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INDUCED HISTOLOGICAL CHANGES IN *PISTACIA VERA* L. COTYLEDONARY TISSUE

SHAISTA JABEEN, NASREEN ZAIDI, FERHA ZAFAR, MUHAMMAD ZAFAR IQBAL*, AND F.H.SHAH
PCSIR Laboratories Complex, Lahore-54600, Pakistan.

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Pistacia vera cotyledonary explants cultured on MS-Murashige and Skoog medium; WP-Woody plant medium; EK-Eriksson medium and SH-Schenk and Hildebrandt medium formed calli of different quantity and quality. Percentage gain in callus mass was maximum on WP medium, relatively double in amount as compared to the rest of the mediums, MS, EK or SH. Calli were white to dark brown in colour and nodular/friable in texture. Active growth of the calli ended in browning of the tissue which led to slow growing new white soft calli. Browning tendency of the cultures was same in case of slow or rapidly growing ones. Effect of replacing macro and micro nutrients of each on browning and callus mass gain was also studied. Interchange of MS and WP macro and micronutrients produced more calli as compared to those of SH and EK. Histological examination of the calli revealed various morphogenetic changes. Wound callus and xylogenesis were observed on combination of MS micro and WP macronutrients. While caulogenesis was observed on MS macro and WP micronutrient combination.

Key words: *Pistacia vera*, Tissue culture, Caulogenesis.

Introduction

Morphogenetic response of cotyledonary tissue of *Pistacia vera* L. seeds was reported in a previous communication [1]. The cotyledonary explants formed callus, roots and embryos; root explants of the seedling formed callus and roots while shoot explants of the same seedling formed callus only [1]. Somatic embryogenesis in *P. vera* cotyledonary explants was also reported by Jabeen *et al.*, [2]. In both instances growth regulators effectively induced juvinality in the mature cotyledons. Therefore, it was planned to explore some other possibilities by manoeuvring macro and micronutrients of different culture media and to observe *in vitro* response of *P.vera* cotyledons in more details.

Materials and Methods

Preparation of explants. Seeds of *P. vera* L. were obtained from dried nut shells treated with 90% alcohol and sterilized with 0.1% (w/v) mercuric chloride solution containing 2-3 drops of Tween-20 per 100 ml. These seeds were washed twice with sterile distilled water after 15 min. soaking in mercuric chloride.

Culture media. Four culture media formulations namely MS- Murashige and Skoog [3] medium; WP - Woody plant medium [4]; EK - Eriksson [5] medium and SH - Schenk and Hildebrandt [6] medium were tried. Macro and Micro nutrients of every basal medium were interchanged. Rest of the supplementation addenda included 3% sucrose 2.0 mg/l of 2,4-D** and KIN** each; 0.2 mg/l of 2ip and IAA** each. pH of the media were adjusted at 5.8 with the help of few drops of 0.1 N HCl/NaOH. pH was maintained before the addition of 0.65% agar. 25 ml of the medium were poured in each 100 ml culture flask, plugged with cotton and autoclaved at 15 lbs psi for 15 min.

Culture conditions. Cultures were incubated at 25 ± 2° for 16 h photoperiod provided by cool white flourescent light of 800 - 1000 Lux intesity.

Histological examination. Culture were fixed in FAA (Formalin + acetic acid + 70% ethanol, 5 : 5: 90 v/v), dehydrated in butanol ethanol series and embedded in paraffin wax. Wax blocks were prepared and cut 10 μm thick section with microtome.The sections were mounted on glass slides, de-waxed and stained with safranin and iron hematoxylin (Heidenhain's Hematoxylin) as suggested by Johansen [7]. Sections were examined under Olympus microscope provided with photomicrographic attachments.

Results and Discussion

The realization of totipotency in plant tissue formulation of a versatile culture medium has remained a favourite subject to plant physiologists. In the history of tissue culture a large number of media are reported for various plant species. Media are generally formulated to meet the nutritional requirements of the tissue. Various concentrations of macro and micro-nutrients which are necessary for growth and development of the plant tissue were employed. Selection of four different media included MS, WP, EK or SH medium for cotyledonary explant cultures of *P. vera*. MS is a very well accepted medium suitable particularly for herbaceous flora and generally tried for all kinds of plants. WP medium is especially designed for woody plant tissues. This medium formulation contained high concentration of $Ca(NO_3)_2$ nec-

*Institute of Chemistry, University of the Punjab, Lahore-54000

essary for high lignin content of the woody plant tissues. SH medium is recognised as low salt medium, while EK medium has been reported suitable for pistachio tissue [8].

The *P.vera* cotyledons cultured on these media formed calli of different quality and quantity, observations were taken after four weeks (Table-1). Maximum gain in callus mass was 816% on WP medium compared with initial weights. Gain in case of EK, MS and SH media was 362%, 313% and 221% respectively (Table 1). Calli were white in colour and nodulated or hyaline in case of Ek and MS medium but honey to dark brown colour without any change in the texture of the calli grown on SH medium. Browning in calli occured in all the experimental plots that were tested. It was tried to control browning by exchanging macro-and-micro nutrient of four media.

TABLE 1.EFFECT OF MEDIA COMPOSITION ON CALLOGENESIS OF EXPLANTS OF *P. VERA.*

Media Formulation	Callus growth		
	Colour	Texture	Gain in fresh weight (%)
MS	White	Nodular	313
WP	" "	" "	816
EK	" "	Hyaline	362
SH	Honey coloured	Soft	221

Effect of replacing micronutrients of MS medium with those of WK, EK or SH medium has less effect on callus gain (Table2). The combinations of macronutrients of MS and WP medium and micronutrients of MS, WP, EK showed much better growth of callus than another combinations. The highest gain of callus was obtained in the combination of MS macro and WP micronutrients (Table 2).

TABLE 2. EFFECT OF INTERCHANGED MACRO AND MICRO NUTRIENTS OF THE FOUR MEDIA ON GAIN IN FRESH WEIGHT (G/EXPLANT) OF CALLUS MASS.

Micro nutrients of four media	Macronutrients and Organic nutrients			
	MS	WP	EK	SH
MS	2.1971	1.2893	1.0731	1.0512
WP	2.7959	2.4120	1.0531	1.0935
EK	1.8213	1.5913	1.0321	0.0372
SH	1.5321	0.9973	0.8928	1.1812

Two growth phases were observed during a period of callus culture. Callus cultures showed vigorous growth in first four to five weeks, then slowed down the growth rate and started browning. Browned callus, however, could be subcultured and indeced new white callus again. It was also seen that brown callus did not die but used to produce new white calli.

Morphological features of callus and histological study. Calli were used to evaluate morphological changes during culture period. Morphological changes in callus were observed mainly on the medium containing WP macronutrient and other four media's micronutrients and vitamins. Loose callus (Fig. 1) tracheal structures (Fig.2) and root primodium-like structure (Fig. 3) were observed on the media containing WP macronutrients and MS micronutrients and vitamins. On the other hand, shoot tip primordium was observed on the medium containing MS macronutrients and WP micronutrients and vitamins.

Loose callus induction. Epidermal region of cotyledonary explants ruptured during early stages of culture. Cracks along the surface are the signs of beginning of browning due to phenolic compounds discharged from the explants. Then callus formation was observed on the abaxial sides of the cotyledons. Therefore, the callus induction might be stimulated by the cracking of epidermal tissue (Fig. 1).

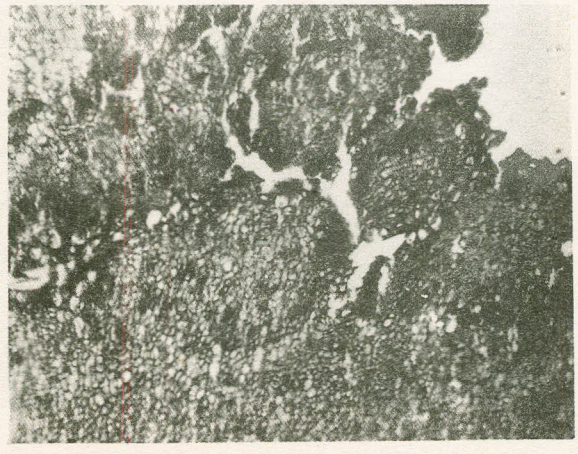

Fig.1. Cross-sections of *in vitro* cultured *P. vera* cotyledonary tissue showing loose callus.

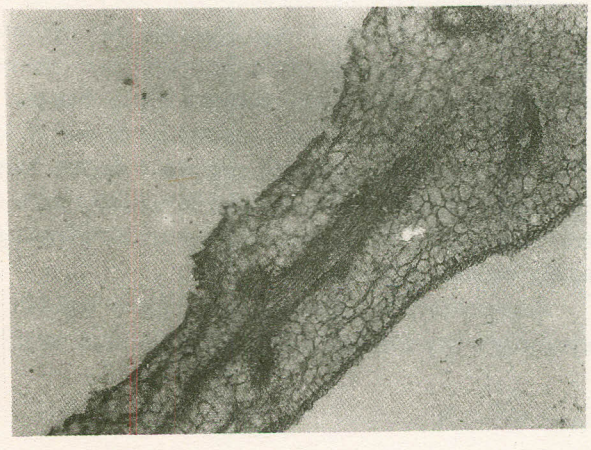

Fig.2 . Cross-sections of *in vitro* cultured *P. vera* cotyledonary tissue showing tracheal elements.

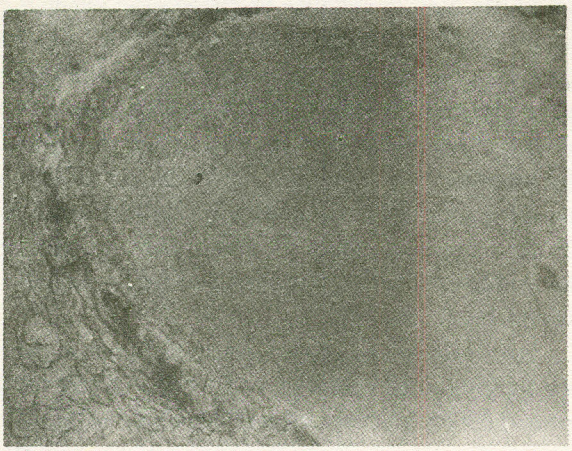

Fig.3. Cross-sections of *in vitro* cultured *P. vera* cotyledonary tissue showing root primordium.

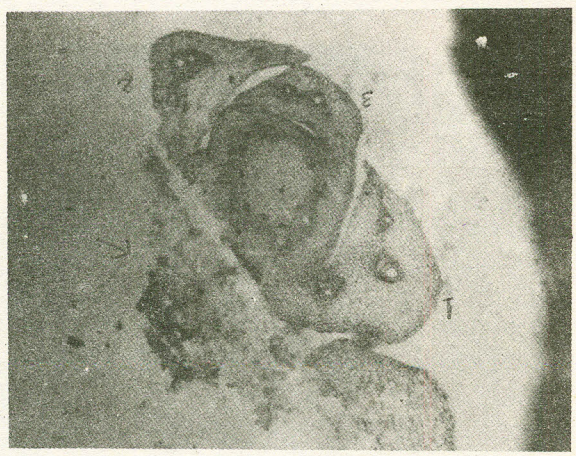

Fig.4. Cross-sections of *in vitro* cultured *P. vera* cotyledonary tissue showing regenerated shoot.

Presence of hormonal balance among 2ip, IAA, KIN and 2,4-D (0.2, 0.2, 2.0 and 2.0 mg/ l) respectively with macro nutrients of WP medium and micronutrients and vitamins of MS medium exhibited further morphogenetic changes.

Tracheal structure. Darkly stained callus next to loose callus formed radially arranged compact columns. Smaller sized cells occassionally formed tracheal structure in the core of column (Fig. 2). Vaccuolated and lightly stained cells grew rapidly without organogensis.

Root primordium-like structure. Figure 3 shows root primordium like structure differentiated in large vacculated cells of callus mass. A close observation of the figure reveals presence of central core of elongated cells forming immature endodermis. Lightly stained small cells concentrically arranged layers around the proximate end of the tip exhibits cortical portion of the root primordium. Between the root tip and the large vacculated tissue there are at least two layers of slightly larger cells covering the cortical portion of the tip which can be identified as poorly developed root cap tissue.

Shoot bud. Transverse section of callus showing *de novo* shoot bud is shown Fig.4. It formed on the abaxial surface of the cotyledonary tissue. There are three sets of large leaves arranged in opposite decussate manner. Each leaf shows distinct vascular zone of mid rib and side veins. Vascular bundles of young attached leaves seem to be the part of the vascular system of the main shoot axis.

The impact of making alteration in macro and micronutrients of the media had pronounced effect on morphogenesis similar to the growth regulators. The *in vitro* cultured cotyledonary tissue of *P. vera* had similar behaviour as exhibited by *Cocos nucifera* explants [9].

***Abbreviations*. 2,4-D-2, 4-Dichlorophenoxy acetic acid; KIN - Kinetin, IAA- Indol acetic acid; 2ip Isopentyladanine; MS - Murashige and Skoog medium; WP - Woody plant medium; EK- Eriksson medium; SH - Schenk and Hildebrandt medium.

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