Saudi Journal of Biological Sciences 29 (2022) 3425–3431

Contents lists available at ScienceDirect

Saudi Journal of Biological Sciences

journal homepage: www.sciencedirect.com

Original article

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

Momin Showkat Bhat [a], Zahoor Ahmad Rather [a,*], Imtiyaz Tahir Nazki [a], Neelofar Banday [a], Tabinda Wani [a], Sadaf Rafiq [a], Iqra Farooq [b], Ahmed Noureldeen [c], Hadeer Darwish [d]

[a] Division of Floriculture and Landscape Architecture, Sher-e-Kashmir University of Agricultural Sciences and Technology of Kashmir, Shalimar, Srinagar 190025, J & K, India
[b] Division of Soil Science, Sher-e-Kashmir University of Agricultural Sciences and Technology of Kashmir, Shalimar, Srinagar 190025, J & K, India
[c] Department of Biology, College of Sciences, Taif University, P.O. Box 11099, Taif 21944, Saudi Arabia
[d] Department of Biotechnology, College of Sciences, Taif University, P.O. Box 11099, Taif 21944, Saudi Arabia

ARTICLE INFO

Article history:
Received 26 December 2021
Revised 7 February 2022
Accepted 9 February 2022
Available online 12 February 2022

Keywords:
Micropropagation
Jasminum nudiflorum
Nodal segments
Winter Jasmine

ABSTRACT

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% $NaOCl_2$ 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% $HgCl_2$ 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}).

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. Flowers appear before leaves on bare branches and hence the name nudiflorum (Harrison, 2012). The shrub appears to be an evergreen in nature due to bright green colour of stem and branches even in winter or early spring when small bright yellow flowers develop on leafless branches. Winter Jasmine is a popular shrub among gardeners with diverse landscape uses. It can be easily trained over walls, fences, arbors and other structures. Winter jasmine is a low maintenance plant growing in thickets along sloping and rocky ravines, which makes it an excellent choice for ground cover and erosion control in the home landscape. It not only adds color to the landscape, but also serves as an important source of forage for pollinators when it blooms in late winter. (Spicer, 2021). It can be grown as a bonsai as it tolerates the wiring methods very well. Besides, it is tolerant to atmospheric pollution.

Sexual and asexual methods are used for the propagation of jasmine species. However, seed setting is generally a rare phenomenon in Winter Jasmine and is usually seen under unusual

* Corresponding author.
E-mail address: zahoornishat@yahoo.com (Z. Ahmad Rather).
Peer review under responsibility of King Saud University.

Production and hosting by Elsevier

https://doi.org/10.1016/j.sjbs.2022.02.011
1319-562X/

Fig 1. (A) Explant preparation (B) Explant inoculation (C) Bud sprouting (D) Explant establishment (E) Explant proliferation (F, G) Rooting with NAA and IBA resp. (H, I) Hardening after 2 and 4 weeks resp.

weather conditions (Shahmoradi and Naderi, 2018). 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/layering 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). Plant tissue culture has emerged as a promising technique to obtain genetically pure elite population under *in vitro* conditions (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 introduction 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

Table 1
Sterilant treatments for culture asepsis and explant survival of nodal explants of Winter Jasmine (*Jasminum nudiflorum* Lindl.).

Treatment	Sterilant used	Concentration (%)	Treatment duration	Culture asepsis (%)	Explant survival (%)
T_1	Mercuric chloride	0.1	10 min	62.44 ± 0.67^{d}	52.00 ± 0.88^{c}
T_2	Sodium Hypochlorite	1.0	10 min	59.23 ± 0.29^{e}	58.33 ± 0.29^{b}
T_3	Mercuric chloride followed by	0.1	10 min	69.44 ± 1.31^{c}	61.11 ± 0.77^{ab}
	Ethyl alcohol	70.0	10 s		
T_4	Sodium hypochlorite followed by	1.0	10 min	63.88 ± 0.97^{d}	63.88 ± 2.78^{a}
	Ethyl alcohol	70.0	10 s		
T_5	Flusilazole followed by	0.01	20 min	72.22 ± 0.29^{b}	44.66 ± 0.58^{d}
	Mercuric chloride followed by	0.1	10 min		
	Ethyl alcohol	70	10 s		
T_6	Flusilazole followed by	0.01	20 min	67.89 ± 0.87^{c}	54.44 ± 0.58^{c}
	Sodium hypochlorite followed by	1.0	10 min		
	Ethyl alcohol	70	10 s		
T_7	Flusilazole followed by	0.02	20 min	77.78 ± 0.97^{a}	42.88 ± 1.13^{d}
	Mercuric chloride followed by	0.1	10 min		
	Ethyl alcohol	70	10 s		
T_8	Flusilazole followed by	0.02	20 min	73.88 ± 0.17^{b}	51.99 ± 0.38^{c}
	Sodium hypochlorite followed by	1.0	10 min		
	Ethyl alcohol	70	10 s		
	C.D $_{(P\leq 0.05)}$			**2.39**	**3.56**

*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.

Table 2
Establishment of nodal explants of Winter Jasmine (*Jasminum nudiflorum* Lindl) under different growth regulator treatments.

Treatment	Growth Regulators	Concentration (mgL^{-1})	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^{e}	13.64 ± 0.60^{a}	1.11 ± 0.07^{c}	4.33 ± 0.04^{a}	7.78 ± 0.07^{a}
2	BAP + Kn	2.0 + 0.5	69.44 ± 0.40^{c}	9.57 ± 0.32^{b}	1.37 ± 0.01^{b}	3.83 ± 0.06^{b}	7.16 ± 0.10^{b}
3	BAP + Kn	3.0 + 0.5	77.78 ± 0.97^{b}	8.04 ± 0.31^{c}	1.59 ± 0.05^{a}	3.51 ± 0.07^{c}	6.44 ± 0.08^{c}
4	BAP + Kn	1.0 + 1.0	63.88 ± 1.30^{d}	10.03 ± 0.58^{b}	1.07 ± 0.04^{c}	3.73 ± 0.09^{b}	7.24 ± 0.10^{b}
5	BAP + Kn	2.0 + 1.0	71.11 ± 0.29^{c}	9.07 ± 0.57^{bc}	1.27 ± 0.01^{b}	3.05 ± 0.07^{d}	6.97 ± 0.09^{b}
6	BAP + Kn	3.0 + 1.0	80.55 ± 0.40^{a}	7.62 ± 0.33^{c}	1.41 ± 0.03^{b}	2.82 ± 0.04^{d}	6.02 ± 0.10^{d}
C.D $_{(P\leq 0.05)}$			**2.28**	**1.47**	**0.13**	**0.21**	**0.29**

*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.

Table 3
Axillary shoot proliferation of nodal explants of Winter Jasmine (*Jasminum nudiflorum* Lindl) under various growth regulator treatments.

Treatments	Growth Regulators	Concentration (mgL^{-1})	Culture Proliferation (%)	Proliferated shoot number	Microshoot length (cm)	Leaves/ proliferated shoot
1	BAP + Kn	1.0 + 0.5	63.88 ± 1.31^{c}	1.79 ± 0.08^{d}	2.61 ± 0.04^{a}	3.95 ± 0.07^{a}
2	BAP + Kn	2.0 + 0.5	66.66 ± 0.96^{bc}	2.03 ± 0.06^{bc}	2.33 ± 0.008^{c}	3.44 ± 0.05^{b}
3	BAP + Kn	3.0 + 0.5	77.78 ± 0.97^{a}	2.41 ± 0.08^{a}	2.25 ± 0.01^{c}	2.85 ± 0.04^{d}
4	BAP + Kn	1.0 + 1.0	66.66 ± 0.96^{bc}	1.72 ± 0.05^{d}	2.44 ± 0.015^{b}	3.86 ± 0.06^{a}
5	BAP + Kn	2.0 + 1.0	69.44 ± 0.97^{b}	1.94 ± 0.83 ^{cd}	2.29 ± 0.04^{c}	3.15 ± 0.08^{c}
6	BAP + Kn	3.0 + 1.0	80.55 ± 0.97^{a}	2.22 ± 0.03^{ab}	2.02 ± 0.03^{d}	2.71 ± 0.09^{d}
C.D $_{(P\leq 0.05)}$			**3.21**	**0.21**	**0.10**	**0.22**

*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.

germplasm. *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 genera, 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

Table 4
Rooting of *in vitro* raised shoots of 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^{b}	8.58 ± 0.25 $(3.10 \pm 0.04)^{e}$	1.09 ± 0.04^{g}	11.03 ± 0.08^{e}
2	NAA	1.0	28.22 ± 0.61^{cd}	14.62 ± 2.80 $(3.92 \pm 0.37)^{d}$	1.90 ± 0.07^{f}	14.28 ± 0.07^{e}
3	NAA	1.5	22.20 ± 0.91^{e}	16.06 ± 3.07 $(4.09 \pm 0.39)^{d}$	2.63 ± 0.34^{de}	18.70 ± 0.05^{d}
4	NAA	2.0	22.00 ± 0.57^{e}	28.97 ± 2.41 $(5.47 \pm 0.22)^{c}$	3.40 ± 0.23^{bc}	24.52 ± 0.11^{c}
5	IBA	0.5	33.27 ± 0.41^{a}	18.51 ± 1.85 $(4.41 \pm 0.205)^{d}$	2.25 ± 0.18^{ef}	18.45 ± 0.04^{d}
6	IBA	1.0	29.63 ± 0.19^{c}	35.18 ± 1.85 $(6.01\ 0.152)^{bc}$	3.13 ± 0.10^{cd}	25.90 ± 0.09^{c}
7	IBA	1.5	29.18 ± 0.46^{c}	44.28 ± 2.86 $(6.72 \pm 0.21)^{b}$	3.83 ± 0.33^{b}	29.94 ± 0.13^{b}
8	IBA	2.0	27.36 ± 0.41^{d}	63.93 ± 1.49 $(8.06\ 0.09)^{a}$	4.74 ± 0.23^{a}	34.67 ± 0.22^{a}
C.D $_{(P\leq 0.05)}$			**1.64**	**0.73**	**0.66**	**3.42**

*Data noted at the end of 4 weeks of culture on MS medium
Figures in the parenthesis are transformed values (Square root transformation).
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.

Table 5
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 $(37.73 \pm 0.52)^{h}$
2	NAA	1.0	48.68 ± 0.90 $(44.23 \pm 0.52)^{g}$
3	NAA	1.5	57.79 ± 0.81 $(49.46 \pm 0.27)^{f}$
4	NAA	2.0	62.56 ± 0.46 $(52.25 \pm 0.48)^{e}$
5	IBA	0.5	67.88 ± 1.23 $(55.46 \pm 0.76)^{d}$
6	IBA	1.0	78.19 ± 0.37 $(62.14 \pm 0.26)^{c}$
7	IBA	1.5	84.72 ± 0.03 $(66.97 \pm 0.66)^{b}$
8	IBA	2.0	89.67 ± 0.83 $(71.22 \pm 0.009)^{a}$
C. D $_{(P\leq 0.05)}$:1.62			

Figures in the parenthesis are transformed values (Arcsine transformation).
*Data noted at the end of 4 weeks of culture in hardening media (Vermiculite + Perlitie = 1:1).
Data are a mean of two replicates with ± SE. Same superscript letters in a column do not differ significantly when compared by Duncan's MRT at 5% level of significance.

running tap water to remove any adhering contaminants. Final washing of nodal explants was done with sterilized water to which 1–2 drops of surfactant (Tween 20) were added and shaken vigorously for about 15 min. Finally the nodal segments were washed with distilled water thrice and taken to inoculation chamber for further sterilization treatment. Treatment with different sterilants including sodium hypochlorite (NaOCl), mercuric chloride ($HgCl_2$), ethyl alcohol (CH_5OH) and Flusilazole was carried out under aseptic conditions of laminar air flow hood. Eight different sterilization treatments were tried for getting optimum aseptic cultures and explant survival, which included T1 = Mercuric chloride @ 0.1% for 10 min; T2 = Sodium hypochlorite @1.0% for 10 min; T3 = Mercuric chloride @ 0.1% for 10 min followed by 70% ethyl alcohol for 10 s; T4 = Sodium hypochlorite @1.0% for 10 min followed by 70% ethyl alcohol for 10 s: T5 = Flusilazole @0.01% for 20 min followed by mercuric chloride @ 0.1% for 10 min followed by 70% ethyl alcohol for 10 s; T6 = Flusilazole @0.01% for 20 min followed by sodium hypochlorite @1.0% for 10 min followed by 70% ethyl alcohol for 10 s; T7 = Flusilazole @0.02% for 20 min followed by mercuric chloride @ 0.1% for 10 min followed by 70% ethyl alcohol for 10 s; T8 = Flusilazole @0.02% for 20 min followed by sodium hypochlorite @1.0% for 10 min followed by 70% ethyl alcohol for 10 s.

2.2. Media preparation and explant inoculation

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℃ 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 rises with double distilled autoclaved water to remove the traces of sterilants. 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 16:8h 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 + 0.5; 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.

2.3. 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. E*x-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 the basic assumptions for analysis of variance, as suggested by Steel and Torrie (1980). 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 T_2 and T_1 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 T_3 and T_4, respectively. However, maximum culture asepsis of 77.78% and 73.88% was recorded with T_7 and T_8, 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 T_4 followed by 61.11% with T_3. Both these treatments were statistically at par with each other but differed significantly from all other treatments. Minimum explant survival of 42.88% was recorded with T_7 followed by 44.66 % with T_5 and 51.99% with T_8. Lowest explant survival was obtained in all these treatments, where a combination of three sterilants was used.

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 D). 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 2). MS media supplemented with BAP + Kn (3.0 + 1.0 mgL^{-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 mgL^{-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 mgL^{-1}) followed by 1.41 recorded in treatment combination BAP + Kn (3.0 + 1.0 mgL^{-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 mgL^{-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 mgL^{-1}) followed by 77.78 % with BAP + Kn (3.0 + 0.5 mgL^{-1}). The highest number of proliferated shoots (2.41) was recorded with growth regulator combination BAP + Kn (3.0 + 0.5 mgL^{-1}) followed by 2.22 in BAP + Kn (3.0 + 1.0 mgL^{-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 mgL^{-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 mgL^{-1}) followed by 44.28% with IBA (1.5 mgL^{-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 mgL^{-1}IBA (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 T_3 and T_4 were observed the best treatments for sterilisation of nodal segments of jasmine, which yielded optimum culture asepsis and higher culture survival but we recommend T_4 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 culture establishment and proliferation. Bhattacharya and Bhattacharyya (2010) also reported higher proliferation rate with highest concentration of BAP 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 micro shoots. 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 perenials (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 mixo-tropic nutrition. These conditions need to be simulated during primary hardening of rooted tissue cultured plants. Highest ex vitro survival (89.67%) was observed in plantlets, which were rooted in MS media supplemented with IBA (2.0 mgL^{-1}) followed by 84.72% in plantlets which came from media supplemented with IBA @ 1.5 mgL^{-1}. 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, T_4 treatment combination (1.0% $NaOCl_2$ 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 mgL^{-1}) as it yielded higher number of microshoots with maximum shoot length, which is very important for subsequent proliferation cycles and successful rooting of microshoots. MS media fortified with IBA (2.0 mgL^{-1}) was found best medium for rooting of micro shoots as it yielded 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 mgL^{-1}).

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 IF 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 appeared to influence the work reported in this paper.

Acknowledgements

The authors would like to thank the Deanship of Scientific Research at Taif University for supporting this work through Researchers Supporting Project number (TURSP - 2020/141), Taif University, Taif, Saudi Arabia.

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