

Effect of Surface Sterilization of *Tagetes erecta* L. Cv. Inca Yellow Hybrid and Orange Hybrid Nodal Explant on Aseptic *in vitro* Propagation

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ABSTRACT

The present investigation was conducted to find out the most suitable surface sterilant and timing for reducing the contaminant in the nodal explants of *Tagetes erecta* L. Cv. Inca Yellow hybrid and Orange hybrid inoculated in MS basal media. Among the various sterilants and timing the explants sterilized with 0.1 % $HgCl_2$ for 10 Min+ 1% NaOCl for 2Min significantly reduced the percentage of contamination and maximum aseptic culture in Inca Yellow hybrid (43.33) and Inca Orange hybrid (40.00). This study is highly helpful and useful for the mass multiplication of true to type disease free planting materials.

Keywords: Basal, *In vitro*, Marigold, Nodal.

INTRODUCTION

The propagation of plant is an age old practice of humankind since time immemorial. Human society could not continue to live without the availability of food, medicine, fibre and amenities provided by the plants, most of which needs man's surveillance for their existence. Plants selection, multiplication and breeding are most useful to promote man's well-being. Plants propagation multiplies these plants and preserve their essential genetic characteristics (Hartmann et al., 2002). The history of selection and improvement of many

floricultural crops contrast with that for plant species, with the shift from exploitation to domestication is much more for commercial plantation, including the use of bulk propagation. Micropropagation and organogenesis are the tissue culture techniques that allows for *in vitro* regeneration of floricultural crops. Plantlets from a large number of other of horticultural crops have been successfully propagated via organogenesis using various explants (Saborio et al., 2001).

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The main advantages of *in vitro* culture are the ability to multiply plants in large quantities and to make improved or selected specimens available for the use in a shorter time than by any other convention means. Marigold (*Tagetes erecta* L.) is an Asteraceous plant of industrial and medicinal importance. This herbaceous plant is native to Mexico, where it is used in traditional medicine and for ornamental purposes. It has been reported that this plant contains bioactive compounds that exhibit nematicidal, fungicidal and insecticidal activity (Vasudevan et al., 1997). The flowers are utilized as a source of pigments for food colouring in the industry, mainly of poultry skin and eggs (Delgado– Vargas et al., 2000). The wide range of uses of this plant underlines the importance of establishing a reliable plant regeneration system for further genetic manipulation.

Marigold has erect stem that can reach 6 to 48 inches in height (depending on the variety). Marigold has oblong and lanceolate leaves with whole margins. Some varieties of marigold have leaves with toothed edges. Leaves are spirally arranged on the branches.

Propagation of *Tagetes erecta* is by seed. Under good conditions about 400 g/ha is required. Germination is within two weeks and usually seedlings are first transplanted into pots before being planted in single or double rows in the field at about 20 cm × 90 cm. *Tagetes erecta* is sometimes intercropped or grown in rotation with commercial crops to reduce diseases and nematode populations.

Marigold requires mild climate for luxuriant growth and flowering. The optimum temperature range for its profuse growth is 18–20°C. Temperatures above 35°C restrict the growth of the plants, which leads to reduction in flower size and number. In severe winter, plants and flowers are damaged by frost. Marigold can be grown in a wide range of soils, as it is adapted in different soil types. French (Dwarf) marigolds are best cultivated in light soil whereas rich well drained, moist soils are best suited for African (Tall) Marigolds.

Sandy loam soil with pH 5.6 to 6.5 is ideal for its cultivation. Commercially this

species is propagated. Conventionally this species is propagated either by means of seeds or by vegetative means.

Due to low seed viability and poor germination it is difficult to fulfil its increasing demand, the multiplicity of uses for *Tagetes* emphasizes its growing importance and demand for improved seeds to satisfy the market's needs. Nevertheless, this demand cannot be fulfilled by growers, given the low viability of seeds and their poor germination rates, which decreases as the seed ages. So tissue culture was selected as an alternative for large scale commercial propagation of this plant. Tissue culture is advantageous in producing multiple copies of a plant species within minimum time and space and callus culture is the primary mean for the indirect organogenesis. Callus or cell suspension culture also could be used for large scale plant cell culture where the bioactive compounds could be extracted. Various *in vitro* propagation studies have been carried out on *Tagetes erecta*, but research is still limited on this valuable ornamental horticultural crop.

Hence there is an urgent need to develop alternative propagation techniques to enhance the reproduction of the quality planting materials of this species few work have been reported *in vitro* multiplication of this species using explants. Therefore, keeping the above facts, the present study was undertaken on surface sterilization of nodal explant of *Tagetes erecta* L.cv. Inca Yellow hybrid and Orange hybrid on aseptic *in vitro* Propagation.

MATERIALS AND METHODS

The present investigation was undertaken during the year 2016-2017 in the Laboratory of Biotechnology-cum-Tissue Culture Centre, Odisha University of Agriculture and Technology, Bhubaneswar. The explants were collected from the nursery of Biotechnology-Cum-Tissue Culture Centre, Odisha University of Agriculture and Technology, Bhubaneswar. The nodal segment of the plant were cut in to 2 to 3 cm size and inoculated after sterilization as per the treatments. The formulation of MS

medium (Murashige & Skoog, 1962) was used as a basal medium for the entire experiment. For preparation of culture medium required quantities of macronutrients, micronutrients, Fe-EDTA, vitamins and growth regulators were taken from the stock solution and mixed in distilled water. Required quantities of sucrose dissolved in water was also added fresh to the solution prepared. The pH of the solution was adjusted to 5.7 ± 0.1 using 0.1 N NaOH or 0.1 N HCL. Then the final volume was made up to one litre with distilled water. After the volume was made up, the acquired quantity of agar was added and boiled in beaker till it got dissolved and looked transparent. The hot medium was distributed to sterilized culture bottles. The bottles with culture medium were autoclaved at 121°C and 15 psi pressure for 20 minutes. The autoclaved medium was kept in a laminar airflow bench for cooling. The working chamber of laminar air flow cabinet was wiped with isopropanol. Filtered air (80-100 cft/min) to ensure that particles do not settle in working area was blown for 5 Min. The sterilized materials to be used (except living tissue) were kept made the chamber and exposed to UV light for 30 minutes. The sterilized explants were inoculated in culture tubes containing the media as per the treatments. Cut ends of explants was be kept in such a way so as to have maximum contact with the medium. All the aseptic manipulations such as surface disinfection of explants, preparation and inoculation of explants and subsequent culturing were carried out in the laminar air flow cabinet. The working table of laminar airflow cabinet and spirit lamp was sterilized by swabbing with absolute alcohol. All the required materials like media, spirit lamp, matchbox, glassware etc., were transferred on to the clean laminar airflow. The UV light will switch on for half an hour to achieve aseptic environment inside the cabinet. The following observations were recorded after 30 days after inoculation (DAI) for surface sterilization and time i.e. Percentage of contamination (Fungal and Bacterial), percentage of explant death and

percentage of aseptic culture. The raw data obtained during the experimental observations were subjected to completely randomized design (Gomez & Gomez, 1984). 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 DISCUSSIONS

The node explants treated with mercuric chloride, sodium hypochlorite, and 70% propanol for varying time in order to establish maximum contaminant free cultures, the results are presented in Table 1 and 2.

From the Table.1 it was revealed that the highest percentage (43.33%) of healthy and contaminant free explants were established when they were exposed to 0.1 % HgCl_2 for 10 Min + 2 Min 1% NaOCl. This was followed by establishment of 33.33 % of the explants when treated with the 0.1% HgCl_2 for 8 min + 2 Min 1% NaOCl. The maximum fungal contamination was recorded at T_0 (86.67 %). This was followed by T_1 (76.67%). Minimum fungal contamination was recorded at T_{12} and T_{13} (10.00 %). This was followed by T_{11} (13.33%). The maximum bacterial contamination was recorded at T_{10} (33.33%). This was followed by T_4 and T_5 (30.00%). Minimum bacterial contamination was recorded at T_{13} (6.66%). This was followed by T_{11} (10.00%). The maximum death was recorded at T_{13} (83.33%). This was followed by T_{12} (76.67%). Minimum death was recorded at T_0 , T_1 , T_6 and T_{10} (0.00%). This was followed by T_2 and T_3 (3.33%).

From the Table.2 it was revealed that the highest percentage (40.00%) of healthy and contaminant free explants were established when they were exposed to 0.1 % HgCl_2 for 10 Min + 2 Min 1% NaOCl. This was followed by establishment of 26.67 % of

the explants when treated with the 0.1% HgCl₂ for 8 min and 10 min. The maximum fungal contamination was recorded at T₀ (76.67 %). This was followed by T₄ (60.00%). Minimum fungal contamination was recorded at T₁₀ (30.00 %). This was followed by T₁₃ (33.33%). The maximum bacterial contamination was recorded at T₃ (46.67%). This was followed by T₂, T₉ and T₁₃ (36.67%). Minimum bacterial contamination was recorded at T₁₁ (20.00%). This was followed by T₁₀ (30.00%). The maximum death was recorded at T₁₃ (26.67%). This was followed by T₁₂ (20.00%). Minimum death was recorded at T₀, T₂, T₄, T₅, T₆, T₉ and T₁₀ (0.00%). This was followed by T₁ and T₇ (3.33%). The results are in alignment with the findings of Gochhayat et al. (2017) in *Lilium*; Rout and Khare, (2018) in *Saraca asoca* (Roxb) De Wil de.

Aseptic culture establishment is first and foremost step for the successful development of micro-propagation protocol on a commercial scale. This results might be due to the toxic effect of chemicals under prolonged duration of treatment (Table.1). All pre-treatments gave significantly better response compared to control, where 100.00 percent contamination was noted. Microbes such as bacteria and fungi were responsible for culture contamination and can completely spoil the cultures. Among the different pre-treatments, highest explant toxicity (83.33%) was recorded with 70% Propanol for 15 min treatment duration (Table 1). These findings are in close confirmation with earlier results reported by Lal et al. (2008) in grape, Verma et al. (2012) in chrysanthemum and Sen et al. (2014) in *Achyranthes aspera* L.

Table 1: Effect of surface sterilization of node explant of *Tagetes erecta* L.cv. Inca Yellow hybrid on contamination and aseptic culture

Media: MS Basal		30 Days			
Treatment	Treatments Details	Fungal %	Bacterial %	Death %	Aseptic %
T ₀	Direct tap water	86.67 (68.53)	13.33 (21.39)	0.00 (2.50)	0.00 (2.50)
T ₁	2 Min. in 0.1% HgCl ₂	76.67 (61.14)	23.33 (28.86)	0.00 (2.50)	3.33 (10.47)
T ₂	4 Min. in 0.1% HgCl ₂	66.67 (54.70)	23.33 (28.86)	3.33 (10.47)	6.67 (14.89)
T ₃	6 Min. in 0.1% HgCl ₂	63.33 (52.71)	26.67 (31.05)	3.33 (10.47)	6.67 (14.89)
T ₄	8 Min. in 0.1% HgCl ₂	53.33 (46.89)	30.00 (33.21)	6.67 (14.89)	10.00 (18.44)
T ₅	10 Min. in 0.1% HgCl ₂	36.67 (37.23)	30.00 (33.21)	13.33 (21.39)	20.00 (26.56)
T ₆	2 Min. in 0.1% HgCl ₂ +2 min in 1% NaOCl	56.67 (48.79)	26.67 (31.05)	0.00 (2.50)	16.67 (24.04)
T ₇	4 Min. in 0.1% HgCl ₂ +2 Min in 1% NaOCl	36.67 (37.23)	26.33 (30.85)	13.33 (21.39)	23.33 (20.86)
T ₈	6 Min. in 0.1% HgCl ₂ +2 Min in 1% NaOCl	33.33 (35.24)	23.33 (20.86)	16.67 (24.04)	26.67 (31.05)
T ₉	8 Min. in 0.1% HgCl ₂ +2 Min in 1% NaOCl	26.67 (31.05)	16.67 (24.04)	23.33 (20.86)	33.33 (35.24)
T ₁₀	10 Min. in 0.1% HgCl ₂ +2 Min in 1% NaOCl	23.33 (28.86)	30.00 (33.21)	3.33 (10.47)	43.33 (41.15)
T ₁₁	5 Min. in 70% Propanol	13.33 (21.39)	10.00 (18.44)	66.67 (54.70)	10.00 (18.44)
T ₁₂	10 Min. in 70% Propanol	10.00 (18.44)	13.33 (21.39)	76.67 (61.14)	0.00 (2.50)
T ₁₃	15 Min. in 70% Propanol	10.00 (18.44)	6.66(14.89)	83.33 (65.88)	0.00 (2.50)
CD at 5%		14.75 (22.55)	13.50(21.56)	7.67 (16.00)	9.07 (17.46)
SEm±		5.32 (13.31)	4.87(12.66)	2.76 (9.46)	3.27 (10.31)

*Figure in pranenthesis are arcsine transformation values

Table 2: Effect of surface sterilization of node explant of *Tagetes erecta* L.cv. Inca Orange hybrid on contamination and aseptic culture

Media: MS Basal		30 Days			
Treatment	Treatments Details	Fungal %	Bacterial %	Death %	Aseptic %
T ₀	Direct tap water	76.67 (61.07)	23.33 (28.86)	0.00 (2.50)	0.00 (2.50)
T ₁	2 Min. in 0.1% HgCl ₂	53.33 (46.89)	36.67 (37.23)	3.33 (10.47)	6.67 (14.89)
T ₂	4 Min. in 0.1% HgCl ₂	56.67 (48.79)	33.33 (35.24)	0.00 (2.50)	10.00 (18.44)
T ₃	6 Min. in 0.1% HgCl ₂	36.67 (37.23)	46.67 (43.05)	3.33 (10.47)	13.33 (21.39)
T ₄	8 Min. in 0.1% HgCl ₂	60.00 (50.77)	26.67 (31.05)	0.00 (2.50)	13.33 (21.39)
T ₅	10 Min. in 0.1% HgCl ₂	46.67 (43.05)	26.67 (31.05)	0.00 (2.50)	26.67 (31.05)
T ₆	2 Min. in 0.1% HgCl ₂ +2 min in 1% NaOCl	46.67 (43.05)	26.67 (31.05)	0.00 (2.50)	26.67 (31.05)
T ₇	4 Min. in 0.1% HgCl ₂ +2 Min in 1% NaOCl	50.00 (45.00)	33.33 (35.24)	3.33 (10.47)	13.33 (21.39)
T ₈	6 Min. in 0.1% HgCl ₂ +2 Min in 1% NaOCl	43.33 (41.15)	33.33 (35.24)	6.67 (14.89)	16.67 (24.04)
T ₉	8 Min. in 0.1% HgCl ₂ +2 Min in 1% NaOCl	43.33 (41.15)	36.67 (37.23)	0.00 (2.50)	20.00 (26.56)
T ₁₀	10 Min. in 0.1% HgCl ₂ +2 Min in 1% NaOCl	30.00 (33.21)	30.00 (33.21)	0.00 (2.50)	40.00 (39.23)
T ₁₁	5 Min. in 70% Propanol	43.33 (41.15)	20.00 (26.56)	13.33 (21.39)	23.33 (28.86)
T ₁₂	10 Min. in 70% Propanol	36.67 (37.23)	36.33 (37.05)	20.00 (26.56)	6.67 (14.89)
T ₁₃	15 Min. in 70% Propanol	33.33 (35.24)	36.67 (37.23)	26.67 (31.05)	3.33 (7.10)
CD at 5%		22.49(28.25)	17.32(24.58)	7.27 (15.56)	12.83 (20.96)
SEm±		8.11 (16.54)	6.25 (14.42)	2.62(9.28)	4.63(12.39)

*Figure in prantthesis are arcsine transformation values

CONCLUSION

From the present studies, it is concluded that by using the standardized sterilization protocols, ornamentally high valued marigold can be taken up for the production of true-to-type, disease free quality planting material in large scale. Explant treated with 0.1 % HgCl₂ for 10 Min+ 1% NaOCl for 2 Min (T₁₀) gave maximum aseptic *in vitro* culture of both varieties. This can also be helpful for long term maintenance of African marigold germplasm, valuable breeding lines and other biotechnological related works.

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