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REGENERATION & CLONAL PROPAGATION OF PISTACIA VERA BY TISSUE CULTURE TECHNIQUE

PROJECT REPORT

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A

REGENERATION THROUGH TISSUE CULTURE

Studies to determine the *in vitro* requirements of cotyledonary explants to enhance callus proliferation remained in progress. Research conducted on tobacco callus cultures suggests that the osmolarity of the medium plays an important role in the induction of morphogenesis. Osmolarity of the medium effects either by itself or through induced changes in the cells of explants. Therefore it was desired to study the effects of various concentrations and combinations of different sugars (sucrose, glucose, fructose and galactose) on growth of callus from cotyledonary explants of *Pistacia vera* culture *in vitro*. Mannitol was added with sucrose to provide a constant molar equivalent of 30g/l sucrose.

Experiment was as follows:.

After removing the shells, seeds were divided into many groups each group consisted of four seeds having weights varying from ± 0.0100 gms to 0.0500 gms only. Average weight of any particular group represented the mean intial fresh weight of the group. In the same way average of the final fresh weights of the explants represented the mean final fresh weight and the difference of the two gave the gain in the weight of explant which is considered due to the the callus formation and one can consider it as weight of callus produced. For each experiment one group of seeds was cultured on the medium containing a Particular sugar concentration.

MS medium was employed throughout the study and all media were supplied with 2.0 mg/l of Kin. in combination with 0.1, 1.0 or 2.0 mg/l of 2,4-D. Two types of experiments were conducted. In one case sugars were used individually at various concentrations and in second case combinations of different sugars was added to the culture medium. Galactose (20 and 30 gm/l)or Glucose (20 and 30 gm/l) or sucrose was employed at a rate of 0, 5, 10, 20 and 30 gm/l in the first case. Where as, in the second type of experiment an alcohol sugar Mannitol (15.3, 13.3, 10.6, 5.3, 0 gm/l) was added to the medium in combination with various amounts of sucrose (0, 5, 10, 20, 30 gm/l) to keep the molar equivalent of the medium in approximately with the sucrose content of the medium i.e. 30 gm/l. Another experiment was done where a combination of glucose and fructose was supplied in the medium. In this case 2.5, 5, 10 and 15 gm/l of glucose was added to the medium in combination with the same quantities of fructose. All explants were grown on the paper bridges in test tubes, which were maintained at 26 ± 1°C under a photoperiod of 16h light provided by cool white flourescent lights. After 4 weeks in-cubation the fresh weight of the explants for each one of the media tested was measured which provided the mean final fresh weight.

RESULTS

Effect of Sucrose Concentration:

The presence of sucrose was not necessary to achieve cell proliferation (Fig.1) as callus formation was observed in sugar free

media also. Effect of sucrose concentration was dependent on the relative auxin/cytokinin ratio in the neutrient media. Maximum growth of callus was achieved in the medium containing a balanced ration of growth regulator. Growth of callus was also dependent on the amount of sucrose present in the media, as the rate of growth increased with the increase in the amount of sucrose present in the medium. Least growth was recorded in explants cultured on media containing 5% sucrose. Termination of growth and beginning of browning of explants was noticed earlier in sucrose less cultures.

These results indicated that presence of sucrose was not necessary for the induction of callus initiation as pistachio cotyledons has got enought sugars stored in it, which not only provide energy for growth but also adds to the movement of growth regulators from the medium to the tissue explant along with water. The osmolarity of the medium due to the sucrose contents of the medium also influenced the availability of the growth regulators. Results also indicate that use of sucrose by the cultured cells also depend on the concentration of 2, 4-D in the medium. Low level of 2, 4-D (0.1 mg/l) in the medium prevented the cultures from utilization of sucrose provided to it while higher concentration of 2, 4-D (2.0 mg/l) increased the availability of sucrose yielding a better culture growth.

Effect of Mannitol Supplements:

It was gathered from previous experiment carried out with various concentrations of sucrose provided different water potentials

in the medium which ultimately influenced availability of growth regulators to the cells. To test this possibility, cotyledonary explants were also cultured on media containing varying levels of sucrose plus mannitol. Mannitol was added to give a 30 gm/l molar equivalent of sucrose at all levels of sucrose (30, 20, 10, 5 and 0 gm/l).

Mannitol was unable to replace sucrose requirement for growth. (Fig 2). Since decreasing the concentration of sucrose significantly reduced calli fresh weight. But the amount of auxin available affected the growth as in the case of sucrose alone. As cotyledonary explants did not seem to use mannitol as a source of carbon and energy, therefore, from results it was concluded that the osmolerity of the medium had an important role in the availability of growth regulators to control growth and differentiation.

Comparative effect of Glucose, Galactose and Sucrose:

The effect of other sources of carbon (glucose and galactose) on induction and growth of calli was also determined. Effect of these sugars was compared with that of sucrose. The results are shown in Fig. 3. Pistachio cotyledonary explants used these sugars and formed calli. Quantity of calli formed due to either of the sugars were markedly different. Glucose was the most effective sugar. But callus showed signs of browning earlier as compared with those calli formed on media supplied with sucrose. Galactose was the least effective sugar in terms of callus initiation and its growth.

Effect of Glucose plus fructose

Sucrose was found to be the most effective sugar for callus growth. Explant tissue also responded to glucose actively. But browning onset was much earlier in the calli cultured on glucose supplemented media as compared to those grown as sucrose supplied ones. The importance of sucrose becomes clear that it is converted in glucose and fructose in first instance and then consumed by the plant cells. Fructose being an isomer of glucose either competes with the glucose transport or its isomerization maintains a balance between intake and utilization. Therefore glucose and fructose were added to the media at a ratio of 1 : 1 i.e. 2.5 + 2.5 gm/l of each for 5 gm/l sucrose, 5 + 5, 10 + 10 and 15 + 15 gm/l of glucose and fructose for 10, 20, 30 gm/l of sucrose. Results are shown in Fig. 4. Explants can grow well in the presence of glucose and fructose but growth was a little lesser than obtained with equivalent amounts of sucrose (Fig. 1). Role of increasing amount of auxin is evident in this case also.

Correlation between osmolarity of and effectiveness of growth regulators

Nature and permeability of sugars contributed in access of growth regulators into the tissue. In Fig 1 - 4 three sets of growth regulators were supplemented with sucrose/galactose and glucose and Fructose. Each contained Kin. 2 mg/l combined with 0.1/1.0/2.0 mg/l of 2, 4-D. Fig 2C & 3C are comparable to each

other as callus formation was slightly more than 30 gm/l glucose than with the same amount of sucrose. Although GRs were same (2, 4-D 2mg/l and Kin 2.0 mg/l) but browning proceeded actively with glucose supplemented media. Callus formation was quite prominant and healthy in a batch supplied with a combination of sucrose and mannitol results are shown in Fig. 2. Presence of mannitol most probably enhanced the inward movement of GRs Fig 2A, 2B and 2C show a markedly improved distribution of GRs with the cells resulting in a balance between sugar consumption and effectiveness of GRs.

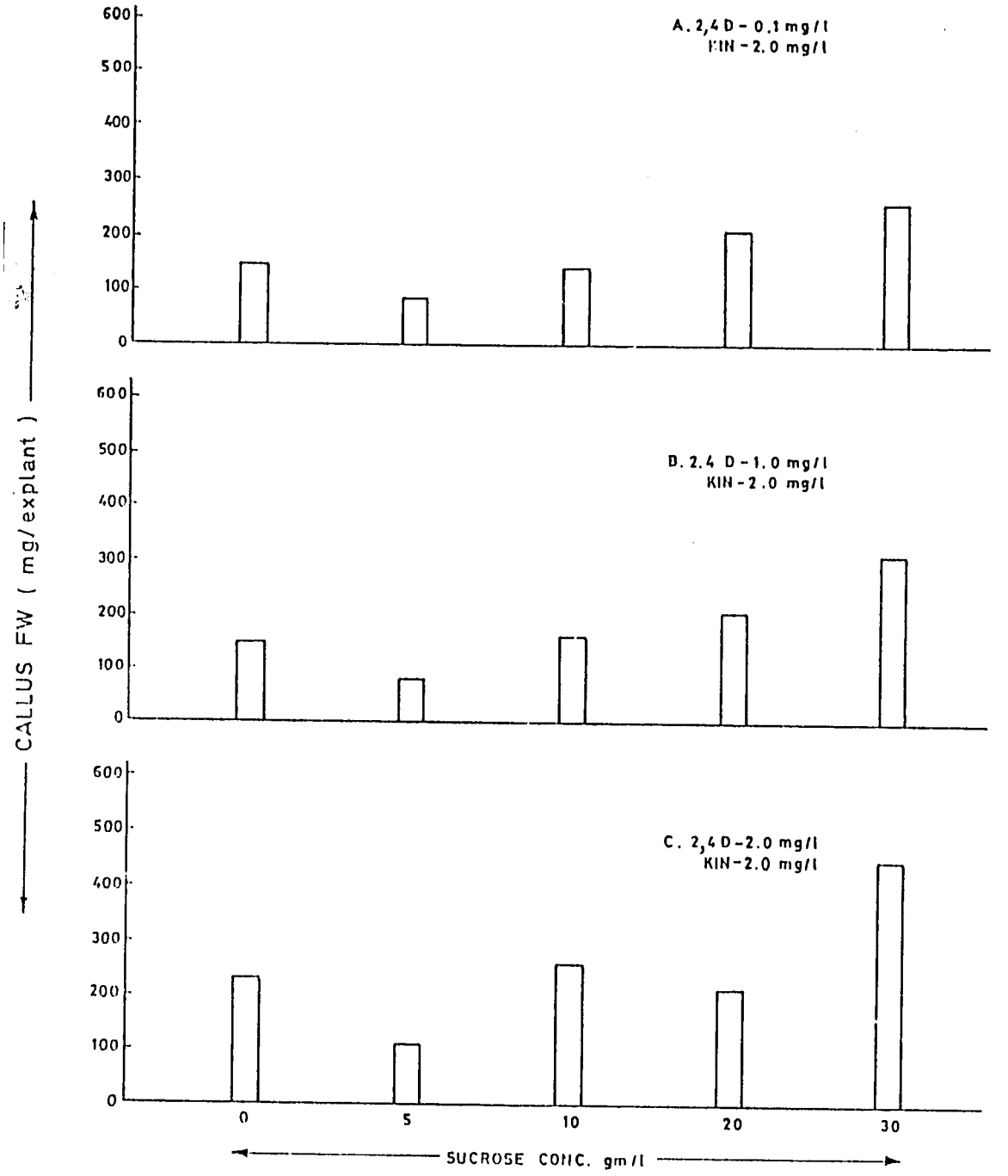


FIG. 1. EFFECT OF SUCROSE CONCENTRATION ON THE GROWTH OF COTYLEDONARY EXPLANTS OF PISTACIA VERA L. CULTURED IN VITRO.

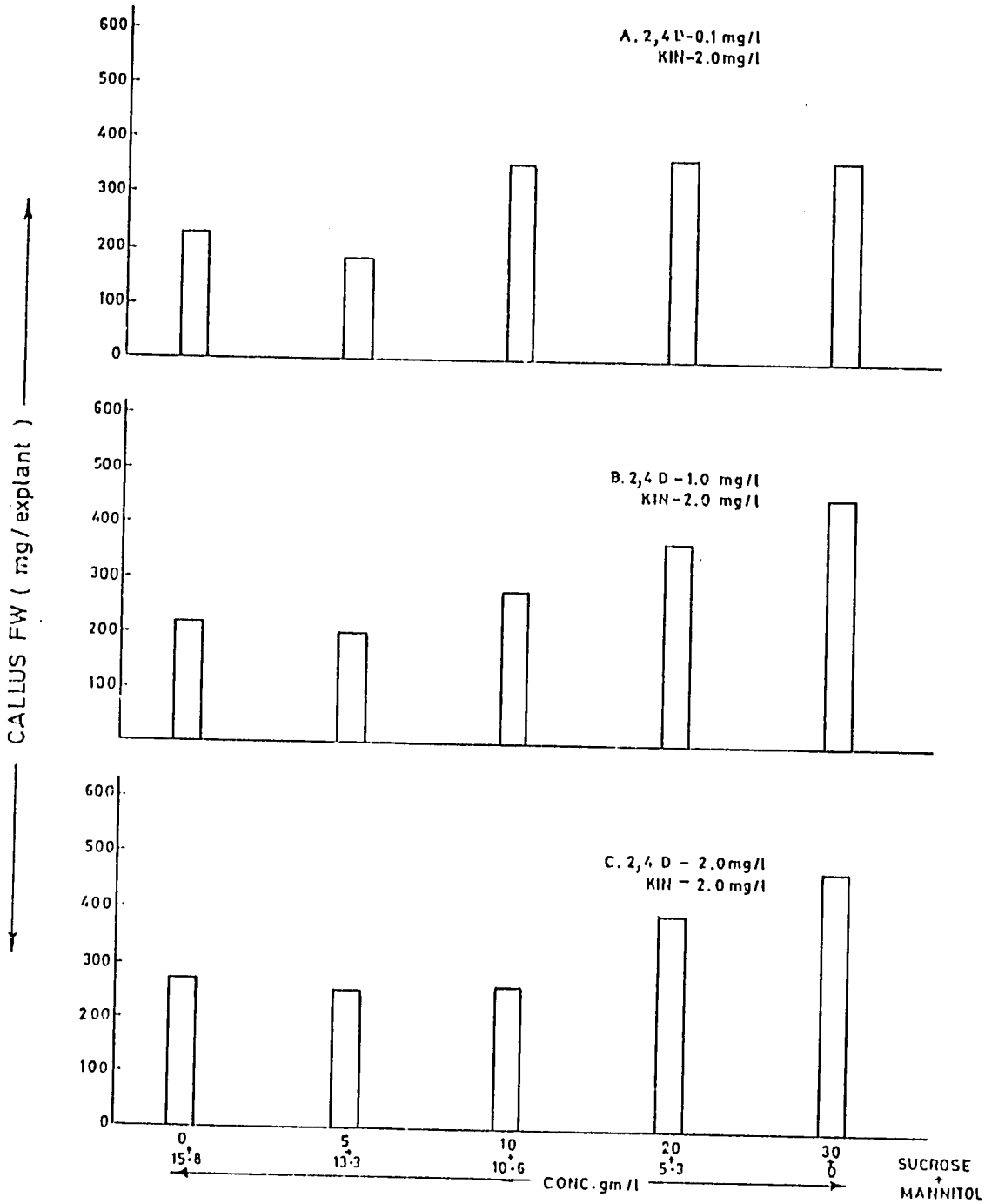


FIG.2. EFFECT OF MANNITOL SUPPLEMENTATION ON THE GROWTH OF COTYLEDONARY EXPLANTS OF PISTACIA VERA L. CULTURED IN VITRO.

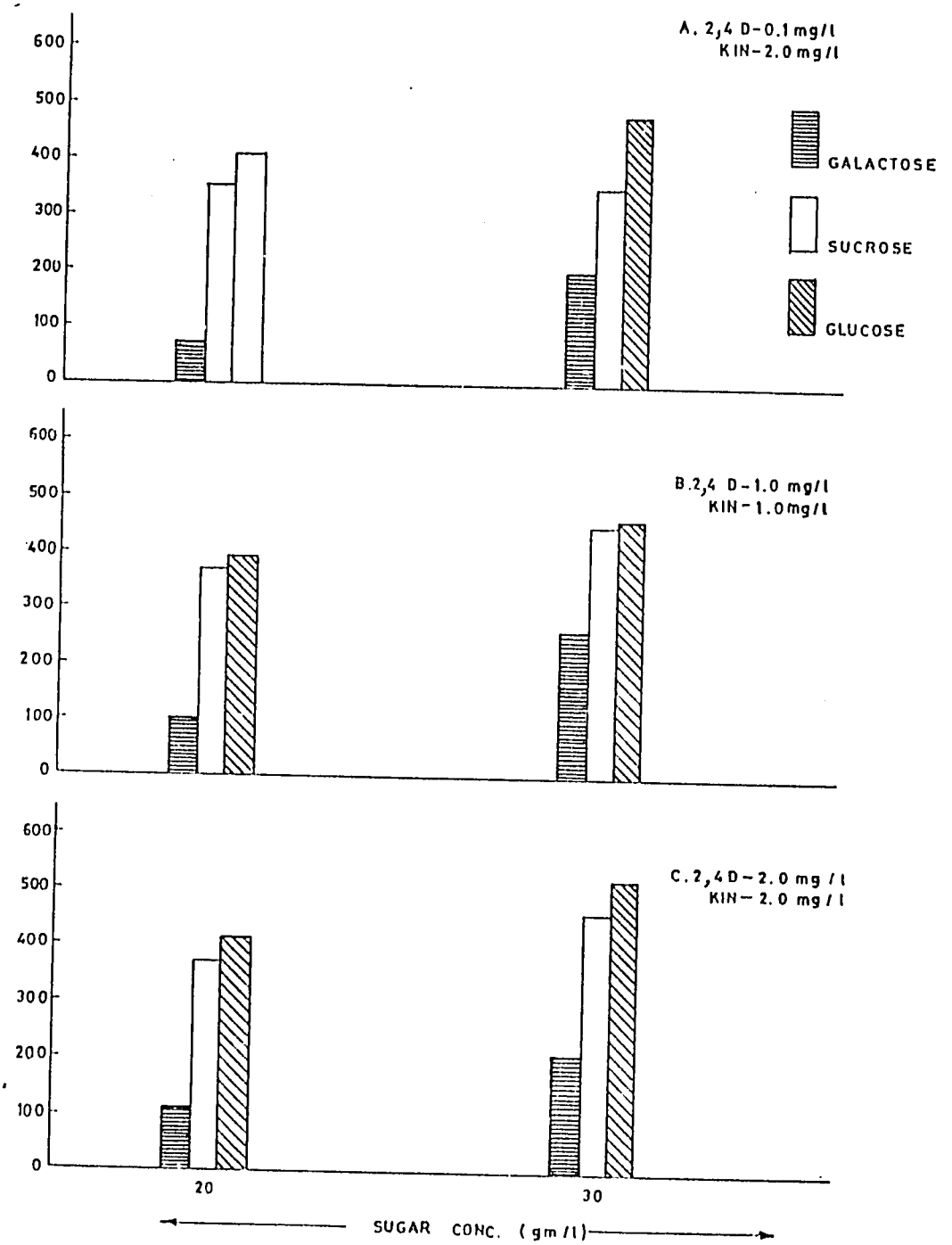


Fig. 3. EFFECT OF CARBON SOURCE ON CALLUS GROWTH FROM COTYLEDONARY EXPLANTS OF PISTACIA VERA L. CULTURED IN VITRO.

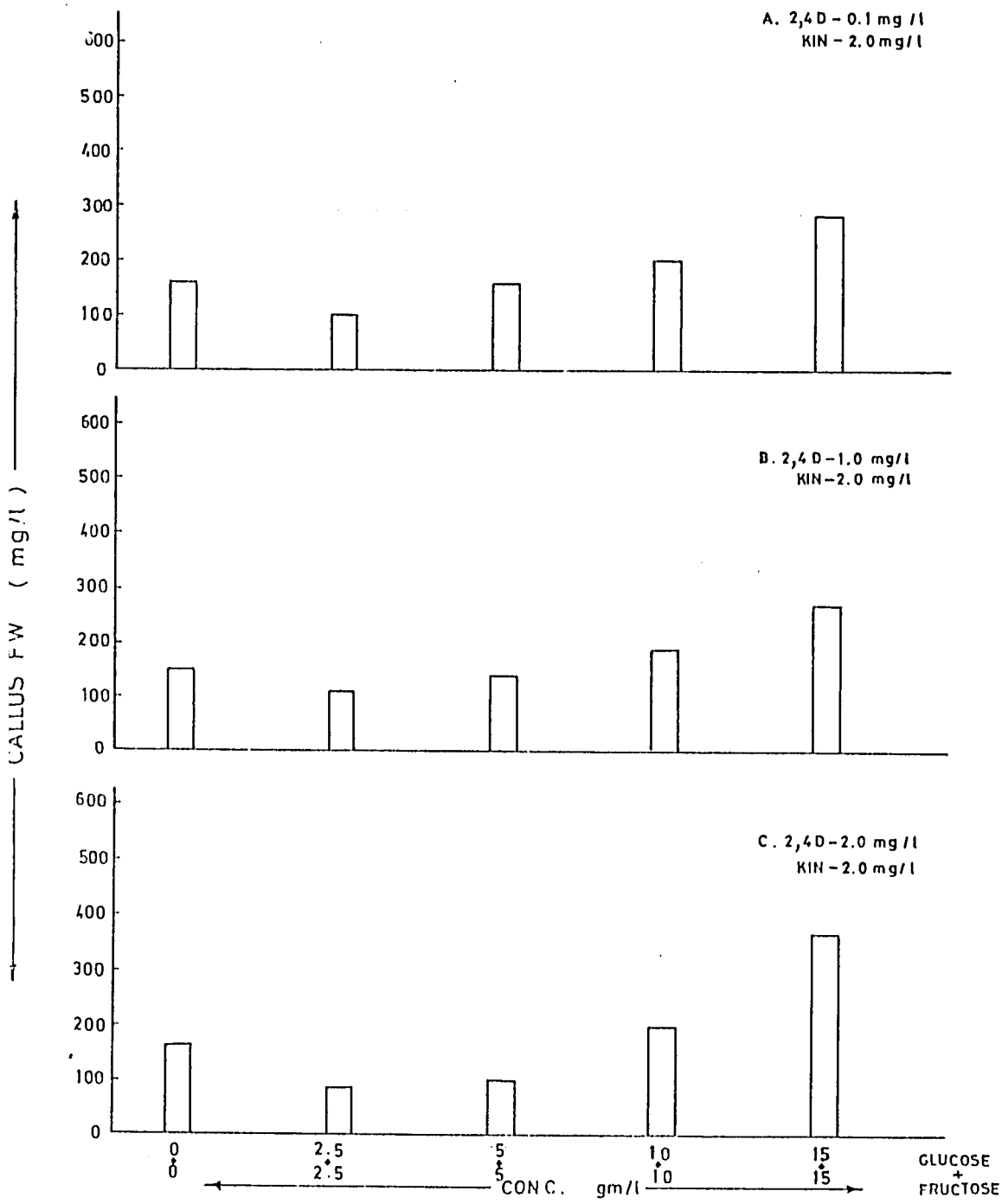


FIG.4. FEFECT OF GLUCOSE PLUS FRUCTOSE SUPPLEMENTATION ON FRESH WEIGHT OF CALLI PRODUCED BY COTYLEDONARY EXPLANTS OF PISTACIA VERA CULTURED IN VITRO.

REJUVINATION OF MATURE P. vera BY MICROGRAFTING TECHNIQUE

In the report for period Oct. 88 - Mar. 89, work on seedlings raising their maintenance under culture conditions for purpose of micrografting was mentioned. Besides using different hormonal conditions, a change in glucose content and growth of seedlings on plain agar, all resulted in necrosis. Thereafter, some success was achieved by taking into consideration of physical factors e.g. humidity. Seedlings so obtained were later given hardening treatment and were transferred to soil in Green-House. The axillary buds of these seedlings were activated using 4 mg/l BAP for the purpose of grafting. Activated buds were grafted to root-stock but were a total failure, mainly due to contamination in first place. Further work on seedling raising was discontinued as similar work was undertaken under section on Nutritional Studies on P. vera in Green House (see present report). These seedlings could also be used as root-stock for grafting studies.

Mature Tree's buds of P.vera from Quetta were brought. The buds were in dormant stage and to activate them, they were cultured. Previous attempts with culture of P. vera vegetative buds all failed, as buds totally balckened in cultures, besides the use of following antioxidants.

1. Citric Acid 1%
2. Ascorbic Acid 1%
3. Citric acid 0.5% + Ascorbic acid 0.5%

Therefore, instead of using antioxidants, the inoculation procedure was modified as following :

Shoots with healthy summer durmant buds were selected. All the leaves were removed from shoots and they were cut into 6 - 8cm pieces, from the internodes. After thoroughly washing under running tap water, the buds alongwith a thin strip of bark was excised and surface sterilized with 0.1% mercuric chloride. After giving 3 - 4 washings with sterilized distilled water under Laminar Flow Cabinit , the buds were held one by one and the hard brown scale leaves were removed by chipping them off with the help of a razor upto a stage when light green scale-leaves with hairs on them were visible. Bark portion was also cut off. Therefore, all the possible extra plant material that may contain much phenols was physically removed. At this stage these buds were inoculated on MS medium.

This method produced 90% green buds and no phenols exudation was seen into the culture medium. The buds were simultaneously provided with the following hormones in the culture medium:

Kinetin	0.25 - 3 mg/l	
BAP	0.25 - 4 mg/l	
AS	5 - 50 mg/l	

All the above hormones were used singly. The main objectives of above study were :

i) Activation and sprouting of mature tree buds under culture conditions,

ii) Unfolding and expansion of leaves,

iii) Shoot proliferation, which will help in producing axillary buds for micrografting purpose.

Activation and sprouting was observed in all hormones and in control. However, unfolding of leaves and their expansion was found only in cultures containing hormonal treatments. Shoot proliferation was found only with BAP and that only at 1 mg/l concentration. Shoot proliferation of cultured buds was necessary for obtaining axillary buds to be used for *in vitro* grafting.

Presently these buds are subcultured on different combinations of BAP + AS, BAP + K and K + AS (Table). The purpose is either to induce shoot proliferation or the buds may remain active for grafting on nursery plants.

Meanwhile, as seedlings of P. vera could not be raised due to non-availability of seeds, as mentioned under Section on Nutritional Studies on *P. vera*. Therefore, *P. khinjik* seeds were procured and sown on culture medium (1/2 MS macro-salts + 1 mg/l NAA) to get seedlings which will serve as rootstock for *in vitro* grafting studies.

P. khinjik seeds were germinated without any difficulty and 90% germination was achieved at 22 - 25^{0}C. The rootstock so obtained will take already cultured buds from mature trees of *P. vera* for *in vitro* grafting and the results will be communicated in the next report.

During Quetta visit for obtaining mature - tree buds (reported above) some work was also done on the following lines.

A. Recognition of Vegetative/Floral buds of P. vera while in dormant stage.

The selection of vegetative buds need a lot of care as the floral buds are also present side by side on the same shoots. The floral buds remain on shoots for the whole year as dormant floral buds and could be misunderstood for vegetative buds. The difference between the floral and vegetative bud is not in the size, as the size varies with the general health (growth stage) of the plant but in the shape of the bud. In floral buds the length to diameter ratio is less as compared to Vegetative bud which are thin and pointed at top.

B. Budding of Vegetative bud on Pistachio trees.

T-budding was used with following variables:

1. Loose-hand tying with plastic tape.
2. Tight-hand tying with plastic tape.
3. Sealing of T-Budding to prevent oxygen supply.
4. Open oxygen supply to T-Budding.

Selective vegetative buds were cut-off with razor. Cutting starts from below the bud and cuts the stem into the wood under the bud, taking the bud thick enough to be pushed under the bark of the root stock wihtout being crumpled, wood was removed

Mature-Tree buds of P. vera

A - VEGETATIVE BUDS B - FLORAL BUDS

Fig 1

taking care that no damage was done to the bud. This was easily achieved by moving the bark too and fro and then pealing it off the wood. Petiole was left intact while leaf pinna was removed. Petiole facilitate the bud handling during budding process.

The root stock was prepared by making a T-shaped incesion through the bark down to the wood. The bark on the upper T-end was flapped and bud was pushed under the root stock bark while holding it by the petiole. The upper end of scion bark was cut to be adjusted with the upper cut end of the root stock bark.

Bud was tightened with plastic strip. Binding started below - upward, so that the bud should slip and come to rest with its upper end and tightely fixed with the bark of root stock. Sixty to 70% success was observed in No. 1, 3 and 4 variables, while variable 2 was effective upto 10% only

Fig 2. T-Budding on P. vera plant (Loose hand tying)

Fig 3. P. vera bud inoculated on MS medium

(Below sprouting)

Fig 4. P. vera bud activation and sprouting on MS medium containing % sucrose and

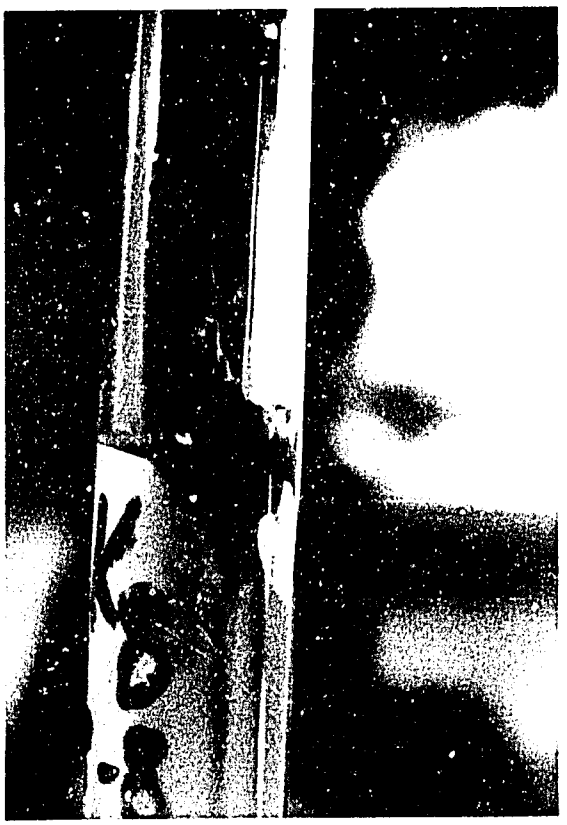


Fig 5. Leaf expansion and unfolding in cultured P. vera bud on MS medium containing % sucrose and

EFFECT OF BAP CONCENTRATIONS ON MATURE A.VERA BUDS

Media contain sucrose 3% + Agar 0.7%, pH 5.7 adjusted with KOH 7 Replicates in each combination

BAP	SECOND WEEK				THIRD WEEK			
	LENGTH Cm	DIAMETER Cm	COLOUR	REMARKS	LENGTH Cm	DIAMETER Cm	COLOUR	REMARKS
	1	2	3	4	1	2	3	4
.25 mg/l	.5	.3	G	All buds healthy and green.	1	.5	G	General increase in size callus initiated bud sprouted.
.5 mg/l	.5	.3	G	All buds healthy and green.	1	.5	G	General increase in size callus initiated six buds sprouted.
1 mg/l	.5	.3	G	All buds healthy and green.	1	.5	G	General increase in size callus initiated Three buds sprouted.
1.5 mg/l	.5	.3	G	All buds healthy and green.	1	.5	G	General increase in size callus initiated Three buds sprouted.
2 mg/l	.5	.3	G	All buds healthy and green.	1	.5	G	General increase in size - no callus Three buds sprouted.
3 mg/l	.5	.3	G	All buds healthy and green.	1	.5	G	General increase in size - no callus Three buds sprouted.
4 mg/l	.5	.3	G	All buds healthy and green.	1	.5	G	General increase in size - no callus Three buds sprouted.

TABLE 1 (CONTINUED)

BAP	FOURTH WEEK				FIFTH WEEK			
	1	2	3	4	1	2	3	4
.25 mg/l	1.3	.7	G	No change. Slight increase in callus.	1.3	.7	Pale G	Callus increased Scale leaf tips dead.
.5 mg/l	1.3	.7	G	Slight increase in size. Slight increase in callus. Leaf in one bud emerging.	1.5	.8	Pale G	Leaves in one bud unfolded. No other change.
1 mg/l	1.4	.7	G	Side bud activated in one bud. Increase in size & callus. No other change.	1.5	.7	Pale G	Increase in Callus. In one bud side bud increased in size. No other change.
1.5 mg/l	1	.5	G	One side bud activated in one. No other change.	1	.5	Pale G	No change. Callus increased in size.
2 mg/l	1	.5	G	No change.	1	.5	Pale G	No change.
3 mg/l	1	.5	G	No change.	1	.5	Pale G	No change.
4 mg/l	1	.5	G	No change.	1	.5	Pale G	No change.

TABLE 1 (CONTINUED)

BAP	SIXTH WEEK				
	1	2	3	4	GENERAL REMARKS
.25 mg/l	1.3	.7	Pale G	Increase in callus. No other change.	Callus formed at base. Scale leaf enlarged and unfolded. Scale leaf tip died in fifth week. No more change.
.5 mg/l	1.6	.8	Pale G	Bunch of leaves in one bud. No other change.	Callus at base. Scale leaves unfolded bud died in 5th week. Bunch of compound leaves in one bud but they do no unfold.
1 mg/l	1.8	1	Pale G	Scale leaf tip dead. In one bud side bud increased. Leaves on side bud healthy.	One side bud activated and increased in length 1.8 cm. Scale leaves unfolded and died in 5th week. No other change.
1.5 mg/l	1	.5	Pale G	Leaves in one bud unfolding. Side bud activated.	In one bud bush of compound leaves appeared but not unfolded. Side buds activated but no growth in them. No other change.
2 mg/l	1	.5	Pale G	No change.	Buds only sprouted. Callus formed. No other change.
3 mg/l	1	.5	Pale G	No change.	Buds only sprouted. Callus formed. No other change.
4 mg/l	1	.5	Pale G	No change.	Buds only sprouted. Callus formed. No other change.

G - Green

TABLE 2

EFFECT OF KINETIN CONCENTRATIONS ON MATURE P. VERA BUDS

Media contain Sucrose 3%, Agar .7%. pH 5.7 adjusted with KOH 7 Replicates in each

Concentrations in mg/l

KINETIN	SECOND WEEK				THIRD WEEK			
	LENGTH	DIAMETER	COLOUR	Remarks	LENGTH	DIAMETER	COLOUR	Remarks
	1	2	3	4	1	2	3	4
0.25	.5	.3	G	All buds healthy & green.	.8	.4	G	Leaf sprouted in one bud. Small callus initiated.
0.5	.5	.3	G	All buds healthy & green.	.7	.3	G	No bud sprouted. Colour mostly green to Dark brown.
1	.5	.3	G	All buds healthy & green.	.6	.3	G	No Change. Callus initiated.
1.5	.5	.3	G	All buds healthy & green.	.7	.4	G	No Change. Callus initiated.
2	.5	.3	G	All buds healthy & green.	1	.4	G	One bud sprouted. Lot of callus.
2.5	.5	.3	G	All buds healthy & green.	1	.5	G	Three buds sprouted. One leaf emerged in on bud. Callus present at base.
3	.5	.3	G	All buds healthy & green.	1	.5	G	Two buds sprouted. Callus present at base.

TABLE 2 (CONTINUED)

KINETIN	FOURTH WEEK				FIFTH WEEK			
	1	2	3	4	1	2	3	4
0.25	.8	.4	8	In one bud one compound leaf emurged Leaf length 1 cm No other change.	1	.5	8	Another bud sprouted. Side buds activated in three buds.
0.5	.7	.3	8	One bud sprouted. Two buds with side buds activated as green spots.	1	.5	8	Bunch of compound leaf emurged. Side bud activated in one bud.
1	.7	.3	8	No change. Only callus formation.	1	.5	8	No change.
1.5	1	.5	8	No change. Only callus formation dark-brown.	1	.5	8	No spouting. Buds swollen. Callus at base.
2	1	.5	8	Two buds sprouted. Lot of callus dark-brown.	1	.5	8	Compound leaf emurged in one bud. Two buds sprouting.
2.5	1	.5	8	One leaf in one bud 1.3 cm Bunch of leaf in other bud Callus brown.	1	.5	8	Compound leaves emurged in one bud. One side bud activated at base.
3	1	.5	8	Three buds sprouted Callus dark brown.	1	.5	8	One compound leaf in one bud. Callus black coloured. Side bud activated in two.

TABLE 2 (CONTINUED)

KINETIN	SIXTH WEEK				
	1	2	3	4	GENERAL REMARKS
0.25	1	.5	B	Compound leaf dry and dead no No change in side buds.	One compound leaf unfolded. 5 leaflets appeared but curled. Callus formed in all. No other change.
0.5	1	.5	B	No change.	Bunch of compound leaves emurged but remain folded. Callus formation. No other change.
1	1	.5	B	No change.	Callus formed. No other change.
1.5	1	.5	B	One sprouting. No other change.	No sprouting buds swollen at base. No other change.
2	1	.5	B	Third bud sprouted. Leaf dead &. side buds activated. No other change.	Three buds sprouted. Bunch of compound leaves emurged in one bud but remain folded. No other change.
2.5	1	.5	B	No change. Callus dark brown.	Compound leaves emurged in one but remain folded. Three buds sprouted. Side buds activated.
3	1	.5	B	No change.	Compound leaves emurged in one but remain folded. Three buds sprouted. Side bud activated.

G - Green
B - Brown

TABLE 3

EFFECT OF ADENINE SULPHATE CONCENTRATION ON MATURE P. VERA BUDS

Media contain sucrose 3%, Agar 0.7%, pH 5.7 adjusted with KOH, 7 Replicates in each

Concentration in mg/l

ADENINE SULPHATE	SECOND & THIRD WEEK 1	2	3	4	FOURTH & FIFTH WEEK 1	2	3	4	GENERAL REMARKS
5	.5	.3	G	All healthy and green. Callus initiated.	.5	.3	B	Small increase in callus. Dark brown callus. No other change.	Different concentrations of Adenine sulphate only increased the callus formation on the cut end of buds. Colour of buds changed from green to dark brown. In only one bud, in 30 mg/l Adenine sulphate, bunch of compound leaves emurged which do not unfolded.
10	.5	.3	G	All healthy and green. Callus initiated.	.5	.3	B	Small increase in callus. Dark brown callus. No other change.	
20	.5	.3	G	All healthy and green. Callus initiated.	.5	.3	B	Small increase in callus. Dark brown callus. No other change.	
30	.5	.3	G	All healthy and green. Callus initiated.	.5	.3	B	Only one showed dark green bunch of leaves emurged. No other change. Callus dark brown.	
40	.5	.3	G	All healthy and green. Callus initiated.	.5	.3	B	Small increase in callus. Dark brown callus. No other change.	
50	.5	.3	G	All healthy and green. Callus initiated.	.5	.3	B	Small increase in callus. Dark brown callus. No other change.	

B = Brown
G = Green

TABLE 4

HORMONE COMBINATIONS

HORMONES USED BAP, KINETIN, ADENINE SULPHATE

Media contains MS, Sucrose 3%, Agar 0.7%, pH 5.7 adjusted with KOH

BAP & Adenine Sulphate		Kinetin + Adenine Sulphate		BAP + Kinetin	
S.No.	Combinations	Sr. No.	Combinations	Sr. No.	Combinations
1	0.25 + 30	1	0.25 + 30	1	4 + .25
2	0.5 + 30	2	0.5 + 30	2	3 + .5
3	1 + 30	3	1 + 30	3	2 + 1
4	1.5 + 30	4	1.5 + 30	4	1.5 + 1.5
5	2 + 30	5	2 + 30	5	1 + 2
6	3 + 30	6	2.5 + 30	6	0.5 + 2.5
7	4 + 30	7	3 + 30	7	0.25 + 3

REGENERATION THROUGH CELL CULTURE

Techniques to develop cell cultures of P. vera were mentioned in period (Oct. 88 - Mar. 89) report. However, the suspension were to initiate each time, from freshly initiated callus cultures. As the browning of P. vera cells in cultures is a general problem. Some work was reported in previous report, to lengthen the growth period of non-grown cellus cells with the help of various combinations of growth hormonies. A paper with the following title and abstract was submitted to Pakistan Journal of Scientific and Industrial Research.

A STUDY OF THE RELATIONSHIP BETWEEN GROWTH REGULATORS AND BROWNING TIME OF PISTACIA VERA CALLI

ABSTRACT

Browring time of Pistacia vera calli depended on concentrations of growth regulators. P. vera calli grown on NAA (5 mg/l); 2, 4-D (1-5 mg/l) + 2iP (1-5 mg/l); 2, 4-D(2-5 mg/l) + Kin (o.5-2 mg/l) remained white/green for a time ranging between 5 - 13 weeks. Thereby permitting further callus proliferation and initiation of suspension culture.

Moreover, due to non-availability of orbital shakers, during the period under report, cell culture work could not be undertaken. In near past, we received our equipment and apparatus for Tissue Culture Project from USA and the same is being installed these days and therefore, work will be initiated in near future.

ANATOMICAL STUDIES OF *in vitro* CULTURED *P. vera* EXPLANTS

In reporting period (Oct. 1988 to Mar. 1989) it was revealed that cotyledonary explants cultured on the hormones supplemented MS full strength media exhibited various degree of dedifferentiation and redifferentiation Results were based on analysis of cotyledonary samples collected at random hormonal concentration. However observations were concluded as wound callus, vaculated callus, meristemetic zones, uniseriate undifferentiated tissue xylogenesis, shoot apex, shoot bud formation. In view of these results it was planned to conduct further studies on the effect of induced behaviour in *in vitro* cultured cotyledonary explants.

Experimental:

Seeds of P. vera were aseptically cultured and incubated with full strength MS medium with auxin , cytokinin alone or in combination.

	Hormones	Concentrations (mg/l)
(1)	IAA	0.5, 1.0, 2.0, 3.0, 4.0, & 5.0
(2)	IBA	0.5, 1.0, 2.0, 3.0, 4.0, & 5.0

(3) IAA	+ IBA	(4) IAA	+ NAA
0.5	0.5	0.5	0.5
"	1.0	"	1.0
"	2.0	"	2.0
"	3.0	"	3.0
"	4.0	"	4.0
"	5.0	"	5.0

In above mentioned combinations concentrations of IAA was kept constant i.e. 0.5 (mg/l) but concentrations of IBA, NAA and 2, 4, D were varied in between 0.5 - 5.0 (mg/l) for each combination. The

seeds were incubated for a total period of eight weeks. After 2,4,6 or 8 weeks intervals cultures were removed from each set. Cotyledons were detached from the emboyes (whether germinated or still dormant) and preserved in FAA. Procedures of dehydration, embedding and dissection of cotyledonary mass were the same as reported in the earlier reports. Anatomical changes observed in the tissue have been tubulated. The work is in continuity therefore conclusion will be presented in the next report.

Data of histological analysis of mature pistacia vera. Cotyledones cultured on MS medium supplemented with specified growth regulators is given along the tables from 1 to 20. Table numbers 7, 11, 23 and 24 are missing due to explant contamination therefore cultures were reincubated with desired growth regulators and definite period prior to the observation.

TABLE 1

GROWTH REGULATOR	-	IAA 0.5 mg/l
AGE OF CULTURE	-	2 weeks

Explant Tissue	Morphogenic Response	UCX Cell Form: Shape	Vol or Dia (mm)	LXW (mm)	REMARKS
(1)	(2)	(3)	(4)	(5)	(6)
Epidermic	+ve	Tubular	-	0.3m - 0.6m x 0.1m - 0.2m	In single layer. Due to the cell div. at right angles to the surface (anticlinally).
	Callogenesis	isodiametric or ray cells shape	0.1m-0.2m	-	Upon the epidermal cells border callus formation in the form of mass meristem. Densly filled with cytoplasm.
Hypodermis	+ve	Spheroidal in shape	0.7-1.0	-	Periclinally divided in 2 to 3 rows with numerous spaces
Storage paren-chyma	+ve	Ovate to hexsagonal in shape	1.0-2.0	-	-
	Rhizogenesis xylogenesis				A root tip like protubrance arises from peripheral zones of cells.
	i) Tracheids	Fusiform shape		2-40x0.5-1.5	With several characteristic patterns. In vertical cylinder scattered and irregularly fused with each other having central metaxylem without bundle sheath.
	ii) Metaxylem	Rounded	1.5-3.0		
	Endedermis or Bundle sheath	-	-	-	Frequence of tracheids and vessels very low. Mostly morphologic response of peripheral region.

TABLE 2

GROWTH REGULATORS - IAA 1.0

AGE OF CULTURE - 2 WEEKS

(1)	(2)	(3)	(4)	(5)	(6)
Epidermic	+ve	Square Shape	0.1-0.2	-	In single layer. anticlinally divided.
Hypodermis	+ve	Tubular to Hexsagonal	- 0.1-0.3	1.3-1.7 -	In many rows, periclinally and anticlinally divided with numerous intercellular spaces
	Callogeneris	Sohercidal to isodismetric	0.3-0.4 0.5-1.2	- -	
Storage - Paranchyma	+ve	Ovate to Hexsagonal	1.2-1.7		Sequence of meristematic cells on epi. is anticlinally and in the hypodermis is in the form of mass meristem , densly filled with cytoplam. Compact arrangement.
	Embryogenesis	Spheroidal	0.7-0.9		Cell div. at different planes with numerous intercellular spaces.
	Callogenesis				Thick walled, densly filled with cytoplasm large nucleus. Shoot tip like structure with leaf primodia.
	Xylogenesis				
	i) Tracheids	Fusiform-shape	-	0.7-1.5 x 0.5 - 0.6	Frequence of tracheids and vessels very low. Vascular bundle without endodermis cells.
	ii) Metaxylem	Rounded	0.5-0.8	-	Arrangement of conducting elements was many types, mostly near the peripheral region.

TABLE 4

GROWTH REGULATORS - IAA 3.0

AGE OF CULTURE - 2 Weeks

(1)	(2)	(3)	(4)	(5)	(6)
Epidermis	+ve	Square shape	-	0.2-0.5	Single layer. Anticlinally divided cells. Thin wall with cytoplasm
Hypodermis	+ve	Hexsagonal to Tubulars	0.5-0.7	- 0.4-0.6 x 0.3-0.5	Many layers. Periclinally divided in vertical rows, compact without spaces clear nucleus and dense cytoplasm.
	Callogenesis	Spheroidal in shape	0.1-0.3	-	Meristematic cells only on the epidermal boider with spaces.
Storage Parenchyma	+ve	Tubular to Ovate	0.8-1.3	-	Hexsagonal parenchyma cells transformed into elongated to ovate shape with anti periclinal division, small spaces.
	Xylogenesis				
	Tracheids	Fusiform	-	0.5-2.0 x 0.1-0.4	Tracheids are in the form of bundles. In between these tracheid xylem parenchyma are present, thin wall and hollow inside.
	Xylem paren-chyma	Spheroidal	0.8-2.0	-	Around the tracheary elements no vascular sheath present.
	Endodermis or bundle sheath	-	-	-	

TABLE 3

GROWTH REGULATORS - IAA 2.0

AGE OF CULTURE - 2 WEEKS

(1)	(2)	(3)	(4)	(5)	(6)
Epidermis	+ve	Spheroidal	0.2-0.4	-	In single layer, thin wall, dense cytoplasm with clear nucleus. Anticlinally divided.
Hypodermis	+ve	Square shape	-	0.2-0.4	
	Callogenesis	Isodiametric to spherical	0.3-0.9		Single layer, compact with cytoplasm periclinally divided. Callus present on epidermis border and inside the central part. These meristematic cells show varied arrangement of cells resulting from varied pattern of cell division.
Storage Parenchyma	+ve	Polyhedral to ovate shape	1.2-1.7	-	Direct transformation to hexagonal to polyhedral and ovate shape with small intercellular spaces.
	Rhizogenesis	-	-	-	Root tip like protubrance appear from the inner side of the epidermis
	Xylogeneris				
	Tracheids	Fusiform shape	-	0.5-1.5 x 0.2-0.5	Frequences of tracheids and vessels are very low. No in the form of cluster or vertical cylinder. Mostly transformation in single tracheid scattered in section. In vasular bundle only tracheid and meta-xylem clear.
	Metaxylem	Rounded	1.3-2.0	-	
	Endodermis or Bundle sheath	-	-	-	

TABLE 5

GROWTH REGULATORS - 4.0

AGE OF CULTURE - 2 weeks

(1)	(2)	(3)	(4)	(5)	(6)
Epidermis & Hypodermis	+ve +ve				Hypodermis and epidermis are completely converted into cluster of meristematic cells
	Calloganesis	Hexasagonal	0.1-0.3	-	These meristematic zones are divided as mass meristem without intercellular spaces. Small portions are in the form of rib meristem.
Storage parenchyma	-ve	Isodiametric in shape	1.5-2.1	-	Morphogenic response was not clear. Cells with numerous spaces,.
	Xylogenesis Tracheids	Fusiform	-	1.2-3.0 x 0.3-0.7	Tracheids single or small, loose clusters scattered in sections. In vascular bundles tracheids and meta-xylem cleared. No bundle sheath around bundle
	Endodermis or Bundle sheath	-	-	-	

TABLE 6

GROWTH REGULATORS - IAA 5.0

AGE OF CULTURE - 2 WEEKS

(1)	(2)	(3)	(4)	(5)	(6)
Epidermis	-ve	-	-	-	Not clear
Hypodermis	+ve	Spherodial in shape	0.5-1.0	-	Rounded cells with varied arrangemt of cells. Spaces between them with cytoplasm.
Storage parenchyma	+ve	Ovate shape		-	Hexsagonal transfer into ovate with numerous spaces.
	Xylogenesis				
	Tracheids	Fusiform	-	0.5-3.0 x 0.1-0.5	Tracheery elements are well developed. Tracheids with vessels are formed in the form of bundles with different arrangemt.
	Vessels metaxylem	Square shape		2-3.5	Vascular bundles with clear metaxylem and without endodermis.
	Endodermis or bundle sheath	Rounded	2.7-3.2	-	
		-	-	-	

TABLE 7

GROWTH REGULATORS - IBA 1.0

AGE OF CULTURE - 2 WEEKS

(1)	(2)	(3)	(4)	(5)	(6)
Epidermis	+ve	-	-	-	Epidermis and Hypodermis is not clear due to the formation of callus
& Hypodermis	+ve	-	-	-	
	Callogenesis	Tubular to spheroidal	0.2-1.0	0.5-1.2 x 0.3-0.8	Meristematic cells on epidermis are divided to various planes but cells inside are periclinally divided in vertical rows. No intercellular spaces between them.
Storage Parenchyma	+ve	Spheroidal, ovate and isodiametric	0.5-1.5	-	Hexsogonal transformation into spherical and ovate without spaces.
	Xylogenesis				
	Tracheids	Fusiform	-	0.2-1.0 x 0.1-0.5	Well developed tracheary elements are present in central region with varied pattern of arrangemt.
	Vessels	Rectangular	-	1.2-1.7 x 0.3-0.6	Around the vascular bundle not endodermis present.
	Metaxylem	Rounded	0.4-0.9	-	
	Endodermis or Bundle Sheath.	-	-	-	

TABLE 3

GROWTH REGULATORS - IBA 2.0

AGE OF CULTURE - 2 WEEKS

(1)	(2)	(3)	(4)	(5)	(6)
Epidermis &	+ve				No distin vision in Epidermis and hypodermis.
Hypodermis	+ve	Tubular		0.3-1.4 x 0.2-0.9	Hypodermis cells are elongated, Anticlinally divided.
	Callogenesis	Tubularis		0.3-1.5 x 0.1-0.7	Meristematic cells are tubular arranged in vertical manner by periclinally division.
Storage parenchyma	+ve	Fusiform to, rectangular & rounded		1.2-1.5 x 0.5-0.7	Large variety of cell shape from Hexsagonal cells developed.
			0.3-1.7	-	Tracheary elements highly developed.
	Xylogenesis				
	Tracheids	Fusiform			Tracheids with vessels in the form of vertical cylinders. Vascular bundles are also well developed in which meta and protoxylem clearly visible. These tracheids and bundles are surrounded with bundle sheath of endodermis like higher plants.
	Vessels	Square	-	0.4-0.7	
	Metaxylem	Rounded	1.7-2.2	-	
	Protoxylem	Hexsagonal	0.4-0.7	-	
	Endodermis or Bundlesheath	Rounded	0.5-0.9	-	
	Embryogenesis	Rounded	0.5-1.0	-	Embryogenic cells with clearly large size nucleus and thick wall present in central region.

TABLE 9

GROWTH REGULATORS - IBA 3.0

AGE OF CULTURE - 2 WEEKS

(1)	(2)	(3)	(4)	(5)	(6)
Epidermis and	+ve	-	-	-	These are completely converted into callus.
Hypodermis	+ve	-	-	-	
	Calloganesis	Spheroidal in shape	0.2-0.8	-	Arrangment of these zonation is rib meristem and mass meristem with not intercellular spaces.
Storage Parenchyma	+ve	Spheroidal to isodiametric	1.2-2.0	-	Direct conversion into rounded cells with numerous spaces.
	Xylogenesis				
	Tracheids	Fusiform	-	1.2-1cm above x 0.3-0.7	Tracheary elements are not well developed
	Vessels	Rectangular	-	1.2-1.5 x 0.4-0.8	Only tracheid with vessels present in vertical bundles which are branched.
	Metaxylem	Rounded	2.0-2.2	-	No advanced vascular bundle present.
	Endodermis or Bundle sheath	-	-	-	Around the tracheid no endermis present.

TABLE 10

GROWTH REGULATOR - IBA 5.0

AGE OF CULTURE - 2 WEEKS

(1)	(2)	(3)	(4)	(5)	(6)
Epidermis and	+ve	-	-	-	Completely covered with callus
Hypodermis	+ve	-	-	-	
	Calloganesis	Spheroidal	0.3-0.5	-	Meristematic zones on Epi and hypodermis in the form of mass and rib meristem. Direct transformation from Hexagonal to spherical or ovate with compact arrangement.
Storage parenchyma	+ve	Spheroidal to ovate	1.5-2.3	-	
	Xylogenesis				
	Tracheid			2.2-1cm above	Tracheary elements well developed. Tracheids in bundles vertically arranged and vascular bundle with tracheids. Meta and Protoxylem. Around there bundle endodermal loose cells layer present. Frequence of V-B is very high.
	Metaxylem	Rounded	1.5-3.5		
	Protoxylem	Rounded	0.2-0.4		
	Endodermis	Square to spheroidal	- 0.5-0.3	1.4-1.5	

TABLE 11

GROWTH REGULATOR - IAA-0.5
IBA-0.5

AGE OF CULTURE - 2 WEEKS

(1)	(2)	(3)	(4)	(5)	(6)
Epidermis	+ve	Tubular	-	0.3-1.3 x	Single layer with anticlinally divided cells.
Hypodermis	+ve	Tubular	-	0.7-2.0 x	Single layer with periclinally divided cells.
	Callogenesis	Hexsagonal	0.4-0.5	-	Upon the epidermal boundry.
Storage Parenchyma		Spheroidal	1.0-1.5	-	Direct transformation into rounded cells with nucleus cells near peripheral region are compact arrangement but in the central are with intercellular spaces.
	Xylogenesis				
	Tracheids	Fusiform	-	1-1 cm above 0.4-0.6	Well developed tracheary elements. Tracheids are in vertical cylinders. Vascular bundles having Meta and protoxylem. Around these endodermis present. Vascular bundles are very rare.
	Metaxylem	Rounded	-	2-4	
	Protoxylem	Hexsagonal	-	0.3-0.9	
	Endrdermis	Spheroidal	1.0-1.7	-	

TABLE 12

GROWTH REGULATOR - IAA 0.5
IBA 1.0

AGE OF CULTURE - 2 WEEKS

(1)	(2)	(3)	(4)	(5)	(6)
Epidermis &	+ve	-		-	No distingsion in epi and hypodermis du to the callus formation.
Hypodermis	+ve	-		-	
	Calloganesis	Tubular Ovate Hexsagonal	- 0.5-1.7 0.7-0.9	1.5-3.0 x 0.5-2.0	Large variety of callus in mass meristem and rib meristem. This variety show tendency of organogenis.
Storage Parenchyma	+ve	Hexsagonal	0.6-2.7	-	Hexsagonal and compact smaller size.
	Xylogenesis				
	Tracheids	Fusiform	-	0.3-0.5 x 0.2-0.4	Only simple Tracheids in scattered and in Bundle form present.

TABLE 13

GROWTH REGULATOR - IAA 0.5
IBA 2.0

AGE OF CULTURE - 2 WEEKS

(1)	(2)	(3)	(4)	(5)	(6)
Epidermis	+ve	Spheroidal	0.1-0.3	-	Single layer anticlinally divided
Hypodermis	+ve	Ovate	0.4-0.7	-	Two to three layer contains callus
					paches, periclinally divided.
	Callaganesis	Spheroidal to	0.4-0.6	-	Meristematic zones with mass
		Ovate	0.7-1.7	-	meristem in the peripheral region.
	Xylogenesis				
	Tracheids	Fusiform	-	0.4-1.2 x 0.1-0.3	Tracheary elements are well developed without endodermis around them.
	Metaxylem	Rounded	0.7-2.0	-	Tracheids in the form of vertical
	Protoxylem	Hexagonal	0.2-0.3	-	bundles. Vascular bundles scattered
	Endodermis	-	-	-	near the peripheral region.
Storage	+ve	Rectangular	-	0.9-3.0 x	Direct transformation into rectangular
Parenchyma		Square	-	0.5-1.0	by anticlinal division.

TABLE 14

GROWTH REGULATOR - IAA 0.5
IBA 3.0

AGE OF CULTURE - 2 WEEKS

(1)	(2)	(3)	(4)	(5)	(6)
Epidermis	+ve	-	-	-	Not clear.
Hypodermis	+ve	Tubular		0.1-0.3 x 0.1-0.2	Anticlinally divided in two to three rows
	Callogenesis	Spheroidal	1.0-1.5	-	Meristematic zones in the pheripheral region in mass meristem form.
Storage Parenchyma	+ve	Fusiform to Ovate	- 0.4-2.0	0.8-1.2 x 0.2-0.7	No inter cellular spaces cells converted into fusiform and ovate
	Xylogenesis				
	Tracheids	Fusiform	-	3.0-1.0 x 0.3-0.7	Only tracheids in vertical cylinder singly in bundles present.

TABLE 15

GROWTH REGULATOR - IAA 0.5
IBA 4.0

AGE OF CULTURE - 2 WEEKS

(1)	(2)	(3)	(4)	(4)	(5)
Epidermis	+ve	Spheroidal	0.5-0.8		Single layer anticlinally divided.
Hypodermis	+ve	Hexsagonal	0.3-0.5		Two to three layer in periclinally divided.
	Calloganesis	Hexsagonal to tubular	0.5-0.7	-	Upon the epidermal border meristematic zones in protubrances form present by dividing in all planes.
				1.0-2.2 x 0.5-0.8	
	Xyloganesis				
	Tracheids	Fusiform	-	0.7-2.0 x 0.1-0.6	Frequence of tracheids very low.
	Metaxylem	Rounded	0.3-2.2	-	Tracheary elements are well developed.
	Protoxylem	Hexsagonal	0.4-0.6	-	Vascular bundle scattered surrounded by endodermis layer.
	Endodermis	Spheroidal	0.7-1.0	-	-
Storage Parenchyma	+ve	Tubular to Ovate	0.5-1.5	1.5-3.0 x 0.5-2.0	Direct change from isodiametric into tubular and ovate.

TABLE 16

GROWTH REGULATOR - IAA 0.5
IBA 5.0

AGE OF CULTURE - 2 WEEKS

(1)	(2)	(3)	(4)	(5)	(6)
Epidermis & Hypodermis	+ve +ve				Epidermis and hypodermis are completely covered with callus.
	Calloganesis	Spheroidal to Square Shape	0.3-0.7 0.2-0.7	- -	Meristematic zones are present on the epidermal border and hypodermis region.
Storage Parenchyma	+ve	Spheroidal	0.5-0.8	-	Storage parenchyma are completely converted into rounded cells. Cells near the peripheral region are compact but in the centre are with small spaces between them.
	Xylogenesis				
	Tracheids	Fusiform	-	1.2-1cm x above 0.4-0.9	Tracheary elements are well developed.
	Vessels	Rectangular	-	0.5-1.0 x 0.2-0.4	Tracheids are in branched bundles with vessels. Vascular bundles are scattered, surrounded with endodermis cells, which are loosely arranged.
	Metaxylem	Rounded	1.2-1.7		
	Endodermis or Bundle sheath	Ovate	0.5-1.2		

TABLE 17

GROWTH REGULATOR - IAA 0.5
NAA 0.5

AGE OF CULTURE - 2 WEEKS

(1)	(2)	(3)	(4)	(5)	(6)
Epidermis	+ve	Spheroidal	0.3-0.7		Single layer anticlinally divided cells without spaces.
Hypodermis	+ve				
	Calloganesis	Spheroidal	0.8-0.9		Meristematic zones are developed on the peripheral parts of sections.
		Isodiametric and Tubular	0.4-1.3	0.6-1.5 x 0.4-1.0	Variety of callus present with mass meristem and rib meristem.
Storage Parenchyma	+ve	Spheroidal	1.2-3.5	-	Direct conversion from hexagonal to spheroidal with compact arrangement.
	Xyloganesis				
	Tracheids	Fusiform	-	1.8-4.0 x 0.1-0.2	Frequence of tracheids is very low. Vascular bundle are scattered and rare in number. Bundle sheaths are present. Cells are rounded with loose arrangement.
	Metaxylem	Rounded	2.0-4.0	-	
	Endodermis or Bundle sheath	Spheroidal	2.0-2.2	-	
	Embryogenesis	Ovate	2.0-2.5	-	Embryogenic cells with one or two nucleus in each cells and thick wall in the central region.

TABLE 18

GROWTH REGULATOR - IAA 0.5
NAA 1.0

AGE OF CULTURE - 2 WEEKS

(1)	(2)	(3)	(4)	(5)	(6)
Epidermis	+ve	Spheroidal	0.4-0.6	-	Single layer, anticlinally divided.
Hypodermis	+ve	Hexsagonal	0.3-0.8		Hypodermal cells are similar with storage parenchyma difference in their sizes and their compact arrangement.
	Xylogenesis				
	Tracheids	Fusiform	-	2.2-2.5 x 0.1-0.3	Tracheary elements are developed.
	Metaxylem	Rounded	2.5-2.9	-	Tracheids and vascular bundle are in low frequence and without endodermis.
	Protoxylem	Isodiametric	0.1-0.3	-	
Storage Parenchyma	+ve	Isodiametric to spheroidal	1.2-2.5 1.4-1.9		Very low frequence of transformation into spheroidal cells.

TABLE 19

GROWTH REGULATOR - IAA 0.5
NAA 2.0

AGE OF CULTURE - 2 WEEKS

(1)	(2)	(3)	(4)	(5)	(6)
Epidermis and Hypodermis	-ve	-	-	-	Epidermis and Hypodermis are not clear due to the formation of compact callus cells.
	+ve	-	-	-	
	Calloganeces	Spheroidal	0.5-0.9	-	Meristematic zones are developed in the peripheral region. Pattern of cell division are in all planes in the form of mass meristem.
Storage Parenchyma	+ve	Spheroidal	0.6-2.0	-	Compact cells arrangement in rounded shape from Hexsagonal.
	Xylogenesis				
	Tracheids	Fusiform	-	1.2-1.0 x 0.1-0.3	Only tracheids are present in scattered and in small bundles in the sections without endodermis.

TABLE 20

GROWTH REGULATOR - IAA 0.5
NAA 3.0

AGE OF CULTURE - 2 WEEKS

(1)	(2)	(3)	(4)	(5)	(6)
Epidermis and Hypodermis	-ve	-	-	-	Epidermis and Hypodermis are not clear due to the callus formation.
	+ve	-	-	-	
	Callogenesis	Isodiametric to Spheroidal	0.5-0.7 1.0-2.5	- -	Arrangement of these meristematic cells are in mass and rib meristem in the central region and on epidermal border.
Storage Parenchyma	-ve	Hexsagonal	1.6-2.0	-	No change.
	Xylogenesis				
	Tracheids	Fusiform	-	5.5-1.0cm above 0.2-0.5	Tracheary elements are not developed.
	Metaxylem	Rounded	1.3-2.9		Tracheids are in bundle form, small vascular bundles scattered in the sections. Endodermis cells are not present.

GREEN HOUSE & NURSERY STUDIES ON P. VERA

A. P. vera plants, raised by seeds in Green-House conditions were reported previously as flourishing well in field conditions, on transfer at the age of 4 months. The plants of lesser age i.e. less than 4 months, did not survived under field conditions probably due to high temperature and humidity.

These plants are now 5 - 6 ft high, healthy and the stem started hardening. The plants and the soil was monthly sprayed with insecticide 'pay-off' as a precautionary measure to avoid any insect attack. However, in humid months of July - August plants in field suffered an attack of mites. Simultaneously a fungus was observed infecting the leaves. In the beginning leaves showed burning scars and later on these scars gradened and damaged whole leaves, which ultimately started growning and falling off.

Mites attack was controlled with periodic sprays of another insecticide 'Danitol'. Whereas the burning sign on leaves were damaging the foliage completely. The leaf samples from the nursery grown plants were taken to Agriculture Department for diagnosis and its effective treatment. A fungicide "Dithane M-45" was suggested by Agriculture Department. The plants were first

weekly sprayed, later by 10 days and now two sprays a month are continued. The disease is under control. New leaves and branches have started sprouting.

B. Leafless branches, which were procured from mature, fruiting trees growing in Quetta. 50% of these branches/stem cuttings were reported to sprout in the previous report. These cuttings are now developed into healthy plants with well developed branches and leaves. A few of them were tried for shifting from Green House to the field. Unfortunately, due to high temperature and humidity these plants died on shifting. The remaining plants which were left in the Green House are healthy and have attained a height of 4 ft, with well developed branches. They will be shifted to the field, as soon as the environmental conditions are favourable.

C. Nutrient Requirements of *P. vera* Plants

Already growing plants in Green-House and Nursery were in a limited numbers and were of variable age and size. Therefore, for study of the nutritional requirements of *P. vera*, it was planned to raise some sized and aged seedlings in a great numbers, to serve as experimental material. The following methodologies were used with results obtained are given:

i) Direct sowing of seeds in the soil

This method was effective, when great numbers of viable seeds are available as observed in our previously reported study on sowing *P. vera* seeds in Green-House beds. During present study we had a limited numbers of seeds in our stock and there were every chance of their being lost in the soil. Therefore, to avoid risk this method was not tried.

ii) Culture in Agar-Tubes

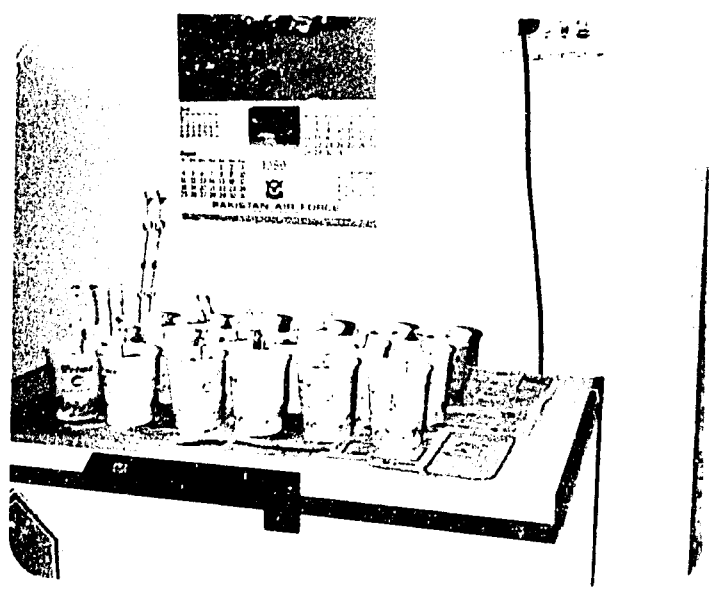
This was a bit safer method with limited number of seeds. Mercuric chloride of 0.1% was used for the surface sterilization of seeds. After sterilization all the mercuric chloride was thoroughly washed away, under aseptic conditions, with sterilized distilled water and the seeds were cultured on 0.7% Agar-tubes. pH of the tubes was set at 5.8. The tubes were incubated at 24°C. Fifty percent of seeds germinated in agar tubes. The seedlings developed from root system, when they were 4 cm large and were given hardening treatment (p.16 Proj.Rep Oct 88 - Mar 89), before transfer to Green-House in plastic bags of size 5" x 18".

iii) Embryo Activation

As the germination percentage was not sufficient enough in method (ii) above, therefore, in further study, the embryos were first activated by soaking seeds of *P. vera* on moist cotton, in petri-dishes, under sterile conditions. The embryos were then isolated from seeds and cultured on MS medium containing 1 mg/l IAA, 0.5 mg/l NAA and 3% sucrose. This method gave enhanced seed germination percentage i.e. it was raised from 50 to 80%.

iv) Pre-Germination

In another experiment, the seeds were Pre-germinated on moist cotton at low temperature (10 - 14^{0}C) and were thereafter sown in sand. The sand pots were kept at room temperature and watered occassionally to keep the sand moist only. This method gave 80 - 90% seedlings.



Seedlings raised by Pre-Germination Method (iv)

By the experiments described above (ii - iv) we were able to germinate P. vera seeds and obtained seedlings at the rate of 80 - 90%. These seedlings were transferred to Green-House for further development. Unfortunately due to electric load-shedding the cooling system of Green-House was not under control. High temperature prevailing in the Green-House killed almost all the seedlings. Moreover, new stock of seeds could not be available before Oct - Nov. Therefore, further studies on raising seedlings and their transfer to Green-House was stopped till the availability of new stock of seeds.

D. Standardization of Growth Conditions

For healthy and equal size growth of P. vera seedlings in G Green-House, the following growth conditions were identified.

1. Soil
   i) Texture
   ii) pH
   iii) Mineral composition.
2. Temperature
3. Illumination
4. Humidity

The soil in the Green-House is all silt and outside in fields it was mostly clay. Therefore, for filling plastic-bags to raise seedlings, the silt, clay and sand was equally mixed and cleared of all gravel etc. This mixed soil was filled in Green-House plastic bags and was used as standard soil.

The texture of standard soil as analysed by hydrometer

gave following compositions, as an average of 3 samplings :

Sand	70%
Silt	18%
Clay	12%

Similarly, the pH found by colour indicator method was 6.8 (an average of 3 samples). Mineral composition for N, P, K, Ca, Mg, Na, Cl, Mn, B & Zn is still to be determined for the standardized soil. Temperature, light and humidity factors could not be ascertained due to the shortcommings as irregular supply of electricity, due to load shedding etc.

ABBREVIATIONS:-

2,4-D-2, 4-Dichloro phenoxy acetic acid;

NAA - Napthalene acetic acid;

IBA - Indole butyric acid;

BAP - Benzyl amino purine;

KIN - Kinetin;

2iP - 2 Isopentenyl adenine;

GR - Growth Regulators;

WPM - Woody plant medium;

MS - Murashige and Skoog medium;

FAA - Formallehyde, Adetic acid, alcohol preservative;

+ve - Positive;

Vol - Volume;

Dia - Diameter;

L - Length;

W - Width;

wt - Weight;

FW - Fresh weight;

GRs - Growth regulators.

FISCAL REPORT FROM 1ST OCTOBER 1987 TO 30TH SEPT. 1989

HEAD	Allocated Budget				Amount Obligated	Amount spent upto 30.9.89	Balance on 1.10.89	Budget Estimate 1.10.89 to 31.3.90	Amount required for next six months
	1st yr	2nd yr	3rd yr	Total for 3 yrs.					
SALARIES AND BENEFITS	165257.00	173689.00	181679.00	520625.00	252909.47	235437.47	17472.53	90000.00	72528.47
CAPITAL ASSETS	500000.00	-	-	500000.00	500000.00	285549.60	214450.00	-	-
CHEMICALS AND GLASSWARE	42500.00	21250.00	21250.00	85000.00	76325.00	72352.00	3972.65	14223.00	10250.35 10250.35
T.A. AND TRANSPORT	25500.00	17000.00	17500.00	60000.00	29547.27	26327.27	3219.73	15000.00	11780.37
MISCELLANEOUS	34000.00	51000.00	-	85000.00	63823.00	51820.04	12002.96	20000.00	7997.09

EXPENDITURE DURING 1ST APRIL 1989 TO 30TH SEPT. 1989

MONTH	Salaries & Benefits	Capital Assets	Chemicals & Glasswares	T.A. and Transport	Miscellaneous	TOTAL
APRIL	14893.00	-	5300.00	1000.00	2060.00	23253.00
MAY	11500.00	-	-	-	13330.00	12833.00
JUNE	11500.00	-	-	1000.00	4550.20	17050.00
JULY	11800.00	-	-	-	9711.85	21511.85
AUGUST	11210.00	5000.00	-	1500.00	-	17710.00
SEPT.	10000.00	3000.00	1728.00	7000.00	18650.00	40834.00
TOTAL	70903.00	5000.00	7028.00	10500.00	36311.05	132742.05