Original Research Article

In vitro Sterilization of *Alstroemeria hybrida* cv. Pluto

ABSTRACT: *The present study was designed to standardize protocol for asepsis of different explants of Alstroemeriahybrida cv. Pluto (rhizome tips, rhizome sections, shoot tips and shoot nodal segments).Carbendazim 200 ppm for 30 minutes + mercuric chloride (0.1 %) dip for 10 minutes and final treatment with ethyl alcohol (70 %) for one minute proved best for culture asepsis of rhizome tips and rhizome sections at 2 and 4 weeks of culture., whereas Carbendazim 200 ppm for 15 minutes followed by mercuric chloride 0.1 % dip for 3 minutes proved better for culture asepsis of shoot tip and shoot nodal segments at 2and 4 weeks of culture. Also Carbendazim 200 ppm for 30 minutes + mercuric chloride (0.1 %) dip for 10 minutes and final treatment with ethyl alcohol (70 %) for one minute proved best for culture survival of rhizome tips and rhizome sections at 4 weeks of culture., whereas Carbendazim 200 ppm for 15 minutes followed by mercuric chloride 0.1 % dip for 3 minutes proved better for culture survival of shoot tip and shoot nodal segments at 4 weeks of culture. In this study protocol for sterilization of different explants leads to the standardization of the explant for tissue culture in Alstroemeriahybrida cv. Pluto.*

Key words: *Sterilization, rhizome tip, rhizome section, shoot tip and shoot nodal segment.*

1. INTRODUCTION:*Alstroemeria hybrida*is a rhizomatous monocot belonging to the family *Alstroemeriaceae*. *Alstroemeria* hybrids are counted among top ten cut flowers globally owing to diversity of colours, low energy cultivation and long vase life. *Alstroemeriahybrida* is a relatively recent introduction into the world's floriculture scene and has become a major cut flower. In India crop was introduced in 2001 by Ministry of Agriculture, GOI, under Food and Agriculture Organization programme at three Model Floriculture Centers Ooty (Tamil Nadu), Chial (Himachal Pradesh) and Srinagar (Jammu & Kashmir). The crop was introduced in SKUAST-Kashmir, Shalimar in 2005-06 under ICAR sponsored Horticulture Mini Mission-I. Legal planting material imported from foreign breeder companies is still very costly ranging from Rs. 500-600/plant. Cultivars introduced in late nineties in India are currently out of the patent regime. However, planting material is not easily available due to constraints imposed on multiplication rate under conventional propagation method of rhizome division. This has been the single major stumbling block that has prevented *Alstroemeriahybrida*from becoming widely adopted by cut flower growers in Kashmir and Himachal Pradesh. *Alstroemeriahybrida*is generally propagated by rhizome division of three year old plants. However, multiplication rate is low. Propagation through seed is not commonly practiced due to variability in hybrids and

long and difficult germination.Attempts have been made by few workers to multiply *Alstroemeria in vitro* through rhizome tips (Hussey *etal.*, 1979; Gabryszewska and Hempel, 1985; Lin and Monette, 1987; Chiari and Bridgen, 2000) but contamination was the major bottleneck to start cultures. *In vitro* multiplication of elite genotypes of Alstroemeria is widely used in commercial multiplication and offers immense opportunities to multiply more number of disease free plants in shortest possible time (Lin *et al*., 2000). *Alstroemeria*can be started with apical shoot tips but there is also a strong dominance effect suppressing the growth of axillary buds (Bond and Alderson, 1993). To promote cultivation of *Alstroemeria* there is a need to establish *in vitro* propagation protocols for the cultivars already available with research institutes and Government departments. This shall not only ensure availability of quality planting material for growers but also open up avenues for cultivar improvement through *in vitro* mutagenesis.Therefore current study involved standardization of protocol for sterilization of various explants of *Alstroemeria* cv.Pluto.

2. MATERIALS AND METHODS

The experiment was conducted in the Division of Floriculture and Landscape Architecture during the year 2012-2013 at SKUAST Kashmir. Rhizometips , rhizome sections,shoot tips and shoot nodal segments of an orange flowered cultivar Pluto were cut from freshly dugout plants and thoroughly washed with tween-20 followed by 200 or 400 ppm Carbendazim treatment for 30 minutes. Further treatment with mercuric chloride 0.1% for 5 or 10 minutes followed by 70% ethanol dip for 30seconds or one minute was done under laminar air flow. Explants were given final 3-4 washings with sterile water before placing on the medium. In all experiments 12 cultures per treatment were maintained each replicated four times. Data recorded was subject to statistical analysis using ANOVA.

2.1. Sterilization of culture media

Culture medium (MS solid and liquid) in test tubes/flasks was sterilized by autoclaving in a horizontal autoclave at 121°C and 1.05 kg cm^{-2} (15 psi) for 20 minutes. This high temperature not only kills bacteria and fungi, but also their heat-resistant spores. Flasks and test tubes were placed in autoclave when it had developed the required pressure. Lid of the autoclave was closed and vacuum valve opened to remove air from the autoclave chamber. Vacuum valve was closed and steam inlet valve opened. Pressure indicator lamp (green lamp) switches off when required pressure (15 psi) is developed inside the autoclave chamber. Time for autoclaving was counted from the switching off of the pressure indicator lamp of the autoclave. After autoclaving the test tubes and flasks for the required time, electric supply of the autoclave was switched off and

exhaust valve opened to release the pressure slowly. Autoclaved medium was allowed to cool overnight at room temperature and used when required but within 10 days.

2.1.1. Culture Environment

All the aseptic manipulations like surface sterilization, preparation and inoculation of explants and subsequent sub-culturing were carried out in the laboratory using laminar air flow cabinet where pure air is forced out continuously so that no contaminant is allowed to enter the cabinet during working time. Pure air is generated by forcing the air to pass through a series of fine filters which remove dust and other potential contaminants. The working table of laminar air flow chamber was first surface sterilized with absolute alcohol and then by ultraviolet light for 30 minutes. The forceps, scalpel and Petri-dishes were first steam sterilized in an autoclave at 121°C for 20 minutes. These instruments were further sterilized at the start of inoculation by pouring some alcohol over them in the laminar hood followed by flaming. During inoculation forceps and blade were flame sterilized several times. Hands were cleaned and wiped with Hi-Care disinfectant gel before working.

2.1.2. Culture incubation

The cultures were generally incubated at 24±1°C in an air conditioned culture room with a 16/8 hour light/dark regime and a light intensity of 3500 lux.

2.1.3. Collection of explants

Different types of explants that were tried during the course of present investigation were collected from plants maintained at green houses in Floriculture fields, Sher-e-Kashmir University of Agricultural Sciences and Technology of Kashmir, Shalimar. The details regarding collection of different explants are as follows.

2.1.4. Rhizome tips and rhizome sections

Healthy plants of the *Alstroemeria* cv. “Pluto” were dug out from the field with the help of spade and disease free rhizome tips and rhizome portions were placed in clean flasks containing distilled water and brought to the laboratory for further processing.

2.1.5. Shoot tips and shoot nodal segments

Disease free shoot tips and shoot nodal segments were separated from the mother plant and cut into smaller pieces which were then placed in clean flasks containing distilled water and brought to the laboratory for further processing.

3. Experimental parameters

A variety of experiments were conducted during the present investigation aimed at the development of a protocol for *in vitro* propagation of *Alstroemeria* cv."Pluto".Details regarding the methodologies adopted are given in the sections that follow.

3.1. Standardisation of disinfection protocol for various explants in *Alstroemeria*

Explant surface sterilization is the first vital step for the initiation of cultures *in vitro.* Final steps in surface sterilization were performed under the aseptic conditions of laminar flow hood. Knives and forceps were flame sterilized before use. While making incisions, knives and forceps were flame sterilized as and when thought necessary to avoid spread of contamination between different parts of the explant. Details of surface sterilization procedures for different explants are given in the following sections.

3.1.1. Rhizome tips and rhizome sections

Explants on arrival in the laboratory were washed with running tap water to remove any adhering dust and debris and cut into manageable size pieces (centimetres). Afterwards the explants were put in a Tween-20 surfactant solution (few drops) along with required quantity of fungicides and given a vigorous shake for 30 minutes to ensure thorough cleaning. There after the surfactant along with the fungicides was washed off under running tap water followed by a final washing with single distilled water. The specimens were later brought to laminar flow hood for treatment with mercuric chloride 0.1%, ethyl alcohol 70% and aseptic inoculation. The details of composition of different sterilant formulations and duration of application are given in Table 1.

3.1.2. Shoot tips and nodal segments

Shoot tips and nodal segments were procured from greenhouse grown plants. These are separated with a sharp scalpel. Explants on arrival in the laboratory were washed with running tap water and thereafter given a vigorous shake for 15 minutes in Tween- 20 surfactant solution fortified with appropriate fungicide and antibiotic concentration. The surfactant and fungicide/antibiotic solution was later removed by placing the explant under running tap water and a final 2-3 rinses with single distilled water. The details regarding the composition of various sterilant formulations and duration of application are given in the Table 2.

Table 1: Composition of sterilant formulations and duration of application for surface sterilization of rhizome tips and rhizome portions in *Alstroemeria* cv. Pluto

Details of the Treatment	
S_1	Mercuric chloride 0.1 % dip for 5 minutes
S_2	Mercuric chloride 0.1 % dip for 10 minutes
S_3	Ethyl alcohol dip (70%) for 30 seconds
S_4	Ethyl alcohol dip (70%) for 1 minute
S_5	Carbendazim 200 ppm for 30 minutes followed by S_1 andS_3
S_6	Carbendazim 200 ppm for 30 minutes followed by S_2 andS_4
S_7	Carbendazim 400 ppm for 30 minutes followed by S_1 andS_3
S_8	Carbendazim 400 ppm for 30 minutes followed by S_2 andS_4

Table 2. Composition of sterilant formulations and duration of application for surface sterilization of shoot tips and shoot nodal segments in *Alstroemeria*cv. Pluto

S_1	Mercuric chloride 0.1% for 3 minutes
S_2	Mercuric chloride 0.1% for 5 minutes
S_3	Carbendazim 200 ppm for 15 minutes + Mercuric chloride 0.1% for 3 minutes
S_4	Carbendazim 200 ppm for 15 minutes + Mercuric chloride 0.1% for 5 minutes
S_5	Carbendazim 400 ppm for 15 minutes + Mercuric chloride 0.1% for 3 minutes
S_6	Carbendazim 400 ppm for 15 minutes + Mercuric chloride 0.1% for 5 minutes

4. RESULTS AND DISCUSSION

Culture asepsis and survival of rhizome tips, rhizome sections, shoot tips and shoot nodal segments

In *Alstroemeria*, rhizome is the main explant an underground structure and carries a significant microbial load. Therefore, achieving culture asepsis and subsequent survival of explants is a significant challenge in *Alstroemeria* .In the current study, rhizome tips and rhizome sections were surface sterilized for 5.0 and 10 minutes in 0.1% mercuric chloride after a 30 minute surfactant treatment fortified with 200 and 400 ppm Carbendazim and final treatment with ethyl alcohol (70 %) for one minute. Significant differences among means

129 in terms of per cent asepsis and subsequent survival were observed for both explants and sterilant combinations
130 in rhizome tips and rhizome sections (Table 3 and 4). Highest culture asepsis was achieved in rhizome tips.
131 Maximum asepsis in rhizome tips may be attributed to its lesser surface area exposed as a result of single cut at
132 the distal end of the rhizome and hence less area for entry of potential infections. This is in comparison to
133 rhizome sections where two cuts are made at the proximal and distal ends and hence more exposed tissue.
134 Further rhizome tips are compact structures with relatively fewer subtending organs like roots and tubercles and
135 hence have less quantity of extraneous material like dirt, soil etc. adhering which may have contributed to higher
136 asepsis in comparison to rhizome sections.

137 Among the sterilant combinations, a 10 minute mercuric chloride (0.1 %) solution dip significantly
138 improved culture asepsis in comparison to 5 minute dip. Ethyl alcohol 70 % treatment alone for one minute
139 proved better (S_4) than a 30 seconds treatment with ethyl alcohol 70 %(S_3).However one minute ethyl alcohol
140 treatment at the end of sterilization procedure involving Carbendazim 200 ppm for 30 minutes followed by
141 mercuric chloride (0.1%) for 10 minutes under laminar flow hood significantly increased culture asepsis
142 (79.20%) (**Fig 1**). The results indicate passing explants through fungicidal treatment followed by mercuric
143 chloride and final treatment with ethyl alcohol is indispensable for culture asepsis in rhizome explants of
144 *Alstroemeria*. Concentrated ethanol is a powerful disinfectant that kills majority of fungal and bacterial spores
145 through instant dehydration. However increasing concentration of Carbendazim from 200 to 400 ppm did not
146 improve the final level of culture asepsis any further. The difference between the two sets of treatments
147 involving 5.0 and 10 minute mercuric chloride dip and 30 seconds and one minute ethyl alcohol (70 %)
148 treatment underlines the importance of minimally adequate time duration of final mercuric chloride (0.1 %) for
149 10 minutes and ethyl alcohol (70 %) for one minute surface sterilization procedure under the laminar flow
150 hood.

151 In the current study, shoot tips and shoot nodal segments were surface sterilized for 3 and 5 minute in
152 0.1% mercuric chloride after a 15 minute surfactant treatment fortified with 200 and 400 ppm Carbendazim.
153 Significant differences among means were observed for both explants. Highest culture asepsis at 2 and 4 weeks
154 of culture was achieved in shoot nodal segments rather than shoot tips (Table 5 and 6) with Carbendazim 200
155 ppm for 15 minutes followed by mercuric chloride (0.1%) for 3 minutes . Lower asepsis of shoot tips may be
156 due to bud morphology that allows accumulation of extraneous material among loosely placed leaves which are
157 also sticky. However, survival was relatively better in shoot tips than shoot nodal segments with Carbendazim
158 200 ppm for 15 minutes followed by mercuric chloride (0.1%) for 3 minutes (Table 8). There was less elusion of

159 exudates from shoot tips in comparison to shoot nodal segments. Less exudates in shoot tips may be due to more
160 proximal position in shoots as most of the exudates flow down and accumulate in the lower portion of flowering
161 shoots which translated into more exudate outflow *in vitro* in the latter and hence less survival. Data also point
162 to the importance of use of carbendazim at 200 and 400 ppm in improving asepsis and survival of the explants.
163 Shoot tips and shoot nodal segments are tender structures and hence use of ethyl alcohol is not advocated.
164 Seyyedyousefi*et al*. (2013) also advocated fragments of stem containing node and internode were washed
165 thoroughly under running tap water for 20 minutes and disinfected

166 Surface sterilization of rhizome tips and rhizome sections with carbendazim 200 ppm for 30
167 minutes followed by mercuric chloride (0.1 %) dip for 10 minutes and ethyl alcohol 70% for one minute
168 resulted in highest per cent aseptic cultures (68.08 per cent in case of rhizome tips and 61.63 per cent in case of
169 rhizome sections at 4 weeks of culture respectively), which is at par with asepsis attained by 5 minute mercuric
170 chloride 0.1 % treatment followed by a 30 second 70% ethyl alcohol dip (Table 3 and 4). Rhizome tips proved
171 to be better for starting *in vitro* cultures. Higher asepsis in rhizome tips may be attributed to its lesser surface
172 area exposed as a result of single cut at the distal end of the rhizome and hence less area for entry of potential
173 infections. At the end of four weeks of culture, 68.08 per cent rhizome tips survived under carbendazim 200
174 ppm for 30 minutes followed by mercuric chloride (0.1 %) dip for 10 minutes and ethyl alcohol 70 % for 1
175 minute (Table 7). Rhizome tips survived better than the rhizome sections which may be due to less injured area
176 in the former and hence lesser quantity of exudates. In the current experiment elusion of exudates from explants
177 was a major cause of physiological mortality *in vitro* **(Fig 2)**. Data reveal the importance of ethyl alcohol in the
178 eventual survival of the aseptic explants recorded at the end of 4 weeks of culture. Use of carbendazim followed
179 by ethyl alcohol dip significantly improved culture survival both in rhizome sections and rhizome tips.
180 Carbendazim decreased mortality due to fungal infections whereas ethyl alcohol may have contributed in
181 survival by not only through sterilization but also due to mitigation of harmful effects of mercuric chloride.
182 Ethyl alcohol being a dehydrant may have played a role in drawing out excess Hg^{2+} ions out of the explant
183 tissues along with the exudates which may have significantly contributed to higher survival in explants under S_6
184 and S_8sterilant treatment. Mohanty*et al*. (2005) advocated use of final wash in 1% hypertonic KCl solution to
185 draw out any traces of Hg^{2+} from the explant tissue. Carbendazim, mercuric chloride and ethyl alcohol have
186 been used by several workers to achieve the desired level of culture asepsis and survival *in vitro*. Hakkart and
187 Versluijs (1988) initially rinsed the rhizomes of *Alstroemeria* cv's Rosario, Toledo, and Jubilee with tap water
188 and later dipped for few seconds in 70% ethyl alcohol. Final sterilization was done in a laminar flow hood with

5 % Ca Hypochlorite (CaOCl) followed by rinsing 3 times with sterile water. In Alstroemeria ,Pierik*et al*. (1988) reported use of 70 % ethyl alcohol for 2-3 seconds followed by 20 minute dip in 1.5 % NaOCl (with few drops of Tween 20). , Jyothi*et al*. (2008) reported use of 1.0 % mercuric chloride for 8 minutes in ginger which is a plant with an underground rhizome similar in architecture to *Alstroemeria* .Sathyagowri and Seran (2011) also reported reasonable level of culture asepsis and survival with Carbendazim (0.3%) + Doxycycline (0.2%) for 10 minutes followed by 70% ethanol for 1 minute in ginger rhizome explants with buds. In the present study 10 minute dip in mercuric chloride (0.1 %) appears to be appropriate for a reasonable level of culture asepsis and to avoid mortality of explants.

Conclusion

Rhizome tips taken during the vegetative growth survived better than the other explants used during study and are considered suitable explant for starting the culture in *Alstroemeria*cv. Pluto. Maximumaseptic growing cultures were obtained with Carbendazim 200 ppm for 30 minutes followed by mercuric chloride (0.1%) treatment for 10 minutes and final treatment with ethyl alcohol (70%) dip for one minute. [A1]

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Table 3. Influence of sterilants on culture asepsis (%) of rhizome tip explant of *Alstroemeria* cv. Pluto

Sterilants		**Percent asepsis of rhizome tips at 2 weeks**	**Percent asepsis of rhizome tips at 4 weeks**
S_1	Mercuric chloride 0.1 % dip for 5 minutes	27.0875 (27.929)	20.84 (24.064)
S_2	Mercuric chloride 0.1 % dip for 10 minutes	41.6625 (40.165)	33.33 (35.181)
S_3	Ethyl alcohol (70%) dip for 30 seconds	6.2725 (8.244)	4.1875 (6.767)
S_4	Ethyl alcohol (70%) dip for 1 minute	37.4975 (37.616)	29.1625 (32.388)
S_5	Carbendazim 200 ppm for 30 minutes followed by S1 and S3	62.4975 (52.382)	54.1625 (47.432)
S_6	Carbendazim 200 ppm for 30 minutes followed by S2 and S4	93.7275 (79.207)	85.4125 (68.083)

S_7	Carbendazim 400 ppm for 30 minutes followed by S1 and S3	81.245 (64.938)	70.83 (57.608)
S_8	Carbendazim 400 ppm for 30 minutes followed by S2 and S4	91.6525 (75.297)	83.33 (66.255)
C.D $_{(P=0.05)}$		**15.66**	**12.62**

Data in parenthesis are arcsine transformed values of the percentage values

Table 4: Influence of sterilants on culture asepsis (%) of rhizome portion explant of *Alstroemeria* cv. Pluto

Sterilants		**Percent asepsis of rhizome portions at 2 weeks**	**Percent asepsis of rhizome portions at 4 weeks**
S_1	Mercuric chloride0.1 % dip for 5 minutes	16.68 (18.127)	12.515 (15.496)
S_2	Mercuric chloride 0.1 % dip for 10 minutes	27.0875 (27.929)	22.9225 (25.379)
S_3	Ethyl alcohol (70 %) dip for 30 seconds	6.2725 (8.244)	4.1875 (6.767)
S_4	Ethyl alcohol (70 %) dip for 1 minute	29.165 (32.631)	27.08 (31.154)
S_5	Carbendazim 200 ppm for 30 minutes followed by S1 and S3	62.4975 (52.382)	54.1625 (47.432)
S_6	Carbendazim 200 ppm for 30 minutes followed by S2 and S4	79.1625 (63.462)	77.08 (61.634)
S_7	Carbendazim 400 ppm for 30 minutes followed by S1 and S3	60.4125 (51.065)	52.08 (46.197)
S_8	Carbendazim 400 ppm for 30 minutes followed by S2 and S4	74.9975 (60.159)	72.9125 (58.842)
C.D $_{(P=0.05)}$		**17.00**	**14.86**

Data in parenthesis are arcsine transformed values of the percentage values

Table 5: Influence of sterilants on culture asepsis (%) of shoot tip explant of *Alstroemeria* cv. Pluto

Sterilants		Percent asepsis of shoot tips at 2 weeks	Percent asepsis of shoot tips at 4 weeks
S_1	Mercuric chloride dip for 3 minutes	74.9975 (60.159)	60.4125 (51.065)
S_2	Mercuric chloride dip for 5 minutes	62.495 (52.264)	52.08 (46.197)
S_3	Carbendazim 200 ppm for 15 minutes followed by S_1	83.33 (66.255)	74.9975 (60.159)
S_4	Carbendazim 200 ppm for 15 minutes followed by S2	79.1625 (63.462)	66.6625 (54.815)
S_5	Carbendazim 400 ppm for 15 minutes followed by S_1	74.9975 (60.159)	62.4975 (52.382)
S_6	Carbendazim 400 ppm for 15 minutes followed by S2	64.58 (53.581)	54.1625 (47.432)
C.D $_{(P=0.05)}$		**7.81**	**7.71**

Data in parenthesis are arcsine transformed values of the percentage values

Table 6 Influence of sterilants on culture asepsis (%) of shoot nodal segment explant of *Alstroemeria* cv. Pluto

Sterilant		Percent asepsis of shoot nodal segments after 2 weeks	Percent asepsis of shoot nodal segments after 4 weeks
S_1	Mercuric chloride dip for 3 minutes	79.1625 (63.462)	64.58 (53.581)
S_2	Mercuric chloride dip for 5 minutes	64.58 (53.581)	47.9125 (43.798)
S_3	Carbendazim 200 ppm for 15 minutes followed by S_1	87.485 (71.959)	79.1625 (63.462)
S_4	Carbendazim 200 ppm for 15 minutes followed by S_2	79.1625 (63.462)	70.83 (57.608)
S_5	Carbendazim 400 ppm for 15 minutes followed by S_1	74.9975 (60.159)	66.6625 (54.815)
S_6	Carbendazim 400 ppm for 15 minutes followed by S_2	64.58 (53.581)	47.9125 (43.798)
C.D $_{(P=0.05)}$		**11.32**	**8.45**

Data in parenthesis are arcsine transformed values of the percentage values

Table 7 : Influence of sterilant treatments on survival of rhizome tip and rhizome section explants of *Alstroemeria* cv. Pluto

Sterilants		Rhizome tip Survival *	Rhizome section Survival*
S_1	Mercuric chloride dip (0.1%) for 5 minutes	20.84 (24.06)	6.27 (8.24)
S_2	Mercuric chloride dip (0.1%) for 10 minutes	33.33 (35.18)	16.68 (18.12)
S_3	Ethyl alcohol (70%) dip for 30 seconds	4.18 (6.76)	4.18 (6.76)
S_4	Ethyl alcohol (70%) dip for 1 minute	29.16 (32.38)	24.99 (29.83)
S_5	Carbendazim 200 ppm for 30 minutes followed by S_1 and S_3	54.16 (47.43)	49.99 (44.99)
S_6	Carbendazim 200 ppm for 30 minutes followed by S_2 and S_4	85.41 (68.08)	74.99 (60.15)
S_7	Carbendazim 400 ppm for 30 minutes followed by S_1 and S_3	70.83 (57.60)	49.99 (44.99)
S_8	Carbendazim 400 ppm for 30 minutes followed by S_2 and S_4	83.33 (66.25)	68.74 (56.13)
$C.D_{(P\leq0.05)}$		**14.31**	**14.87**

Values in parenthesis are arcsine transformed data in per cent

*Data recorded at 4 weeks of culture

Table 8 : Influence of sterilants on survival percent of shoot tip and shoot nodal segments of *Alstroemeria* cv. pluto

Sterilants		Shoot tips survival (%)	Shoot nodal segments survival (%)
S_1	Mercuric chloride 0.1% dip for 3 minutes	41.22 (39.77)	42.56 (40.58)
S_2	Mercuric chloride 0.1% dip for 5 minutes	39.78 (32.11)	40.49 (35.87)
S_3	Carbendazim 200 ppm for 15 minutes followed by T_1	68.7475 (56.13)	62.4975 (52.38)

S_4	Carbendazim 200 ppm for 15 minutes followed by T2	50.23 (44.63)	54.1625 (47.43)
S_5	Carbendazim 400 ppm for 15 minutes followed by T_1	54.1625 (47.43)	58.33 (49.83)
S_6	Carbendazim 400 ppm for 15 minutes followed by T2	45.83 (42.56)	47.58 (39.37)
C.D		**9.33**	**9.06**

Data in parenthesis are arcsine transformed values of the percentage values

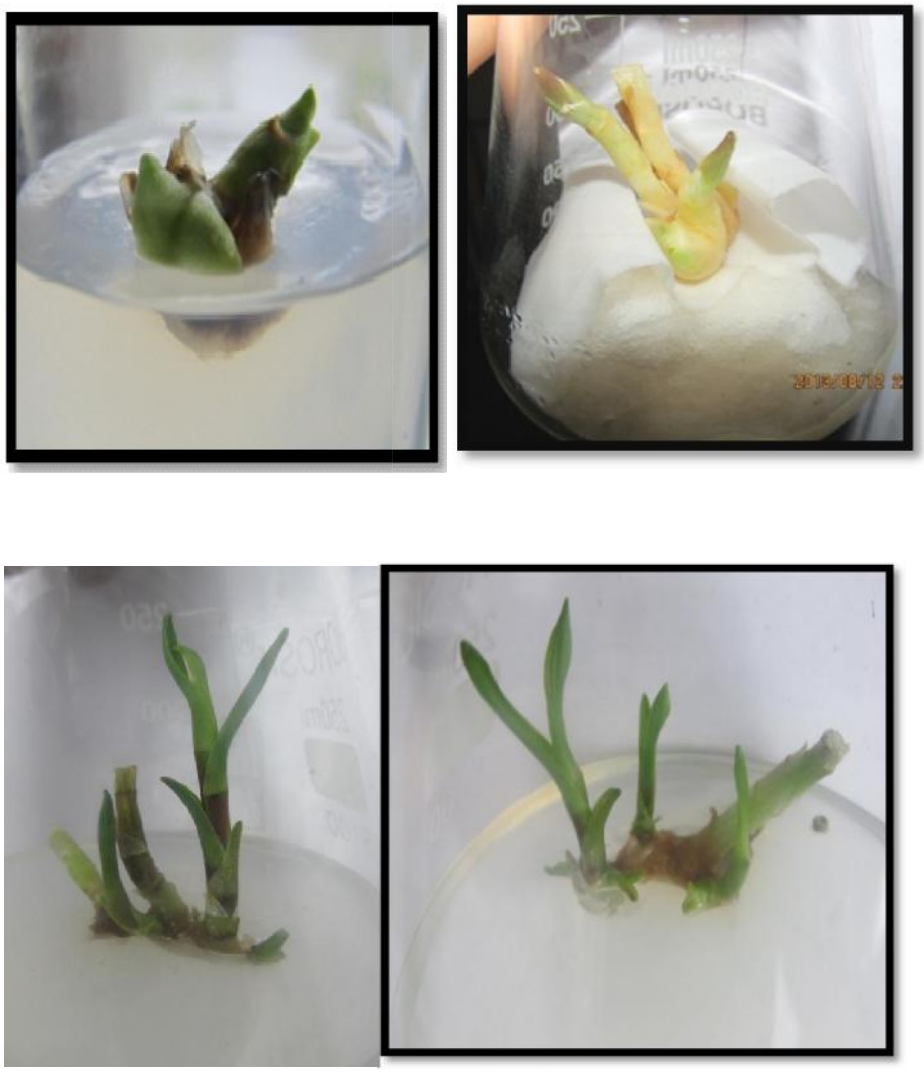

Fig 1) Aseptic cultures[A2]

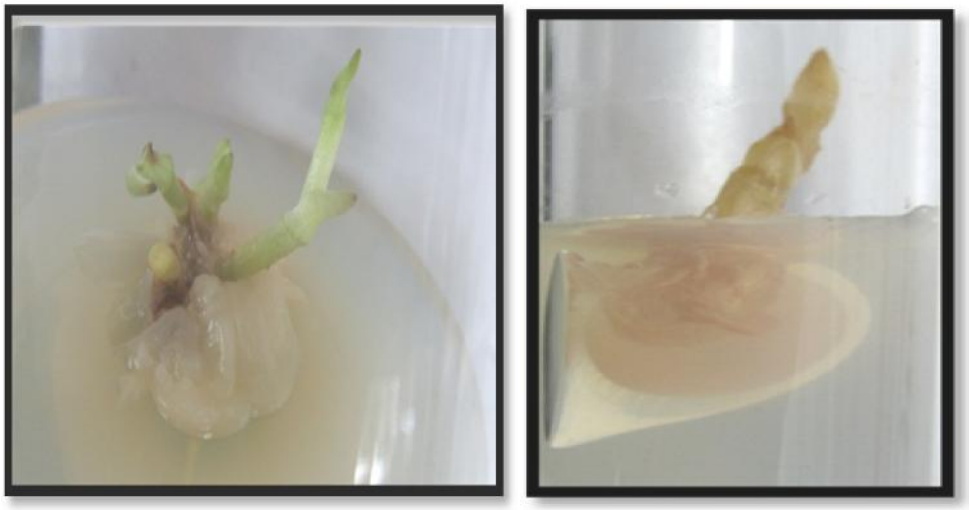

Fig 2) Exudate production from explants[A3]