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STANDARDIZATION OF *IN VITRO* REGENERATION OF HYBRID LILIUM Cv. TRESOR

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ABSTRACT
An efficient protocol for *in vitro* conditions from bulb scale explants for culture establishment in hybrid Lilium Cv. Tresor on Murashige and Skoog basal medium supplemented with plant bioregulators at the Biotechnology-cum-Tissue Culture Centre, OUAT, Bhubaneswar. The calli mass derived from bulb scale explants were cultured in MS media supplemented with growth regulators in combination of BAP (0.5 mg/l) and (1.0mg/l) with 2,4-D (0.5,1.0,1.5,2.0 and 2.5mg/l) and 2,4-D (0.5,1.0,1.5,2.0 and 2.5mg/l) alone with 8% (w/v) agar, 30% (w/v) sucrose with three replications each. As the results revealed after 120 days of culture among the treatments, basal media supplemented with BAP 0.5mg/l + 2,4-D 1.5mg/l gave early swollen mass initiation (10.33), early bulb initiation (17.33), early shoot mass initiation (18.00) and BAP 0.5 mg/l + 2,4-D 0.5mg/l earliest shoot proliferation (45.00). MS + BAP 1 mg/l + 2,4-D 1.5 mg/l gave significantly maximum shoots per mass (3.33), height of shoot mass (5.04), followed by 150 days of culture MS + BAP 1mg/l + 2,4-D 1.5mg/l gave highest number of shoots per shoot mass (6.33), maximum number of bulbs per shoot mass (4.00), maximum height of the plantlet (7.16), and the maximum size of the bulb (0.60 cmx0.46 cm) producing deep green color compact plantlets was concluded as the best combination. Thus protocol opened the prospects for developing an indirect means by in vitro regeneration of hybrid Lilium Cv. Tresor mass multiplication of true to type, for disease free plantlets of this high value ornamental cut flower.

KEYWORDS: Bulb scale, Callus, $HgCl_2$, propagation, Tresor.

INTRODUCTION
Lillium species, for centuries have been one of the horticultural most important genera for cut flower, pot plant production and is taken as the king of ornamental bulb plants. The genius Lillium comprises of about 85 species, and as it is derived from interspecific hybridization, acquires a great importance in the commercial market being a monocot bulbous crop. *Lilium* is also gaining popularity due to its attractive large flowers, capacity to rehydrate after long transportation and emerging as the largest income contributor[1]. Apparently, 80 species of Lillium are found in the temperate and subtropical zones of northern hemisphere [2]. A good number of researchers[3] did carry out studies on inter-specific hybridization, for example *L. longiflorum* x Asiatic hybrid and have had great success in early 1990's introducing a new class of lily called as LA (Longiflorum x Asiatic) hybrids to the market. Tresor belonging to *Asiatic* hybrids variety were bred for the numerous desirable characteristics such as vigorous growth of bulb, healthier leaves, stems, larger flowers and elegant fragrance. There are many techniques available for the conservation of plant genetic resources of rare and endangered species. These include micro propagation, seed germination, Regeneration from callus, embryo rescue, micrografting, and cryopreservation [4]. Flower bulbs have been appreciated and cultivated for thousands of years and long before they were widely grown commercially or extensively researched. Previous literature suggests that explants from flower organs to bulb scales of Lilium could be easily manipulated and regenerated using tissue culture techniques[5]. Lilium propagation is usually done by vegetative means which produces 3-4 bulbs per bulb scale depending on size and variety. Through micropropagation of bulb plant such as Lilium, an alternative way has been found to the conventional methods of vegetative propagation because of its various advantages, such as increasing the multiplication level in less time and enables material free from viruses and other diseases to be obtained[6].Therefore, there is a need to develop a protocol for its mass propagation. Through tissue culture, there is not only a continuous supply of bulblets but true-to-type and disease free plants can be obtained. Therefore, the objective of the present research was to establish a protocol for standardization of sterilization time and Plant bioregulators for callus formation in Lilium hybrid *Cv*. Tresor.

MATERIALS AND METHODS

The present investigation was carried out during the year 2013-14 at Biotechnology-cum-Commercial Tissue Culture Centre, OUAT, Bhubaneswar, Odisha.

Source of Explants

Healthy and disease-free bulbs of hybrid Lilium Cv. Tresor was collected from plants grown under poly-house in the of Biotechnology-cum-Commercial Tissue Culture Center, OUAT, Bhubaneswar, maintained as mother plant for conducting *in vitro* studies in the laboratory. The bulb scale was taken as explant for *in vitro* regeneration (Fig.1).

FIGURE 1: View of Hybrid Lilium Cv.Tresor

Stock solution, Media preparation and Sterilization

The chemicals used for the present study were analytical reagents of excel R grade of Titan Biotech Ltd., Ranbaxy Laboratory Ltd., Merck (India), Qualigen Fine Chemicals, and Himedia Laboratories Ltd. (India). Auxins, Cytokinin, myo-inositol and Fe-EDTA that were supplied by Sigma (USA) and Agar from Ranbaxy Laboratory Limited. MS Medium[7] was used throughout the investigation, required quantities of macronutrients, micronutrients, Fe-EDTA, vitamins and plant bioregulators were taken from the stock solution and required quantity of sucrose dissolved in distilled water was added fresh to the medium. The pH of the solution was adjusted to 5.7+ 0.1 using 0.1N NaOH or 0.1 N HCL. Then volume was made up to 1L with distilled water. Agar (0.6% w/v) was added to the medium boiled and poured into the culture tube followed by plugging. Culture tube containing culture medium was autoclaved for the 20 minutes at 121^0C and 15Psi pressure. The autoclaved medium was kept in laminar air flow bench for cooling. All the glassware were dipped in detergent solution for overnight and washed under running tap water. They were rinsed with distilled water and then dried in oven for 2hrs at 150^0C. Forceps, petridish and scalpel were thoroughly cleaned with iso-propanol, rapped with paper and kept in a clean sterilized autoclave at 15psi and 121^0C for 20 minutes. The working chamber of laminar air flow cabinet was wiped clean with iso-propanol. Filtered air (80-100 cft/min) was blown for 5 minutes, to ensure that particles do not settle in working area. The sterilized materials to be used (except living tissue) were kept in the laminar chamber being exposed to UV light for 30 minutes.

Inoculation

For callus formation study, bulb scale explants were treated with 0.1% $HgCl_2$ for 5 min, followed by culturing in MS media supplemented with plant bioregulators for two culture periods consisting of 60 days giving sufficient amount of profuse calli mass serving as explant for *in vitro* culture of plantlets. After that each section of the bulb scale (7 × 7 mm), with the dorsal side in contact with the medium, was placed in a culture tube with MS (Murashige and Skoog, 1962)[7] medium containing 2,4-D (0.5, 1.0, 1.5, 2.0 and 2.5 mg/l) alone and BAP (0.5and 1.0 mg/l) in combination with 2,4-D (0.5, 1.0, 1.5, 2.0 and 2.5 mg/l) and a control. Observation on days to swollen mass initiation, days to bulb initiation, days to shoot mass initiation, days to shoot proliferation recorded from 120 DAI and recorded at 150 DAI.

Establishment of culture

After inoculation, the culture were kept at 25 $\pm 2^0$C in an air conditioned room with 16 hours light period (3000-3200 lux) supplied by fluorescent tubes and 80% relatively humidity[5].

Statistical analysis

The raw data obtained during the experimental observations were subjected to statistical analysis as per method by Gomez and Gomez, (1984)[8]. The significance and non-significance of the treatment effect were judged with the help of 'F' variance ratio test. Calculated 'F' value was compared with the table value of 'F' at 5% level of significance. The data were transferred from where ever required before suitability of Analysis of Variance (ANOVA) analyzed in statistical package SAS version 7.0.

RESULT AND DISCUSSION

In perusal of data presented in Table.1, Fig. 2 and 3 it was revealed that for Lilium hybrid cultivar Tresor T_9 (MS + BAP 0.5 mg/l + 2,4-D 1.5 mg/l) gave the earliest shoot mass initiation with 10.33 days, significant early bulb initiation with 13.66 days was seen in T_{11} (MS + BAP 0.5 mg/l + 2,4-D 2.5 mg/l). The bulb scalb in particular, a specialized and succulent form of containing nutrients and water reservoirs, respond well to lower concentration of bioregulators[9]. The

treatment T_9 showed equally stable results for shoot mass initiation as 18.00 days whereas earliest shoot proliferation from shoot mass being 45.00 days significantly seen in T_7 (MS + BAP 0.5mg/l +2,4-D 0.5mg/l). T_{14} (MS + BAP 1mg/l + 2,4-D 1.5mg/l) gave significantly maximum value for height of shoot mass(5.04) and T_{10} (MS + BAP 0.5mg/l + 2,4-D 2mg/l) gave maximum number of shoots per mass(4.0). As from the data represented in Table -2 it was clearly seen, T_{14} (MS + BAP 1mg/l + 2, 4-D 1.5mg/l) gave significantly high number of shoots per shoot mass(6.33), maximum number of bulbs per shoot mass (4.00), maximum height of the plantlet (7.16), and lastly the maximum size of the bulb (0.60x0.46) producing deep green color compact plantlets (Fig.4,5). The growth and development processes of the plant since influenced by the exogenous application of bioregulators such as cytokinin and auxins [10,11,12, 13].

TABLE 1: Effect of Plant bio-regulators on days to swollen mass emergence, bulb formation, shoot and plantlet formation from developed callus in Hybrid Lilium Cv.Tresor

Basal Medium- MS — Culture period - 120 days

Characters Treatments	BAP	2,4-D	Day to swollen mass initiation	Days to bulb initiation	Days to shoot mass initiation	Days to shoot proliferation	Number of shoots/ mass	Height of shoot mass	Number of leafs per shoot Mass
T_1	-	-	11.00	25.33	20.00	52.00	2.00	2.87	1.00
T_2	-	0.5	14.66	25.33	25.33	54.00	2.33	3.21	1.00
T_3	-	1.0	16.33	27.33	27.33	54.66	3.00	3.23	1.00
T_4	-	1.5	18.33	19.33	29.33	55.33	2.66	3.28	1.00
T_5	-	2.0	15.33	16.66	22.66	54.33	2.33	3.24	1.00
T_6	-	2.5	13.33	14.33	20.33	52.33	2.33	3.10	1.00
T_7	0.5	0.5	12.66	27.33	20.66	45.00	2.00	1.36	1.00
T_8	0.5	1.0	12.33	26.33	20.00	47.33	2.66	1.84	1.00
T_9	0.5	1.5	10.33	17.33	18.00	50.33	3.00	2.03	1.00
T_{10}	0.5	2.0	14.00	16.00	17.66	53.00	4.00	3.14	1.00
T_{11}	0.5	2.5	8.66	13.66	16.33	46.00	2.00	1.25	1.00
T_{12}	1.0	0.5	14.33	46.00	48.00	53.00	2.33	3.68	1.00
T_{13}	1.0	1.0	20.33	47.33	57.66	58.00	2.66	4.56	1.00
T_{14}	1.0	1.5	21.66	48.33	64.33	58.00	3.33	5.04	1.00
T_{15}	1.0	2.0	17.66	47.33	47.66	52.33	3.33	4.20	1.00
T_{16}	1.0	2.5	17.66	45.66	46.33	49.66	2.33	3.63	1.00
SE(m)±			0.31	0.45	1.74	0.31	-	0.18	-
CD 5%			0.89	0.27	4.92	0.88	-	0.52	-

TABLE 2: Effect of Plant bio-regulators on bulb formation, shoot and plantlet formation from developed callus in Hybrid Lilium Cv. Tresor

Basal Medium- MS — Culture period - 150 days

Characters Treatments	BAP	2,4-D	Number of shoots per shoot mass	Number of bulbs per shoot mass	Height of the plantlet	Size of the bulb
T_1	-	-	2.00	1.00	3.22	0.43×0.16
T_2	-	0.5	3.33	1.33	4.31	0.36×0.16
T_3	-	1.0	4.33	1.33	4.15	0.40×0.13
T_4	-	1.5	4.66	1.00	4.50	0.26×0.13
T_5	-	2	3.66	1.33	4.20	0.33×0.13
T_6	-	2.5	3.33	1.33	4.16	3.66×0.13
T_7	0.5	0.5	3.00	1.33	4.15	0.30×0.13
T_8	0.5	1	4.33	1.00	3.28	0.20×0.10
T_9	0.5	1.5	5.00	1.00	3.22	0.10×0.07
T_{10}	0.5	2	5.33	1.00	3.19	0.10×0.10
T_{11}	0.5	2.5	3.33	1.00	2.81	0.16×0.10
T_{12}	1	0.5	4.66	1.33	5.04	0.50×0.36
T_{13}	1	1	6.00	2.66	6.95	0.56×0.43
T_{14}	1	1.5	6.33	4.00	7.16	0.60×0.46
T_{15}	1	2	4.66	3.66	5.18	0.50×0.40
T_{16}	1	2.5	4.33	3.00	5.18	0.50×0.30
SE(m)±			0.63	0.51	0.19	
CD 5%			0.78	1.45	0.53	

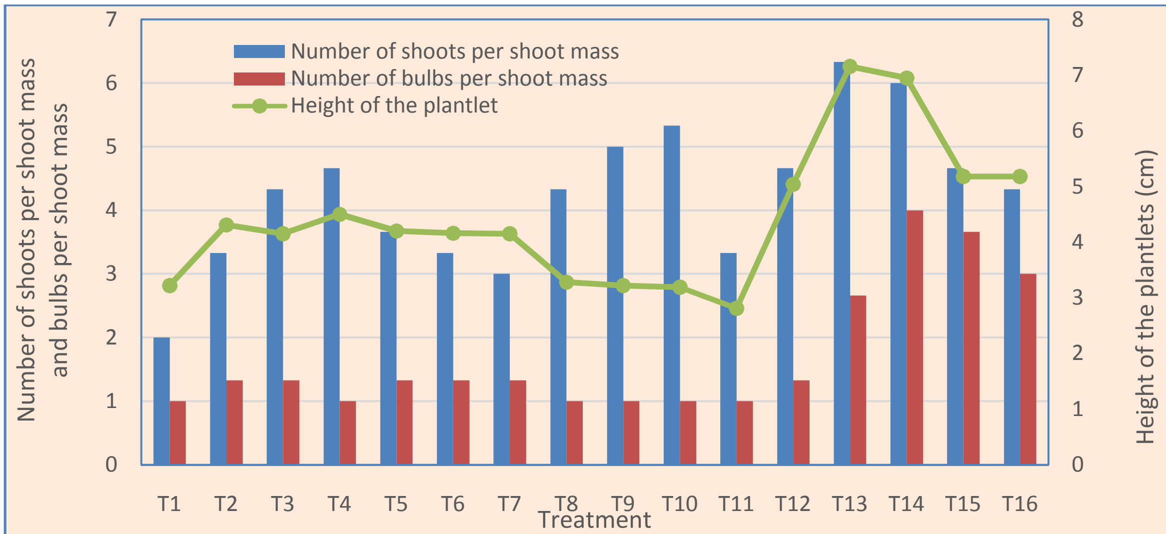

FIGURE 2: Effect of Plant bio-regulators on bulb formation, shoot and plantlet formation from developed callus in Hybrid Lilium Cv. Tresor

FIGURE 3: Shoot mass initiation

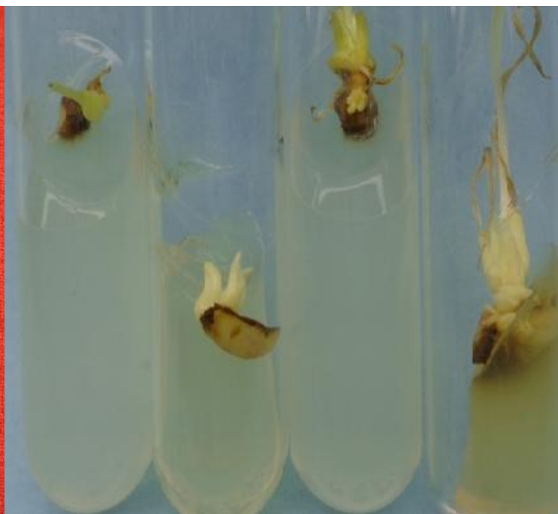

FIGURE 4: Bulb mass initiation

FIGURE 5: Plantlet formations in. Hybrid Lilium Cv.Tresor

CONCLUSION

The study demonstrated that hybrid Lilium Cv. Tresor bulb scale explants can be used to produce calli mass *in vitro* which further can successfully produce healthy plantlets under the aseptic conditions on a large number and less time. Hence this investigation will help form a mass production of planting material using the biotechnological approach of such a valuable ornamental crop.

REFERENCES

[1]. Gupta, P., Sharma, A.K. and Charturvedi, H.C. (1978) Multiplication of *Lilium longiflorum* Thumb by aseptic culture of bulb-scales and heir segments. *Indian Journal of Experimental Biology* 16:940-942.

[2]. Blom-Barnhoorn, G.J. and J. van Aartrijk (1985) The regeneration of plants free of LSV and TBV from infected *Lilium* bulb-scale explants in the presence of virazole. *Acta Hortic*. 164: 163-168.

[3]. Van Tuyl, J.M., Van Diën, M.P., Van Creij, M.G.M., Van Kleinwee, T.C.M., Franken. J. and Bino, R.J. (1991) Application of *in vitro* pollination, ovary culture, ovule culture and embryo rescue for overcoming incongruity barriers in inter-specific *Lilium* crosses. *Plant Sci*. 74:115-126.

[4]. Stanilova, M.I., Ilcheva, V.P. and Zagorska, N.A. (1994) Morphogenetic potential and in *vitro* micro-propagation of endangered plant species *Leucojum aestivum* L. and *Lilium rhodopaeum* Delip. *Plant Cell Rep* 13:451–453.

[5]. Beura, S., Sahu, A., Rout, S., Beura, R. and Jagadev, P.N. (2017) Standardization of plant bioregulators for *in vitro* shoot proliferation of *Curcuma longa* L. Cv. Roma. *Int. Journal of Current Microbiology and applied science*. 6(5):386-394.

[6]. Van Aartrijk, J., Blom-Barnhoorn, G.J., and PCG, van der Linde (1990) Lilies. In P.V. Ammirato, D.A. Evans, W.R. Sharp, and Y.P.S. Bajaj (eds.), Handbook of Plant Cell Culture V5. Collier Macmillan Publishers, London, pp. 535-576.

[7]. Murashige, T. and Skoog, F. (1962) A revised medium for rapid growth and bioassays with tobacco cultures. *Physiologia Plantarum*. 15(3):473-497.

[8]. Gomez, K.A. and Gomez, A.A. (1984) Statistical procedures for agricultural research 3rd edn, John Wiley & Sons, Singapore. 680p.

[9]. Mc Rae, E.A. (1998) Lilies: A guide for flower grower and collectors. Timber press, Oregon.

[10]. Park, S.Y., Kim, Y.M., Moon, H.K., Murty, H.N., Choi, Y, Cho, H.M. (2008) Micro propagation of *Salix Pseudolasiogyne* from the nodal explants. *Plant tissue organ Culture*.93:341-346.

[11]. Barpanda, S., Beura, S; Rout, S; and Jagadev. P.N. (2017) Studies on *in vitro* regeneration of Sandalwood (*Santalum album* Linn) from leaf disc explants. *Journal of Pharmacognosy and Phytochemistry*, 6(6):892-896.

[12]. Beura, S., Mohanty, P; Rout, S. and Beura, R. (2016). *In vitro* Clonal propagation of an Ornamental Garden Plant *Bauhinia galpinii*, *Journal of Tropical Forestry*. 32 (2):71-82.

[13]. Rout, S. and Khare. N. (2018) Effect of various surface sterilization on contamination and callus regenerations of Ashoka (*Saraca asoca*) from leaf segment explants. *Int. Jour. Curr. Microbilogy app. Sci*.7(7):2027-2033.