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Standardization of Surface Sterilization Technique for Aseptic *in vitro* Establishment and Survival of Leaf Explants in *Gerbera jamesonii* cv. Rosalin

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Abstract—*Surface sterilization is the most important step in explant preparation for micropropagation as it controls the fungal and bacterial contamination from field sources. The newly emerging hairy leaf explants of Gerbera jamesonii cv. Rosalin were collected, washed repeatedly using Tween–20 in fresh running water and then treated with 12 different combinations of Sodium hypochlorite (1.5%), Mercuric chloride (0.1%) and ethyl alcohol (70%) with varying exposure durations. Surface sterilized explants were then inoculated in MS medium supplemented with various concentrations and combinations of 2,4-D and BAP. Lowest rate of contamination (13.44 %) was observed when the explants were treated in Mercuric chloride (0.1%) for 2 minutes + ethyl alcohol (70%) for 30 seconds. Lowest rate of explant mortality (16.56 %) was observed when the explants were treated with Sodium hypochlorite (1.5%) for 10 minutes but the contamination rate was found maximum (45.56 %) in this treatment. However, highest rate of explant survivability (64.89 %) was observed when the explants were treated with Mercuric chloride (0.1%) for 1 minute + ethyl alcohol (70%) for 30 seconds. The results indicated that for surface sterilization of gerbera leaf explants, Mercuric chloride (1-2 minutes) + 70 % ethyl alcohol (30 seconds) was found optimum for gerbera leaf explant surface sterilization, but as per the health hazard factors Sodium hypochlorite (1.5%) for 15 minutes + ethyl alcohol (70%) for 30 seconds was found optimum and can be recommended for gerbera leaf explant surface sterilization.*

Keywords*: Gerbera, contamination, in vitro, surface sterilization*

1. INTRODUCTION

Gerbera (*Gerbera jamesonii*) also known as Transvaal Daisy or Barberton Daisy or African Daisy is one of the most valuable ornamental species grown as a potted plant as well as for cut flowers. It is considered one of the most economically important cut flowers in the world. Propagation of gerbera through vegetative method is very slow and is inadequate for the production of large number of uniform propagules for commercial cultivation. So, tissue culture is the only technique used for rapid and large scale multiplication (Kumar *et al*., 2004). Micropropagation not only accelerates plant production, but it also generates virus-free plantlets (Kozai *et al*., 1997). *In vitro* propagation consists of various stages *viz.*, selection of explants, aseptic culture establishment, multiplication, rooting and acclimatization of plants. Since the plant tissues inherently have various pathogens on the surfaces and natural openings when sourced directly from the field grown plants, microbial contamination (either fungal or bacterial) presents a major challenge to the initiation and maintenance of viable *in vitro* cultures. The most important step for aseptic culture establishment is sterilization of explants. Since microbial contamination by fungi or bacteria is one of the most serious problems in plant tissue culture (Leifert and Cassells 2001; IAEA-TECDOC 2004) that raise the costs of large-scale micropropagation (Cassells 1997; Kozai *et al*. 1997), it is efficient to start with an efficient plant sterilization step (Traore *et al*., 2005).

2. MATERIALS AND METHODS

Explant source

The experiment was conducted at plant tissue culture laboratory, Department of Floriculture, Medicinal and Aromatic Plants, UBKV, Cooch Behar during April 2015 and June 2015. Newly emerging hairy leaf explants of commercial gerbera cultivar Rosalin was collected from the polyhouse of the department.

Surface sterilization

Initially the healthy, diseased-free and undamaged explant sources were washed under running tap water to remove the adhered soil particles and dirt from the explant surfaces and then were washed again thoroughly under running tap water using 2-3 drops of Tween-20 detergent for about 20 minutes. The explants were then rinsed with double sterilized distilled water for 4-5 times to remove the detergent remnant from the explant surface. The washed explants were then brought to

laminar flow cabinet and were reduced in size (0.5-1.0 cm) with the help of well sterilized surgical blade and forceps before inoculation. The explants were then subjected to surface sterilization in different sterilants and their combinations for varying time durations as shown in Table 1, followed by a 5 minutes rinse in sterile distilled water under aseptic conditions in the laminar flow chamber. The solutions were removed and surface sterilized explants were washed thoroughly with sterile, distilled water for two to three times under aseptic conditions in a laminar airflow hood and placed on a sterilized petridish covered with autoclaved filter paper to remove excess moisture present on the surface of explants. Prior to commencement of the work, the table surface of laminar flow cabinet was first swabbed with 95% (v/v) ethanol and all the required materials except the excised explants were kept inside the chamber and exposed to UV light for 60 minutes. The laminar flow was switched on 10 to 20 minutes prior to inoculation. The explants were then put on Murashige and Skoog (1962) medium supplemented with 30g/l sucrose, vitamins, agar (7 g/l) and appropriate plant hormones such as 2,4-D (2 mg/l) and BAP (0.5 mg/l) with pH adjusted to 5.8 before autoclaving the media at 121^{o}C and 1.5 atm. for 15 minutes. The explants were placed in such a manner that conformed to the original polarity and exposed above the surface of growing medium. The cultures were then kept in a growth chamber for about three weeks at 25±2^{0} C with 16 hours photoperiod and 3500 lux of light intensity. After three weeks of inoculation, the percentage of contaminated, necrotic and survived explants was recorded. Ten explants were used in each sterilization treatment and each treatment was done in 3 replications. The data generated was subjected to ANOVA in complete randomized design at 5% level of significance. To satisfy model, assumptions of experiments were subjected to square root transformations.

3. RESULTS AND DISCUSSION

The results showed that the effect on various sterilants used on explants on contamination, necrotic and survival of the explants (Table 2) was highly significant. Among the different disinfecting treatments of explants for tissue culture, lowest rate of contamination (13.44 %) was obtained when the explants were treated in mercuric chloride (0.1%) for 2 minutes + ethyl alcohol (70%) for 30 seconds, while lowest necrotic rate of explants (16.56 %) was observed in Sodium hypochlorite (1.5%) treated for 10 minutes time duration which was significantly at par with the 1 minute treatment of the explants in 0.1 % Mercuric chloride (17.00 %). However, the highest rate of survivability (64.89%) was obtained when the explants were subjected to 0.1% mercuric chloride for 1 minute + ethyl alcohol (70%) for 30 seconds followed by Sodium hypochlorite (1.5%) for 15 minutes + ethyl alcohol (70%) for 30 seconds with a survival rate of 61.67%. Though the necrosis percentage was the least when the explants were treated in Sodium hypochlorite (1.5%) for 10 minutes, the contamination percentage was much higher (45.56%) that eventually declined in the rate of survivability. This might be due to weaker chemical reaction and shorter exposure duration that unable the pathogens to decimate from the explant surface (Thokchom and Maitra, 2016). Highest rate of explant mortality (47.78%) was observed in 0.1% Mercuric chloride (3 minutes) + 70% (v/v) ethyl alcohol (30 seconds) might be due to the fact that the exposure duration was much higher which leads to the internal injury of the explant tissues that ultimately dries out after becoming necrotic (Jan *et al*., 2013; Thokchom and Maitra, 2016). Over sterilization increased the tissue mortality of explants (Majid *et al*., 2014).

4. CONCLUSION

From the above findings, it can be concluded that treatment of newly emerging hairy leaf explants of gerbera in Mercuric chloride (0.1%) + ethyl alcohol (70%) (1 minute + 30 seconds) was found to be the best in terms of explant survivability. However, in terms of the health hazard factors, Sodium hypochlorite (1.5%) for 15 minutes + ethyl alcohol (70%) for 30 seconds was found optimum and can be recommended for gerbera leaf explant surface sterilization.

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REFERENCES

[1] Cassells A. C. 1997. Pathogen and microbial contamination management in micropropagation—an overview. Kluwer Academic Publishers, Dordrecht.

[2] IAEA-TECDOC. 2004. Low cost options for tissue culture technology in developing countries. IAEA-TECDOC-1384 IAEA. http://www.pub.Iaea.org/MTCD/publications/PDF/te1384web.pdf. Accessed 05 March 2011.

[3] Jan, A.; Bhat, K.M.; Bhat, S.J.A.; Mir, M.A.; Bhat, M.A.; Imtiyaz A.;Waniand Rather, J.A. (2013). Surface sterilization method for reducing microbial contamination of field grown strawberry explants intended for *in vitro* culture. *African Journal of Biotechnology*, 12(39): 5749-5753.

[4] Kozai T.; Kubota C.; Byoung R. J. 1997. Environmental control for the large-scale production of plants through in vitro techniques. Plant Cell Tissue Organ Cult 51: 49–56.

[5] Kumar S., Kanwar J.K., Sharma D.R., 2004. *In vitro* regeneration of *Gerbera jamesonii* from leaf and petiole

explants. *Journal of Plant Biochemistry and Biotechnology*, **13**: 73–75.

[6] Leifert C.; Cassells A. C. 2001. Microbial hazards in plant tissue and cell cultures. *In Vitro Cell Dev Biol Plant* 37: 133–138.

[7] Murashige T. and Skoog F. 1962. A revised medium for rapid growth and bioassays with tobacco tissue cultures. *Physiol Plant* 15: 473– 497.

[8] Majid B N, Roopa G, Sampath KKK, Kini RK, Prakash HS, Abbagani S, Mehdi K and Geetha N. 2014. Establishment of an efficient explant surface sterilization protocol for *in vitro m*icropropagation of *Salacia chinensis* L., an endangered anti-diabetic medicinal plant. *World Journal of Pharmacy and Pharmaceutical Sciences*, **3**(12): 1266-1274**.**

[9] Traore, A.; Xing, Z.; Bonser, A. and Carlson, J. (2005). Optimizing a protocol for sterilization and *in vitro* establishment of vegetative buds from immature Douglas fir trees. *Hort Science*, 40(5): 1464-1468.

[10] Thokchom R. and Maitra S. 2016. Standardization of Surface Sterilization Technique for *In-vitro* Propagation of Anthurium (*Anthurium andraeanum Lind*.) cv. Jewel. *International Journal of Current Research and Academic Review*. 4(2): 335-339.

Table 1: Different Sterilants and their Combination for Varying Time Duration

Sterilants and their combination	Time duration
Mercuric chloride (0.1%)	1 min
Mercuric chloride (0.1%)	2 min
Mercuric chloride (0.1%)	3 min
Sodium hypochlorite (1.5%)	10 min
Sodium hypochlorite (1.5%)	15 min
Sodium hypochlorite (1.5%)	20 min
Mercuric chloride (0.1%) + ethyl alcohol (70%)	1 min + 30 s
Mercuric chloride (0.1%) + ethyl alcohol (70%)	2 min + 30 s
Mercuric chloride (0.1%) + ethyl alcohol (70%)	3 min + 30 s
Sodium hypochlorite (1.5%) + ethyl alcohol (70%)	10 min + 30 s
Sodium hypochlorite (1.5%) + ethyl alcohol (70%)	15 min + 30 s
Sodium hypochlorite (1.5%) + ethyl alcohol (70%)	20 min + 30 s

Table 2: Influence of Different Sterilants on Percent Contaminated, Mortality and Survivability of Cultures in *Gerbera jamesonii* cv. Rosalin

Time duration	Contaminated	Necrosis	Survival
Mercuric chloride (0.1%) (1 min)	33.78 (35.50)	17.00 (24.28)	47.00 (43.28)
Mercuric chloride (0.1%) (2 min)	21.11(27.33)	26.33 (30.87)	53.67 (47.11)
Mercuric chloride (0.1%) (3 min)	19.33 (26.08)	39.67 (39.04)	41.00 (39.81)
Sodium hypochlorite (1.5%) (10 min)	45.56 (43.02)	16.56 (23.98)	36.89 (37.38)
Sodium hypochlorite (1.5%) (15 min)	30.78 (33.69)	31.33 (34.02)	37.89 (37.98)
Sodium hypochlorite (1.5%) (20 min)	20.44 (26.86)	46.67 (43.09)	32.889 (34.99)
Mercuric chloride (0.1%) + ethyl alcohol (70%) (1 min + 30 s)	17.00 (24.34)	18.11 (25.17)	64.89 (53.66)
Mercuric chloride (0.1%) + ethyl alcohol (70%) (2 min + 30 s)	13.44 (21.35)	28.89 (32.51)	60.78 (51.23)
Mercuric chloride (0.1%) + ethyl alcohol (70%) (3 min + 30 s)	13.56 (21.59)	47.78 (43.73)	38.67 (38.44)
Sodium hypochlorite (1.5%) + ethyl alcohol (70%) (10 min + 30 s)	21.00 (27.25)	20.22 (26.71)	58.78 (50.06)
Sodium hypochlorite (1.5%) + ethyl alcohol (70%) (15 min + 30 s)	17.56 (24.77)	20.56 (26.96)	61.67 (51.75)
Sodium hypochlorite (1.5%) + ethyl alcohol (70%) (20 min + 30 s)	18.11 (25.18)	33.11 (35.12)	48.78 (44.30)
S.Em±	0.98	0.89	1.00
CD at 5%	2.86	2.61	2.92