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Identification of Best Exposure Timings of Sodium Hypochlorite in Surface Sterilization of Different Explants in *invitro* Propagation of *Annona muricata*

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Abstract: *The most crucial and delicate stage in plant tissue culture is the surface sterilization of the explant. An inappropriate concentration of sterilant may have deadly effects on explants by altering cell division and other metabolic processes that limit the growth and development of explants. Considering the explants observations and sterilization treatments of Sodium hypochlorite (4%), T_6 (7 min), T_7 (8 min) and T_8 (9 min) are selected for green nodal explants as highest response, least days for initiation of response, good health with less contamination and moderate survival of explants. Whereas, T_5 (6 min) T_6 (7 min) and T_7 (8 min) are selected for leaf with lower contamination with good health and survivability. Internode was not selected due to no response with higher contamination and less survivability. Brown nodal explants responded very less and less survivability with higher contamination as compared to other explants.*

Keywords: Annona muricata, Sodium hypochlorite, surface sterilization, invitro propagation

I. INTRODUCTION

The genus *Annona* is the most economically important containing 120 species.Five edible species have been identified, four of which are from South or Mesoamerica and one from Eastern Africa(Pinto et al., 2010).Soapsop (*Annona muricata* L) is a native of South America is widely distributed throughout tropical and subtropical regions of the world, including India, Malaysia and Nigeria. It is also known as graviola, guanabana, paw-paw and sirsak.*A. muricata* is the herb that is most frequently used to treat most cancers in Jamaica and Trinidad. Moreover, it has been described as anticrustacean, antiparasitic, cytotoxic (acetogenins), antiamoebic, antibacterial, antifungal, spasmogenic, vasodilator, smooth muscle relaxant, anti-inflammatory and anti-microbial (Rady *et al.,* 2018).This family has a considerable number of tropical and subtropical habitats around the world, but further geographical development is constrained by the Annonaceae's climatic requirements to regions with extremely precise slope, temperature, relative humidity and soil characteristics (Encina*et al.,*2014). In addition to traditional techniques for growing plants, cherimoya and other *Annona* species can benefit from the effective application of *in vitro* tissue culture techniques for micropropagation to get over those issues (Santana *et al.,* 2003). *In vitro* propagation is a method, possibly to grow identical plants from superior specimen quickly.

II. MATERIAL AND METHODS

In the present study, experiments were carried out on sterilization and best explant of soursop for *in vitro* micro propagation during 2020 to 2022 at Tissue culture laboratory, College of Horticulture, Sirsi (University of Horticultural

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Sciences, Bagalkot), Uttar Kannada district, Karnataka. Annona muricata seedlings were brought from a nursery in Shivamogga, and mother plants were kept in the nursery of the Department of Fruit Science at COH, Sirsi. Four different kinds of explant were used for direct regeneration.

Plant tissue culture losses are attributed to the formation of *in vitro* cultures from mother plants that have been grown in the field and are susceptible to microbial contamination. It is essential to choose the right sterilising chemicals and exposure duration, which will remove only impurities from the living material but not its viability. Hence, sterilization was standardised in the current experiment, where all the explants were washed under tap water for 20 minutes, then washed with distilled water containing two drops of Tween-20 and soaking in Carbendazima (2 mg/L) and Cetrimide (0.5 mg/L) solutions before treating with surface sterilants. The observations were recorded at weekly intervals. Green nodal, brown nodal, internode and leaf explants of *Annona muricata* were used to standardise the procedure for sterilization for direct regeneration. Utilizing practical and efficient disinfectants such as sodium hypochlorite, mercuric chloride and ethanol has made tissue culture practicable (Krikorian 1962).

2.1 Procedure

Surface sterilization and preparation ofexplant (green wood, internode, brown wood and leaves)

Nodal explants and leaves from the seedlings of *Annona muricata* grown in a greenhouse were sterilized by the following procedure.

To lower the bacterial and fungal load, the mother plant was frequently sprayed with Carbendazim (0.2%) and cetrimide (0.5 g/L) at weekly intervals and streptomycin sulphate (0.5 g/L) at 10 days interval.

Using a scissors that had been swabbed with cotton dipped in 70 per cent ethanol, the healthy and freshly sprouted stem was removed from the mother plant that was grown in a polybag under greenhouse conditions.

Leaves of the stem were removed to their half and then nodal explants were prepared.

Stems measuring 10 cm were washed for 10 minutes under running tap water, then soaked for three hours in solution containing Carbendazim (0.2%), cetrimide (0.5g/ L) and two drops of Tween 20.

Initial sterilization with Sodium hypochlorite or mercuric chloride has been taken up and then washed with distil water for four times. Further sterilization was carried out under sterile laminarhood, as per the treatments. Explants were subjected to sterilization treatments in a closed container and repeatedly washed with sterile water inside the laminar air flowchamber for five times.

Treated nodal explants were cut with sterile blades to remove any dead tissues and were fragmented into 3-4 cm length containing two nodes. Leaves were fragmented then both the explants were placed over the MS media containing BAP (1.5mg/L) (Plate 1).

2.2 Treatment details

Experiment was done with exposure time of Sodium hypochlorite (4%) on explants.The experimental design used was CRD with 11 treatments, three replications and 10 explants per replication and following treatments were used.

Treatment No.	Treatment
T_1	Control
T_2	4% NaOCl for 3 min
T_3	4% NaOCl for 4 min
T_4	4% NaOCl for 5 min
T_5	4% NaOCl for 6 min
T_6	4% NaOCl for 7 min
T_7	4% NaOCl for 8 min
T_8	4% NaOCl for 9 min
T_9	4% NaOCl for 10 min

NaOCl- Sodium hypochlorite

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2.3 Statistical analysis

As all the studies were conducted in the laboratory under well-defined condition of medium of growth, temperature and light, complete randomizeddesign(CRD)wasemployedforanalysisoftheexperiments. Critical difference (C.D.) values given in the table were at one per cent level of significance, where the F test was significant and used to compute the means. Values in percentages were subjected to arc sine transformation to ensure homogenity. Wherever values were 0 per cent or 100 per cent, arcsin (1/4n) and arcsin (100-1/4n), where n is the number of observations that make up the percentage, were substituted respectively (Panse and Sukhatme 1967). The significance of differences among means was carried out using Duncan's multiple-range test at P= 0.01.

III. RESULTS

3.1 Contamination (%)

There was only fungal contamination was recorded in all the treatments in all the explants.The data on the contamination in green nodal, leaf, brown nodal and internode explants affected by various Sodium hypochlorite(4%) treatment timings are represented in Tables1 and 2 respectively.Significantly the lowest level (20.00% - 30.00%) of fungal contamination was recorded in T_9 (NaOCl 4 % for 10 min) and the highest was noted in T_1 (control) with 100 per cent contamination for all the explants (green, brown nodal, leaf and internode).

3.2 Health (+, ++, +++)

The data pertaining to health of green nodal, leaf, brown nodal and internodal explant influenced by various Sodium hypochlorite(4%) treatment timings are presented in the Tables 3, 4, 5 and 6 respectively.Health was maintained good to moderate in all the explants in treatment from T_5 to T_8 (NaOCl 4 % for 6 min to 9 min).

3.3 Initiation of Response (%)

In the present experiment of sterilization explants with different time of Sodium hypochlorite (4%), no response was seen in leaf and internode explants and significantly the highest initiation of response (93.33%) was noted in T_8 (NaOCl 4% for 9 min) in green nodal explants (Table 7). Whereas, brown nodal explants showed response (66.66%) in T_6 (NaOCl 4% for 7 min) (Table 8), which was represented in Plate 2.

3.4 Days taken for shoot initiation

Significantly the least days (14.33 to 15.67)taken for initiation of response in green nodal explants was recorded in T_6 to T_8(NaOCl 4% for 7 to 9 min) (Table 7). 24.50 days were taken for shoot emergence in brown nodal explants in T_6 (NaOCl 4% for 7 min) (Table 8).

3.5 Survival (%)

The information in Tables 3, 4, 5 and 6 relates to the survival of explants in green nodal, leaf, brown nodal and internodal explants respectively, as affected by various Sodium hypochlorite(4%) treatments.Significantly the highest percentage of survival was recorded in T_8 (NaOCl 4 % for 9 min) in internode and green nodal explants (80.00%) and in T_7 (NaOCl 4 % for 8 min) in leaf and brown wood explants (56.67%).

IV. DISCUSSION

The pre-treatment in the greenhouse stock plants with systemic fungicides was not enough to avoid all the endogenous pollutants. The disinfection treatments with Sodium hypochlorite helped to sanitize all surface of the explants (Bridg 2000). As it is a highly powerful disinfectant and known as a bacterial killer, ensures a large drop in microbial population even at very small amounts (Odutayo *et al.,* 2007; Eed *et al.,* 2010).

Increasing the exposure duration and concentration of sterilizing agents had reduced the contamination rate because sterilizing chemical ruin the shape and functions of microbe's enzymes (George *et al.,* 2008).Numerous species of bacteria have been controlled well with Sodium hypochlorite treatment (Tiwari *et al.,* 2012). Bacterial populations can be decreased

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by very low concentrations of Sodium hypochlorite (Nakagawara *et al.,* 1998) as the percentage of contamination decreases with increasing Sodium hypochlorite concentration and immersion time. It has been shown that Sodium hypochlorite is highly effective against a variety of bacteria even low doses are sufficient to dramatically lower/no bacterial populations. Bridg (2000) obtained high aseptic cultures in nodal explants with low (45%) contamination (bacteria- 30% and fungi- 55%) by treating Sodium hypochlorite (3%) for 15 min with tween 20 as a wetting agent in *A. cherimola* and *A. muricata*. Treating Agrymicin and Benomyl each 30 min and Sodium hypochlorite(1%) for 15 min recorded zero contamination in pitahaya (Vinas *et al.,* 2012).These findings are consistent with those of Badoni and Chauhan's (2010) tests,Oros *et al.* (2020), Feleke*t al.* (2015) and Nelson *et al.* (2015).Similar findings were found by Bridg (2000) as treating of Sodium hypochlorite (3%) for 15 min with tween 20 as a wetting agent in *A. cherimola* and *A. muricata* recorded aseptic cultures with green explants of 55 per cent. Treating Agrymicin and Benomyl each 30 min and Sodium hypochlorite (1%) for 15 min had least damage (16%) in pitahaya (Vinas *et al.,* 2012).Feleke*t al.* (2015) sterilized nodal explants of peach using Sodium hypochlorite (0.25%) for 15 min found least tissue death (4.71%). Higher treatment of Sodium hypochlorite for 10 and 15 minutes did not significantly differ health from one another and effectively managed contamination (Jaskani *et al.,* 2008).

Negative results for increased exposure time were found in explants. Acheampong *et al.*(2015) found that the length of time in pineapple explants were exposed to Sodium hypochlorite improved sterilisation, but exposures that lasted longer than 20 minutes were harmful to the explants. The increasing exposure duration and concentration of sterilant above certain optimum limit cause loss of explants because of the oxidant chemical ingredient ruin the plant tissues (Danso *et al.,* 2011). These findings are similar of the negative effects of Sodium hypochlorite at high concentration were recorded by Colgecen *et al.*(2011).

Similar results in the present experiment were obtained by Bridg (2000) as treating NaOCl (3%) for 15 min with tween 20 as a wetting agent in *A. cherimola* and *A. muricata* recorded aseptic cultures with 45 per cent bud sprouting. Similarly, Nelson *et al.* (2015) treated buds of pineapple with NaOCl (2%) for 20 min recorded response of 40 per cent. According to Estrela *et al.*(2003) the efficacy of Sodium hypochlorite (2%) increased with longer exposure times. In another study, Sodium hypochlorite was shown to be better with more survived explants, because of their more fragile and sensitive cuticles in mother plants cultivated in greenhouse (Sisko 2011).Sour cherry shoots with axillary winter buds found maximum survival (80%) when treated with Sodium hypochlorite (1%) for 20 min with few drops of tween 20 (Mihaljevic *et al.,* 2013). Feleke*t al.* (2015) sterilized nodal explants of peach using Sodium hypochlorite (0.25%) for 15 min recordedthe maximum survival (85.71%).In contrary to above results, longer immersion times and higher Sodium hypochlorite concentrations had a detrimental effect on the seeds, turning them a dark shade of black and lowering their germination rates (Pinto *et al.,*(2010) Garcia *et al.,* 2011).

V. CONCLUSION

In the present study considering the explants observations and sterilization treatments,T_6 (7 min), T_7 (8 min) and T_8 (9 min) are selected for green nodal explants as highest response, least days for initiation of response, good health with less contamination and moderate survival of explants.Whereas, T_5 (6 min) T_6 (7 min) and T_7 (8 min) are selected for leaf with lower contamination with good health and survivability. Internode was not selected due to no response with higher contamination and less survivability. Brown nodal explants responded very less and less survivability with higher contamination as compared to other explants. Some woody plants have been successfully reproduced *in vitro*, although the development of some species has been hampered by challenges in creating aseptic cultures from mature explants and browning of explants by Abbott (1977) and Barghchi and Alderson (1983).

Considering the sterilization procedure and response of explants of above experiment, green nodal and leaf were the best two explants selected for further experiments. Among sodium hypochlorite (4%) treatment timings T_6 (7 min), T_7 (8 min) and T_8 (9 min) are selected for green nodal explants. Whereas, T_5 (6 min) T_6 (7 min) and T_7 (8 min) are selected for leaf explants (Plate 2 and 3).

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Treatment		Contamination of green nodal (%)				Contamination of leaf (%)			
Sodium hypochlorite (4%)	Time (min)	1st week	2nd week	3rd week	4th week	1st week	2nd week	3rd week	4th week
T_1	0	36.67 (37.26)^{a}	53.33 (46.92)^{a}	70.00 (56.83)^{a}	100.00 (90.00)^{a}	0.00 (0.29)	0.00 (0.29)	50.00 (45.00)^{a}	100.00 (90.00)^{a}
T_2	3	33.33 (35.26)^{ab}	50.00 (45.00)^{a}	63.33 (52.75)^{ab}	83.33 (66.09)^{b}	0.00 (0.29)	0.00 (0.29)	45.00 (42.13)^{b}	55.00 (47.87)^{b}
T_3	4	30.00 (33.20)^{bc}	43.33 (41.17)^{b}	56.67 (48.84)^{bc}	70.00 (56.83)^{c}	0.00 (0.29)	0.00 (0.29)	41.00 (39.81)^{c}	52.00 (46.15)^{b}
T_4	5	26.67 (31.08)^{cd}	36.67 (37.26)^{c}	50.00 (45.00)^{cd}	60.00 (50.78)^{d}	0.00 (0.29)	0.00 (0.29)	37.50 (37.76)^{d}	40.60 (39.58)^{c}
T_5	6	23.33 (28.88)^{de}	26.67 (31.08)^{d}	46.67 (43.09)^{de}	53.33 (46.92)^{e}	0.00 (0.29)	0.00 (0.29)	26.50 (30.98)^{e}	37.00 (37.46)^{cd}
T_6	7	20.00 (26.56)^{e}	23.33 (28.88)^{d}	40.00 (39.23)^{ef}	46.67 (43.09)^{f}	0.00 (0.29)	0.00 (0.29)	24.00 (29.33)^{ef}	35.25 (36.42)^{d}
T_7	8	13.33 (21.41)^{f}	16.67 (24.09)^{e}	33.33 (35.26)^{fg}	43.33 (41.17)^{f}	0.00 (0.29)	0.00 (0.29)	22.00 (27.97)^{fg}	30.00 (33.21)^{e}
T_8	9	10.00 (18.43)^{g}	13.33 (21.41)^{ef}	26.67 (31.08)^{g}	36.67 (37.26)^{g}	0.00 (0.29)	0.00 (0.29)	20.50 (26.91)^{gh}	25.00 (29.99)^{f}
T_9	10	6.67 (14.59)^{h}	10.00 (17.99)^{f}	16.67 (23.55)^{h}	30.00 (33.10)^{g}	0.00 (0.28)	0.00 (0.28)	17.60 (24.69)^{h}	20.00 (26.56)^{g}
S.Em±		0.70	0.89	1.17	0.90	1.33	1.54	1.85	2.14
LSD at 0.01		2.90	3.67	4.85	3.71	NS	NS	7.64	8.85
CV (%)		4.43	4.712	4.87	3.01	8.15	8.21	7.60	7.10

Table 1: Effect of exposure time of sodium hypochlorite (4%) on contamination in green nodal and leaf explants of *Annona muricata*

Figures above paranthes is indicate the actual values and figures in paranthesis are arc sine transformed values. Values with the same letter are satistically non-significant at LSD *($p \leq 0.01$).*

Treatment		Contamination of brown nodal (%)				Contamination of internodal (%)			
Sodium hypochlorite (4%)	Time (min)	1st week	2nd week	3rd week	4th week	1st week	2nd week	3rd week	4th week
T_1	0	66.67 (54.76)^{a}	70.00 (56.83)^{a}	73.33 (58.97)^{a}	100.00 (90.00)^{a}	36.67 (37.26)^{a}	53.33 (46.91)^{a}	73.33 (58.97)^{a}	100.00 (90.00)^{a}
T_2	3	56.67 (48.84)^{b}	63.33 (52.75)^{a}	70.00 (56.83)^{a}	100.00 (90.00)^{a}	33.33 (35.26)^{ab}	50.00 (45.00)^{a}	66.67 (54.76)^{ab}	83.33 (65.91)^{b}
T_3	4	53.33 (46.91)^{b}	50.00 (45.00)^{b}	56.67 (48.84)^{ab}	90.00 (72.15)^{b}	30.00 (33.20)^{abc}	43.33 (41.17)^{ab}	56.67 (48.84)^{bc}	73.33 (58.97)^{bc}
T_4	5	36.67 (37.26)^{c}	43.33 (41.17)^{b}	50.00 (45.00)^{bc}	83.33 (66.09)^{b}	26.67 (31.08)^{bcd}	36.67 (37.26)^{bc}	50.00 (45.00)^{cd}	66.67 (54.76)^{cd}
T_5	6	26.67 (31.08)^{d}	40.00 (39.23)^{b}	46.67 (43.09)^{bc}	53.33 (46.91)^{c}	23.33 (28.88)^{cde}	26.67 (31.08)^{cd}	46.67 (43.09)^{cde}	53.33 (46.91)^{de}
T_6	7	20.00 (26.56)^{d}	23.33 (28.88)^{c}	40.00 (39.23)^{bcd}	46.67 (43.07)^{cd}	20.00 (26.56)^{de}	23.33 (28.88)^{de}	40.00 (39.23)^{de}	46.67 (43.07)^{ef}
T_7	8	13.33 (20.91)^{e}	16.67 (23.55)^{cd}	33.33 (34.74)^{cd}	43.33 (41.12)^{cde}	16.67 (24.09)^{e}	16.67 (23.55)^{ef}	33.33 (35.26)^{ef}	43.33 (41.12)^{efg}
T_8	9	10.00 (17.99)^{ef}	13.33 (20.91)^{d}	26.67 (30.51)^{de}	36.67 (37.17)^{de}	16.67 (23.55)^{e}	13.33 (20.91)^{f}	26.67 (30.51)^{fg}	36.67 (37.17)^{fg}
T_9	10	6.67 (14.59)^{f}	10.00 (17.99)^{d}	16.67 (23.55)^{e}	30.00 (32.65)^{e}	6.67 (14.59)^{f}	10.00 (17.99)^{f}	16.67 (23.55)^{g}	30.00 (32.65)^{g}
S.Em±		1.27	1.49	2.38	2.14	1.33	1.54	1.85	2.14
LSD at 0.01		5.25	6.17	9.83	8.84	5.50	6.37	7.64	8.85
CV (%)		6.63	7.13	9.74	6.43	8.15	8.21	7.60	7.10

Table2: Effect of exposure time of sodium hypochlorite (4%) on contamination in brown nodal and internodal explants of *Annona muricata*

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Treatment		Health				Survival (%)			
Sodium hypochlorite (4%)	Time (min)	1st week	2nd week	3rd week	4th week	1st week	2nd week	3rd week	4th week
T_1	0	+++	+++	++	+	60.00 (51.22)^{d}	50.00 (45.03)^{e}	33.33 (34.95)^{f}	0.00 (0.28)^{f}
T_2	3	+++	+++	++	++	70.00 (56.83)^{cd}	63.33 (52.75)^{d}	50.00 (45.00)^{e}	33.33 (35.26)^{e}
T_3	4	+++	+++	++	++	80.00 (63.56)^{bc}	66.67 (54.76)^{d}	63.33 (52.75)^{d}	50.00 (45.00)^{d}
T_4	5	+++	+++	+++	+++	80.00 (63.56)^{bc}	73.33 (58.97)^{cd}	66.67 (54.76)^{cd}	53.33 (46.92)^{d}
T_5	6	+++	+++	+++	+++	90.00 (72.15)^{ab}	80.00 (63.56)^{bc}	70.00 (56.83)^{bcd}	63.33 (52.75)^{c}
T_6	7	+++	+++	+++	+++	90.00 (72.15)^{ab}	86.67 (68.89)^{ab}	76.67 (61.20)^{ab}	73.33 (58.97)^{ab}
T_7	8	+++	+++	+++	+++	90.00 (72.15)^{ab}	90.00 (72.15)^{a}	73.33 (58.97)^{abc}	70.00 (56.83)^{abc}
T_8	9	+++	+++	+++	+++	90.00 (72.15)^{ab}	86.67 (68.89)^{ab}	80.00 (63.56)^{a}	76.67 (61.20)^{a}
T_9	10	+++	++	++	++	95.00 (77.08)^{a}	90.00 (73.17)^{a}	73.33 (59.03)^{abc}	66.67 (55.03)^{bc}
S.Em±						2.08	1.76	1.37	1.31
LSD at 0.01						8.60	7.34	5.65	5.40
CV (%)						5.40	4.96	4.37	4.95

Table 3: Effect of exposure time of sodium hypochlorite (4%) on health and survival in green nodal explants of *Annona muricata*

Figures above paranthesis indicate the actual values and figures in paranthesis are arc sine transformed values for survival. Values with the same letter are satistically non-significant at LSD *($p \leq 0.01$)*.

Treatment		Health				Survival (%)			
Sodium hypochlorite (4%)	Time (min)	1st week	2nd week	3rd week	4th week	1st week	2nd week	3rd week	4th week
T_1	0	++	++	++	+	60.00 (51.22)^{b}	50.00 (45.03)^{e}	33.33 (34.95)^{f}	0.00 (0.28)^{f}
T_2	3	+++	++	++	+	70.00 (56.83)^{b}	63.33 (52.75)^{d}	50.00 (45.00)^{e}	43.33 (41.17)^{e}
T_3	4	+++	+++	++	+	70.00 (56.83)^{b}	66.67 (54.76)^{d}	63.33 (52.75)^{d}	60.00 (50.78)^{d}
T_4	5	+++	+++	++	++	75.00 (60.07)^{b}	73.33 (58.97)^{cd}	66.67 (54.76)^{cd}	63.33 (52.75)^{cd}
T_5	6	+++	+++	++	++	90.00 (72.15)^{a}	80.00 (63.56)^{bc}	70.00 (56.83)^{bcd}	70.00 (56.83)^{bc}
T_6	7	+++	+++	++	++	90.00 (72.15)^{a}	86.67 (68.89)^{ab}	76.67 (61.2)^{ab}	76.67 (61.20)^{ab}
T_7	8	+++	+++	++	++	90.00 (72.15)^{a}	90.00 (72.15)^{a}	73.33 (58.97)^{abc}	80.00 (63.56)^{a}
T_8	9	+++	++	+	+	90.00 (72.15)^{a}	86.67 (68.89)^{ab}	80.00 (63.56)^{a}	66.67 (54.76)^{cd}
T_9	10	++	++	+	+	95.00 (77.08)^{a}	90.00 (73.17)^{a}	73.33 (59.03)^{abc}	63.33 (52.93)^{cd}
S.Em±						1.46	2.13	1.78	1.37
LSD at 0.01						6.04	8.78	7.34	5.65
CV (%)						4.21	5.61	4.96	4.37

Table 4: Effect of exposure time of sodium hypochlorite (4%) on health and survival in leaf explants of *Annona muricata*

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Treatment		Health				Survival (%)			
Sodium hypochlorite (4%)	Time (min)	1st week	2nd week	3rd week	4th week	1st week	2nd week	3rd week	4th week
T_1	0	+++	++	++	+	60.00 (51.22)^{c}	56.67 (49.10)^{e}	46.67 (43.02)^{cd}	0.00 (0.28)^{f}
T_2	3	+++	++	++	+	70.00 (56.83)^{bc}	63.33 (52.75)^{de}	50.00 (45.00)^{cd}	0.00 (0.29)^{f}
T_3	4	+++	+++	++	++	70.00 (56.83)^{bc}	66.67 (54.76)^{de}	56.67 (48.84)^{bc}	3.33 (10.52)^{e}
T_4	5	+++	+++	++	++	80.00 (63.56)^{ab}	73.33 (58.97)^{cd}	56.67 (48.84)^{bc}	10.00 (18.43)^{d}
T_5	6	+++	+++	+++	++	90.00 (72.15)^{a}	80.00 (63.56)^{bc}	40.00 (39.23)^{d}	13.33 (21.41)^{cd}
T_6	7	+++	+++	+++	+++	90.00 (72.15)^{a}	86.67 (68.89)^{ab}	73.33 (58.97)^{a}	46.67 (43.09)^{a}
T_7	8	+++	+++	+++	++	90.00 (72.15)^{a}	90.00 (72.15)^{a}	70.00 (56.83)^{ab}	26.67 (31.08)^{b}
T_8	9	+++	+++	+++	++	90.00 (72.15)^{a}	86.67 (68.89)^{ab}	80.00 (63.56)^{a}	16.67 (23.88)^{c}
T_9	10	+++	++	++	+	90.00 (71.57)^{a}	83.33 (66.31)^{abc}	50.00 (45.00)^{cd}	0.00 (0.29)^{f}
S.Em±						2.11	1.75	1.98	0.85
LSD at 0.01						8.72	7.23	8.17	3.51
CV (%)						5.59	4.91	6.86	8.87

Table 5: Effect of exposure time of sodium hypochlorite (4%) on health and survival in brown nodal explants of *Annona muricata*

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Treatment		Health				Survival (%)			
Sodium hypochlorite (4%)	Time (min)	1st week	2nd week	3rd week	4th week	1st week	2nd week	3rd week	4th week
T_1	0	+++	++	++	+	60.00 (50.77)^{c}	50.00 (45.00)^{e}	33.33 (35.26)^{d}	0.00 (0.29)^{g}
T_2	3	+++	+++	++	++	70.00 (56.83)^{c}	56.67 (48.84)^{de}	36.67 (37.26)^{d}	26.67 (31.08)^{e}
T_3	4	+++	+++	++	++	70.00 (56.83)^{c}	60.00 (50.78)^{cd}	46.67 (43.09)^{c}	40.00 (39.23)^{d}
T_4	5	+++	+++	+++	+++	70.00 (56.83)^{c}	63.33 (52.75)^{cd}	56.67 (48.84)^{b}	40.00 (39.23)^{d}
T_5	6	+++	+++	+++	+++	80.00 (63.56)^{b}	73.33 (58.97)^{ab}	60.00 (50.78)^{ab}	46.67 (43.09)^{c}
T_6	7	+++	+++	+++	++	90.00 (72.15)^{a}	80.00 (63.56)^{a}	63.33 (52.75)^{ab}	50.00 (45.00)^{bc}
T_7	8	+++	+++	+++	++	80.00 (63.56)^{b}	76.67 (61.20)^{a}	66.67 (54.76)^{a}	53.33 (46.92)^{ab}
T_8	9	+++	+++	+++	++	70.00 (56.83)^{c}	66.67 (54.76)^{bc}	56.67 (48.84)^{b}	56.67 (48.84)^{a}
T_9	10	+++	+++	++	+	80.00 (64.12)^{b}	60.00 (50.98)^{cd}	46.67 (43.06)^{c}	20.00 (26.37)^{f}
S.Em±						2.13	1.46	1.28	1.22
LSD at 0.01						8.78	6.04	5.27	5.02
CV (%)						5.61	4.21	4.09	4.57

Table 6: Effect of exposure time of sodium hypochlorite (4%) on health and survival in internodal explants of *Annona muricata*

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Table 7: Effect of exposure time of sodium hypochlorite (4%) on initiation of response and days taken for shoot induction in green nodal explants of *Annona muricata*

Treatment		Initiation of response (%)				Days taken for shoot induction
Sodium hypochlorite (4%)	Time (min)	1st week	2nd week	3rd week	4th week	
T_1	0	0.00 (0.29)^{f}	0.00 (0.29)^{g}	0.00 (0.29)^{f}	0.00 (0.29)^{f}	0.00 (1.00)^{c}
T_2	3	6.67 (14.96)^{d}	10.00 (18.43)^{f}	26.67 (31.08)^{e}	60.00 (50.78)^{e}	22.67 (4.86)^{a}
T_3	4	10.00 (18.43)^{c}	13.33 (21.41)^{e}	56.67 (48.84)^{c}	73.33 (58.97)^{d}	21.00 (4.69)^{a}
T_4	5	13.33 (21.41)^{b}	16.67 (24.09)^{de}	60.00 (50.78)^{bc}	83.33 (66.09)^{cd}	18.67 (4.43)^{ab}
T_5	6	10.00 (18.43)^{c}	20.00 (26.56)^{cd}	63.33 (52.75)^{bc}	85.00 (67.21)^{bc}	18.00 (4.36)^{ab}
T_6	7	16.67 (24.09)^{a}	33.33 (35.26)^{a}	66.67 (54.76)^{b}	90.00 (71.57)^{abc}	14.33 (3.92)^{b}
T_7	8	16.67 (24.09)^{a}	26.67 (31.08)^{b}	73.33 (58.97)^{a}	93.00 (74.66)^{ab}	15.33 (4.04)^{b}
T_8	9	13.33 (21.41)^{b}	23.33 (28.88)^{bc}	66.67 (54.76)^{b}	93.33 (77.08)^{a}	15.67 (4.08)^{b}
T_9	10	3.33 (10.34)^{e}	13.33 (21.22)^{ef}	40.00 (39.16)^{d}	83.33 (66.04)^{cd}	19.00 (4.43)^{ab}
S.Em±		**0.41**	**0.67**	**1.01**	**1.81**	**0.13**
LSD at 0.01		**1.67**	**2.80**	**4.16**	**7.48**	**0.54**
CV (%)		**4.12**	**5.09**	**4.01**	**5.30**	**5.64**

Figures above paranthesis indicate the actual values and figures in paranthesis are arc sine transformed values for initiation of response and square root transformed values for days taken for shoot induction. Values with the same letter are satistically non-significant at LSD *(p ≤ 0.01)*

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Table 8: Effect of exposure time of sodium hypochlorite (4%) on initiation of response and days taken for shoot induction in brown nodal explantsof *Annona muricata*

Treatment		Initiation of response (%)				Days taken for shoot induction
Sodium hypochlorite (4%)	Time (min)	1st week	2nd week	3rd week	4th week	
T_1	0	0.00 (0.29)	0.00 $(0.29)^b$	0.00 $(0.29)^b$	0.00 $(0.29)^b$	0.00 $(1.00)^b$
T_2	3	0.00 (0.29)	0.00 $(0.29)^b$	0.00 $(0.29)^b$	0.00 $(0.29)^b$	0.00 $(1.00)^b$
T_3	4	0.00 (0.29)	0.00 $(0.29)^b$	0.00 $(0.29)^b$	0.00 $(0.29)^b$	0.00 $(1.00)^b$
T_4	5	0.00 (0.29)	0.00 $(0.29)^b$	0.00 $(0.29)^b$	0.00 $(0.29)^b$	0.00 $(1.00)^b$
T_5	6	0.00 (0.29)	0.00 $(0.29)^b$	0.00 $(0.29)^b$	0.00 $(0.29)^b$	0.00 $(1.00)^b$
T_6	7	0.00 (0.29)	16.67 $(24.09)^a$	33.33 $(35.26)^a$	66.66 $(54.74)^a$	24.50 $(5.05)^a$
T_7	8	0.00 (0.29)	0.00 $(0.29)^b$	0.00 $(0.29)^b$	0.00 $(0.29)^b$	0.00 $(1.00)^b$
T_8	9	0.00 (0.29)	0.00 $(0.29)^b$	0.00 $(0.29)^b$	0.00 $(0.29)^b$	0.00 $(1.00)^b$
T_9	10	0.00 (0.29)	0.00 $(0.29)^b$	0.00 $(0.28)^b$	0.00 $(0.29)^b$	0.00 $(1.00)^b$
S.Em±		0.00	0.06	0.10	0.21	0.01
LSD at 0.01		NS	0.26	0.42	0.85	0.05
CV (%)		0.00	3.73	4.19	5.63	1.47

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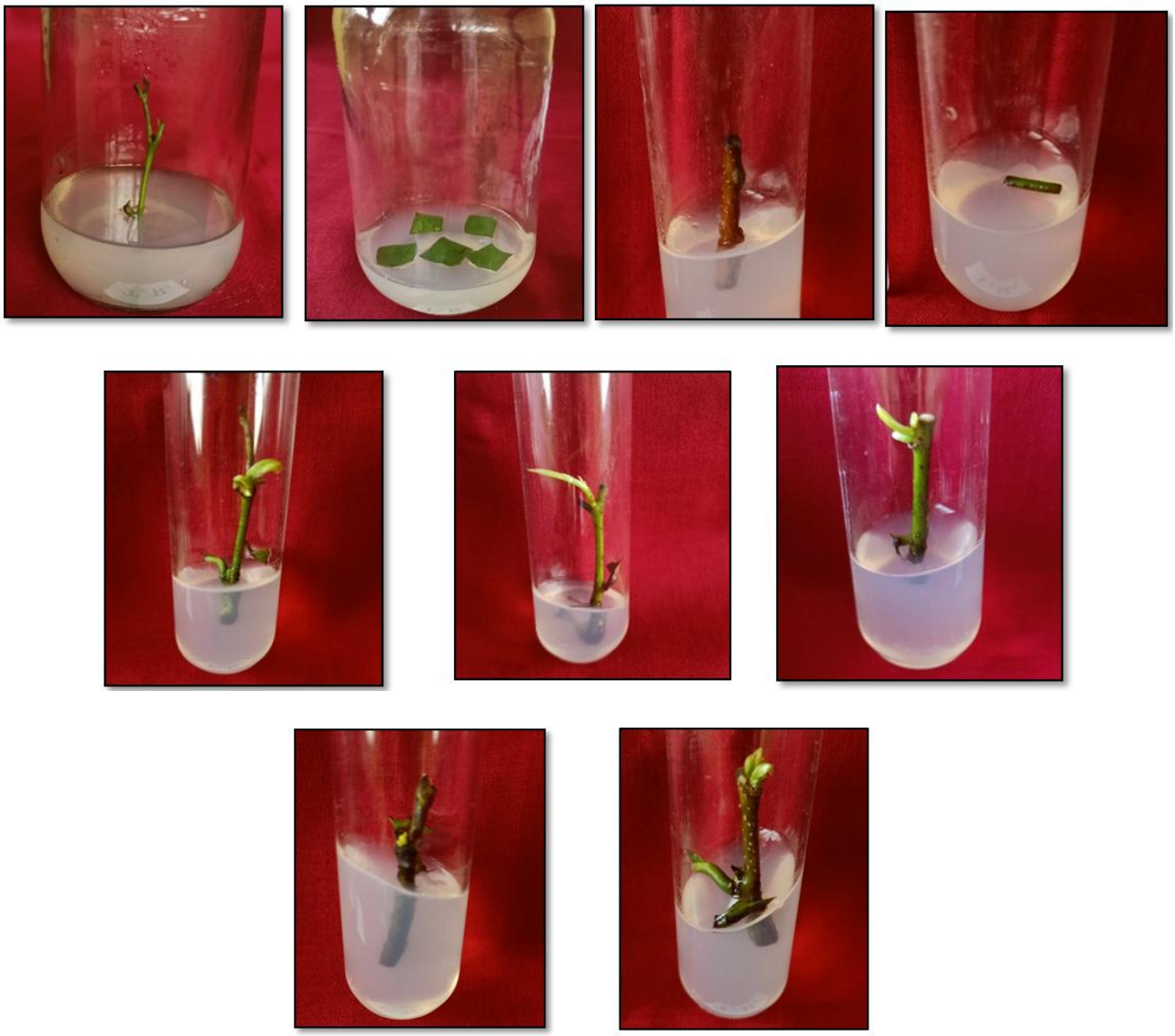

Plate 2: Different types of explants used for *in vitro* propagation of *Annona muricata* (a) green nodal, (b) leaves, (c) brown nodal, (d) internode, effect of exposure time of sodium hypochlorite (4%) on response of green and brown nodal explants; (e), (f) and (g)- initiation of response observed in T_8,T_7 and T_6(4% NaOCl for 9, 8 and 7 min respectively) in green nodal explants.(h) and (i) initiation of response observed in T_6(4% NaOCl for 7 min) in brown nodal explants after 30 days of initiation

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