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EFFECT OF SURFACE STERILIZATION ON *IN VITRO* SURVIVAL OF EXPLANTS OF NONI (*Morinda citrifolia* L.)

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Abstract: *In vitro studies were undertaken to investigate the effect of different concentrations of mercuric chloride, sodium hypochlorite and calcium hypochlorite in combination with different time durations on the survival percentage of nodal segment, shoot tip and leaf bit explants of noni (Morinda citrifolia* L.*). Surface sterilization with mercuric chloride @ 0.1 per cent for 4 minutes recorded the highest survival of 82.59, 84.26, and 76.53 per cent in nodal segment, shoot tip and leaf bit explants respectively and was found to be a strong surface sterilant. Treatment with sodium hypochlorite @ 2 per cent for 10 minutes resulted in survival of 71.99, 78.79 and 66.15 per cent in nodal segment, shoot tip and leaf bit explants respectively. Calcium hypochlorite @ 5 per cent recorded the survival of 64.23, 70.17, and 60.57 per cent in nodal segment, shoot tip and leaf bit explants and was found to be a weak surface sterilant for surface sterilization of noni.*

Key words: *Morinda citrifolia* L., explants, surface sterilization, *in vitro* survival percentage

I. INTRODUCTION

Morinda citrifolia L, popularly known as noni or Indian mulberry, is an important medicinal plant belongs to the family rubiaceae. Native to Polynesia, this evergreen tree is found growing in tropical and subtropical regions of the world. Various parts of the plant including roots, barks, leaves and fruits have been used for over 2000 years to treat several diseases such as high blood pressures, diabetes, eye problems, skin wounds, throat problems, respiratory ailments, constipation, and stomach pains. Realising importance of its medicinal value, it is commercially grown now. In India it is cultivated in an area of 653 acres in the states of Andaman & Nicobar, Maharashtra, Karnataka, Madhya Pradesh, Odisha and Andhra Pradesh (Anon.2015). The conventional seed propagation is difficult and seedlings raised are not true to type. Hence *in vitro* culture is used for rapid multiplication of elite genotypes. In tissue culture, maintenance of aseptic or sterile conditions is essential for successful culture of explants. Surface sterilization makes the explants contamination free. Various sterilization agents are used to decontaminate the tissues. These sterilants at higher concentrations are also toxic to plant tissues. Hence proper concentration of sterilants and duration of their exposure to explants have to be standardized to minimise explant injury and to achieve better survival. The present study was conducted to standardize the best sterilization protocol for *in vitro* culture of noni.

II. MATERIALS AND METHODS

Actively grown young branches of noni with 5-6 nodes were collected from field grown plants. Nodal segments, shoot tips and leaf bits were excised from them. These explants were washed in running tap water. They were then surface sterilized with 70 per cent ethanol for 1 minute. Three separate experiments were carried out. Each experiment comprised of 10 treatments. The first experiment involved surface sterilization of explants with mercuric chloride @ 0.05, 0.1 and 0.2 per cent for 2, 4 and 8 minutes respectively. In the second experiment sodium hypochlorite was used in the concentration of 1, 2 and 3 per cent for 5, 10 and 15 minutes respectively and the third experiment comprised

of treatment of explants with calcium hypochlorite @ 3, 5 and 7 per cent for 5, 10 and 15 minutes respectively. Nodal segments, shoot tips and leaf bits were cultured on the agar solidified MS basal medium. The experiment was arranged in completely randomized design repeated three times with 20 explants in each replication. The data were subjected to statistical analysis using IRRISAT programme as per the method suggested by Panse and Sukhatme (1967).

III. RESULT AND DISCUSSION

3.1. Effect of mercuric chloride on survival percentage of explants.

The data presented in the Table 1 shows the effect of different concentrations of mercuric chloride (0.05, 0.1 and 0.2 per cent) in combination with various exposure periods (2, 4 and 8 minutes) on the survival of different explants (nodal segment, shoot tip and leaf bits). The mean survival of explants ranged from 6.15 to 84.26 per cent. The highest survival of 82.59, 84.26, and 76.53 per cent was observed in nodal segment, shoot tip and leaf bits respectively in treatment with 0.1 per cent mercuric chloride for 4 minutes and was followed by survival of 75.25, 77.46, and 68.63 per cent in nodal segment, shoot tip and leaf bits respectively in treatment with 0.05 per cent mercuric chloride for 2 minutes while the lowest survival of 8.68, 9.51, and 6.15 per cent in nodal segment, shoot tip and leaf bits respectively were recorded in the control. The survival of nodal explants when compare to shoot tip was lower which might be due to the aged nature of the nodal explants which may be heavily contaminated. When the concentration of mercuric chloride was increased the survival percentage was low which might be due to tissue damage by mercuric chloride in higher doses. The results of our study is in accordance with the findings of Anoop Badoni and Chauhan (2010); Gajakosh *et al.* (2010); Rattanpal *et al.* (2011); Aarifa *et al.* (2013); Sreeranjini and Siril (2014); and Saini and Patel (2015).

3.2. Effect of sodium hypochlorite on survival percentage of explants.

The treatment of various explants with different concentrations of sodium hypochlorite (1, 2 and 4 %) in combination with various exposure periods (5, 10 and 15 minutes) have resulted in survival of explant ranging from 5.12 to 78.79 per cent. The treatment with 2 % sodium hypochlorite for 10 minutes recorded the highest survival of 71.99, 78.79 and 66.15 per cent in nodal segment, shoot tip and leaf bits respectively and was followed by treatment with 2 % sodium hypochlorite for 5 minutes which recorded the survival of 67.28, 71.51 and 60.21 per cent in nodal segment, shoot tip and leaf bits respectively. Control recorded the lowest survival of 8.76, 9.19 and 5.12 per cent in nodal segment, shoot tip and leaf bits respectively (Table 2). Sodium hypochlorite has turned out to be a good sterilant than calcium hypochlorite due to bleaching effects of the latter. Our results are in accordance with findings of Roshan Zamir *et al.* (2004) and Elio Jiménez *et al.* (2011)

3.3. Effect of calcium hypochlorite on survival percentage of explants.

The effect of different concentrations of calcium chloride (3, 5 and 7 per cent) in combination with different exposure periods (5, 10 and 15 minutes) on the survival of explants cultured from nodal segments, shoot tip and leaf bits are shown in the table 3. The mean survival of explants ranged from 3.26 to 70.17 per cent. The highest survival of 64.23, 70.17, and 60.57 per cent was observed in nodal segment, shoot tip and leaf bits respectively in treatment with 5 per cent calcium hypochlorite for 15 minutes and was followed by survival of 60.26, 65.56, and 54.9 per cent in nodal segment, shoot tip and leaf bits respectively in treatment with 5 per cent calcium hypochlorite for 10 minutes while the untreated control recorded the lowest survival of 8.71, 8.92, and 3.26 per cent in nodal segment, shoot tip and leaf bits respectively. Calcium hypochlorite is a weak surface sterilant and hence explants are to be soaked in it for longer period. The result of our study are in conformity with results of Assareh and Sardabi (2005) and Ines Mihaljević *et al.* (2013)

Table 1: Effect of mercuric chloride on survival of nodal, shoot tip and leaf bit explants

Treatments	Nodal segment explant Survival (per cent)	Shoot tip Explant Survival (per cent)	Leaf bit Explant survival (per cent)
T_1 -Control	8.68(16.88)	9.51(17.77)	6.15(14.03)
T_2 -$HgCl_2$ 0.05% for 2 minutes	25.48(30.32)	28.21(32.07)	18.41(25.39)
T_3- $HgCl_2$ 0.05% for 4 minutes	32.44(34.71)	35.84(36.77)	25.84(30.53)
T_4-$HgCl_2$ 0.05% for 8 minutes	38.61(38.39)	42.57(40.73)	32.49(34.73)
T_5- $HgCl_2$ 0.1% for 2 minutes	75.25(60.27)	77.46(61.74)	68.63(55.98)
T_6- $HgCl_2$ 0.1% for 4 minutes	82.59(65.37)	84.26(66.65)	76.53(61.03)
T_7- $HgCl_2$ 0.1% for 8 minutes	68.38(55.80)	71.79(57.93)	59.68(50.59)
T_8- $HgCl_2$ 0.2% for 2 minutes	59.68(50.60)	63.24(52.69)	50.92(45.54)
T_9- $HgCl_2$ 0.2% for 4 minutes	53.23(46.86)	55.49(48.16)	43.80(41.43)
T_{10}- $HgCl_2$ 0.2% for 8 minutes	45.61(42.48)	48.32(44.03)	35.52(36.59)
S.Ed.	1.90	1.70	1.70
CD(0.05)	3.97	3.54	3.55

Values in parenthesis are arcsine transformed

Table 2: Effect of sodium hypochlorite on survival of nodal, shoot tip and leaf bit explants

Treatments	Nodal segment explant Survival (per cent)	Shoot tip Explant Survival (per cent)	Leaf bit Explant survival (per cent)
T_1-Control	8.76(17.11)	9.19(17.58)	5.12(12.82)
T_2-NaOCl 1% for 5 minutes	16.29(23.80)	14.52(22.37)	7.68(16.05)
T_3-NaOCl 1% for 10 minutes	23.01(28.66)	22.66(28.43)	13.64(21.67)
T_4-NaOCl 1% for 15 minutes	30.15(33.28)	31.52(34.13)	21.57(27.64)
T_5-NaOCl 2% for 5 minutes	67.28(55.14)	71.51(57.75)	60.21(50.89)
T_6-NaOCl 2% for 10 minutes	71.99(58.05)	78.79(62.59)	66.15(54.43)
T_7-NaOCl 2% for 15 minutes	58.93(50.15)	61.79(51.83)	51.05(45.59)
T_8-NaOCl 4% for 5 minutes	50.90(45.52)	54.70(47.70)	42.36(40.61)
T_9-NaOCl 4% for 10 minutes	44.74(41.97)	47.69(43.68)	35.05(36.28)
T_{10}-NaOCl 4% for 15 minutes	36.85(37.37)	39.39(38.88)	28.43(32.22)
S.Ed.	1.20	1.07	1.28
CD (0.05)	2.50	2.24	2.66

Values in parenthesis are arcsine transformed

Table 3: Effect of calcium hypochlorite on survival of nodal, shoot tip and leaf bit explants

Treatments	Nodal segment explant Survival (per cent)	Shoot tip Explant Survival (per cent)	Leaf bit Explant survival (per cent)
T_1-Control	8.71(17.12)	8.92(17.26)	3.26(9.69)
T_2-$Ca(OCl)_2$ 3% for 5 minutes	17.33(24.60)	21.41(27.55)	5.57(13.63)
T_3-$Ca(OCl)_2$ 3% for 10 minutes	24.63(29.73)	27.41(31.56)	10.74(19.12)
T_4-$Ca(OCl)_2$ 3% for 15 minutes	30.60(33.59)	34.28(35.84)	16.59(23.95)
T_5-$Ca(OCl)_2$ 5% for 5 minutes	54.79(47.74)	56.63(48.80)	43.44(41.22)
T_6-$Ca(OCl)_2$ 5% for 10 minutes	60.26(50.94)	65.56(54.07)	54.9(47.81)
T_7-$Ca(OCl)_2$ 5% for 15 minutes	64.23(53.27)	70.17(56.92)	60.57(51.12)
T_8-$Ca(OCl)_2$ 7% for 5 minutes	47.51(43.57)	50.78(45.46)	34.55(35.98)
T_9-$Ca(OCl)_2$ 7% for 10 minutes	41.65(40.20)	43.70(41.38)	29.16(32.67)
T_{10}-$Ca(OCl)_2$ 7% for 15 minutes	36.67(37.29)	40.24(39.37)	22.49(28.31)
S.Ed.	0.95	1.16	1.73
CD(0.05)	1.98	2.42	3.62

Values in parenthesis are arcsine transformed

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