In vitro and *in vivo* Propagation of Monotheca buxifolia (falc.): A comphrensive study

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Abstract

Monotheca buxifolia an economic and medicinal plant is restricted to limited areas due to deforestation, overgrazing, low regeneration, slow rate of germination, unsuccessful germination from cuttings and dormancy. Hence to cope with the issue of seed dormancy various *invitro* and *invivo* experiments were designed. The accidental breaking of seed through fungi (*Rhizophous stenolifer*) is also reported for the first time in current study. Different techniques used for breaking of dormancy are mechanical, chemical treatments i.e. (priming, scarification, stratification, ethanol, sulphuric acid, organic matter treatment, hot water) and tissue culture. Seeds were treated through various experiments in field, green house and laboratory. The scarified seeds placed on Murashige and skoog medium for proliferation and callus production was 60 % germination. Explants (Meristematic tissues) from field and tubes were inoculated on M. S (Murashige and skoog medium) + 2,4-D+ Kinetin. Meristematic tissues collected from field shows 10 % callus formation and meristematic tissues from tubes show significant result (70% callus formation). Our study concludes that the best suitable media for callus preparation of *Monotheca* is Murashige and skoog medium. Moreover, propagation of *Monotheca* via cutting is not possible. Development of callus explant from test tube was found to be more promising than field. Interestingly, *Rhizophous stenolifer* fungi can break dormancy of *Monotheca seeds* and found to promote growth significantly

Key words: *Monotheca buxifolia*, *in vivo* propagation, *in vivo* propagation, Fungi

INTRODUCTION

Monotheca buxifolia (Falc.) belongs to family Sapotaceae. A family of 35-75 well defined genera and 800 species, distributed in tropical regions of the world, represented in Pakistan by 6 genera and 7 species, (Nasir & Ali, 1972) only *Monotheca buxifolia* is native. *Monotheca* is a small tree or large thorny shrub. The two types of the plant are distinguished on fruit color. The first type has dark-colored fruit. The second type 'Himyamtah' bore yellowish-green fruit with red bluish. The roots are extensive and deep in the soil, enabling the plant to survive in arid mountains (Al-Yahyai & Al-Nabhani, 2006). Himyamtah occurs in dry Olea and Juniper forest and is evergreen shrub. This was first included in Myrsinaceae but Radlkoger kept it in family sapotaceae. The plant differs from other members of Sapotaceae by having no latex and larger stamens (Alfarhan, Al-Turki, & Basahy, 2005). The flowering period is from April to May. Fruiting stage is from June to August (Ali, Purohit, Mughal, Iqbal, & Srivastava, 1996). The extended fruits harvesting season was due to influence of elevation on fruit maturity and ripening.

The genus of *Monotheca* is distributed in North West Pakistan, Afghanistan, Oman, N. Somalia, S. Somalia and Ethopia (Ghazanfar & Fisher, 2013). This species is represented by either pure or mixed with *Olea ferrugenia*, *Acacia modesta*, *Punica granatum*, *Ficus palmata* and *Quercus baloot* which is rarely found in association with this species (Champion, Seth, &

Khattak, 1965). At some locations *Dalbergia sisso* is also sporadically associated with *Monotheca.* In spite other species, *Monotheca* is the most preferred species in hilly areas (Khan, Ahmed, Wahab, Ajaib, & Hussain, 2010). Loss of native species in Oman has been reported and attributed to many factors including invasive species, over grazing and human related activities (Al-Rawahy, Al-Dhafri, & Al-Bahlany, 2003). Global climate change may influence decline and lack of regeneration of junipers trees in the Mountains of Arabian peninsula (Fisher, 1997). The status of the species has not been assessed by International union for conservation of Nature (IUCN). The species was once available in Peshawar but now due to global climate change it became extinct. It is now restricted to hilly temperate zones of Pakistan. It has become endangered in Darra Adam Kheil (R. Ullah et al., 2010). The phytochemical studies revealed that leaves of *Monotheca* are chemically enriched with flavonoids, terpenoids (Rehman, Khan, Farid, Kamal, & Aslam, 2013) saponines, Anti-inflammatory (I. Ullah et al., 2016) anthroquinones, (Huma, Abdur , Ibrar, Barkatullah, & Ishfaq, 2010) cardiac glycosides, tannins and reducing sugars. Hence, it is concluded that aqueous fraction is rich in strong anti-oxidants (Rehman, et al., 2013) *Monotheca* is a mountain inhabitant, particularly in areas with rough terrain where conventional horticultural or agronomic cropping is limited (Al-Yahyai & Al-Nabhani, 2006). Its extract is used for tired eyes and stomachache (Zaman & Hazrat, 2013) as a fuel, fodder, timber (R. Ullah, et al., 2010) laxative, digestive and are used in urinary tract diseases (Marwat et al., 2011). Moreover, it's also used to Retain original taste of milk (ul Hassan, Murad, Tariq, & Ahmad, 2014).

The current study was aimed to perform *invivo* and *invitro* propagation of *Monotheca buxifolia*. The (Murashige and Skoog medium) M.S medium was used for micro propagation of the plant while different treatments (Control, Priming, Scarification, Stratification, Hot water treatment, Chemical treatment (Ethanol (C_2 H_6 o) & Sulphuric acid (H_2 So_4)), Fungi (for breaking dormancy) and Cuttings), were performed for dormancy breaking (main reason behind limited propagation of the plant. The accidental attack of Fungi (*Rhizopus stolonifer*) was successful attempt to break dormancy.

MATERIALS and METHODS

In vitro experiments

Experimental studies were conducted on *Monotheca buxifolia* of family Sapotaceae on various aspects to break its dormancy in Centre of Plant Biodiversity and Botanical Garden Azakheil, University of Peshawar, Institute of Biotechnology and Genetic Engineering (IBGE) and Plant Pathology Department, University of Agriculture Peshawar. Two experiments were conducted on dormancy breaking i.e. in field and green house. The protocol (Huma, et al., 2010) has been followed with slight modification.

Control

The parameters used in both experiments were Control (seeds were used soaked for 30 minutes in tap water in both experiments (Table. 1 & 2)),

Priming

Priming (the seeds were kept in water for 24 hours before germination in experiment no. 1 (Table. 1). While in experiment no. 2 seeds were kept in water for 24, 48 & 72 hours respectively before germination as shown in table 2.) ,

Scarification

Scarification (The outer coat of seed is slit or softens for germination). In scarification method the outer wall is ruptured either by sand paper or with other hard substance. The seed were rubbed with sand paper and put it in water for Thirty (30) minutes in experiment no. 1 (Table. 1) and seeds were rubbed with sand paper and put in water for 24, 48 and 72 hours respectively in experiment no. 2. (Table.2).

Stratification

Stratification (Treatment of seeds at low temperature). The seeds were treated at low temperature for 24 hours in experiment no.1. (Table.1). In 2nd experiment the seeds were kept for 30 and 60 days respectively at freezing temp and for 30 and 60 days respectively at 10^{o}C. (Table.2).

Hot water treatment

Hot water treatment (The seeds were kept for 70 ^{0}C for 30 minutes in 1st step and in 2nd step treated seeds were again putted in tap water for 30 minutes in experiment No. 1. (Table.1). The seeds were soaked in hot water of 50^{0}C for 30, 60 and 90 minutes respectively in experiment no. 2. (Table. 2)) ,

Chemical treatment

Chemical treatment (Various chemicals are also used to break seed dormancy; the dormancy is broken through various chemicals like ethanol and sulphuric acid used at various ratios. Sulphuric acid (H_2SO_4) of 100 % concentration were used for treatment seeds in 1st step and in 2nd step H_2SO_4 treated seeds were soaked in tap water for 30 minutes . Ethanol (C_2H_6O)100% concentration were used for treatment of seeds in 1st step and in 2nd step ethanol treated seeds were soaked in tap water for 30 minutes were used in experiment No 1. (Table.1). sulphuric acid (H_2SO_4) of 25%, 50% and 100% concentration were used for treatment seeds in 1st step respectively and in 2nd step H_2SO_4 treated seeds were soaked in tap water for 30 minutes. Ethanol (C_2H_6O) 25%, 50% and 100% concentration were used for treatment of seeds in 1st step and in 2nd step Ethanol treated seeds were soaked in tap water for 30 minutes were used in experiment No 2. (Table.2). Beaker was used in different stages for treatment of seeds.) (Ethanol ($C_2 H_6 O$) & sulphuric acid ($H_2 So_4$)),

Rhizophous stenolifer fungi and Cuttings of the plant

Fungi (Petri dish & Potato Distil Agar media) (Fig. 4 & 5) and cuttings (a part such as stem, leaf or root, removed from plant to propagate a new plant known as cutting. In presence experiment plant stem was treated with 1Mg/ml of IBA and NAA and were put in tubes in green house for results.

Evaluation of accidental attack of Fungi *Rhizophous stenolifer* on Seeds

The breaking of dormancy through fungi is a new experience as performed. The seeds of *Monotheca buxifolia* were put in petri dish although it was not expecting but fungi attack over seeds in petri dish and seeds accidentally rupture the wall and germinate. Seeds were inoculated on PDA (Potato dextrose agar media) media.

Before inoculation seeds were treated with water, $HgCl_2$ and fungicides, while some seeds were used untreated. By inoculation of seeds on 500 ml Potato Dextrose Agar media, again Fungi attacked over it, break its dormancy and germinate. Further studies were carried out in Plant Pathology lab. Agriculture University so it was ensured that a fungus is responsible for breaking of dormancy of seeds. The effected seeds were transferred to the field and they germinated there. The above results were found in Delgado-Sanchezhe experiment who reported that *Penicillium chrysogenum* and *Phoma* spp has the ability to break dormancy of *Opuntia streptacantha* seeds.(Abbaszaadeh, Ghobadian, Omidkhah, & Najafi, 2012)

***In vivo* experiments**

Plant material

The plant material was taken from new germinating seed in field in Centre of Plant Biodiversity University of Peshawar and second explant was taken from scarified seeds put over MS media which germinate with in less than 2 month time in Institute of Biotechnology and Genetic Engineering (IBGE). The buds explants were collected from District Karak. The buds present on the branches were collected through Spatula.

Culture methods:

Cleaning of Glass ware

All the Glass ware washed with the detergent (Surf), then washed with running tap water and after that rinsed with distilled water. The petri dishes, Forceps, Scapula were sterilized before use in dry sterilization oven at temperature of about 121 ^{0}C for 1 hour 15 minutes.

Culture media:

The basal media used was of Murashige and skoog's (MS 1962).

Agar

The medium was solidified by using Agar. For all cultures the pH of the medium was adjusted to 5.8 in different media with 0.1 NHCl or 0.1 N NaOH and flask of stirrer with magnetic balance was used to prevent it from solidifying before balancing of its pH. The agar was then added and put in micro wave oven to mix the media completely.

Media produced for seeds rooting and shooting, buds for rooting shoots explant from tube and field explant (Callus production) was 250 ml. vitamins used in the media for callus formation were 2,4-D and kinetin 1mg/500ml each and growth regulators for rooting was IBA 0.25ml. The below formula was adopted to find the various parameters of callus.

$$Average\ growth = \frac{length\ X\ Width}{Total\ Growth}$$

Cultural Conditions

In first experiment explant was taken from field. In was sub cultured 3 times. In second experiment simple MS Media having seeds observed that germinated and were taken as ex plants. The size of explants was 3-4mm. After heating and dissolving the agar, the hot media was then dispersed in test tubes and up to half of its length were filled. The test tubes neck was covered with aluminum foil and was autoclaved for 121 ^{0}C for 1 hour 15 minutes.

Aseptic manipulation of plant material

This is obtained in two steps.

Sterilization of plant material

The surface of seeds carries a wide range of contaminants. To eliminate these, the seeds were soaked in 0.2 % $HgCl_2$ (Mercuric chloride) for 2-3 minutes. They were than washed with 3-4 washing of sterilized distilled water. The seeds were then soaked in distilled water for 30 minutes before inoculating them onto the culture medium supplemented with appropriate growth hormones. Single seed was cultured in a one test tube. After its germination it was divided into explants of 3-4 mm and was put on media. While the field specimen was first divided into explants of 3-4 mm and was then sterilized for about 3-4 times soaked in 0.2 % $HgCl_2$ (Mercuric chloride) and then washed with Distilled water. One explant of 3-4 mm was cultured per test tube. Buds from mature plants collected from District karak were also culture with same methodology.

Sterilization of transfer area

The sterile environment is basic need and condition for successful tissue culturing. Prevention of entry of microbes in the culture test tubes, flasks and petri dishes along with other accessories i.e. Forceps and spoons during Inoculation are very important. To ensure all these aseptic conditions transfer operation was carried out in strict aseptic conditions and this was achieved by carrying all the inoculation in the laminar air flow cabinet containing high efficiency of particulate air filter with positive air pressure blowing outward from rear of the chamber to prevent any spares entering from entrance of the cabinet. The instruments were dipped in alcohol and flamed before every use to prevent damage to delicate tissue. The arms were thoroughly sprayed with alcohol and allowed to dry before lighting the lamp.

In order to prevent the entry of contaminants into the culture flask, the neck of the flask were flamed before and after inserting the material into it. The instruments should not be dipped in the alcohol immediately after heating as the alcohol being highly inflammable may catch fire.

Growth conditions

The culture was kept in shelves with 16 hours light cycle in every 24 hours. The temperature was regulated at 25±1 ^{0}C

Media for seeds germination

The same M.S media followed for treated seed germination. The growth of new seedlings was measured by mathematical formula i.e. (Gr) = [Nr/N×100].

RESULTS & DISCUSSION

Invitro:

Dormancy is a biological phenomenon that blocks a viable seed from germinating seed under favorable conditions. Physiological dormancy is also discussed in terms of the structures with in the seed that are responsible for the seed dormancy, such as embryo dormancy, testa dormancy, and pericarp dormancy(Finch-Savage & Leubner-Metzger, 2006) (Brunick, 2007).

Seeds of *Monotheca buxifolia* were subjected to various experimental treatments to overcome seeds dormancy. The pre sowing treatments involve in earlier germination and growth of seedling (Karaguzel, Cakmakci, Ortacesme, & Aydinoglu, 2004).

The first experiment was conducted in field and different treatments were applied before sowing. These treatments were Priming, Scarification, Sulphuric acid treatment, Stratification, Ethanol and Hot water treatment. Results show 17% germination only in control. The result was opposite to (Huma, et al., 2010) as they applied all these treatments to *Monotheca buxifolia* seeds and experiment conducted in incubator and obtained 88% success in (Hot water + sand paper scarification) treatment, (76 % , IAA+ Sand paper scarification) treatments, and (control 4%).

Second experiment was conducted in the green house and same treatments like Priming, Scarification, Sulphuric acid treatment, Stratification, Ethanol and Hot water treatment were applied before sowing (Fig.02).The results of second experiment was same to the first experiment, even a single seed was not germinate. The results were contrasted to Al- Yahyai and Al Nabhani who applied scarification, stratification, primed and untreated where the results were up to 90% in primed, 60% untreated and scarified up to 60%.

Stem cutting did not form root or shoots two months after planting with or without Indole-3-butyric acid (IBA) rooting hormones (Al-Yahyai & Al-Nabhani, 2006). The 1000 ppm NAA, IBA were to shoots and confirmed and the results were concluded to be true. The results shows that *Monotheca* cannot be propagate by cutting.

The dormancy breaking through priming is 90%, shows to be successful technique for germination. It is impossible to propagate Monotheca through cutting.

Invivo:

Although research work on tissue culture has been started (Street, 1973) Haberlandt and continue till present but(Gautheret, 1940) Gautheret started tissue culturing of woody plants in 1934. Various woody plants are propagated using tissue culture techniques but no such type of work is done on *Monotheca buxifolia.*

Media for callus production

Callus induction require the presence of Auxin or cytokinine or both in the nutrient media depending upon the source of explant (MS+2,4-D) rapid callus growth was observed (Sharma, 2007). Two types of ex plants were used for callus production (Meristematic tissue from field plant and meristematic tissues from test tube plants) in the present study. Basal MS media with addition of PGRs (2,4-D and Kinetine) were used for both Explant . Ten replicates having MS media and Explant (Meristematic tissue from field plant) were made. After 3 weeks callus formation was observed in only one replicate (Fig 8). Two time sub culturing of this callus shows no significant results. Third time sub culturing of this callus leads to increase size up to 1.5 cm lengths and 1 cm width. This conform the results of (Arora, Sharma, Srivastava, Ranade, & Sharma, 2010). Arora *et al* (Shrivastava & Rajani, 1999) also reported that addition of 2,4-D induce a thin layer of granular callus after 4 weeks of culture. Another Media (MS media+ IBA+ BAP) were used for root and shoot formation but no result were observed (Pl. 2, Fig 1).

This results was opposite to (Gupta et al., 2005), reported that MS media supplemented with BAP induced shoot formation in *Bacopa moneiri* L. Ten replicates having MS media and Explant (Meristematic tissue from test tube plant) were made. After three weeks callus formation was observed in 7 replicates and growth speed of these callus was fast as compare to callus of field ex plant. Average growth speed was 1.86 cm. (Table 7). Formula used:

Thomas *et al* (Thomas & Yoichiro, 2010) reported M.S media supplemented with 2,4-D and kinetin induced callus in root segment, leaf segment and hypocotyl**.**

Media for buds

Buds used as explant were inoculated in MS Media having IBA with concentration of 0.25 ml for rooting. But the Explants died. This results was opposite to results of (Snir & Harpaz, 2006) whose reported that M.S media supplemented with IBA induce propagation of lateral bud.

Media for seeds germination

The seeds were first rubbed with sand paper (scarified) and were then soaked in tap water for an hour then inoculated on the M.S with no growth hormones. It was observed that after 17 days, seedling appears on the media. Out of 10 only 6 plants germinated (Fig 1). So the average germination percentage was 60% table 9.

In 2nd experiment scarified and un scarified seed both were used. 50% germination occurred in scarified seeds while only 0% germination (Fig. 6). Both scarified and un scarified seeds were soaked in tap water for about an hour before inoculation. Percentage of un scarified give no results.

Although MS media supplemented with BA, IAA, 2,4-D was used for embryogenesis, organogenesis, rhizogenesis by (Rout, 1999) for seeds, but it was not found anywhere in the literature that Simple MS media was used for seed culturing**.**

It was concluded from Invivo propagation that the best media for callus formation of Monotheca plant is M.S media. We can propagate the embryogenesis and organogenesis of seed by M.S media which is relatively fast for germination than field.

After failure of both experiments in field we were supposed to check the viability of the seeds, some of which were placed in petri dishes at room temperature It was amazing to see that one seed which was contaminated by fungi shows emergence. Success was found in its growth whenever it was transferred to the field (Fig.2). It was concluded that it was the fungi that possibly release some chemical to break/decompose the hard seed coat of *Monothec,* and it started germination. Latter on it was identified as *Rhizophous stonilifer* (Table.5). By transferring to field only 10% germination was detected, and only contaminated seeds show germination. It was further modified by placing some nutrient media (PDA) instead of tissue paper and was used and repeats the experiment. To confirm this hypothesis another experiment was planned using PDA (Potato Dextrose Agar) media (Fig.4). Some seeds were treated before inoculation to media using Fungicides, $HgCl_2$ and water (Fig.5). These treated seeds show no results and germination failed, while some seeds were spared untreated. These untreated seed shows 66.6% germination (Table.6).

The results suggested that seeds of *Monotheca* seed dormancy could be effectively broken by fungi (*Rhizophus stonilifer*). The dormancy breaking of *Monotheca* seed through fungi was first report which has not been mentioned previously in the literature.

Conclusion

Dormancy is one of the reasons behind limited propagation of *Monotheca buxifolia.* The above result shows that most effective technique for the breaking dormancy was priming. The in vivo result shows that M.S media is best suitable media for Callus formation. Propagation of the plant through cutting is not possible. For fast propagation the embryogenesis and organogenesis of seed by M.S media field is most suitable. **The above study is also providing protocol for the callus formation of Monotheca plant.** The most interesting thing happen during the research is that the dormancy of the seed was break through fungi (*Rhizophous stenolifer*) previously not been mentioned in the literature. This was first report as per available in online literature that for effective dormancy breaking of *Monotheca buxifolia* fungi is helpful.

TABLES

Table1. Experiment conducted in field

Methods	Treatments
Stratification	Put in freezer for 24 hours
Ethanol	30 minutes treated with ethanol and 30 minutes soaked in tap water
Hot water	70 ^{0}c minutes treated with hot water and 30 minutes soaked in tap water
H_2so_4	30 minutes treated with H_2so_4 and 30 minutes soaked in tap water
Control	30 minutes soaked in tap water
Scarification	Sand paper and soaked in tap water for 30 minutes
Priming	Soaked in tap water for 24 hours

Table2. Experiment conducted in Green House

Methods	Treatment 1	Treatment 2	Treatment 3

Stratification	1 month 18 days in freezer	2 months 18 days in freezing temperature	2 months 18 days in cool temperature
Ethanol	30 mint in water 25 % ethanol	30 mint in water 50 % ethanol	30 mint in water 100 % ethanol
Hot water	50^0 c and 30 mint in water	50^0 c and 60 mint water	50^0 c and 120 mint in water
H_2so_4	30 mint in water 25 % H_2so_4	30 mint in water 50 % H_2So_4	30 mint in water 100% H_2So_4
Control	30 mint	30 mint	30 mint
Scarification	Sand paper 30 mint in water	Sand paper 60 mint in water	Sand paper 120 mint in water
Priming	24 hours in water	48 hours in water	72 hours in water

Table3. Effect of experiment conducted in field on germination

Methods	**Germination results**
Stratification	0%
Ethanol	0%
Hot water	0%
H_2so_4	0%
Control	17 %
Scarification	0%
Priming	0%

Methods	**Treatment 1**	**Treatment 2**	**Treatment 3**
Stratification	0 %	0 %	0 %

Ethanol	0 %	0 %	0 %
Hot water	0 %	0 %	0 %
H_2so_4	0 %	0 %	0 %
Control	0 %	0 %	0 %
Scarification	0 %	0 %	0 %
Priming	0 %	0 %	0 %

Table4. Effect of experiment conducted in Green House on germination.

Table. 5. Results of PDA Media

Treatments	**R1**	**R2**	**R3**
Fungicides	0	0	0
Hg Cl_2	1 green	1 bacteria	1 green
Water	1 greenish	0	0
Untreated	1 Rhizopus	1 Rhizopus	1 Black

Table 6. Effect of PDA Media on germination

Teatments	R1	**R2**	**R3**
Fungicides	0 %	0 %	0 %
Hg Cl_2	0 %	0 %	0 %
Water	0 %	0 %	0 %
Untreated	33.3 %	33.3 %	0 %

Fig 2. Attack & Germination of Rhizophus stonilifer on Monotheca buxifolia seeds in petri dish	
Seeds	*Monotheca buxifolia*
Pathogen	Rhizophus stolonifer
Observation time	5 Days
Nutritional status	Nill

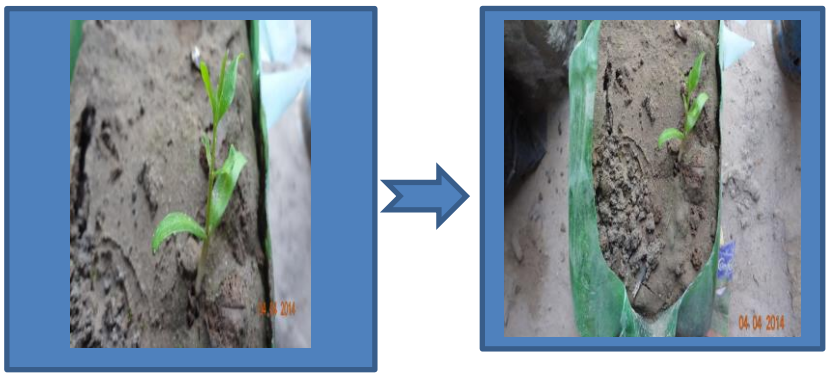

Fig. 03. Germination of Rhizophus effected seeds of Monotheca Buxifolia in Field	
Seeds	*Monotheca buxifolia*
Pathogen	Rhizophus stolonifer
Observation time	03 Month
Nutritional status	Field

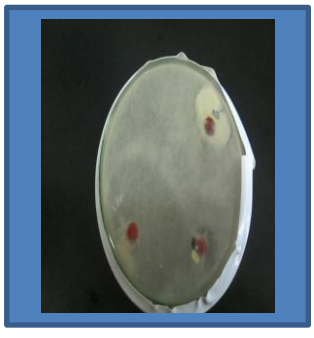

Fig. 05. $HgCl_2$, Untreated, Water and Fungicide plates used in lab. Experiment.	
Seeds	*Monotheca buxifolia*
Pathogen	Rhizophus stolonifer
Observation time	03 Month
Nutritional status	PDA meda

TABLES

Table 7. Effect of different Plant growth regulators on callus growth and proliferation.

Media	Explant	Types of regeneration			Culture period	Remarks
		GC	RG	SG		
MS MediA + 2,4-D+ Kinetine (ml.....)	Meristematic tissues from field	+	_	_	3 Weeks	Micro callus formed
M.S Media + 2,4-d+ Kinetine	Meristematic from tubes	++	_	_	3 Weeks	Maximum callus formed
MS Media + IBA	Bud	_	_	_	3 Weeks	Failed
MS Media	Seeds	_	++	++	2 months	Healthy shoot with hairy roots were regenerated
MS Media IBA+ BAP.	Meristematic tissues from field	_	_	_	3 Weeks	Failed

+	low	GC	Green Callus
+ +	good/ optimal	RG	Root Growth
-	Nill	SG	Shoot Growth

Table 8. The average growth observed in the callus production was

Test tube No.	Length in Cm	width in Cm L×W
1	1.2	0.6
2	1.1	0.7
3	1.6	0.6

4	1.5	1
5	1.5	1
6	1.5	1
7	2.5	1.6
8	2.6	1.5
		14.86

Now average growth can be fined as:

$$\frac{length\ X\ Width}{Total\ Growth} = \frac{14.86}{8} = 1.86$$

Table 9. Effect of MS media on seed germination

Treatment	**Media**	**%age of germination**
Scarification+ priming	Basal M.S	60%
Scarified	Basal M.S	30%
Un scarified	Basal M.S	0%

Fig 6. Effect of MS media. Seed germination.

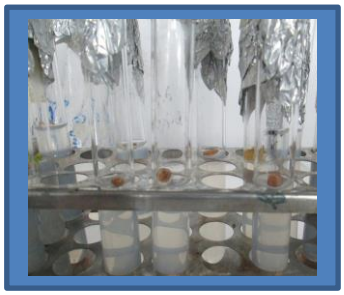

Fig 7. Effect of MS media on. rooting & shooting.

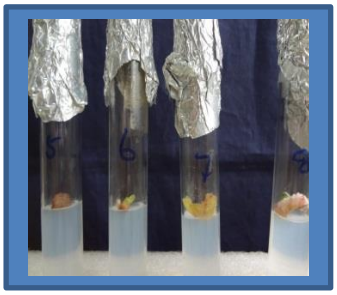

Fig8. Development of callus.

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