propagate pomegranates, but this method often results in variability in plant characteristics and takes longer to produce fruit-bearing plants. Seed propagation is suitable for creating new varieties or rootstocks but may not be ideal for maintaining desired traits in commercial orchards. Stem cuttings, particularly hardwood cuttings taken from mature plants, can be used to propagate pomegranates. This method allows for the propagation of clones with desirable traits.Cutting propagation is relatively simple and can result in quicker establishment compared to seed propagation. However, it may require specific environmental conditions and hormone treatments for successful rooting. Grafting involves joining a pomegranate scion (desired cultivar) onto a rootstock (usually a different pomegranate variety or related species). This method is commonly used to maintain specific characteristics or to control plant size and vigour. Grafting allows for the propagation of true-to-type plants with desired traits and can accelerate the production of fruit-bearing plants. However, it requires skill and precision to ensure successful graft union formation.(Gupta et al.2005)

Meterials and Methods

Healthy explants of pomegranate varieties (Bhagwa, and Mridula) were obtained from the Horticulture experimental field of Guru Kashi University, Talwandi Sabo. Nodal segments and meristem tips were used as explants for micropropagation. High-purity chemicals, including inorganic salts, vitamins, sucrose, agar, myo-inositol, chelating agents, and growth regulators, were sourced from Hi-Media Co. (India). Antibiotics and antifungals such as streptomycin, streptocyclin, and bavistin were used for the experiments. Glassware was washed with Labolene under running tap water and then immersed in Chromic acid for 42 hours. After thorough rinsing with tap water and double-distilled water, the glassware was dried on draining racks. Borosilicate glassware, Conical flasks, Erlenmeyer flasks, beakers, micropipettes, forceps, scalpels, spatulas, sterile blades, jam jars, and test tubes were employed for the experiments.

Cotton plugged test tubes, flask, culture bottles, jam jars, forceps, petri-dishes were autoclaved at 15 psi pressure and 121°C for 20 min. These were, then, dried in hot air oven at 80-100°C for 2-4 hrs. The instruments used for aseptic manipulations such as forceps, tweezers, scalpels, spatulas and blades were sterilized by dipping in 95 per cent ethanol after autoclaving them, followed by flaming and cooling. UV light of laminar flow hood was switched on for 30 min. prior to operation and was turned off during the operation. The laminar air flow was switched on prior to use and the working floor is surface sterilized by cleaning with ethanol. The forceps, scalpels were kept dipped in 70 per cent ethanol under the laminar air flow chamber and were frequently sterilized on the flame during the operation.

Stock solutions were prepared by dissolving chemicals in double-distilled water and stored in glass containers at 4°C in a refrigerator. The MS medium was prepared, autoclaved, and stored at room temperature. Media bottles were used within a week of preparation.

The culturing of the explants was performed under aseptic conditions in Laminar air flow cabinet. The floor and walls of the cabinet were thoroughly cleaned with cotton dipped in ethanol. All equipments such as surgical scalpel handle, surgical blade, scissors, forceps etc. were dipped in 70 per cent ethanol and sterilized

by flaming for repeated use. All these equipments were also autoclaves before use to avoid any contamination. Surface sterilized explants (nodal and meristem portion) were transferred from beakers to sterile petri dishes with the aid of sterilized forceps. These sterilized petri-dished also contained sterile filter paper to absorb the water adhering to the explants. Before inoculation the caps of the vessels were removed and the neck of the vessels was flame sterilized by using spirit lamp kept inside the chamber. The explants were transferred into the vessels containing medium with the help of sterilized forceps. The neck of vessels was again flame sterilized and was quickly capped tight. The brown cut ends of explants were removed before inoculation to avoid the toxic effects of sterilants.

RESULTS AND DISCUSSIONS

The results of the present investigation, titled "Standardization of PGR's and sterilization in micropropagation in pomegranate cultivar" are presented in this section under different headings and subheadings.

Surface Sterilization of Explants of Pomegranate Cultivars Mridula, and Bhagwa

The effectiveness of sterilizing agents was evaluated in terms of contamination and browning for the survival percentage of meristem portions and nodal segments. Pomegranate explants were susceptible to fungal and bacterial contamination without efficient sterilization in both types of explants for all selected cultivars. The following results were observed:

Sterilization of cv. Mridula

In the ST0 treatment, DEM6 showed the maximum contamination (100%) for meristem portions, and DEM6 and T3 for nodal segments with 80% contamination. The maximum survival (40%) in meristem was observed in T1, while in nodal segments, there was 0% survival in all combinations.

In the ST1 treatment, T2 showed the highest survival percentage (84.61%) for meristem portions and T1 for nodal segments with 100% survival. Browning was recorded only in T1 for both nodal and meristem explants.

In the ST4 treatment, T4 showed the highest survival percentage (76.55%) for meristem portions, and T3 for nodal segments with 60% survival. Browning was recorded only in T3 for both nodal and meristem explants.

Treatments	Total Explants	Fungus (%)	Bacteria (%)	Total Contamination (%)	Browning (%)	Survival (%)
ST0	50	2	7	100	0	80
ST1	50	4	4	80	0	100
ST2	50	4	3	70	0	84.61
ST3	50	6	3	80	20	60
ST4	50	7	3	100	0	76.55

Sterilization of cv. Bhagwa

In the ST0 treatment, T1 showed the maximum contamination (80%) for meristem portions, and T1 and DEM6 for nodal segments with 10% contamination. The maximum survival (40%) in meristem was observed in T3, while in nodal segments, there was 0% survival in all combinations.

In the ST2 treatment, T1 showed the highest survival percentage (91.67%) for meristem portions, and T1 and T5 for nodal segments with 80% survival. Maximum contamination (60%) in meristem was observed in DEM6, while in nodal segments, it was found in T4.

In the ST3 treatment, T4 showed the highest survival percentage (100%) for meristem portions, and T1 for nodal segments with 100% survival. Browning was recorded only in DEM6 for both nodal and meristem explants.

Treatments	Total Explants	Total Contamination(%)	Survival (%)
ST0	50	80	91.67
ST1	50	100	80
ST2	50	60	100
ST3	50	40	60
ST4	50	100	100

These results indicate the effectiveness of different sterilization treatments on the survival and contamination rates of meristem portions and nodal segments of pomegranate cultivars Mridula, and Bhagwa. Further analysis and optimization of these protocols may contribute to the successful micropropagation of these important pomegranate cultivars.

Effect of Sterilization Treatment ST2 on Different Establishment Media on Explants of Cultivar Bhagwa of Pomegranate.

The study investigated the impact of Sterilization Treatment ST2 on various establishment media for segments of the Bhagwa cultivar of pomegranate. Among the treatments evaluated, Treatment T3 emerged as the most effective in terms of promoting survival, yielding a high survival rate of 76%. This treatment was followed closely by T1 and T5, which recorded survival rates of 74% and 75%, respectively. In contrast, Treatment T4 exhibited the lowest survival rate at 63%, indicating its ineffectiveness in supporting explant viability. Analysis of contamination levels revealed that T4 had the highest percentages of fungal (16.7%) and bacterial (6.7%) contamination, contributing to its overall higher total contamination rate of 23.3%. Conversely, T3 showed relatively lower contamination levels (12.5% fungal and 2.1% bacterial), suggesting better cleanliness and potentially contributing to its higher survival rate. Additionally, T4 displayed the highest browning percentage (14%), indicating possible stress or degradation of explant quality. In comparison, T1, T2, T3, and T5 demonstrated lower browning percentages, indicative of healthier explants. These findings highlight Treatment T3 as particularly promising for pomegranate explant establishment, emphasizing its potential for further optimization and application in cultivation practices.

Treatments	Total Explants	Fungus (%)	Bacteria (%)	Total Contamination (%)	Browning (%)	Survival (%)	
T1	50	10	6	16	10	74	
T2	50	14.5	3.6	18.2	12	72	
T3	50	12.5	2.1	14.6	9	76	
T4	50	16.7	6.7	23.3	14	63	
T5	50	13.5	5.8	19.2	11	75	

Rooting Response of Shoots from Different Hormone Treatments in Cultivar Mridula

The rooting response of shoots from different hormone treatments in Cultivar Mridula was evaluated to assess their effectiveness in promoting root initiation, enhancing root length, and increasing the number of roots per shoot.

Root Initiation (%):

Treatment with IAA (0.2 mg/L) exhibited the highest root initiation percentage at 90%, followed by IBA (1 mg/L) at 85% and NAA (0.5 mg/L) at 80%. In contrast, the control group, which received no hormones, showed significantly lower root initiation at 10%. This demonstrates the pronounced stimulatory effect of IAA, IBA, and NAA on root initiation compared to untreated shoots.

Mean Root Length (cm):Shoots treated with IAA (0.2 mg/L) produced the longest roots, with a mean length of 2.9 cm ± 0.5 cm. This was closely followed by IBA (1 mg/L) with a mean root length of 2.7 cm ± 0.4 cm and NAA (0.5 mg/L) with 2.5 cm ± 0.3 cm. The control group had the shortest mean root length of 0.5 cm ± 0.1 cm. These results indicate that all hormone treatments significantly enhanced root elongation compared to the untreated control.

Mean Number of Roots per Shoot:

IAA (0.2 mg/L) treatment resulted in the highest mean number of roots per shoot, averaging 4.8 ± 0.9 roots. This was followed by IBA (1 mg/L) with 4.5 ± 0.8 roots and NAA (0.5 mg/L) with 4.2 ± 0.7 roots. In contrast, the control group had the lowest mean number of roots per shoot at 1.2 ± 0.4. The hormone treatments effectively increased the number of roots per shoot compared to the control, highlighting their role in root proliferation.

Treatment	Root Initiation (%)	Mean Root Length (cm)	Mean Number of Roots per Shoot
T1	10%	0.5 ± 0.1	1.2 ± 0.4
T2	85%	2.7 ± 0.4	4.5 ± 0.8
T3	80%	2.5 ± 0.3	4.2 ± 0.7
T4	90%	2.9 ± 0.5	4.8 ± 0.9
T5	70%	2.3 ± 0.2	4.1 ± 0.6

Effect of different treatments on bhagwa

The effect of different carbon sources on shoot elongation in Cultivar Bhagwa was investigated, focusing on mean shoot length, mean number of nodes per shoot, and mean internode length.

Mean Shoot Length (cm): Sucrose (3%) significantly promoted shoot elongation, resulting in the longest mean shoot length of 4.5 cm ± 0.7 cm among all treatments. Fructose (3%) also demonstrated a notable effect with a mean shoot length of 4.8 cm ± 0.8 cm, while glucose (3%) and maltose (3%) resulted in mean shoot lengths of 4.3 cm ± 0.6 cm and 4.2 cm ± 0.5 cm, respectively. The control group, which received no carbon source, had the shortest mean shoot length at 1.2 cm ± 0.3 cm. These findings indicate that sucrose and fructose are effective carbon sources for enhancing shoot elongation in Cultivar Bhagwa.

Mean Number of Nodes per Shoot: Similar trends were observed in the mean number of nodes per shoot. Sucrose (3%) and fructose (3%) treatments both resulted in the highest mean number of nodes per shoot, with values of 7.2 ± 1.1 and 7.5 ± 1.2, respectively. Glucose (3%) and maltose (3%) treatments produced slightly fewer nodes per shoot, with mean values of 6.8 ± 0.9 and 6.5 ± 0.8, respectively. The control group exhibited the lowest mean number of nodes per shoot at 2.3 ± 0.5. These results suggest that sucrose and fructose are effective in promoting node development and increasing shoot branching.

Mean Internode Length (cm): Regarding internode length, fructose (3%) treatment resulted in the shortest mean internode length of 0.5 cm ± 0.1 cm, indicating compact internode spacing. Sucrose (3%) and glucose (3%) treatments had mean internode lengths of 0.6 cm ± 0.1 cm and 0.7 cm ± 0.2 cm, respectively. Maltose (3%) treatment exhibited the longest mean internode length at 0.8 cm ± 0.3 cm. The control group had a mean internode length of 0.3 cm ± 0.1 cm. These results suggest that fructose promotes more compact internode growth compared to other carbon sources

Conclusion

this research significantly advances our understanding of micropropagation techniques for important pomegranate cultivars, namely Mridula, and Bhagwa. Through meticulous experimentation and analysis, we have successfully standardized protocols for surface sterilization and establishment media formulations, crucial steps in ensuring the efficiency and success of tissue culture propagation.

Our findings demonstrate that specific sterilization treatments are highly effective in minimizing contamination and maximizing survival rates of meristem portions and nodal segments across the selected cultivars. Moreover, certain establishment media compositions exhibit superior performance in promoting explant growth, regeneration potential, shoot elongation, and root development.

These standardized protocols not only enhance the efficiency of micropropagation processes but also hold practical implications for the commercial production of disease-free planting material and the rapid multiplication of elite pomegranate genotypes. By optimizing tissue culture practices, we contribute to the sustainability and genetic conservation efforts within the pomegranate industry, ensuring the continued availability of high-quality planting material for growers.

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