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Research Paper

In vitro sterilization and establishment protocol for hybrid tea rose cvs."Hot Shot" and "Avalanche"

Tanzeela Yaseen*, I.T. Nazki[1], Neelofar Banday[1],
F.A.Khan[3], M.D. Shah[4] and Insha Nazir[1]

*[1] Division of FLA; [2] Division of Plant Biotechnology; [3] Division of BSH; [4] Division of Plant Pathology,*Division of FLA, Sher-e- Kashmir University of Agricultural Sciences and Technology of Kashmir, Shalimar, Srinagar- 190025, Jammu and Kashmir (India)*
*e-mail: *syed.tanzella@gmail.com*

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ABSTRACT

The experiment was undertaken for the purpose of standardizing the protocolfor *in vitro* propagation ofHybrid Tea Rose cultivars"Hot Shot" and "Avalanche". Nodal explants from actively growing roses under polyhousewere used as explants for *in vitro* propagation. Sterilization treatment combination S_6 (Mercuric chloride 0.15% for 10 minutes followed by ethyl alcohol 70% for 30 seconds) exhibited the highest percentage of explant survival and culture asepsis(91.66%, 92.08% and 95.83%, 95.79%) in cultivars "Hotshot" and "Avalanche". Highest culture establishment per cent (87.49% and 90.41%) was recorded in T_6 (BAP 2.0 mg L^{-1}+ GA_3 2.0 mg L^{-1}).

Key words: Culture asepsis, establishment, micropropagation, nodal cuttings, rosa hybrida, sterilants

The rose is a perennial flowering plant that is a member of the Rosaceae family, which has about 100 genera and 2,000 species, as stated by Arne *et al.* (1993).About 200 species and thousands of cultivars make up the genus Rosa, of which more than 150 species have been identified through cataloging (Scott and Hansen, 1982). Just 11 of the 200 species of roses are known to have influenced the development of contemporary cultivars (Wylie, 1955). Rose is indigenous to temperate regions of the Northern Hemisphere. Rose regarded as the "Queen of flowers" holds a significant demand for cut flowers and as potted plants.No other plant has captivated attention and has such widespread appeal throughout history as the rose.

Roses are shrubs that grow upright, climb, or trail; their stems have prickles that come in all sizes and forms. The leaves are complex, pinnately arranged, and typically have oval leaflets. While cultivated roses often have doubleblossoms, wild roses typically have five petals. Roses come in a variety of sizes, from miniatures with a diameter of 1.25 cm to hybrid blossoms larger than 17.5 cm. A berrylike "fruit" is known as hip which is rich in vitamin C.

Generally, roses are propagated using vegetative techniques like grafting and budding. In breeding programs, seeds are utilized to improve crops and produce rootstocks (Horn, 1992). Vegetative propagation does not guarantee healthy, disease-free plants. In addition, seasonality and poor multiplication rates are significant barriers to conventional propagation. In view of the importance of rose as top ranked commercial cut flower and the need of proper sterilization techniques for the establishment of explants*in vitro*, study was conducted during 2020–2023at SKUAST-K, Shalimar, in the Division of Floriculture and Landscape Architecture's tissue culture laboratory.The goal of the proposed study was to create a methodology for sterilization and*in vitro*establishment of Hybrid Tea Rose cultivars"Hot Shot" and "Avalanche".

MATERIALS AND METHODS

Collection of explant and surface sterilization: The explant was obtained from stock plants of Hybrid Tea Rose cultivars"Hot Shot" and "Avalanche" (Fig.1) growing in a polyhouse at the Division of Floriculture and

Landscape Architecture's experimental farm in Shalimar, SKUAST-K. Upon arriving at the laboratory, explants were carefully cut into smaller segments, each bearing a node or two, and thoroughly washed with tap water to get rid of any leftover particles and grime.After that, thoroughly stir in Tween-20 (surfactant) for 30 minutes. After rinsing the surfactant with running tap water, distilled water was used for the last rinse. Three sterilizing chemicals were utilized: 70% ethyl alcohol, 0.2% and 0.4% sodium hypochlorite, and 0.1% and 0.15 percent mercuric chloride. A laminar flow containing varying concentrations of these surface sterilants was used to sterilize the explants. Before being placed into culture media, the explant was cleaned on three separate occasions with sterile water after receiving sterilant treatment. Following the 4th week of culture, the percentage of explant survival and asepsis was noted.

Preparation of culture media and incubation: The study made use of the macro, micro and vitamin-rich media developed by Murashige and Skoog in 1962. Growth regulators, myo inositol, and sucrose (usually 30 g/1), were added in accordance with the needs of the treatment. After then, the pH of the medium was adjusted to 5.7. Using a microwave oven, the medium was lightly heated before the required amount of agar was added, stirred, and heated to boiling in order to completely dissolve it. The media was spread into culture containers and covered with cotton plugs after cooling for a few minutes. Test tubes and flasks containing media were autoclaved at 121°C and 15 psi , to ensure sterility.

The cultures were kept at 24°C in a cool room with a 16/8 light/dark cycle and 3500 lx of light intensity. Different PGR concentrations and combinations were investigated to ensure optimal culture establishment, proliferation, and roots. Nodal cuttings were sterilized and deposited on MS medium containing various growth regulator combinations BAP (6-Benzylamino Purine) and GA_3 for establishment according to the polarity of whence they originated.Twelve explants were assigned to each treatment combination, one for each test tube, and the replication was carried out three times. Data on the percentage of established shoots, total number of shoots / explant, shoot length (cm), and the total number of leaves on established shoots were obtained following a four-week culture period.

Statistical analysis: Two factorial CRD (Completely Randomized Design) with three replications per treatment was applied to the data for different parameters gathered for this investigation, and the square root transformation was used to the % data.

RESULTS AND DISCUSSION

Aseptic culturepercent and survival percent: According to the findings in (Table 1), the percentage of survival and culture asepsis were significantly impacted by the application of sterilants, either alone or in combination. Interaction between the two factors cultivar and treatment was observed to be non- significant (Table 2.1). Higher mean culture asepsis per cent in nodal explants was recorded in the cultivar‘ Avalanche’ (68.23%) than ‘Hotshot’ (64.37%).The experiment involving S_6 (Mercuric chloride 0.15% for 10 minutes, and ethyl alcohol 70% for 30 seconds)yielded the highest aseptic culture percentage (91.66% and 95.83%)in cultivars “Hotshot” and “Avalanche”.Significantly lowest culture asepsis in (45.83%and 50.00%) was recorded in S_4 (Sodium hypochlorite 0.2% for 10 minutes). Highest survival per cent (92.08 % and 95.79%) was also recorded with S_6 (Mercuric chloride 0.15% for 10 minutes, and ethyl alcohol 70% for 30 seconds) while as lowest survival per cent (74.12 % and 72.37%) was recorded in S_7 (Sodium hypochlorite 0.4% for 10 minutes and ethyl alcohol 70% for 30 seconds).

A successful protocol for *in vitro* propagation must be developed, and selecting the right explant with the least amount of delay is crucial to achieving the desired results of any tissue culture method.The complete loss of explants has been caused by inadequate sterilization techniques, and their incubation on the medium containing sucrose has increased microbial growth even more. Additionally, during sub-cultures, it raises the possibility that viruses will infect healthy explants. Explant survival is decreased by even a small amount of contamination following sterilization, such as a single live spore or bacterial cell. The rate of multiplication and regeneration would be lower in such cultures. Additionally, the quality of plant genetic resources would decline (Wangand Valkonen, 2009).

The data revealed that combination of Mercuric chloride and ethyl alcohol resulted in higher culture asepsis and survival percentin explants. Mercuric chloride is an antimicrobial agent that is effective against both fungi and bacteria. Additionally, adding a final 10-second ethyl alcohol dip greatly increased explant survival. Ethyl alcohol eliminates any remaining Hg^{2+} ions that remain in explant material in addition to water. According to Verma*et al*. (2011). the greatest survival rate was obtained by surface sterilization using 0.1% Mercuric chloride. When compared to single-chemical sterilisation, the simultaneous use of two or more sterilants proved to be more successful in sterilising buds or explants (Rather, 2014). However, sodium hypochlorite comparatively proved to be less effective (Abbasi *et al.,* 2016).

Table 1: Influence of sterilant treatments on culture asepsis (%) and survival (%) of Hybrid Tea Rose cultivars "Hotshot" and "Avalanche"

Treatments		Culture asepsis (%)		Survival (%)	
		Hotshot	Avalanche	Hotshot	Avalanche
S_1	Mercuric chloride 0.1 % for10 min	54.16 (7.41)	58.33 (7.68)	80.45 (9.02)	83.74 (9.18)
S_2	Mercuric chloride 0.15 % for 10 min	62.49 (7.95)	66.66 (8.19)	70.91 (8.46)	74.24 (8.66)
S_3	Sodium hypochlorite 0.2 % for 10 min	45.83 (6.82)	50.00 (7.13)	80.16 (9.00)	81.16 (9.06)
S_4	Sodium hypochlorite 0.4 % for 10 min	48.33 (6.98)	54.16 (7.41)	78.45 (8.91)	80.87 (9.04)
S_5	S_1 +Ethyl alcohol 70 % for 30 sec	87.49 (9.40)	91.66 (9.61)	89.99 (9.53)	90.29 (9.55)
S_6	S_2 +Ethyl alcohol 70% for 30 sec	91.66 (9.61)	95.83 (9.83)	92.08 (9.64)	95.79 (9.83)
S_7	S_3 +Ethyl alcohol 70% for 30 sec	58.33 (7.68)	62.49 (7.95)	76.20 (8.78)	80.87 (9.03)
S_8	S_4 + Ethyl alcohol 70% for 30 sec	66.66 (8.19)	66.66 (8.19)	70.62 (8.44)	74.12 (8.65)
	Mean	64.37 (8.00)	68.23 (8.25)	79.86 (8.97)	82.64 (9.12)
	LSD ($P_{=0.05}$) Cultivar (C)	**N.S**		**N.S**	
	Sterilants (S)	**9.87**		**7.58**	

Culture establishment and budsprouting: During the experiment, eight combinations of plant growth regulators were examined, and all of them produced good culturedevelopment(Table 2, Fig. 2). Data analysis showed that distinct PGR concentrations, cultivars, and their interactions had a major impact on the establishment percentage of nodal explants in both cultivars. 'Avalanche' resulted in significantly highest mean establishment of (67.67%) than Hotshot (64.58%).Highest establishment per cent (87.49 and 90.41%) was recorded in T_6 (BAP 2.0 mg L^{-1}+ GA_3 2.0 mg L^{-1}) in cultivars "Hotshot" and "Avalanche" and lowest establishment per cent of (41.66% and 45.83%) was observed in T_4 (BAP 3.0 mg L^{-1}+ GA_3 1.0 mg L^{-1}). T_7 (BAP 2.5 mg L^{-1}+ GA_3 2.0 mg L^{-1}) recorded least number of days (4.02 and 5.23 days) taken for sprouting of budsin cultivars "Hotshot" and "Avalanche". Among cultivars 'Avalanche' resulted in lower mean minimum days for sprouting of buds (5.88) than Hotshot (5.89). However the interaction effect was non-significant(Table 2.1).

An essential step in creating an effective procedure for the in vitro multiplication of any plant is the establishing of the explants following surface sterilization. A dopting an explant to a heterotrophic mode of nutrition could be referred to as establishment. MS media fortified with T_6 BAP + GA_3 (2.0 + 2.0 mg L^{-1}) showed that they were among the most effective in establishing culture. For bud sprouting cytokinins plays an important role by promoting bud break and reducing the apical dominance. This could also be because there is a strong sink/growing area with a higher likelihood of continuing to grow and sprout because the growing tip is still intact and a source of auxin. Rout *et al,* (1990) and Afrin, S. *et al*, (2022)were successful in getting maximum number of shoots explant^{-1} in Rose cultivars.

Number of shoots explant^{-1}, lengthof shoots and number of leaves shoot^{-1}: From established cultures (Table 2) proliferating shoots were divided and added to MS media with varying concentrations of BAP+GA_3 to facilitate their proliferation and multiplication. A noteworthy interaction was found between the two components cultivars and PGRs (Table 2.1).Avalanche showed a considerably higher mean average number of shoots initiated per explant (2.81) than Hotshot (2.57). Treatment T_7BAP + GA_3 (2.5 + 2.0 mg L^{-1}) recorded highest number of shoots explant^{-1} (3.62 and 4.17), maximum length of established shoots (4.50 cm and 4.75 cm) and number of leaves shoot^{-1}(4.08 and 4.45). Cytokinins are most widely used PGRs in plant tissue culture for shoot proliferation. They promote lateral shoot growth (Taji and Williams, 1996).BAP 1.5-2.5 mg/l has been proven to be essential to shoot development and growth in nodal culture. For shoot elongation of rose cultivarsthe amalgamation of BAP and GA_3 was tested in MS media. By further increase in concentrations of GA_3 from 1.5 mg/l to 2 mg/l, average length of shoots was increased and BAP 2.0- 3.0 mg/l + GA_3 gave the best results. According to Patil*et al*,(2005) the chrysanthemum cv. "PKV-Shubra" shoot tip explant inoculated on MS + BAP 2 mg + NAA 0.5 mg/ lperformed better in terms of response to shoot initiation. For shoot elongation of rose, the combination of BAP and GA_3 were examined and combining BAP 3.0 mg $L^{-1}$with GA_3gave the best results (Asad*et al.,* 2010, Chhalgri*et al.,* 2020 and Kaythi, O.*et al.,* 2021).

Table 2: Influence of growth regulators on establishment of Hybrid Tea Rose cultivars "Hotshot" and "Avalanche"

	Treatments	Conc. (mg L^{-1})	Establishment (%)		Days to bud sprouting		Number of shoots explant^{-1}		Length of shoots explant^{-1}		Number of leaves explant^{-1}	
			Hotshot	Avalanche	Hotshot	Avalanche	Hotshot	Avalanche	Hotshot	Avalanche	Hotshot	Avalanche
T_1	BAP+ GA_3	1.5 + 1.0	66.66^{c} (8.19)	70.83^{c} (8.46)	5.49^{a}	6.95^{a}	1.25^{d}	1.52^{d}	1.50^{d}	1.87^{c}	3.29^{d}	2.75^{cd}
T_2	BAP+ GA_3	2.0 + 1.0	70.83^{bc} (8.46)	79.16^{bc} (8.94)	4.95^{b}	6.54^{b}	2.00^{cd}	2.27^{cd}	2.37^{cd}	2.50^{b}	3.36^{d}	3.17^{d}
T_3	BAP+ GA_3	2.5 + 1.0	54.16^{d} (7.41)	54.16^{d} (7.41)	4.87^{bc}	5.31^{bc}	2.27^{bc}	2.41^{c}	2.47^{c}	2.55^{b}	3.76^{c}	3.57^{c}
T_4	BAP+ GA_3	3.0 + 1.0	41.66^{e} (6.50)	45.83^{e} (6.82)	5.48^{a}	5.93^{a}	2.21^{c}	2.40^{c}	2.40^{c}	2.50^{b}	3.64^{c}	3.52^{c}
T_5	BAP+ GA_3	1.5 + 2.0	79.16^{b} (8.94)	80.16^{b} (8.99)	5.08^{c}	5.32^{c}	3.16^{b}	3.51^{bc}	3.12^{bc}	3.25^{bc}	3.86^{bc}	3.63^{bc}
T_6	BAP+ GA_3	2.0 + 2.0	87.49^{a} (9.40)	90.41^{a} (9.55)	4.08^{d}	5.25^{d}	3.61^{a}	4.12^{ab}	4.00^{b}	4.50^{ab}	3.91^{ab}	4.10^{ab}
T_7	BAP+ GA_3	2.5 + 2.0	62.49^{cd} (7.95)	62.49^{cd} (7.95)	4.02^{d}	5.23^{d}	3.62^{a}	4.17^{a}	4.50^{a}	4.75^{a}	4.08^{a}	4.45^{a}
T_8	BAP+ GA_3	3.0 + 2.0	54.16^{d} (7.41)	58.33^{d} (7.68)	5.04^{ab}	5.30^{ab}	3.61^{a}	4.15^{a}	4.43^{a}	4.62^{a}	3.97^{b}	4.44^{a}
		Mean	64.58^{b} (8.03)	67.67^{a} (8.22)	5.89^{a}	5.88^{b}	2.57^{a}	2.81	2.79^{b}	2.98^{a}	3.72^{a}	3.61^{b}
		LSD ($P_{=0.05}$)	**N.S**		**N.S**		**0.22**		**0.19**		**N.S**	
		Cultivar (C)	**9.04**		**0.87**		**0.43**		**0.38**		**0.39**	
		Treatment (T)										

*Figures in the parentheses are square root transformed values of percentage data

Table 2.1: Interaction effect of cultivars and Sterilants / PGR's on culture asepsis and establishment of Hybrid Tea Rose cultivars "Hotshot" and "Avalanche".

Treatment	Culture asepsis (%)	Survival (%)	Establish ment (%)	Days to bud sprouting	Number of shoots explant^{-1}	Length of shoots explant^{-1}	Number of leaves explant^{-1}
C×T$_1$	56.25 (7.54)	82.10 (9.10)	68.74 (8.32)	6.22	1.39	1.69	3.02
C×T$_2$	64.58 (8.07)	72.58 (8.56)	74.99 (8.70)	5.75	2.14	2.44	3.27
C×T$_3$	47.92 (6.98)	80.66 (9.03)	54.16 (7.41)	4.59	2.34	2.49	3.67
C×T$_4$	51.25 (7.20)	79.66 (8.97)	43.75 (6.66)	5.11	2.30	2.45	3.58
C×T$_5$	89.58 (9.50)	90.14 (9.54)	79.66 (8.97)	5.19	3.34	3.19	3.74
C×T$_6$	93.75 (9.72)	93.93 (9.74)	88.96 (9.47)	4.66	3.87	4.25	4.01
C×T$_7$	60.41 (7.81)	78.54 (8.90)	56.25 (7.54)	4.42	3.89	4.62	4.27
C×T$_8$	66.66 (8.19)	72.37 (8.55)	62.49 (7.95)	5.14	3.88	4.53	4.10
LSD ($P_{=0.05}$)	**N.S**	**N.S**	**N.S**	**N.S**	**0.65**	**0.54**	**N.S**

Hotshot

Avalanche

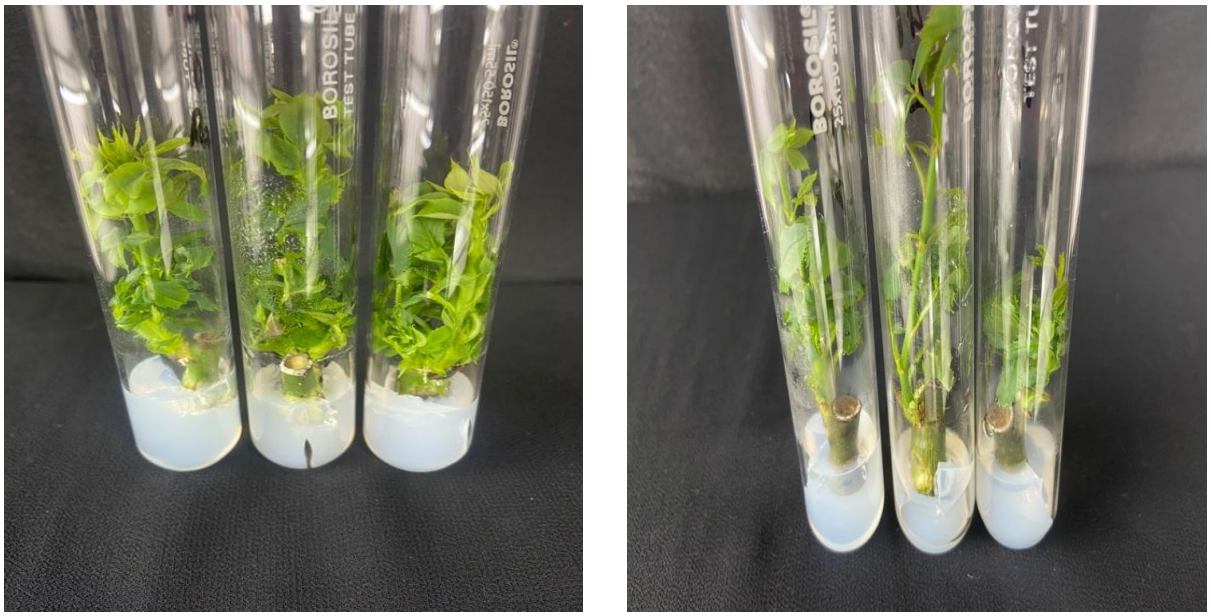

Fig. 2: Establishment of nodal explants of Hybrid Tea Rose cultivars "Hotshot" and "Avalanche" after weeks of culture

CONCLUSION

The maximum aseptic cultures and explant survival rate in cultivars was found in plants surface sterilized using S_6 (mercuric chloride 0.15% for 10 minutes, subsequently followed by ethyl alcohol 70% for 30 seconds)Avalanche and Hotshot. The treatment combination that produced the greatest establishment culture percent was T_6 (BAP 2.0 mg L-1 + GA 2.0 mg L-1). Treatment T_7recorded the highest number ofshoots, leaves per explant^{-1} and shoot lengthoverall.It is therefore acknowledged as the most effective treatment out of all the other PGR combinations for establishment.

Genetics plays a significant part in the establishment, proliferation and days to bud sprouting. The type of cultivar had a significant impact on explant establishment. Among the two cultivars, 'Hotshot'and 'Avalanche', highest establishment rates, minimum days to bud initiation and the most shoots per explant were reported in Avalanche.

REFERENCES

Abbasi, Z., Singh, R. P., Gautam, D. N. S. 2016. A novel aseptic technique for micropropagation of Aloe vera mill. *Advanced Herbal Medicine,* **2:** 47-60.

Afrin, M. A., Rahman, M., Khalekuzzaman, M. M., HasanA. H. F., Fahimand Alam M. A. 2022.Study on *in vitro* micropropagation of Rosa. *BangladeshJournal of Agriculture,* **47**: 66-74

Arene, L., Pellegrino, C. and Gudin, S. A. 1993. Comparison of the somaclonal variation level of *Rosa hybrida* L. cv."Meirutral" plants regenerated from callus or direct induction from different vegetative and embryonic tissues. *Euphytica,* **71**:83-90.

Asad S, Hameed N, Ali A, Bajwa R, Vecherko NA, Mursalieva VK. 2010. Factors affecting the growth anddevelopment of roses *in vitro*. *Journal ofBiotechnol*ogy, **1**:41-52.

Chakrabarty D, Mandal AK, Data SK. 2000. *In vitro* propagation of rose cultivars. *Indian Journal of Plant Physiology***5(2)**:189-192.

Chhalgri, M., Yasmeen, S., Khan, I., Aslam, M., Rajpar, A., Tayyaba, Iqbal, R., Panhwar, M., Siddiqui, M., Khan, T., Nizamani, M. R. and Nizamani, F. 2020. Effect of Plant Growth Hormones on Shoot and Root Regeneration in Rose under In Vitro Conditions. *Life sciences,* **8**: 93-97

Horn, W. A. H. 1992. Micropropagation of rose *(Rosa L.)* pp. 320–342 In: Biotechnology in agriculture and forestry Vol. 20 High-tech and micropropagation IV (Eds. Y. P. S Bajaj), Springer Germany7.

Kaythi, O., Lwin, K. M. and Khai, A.A. (2021). *In vitro* Micropropagation of Rose through Plant Tissue Culture Technique. *Journal of Scientific and Innovative Research,* **10:** 1-4.

Nizamani, F., Nizamani, G. S., Nizamani, M. R., Ahmed, S. and Ahmed, A. 2016. Propagation of Rose (Rosa Hybrida L.) Under Tissue Culture Technique. *International Journal of Biology Research,* **1:** 23-27.

Ozel, C. and Arslan, O. 2006. Efficient Micropropagation of English Shrub Rose "Heritage" Under in Vitro Conditions. *International Journal of Agriculture and Biology*. **8** (5): 626-629.

Patil, S. R., Sripriya, B., Anitha, C. and Chopde, M. 2005. *In vitro* propagation of chrysanthemum (*Dendranthema grandiflora*). *Journal of Soils and Crops,* **15**(2): 287-289.

Rather, Z. A., Nazki, I. T., Qadri, Z. A., Mir, M. A., Bhat, K. M., and Hussain, G. 2014. In vitro propagation of herbaceous peony (*Paeonialactiflora* Pall.) cv. Sara Bernhardt using shoot tips. *Indian Journal of Horticulture,* **71**(3): 385-389.

Rout, G.R., Debata, B.K., Das, P. 1990. *In vitro* clonal multiplication of roses. Proc Natl Acad Sci India, **60**:311-8.

Scott, H. and Hansen, M. 1982. Old garden roses and their place in the development of modern roses. *American Rose Society Annual,* **6**7:106-123.

Taji, A. M. and Williams, R. R. 1996. *Overview of plant tissue culture,* **In:** *Tissue Culture of Australian Plants*: *past, present and future* (Eds. A.M. Taji and R.R. Williams), University of New England Press, Armidale, Australia.pp.1-15

Verma, S., Yadav, K. and Singh, N. 2011. Optimization of the protocols for surface sterilization, regeneration and acclimatization of *Stevia rebaudiana*Bertoni. *American Eurasian Journal of Agricultural Environmental Science,* **11**: 221-227.

Wang, Q., and Valkonen, J. P. 2009. Cryotherapy of shoot tips: novel pathogen eradication method. *Trends in Plant Science,* **14**: 119-122.

Wojtania, A. and Matysiak, B. 2018. In vitro propagation of *Rosa 'Konstancin'* (*R. rugosa*×*R. beggeriana*), a plant with high nutritional and pro-health value. *Folia Horticulturae,* **30**: 259-267.

Wylie, A. P. 1955. The history of garden roses, Part 2. *Journal of Royal Horticultural Society* **79**: 8–24.