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Micropropagation of Miniature Rose cv. Ice Fairy: Impact of growth regulators on *in-vitro* shooting and rooting

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Abstract

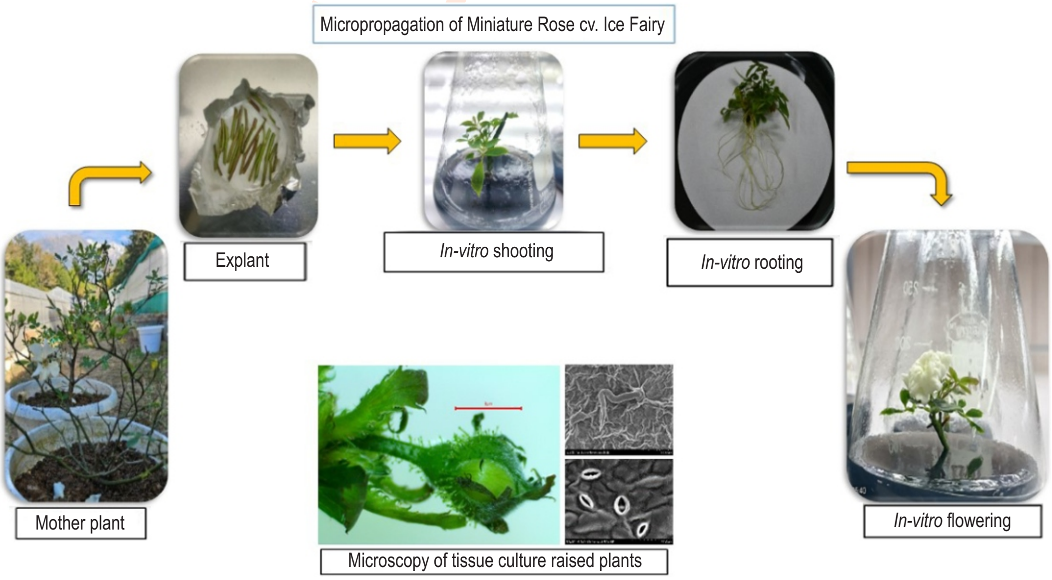
Aim: The aim of this experiment was to optimize a procedure for *in-vitro* micro-propagation of Miniature Rose cv. Ice Fairy (*Rosa hybrida* L.) utilizing the nodal segments as an explant.

Methodology: The impact of cytokinins and gibberellins was investigated under *in-vitro* conditions. Different levels of BAP (6- Benzylaminopurine) (1, 1.5, 2, 2.5 mg l^{-1}), focusing on the developmental stages of explant whereas GA$_3$ (Gibberellic acid) was employed for shoot elongation and flowering. Activated charcoal (1 g l^{-1}) was added into the medium to inhibit phenolic oxidation.

Results: The highest shoot initiation was found in treatment T$_4$ (94 %) with culture survival rate of 86 % when Murashige and Skoog (MS) medium was supplemented with 2.5 mg l^{-1} BAP and 1 g l^{-1} activated charcoal. The highest number of elongated shoots were noted in the medium containing 2.5 mg l^{-1} BAP and 1 mg l^{-1} GA$_3$. It was observed that MS media supplemented with BAP was successful for shooting as well as rooting. *In-vitro* plantlets with 5 to 6 roots, each measuring 4-5 cm in length were carefully removed and transplanted into sterilized glass jars. The jars had a mixture of sand, coco-peat and soil in 1:1:2 (v/v) ratio, which was moistened with 30 % of MS basal liquid medium.

Interpretation: These findings may therefore contribute towards the commercial micropropagation of miniature roses.

Key words: 6-Benzylaminopurine, *In-vitro* propagation, Miniature rose, MS media, Phenolic oxidation



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Introduction

Rose commonly referred to as the queen of flowers, is an iconic symbol of beauty and elegance in horticulture. It is a type of woody perennial flowering plant within the Rosaceae family. While most species are indigenous to Asia, some are also native to Europe, North America and NorthWestern Africa (Sharma and Pathak., 2024). The primary rose classes include hybrid tea, floribunda, grandiflora, climbers, shrubs, miniatures, etc. Each class offers distinct traits ranging from size and shape of the flowers to the growth patterns and hardiness of the plants (Afrin *et al.,* 2022). Miniature roses are popular floriculture crop, known for their compact size, vibrant colours and ornamental appeal. They are often used in both landscaping and as house plants making them a subject of great interest in both horticultural research and plant tissue culture studies. Micropropagation techniques are widely employed for the large scale propagation of miniature roses (Riya *et al.,* 2024). Some of the earliest miniature roses have been derived from species like China rose (*Rosa chinensis*) and Tea rose (*Rosa odorata*), both known for their smaller blooms and compact growth habits. Roses offer a range of medicinal benefits including anti-inflammatory, anti-diabetic, anti-ageing and anti-depressant properties (Wang, 2024).

In addition to its medicinal benefits, roses are highly valuable in the industrial sector, especially for making cosmetics and perfumes (Muiruri *et al.,* 2011). This global fascination has driven continuous efforts to enhance their characteristics such as improvement for diverse array of colors, increased disease resistance, and prolonged flowering periods (Oo *et al.,* 2021). More than 200 Rose species and approximately 25,000 cultivars of modern roses are available (Sadar *et al.,* 2022, Kumari *et al.,* 2022). Major constraints in rose improvement include slow progress of conventional breeding methods due to the perennial nature of the crop. Conventional propagation methods involve challenges like seasonal variations and inherently low rates of multiplication (Rahman *et al.,* 2023). Additionally, a high degree of sterility has been also observed, due to mismatched chromosome numbers. The crop also suffers significant losses from pest infestations, fungal (black spot, powdery mildew, dieback) and viral diseases. To overcome these limitations, tissue culture techniques have emerged as a transformative approach in rose cultivation (Saklani *et al.,* 2015). The utilization of this technique has helped to enhance the traits of rose, aiming to improve the quality and durability of rose varieties (Attia *et al.,* 2012; Tolembetoba *et al.,* 2017). Tissue culture is widely being utilized for the mass propagation of superior varieties, enabling propagation of genetically uniform and disease-free plants (Pati *et al.,* 2010).

Additional to micro propagation, plant tissue culture techniques have been used for somatic hybrid development, improvement of plant, ploidy manipulation and synthetic seed production (Mehbub *et al.,* 2022). *In vitro* propagation offers numerous advantages for miniature roses, including accelerated growth, early flowering and production of compact, well branched plants, which are ideal for ornamental purposes. The conventional propagation of Miniature Rose cv. 'Ice Fairy' proved challenging due to its low success rate. Hence, the present study aims to optimize an effective micro-propagation protocol for the efficient bulk production of Miniature Rose cv. Ice Fairy using nodal segments.

Materials and Methods

Plant material: Nodal explants bearing lateral branches of Miniature Rose cv. Ice Fairy (*Rosa hybrida* L.) were obtained from the Rose germplasm preserved at the CSIR-Institute of Himalayan Bioresource Technology, located in Palampur, India.

Determination of survival percent and contamination rate: These provide a quantitative measure to assess the survival rate of cultures. Both the metrics were calculated by the following formulas:

$$\text{Survival (\%)} = \frac{\textit{No of survived explants}}{\textit{Total no of cultured explants}} \times 100$$

$$\text{Microbial contamination (\%)} = \frac{\textit{No of contaminated explants}}{\textit{Total no of cultured explants}} \times 100$$

Preparation of explants: The nodal explants were sectioned into 3-4 cm long segments and washed thoroughly undertap water for 20-30 min to eliminate surface dust followed by a detergent, Tween-20 for 3 min and surface sterilized with 70 % ethanol for 60 sec. Prior to culture, the explants were treated with 1 % Bavistin for 20 min with continuous shaking and rinsed subsequently three to four times with distilled water, followed by a wash in 0.1 % $HgCl_2$ for 30 sec inside a horizontal laminar air flow. Explants were thoroughly washed with distilled water to remove any traces of disinfectant and dried on an autoclaved Whatman filter papers (90 mm).

Media preparation for *in-vitro* propagation: A full-strength MS media comprising sucrose (30 g l^{-1}), activated charcoal (1 g l^{-1}), agar (8 g l^{-1}) was prepared. A total of five treatments were designed with 5 replicates having 4 explants in a flask each following a completely randomized design for the study. The treatments were T_1 (MS + 1 mg l^{-1} BAP), T_2 (MS +1.5 mg l^{-1} BAP), T_3 (MS +2 mg l^{-1} BAP), T_4 (MS +2.5 mg l^{-1} BAP), Control (MS media without growth hormone). Varied concentrations of 6-Benzylaminopurine (1, 1.5, 2, 2.5 mg l^{-1}) were used for initiation and multiplication of shoot and root. GA_3 (1 mg l^{-1}) was administered for promoting shoot elongation and flowering. The pH of each media was adjusted to 5.8 with1N HCl and 1N NaOH before incorporating 0.8 % (w/v) agar. The medium was sterilized by autoclaving the media at 121°C and 15 psi pressure for 15 min. After autoclaving, the media were allowed to solidify undisturbed for 48 hr. Nodal explant comprising laterals were cultured for a month on shoot initiation media. The media consisted of full-strength medium comprising different concentrations of hormone BAP (1, 1.5, 2, 2.5 mg l^{-1}) and 1 g l^{-1} activated charcoal, as it inhibits phenolic oxidation. Root initiation and multiplication were achieved in the presence of BAP. For initiating multiplication of shoots and shoot elongation, shoots having roots were transferred to full strength MS medium containing different concentrations of BAP (1, 1.5, 2, 2.5 mg l^{-1}) with 1 mg l^{-1} GA_3 and 1 g l^{-1} activated charcoal for additional 4 weeks. *In-vitro* cultures were kept in a culture room at 26±2°C and were subjected to 16 hr light and 8 hr dark period, with a light intensity of 3000 lux sourced from a white fluorescent light.

Acclimatization: *In-vitro* plantlets with 5 to 6 roots, were carefully extracted from 250 ml conical flaks after third subculture and was thoroughly washed with double distilled water to remove agar residue and any remnants of growth medium. Subsequently, the roots were immersed in 0.1 % Bavistin solution for 30 sec and then transplanted into autoclaved glass jars. These jars were filled with a mixture of sand, coco-peat and soil (1:1:2 v/v), and moistened with 30 % MS basal liquid medium. Plantlets then were transferred to pots containing media comprising of sand, soil, FYM (1:1:1 v/v) and NPK fertilizer (19:19:19 v/v) once a month and were covered with a transparent polyethylene sheet for the first ten days of growth. The plants were transplanted to 25 cm pots containing media soil, sand, cocopeat and FYM (1:1:1:2 v/v) after 8 weeks for acclimatization.

Microscopic analysis: Microscopic observations, from the day of shoot initiation to budding, were conducted using a Magnus TZM6 microscope (Magcam DC10) with the assistance of Magvision software. The microscopic data showed shoot initiation, elongation, root multiplication and bud having trichomes (delicate outgrowths that aid in plant development and offer protection against diseases and pests). Further microscopic analysis was conducted using a HITACHI S-3400N Scanning electron microscope (SEM), with a magnification range 10x to 300,000x. The leaf samples were taken from the mother plant and from the tissue cultured raised plants. The samples were mounted on an aluminum stub and prepared by coating them with a gold palladium using a HITACHI E-1010 ION SPUTTER.

Statistical analysis: The experiments were conducted with three replicates each, following a completely randomized design (CRD). Data were collected every week after culturing from various experiments laid out to check the efficacy of hormones in in-vitro propagation. Statistical analysis involved performing ANOVA and separation of means by DMRT, with significance determined at $p \leq 0.05$ level. Data was analyzed by SPSS software (IBM Statistics software SPSS 20).

Results and Discussion

Survival percent shows that the rate of explants that survived under *in-vitro* conditions, while contamination rate indicates the cultures which were contaminated. The highest survival percent was observed in T_4 treatment, having BAP at 2.5 mg l^{-1} and activated charcoal at 1 g l^{-1}. This treatment achieved a survival rate of 86 % with lowest contamination rate (14%). The minimum response with respect to explant survival was observed in growth regulator free MS medium (control) (Fig. 1) with highest contamination rate. Similar results were reported by Namita *et al.* (2015) in rose cv. Happiness, where the maximum number of shoots were observed with treatment containing 2.5 mg l^{-1} BAP. The optimal dose of growth regulators results in effective shoot prolifertaion. The beneficial effects of BAP on various metabolic processes are well-documented and influence plant metabolism (Kulaeva, 1980). BAP is a synthetic cytokinin, a plant hormone essential for regulating cell division, shoot formation and bud development. The enhanced shoot proliferation in tissue culture is due to optimal BAP concentrations that stimulate axillary branching. By encouraging the growth of axillary buds, BAP facilitates the development of multiple shoots from a single explant, leading to shoot proliferation.

Shoot formation: The data demonstrated the effectiveness of BAP as a hormone promoting shoot formation as it plays a crucial role in plant metabolism by enhancing cell division and promoting the development of shoots, thus influencing the overall growth and regeneration processes. Increasing the BAP concentration from 2 to 2.5 mg l^{-1} accelerated shoot development, reducing the time required for shoot formation. The optimal shoot formation was achieved at 2.5 mg l^{-1} of BAP, occurring 6.1 days after inoculation (Table 1; Fig. 2,3). As the BAP concentration decreased, both the number of shoots per explants decreased and the time required for shoot initiation was increased.

Table 1: Effect of varied BAP concentrations on shoot initiation from nodal explants of Miniature Rose cv. Ice Fairy

Treatments	Shoot formation observed in flasks	Days taken to shoot formation	No. of leaves/ shoot
T_1	4.6±0.40	9.2 ± 2.47^{c}	2.2 ± 0.58^{ab}
T_2	5.4±0.25	8.59 ± 2.95^{c}	1.4 ± 0.6^{b}
T_3	7.2±0.33	7 ± 3.2^{b}	2.6 ± 0.81^{ab}
T_4	9.4±0.21	6.1 ± 0.37^{a}	3.8 ± 0.48^{a}
Control	4.4±0.55	10.4 ± 3.8^{d}	0.8 ± 0.58^{b}
CD (0.05)	NS	1.981	1.838

Values are mean of three replicates ± S.E. Different letters accompanying values indicates substantial changes ($p \leq 0.05$), determined through Duncan's Multiple Range Test. T_1 (MS + 1 mg l^{-1} BAP), T_2 (MS + 1.5 mg l^{-1} BAP), T_3 (MS + 2 mg l^{-1} BAP), T_4 (MS + 2.5 mg l^{-1} BAP), Control (MS media without growth hormone)

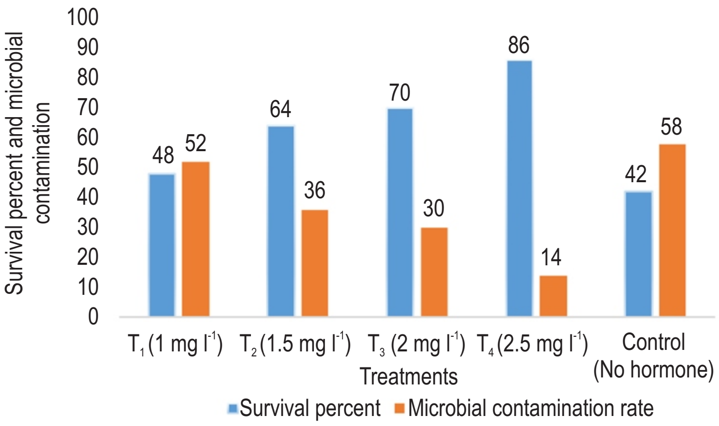

Fig. 1: Graph showing survival percenatge and microbial contamination rate of Miniature Rose cv. Ice Fairy different treatments where T_1 (MS + 1 mg l^{-1} BAP), T_2 (MS + 1.5 mg l^{-1} BAP), T_3 (MS + 2 mg l^{-1} BAP), T_4 (MS + 2.5 mg l^{-1} BAP), Control (MS media without growth hormone).

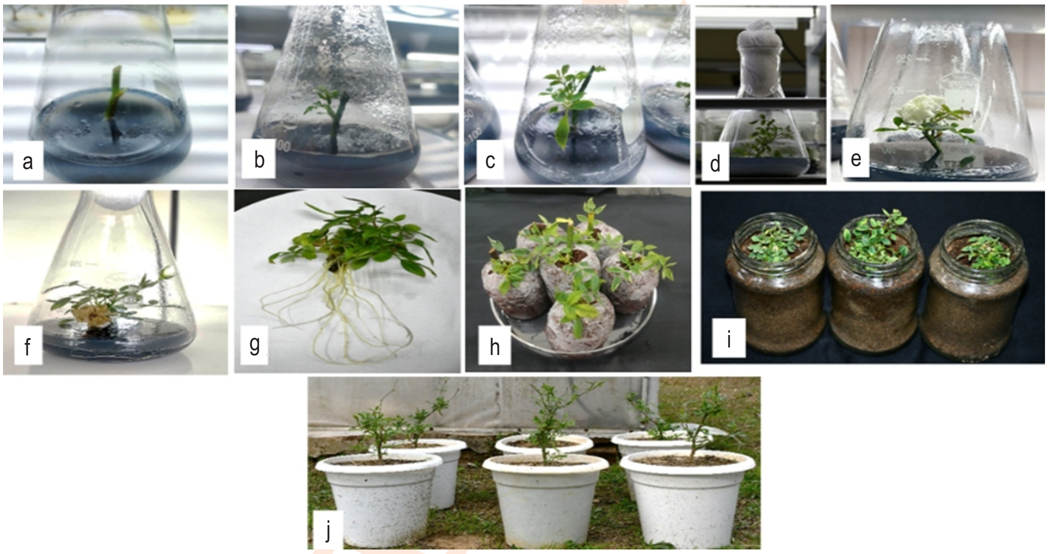

Fig. 2: Micropropagation of Miniature Rose cv. Ice Fairy. (a) explant cultured; (b) culture showing shoot initiatian; (c) leaves appear on shoot after a week; (d) after first subculturing; (e) apperaence of first flowering after second subculturing; (f) flask showing appearance of second bud; (g) plant with elongated shoots and roots after third subculturing; (h) healthy plants tranferred to cocopeat pellets; (i) plants transferred to autoclaved jars containing sand: coco-peat: soil for a week; (j) acclimatized plants in pots.

At 1.5 mg l^{-1} BAP, shoot initiation occurred after 8.59 days, while at 1 mg l^{-1}, it took 9.2 days for shoot formation. A successful micro-propagation protocol consists of several steps, each with their own set of requirements. Pati *et al.* (2010) defined these as: establishing disease-free cultures; multiplication of shoot; initiation of root and acclimatization and transferring the *in-vitro* raised plants to field. The objective of this study was optimization of culture conditions for the *in-vitro* micro-propagation of miniature rose using various concentrations of growth hormones either individually or in combination. The findings of this study indicate that shoot formation, multiplication and root development were successfully accomplished using medium supplemented with varying BAP concentrations. Specifically, the maximum rate of shoot formation from nodal explants occurred when BAP concentrations ranging 2.5 mg l^{-1} were tested in MS medium at 26°C. Whereas, lower concentrations of BAP resulted in less shoot proliferation. According to Khoshkhui and Jabbarzadeh (2005), the optimal treatment for proliferation involved amalgamation of 2.5-3 mg l^{-1} BAP and 0.1 mg l^{-1} of IBA. Thi *et al.* (2008) proved that media containing with 3 mg l^{-1} BAP was optimal for both shoot initiation and multiplication in roses. In another study, Houng *et al.* (2021) was found that when the media was supplemented with 1.5 mg l^{-1} BAP

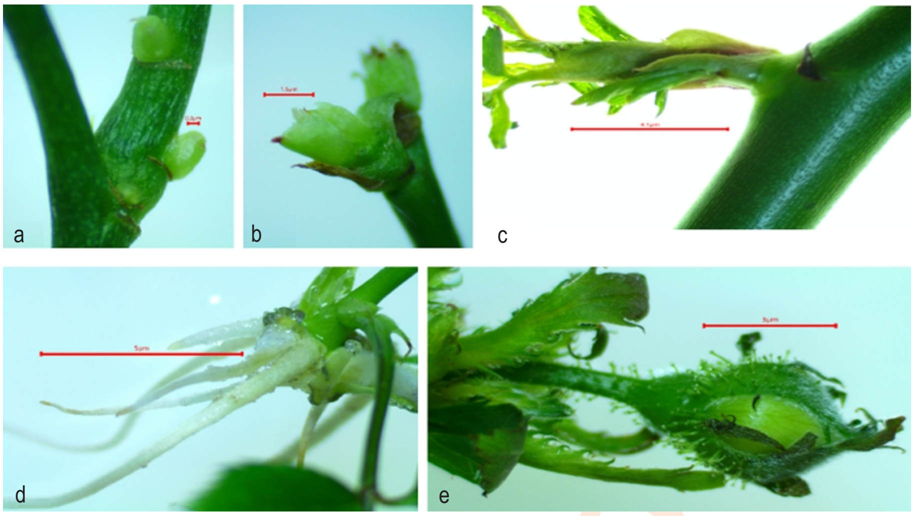

Fig. 3: Microscopy of micropropagation of Miniature Rose cv. Ice Fairy. (a) shoot initiation (scale bar=0.3 µm); (b) shoot formation (scale bar=1.5 µm); (c) complete shoot formation showing leaves (scale bar=4.1 µm); (d) healthy roots after second subculturing (scale bar=5 µm); (e) bud showing trichomes (scale bar=3 µm).

Table 2: Effect of different BAP concentrations on root formation from nodal explants of Miniature Rose cv. Ice Fairy

Treatments	Root formation seen in flasks	Days taken to root formation	Root length (cm)
T_1	3.2±0.11^d	9.6±3.93^b	3.23^c
T_2	5.1±0.10^b	10.75±4.37^c	4.76^{ab}
T_3	6.5±0.11^{ab}	9.55±4.3^b	5.34^a
T_4	7.2±0.19^c	8.7±2.28^a	5.61^a
Control	2.7±0.21^e	11.70±4.39^{cd}	2.12^d
CD (0.05)	0.53	0.59	0.59

Values are mean of three replicates ± S.E. Different letters accompanying values indicates substantial changes ($p \leq 0.05$), determined through Duncan's Multiple Range Test. T_1 (MS + 1 mg l^{-1} BAP), T_2 (MS + 1.5 mg l^{-1} BAP), T_3 (MS + 2 mg l^{-1} BAP), T_4 (MS + 2.5 mg l^{-1} BAP), Control (MS media without growth hormone)

and 6 parts per million (ppm) Nano silver the regeneration of shoot rate exceeded by 95.56 %. Among various cytokinins tested for rose proliferation, BAP consistently yielded the highest proliferation rates.

Root formation: In the present study the earliest root induction was observed with 2.5 mg l^{-1} BAP within 15 days of sub culture (Table 2; Fig. 2,3). The induction of roots during shoot induction process suggests interaction between BAP and the endogenous hormonal balance of the plant, especially under *in-vitro* conditions. This interaction likely facilitated root development. Similar results were reported by Khairudin *et al.* (2020) who observed spontaneous root induction during the process of shooting in which the earliest root induction was observed in the treatments using 1.5, 2.0 and 3.0 mg l^{-1} BAP. The maximum number of roots per shoot were seen when BAP concentration increased from 2 to 2.5 mg l^{-1}. In this study, BAP alone demonstrated greater efficacy in promoting root formation. Data shown in Table 2 depicts that the number of days taken for root formation were less in treatment T_4 which contained 2.5 mg l^{-1} BAP. The percent of root formation was positively correlated with increasing concentrations of BAP. As shown in Fig. 3, the roots were formed after 2nd sub culture with differentiated root primordia and the scale bar 5 µm. As illustrated in Fig. 2, the rooting was completely achieved after 3rd sub culture in media containing BAP. Otiende *et al.* (2021) reported that higher levels of IAA and an increased auxin to cytokinin ratio in the stem base contribute to the positive acropetal gradient that enhances rooting capacity in leafy single-node stem cuttings of rose. BAP might have contributed to root induction in tissue culture by interacting with the rose nodal explant's endogenous hormonal balance, which might have stimulated root formation (Kochuthressia *et al.,* 2010). The maximum rooting percent observed likely attributed to the optimal concentration of BAP, which possibly enhanced cambial growth at the base of explants, thereby facilitating differentiation of root primordia (Muttaleb *et al.,* 2017).

Table 3: Effect of varying concentrations of BAP with GA_3 on the shoot elongation from nodal explants of Miniature Rose cv. Ice Fairy

$BAP+GA_3$ (mg l^{-1}) concentration in MS medium	No. of shoots/Explant	Shoot length (cm)
1+1	1.6 ± 0.50^{b}	1.69 ± 0.53^{bc}
1.5+1	1.2 ± 0.58^{b}	1 ± 0.45^{bc}
2+1	1.8 ± 0.58^{ab}	2.34 ± 0.60^{b}
2.5+1	3.2 ± 0.37^{a}	4.02 ± 0.29^{a}
No hormone	0.8 ± 0.48^{b}	0.68 ± 0.44^{c}
CD (0.05)	1.52	1.41

Values are mean of three replicates ± S.E. Different letters accompanying values indicates substantial changes ($p\leq0.05$), determined through Duncan's Multiple Range Test

Table 4: The impact of BAP and GA_3 concentrations on the flowering from nodal explants of Miniature Rose cv. Ice Fairy in the *in-vitro* conditions

$BAP+GA_3$ (mg l^{-1}) concentration in MS medium	Days to bud initiation	Days to flowering	No. of buds/shoot
1+1	13.4±5.49	22.8±6.64	0.4±0.2
1.5+1	13.9±5.68	21.1±6.69	0.6±0.2
2+1	14.5±5.92	23.1±7.22	0.8±0.2
2.5+1	11.4±2.86	19.1±4.28	1.4±0.4
No hormone	14.4±5.91	25.5±6.3	0.2±0.2
CD (0.05)	NS	NS	NS

Values are mean of three replicates ± S.E. Different letters accompanying values specifies substantial changes ($p\leq0.05$), determined through Duncan's Multiple Range Test

Shoot elongation: Various combinations of cytokinin (BAP) with respect to gibberellin (Ga_3) in Table 3. The present of data revealed that the combination of BAP with GA_3 yielded good results in achieving high number of shoots per explant. The maximum recorded shoots per explant (3.2) and shoot length (4.02) was recorded in T_4 treatment containing BAP @ 2.5 mg l^{-1} with 1 mg l^{-1} GA_3, while the least no of shoot per explant (0.8) and minimum shoot length (0.68 cm) were observed in growth regulator free MS medium (control). Combinations of BAP and GA_3 significantly enhanced shoot number and length, with higher BAP concentrations paired with GA_3 yielding positive results in shoot formation and elongation is contrary to the finding of Shabbir *et al.* (2009), who reported that higher BAP and GA_3 concentrations promoted bud growth but inhibited shoot proliferation in *Rosax hybrida* cultivars. In the present study increased BAP concentration in combination with GA_3 correlates with enhanced shoot multiplication. These findings are some what related with the findings of Baskaran and Staden (2013) where long term exposure of *Agapanthus praecox* to combinations of cytokinin and IAA in the medium promoted adventitious shoot proliferation and resulted in the production of healthy shoots. Among the cytokinin, BA facilitated better shoot regeneration, with an average of 10.5 shoots/explant, compared to others.

***In-vitro* flowering:** The influence of varying BAP concentrations in combination with GA_3 on *in-vitro* flowering indicated that *in-vitro* flowering was achieved after second sub-culture. Furthermore, increasing BAP concentration from 2-2.5 mg l^{-1} GA_3, led to a higher rate of flowering. However, this increase in BAP concentration did not significantly affect the number of days required for flowering to occur. Data showed that the highest number of buds was noted with the amalgamation of 2.5 mg l^{-1} BAP and 1 mg l^{-1} GA_3 (Table 4; Fig. 2, 3). This demonstrates the effectiveness of BAP in combination with GA_3 as *in-vitro* flower formation offers a model system for studying flower initiation and development, which can be applied to *in-vitro* breeding particularly for plants with long vegetative growth periods as they are prone to mutations (Wang *et al.,* 2002). Additionally, microscopy results revealed the presence of trichomes in the buds (Fig. 3). Inflorescence buds were only formed in the presence of GA_3 in combination with 2.5 mg l^{-1} BAP, whereas no differentiation was observed in the control (MS media without hormone). These results suggest that GA_3 is a vital plant growth promoting hormone for transition from a vegetative to a reproductive stage. Similar results were reported by Naor *et al.* (2004) in Calla lily. They studied the effect of cytokinins (BA, 0.4-13.3 µM) and gibberellins (GA_3 5.8-2900 µM) on the inflorescence development in Calla lily plantlets regenerated through tissue culture.

Microscopic analysis: Different stages of growth of the rose

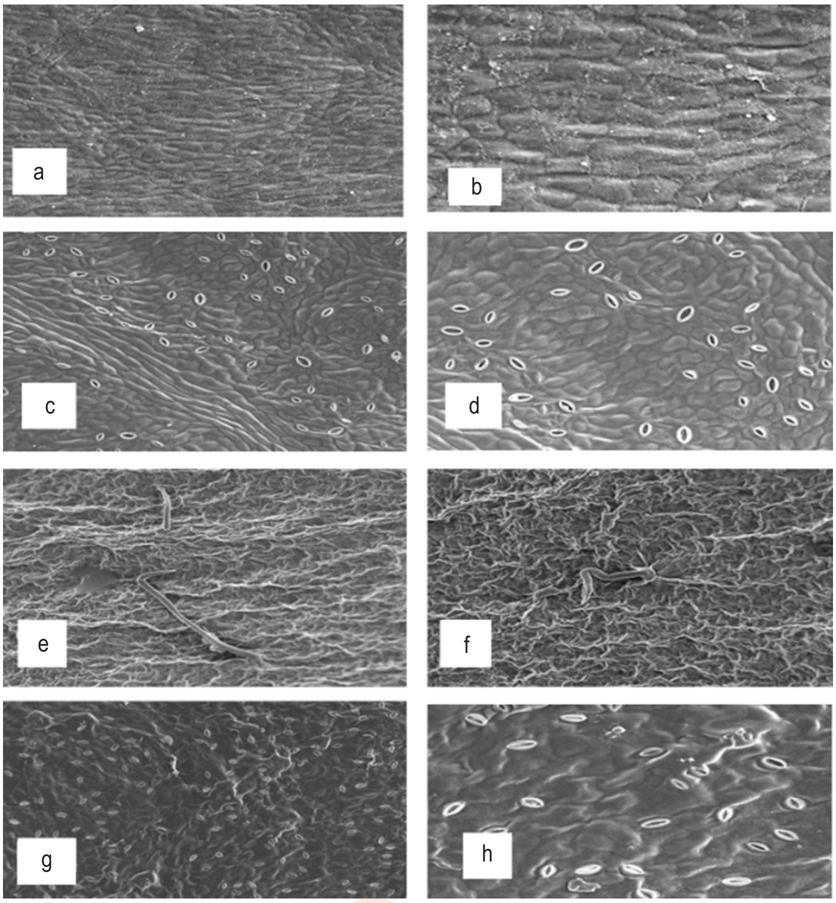

Fig. 4: SEM of Miniature Rose cv. Ice Fairy. (a-d) Abaxial and Adaxial epidermis cells (mother plant leaf sample); (e-h) Abaxial and Adaxial epidermis cells (Tissue culture raised plants leaf sample).

plant from shoot initiation to bud stage is shown in Fig. 3. Microscopic analysis showed transition of rose plant from vegetative to reproductive stage in the *in-vitro* conditions. The shoot, root and shoot bud initiation were noticed under a stereozoom microscope. It was observed that the top portion of the shoots extended much faster than the basal portion (Fig. 3b). In the next 2-weeks, the shoot buds further transformed into shoots with distinct leaf primordia (Fig. 3c). After 15 days of subculture, spontaneous root induction was seen (Fig. 3d) and formation of bud was observed after second subculture (Fig. 3e). Bose *et al.* (2017) reported that the combination of BA along with NAA performed well for inducing organogenetic response of root explants in Limonium hybrid Misty blue, however, in this study cytokinin showed efficacy in the induction of shoot as well as for the process of spontaneous root induction in rose cv. Ice Fairy. Similar microscopic analysis was also performed by Gantait and Sinniah (2014) for *Gerbera jamesonii* to induce roots and gradual developmental stages of rhizogenesis.

The samples were studied with the help of SEM to examine the micro-morphological physiognomies of leaves, including stomata, trichomes, abaxial epidermis cells and adaxial epidermis cells, as shown in Fig. 4. The results obtained from the scanning electron microscopy revealed distinct morphological characteristics of leaf epidermis of the leaves (Fig 4e,f). It was observed that the stomata were kidney shaped, however, those on the surface of leaves from tissue-cultured plants were closed (Fig. 4g,h). While both exhibited kidney-shaped stomata, the leaves of tissue cultured plants showed closed stomata with unique trichome characteristics not observed in leaves of mother plant. Johansson *et al.* (1992) in their study of micro propagated roses, particularly the Mme Isaac Pereire variety, the research on leaf structure highlighted various morphological and anatomical changes at different stages of growth, particularly with respect to the transition from *in-vitro* conditions to normal growth environments.

They observed that trichomes were more prominent on *in -vitro* leaves comparison to the weaned and stock plants. In conclusion, these findings indicate notable differences between *in-vitro* raised plants and mother plant. It was observed that the plants underwent some morphological changes after the tissue cultured raised plants were acclimatized to adapt to the environmental conditions. In a study, Bag *et al.* (2019) elucidated that acclimatization not only affects stomatal and trichome development but also leads to broader morphological changes. In *Camellia sinensis,* the leaves from *in-vitro* plants exhibited thicker laminae with larger parenchymatous cells, while acclimatized plants showed well-developed vascular systems and improved stomatal characteristics. These findings underscore the importance of acclimatization in bridging the physiological gap between *in-vitro* and field conditions, ensuring the survival and growth of tissue-cultured plants in natural environments. This suggests that tissue cultured plants when acclimatized may influence stomatal function and trichome development for adapting to the environment, distinguishing them morphologically from their mother plant.

This study presents a successful *in-vitro* propagation protocol for miniature rose cv. Ice Fairy using nodal explants, consisting of four key growth stages: shoot initiation, shoot multiplication, shoot elongation and rooting. The optimal concentration for shooting and rooting was 2.5 mg l^{-1} BAP, while GA_3 (1 mg l^{-1}) enhanced shoot elongation. The method ensures disease free, uniform plants with consistent quality traits such as flower size, color and compactness. This technique provides an efficient approach for mass production of miniature roses, ideal for commercial and ornamental purposes.

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