Original article

Standardization of in vitro micropropagation of Winter Jasmine (Jasminum nudiflorum) using nodal explants

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A B S T R A C T

Present investigation was carried out to arrive at an effective micropropagation protocol for Winter Jasmine (Jasminum nudiflorum) using nodal segments from actively growing plants as explants. Explants were collected from current season shoots during April-May just after the initiation of new flush. Combined sterilization treatment of explants with 1.0% NaOCl for 10 min followed by 70% ethanol for 10 s recorded highest culture survival (63.88%) and optimum culture asepsis (63.88%) followed by the treatment containing 0.1% HgCl2 for 10 min followed by 70% ethanol for 10 s with culture survival (61.11%) and culture asepsis (69.44%). Highest culture establishment (80.55%) and minimum days to bud sprouting (7.62 days) was recorded with Benzyl adenine + Kinetin (3.0 + 1.0 mgL−1) but maximum length (4.33 cm) and leaf number (7.78) of established micro shoots was recorded with Benzyl adenine + Kinetin (1.0 + 0.5 mgL−1). Maximum proliferated shoots (2.41) and an optimum proliferation percentage (77.78 %) was recorded with Benzyl adenine + Kinetin (3.0 + 0.5 mgL−1). Minimum size of proliferated shoots (2.02 cm) was recorded with Benzyl adenine + Kinetin (3.0 + 1.0 mgL−1) followed by 2.25 cm recorded with Benzyl adenine + Kinetin (3.0 + 0.5 mgL−1). Highest rooting (63.93%), primary root number/microshoot (4.74) and longest primary roots (34.67 mm) were recorded with IBA (2.0 mgL−1). IBA yielded better results than NAA in terms of higher rooting percentage and root number. However, days to root initiation were found minimum (22.00) with 2.0 mgL−1 of NAA. Highest ex vitro survival of rooted microshoots (89.67%) was recorded with IBA (2.0 mgL−1).

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1. Introduction

Jasmine belongs to family Oleaceae and the genus Jasminum that contains around 200 species of flowering plants (Saecuianu, 2021). Jasminum nudiflorum commonly known as winter jasmine or winter flowered jasmine is a semi-evergreen shrub from China which flowers immediately after winter. It is a medium-sized plant with dark green trifoliate foliage and long arching branches. Flow-
Further sexual propagation is not a reliable method because of poor seed germination under natural conditions and high mortality rate of seedlings (Reddy and Gupta, 2013). It is traditionally propagated by cutting and layering but these methods of propagation restrict the quantity of plant material produced. Layering is a very cumbersome and time consuming method of propagation with a low multiplication ratio. Continuous production of plants through cutting/lowering has been found to result in lower flower production, varietal degeneration and lack of resistance (Cai et al., 2007). Due to genetic variability manifestation of seeds, the population propagated by normal seed development method may not show clonal population reliability (El-Sadat and Hewidy, 2020).

Micro-propagation provides a fast and dependable method of production of a large number of uniform plantlets in a short time and the stock of germplasm can be maintained for many years (Khan et al., 2020). It is the successful method for production of true to type quality planting material with reduced cost of production (Chaitanya et al., 2018). Micro-propagation is the practice of accelerated production of progeny plants under controlled conditions irrespective of the season using modern plant tissue culture method (Malik, 2007; Ahmad et al., 2007; Bhat et al., 2012; Jamsheed et al., 2013; Sarkar et al., 2016). It is regarded as a suitable technology for crop improvement with high efficiency in terms of somaclonal/gametoclonal variants development and cultivate materials of higher quality (Ahmed et al., 2021). It can also be used for the introdution of genetic diversity and selection of beneficial variants of high yield with improved tolerance to biotic or abiotic stresses (Hussain et al., 2012).

To overcome all the issues and problems related to propagation of Winter Jasmine, micropropagation is a reliable alternative. Micropropagation work on different species of Jasminum has been successfully carried out through direct or indirect shoot regeneration, using shoot tips (U-Kong et al., 2012), young leaves (U-Kong et al., 2012; Sapra and Pandya, 2017; Thenmozhi, 2019), nodal & internodal segments (Farzinebrahimi et al., 2014; Salim, 2016), and apical/axillary shoots (Biswal et al., 2016). Probability and prospects of in vitro propagation of different species of Jasminum has been reviewed by Rahman et al. (2018) and Chaitanya et al. (2018). Some tissue culture related studies have been conducted on Winter Jasmine related to callus induction and regeneration (Davallo et al., 2014; Lu et al., 2019), protoplast culture (Ahmed et al., 2021) and mutagenic sensitivity to EMS (Ghosh et al., 2018) but no work has been conducted for developing a full-fledged micropropagation protocol through nodal segments. Colour diversity in Jasminum species is restricted to yellow and white which can be widened by bringing in mutations in existing weather conditions (Shahmoradi and Naderi, 2018).
In vitro mutagenesis has tremendous potential in creating new mutations in colour, shape and form in plants and development of a micropropagation protocol is a pre-requisite for in vitro mutagenesis. Keeping in view the difficulties encountered in propagation techniques presently used for multiplication of Winter Jasmine and narrow colour diversity available in the genus, experiment was carried out to standardize an efficient micropropagation technique for large scale multiplication of quality planting material of *Jasminum nudiflorum* and pave way for developing new colour variants of this versatile landscape plant through in vitro mutagenesis.

### 2. Materials and methods

#### 2.1. Plant material collection and sterilisation

Studies were carried out in the Laboratory of Plant Tissue Culture of The Division of Floriculture and Landscape Architecture, Sher-e-Kashmir University of Agricultural Sciences and Technology of Kashmir, Shalimar, during 2019–2020. The current year’s shoots of mature *Jasminum nudiflorum* plant were excised, leaves removed and cut to make smaller nodal segments in the laboratory (Fig. 1 A). Nodal segments were placed in a beaker and washed with

| Treatment | Sterilant used | Concentration (%) | Treatment duration | Culture asepsis (%) | Explant survival (%) |
|-----------|----------------|-------------------|-------------------|---------------------|---------------------|
| T1        | Mercuric chloride 0.1 | 10 min | 62.44 ± 0.67<sup>d</sup> | 52.00 ± 0.88<sup>c</sup> |
| T2        | Sodium hypochlorite 1.0 | 10 min | 59.23 ± 0.29<sup>e</sup> | 58.33 ± 0.29<sup>b</sup> |
| T3        | Mercuric chloride followed by Ethyl alcohol 70.0 | 10 s | 69.44 ± 1.31<sup>c</sup> | 61.11 ± 0.77<sup>ab</sup> |
| T4        | Sodium hypochlorite followed by Ethyl alcohol 70.0 | 10 s | 63.88 ± 0.97<sup>d</sup> | 63.88 ± 2.78<sup>a</sup> |
| T5        | Flusilazole followed by Ethyl alcohol 70 | 10 s | 72.22 ± 0.29<sup>b</sup> | 44.66 ± 0.58<sup>d</sup> |
| T6        | Flusilazole followed by Sodium hypochlorite followed by Ethyl alcohol 70 | 10 s | 67.89 ± 0.87<sup>c</sup> | 54.44 ± 0.58<sup>b</sup> |
| T7        | Flusilazole followed by Mercuric chloride followed by Ethyl alcohol 70 | 10 s | 77.78 ± 0.97<sup>a</sup> | 42.88 ± 1.13<sup>d</sup> |
| T8        | Flusilazole followed by Sodium hypochlorite followed by Ethyl alcohol 70 | 10 s | 71.88 ± 0.17<sup>b</sup> | 51.99 ± 0.38<sup>c</sup> |

*Data noted at the end of 4 weeks of culture on MS medium. Data are a mean of three replicates with ± SE. Same superscript letters in a column do not differ significantly when compared by Duncan’s MRT at 5% level of significance.*

| Treatment | Growth Regulators (mgL<sup>-1</sup>/C<sub>0</sub>) | Culture Establishment (%) | Days to bud sprouting | Shoot Number | Shoot length (cm) | Leaf number/shoot |
|-----------|---------------------------------|--------------------------|----------------------|--------------|-----------------|------------------|
| 1         | BAP + Kn 1.0 + 0.5              | 61.11 ± 0.40<sup>a</sup> | 13.64 ± 0.60<sup>a</sup> | 1.11 ± 0.07<sup>c</sup> | 4.33 ± 0.04<sup>d</sup> | 7.78 ± 0.07<sup>a</sup> |
| 2         | BAP + Kn 2.0 + 0.5              | 69.44 ± 0.40<sup>c</sup> | 9.57 ± 0.32<sup>b</sup> | 1.37 ± 0.01<sup>b</sup> | 3.83 ± 0.06<sup>c</sup> | 7.16 ± 0.10<sup>bc</sup> |
| 3         | BAP + Kn 3.0 + 0.5              | 77.78 ± 0.97<sup>b</sup> | 8.04 ± 0.31<sup>b</sup> | 1.59 ± 0.05<sup>d</sup> | 3.51 ± 0.07<sup>c</sup> | 6.44 ± 0.08<sup>c</sup> |
| 4         | BAP + Kn 1.0 + 1.0              | 63.88 ± 1.30<sup>b</sup> | 1.03 ± 0.58<sup>b</sup> | 1.07 ± 0.04<sup>d</sup> | 3.73 ± 0.09<sup>b</sup> | 7.24 ± 0.10<sup>d</sup> |
| 5         | BAP + Kn 2.0 + 1.0              | 71.11 ± 0.20<sup>c</sup> | 9.07 ± 0.57<sup>b</sup> | 1.27 ± 0.01<sup>b</sup> | 3.05 ± 0.07<sup>d</sup> | 6.97 ± 0.09<sup>c</sup> |
| 6         | BAP + Kn 3.0 + 1.0              | 80.55 ± 0.40<sup>a</sup> | 7.62 ± 0.33<sup>c</sup> | 1.41 ± 0.03<sup>b</sup> | 2.82 ± 0.04<sup>d</sup> | 6.02 ± 0.10<sup>d</sup> |

*Data noted at the end of 4 weeks of culture on MS medium. Data are a mean of three replicates with ± SE. Same superscript letters in a column do not differ significantly when compared by Duncan’s MRT at 5% level of significance.*

| Treatment | Growth Regulators (mgL<sup>-1</sup>/C<sub>0</sub>) | Microshoot length (cm) | Leaves/ proliferated shoot |
|-----------|---------------------------------|------------------------|---------------------------|
| 1         | BAP + Kn 1.0 + 0.5              | 63.88 ± 1.31<sup>c</sup> | 1.79 ± 0.08<sup>d</sup> |
| 2         | BAP + Kn 2.0 + 0.5              | 66.66 ± 0.96<sup>bc</sup> | 2.01 ± 0.06<sup>b</sup> |
| 3         | BAP + Kn 3.0 + 0.5              | 77.78 ± 0.97<sup>b</sup> | 2.41 ± 0.08<sup>a</sup> |
| 4         | BAP + Kn 1.0 + 1.0              | 66.66 ± 0.96<sup>bc</sup> | 1.72 ± 0.05<sup>d</sup> |
| 5         | BAP + Kn 2.0 + 1.0              | 69.44 ± 0.97<sup>b</sup> | 1.94 ± 0.83<sup>ed</sup> |
| 6         | BAP + Kn 3.0 + 1.0              | 80.55 ± 0.97<sup>a</sup> | 2.22 ± 0.03<sup>ab</sup> |

*Data noted at the end of 4 weeks of culture on MS medium. Data are a mean of three replicates with ± SE. Same superscript letters in a column do not differ significantly when compared by Duncan’s MRT at 5% level of significance.*
Lindl Plantlet survival during primary hardening in Winter Jasmine (Jasminum nudiflorum Lindl) with different types of auxins.

| Treatment | Growth regulator | Concentration (mgL⁻¹) | Root initiation (day) | Rooting (%) | Primary roots/shoot | Root length (mm) |
|-----------|------------------|------------------------|-----------------------|-------------|---------------------|-----------------|
| 1         | NAA              | 0.5                    | 31.42 ± 0.49          | 8.58 ± 0.25 | 1.09 ± 0.04         | 11.03 ± 0.08    |
| 2         | NAA              | 1.0                    | 28.22 ± 0.61          | 14.62 ± 2.80| 1.90 ± 0.07         | 14.28 ± 0.07    |
| 3         | NAA              | 1.5                    | 22.20 ± 0.91          | 16.06 ± 3.07| 2.63 ± 0.34         | 18.70 ± 0.05    |
| 4         | NAA              | 2.0                    | 22.00 ± 0.57          | 28.97 ± 2.41| 3.40 ± 0.23         | 24.52 ± 0.11    |
| 5         | IBA              | 0.5                    | 33.27 ± 0.41          | 18.51 ± 1.85| 2.25 ± 0.18         | 18.45 ± 0.03    |
| 6         | IBA              | 1.0                    | 29.63 ± 0.19          | 35.18 ± 1.85| 3.13 ± 0.10         | 25.90 ± 0.09    |
| 7         | IBA              | 1.5                    | 29.18 ± 0.46          | 44.28 ± 2.86| 3.83 ± 0.33         | 29.94 ± 0.13    |
| 8         | IBA              | 2.0                    | 27.36 ± 0.41          | 63.93 ± 1.49| 4.74 ± 0.23         | 34.67 ± 0.22    |
| C.D (P₂₀,₀₅) | 1.64             | 0.73                   |                       |             |                     |                 |

Table 4

Plantlet survival during primary hardening in Winter Jasmine (Jasminum nudiflorum Lindl).

| Treatment | Growth regulators | Concentration (mgL⁻¹) | Plantlet Survival (%) |
|-----------|-------------------|-----------------------|-----------------------|
| 1         | NAA               | 0.5                   | 37.48 ± 0.88          |
| 2         | NAA               | 1.0                   | 48.68 ± 0.90          |
| 3         | NAA               | 1.5                   | 57.79 ± 0.81          |
| 4         | NAA               | 2.0                   | 62.56 ± 0.46          |
| 5         | IBA               | 0.5                   | 67.88 ± 1.23          |
| 6         | IBA               | 1.0                   | 78.19 ± 0.37          |
| 7         | IBA               | 1.5                   | 84.72 ± 0.03          |
| 8         | IBA               | 2.0                   | 89.67 ± 0.03          |
| C.D (P₂₀,₀₅) | 1.62             |                       |                      |

Table 5

Murashige and Skoog (1962) media was used for various experiments. The required quantity of sucrose (3%), myo-inositol (100 mg L⁻¹) and all other macro & micro nutrients and vitamins were mixed in a beaker. The growth regulators were added as per the treatments and pH was adjusted at 5.7 by adding few drops of sodium hydroxide or sulphuric acid. After heating the mixture in a microwave oven, 7 gL⁻¹ agar was added. The nutrient mixture was stirred well and again heated till boiling in order to completely dissolve the agar. Prepared culture media was poured into flasks or test tubes, plugged with cotton plugs and covered with clean papers using rubber bands. The media was autoclaved at a temperature of 121°C and a pressure of 15 psi for 15–20 min. Washed nodal explants were surface sterilized with different sterilants under Laminar Air flow hood followed by three final rinses with double distilled autoclaved water to remove the traces of sterils. The nodal explants were properly prepared and inoculated in the culture establishment medium conforming to their original polarity (Fig. 1 B–C). All the cultures were kept in the incubation room at a temperature of 24 ± 1°C with photoperiod of 16:8 light/dark cycles plus 3500 lx intensity of light.

For standardizing the growth regulator concentrations and combinations for optimum culture establishment, six treatment combinations consisting of Benzyl Amino Purine (BAP) and Kinetin (Kn) in different combinations (1.0 + 0.5; 2.0 + 0.5; 3.0 + 5.0; 1.0 + 1.0; 2.0 + 1.0 and 3.0 + 1.0) were used. Data pertaining to percent establishment, days to bud sprouting, number of shoots/explant, shoot length (cm) and number of leaves per established explant was noted four weeks after inoculation. Explants were sub-cultured after every 4 weeks. Uniform micro-shoots excised from the established cultures under aseptic conditions were inoculated individually in test tubes containing growth regulator supplemented media (same as establishment media) for proliferation. Proliferated micro-shoots of uniform size were cut and placed in rooting medium augmented with IBA or NAA (0.5, 1.0, 1.5 and 2.0 mgL⁻¹). Data related to all rooting parameters including percent rooting, days to root initiation, root length and number of roots per shoot was recorded after 4 weeks of culture.

2.2. Media preparation and explant inoculation

2.3. Hardening and acclimatization of plantlet

The in vitro rooted plantlets were washed thoroughly with distilled water to remove the adhering agar gel and planted in the polypropylene glasses filled with the previously prepared autoclaved hardening media containing vermiculite and perlite in 1:1 ratio. Plants were watered with double distilled water. Similar polypropylene glass was inverted over the first one and the joint...
of the two glasses was sealed with a strip of parafilm to maintain optimum humidity around the young rooted tissue cultured plant. These polypropylene containers containing in vitro rooted plantlets for hardening were kept in the same incubation room under similar conditions. Upper polypropylene glasses were perforated when the plants showed new leaf growth (about 10 days after transfer). The size and number of the holes was gradually increased after the signs of establishment of plant. The upper inverted glass was completely removed when the plants showed proper establishment. Ex-vitro survival of the rooted plantlets was recorded after 4 weeks.

2.4. Statistical analysis

Completely randomized design with 3 replications was used for various experiments. During present investigation, the data obtained for different experiments was subjected to analysis of variance for completely randomized design. The percentage data was subjected to Angular or square root transformation to satisfy variance for completely randomized design. The percentage data for various experiments. During present investigation, the data was analyzed using OPSTAT, a free online Agriculture Data Analysis Tool created by O.P. Sheoran, Computer Programmer at CCS HAU, Hisar, India.

3. Experimental results

3.1. Aseptic and surviving cultures

Sterilant treatments had a significant effect on culture asepsis. There was increase in culture asepsis when the sterilants were used in combination or when their concentration was increased (Table 1). Minimum aseptic cultures of 59.23% and 62.44% were recorded with T2 and T1 respectively, when single sterilant was used. There was improvement in culture asepsis when explants were treated with two sterilants together, resulting in 69.44% and 63.88% aseptic culture with T1 and T3 respectively. However, maximum culture asepsis of 77.78% and 73.88% was recorded with T4 and T3 respectively when explants were treated with three sterilants. Both these treatments differed significantly from each other and were superior to all other sterilant treatments. Generally increasing the concentration of sterilants or using multiple sterilants in a sterilization programme results in reduced explant survival because of phytotoxic effects of sterilants. Maximum explant survival of 63.88% was obtained with T4 followed by T4 because of phytotoxic effects of sterilants. Maximum explant survival of 63.88% was obtained with T4 followed by T3 and T1 respectively. However, increasing the concentration of sterilants or using multiple sterilants. Both these treatments differed significantly from each other but differed significantly from all other treatments. Minimum explant survival of 42.88% was recorded with T7 followed by IBA (1.5 mg L⁻¹) followed by 44.28% with IBA (1.5 mg L⁻¹). Maximum size of proliferated microshoots (2.61 cm) and highest number of leaves (3.95) was recorded with growth regulator combination of BAP + Kn (1.0 + 0.5 mg L⁻¹).

3.2. Culture establishment

Murashige and Skoog medium fortified with six different combination of growth regulators was used for the establishment of nodal segment explants (Fig. 1 C). There was increase in culture establishment and reduction in the days to sprouting with the increase in overall concentration of cytokinins in the media (Table 5). MS media supplemented with BAP + Kn (3.0 + 1.0 mg L⁻¹) was found optimum for culture establishment recording maximum explant establishment (80.55%) and minimum days to bud sprouting (7.62 days) followed by BAP + Kn (3.0 + 0.5 mg L⁻¹) with explant establishment (77.78 %) and days to bud sprouting (8.04 days). However, number of shoots per established explant was recorded maximum (1.59) with BAP + Kn (3.0 + 0.5 mg L⁻¹) followed by 1.41 recorded in treatment combination BAP + Kn (3.0 + 1.0 mg L⁻¹). Increase in cytokinin concentration in media resulted in reduction of shoot length and leaf number of established shoots. Maximum leaf number (7.78) and shoot length (4.33 cm) of established shoots was recorded with BAP + Kn (1.0 + 0.5 mg L⁻¹).

3.3. Culture proliferation

Single node cuttings of established shoots were put in growth regulator supplemented media for further multiplication (Fig. 1 E). Data presented in Table 3 shows that highest culture proliferation (80.55%) was recorded on MS media containing BAP + Kn (3.0 + 1.0 mg L⁻¹) followed by 77.78 % with BAP + Kn (3.0 + 0.5 mg L⁻¹). The highest number of proliferated shoots (2.41) was recorded with growth regulator combination BAP + Kn (3.0 + 0.5 mg L⁻¹) followed by 2.22 in BAP + Kn (3.0 + 1.0 mg L⁻¹). Maximum size of proliferated microshoots (2.61 cm) and highest number of leaves (3.95) was recorded with growth regulator combination of BAP + Kn (1.0 + 0.5 mg L⁻¹).

3.4. Rooting and hardening

Healthy individual shoots of uniform size and thickness from proliferated cultures were taken and put in rooting media supplemented with different auxin types (IBA & NAA) and concentrations (0.5, 1.0, 1.5, 2.0 mg L⁻¹). There was increase in all rooting parameters with the increase in concentration of auxins (Table 4). However, performance of IBA was superior in comparison to NAA (Fig 1 F-G). Highest rooting (63.93%) was recorded with IBA (2.0 mg L⁻¹) followed by 44.28% with IBA (1.5 mg L⁻¹). Maximum length (34.67 mm) and number (4.74) of primary roots/shoot was also observed in medium supplemented with IBA (2.0 mg L⁻¹) followed by primary root number of 3.83 and root length of 29.94 mm with IBA (1.5 mg L⁻¹). However, days to root initiation were found minimum (22.00) with 2.0 mg L⁻¹ of NAA. Rooted plantlets after four weeks of culture in the rooting media were taken out of agar gel media and after washing with sterile water transferred into polypropylene glasses containing rooting media (perlite and vermiculite in the ratio of 1:1) for primary hardening (Fig 1 H-I). During primary hardening, maximum plant survival of 89.67% was observed in plants which came from rooting media containing 2.0 mg L⁻¹IBA (Table 5).

4. Discussion

Fungal and bacterial contaminations are one of the most important limiting factors, particularly in woody plants during in vitro cultures (Ahmadpoor et al., 2022). Hence in vitro sterilization is a primary step of plant tissue culture and the ultimate results of in vitro culture are directly depended on the efficiency of the sterilization (Hesami et al., 2019). Being successful in plant tissue culture and releasing plant regeneration protocols are highly dependent on the efficiency of the sterilization stage. (Da Silva et al., 2016; Hesami et al., 2018). Inappropriate concentrations of sterilants have lethal effect on cell division and it restricts growth and development of explant. Therefore, suitable concentration, combinations and duration of exposure of sterilant is essential to raise in vitro cultures successfully (Bhadane and Patil, 2016). Surface sterilisation of explants is carried out to obtain sizeable number of surviving aseptic cultures. However, increasing the sterilant number or their concentration increases explant asepsis but reduces their survival. The objective of standardizing sterilization protocol should be to obtain optimum culture asepsis and optimum culture survival. During this study, treatment of nodal segment explants of jasmine with combination of three sterilants at higher concentrations yielded maximum culture asepsis (77.78%).
but minimum culture survival (42.88%). Combined sterilant treatments aggravate the phyto-toxic effects leading to reduced explant survival. Similar results were recorded by Rather et al. (2014) and Rafiq et al. (2021) with underground buds of herbaceous peony and Oriental Lilium cv. Ravenna, respectively.

Many scientists have also reported a negative correlation with the high concentration of the disinfectants and the rate of explant viability (Nongalleima et al., 2014; Da Silva et al., 2016; Hesami et al., 2018).

Sterilization of explants with T3 and T4 were observed the best treatments for sterilisation of nodal segments of jasmine, which yielded optimum culture asepsis and higher culture survival but we recommend T4 because of hazardous effects of mercury chloride on the environment and the humans.

Cytokinins particularly BAP have been found indispensable for axillary shoot proliferation in plant tissue culture. These growth hormones generally suppress apical dominance and force axillary shoot production. Cytokinins are one of the most important factors in plant tissue culture especially in proliferation stage. It plays multiple roles in the plant development including cell division and cell expansion, plant protein synthesis stimulation and the activities of some enzymes (Arab et al., 2014). Use of cytokinins during multiplication stage increases the proliferation rate as well as shoots quality (Hassan et al., 2019). Among the cytokinins, the role of Benzyl adenine as an indispensable hormone for shoot induction and proliferation was proposed by Economou and Read (1980). Nikhat (2004); Sonali (2004) obtained multiple shoot formation on medium fortified with BAP while other plant growth hormones were found ineffective to induce multiple shoots. During present studies, highest concentration of BAP yielded maximum performance on medium fortified with BAP + Kin (2.0 + 0.5 mg/l). Arriving at an appropriate cytokinin level for mass propagation of any species is very important for optimum shoot multiplication. Proliferation characteristics of IBA in Jasminum officinale. BAP in general has been reported most effective for axillary shoot proliferation, meristem culture and shoot-tip culture of various species (Bhattacharya and Bhattacharyya, 1997; Cai et al., 2007; Sun et al., 2009). Sometimes combined use of two types of cytokinins shows better results than a single cytokinin in inducing axillary shoots. Kharde and Kshirsagar (2014) reported earlier shoot initiation, higher shoot number, shoot length and leaf number in Rosa hybrida with BAP + Kin (2.0 + 0.5 mg/l). Arriving at an appropriate cytokinin level for mass propagation of any species is very important for optimum shoot multiplication. Proliferation characteristics like shoot length and leaf number showed a reduction with increase in cytokinin concentration in media. This reduction may be attributed to production of higher number of axillary shoots at higher levels of cytokinin, leading to utilization of more nutrients and energy in inducing more axillary branches but of smaller length.

The in vitro rhizogenesis of microshoots is a deciding factor in establishing a successful micropropagation protocol. Success in micropropagation is thus dependent on the production of good quality adventitious roots, (Kevers et al. 1997; De Klerk et al. 1999). It is well established that auxins play a central role in the determination of rooting capacity of cuttings (Fogaça and Fett-Neto, 2005). Auxins are important factors involved in rooting because they promote adventitious roots formation in the vast majority of species (De Klerk, 2002). Auxins are potent regulators of cell division and cell differentiation which promote root initiation and production of adventitious roots in cuttings and microshoots. Auxins affect cell differentiation and promote starch hydrolysis and the mobilization of sugars and nutrients to the cutting base, all of which influence the rooting capacity, producing higher rooting percentage and higher number of roots per rooted cutting (Leakey, 2004). In adventitious root formation of many woody species, auxin has been found to be the effective inducer (Selby et al., 1992; Diaz-Sala et al., 1996; Goldfarb et al., 1998; De Klerk et al., 1999). It also plays an important role in modulating lateral root formation in a number of woody perennials (Ruegger et al., 1998; Rogg et al., 2001). For induction of adventitious rooting, higher auxin concentration is required (De Klerk et al., 1999). During present study, highest concentration of IBA yielded maximum rooting percentage compared to NAA. Better performance of IBA in comparison to NAA has also been reported by Sessou et al. (2020) and Kabir et al. (2015). The better rooting responses with IBA could be attributed to its higher stability than other auxins (Nissen and Sutter, 1990). According to Kozai et al. (1997), tissue cultured plants are very delicate and tender due to the conditions under which they develop including high humidity in vessels (80–90%), controlled temperatures (25 + 2 °C), low light intensity and mico-tropic nutrition. These conditions need to be simulated during primary hardening of rooted tissue cultured plants. Higest ex vitro survival (89.67%) was observed in plantlets, which were rooted in MS media supplemented with IBA (2.0 mg/l) followed by 84.72% in plantlets which came from media supplemented with IBA @ 1.5 mg/l. IBA treated plantlets recorded higher survival rate than NAA treated plantlets because of good rooting characteristics.

5. Conclusion

Taking culture asepsis and explant survival into consideration, T4 treatment combination (1.0% NaOCl for 10 min + 70% ethanol for 10 s) is best treatment combination for explant sterilization of Jasminum nudiflorum as it recoded optimum culture asepsis and highest explant survival. For culture establishment and proliferation, best growth regulator combination was found to be BAP + Kin (3.0 + 0.5 mg/l) as it yielded higher number of microshoots with highest shoot length, which is very important for subsequent proliferation cycles and successful rooting of microshoots. MS media fortified with IBA (2.0 mg/l) was found best medium for rooting of micro shoots as it rooted plantlets with better rooting characteristics. Highest ex-vitro plantlet survival in standardized hardening media (1:1 perlite and vermiculite) was also observed in rooted plants which came from rooting media supplemented with IBA (2.0 mg/l).

The authors have no conflicts of interest to declare.

6. Consent to participate

All authors consent to participate in the manuscript publication

7. Consent for publication

All authors approved the manuscript to be published

8. Availability of data and material

The data supporting the conclusions of this article are included within the article. Any queries regarding these data may be directed to the corresponding author.

Author Contributions

MSB designed the research and conducted experiments. ZAR edited the manuscript; ITN and NB helped in methodology and project administration. TW and SR helped in conducting experiments and IP helped in writing the manuscript. AN and HD revised the manuscript.
Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have influenced the work reported in this paper.

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References

Ahmad, P., Sharma, S., Sivastava, P.S., 2007. In vitro selection of NaHCO3 tolerant cultivars of Morus alba (Local and Sujanpur) in response to morphological and biochemical parameters. Hortic. Sci. Prague 34 (3), 115–123.

Ahmed, M.A., Miao, M., Pratinsakis, E.D., Zhang, H., Wang, W., Yuan, Y., Wu, B., 2021. Protoplast Isolation, Fusion, Culture and Transformation in the Woody Plant Jasminum sambac spp. Agriculture 11 (8), 695.

Ahmadpoor, F., Zare, N., Asghari, R., Reiekhzadeh, P., 2022. Sterilization protocols and the effect of plant growth regulators on callus induction and secondary metabolites production in in vitro cultures of Melia azedarach L. A MB Exp 12 (1).

Arab, M.M., Yadollahi, A., Shojaieyan, A., Shokri, S., Ghajah, S.M., 2014. Effects of nutrient media, different cytokinin types and their concentrations on in vitro multiplication of G. N15 (hybrid of almond peach) vegetative rootstock. J. Genetic Eng. Biotechnol. 12 (2), 81–87.

Bhat, M.A., Jamshed, S., Mujib, A., Aozoo, M.M., Mahmooduzzafar, A., Ahmad, J., 2012. Plant Tissue Culture: A useful measure for the screening of salt tolerance in plants. In: Ahmad, P., Aozoo, M.M., Prasad, M.N.V. (Eds.), Salt stress in Plants: Omics, Signalling and adaptations. Springer, Dordrecht Heidelberg London, NY, pp. 465–496.

Bhattarcharya, S., Bhattarcharya, S., 2010. In Vitro Propagation of Jasminum officinale L.: A Woody Ornamental Vine Yielding Aromatic Oil from Flowers. In: Jain, S., Ochatt, S. (Eds.), Protocols for In Vitro Propagation of Ornamental Plants. Methods in Molecular Biology (Method and Protocols), p. 589.

Bhattarcharya, S., Bhattarcharya, S., 1997. Rapid multiplication of Jasminum officinale L. in by in vitro culture of nodal explants. Plant Cell Tissue Organ Culture 51, 57–60.

Bhadane, B. S., & Patil, R. H. 2016., Data on the cost effective surface sterilization method for C. cardaric (L.) seeds and callus induction from aseptic seedling. Data in brief, 7, 85–90.

Cai, H., Chen, X.Q., Xiong, Z.M., Xie, L.L., Zhao, L.J., 2007. Techniques of in vitro micro-propagation and sugar-free rooting of jasmine (Jasminum sambac). Jangsu J. Agri. Sci. 23, 464–468.

Chattaboy, H.S., Nataraj, S.K.M., Krishnappaa, M., 2018. Review on Propagation Techniques of Jasminum sambac (L.). J. Pharmacogn. Phytochem. 76, 593–596.

de Klerk, G.-J., van der Krieken, W., de Jong, J.C., 1999. Review of the formation of adventitious roots: new concepts, new possibilities. In Vitro Cell. Developmental Biol. Plant 35 (3), 189–199.

Klerk, G.-J., 2002. Rooting of microcuttings; theory and practice. In Vitro Cell. Developmental Biol. Plant 38 (5), 415–422.

Da Silva, J.A.T., Kulus, D., Zhang, X., Zeng, S., Ma, G., Piqueras, A., Zhang, X., Zeng, S., Ma, G., Piqueras, A., 2016. Disinfection of explants for saffron (Crocus sativus) tissue culture. Environ. Exp. Biol 14, 183–198.

Diaz-Sala, C., Hutchison, K.W., Goldfarb, B., Greenwood, M.S., 1996. Maturation-related loss in rooting competence by loblolly pine stem cuttings: The role of auxin transport, metabolism and tissue sensitivity. Physiologia Plantarum 97 (3), 481–490.

Economou, A.S., Read, P.E., 1980. Effect of benzyladenine pretreatments on shoot proliferation from petunia leaf segments cultured in vitro. In: Proceedings of the Plant Growth Regulator Working Group; annual meeting (USA).

El-Sadat, N.H.A., Hewidy, M., 2020. In vitro propagation protocol of Jasminum nudiflorum. Biotechnology 99 (1), 49–57.

Hata, K., Hoahangzade, R., Tofidfar, M., 2019. Modeling and Optimizing in vitro Stabilization of Chrysanthemum via Multilayer Perceptron-Non-dominated Sorting Genetic Algorithm-II (MLP-NSGAII). Front. Plant Sci. 10, 282.

Hesami, M., Naderi, R., Yousefzadeh-Najafabadi, M., 2018. Optimizing sterilization conditions and growth regulator effects on in vitro shoot regeneration through direct organogenesis in Chenopodium quinoa. BioTechnology 99 (1), 49–57.

Kharde, A.V., Kshirsagar, A.B., 2014. Effect BAP and Kinetin on nodal culture of Rosa Chinensis cv. Nankotsu. J. In Vitro Biotechnol. 20 (51) 124–127.

Kozai, T., Kubota, C., Jeong, B.R., 1997. Environmental control for the large-scale production of plants in vitro technique. Plant Cell Tissue Organ Culture 51, 49–56.

Leeley, F.R., 2004. Physiology of vegetative reproduction. Academic Press.

Lu, Y., Liu, Z., Lyu, M., Yuan, Y., Wu, B., 2019. Characterization of JswOx1 and JswOx4 during callus and root induction in the shrub species Jasminum Sambac. Plants 8 (4), 79.

Makil, G. C. 2007 Applications of biotechnology innovations in pharmaceutics and nutraceutics in multitherapeutic medicinal and special plants Vol II ed Karan singh, ML jahdon and D singh. Aavishkar publishers, Jaipur, 2, 243–265.

Murashige, T., Skoog, F., 1962. A revised medium for rapid growth and bio assays with tobacco tissue cultures. Physiologia Plantarum 15 (3), 473–497.

Nongalleima, K., Dikash Singh, T., Amitabha, D., Deb, L., Sunitibala Devi, H., 2014. Optimization of surface sterilization protocol, induction of axillary shoots regeneration in Zingerib zerumbet (L.) Sm. as affected by season. Biocol. Res 45, 317–324.

Rafie, S., Rather, Z., Bhat, R.A., Naki, I., Al-Harbi, M.S., Randay, N., Farooq, I., Samra, B.N., Khan, M., Ahmad, A.F., 2021. Standardization of in vitro micropropagation procedure of Oriental Lilium Hybrid cv. ‘Ravenna’. Saudi J. Biol. Sci. 28, 7581–7587.

Rahman, M.S., Mourin, N., Nandi, N., N, Khan, M., 2018. In vitro micropropagation of Jasminum grandiflorum L. Bangladesh J. Scientific Industrial Res. 53, 277–282.

Rahmat, Z., Naki, I., Qadir, Z., Mir, M., Bhat, K., Hussain, G., 2014. In vitro propagation of beach peony (Paeonia lactiflora Pall.) cv. Sara Bernhardt using shoot tips. Indian J. Horticulture 71, 385–389.

Rogge, L.E., Lasswell, J., Bartel, B., 2001. A gain-of-function mutation in IAA28 suppresses lateral root development. Plant Cell 13, 465–480.

Ruegger, M., Dewey, E., Gray, W.M., Hobbie, L., Turner, J., Estelle, M., 1998. The TIR1 protein of Arabidopsis functions in auxin response and is related to human SKP2 and yeast grp1. Genes Develop. 12, 198–207.

Sapra, N. P., & Pandya, H., 2017. An in vitro analysis and Ethnobotanical Profile of Jasminum sambac L.. J. Ethnopharmacology 212, 2017 69–80.

Sarkar, M.K.I., Janhan, M.A.A., Roy, C.K., Hossain, K., Rahman, S.S., Mia, M.R., Islam, M.R., Azim, D.A., 2016. In vitro Shoot Micro Propagation of Medicinal Applications and Ornamental Value of Centrum nuciforum L. Indians. J. Sci. Technology 9, 789–795.

Steel, R.G., Torrie, J., 1980. Principles and procedures of statistics: a biometrical approach. 2nd ed. McGraw-Hill Inc.

Sun, Y., Tang, F., Fang, W., Tan, G., 2009. Rapid propagation in vitro of jasmine. Acta Phytotax. Geographica Sinica 48, 390–394.