J. Hortic. Sci.
Vol. 18(1) : 173-180, 2023

https://doi.org/10.24154/jhs.v18i1.2160

Original Research Paper

Standardization of sterilization protocol for explants and its suitability for direct organogenesis in tuberose cv. Arka Vaibhav

Mahananda[1], Bharathi T.U.[2]*, Usharani T.R.[2], Kumar R.[2] and Kulkarni B.S.[1]

[1]College of Horticulture, University of Horticulture Sciences Campus, GKVK, Bengaluru - 560065, Karnataka, India
[2]ICAR-Indian Institute of Horticultural Research, Bengaluru-560089, Karnataka, India
*Corresponding author Email : ushabharathi.t@icar.gov.in

ABSTRACT

A study was carried out to standardize the sterilization protocol for different explants (terminal stem scale, immature flower bud and tepal segment) and to select the suitable explant for the direct organogenesis of tuberose cv. Arka Vaibhav. The highest survival per cent (100) and uncontaminated cultures (0.00) of terminal stem scale explant was observed in pre-treatment with overnight soaking of terminal stem scale in the solution comprising carbendazim (0.1%), chlorothalonil (0.05%) and myristyl trimethyl ammonium bromide (cetrimide) (0.05%) and subsequently surface sterilization with 70% ethanol (1 min), 4% sodium hypochlorite (10 min) followed by 0.1% $HgCl_2$ (15 min). The explant immature flower bud recorded the highest survival per cent (100) and maximum aseptic cultures in the treatment T_1 comprised of 1.0 drop Tween-20 + 70% ethanol (30 sec) and 1% sodium hypochlorite (3 min). Pre-treatment of tepal segment explant in 0.1% carbendazim (30 min) solution followed by surface sterilization with combination of 1.0 drop Tween-20 + 70% ethanol (30 sec) followed by 1% sodium hypochlorite (3 min) registered 91.66% of survival with the minimum contamination (10%) in the treatment. Among the three explants used, the terminal stem scale was found suitable for direct organogenesis with early greenness (5.72 days) and highly responsive to shoot induction (100%) in MS medium supplemented with 4 mg/L BAP + 0.1mg/L IAA. Other two explants *viz.,* immature flower bud and tepal segment failed to respond for direct organogenesis by shoot induction instead produced profuse callus.

Keywords : Aseptic culture, direct organogenesis, explants, surface sterilization

INTRODUCTION

Tuberose (*Agave amica* (Medik.) Thiede & Govaerts.) is an important bulbous flower crop valued for its pleasant fragrance and widely grown for loose flower as well as cut flower purpose. The commercial cultivation of tuberose in India is confined to West Bengal, Karnataka, Maharastra, Tamil Nadu, Haryana, Punjab, Gujarat, Rajasthan, Andhra Pradesh including Assam. It is cultivated in an area of about 21.77 ('000 ha) with loose flower production of 117.14 ('000 metric tons) and cut flower production of 102.25 lakh numbers of cut stems (Anon., 2023). Tuberose concrete and absolute are highly priced in international market and their extraction has been established as export oriented agro-industry in India. The consumer preference for natural products increased the market demand for the tuberose floral extract which intern upsurge the planting material requirement. Tuberose is propagated through bulbs and rapid multiplication of quality planting material through conventional propagation is highly time consuming.

Micro-propagation is a tool to augment the supply of planting material in a short period of time, need less space and time for large populations, and provides the opportunity to keep the plant material disease-free. Contamination is a major problem in the selection and preparation of explant and may lead to significant loss of cultures (Pandey *et al*., 2009; Mir *et al*., 2012). Contamination in tissue culture can originate from two sources, either through carryover of microorganisms from the surface of the explant or from within the tissue itself (endophytic micro-organisms). Although, in meristem culture, depending on meristem size most of the microorganisms can be eliminated, whereas, in leaf, petiole and stem explants, the infection would carry over to the cultures (Leifert and Cassells, 2001). Bacterial contamination was the major limitation in the sterilization process (Ray and Ali, 2016). In most of the studies, sodium hypochlorite or mercuric chloride

($HgCl_2$ 0.1%) has been used for sterilization. In some cases, treatment with fungicides such as carbendazim and chlorothalonil has been used and for inhibition of growth of bacteria in plant tissue culture antibiotics were used (Taha *et al*., 2018). Standardization of rapid multiplication techniques through tissue culture in tuberose is essential for the large scale production and distribution of quality planting material to the farmers. Keeping the above in view, present study was carried out to standardize the surface sterilization for different explants and to find the right explants for the direct organogenesis of tuberose cv. Arka Vaibhav.

MATERIAL AND METHODS

The present investigation was carried out in the Division of Flower and Medicinal Crops, ICAR-Indian Institute of Horticultural Research, Bengaluru during the year 2019-2020 using tuberose commercial cultivar Arka Vaibhav (double type). The variety Arka Vaibhav produces multi-whorled flower on the spikes having cut flower value. Three different explants *viz*., terminal stem scale (5 mm), immature flower bud (10-15 mm) and tepal segments (10-15 mm) were used to standardize the surface sterilization and to identify the best responsive explant for direct organogenesis (Fig. 1 & 2). As the terminal stem scales were obtained from the soil, in order to avoid heavy contamination due to soil borne diseases, seven different treatments were designed using completely randomised block design (CRD) and replicated thrice for the production of aseptic culture of terminal stem scales with pre-treatment followed by surface sterilization. The immature flower buds were separated from the spike and tepal segments were obtained from freshly opened flowers. Six different treatments were followed for the surface sterilization of immature flower bud and tepal segment explants using CRD with three replications. The parameters such as contamination per cent and survival per cent were recorded. To study the suitability of different explants for the direct organogenesis of tuberose cv. Arka Vaibhav, an

a. Terminal stem sacle (0.5-1.5 cm) b. Immature flower bud (10-15 mm) c. Tepal segment (10-15 mm)

Fig. 1 : Different explants with different sizes used to initiate the culture before inoculation into culture media

A B C

Fig. 2 : Terminal stem scale explants of tuberose cv. Arka Vaibhav during and after surface sterilization in LAF

experiment was conducted for direct organogenesis in CRD with six treatments and three replications using three different explants *viz*., terminal stem scale, immature flower bud and tepal segment. The explants were cultured on MS medium containing different combinations of growth regulators (auxins @ 0.1 mg and cytokinin @ 2.0, 4.0 mg). The observations on days to complete greenness and shoot induction per cent response were recorded. The collected data were subjected to statistical analysis using OPSTAT software.

RESULTS AND DISCUSSION

Establishment of aseptic culture for terminal stem scale explants

The data presented in Table 1 revealed that pre-treatment of terminal stem scale explants with the treatment (T_6) comprising of overnight soaking with 0.1% carbendazim + 0.05% chlorothalonil + 0.05% myristyl trimethyl ammonium bromide (cetrimide) and surface sterilization with 70% ethanol (1 min) + 4% sodium hypochlorite (10 min) + 0.1% $HgCl_2$ (15 min) resulted in 0.00% contamination, while, the control treatment with distilled water wash for 30 minutes recorded the maximum contamination of 100.00%.

The per cent survival of explant significantly varied among the different treatments (Table 1). The maximum survival (100%) of terminal stem scales was recorded in T_6 treatment with the overnight soaking of terminal stem scale explants in 0.1% carbendazim + 0.05% chlorothalonil + 0.05% myristy trimethyl ammonium bromide (cetrimide) followed by surface sterilization with 70% ethanol (1 min) + 4% sodium hypochlorite (10 min) + 0.1% $HgCl_2$ (15 min), followed by T_5 treatment with the overnight soaking of terminal stem scale explants in 0.1% carbendazim + 0.05% chlorothalonil + 0.05% myristyl trimethyl ammonium bromide (cetrimide) and surface sterilization with 70% ethanol (1 min) + 4% sodium hypochlorite (10 min) which recorded 96.66% of explants survival. The lowest per cent survival of explant was observed in control (40%).

Table 1 : Effect of surface sterilization on terminal stem scale explant of tuberose cv. Arka Vaibhav

Treatment	Contamination of explant (%)	Survival of explant (%)
T_0: Distilled water wash for 30 min (control)	100.00 (90.00)	40.00 (39.13)
T_1 : Overnight soaking with 0.05% carbendazim	86.66 (68.83)	50.00 (44.81)
T_2: Overnight soaking with 0.05% carbendazim + 0.05% chlorothalonil	83.33 (66.12)	63.33 (52.84)
T_3: Overnight soaking with 0.1% carbendazim + 0.05% chlorothalonil + 0.05% myristyl trimethyl ammonium bromide (cetrimide)	46.66 (43.06)	76.66 (61.11)
T_4: T_3 + 70% ethanol (1 min)	33.33 (35.20)	93.33 (77.69)
T_5: T_3+ 70% ethanol (1 min) + 4% sodium hypochlorite (10 min)	13.33 (21.14)	96.66 (83.85)
T_6: T_3 + 70% ethanol (1 min) + 4% sodium hypochlorite (10 min) + 0.1% $HgCl_2$ (15 min)	0.00 (0.00)	100.00 (90.00)
Mean	51.90 (46.34)	74.28 (64.23)
SEm±	2.82	5.49
CD (p=0.05)	8.54	16.66
CV (%)	9.40	12.80

Values in the parenthesis are angular transformed values

The success of micro-propagation mainly depends upon the initiation of aseptic culture and hence, the explants were initially pre-treated with fungicide and bactericide solution to reduce considerable amount of microbial load. The terminal stem scale explants obtained from underground parts of field grown plants are readily exposed to soil borne pathogens, therefore, decontamination of explants become a difficult task. The pre-treatment of terminal stem scale explants with combination of fungicides and bactericide solution is extremely necessary before surface sterilization with sterilizing agents. Krishnamurthy *et al*. (2001) reported that combined use of carbendazim (1000 ppm) and citrimide (500 ppm) helps in maximum control of *in vitro* culture contaminations in tuberose. Kanchana *et al*. (2019) and Copetta *et al*. (2020) also reported similar sterilization procedures in tuberose micropropagation. Decontamination of explants obtained from underground parts has been reported (Gajbhiye *et al.*, 2011). Similarly, Aslam *et al*. (2013) also reported the suitability of $HgCl_2$ as an ideal surface sterilant for disinfection of underground plant parts in lilum. The fungicidal and bactericidal compounds have to be used at non-phytotoxic levels to get the desired results.

Establishment of aseptic culture for immature flower bud and tepal segment explants

The per cent contamination of immature flower bud and tepal segment was significantly influenced by different treatments (Table 2). The results revealed that the per cent contamination of explants was 0.00 % in the treatment T_1 comprising of 1.0 drop Tween- 20 + 70% ethanol (30 sec) + 1% sodium hypochlorite

Table 2 : Effect of surface sterilization on immature flower bud and tepal segment explant of tuberose cv. Arka Vaibhav

Treatment	Immature flower bud		Tepal segment	
	Contamination of explant (%)	Survival of explant (%)	Contamination of explant (%)	Survival of explant (%)
T_0: Distilled water wash for 15 min (control)	76.66 (61.11)	23.33 (28.84)	86.66 (68.83)	16.67 (23.85)
T_1: 1.0 drop Tween- 20 + 70% ethanol (30 sec) + 1% sodium hypochlorite (3 min)	0.00 (0.00)	100.00 (90.00)	73.33 (58.98)	28.33 (32.13)
T_2: 1.0 drop Tween- 20 + 70% ethanol (30 sec) + 2% sodium hypochlorite (3 min)	13.33 (21.14)	86.66 (68.64)	56.66 (48.83)	35.66 (36.58)
T_3: 1.0 drop Tween- 20 + 70% ethanol (30 sec) + 2% sodium hypochlorite (5 min)	26.66 (30.98)	73.33 (58.91)	36.66 (44.98)	43.33 (41.14)
T_4: 0.1% carbendazim (30 min) + 1.0 drop Tween- 20 + 70% ethanol (30 sec) + 2% sodium hypochlorite (10 min)	10.00 (6.14)	88.33 (70.09)	10.00 (6.14)	91.66 (73.37)
T_5: 0.2% carbendazim (30 min) + 1.0 drop Tween- 20 + 70% ethanol (30 sec) + 2% sodium hypochlorite (10 min)	21.66 (27.70)	75.00 (59.98)	23.33 (28.07)	50.66 (45.37)
Mean	24.71 (24.51)	74.44 (62.74)	47.77 (42.64)	44.38 (42.07)
SEm±	2.80	1.36	6.53	3.04
CD (P=0.05)	8.65	4.19	20.11	9.36
CV (%)	20.50	3.16	23.12	11.80

Values in the parenthesis are angular transformed values

(3 min) followed by 10.00 % in treatment T_4 comprising of 0.1% carbendazim (30 min) + 1.0 drop Tween-20 + 70% ethanol (30 sec) + 2% sodium hypochlorite (10 min), while, the control treatment recorded the maximum per cent contamination of explants (76.66%). Significant difference was recorded among the treatments for the per cent survival of explants. The per cent survival of explants was recorded maximum (100%) in T_1 treatment consisting of 1.0 drop Tween- 20 + 70% ethanol (30 sec) + 1% sodium hypochlorite (3 min), followed by T_4 treatment containing 1.0 drop Tween- 20 + 70% ethanol (30 sec) + 2% sodium hypochlorite (10 min) + 0.1% carbendazim (30 min) showing 88.33% of explant survival. However, minimum survival of explants was recorded in control (23.33%).

Pre-treatment is not required for the immature flower bud as the explants are not readily in contact with any soil borne pathogens. In the present study, sequential application of 1 drop Tween-20 + 70 % ethanol for 30 seconds and 1 % sodium hypochlorite (NaOCl) for 3 minutes were found to be optimum for obtaining contamination free cultures. The bactericidal action of NaOCl is due to HOCl (hypochlorous acid) and OCl^- (hypochlorite ion) ions, which are highly effective with less phytotoxic effect. The level of surface sterilants, their exposure time and combination have great influence on the culture establishment. Further, the increase in concentration and prolonged exposure to sterilant led to the death of explants and excessive contamination of cultures. The probable reason may be due to metal contamination proving phytotoxic for the survival of the explants. The results are in accordance with the findings of Rather (2010) in peony. The phytotoxicity of sterilant mainly depend upon the nature, age and type of plant parts. The efficacy of these sterilizing agents on bulbous explants has been earlier reported by Aslam *et al*. (2013) who mentioned that the combination of chlorox (sodium hypochlorite) and mercuric chloride ($HgCl_2$ 0.1%) helps to reduce bacterial contamination in *in vitro* culture. Mercuric chloride has been one of the most used sterilant, while, sodium hypochlorite is also efficient for tuberose (Krishnamurthy *et al*., 2001; Mishra *et al*., 2005). Chen *et al.* (2005) also reported minimum contamination of cultures using NaOCl and ethanol for unopened flower buds of Narcissus.

Significant differences were observed among the treatments for the per cent contamination of tepal segment explants (Table 2). Sterilization of tepal segment explants with T_4 treatment consisting of 1 drop Tween- 20 + 70% ethanol (30 sec) + 2% sodium hypochlorite (10 min) + 0.1% carbendazim (30 min) recorded less contamination (10%), followed by T_5 treatment consisting of 1 drop Tween- 20 + 70% ethanol (30 sec) + 2% sodium hypochlorite (10 min) + 0.2% carbendazim (30 min) which recorded 23.33% contamination and maximum contamination (86.66%) was observed in control treatment. The per cent survival of tepal explants significantly differed among the six treatments studied and the maximum (91.66%) tepal explants survived in T_4 treatment comprising of 1 drop Tween- 20 + 70% ethanol (30 sec) + 2% sodium hypochlorite (10 min) + 0.1% carbendazim (30 min), followed by T_5 treatment containing 1 drop Tween- 20 + 70% ethanol (30 sec) + 2% sodium hypochlorite (10 min) + 0.2% carbendazim (30 min) which recorded 50.66% explant survival, while, lowest survival of explants (16.67%) was observed in control treatment. This might be due to the optimum concentration and duration of exposure to sterilizing agents which resulted in minimum contamination and maximum survival. Combined use of chemicals greatly contributed to the contamination free culture than the individual sterilizing agents. The results are in agreement with the findings of Kadam *et al*. (2010) who reported that pre-treatment of explants with 0.1% each of carbendazim, mancozeb and 200 mg/L of 8-HQC for 2 h recorded the maximum culture survival in petal segment (87.5%) with minimum fungal and bacterial contamination in tuberose. Mishra *et al*. (2005) in tuberose, Bora and Paswan (2003) in heliconia and Dilta *et al*. (2000) in lily reported that ethanol and sodium hypochlorite found effective for surface sterilization, however, Sangavai and Chellapandi (2008) found that combination of ethanol (70%) and sodium hypochlorite (10 min) resulted in 88.35% aseptic culture in tuberose.

Suitability of explants for the direct organogenesis

Days taken to complete greenness of explants such as terminal stem scale, immature flower bud and tepal segment were significantly differed (Table 3). The treatment T_5 consisting of MS + 4 mg/L BAP + 0.1 mg/L IAA recorded minimum days to complete greenness in all the three explants *viz*., terminal stem scale (3.60 days), immature flower bud (4.67 days) and tepal segment (5.83 days). This might be due to

Table 3 : Effect of different media combinations on days to complete greenness in different explant of tuberose cv. Arka Vaibhav

Treatment	Days to complete greenness		
	Terminal stem scale	Immature flower bud	Tepal segment
T_0 - Half MS media	7.40	8.00	10.00
T_1 - Basal MS media	6.20	7.67	8.33
T_2- Basal MS + 2.0 mg/L BAP	5.40	6.33	7.67
T_3 - Basal MS + 4.0 mg/L BAP	4.27	6.00	6.67
T_4 - Basal MS + 2.0 mg/L BAP + 0.1 mg/L IAA	4.40	5.33	6.00
T_5 - Basal MS + 4.0 mg/L BAP + 0.1 mg/L IAA	3.60	4.67	5.83
Mean	5.21	6.33	7.42
SEm±	0.13	0.36	0.25
CD (P=0.05)	0.39	1.11	0.76
CV (%)	4.24	9.84	5.73

higher concentration of cytokinin's (BAP @ 4.0 mg/L) inducing early bud emergence. The half MS medium which is devoid of growth regulators exhibited maximum number of days to complete greenness in all the three explants such as terminal stem scale (7.40 days), immature flower bud (8.00 days) and 10.00 days in tepal segment. Earlier findings reported that the days taken for greenness in tuberose decreased with increasing concentration of BAP. However, in the present study, a different trend was observed wherein minimum days taken for complete greenness of explants with 4 mg/L BAP, which indicated that there is no antagonistic effect of higher levels of BAP for all the types of explants used. These results are in conformity with the findings of Jyothi *et al*. (2008) in tuberose.

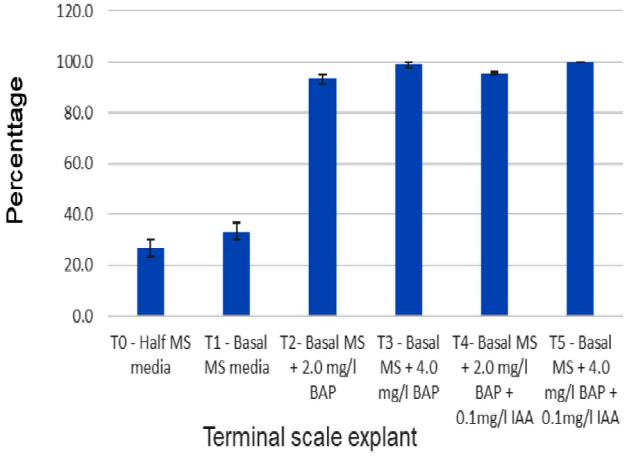

Fig. 3 : Influence of different media combinations on per cent response to shoot induction in tuberose cv. Arka Vaibhav

Among the three different explants used for the study, terminal stem scale explant alone responded very well for the shoot induction and other two explants like immature flower bud and tepal segment has not responded for shoot induction even after repeated inoculation on different combination of media. Media treatments differed significantly with respect to per cent response to shoot induction and culture establishment (Fig. 3). The results revealed that the maximum per cent response of 100% shoot induction was observed in terminal stem scale explants cultured in the T_5 media comprised of full MS + 4 mg/L BAP + 0.1 mg/L IAA, followed by T_3 media consist of MS + 4 mg/L BAP which recorded 98.88% of response to shoot induction. The least response of 78.87% was observed in control with half MS media.

The appropriate ratio of auxins and cytokinin's in the plant system has major role in culture establishment and the finest balance determines the direct and indirect organogenesis. Among the different explants studied for *in vitro* regeneration response, only terminal stem scale explants responded very well for direct shoot formation (Fig. 4). As it is the sole propagating part of tuberose which contain more totipotent cells and meristematic cells resulted in high response for direct organogenesis. Surendranath *et al*. (2016) in tuberose also reported that the axillary buds responded for shoot induction with the response rate of 95.00% and 92.50% in Arka Prajwal and Arka Suvasini, respectively.

Fig. 4 : Successful established cultures of terminal stem scale explant

CONCLUSION

The terminal stem scale explant pre-treated with 0.1% carbendazim + 0.05% chlorothalonil + 0.05% cetrimide and surface sterilization with 70% ethanol (1 min) + 4% sodium hypochlorite (10 min) + 0.1% $HgCl_2$ (15 min) resulted in contamination free aseptic culture. Pre-treatment of immature flower bud and tepal segment with 1 drop Tween-20 + 70% ethanol (30 sec) + 1% sodium hypochlorite (3 min) and 0.1% carbendazim (30 min) + 1 drop Tween-20 + 70% ethanol (30 sec) + 2% sodium hypochlorite (10 min) recorded contamination free culture. The study concluded that the terminal stem scale explant is the best suited and most viable explant compared to immature flower bud and tepal segment for direct organogenesis in tuberose.

REFERENCES

Anonymous, 2023. Indiastat; 2021-22 3rd advance estimates. https://www.indiastat.com/table/agriculture/area-production-tuberose-india-2011-2012-2021-2022/964510.

Aslam F., Naz S., Tariq A., Ilyas S. and Shahzadi K. 2013. Rapid multiplication of ornamental bulbous plants of *Lilium orientalis* and *Lilium longiflorum*. *Pak. J. Bot.,* **45**(6): 2051-2055.

Bora, S. and Paswan, L. 2003. *In vitro* propagation of *Heliconia psittacorum* through axillary bud. *J. Ornam. Hortic.,* **27**(5): 450-452.

Chen, L., Zhu X., Gu, L. and Wu, J. 2005. Efficient callus induction and plant regeneration from anther of Chinese narcissus (*Narcissus tazetta* L. var. Chinensis Roem). *Plant Cell Rep.*, **24**(7): 401-407.

Copetta, A., Marchioni, I., Mascarello, C., Pistelli, L., Cambournac, L., Dimita, R. and Ruffoni, B. 2020. *Polianthes tuberosa* as Edible Flower: *In vitro* propagation and nutritional properties. *Int. J. Food Eng.*, **6**(2): 57-62.

Dilta, B. S., Sehgal, O. P., Pathania, N. S. and Chander, S. 2000. *In vitro* effect of NAA and BA on culture establishment and bulblet formation in lily. *J. Ornam. Hortic.*, **3**(2): 65-70.

Gajbhiye, S. S., Tripathi, M. K., Vidya Shankar, M., Singh, M., Baghel, B. S. and Tiwari, S. 2011. Direct shoot organogenesis from cultured stem disc explants of tuberose (*Polianthes tuberosa* Linn.). *J. Agric. Sci. Technol*., **7**(3): 695-709.

Jyothi, R., Singh, A. K. and Singh, K. P. 2008. *In vitro* propagation studies in tuberose *(Polianthes tuberosa Linn.*). *J. Ornam. Hortic*., **11**(3): 196-201.

Kadam, G. B., Singh, K. P., Singh, A. K. and Jyothi, R. 2010. *In vitro* regeneration of tuberose through petals and immature flower buds. *Indian. J. Hortic*., **67**(1): 76-80.

Kanchana, K., Kannan, M., Hemaprabha, K. and Ganga, M. 2019. Standardization of protocol for sterilization and *in vitro* regeneration in tuberose (*Polianthes tuberosa* L.). *Int. J. Chem. Stud.*, **7**(1): 236-241.

Krishnmurthy, K. B., Mythili, J. B. and Meenakshi, S. 2001. Micropropagation studies in single vs double types of tuberose (*Polianthes tuberosa* L.). *J. Appl. Hortic*., **3**(2): 82-84.

Leifert, C. and Cassells, A. C. 2001. Microbial hazards in plant tissue and cell cultures. *In vitro Cell. Dev. Biol. Plant.*, **37**(2):133-138.

Mir J. I., Ahmed N., Itoo H., Sheikh M. A., Rizwan R. and Wani S. A. 2012. *In vitro* propagation of Lilium (*Lilium longiflorum*). *Indian J. Agric. Sci.*, **82**(5): 455-458.

Mishra, A., Pandey, R. K. and Gupta, R. K. 2005. Micro-propagation of tuberose (*Polianthes tuberosa* L.) cv. Calcutta Double. *Prog. Hortic.,* **37**(2): 331-335.

Pandey R. K., Singh A. K., Mamta S. 2009. *In vitro* propagation of *Lilium*. *Biol. Forum-An Int. J.*, **1**(2): 26-28.

Rather, Z.A., 2010. Studies on *in vitro* propagation of Peony (*Paeonia* spp.). *Ph.D. Thesis* submitted to Sher-e-Kashmir University of Agricultural Sciences and Technology, Kashmir, Shalimar.

Ray, S. S. and Ali, N. 2016. Biotic contamination and possible ways of sterilization: A review with reference to bamboo micropropagation. *Braz. Arch. Biol. Technol.*, **59**: e160485.

Sangavai, C. and Chellapandi, P. 2008. *In vitro* propagation of a tuberose plant (*Polianthes tuberosa* L.). *Electronic J. Biol.*, **4**(3): 98-101.

Surendranath, R., Ganga, M. and Jawaharlal, M. 2016. *In vitro* propagation of tuberose. *Environ. Ecol.,* **34**(4D): 2556-2560.

Taha L. S., Sayed S. S., Farahat M. M., El-Sayed I. M. 2018. *In vitro* culture and bulblet induction of Asiatic hybrid lily 'Red Alert' *J. Biol. Sci.*, **18**(2): 84-91.

(Received : 25.01.2023; Revised : 16.06.2023; Accepted 18.06.2023)