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# CALLUS FORMATION FROM THE MESOCARP TISSUE OF *PISTACIA VERA* L.

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This communication reports callogenesis from mesocarp tissue of *P. vera* and a solution of polyphenol oxidation problem by serial subculturing of the explants. Immature fruits were obtained from Department of Agriculture Quetta and stored in cool dry place at 5°. 7-8 mm pieces of mesocarp tissue were peeled off from the stones, followed by surface sterilization with ethanol for 1 minute. Explants were immersed in 0.1% $HgCl_2$ (containing few drops of Tween 20) for 15 minutes, then rinsed three times with distilled sterilised water and incubated on culture media. The culture medium contained MS (Murashige and Skoog [5]) macro and micro elements, sucrose 3%, and Merck agar 0.7%. The concentration and combinations of growth regulators mentioned in Table were selected from the results collected during studies conducted on *P. vera* mature cotyledons to obtain friable calli for cell culture purposes (Ahmad *et. al.* [8].) The pH was adjusted to 5.7 before autoclaving and 20-25 ml of medium was dispensed per 100 ml flasks. Cultures were maintained at 26 ± 1° under 3 Klux of light for 16 hrs from cool white fluorescent tubes. Explants were subcultured after every 9-10 days.

TABLE 1. CALLUS INDUCTION FROM MESOCARP TISSUE.

| Composition of growth regulators | | | | |
|---|---|---|---|---|
| NAA (mg/l) | 2, 4-D (mg/l) | Kin (mg/l) | Quantity | Remarks |
| 0 | 0 | 0 | – | |
| 2 | – | – | – | |
| 5 | – | – | – | |
| 2 | 4 | – | – | |
| 1 | 4 | 2 | + | |
| 2 | 4 | 2 | +++ | Nodular: compact callus. |
| – | 3 | 2 | ++ | Greyish white callus. |
| – | 2 | 2 | + | |
| 2 | – | 2 | – | |

+ Fair; ++ Good; +++ Very Good; – Absent

Mesocarp explants did not form any cellus on medium devoid of any growth regulators and containing only 2, 4-D or NAA (Table 1). The addition of Kinetin was observed to promote callogenesis. Calli were greyish-white, nodular and compact. True calli were formed with 4 mg/1 2, 4-D + 2 mg/1 NAA + 2 mg/1 Kinetin (Fig. 1). The absence of NAA slightly retarded the rate of callogenesis. Calli were also obtained with 3 mg/1 2, 4-D + 2 mg/1 Kin as shown in Fig. 2 and 2, 4-D (2 mg/1) + Kin (2 mg/1). The initiation of callus took place in approximately 70-80 days.

Culture establishment of *P. vera* is considered difficult (Zimmerman [7]). One of the reason being the discharge of polyphenolic compounds as a reaction to wound/cutting. The inhibitory effect of polyphenols causes localized death of tissue coming in contact with these substances. Dodds and Roberts [4] have recommended the subculturing of the explants to a fresh medium on signs of enzymatic browning. Similarly the tissue and medium discoloration in thornless blackberry culture were effectively controlled by Broome and Zimmerman [3] when the shoot tip explants were transferred to fresh medium 1-2 day after initial cul-

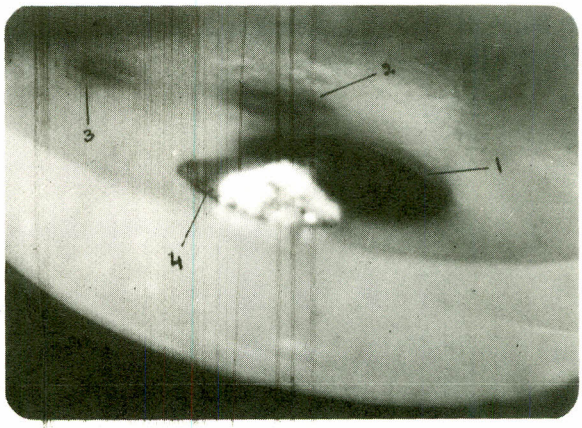


Fig. 1. (a) Callus formation from mesocarp tissue on medium containing 2, 4-D (4 mg/l) + NAA (2 mg/1) + Kinetin (2 mg/1).
(b) Dark spots in the back-ground indicates the number of subcultures (1-4).

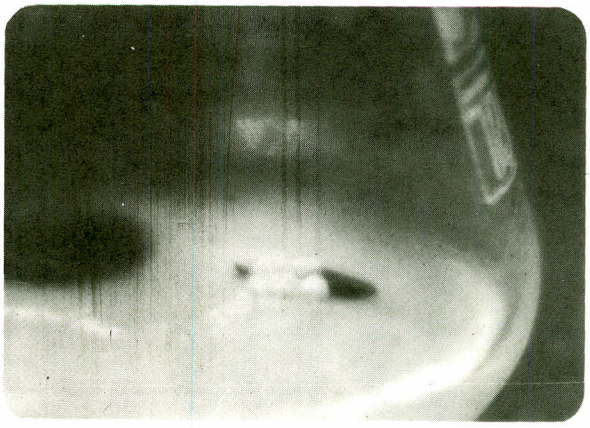

Fig. 2. Callus formation from mesocarp tissue on medium containing 2, 4-D (3 mg/l) + Kinetin (2 mg/l).

turing. Repeated subculturing of the *P. vera* explants proved to be effective to overcome the problem. Amount of polyphenolic discharge from the explants during incubation reduced after each subculturing of 9-10 days. It was judged from the fact that the area of dark brown stain on nutrient medium in contact with the explant reduced from approximately 1 cm dia to non detectable size after third or fourth subculture (Fig. 1). The inhibitory effect of polyphenols was confirmed by the fact that, it was only the oxidation of polyphenols that stopped those physiomorphogenic processes which lead to the formation of callus. It was observed that polyphenols were inhibitory to callogenesis but definitely not toxic to pistachio explants. Callus initiation took place in 70-80 days. The sutdy was in continuation of a programme to establish cell culture technique for clonal propagation of *P. vera.*

*Key words:* Mesocarp callus, Polyphenoles, *Pistacia vera.*

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