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Basic Information of the Project:

Name of the project	Micropropagation of Date Palm through Tissue Culture
Project period (from-to)	1st June 2010 to 31st Jan 2016
Total project duration	66 Months
Total Project cost	Rs. 24.061 Millions
Total Expenditures	Rs. 18.845074 Millions
Name of the Project Manager with designation	Dr. Muhammad Azhar Bashir Assistant Horticulturist
Phone and Email	062-9255432 azharbwp67@yahoo.com
Host Institute	Horticultural Research Station Bahawalpur
Name and Designation of the Team Leader with Name of the Collaborating institute	Dr. Aish Muhammad PSO, National Agricultural Research Centre Islamabad
Overseas cooperating scientist and organization	Nil

Executive Summary

Date is an important fruit of Pakistan and is cultivated over an area of 91500 hectares with annual production of 537800 tonnes. Due to its ability to tolerate adverse environmental conditions, the arid climate of Southern Punjab, upper Sindh and Balochistan is quite suitable for its cultivation. There are three techniques to propagate date palm: Seed propagation, offshoot propagation (traditional methods), and the recently developed tissue culture techniques. Plants propagated through seeds differ considerably with regard to production potential, fruit quality and harvesting time, making them very difficult to market as one harvest. Suckers are mainly produced in a limited number during the early life of the palm depending on the variety and on prior fertilization treatment, irrigation and earthing up around the trunks. Tissue culture has advantage over conventional propagation including; Propagation of healthy and uniform plants from selected female cultivars and ensure an easy and fast exchange of plant material between different regions of a country or between countries without any risk of the spread of diseases and pests. During present study in vitro cultures were established from field grown suckers of cvs. Halavi, Asil, Zahidi and Dhakki provided by Horticultural Research Station Bahawalpur and Date Palm Research Sub-Station, Jhang. Different media/hormonal combinations were exploited for culture initiation and multiplication. As the success of propagating monocotyledons woody plants through *in vitro* techniques has been limited, extensive research work was required to establish in vitro cultures. During project life 18000 in vitro shoots and 200 rooted plants were produced. When rooted plants were shifted on hardening, these could not survive which might be due to poor

rooting system or acclimatization environment. Slow growth, variable tissue behavior and systemic infections remained major issues during project execution. On the sidelines of tissue culture activities typing system for varietal identification based on single nuclear polymorphism (SNP) for date varieties used in this study was also developed which is integral part of micropropagation. Results were presented at national/international conferences and published in PARB reports and journals of international repute. The technology was visited by scientists, bureaucrats, policy makers and farmers. Keeping in view the demand of this technology Punjab and Sindh governments has initiated development projects for the establishment of tissue culture labs and/or import of tissue culture raised date plants and distribution among farmers.

Suckers of date varieties i.e. Aseel, Hallavi, Zahidi and Dhakki were provided by the host institute Horticultural Research Station, Bahawalpur to NARC, Islamabad for micropropagation.

Potting media ingredients (Field soil, silt, sewage sludge, leaf mold and organic manure combinations were made in different ratio and black plastic bags were filled with this media. Seedlings of Zahidi, Asil and Halavi varieties were raised at HRS, Bahawalpur for media standardization. These seedlings were transplanted under lath house for hardening assessment before arrival of tissue-cultured plantlets in soil media consisting of different combination of peat moss, leaf mold, sewage sludge and organic fertilizer to get rapid growth and better survival. A seminar/farmer's day for awareness of modern production technology of date palm among farmers community was held at HRS, Bahawalpur on 22.12.2015.

PROGRESS OF RESEARCH WORK

1. Introduction:

Date is an important fruit of Pakistan and is cultivated over an area of 84400 hectares with annual production of 426300 tonnes. Due to its ability to tolerate adverse environmental conditions, the arid climate of Southern Punjab, upper Sindh and Balochistan is quite suitable for its cultivation.

There are three techniques to propagate date palm: Seed propagation, offshoot propagation (traditional methods), and the recently developed tissue culture techniques.

Seed is convenient material for the propagation but this method cannot be used commercially for propagating the cultivars of interest in a true-to-type manner due to heterozygous characteristics of seedlings which are related to the dioeciously nature of the date palm. Seedlings differ considerably with regard to production potential, fruit quality and harvesting time, making them very difficult to market as one harvest.

Conventionally, Date Palm (*Phoenix dactylifera* L.) is propagated through offshoots which are produced from auxiliary buds situated on the base of the trunk during its juvenile phase of the palm. Offshoots are mainly produced in a limited number during the early life of the palm depending on the variety and on prior fertilization treatment, irrigation and earthing up around the trunks. Zahidi, Berim and Hayani varieties are known to produce large numbers of offshoots, while Mektoum and Barhee varieties produce relatively low numbers of offshoots.

Tissue culture is a recent technique mainly used for rapid propagation of several perennial fruit trees including date palm. The application of tissue culture techniques for date palm, also called *in vitro* propagation, has many advantages (in comparison to the above two techniques) and enables the following:

- Propagation of healthy selected female cultivars (disease and pest-free), Bayoud resistant cultivars, or males having superior pollen with useful metaxenia characteristics which can easily and rapidly be propagated.
- Large scale multiplication.
- No seasonal effect on plants because they can be multiplied under controlled conditions in the laboratory throughout the year.
- Production of genetically uniform plants.
- Clones to be propagated from elite cultivars already in existence, or from the F1 hybrids of previous selections, and seed-only originated palms.
- Ensure an easy and fast exchange of plant material between different regions of a country or between countries without any risk of the spread of diseases and pests.
- Economically reliable when large production is required.

Normally, date palm is propagated *in vitro* by two methods: the first method is by embryogenesis in which vegetative embryos can continuously be formed from embryogenic callus. The second procedure is organogenesis which produces date palm buds that eventually gives plantlets without passing through the callus stage.

The present project titled ‘micropropagation of date palm through tissue culture’ mainly focused on direct organogenesis for mass propagation of good quality date varieties through tissue culture to meet the ever increasing demand of the growers by micropropagation technique as rapid clonal production of selected cultivars which is cheaper than the offshoot method.

2. Project Objective:

Mass Multiplication of Date Palm through Tissue Culture.

3. Outputs planned for the project: (As per project document)

a) Host Institute: Horticultural Research Station Bahawalpur)

Output 1: Provision of explant source of 4 varieties of date palm to NARC

Output 2: Installation of lath house

Output 3: Hardening of date palm plants

Output 4: Field transplantation of tissue plants of targeted varieties

Output 5: Distribution of tissue plants to farmers

Output 6: Field evaluation of plants developed through tissue culture

Output 7: Dissemination of production technology of date palm plants developed through tissue culture

Output 8: Project's final report submission to PARB

4. Detailed component wise methodology adopted, data analyzed and results obtained (Host Instt. HRS Bahawalpur)

1. : Provision of explant source of 4 varieties of date palm to NARC

The major project activities were started in June 2010. . In first year explants (offshoots/suckers) of 4 varieties of interest (Aseel, Hallavi, Zahidi and Dhakki) from Horticultural Research Station, Bahawalpur as well as Date palm Research Sub-Station, Jhang were sent to Biotech Lab NARC, Islamabad which were employed to standardize the tissue culture protocols. Major focus during this time span was to standardize tissue culture protocol and to determine the best combinations of growth hormones for mass propagation of four varieties. In the first year of research problems like contamination and browning could not be resolved due to lack of man power for proper surveillance of growing cultures. In

the second year, the 1st year activities were rescheduled and standardized protocols were used for further experiments to achieve the targets by overcoming the 1st year problems.

Output 2: Installation of lath house

It was established at HRS, Bahawalpur

Output 3: Hardening of date palm plants

Fungicide treated 400 stones of Zahidi date cultivar were sown during September, 2011 in different combination of media ingredients. Soil medium containing 25% leaf mold, 25% sewage sludge and 50% organic fertilizer (T6) showed the maximum survival (88%), attained maximum seedling height (38 cm), and obtained maximum number of leaves (6) at the end of June, 2013. Established seedlings of Zahidi variety were uprooted and replanted in potting media consisting of same ingredients. The minimum mortality (27-33%) was recorded in media consisting leaf mold, sewage sludge and organic fertilizer (Table 1).

Established seedlings of Zahidi date cultivar were transplanted during 2013-14 till the availability of tissue cultured plants as approved in Project activities. The different media ingredients consisting of peat moss, leaf mold, sewage sludge and organic fertilizer were prepared by making different combinations by volume, the media were bagged, sealed and dumped for its decomposition for a period of two months before transplanting. The media were sterilized with solar radiations by sealing in white polythene covering for one month before transplanting. Each medium was analyzed for N, P & K, pH and E.C. etc. Then the media were filled in black plastic bags in equal quantity by volume. Soil analysis of media composition and data on number of days to initiate growth, seedling height and number of leaves per plant were recorded.

Highest survival (96%) with minimum days (59 days) to initiate growth, maximum plant height (26.3cm) and number of leaves/plant (6) were recorded from the plants grown in 100% peat moss (T2) (Table 2).

Tissue-cultured plants of date palm need appropriate soil media during hardening. Therefore, established seedlings of Asil date cultivar were transplanted during 2013-14 till the availability of tissue cultured plants to find out best potting media to get rapid growth and better survival of tissue-cultured plantlets or nursery of date palm. The treatments consisted of combinations of ingredients as T_1 (25% leaf mold +25% sewage sludge+50% organic fertilizer), T_2 (100% peat moss), T_3 (50% peat moss+25% leaf mold+25% sewage sludge), T_4 (50% peat moss+25% leaf mold +25% organic fertilizer), T_5 (50% peat moss+25% sewage sludge+25% organic fertilizer) and T_6 (25% peat moss+25% leaf mold +25% sewage sludge +25% organic fertilizer). Highest survival (92%) with minimum days (39 days) to initiate growth, maximum plant height (29 cm) and number of leaves/plant (5) were recorded from the plants grown in 100% peat moss (T_2). It was concluded from the study that seedlings of date palm grow and survive better in the medium containg 100% peat moss than that of other media as peat moss has highest NPK percentages and lowest pH and EC value that favour growth parameters. Maximum temperature range (15-26^{o}C) and maximum relative humidity range (72-84%) under lath house conditions also favour the growth parameters (Table 3).

Fig 1: PARB Team visiting potting media experiment (Horticultural Research Station, Bahawalpur)

Fig 2: A seminar / farmer day on 'Modern ProductionTechnology of Date palm' (Horticultural Research Station, Bahawalpur)

Table 1: Standardization of Potting Media for Date palm Nursery (Cv. Zahidi)

Treatments	**Field Soil %**	**Silt %**	**Leaf mold %**	**Sewage Sludge %**	**Organic Fertilizer %**	**Germin-ation %**	**No. of days to germinate**	**Survival %**	**Seedling Height (cm)**	**No. of leaves per plant**	**Mortality (%)**
T1 (control)	25	25	25	25	-	89.5	84	76	27.72	4.76	40.00
T2	-	25	25	50	-	98.8	80	74	28.95	4.90	76.66
T3	-	25	50	25	-	62.9	90	84	26.51	4.24	60.00
T4	-	50	25	25	-	58.4	80	74	28.53	4.54	63.33
T5	-	25	25	25	25	37.2	92	86	34.66	5.64	53.33
T6	-	-	25	25	50	36.8	94	88	37.93	6.10	33.33
T7	-	-	50	25	25	53.7	92	84	33.46	5.52	33.33
T8	-	-	25	50	25	66.6	92	84	32.39	5.26	26.66

Table 2: Standardization of Potting Media for Date palm Nursery (Cv. Zahidi)

Treatments	Pot Media Ingredients				Soil Analysis					Parameters		
	Peat moss %	Leaf mold %	Sewage Sludge %	Organic Fertilizer %	N%	P%	K%	pH	E.C. (dSm^{-1})	No. of days to initiate growth	Seedling Height (cm)	No. of Leaves per Plant
T1 (control)	-	25	25	50	1.11	0.009	0.44	7.5	0.36	95.6	35.8	4.0
T2	100	-	-	-	7.16	0.030	0.75	5.7	0.12	59.0	58.0	6.0
T3	50	25	25	-	2.71	0.011	0.47	6.2	0.24	66.2	51.6	5.8
T4	50	25	-	25	2.83	0.014	0.53	6.7	0.28	73.0	52.2	5.4
T5	50	-	25	25	2.30	0.013	0.44	7.1	0.25	82.2	50.6	5.2
T6	25	25	25	25	1.66	0.010	0.43	7.3	0.32	93.4	39.6	4.4

Table 3: Hardening of Plantlets of Asil Date Cultivar under Lath House in Various Potting Media

Treatments	Pot Media Ingredients				Parameters				Meteorological data				
	Peat moss %	Leaf mold %	Sewage Sludge %	Organic Fertilizer %	Survi-val %	Days to initiate growth	Plant Height (cm)	No. of Leaves per Plant	Month	Avg. Max. Temp. (^{o}C)	Avg. Min. Temp. (^{o}C)	Avg. Max. R.H. (%)	Avg. Min. R.H. (%)
T1 (control)	-	25	25	50	76	55.7	15.7	2.0	**Dec., 13**	16	8	87	68
T2	100	-	-	-	96	38.8	26.3	3.3	**Jan., 14**	18	10	85	63
T3	50	25	25	-	87	46.2	21.5	3.1	**Feb., 14**	20	12	81	65
T4	50	25	-	25	91	43.4	23.3	3.0	**Mar., 14**	23	15	80	60
T5	50	-	25	25	84	52.3	19.5	2.9	**April, 14**	33	21	79	57
T6	25	25	25	25	80	58.3	18.4	2.4	**May, 14**	40	23	77	55

3. **Outputs planned for the project:** (As per project document)

b) Collaborating Institute: NARC, Islamabad

Output 1: In vitro culture establishment

Output 2: Procurement of chemicals and equipment

Output 3: Recruitment of project staff

Output 4: Production of in vitro rooted plants

4. **Detailed component wise methodology and Results:**
   (Collaborating Institute: NARC, Islamabad)

Explants collection:

Field grown suckers and female flowers were used as explant source for the initiation of cultures. Suckers (100 for each variety) and flowers of varieties i.e., Halavi, Asil, Zahidi and Dhakki were provided by Horticultural Research Station Bahawalpur (Asil & Zahidi), Date Palm Research Sub-Station, Jhang (Halavi & Dhakki).

Explant Preparation and Sterilization:

For explant excision, outer leaves were excised with the help of a sharp knife, and shoot tips of 2x2cm were dissected from each sucker. Total number of plants used were 150 including shoot tips and auxiliary buds. Explants were dipped in antioxidant solution containing ascorbic acid (1g/L) and citric acid (1g/L) for thirty minutes. Explants were surface sterilized under aseptic environment (laminar flow cabinet) with 4g/l antifungal metalaxyl mancozeb (Ms. Jiangsu Baoling Chemical Co. Ltd China) for 30 minutes followed by washing with autoclaved distilled water. Explants were then dipped in 1.6% NaOCl

solution with 2-3 drops of Tween-20 for 20 minutes. Bleach solution was removed by rinsing explants in autoclaved distilled water. Tissues damaged by sodium hypochlorite were trimmed. Explants were treated with different concentrations of cefotaxime and streptomycin sulfate ranging from 500- 3000 mg/L.

Suckers of *Phoenix dactylifera* were collected, excised and surface sterilized for *in vitro* culture initiation. Explants were excised and submerged in a chilled solution of 1g/L ascorbic acid and citric acid for thirty minutes. We found that exposure of explants immediately after cutting to 1g/L of chilled ascorbic acid and citric acid each for half an hour was very effective for prevention against browning. This was in contrast to Zaid, 1987 and Khan, 2012 who reported that browning was due to phenolic compounds and addition of antioxidants in the medium will help in getting rid of harmful effect of phenols but was in accordance with Tao *et al.* (2007) who claimed that soaking of explants in vitamin C solution before culturing reduces browning. Explants were then treated with NaOCl (1.6%) used as bleaching agent for 20 minutes. Higher concentrations of NaOCl resulted in tissue death of explants. Hypochlorite present in NaOCl releases chlorine slowly and acts as bleaching agent. Free oxygen reacts with water and forms hypochlorous acid which produces nascent oxygen that is responsible for microbial destruction. Chlorine combines with proteins of the enzymes that result in the death of tissues (Dennis *et al.,* 1979).

Fig. 3: Chief executive PARB, parliamentarians, PARB members during monitoring of project activities

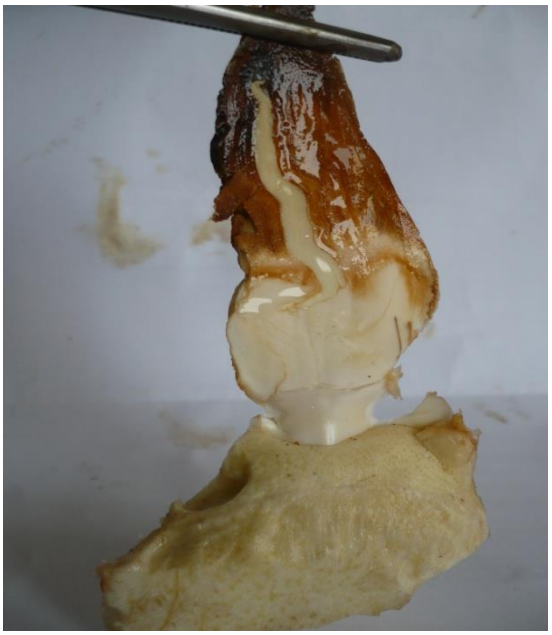

Fig. 4: Different steps for explant excision from suckers

Table 4: Survival of explants on different concentrations of Streptomycin sulfate and Cefotaxime used for control of bacterial contamination.

Tr	Cefo (mg/L)	Survival	Tr	Strep (mg/L)	Survival	Tr	Cefo: Strep (mg/L)	Survival
T1	500:0	36.67±4.01 GF	T5	0:500	53.33±4.01E	T9	500:500	93.33±4.01BC
T2	1000:0	53.33±4.01E	T6	0:1000	66.67±4.01D	T10	1000:1000	91.67±4.01C
T3	2000:0	43.33±4.01 EF	T7	0:2000	68.33±4.01D	T11	2000:2000	103.33±4.01B
T4	3000:0	96.67±4.01 BC	T8	0:3000	81.25±3.47C	T12	3000:3000	125.00±4.91A
		T0 control (Without antibiotics), 16.67±4.01 G						

Tr. Treatment Cefo. Cefotaxime Strep. Streptomycin

***In vitro* stock establishment:**

Cultures were initiated from meristem tip and auxiliary buds excised from the suckers. Different antibiotics/ fungicides were used in the medium to control systemic infections. MS medium containing different plant growth regulators were exploited to initiate the *in vitro* cultures. Phenolic secretions were controlled by the addition of antioxidants in the medium and frequent sub culturing. Explants were cultured on initiation medium comprising of basal MS medium supplemented with ascorbic acid, citric acid and activated charcoal and incubated at 27±2^{o}C. Survival efficiencies were recorded after 3 weeks. Cultures were incubated at photoperiod of 16/8 h after five weeks. To evaluate regeneration efficiencies of various explants experiments were performed thrice. Media were autoclaved at 121^{0}C, at 15 Psi for 15 minutes. pH of medium was adjusted at 5.7.

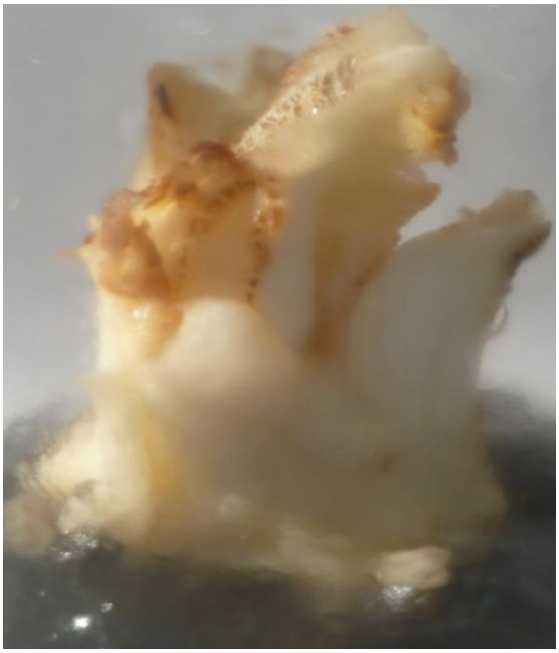

Fig. 5: Culture initiation, systemic infections, tissue browning and sudden death of explants

Within one week of culturing brown colored exudates are released into the medium from cut end that converts the color of whole medium into brown ultimately retards the growth of explants and leads to death.

Browning results from oxidation of phenolic compounds that are released from cut ends of explants by polyphenoloxidases and peroxidases as result quinones are produced, quinones inhibit enzyme activity of plants. Among various strategies

that results in neutralization of these toxic substances we selected the use of absorbing agents PVP and activated charcoal and antioxidants ascorbic acid and citric acid in medium.

Table 5 showing different media compositions B1-B5 which comprises basal MS with adenine sulfate. $2H_2O$ (50 mg/L), myoinisitol (100 mg/L), thiamine HCL (1mg/L), glutamine (0.2 mg/L) nicotinic acid (1mg/L). Morphogenic response of different explants against browning was investigated as shown in table 4 shoot tips of 2.2 mm were cultured, dome shaped shoot tips were without auxiliary tissues. Shoot tips of 5.5mm were cultured in which few auxiliary leaves remained attached with the shoot tips. Primordial leaves were also cultured. Table 3 showing that in B1 absorbing agent PVP was added in B2 activated charcoal, B3 citric acid and ascorbic acid and PVP, B4 citric acid, ascorbic acid and activated charcoal, B5 citric acid, ascorbic acid, activated charcoal and PVP. In medium B5 all the three types of explants showed maximum survival efficiency against browning and sudden death. Primordial leaves were less sensitive against harmful effects of bleaching agents as compared to shoot tips. Survival efficiency of primordial leaves was high even in presence of PVP or activated charcoal only. Shoot tips were more sensitive against harmful effects of phenolic compounds for maximum survival of shoot tips both antioxidants and absorbing agents are to be added. We stored explants at 4^oC in 1000mg/L ascorbic acid

and 1000mg/L citric acid for half an hour before culturing. With the addition of ascorbic acid, citric acid, PVP and activated charcoal in medium browning problem was resolved this was almost in according to Khan, 2012 who reported the same morphogenic response of different explants. The adsorptive capacities of AC have also been reported that thiamine HCl and nicotinic acid are removed from media by AC, whereas inositol and sucrose are not in our study increased amount of nicotinic acid and thiamine HCl was added to compensate this loss.

Shoot tips when trimmed and excised leaf primordia were present and attached with the shoot tips, when all the leaves were removed a dome shaped shoot tip appeared that was completely meristematic in nature. Both properly excised shoot tips and as well as shoot tips with few attached leaf primordia were cultured. Some leaf primordia were cultured separately too. It was observed that shoot tips were efficient in callus formation as well as for direct shoot regeneration. But regeneration time depends upon size of shoot tips. In shoot tips of 2mm.2mm auxiliary leaves surrounding the meristem gave more rapid response.

***In vitro* multiplication:**

After culture initiation, these cultures were shifted to multiplication medium for shoot multiplication. For shoot proliferation and elongation three different types of media with difference in concentrations of kinetin, Benzyl amino purine (BAP), Naphthalene Acetic Acid (NAA) and Indolebutyric Acid (IBA) were used (Table 6). After 3 weeks shoot number and length of the cultures were recorded. This step is the rate limiting step in plant production and focus was on achieving higher rate of multiplication. Different physical phases of medium (solid or liquid) were used. When liquid medium used, cultures were incubated on continuous shaking at 50 rpm. For shoot proliferation and elongation different concentrations of kinetin, BAP, NAA and IBA were used (Table 6). After 3 weeks it was

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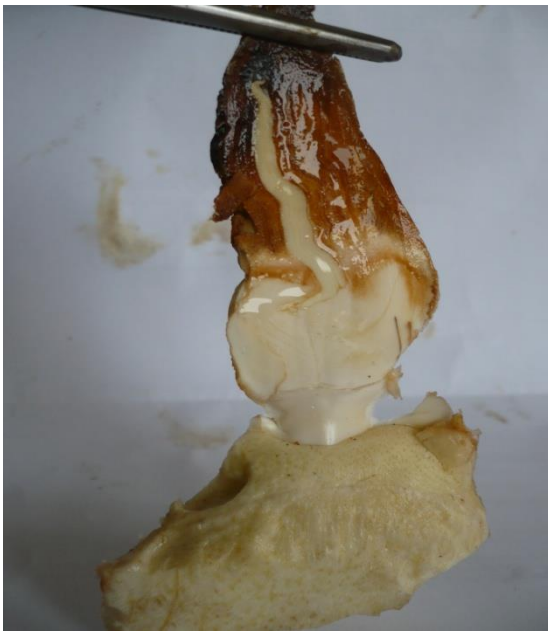

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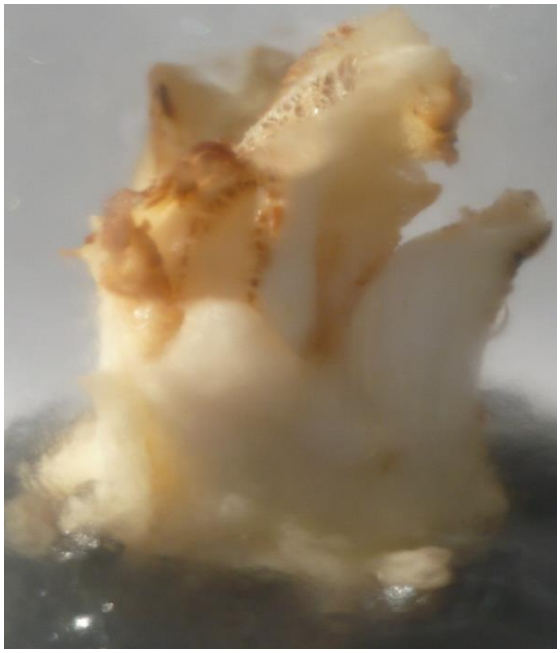

Fig. 5: Culture initiation, systemic infections, tissue browning and sudden death of explants

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Browning results from oxidation of phenolic compounds that are released from cut ends of explants by polyphenoloxidases and peroxidases as result quinones are produced, quinones inhibit enzyme activity of plants. Among various strategies

that results in neutralization of these toxic substances we selected the use of absorbing agents PVP and activated charcoal and antioxidants ascorbic acid and citric acid in medium.

Table 5 showing different media compositions B1-B5 which comprises basal MS with adenine sulfate. $2H_2O$ (50 mg/L), myoinisitol (100 mg/L), thiamine HCL (1mg/L), glutamine (0.2 mg/L) nicotinic acid (1mg/L). Morphogenic response of different explants against browning was investigated as shown in table 4 shoot tips of 2.2 mm were cultured, dome shaped shoot tips were without auxiliary tissues. Shoot tips of 5.5mm were cultured in which few auxiliary leaves remained attached with the shoot tips. Primordial leaves were also cultured. Table 3 showing that in B1 absorbing agent PVP was added in B2 activated charcoal, B3 citric acid and ascorbic acid and PVP, B4 citric acid, ascorbic acid and activated charcoal, B5 citric acid, ascorbic acid, activated charcoal and PVP. In medium B5 all the three types of explants showed maximum survival efficiency against browning and sudden death. Primordial leaves were less sensitive against harmful effects of bleaching agents as compared to shoot tips. Survival efficiency of primordial leaves was high even in presence of PVP or activated charcoal only. Shoot tips were more sensitive against harmful effects of phenolic compounds for maximum survival of shoot tips both antioxidants and absorbing agents are to be added. We stored explants at 4^oC in 1000mg/L ascorbic acid

and 1000mg/L citric acid for half an hour before culturing. With the addition of ascorbic acid, citric acid, PVP and activated charcoal in medium browning problem was resolved this was almost in according to Khan, 2012 who reported the same morphogenic response of different explants. The adsorptive capacities of AC have also been reported that thiamine HCl and nicotinic acid are removed from media by AC, whereas inositol and sucrose are not in our study increased amount of nicotinic acid and thiamine HCl was added to compensate this loss.

Shoot tips when trimmed and excised leaf primordia were present and attached with the shoot tips, when all the leaves were removed a dome shaped shoot tip appeared that was completely meristematic in nature. Both properly excised shoot tips and as well as shoot tips with few attached leaf primordia were cultured. Some leaf primordia were cultured separately too. It was observed that shoot tips were efficient in callus formation as well as for direct shoot regeneration. But regeneration time depends upon size of shoot tips. In shoot tips of 2mm.2mm auxiliary leaves surrounding the meristem gave more rapid response.

***In vitro* multiplication:**

After culture initiation, these cultures were shifted to multiplication medium for shoot multiplication. For shoot proliferation and elongation three different types of media with difference in concentrations of kinetin, Benzyl amino purine (BAP), Naphthalene Acetic Acid (NAA) and Indolebutyric Acid (IBA) were used (Table 6). After 3 weeks shoot number and length of the cultures were recorded. This step is the rate limiting step in plant production and focus was on achieving higher rate of multiplication. Different physical phases of medium (solid or liquid) were used. When liquid medium used, cultures were incubated on continuous shaking at 50 rpm. For shoot proliferation and elongation different concentrations of kinetin, BAP, NAA and IBA were used (Table 6). After 3 weeks it was