RESEARCH ARTICLE

EFFECT OF MERCURIC CHLORIDE ON STERILIZATION OF DIFFERENT EXPLANTS OF *LILIUM LONGIFLORUM* CULTIVARS ELITE, BRUNELLO, CORDELIA

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Abstract: Aqueous solution of mercuric chloride (0.1%) was used to study its effect on sterilization of different explants of three cultivars of *lilium longiflorum* i.e. Elite, Brunello and Cordelia. From each cultivar three types of explants i.e. shoot tips, scales and nodal segment were treated. The treatment duration varied from one to ten minutes in all three types of explants from each cultivar. It was observed that the survival rate of scales was maximum in all the three cultivars and minimum in nodal segments. The best survival was observed when seven minutes' treatment was given to all the cultivars used. The survival rate started to decline when time duration was more than seven minutes. For treatment duration of one minute and two minutes the survival rate was zero in all three explants used of cultivar Elite and Cordelia. In case of cultivar Brunello there was no survival in any explants for one-minute treatment duration.

Keywords: *Lilium Longiflorum*, Mercuric Chloride, Shoot Tips, Scales, Nodal Segment

INTRODUCTION

Lilium (*Lilium Longiflorum Thunb*.) belongs to the family Liliacae and is a native of Northern Hemisphere (up to South Canada and Siberia). It also has the southern limits in Florida and the Nilgiri mountains of India. The diploid chromosome number of *L. longiflorum* is 24. Lilium is one of the top ten ornamental bulbous crops in the world. Due to large flowers, diverse array of colours, wide range of variability, long vase life and capacity to rehydrate after long transportation, it has become an economically important floriculture crop. Hybrid lilies are exceptionally useful for use as cut-flowers and pot plants. Materova *et al*. (1987) isolated lilaline, a favonoid alkaloid from the flowers of *Lilium candidum*.

Conventionally Lilium can be easily propagated by sexual and asexual methods of propagation but these prevalent methods are not capable of meeting the increasing demand in domestic and global market. So, In vitro multiplication of Lilium is utmost important. With the advent of tissue culture technique, micro propagation has been done in Lilium.

Microbial contamination is serious problem in micro propagation. The explants may be contaminated with microorganisms include bacteria, viruses, yeast, fungi etc (Omamor *et al*. 2007). These microbes usually result inincreased culture mortality and can also result in tissue necrosis, variable growth, and reduced rooting, reduced shoot proliferation (Kane 2003). So, in micro propagation determination of most effective surface sterilization procedure is one of the most important steps to avoid contamination. Mercuric Chloride at low concentrations (up to 0.1%) is highly antimicrobial with action against both bacteria and fungi. Treatment duration of aqueous solution of Mercuric Chloride for effective sterilization may vary for the same species, or the same variety, or the different explants of same variety. This is due to the type and quantity of microorganism on explants that is dependent on seasons. Therefore the aim of present study was to determine the most effective treatment duration of mercuric chloride for three different explants of three Lilium Cultivars used, where the survival rate is maximum.

MATERIALS AND METHODS

The present investigation was carried out in the Tissue Culture Laboratory of the Centre for Research and Application in Plant Tissue Culture, Department of Science and Technology, Government of Haryana, located at CCS Haryana Agricultural University, New Campus, Hisar. The experiment was laid out in a C.R.D. (Factorial) with three replications. There were ten treatments in which treatment duration of aqueous solution of mercuric chloride (0.1%) varies from one minute to ten minutes in three explants of each cultivar.

Different explants such as scales, shoot tip and nodal segment were thoroughly washed in running tap water for 30 minutes and then dipped in Teepol (1%) for 10 minutes. The explants were then rinsed in distilled water.

The explants were treated with 0.1% mercuric chloride ($HgCl_2$) for different time intervals (1-10 minutes) and rinsed 4-5 times with sterilized distilled water. The explants were then inoculated on to the culture media containing different concentration of growth regulator under sterilized conditions and incubated in the culture room. Different auxins and cytokinins were initially tried for culture establishment. NAA and BAP having better results

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were further used for regeneration. Cultures were maintained at 25±2˚C under dark and light photoperiod (8/16 h) light intensity 50 u^2 Ems^{-1} as required. For different treatment duration of aqueous solution of mercuric chloride percent survival was calculated for each explant of three cultivars.

RESULTS AND DISCUSSION

Effective disinfection of stating material is an important step towards final goal achievement of producing disease free planting material through plant tissue culture. While disinfecting the planting material it is important to make sure that disinfectants used do not cause mortality to explants. For this purpose a combination of different disinfectants were used for different time intervals for successful sterilization of lilium bulb, scale, shoot tip and nodal segment explants. Initially tap water washing was done to remove dirt etc. Followed by detergent washing using Teepol (10%) for 10 min was carried out followed by washing using distilled water. These explants were further treated with 0.1 per cent mercuric chloride for eight minutes. These treatments were carried out under aseptic conditions and explants were washed 4-5 times using sterilized distilled water prior to culturing over different media for further studies. Mercuric chloride has been used successfully for effective sterilization in lilium by several workers (Dilta *et al*., 2000 and Priyadarshini and Sen, 1992).

Surface sterilization of different explants of *Lilium longiflorum* cv. Elite. Table 1 indicates the effect of $HgCl_2$ on sterilization of different explants of *Lilium longiflorum* cv. Elite. Three types of explants were used i.e. shoot tips, scales and nodal segments. The treatment duration varied from one to ten min in all three types of explants. It was observed that survival rate was zero up to two minutes in all explants. The maximum survival rate in different explants was observed when seven minutes treatment was given. Among the different explants used scales showed the maximum (70.6%) and nodal segment the minimum (66.9%) survival rate. The survival rate started to decline when the treatment time was increased more than seven minutes.

Table 1. Effect of mercuric chloride (0.1%) on sterilization of different explants of *Lilium longiflorum* cv. Elite

Treatment time(min)	**Per cent survival**			**Mean**
	Shoot tip	**Scales**	**Nodal segment**	
1.	0.0±0.0	0.0±0.0	0.0±0.0	0.0
2.	0.0±0.0	0.0±0.0	0.0±0.0	0.0
3.	0.0±0.0	14.20.3	0.0±0.0	4.7
4.	32.2±0.3	32.9±0.3	32.1±0.3	31.4
5.	38.3±0.2	39.7±0.2	37.1±0.3	38.4
6.	48.9±0.1	49.9±0.3	49.6±0.2	49.5
7.	69.2±0.4	70.6±0.2	66.9±0.4	68.9
8.	55.2±0.2	56.4±0.2	55.4±0.4	55.7
9.	44.1±0.3	44.3±0.3	43.7±0.2	44
10.	30.8±0.3	32.3±0.2	29.7±0.3	30.9
Mean	31.9	34.0	31.5	

C.D. at 5% level
Treatment = 0.40
Cultivar = 0.22
Treatment × Cultivar = 0.69

Surface sterilization of different explants of *Lilium longiflorum* cv. Brunello. Table 2 indicates the survival rate of different explants of *Lilium longiflorum* cv. Brunello. The table shows that the maximum survival rate was observed when seven minutes $Hgcl_2$ (0.1%) treatment was given to all three type of explants i.e. shoot tips, scales and nodal segments. The highest (82.4) survival rate was observed in scales and lowest (69.2%) in nodal segments. It was observed that there was no survival when the treatment time was one minute in all type of explants. The rate of survival was reduced when time duration was increased more than seven minutes.

Table 2. Effect of mercuric chloride (0.1%) on sterilization of different explants of *Lilium longiflorum* cv. Brunello

Treatment time(min)	**Per cent survival**			**Mean**
	Shoot tip	**Scales**	**Nodal segment**	
1.	0.00.0	0.00.0	0.00.0	0.0
2.	17.20.5	20.30.2	16.70.3	18.1
3.	31.40.3	32.20.2	30.90.2	31.5
4.	35.40.3	37.30.2	33.90.2	35.6
5.	44.10.2	36.30.4	43.60.3	44.7
6.	53.30.2	55.80.3	55.40.4	53.2
7.	70.20.5	82.40.3	69.20.4	73.9
8.	56.90.2	57.70.4	55.30.4	56.6
9.	46.40.3	49.30.4	46.40.3	47.4
10.	40.60.3	40.50.4	39.90.3	40.4
Mean	39.6	42.2	38.6	

C.D. at 5% level
Treatment = 0.51
Cultivar = 0.28
Treatment × Cultivar = 0.88

Surface sterilization of different explants of *Lilium longiflorum* cv. Cordelia. Table 3 shows the survival rate of different explants of *Lilium longiflorum* cv. Cordelia when treated with $Hgcl_2$ (0.1%). It was observed that there was no survival when all the three type of explants were treated up to two minutes. The maximum survival rate in different explants used was observed at seven minutes treatment. Among the different explants used the maximum (79.1%) survival rate was shown by scales and minimum (68.9%) by nodal segments. The survival rate continuously reduced when treatment duration was more than seven minutes.

Table 3. Effect of mercuric chloride (0.1%) on sterilization of different explants of *Lilium longiflorum* cv. Cordelia

Treatment time(min)	**Per cent survival**			**Mean**
	Shoot tip	**Scales**	**Nodal segment**	
1.	0.0±0.0	0.0±0.0	0.0±0.0	0.0
2.	0.0±0.0	0.0±0.0	0.0±0.0	0.0
3.	24.1±0.3	29.3±0.1	22.6±0.2	25.3

4.	34.2±0.2	34.5±0.2	32.3±0.3	33.7
5.	40.9±0.2	42.9±0.1	40.5±0.2	41.4
6.	69.7±0.4	53.1±0.1	51.1±0.2	57.9
7.	71.2±0.5	79.1±0.5	68.9±0.3	73.1
8.	56.9±0.2	58.6±0.4	56.3±0.1	57.3
9.	47.3±0.2	51.3±0.1	48.9±0.2	49.2
10.	38.2±0.1	43.0±0.2	39.4±0.5	40.2
Mean	38.3	39.2	36.0	

C.D. at 5% level
Treatment = 0.40
Cultivar = 0.22
Treatment × Cultivar = 0.70

CONCLUSIONS

Among all the cultivars i.e. Elite, Brunello, Cordelia used, the maximum survival rate was observed at seven minute treatment duration of aqueous solution of $HgCl_2$ (0.1%). It has been also observed that the survival rate of scales was maximum in all the three cultivars and minimum in nodal segments.

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