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Anatomy study of shoot tips, shoots, flowers and immature fruits of *Salvadora persica* in vitro

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Abstract

Shoot tips, Shoots, flowers and immature fruits were used as explants. They were inoculated on MS medium supplemented with different concentrations of some growth hormones. In case of using shoot tips on MS +NAA (0.1 mg/l) + BA (1mg/l), groups of parenchyma cells were formed meristematic centers and small buds were regenerated after 8 weeks of culture. When they cultured on MS+NAA (0.1 mg/l) + BA (1.5mg/l) small parenchyma cells contain prominent nuclei, tracheids were observed. Shoots on MS+Z (0.5mg/L) + AC (0.5mg/l) only small size of parenchyma cells surrounded by thin cell wall. When flowers were cultured on MS+ NAA (0.5mg/l) + BA (0.5mg/l) different sizes and shapes of parenchyma cells and tracheids were detected. For immature fruits on MS+2,4-D (0.5mg/l) small size of parenchyma cells contain thin cell wall and schlerenchyma cells were seen, On MS+NAA (0.5) + BA (1mg/l) only small parenchyma cells surrounded by thin cell wall were observed.

Keywords

Savadora perscia, Shoot Tips, Shoots, Flower, Immature Fruits, In Vitro

1. Introduction

Salvadora persica belongs to family of salvadoraceae as evergreen shrubs or small trees with crooked trunks growing up to 5 m tall with gray or whitish stems, bark slightyroug their leaves oblong-elliptic to circular and opposite pairs, flowers greenish to yellow. Those trees avalible in different areas of the world as in Pakistan, Syria, Palestine, Cameron Chad, Nigeria, Sengal, Algeria, Angola, Egypt, Eritrea, Ethiopia, India, Iran, Jordan, Kenya, Libya, Malawi, Mali, Mauritania, Mozambique, Niger, Oman, Saudi Arabia, Somalia, Southand Africa, Sri Lanka, Sudan, Tanzania, Uganda,Yemen, and Zimbabwe (1). The *Salvadora persica*is widespread grow in valleys, on dunes and river banks.They prefer areas where groundwater is availble and adapted to alkaline or very saline soils, such as clay rich and soils without salt and drought resistance (2).

The *S.persica* contains a number of medical beneficial properties being brasive,anticeptics, astringent, detergent, and enzyme inhibitors (3). The chemical analysis of *S. perscia* revelaed the presence of carbohydrates, alkloids, sulfur, vitaminC, glycosides, silica, tannins, saponnins, flavonoids, sterols and volatile oils also there large amount of salts contain chlorine, resins, salvadourea, B-sitosterol, trimethylamine (4), (5).

The *Salvadora persica* used in agroforestry system as a shelterbelts and windbreak to protect farm habitation, gardens and orchards and help in land reclamation (6), (7). The studied plants also produce non adible oil that used in soap manufacture (8). The viability is very low (30%) and has high genetic variability (9).

There have been several reports concerning medical uses of *Salvadora perscia* parts: roots used for headaches, general body pain, gonorrhea, back pain, chest disease, stomach aches, rheumatism, fruits and leaves as astringent, anthelmintic, , liver tonic, caminative, deobstruent, aphorodisiac (3). Young stems used as tooth brushes siwak (5).

The aim of present work is to study internal strcture obtained from segement of shoot tips, shoots, flowers and

immature fruits were cultured on different combinations of MS medium of *Salvadora persica* in vitro.

2. Materials and Methods

Salvadora persica L plants were collected from Ghat region Wadi Tanzift, Akakos, 600 km south of Sebha city in Libya. The parts of shoots, flowers, immature fruits were sterilized in laminar flow using 70% ethyl alcohol(v/v) for 1 min,then transferred to a petri-dish 1% sodium hypochlorite for 5 min. later they washed with sterile distilled water three times for 5 min each. The shoot tips were separated under binocular microscope 0.5 cm length.The explants were inoculated on Murashige and Skoog medium (10) supplemented with different concentrations of 2,4-dichlorophenoxy acetic acid (2,4-D), Naphthalene acetic acid (NAA), Benzyl adenine (BA), Zeatin (Z) and activated charcoal (AC). The medium supplemented with sucrose 30g/l, agar 8 g/l. pH was adjusted to 5.7-5.8 befor autoclaving at 121°C for 20 min. The culture were incubated in a dark growth chamber for 7 days at 25±2°C and they transferred to 8/16 hrs light regime (11).

Histological study was performed by fix the fragments of explants in formalin alcohol-acetic acid (FAA) for 24h and dehydrated by using series of alcohol and embedded in paraffin wax, later they sectioned by microtome(12µm), then stained with safranin, aniline blue and mounted in candabalsm.They photographed under microscope with Sony digital camera.

3. Results

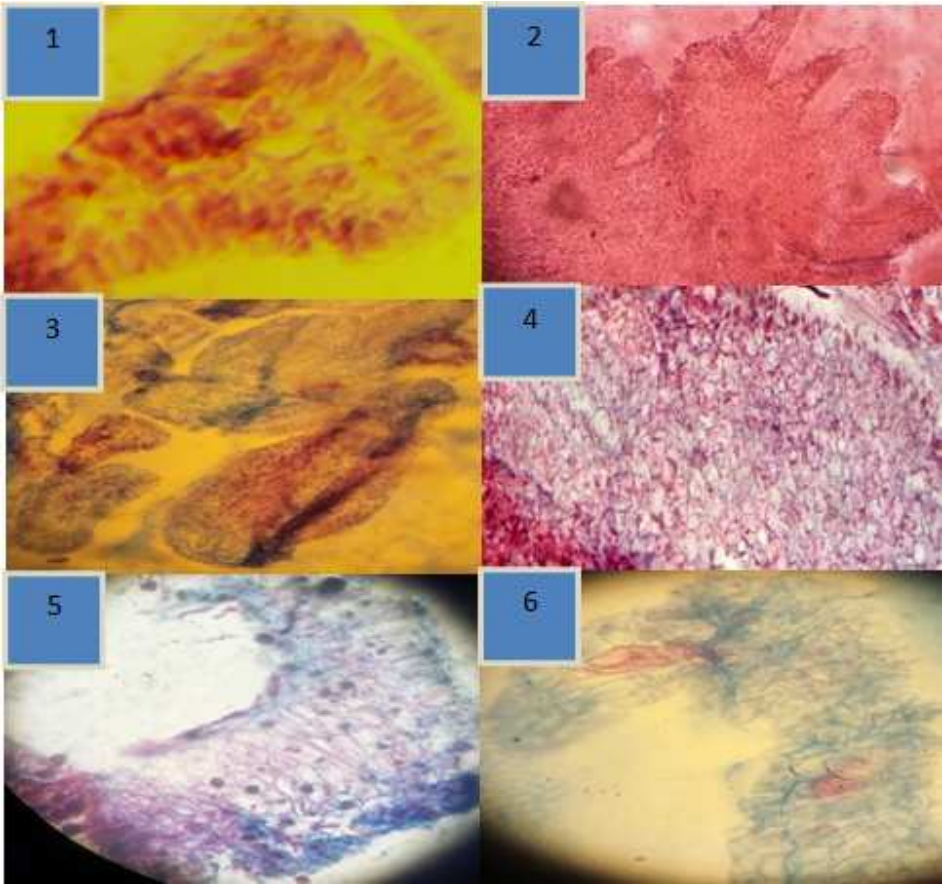

Fig. 1. *Globular buds on MS+NAA(0.1mg/l)+BA(1.5mg/l) from shoot tips. Fig. 2.buds and meristematic centres formed from shoot tips on MS+NAA(0.1)+BA(1mg/l).Fig.3.buds regenerated from shoo tips and tracheids on MS+NAA(0.1mg/l)+ BA(1.5).Fig. 4.small parenchyma cells with thin cell wall from shoots on MS+Z(0.5mg/l)+AC(0.5mg/l).Fig. 5.different size and shapes of parenchyma cells, trachedis from flowers on MS+NAA)0.5mg/l)+BA(0.5mg/l).Fig 6. Small cells with thin cell wall on MS+2,4-d(0.5 mg/l) from immature fruits.*

3.1. Shoot Tips

1. MS+ NAA (0.1mg/l) + BA(1mg/l).

Meristematic centres were formed from groups of parenchyma cells, they have a dense cytoplasm (fig1 ,table 1), buds were regenerated from prmodium leaves of explants after 8 weeks of culture (fig 2 ,table 1), also different size and shapes of parenchyma cells were observed.

2. MS + NAA(0.1mg/l) + BA(1.5mg/l). A small parenchyma cells contain prominent nuclei in outer part of the section, tracheids were observed in sections and small globular buds were formed from long

parenchyma cells (fig3, table1).

3.2. Shoots

1. MS+Zeatin (0.5mg/l)+Activated charcoal (0.5mg/l) small size of parenchyma cells contain thin cell wall were distributed through the sections (fig4, table1).

3.3. Flowers

1. MS+NAA (0.5mg/l)+BA (0.5mg/l), different size and shapes of parenchyma cells, tracheids were observed in sections, some of parenchyma cells showed dense stained with aniline blue stain, other cells showed weak stained. Small cells contain nuclei arranged in groups were seen in center of sections (fig5, table1).

3.4. Immature Fruits

1. MS+2,4-D (0.5mg/l), small size of parenchyma cells surrounded with thin cell wall contain different amounts of cytoplasm were seen in the centre of sections, longitudinal cells were also observed, schlerenchyma cells and tracheids also seen (fig6, table1).
2. MS+NAA (0.5mg/l) + BA (1mg/l), small parenchyma cells with thin cell walls through the section , some of them have dense stain, but others showed less staining.

Table 1. *Effect of concentrations of growth regulatros on shoot tips, shoots,flowers, immature seed(mg/l).*

Explants	NAA	BA	Zea	2,4-D	Ac	results
Shoot tips	0.1	1	-	-	-	meristematic centers,buds
Shoot tips	0.1	1.5	-	-	-	buds,trachids
Shoots	-	-	0.5	-	0.5	different size of parenchyma cells
Flowers	0.5	0.5	-	-	-	different size of parenchyma cells, tracheids
Immature fruits	-	-	-	0.5	-	schlerenchyma
Immature fruits	0.5	1	-	-	-	dense stain parenchyma cells

4. Discussion

According to literature data that application of micropropation technique on woody plants proved difficulties, that due to endogenous and exogenous level of hormones (12), (13) as our study plant considered desert plants.

The addition of BA to medium showed ability to form lateral branches in many plants, *Trchosanthes dioica* (14), *Rosmarius officinales* (15), *Morifolium* (16).

High concentration of 2,4-D increases the amount of callus induction in *calligonum comosum*.(17)

In the present study the Shoot tips proved the best explant among others used, as buds and meristematic centres were observed in this study.

Many reports concerning the shoot tips used as explants in different plants such as*phyllanthus amarus* (18), *Carica papaya*(19), *Vigna* (20).

Many workers confirmed that cotyledonary nodes play important role in induction of shoot when used as explants as they supply endogenous growth regulators to culturs (21),

Sen and sharma (22) used different concentration of NAA, BA to MS medium when different responses were recorded. Our study agree with (5) on *Salvador aperscia*, when used MS+NAA (0.5mg/l) + BA (0.5mg/l).

Saad and Megna (23) reported high amount of callus were obtained in the case of culturing shoot tips.

The addition of Zeatin and activated charcoal in present study gave good responses on MS+ Z (0.1,0.5 mg/l) + AC (0.5mg/l), that agree with study done by (17) on *Calligonum comosum*, *Maerua crssifolia* (24) .

5. Conclusion

It may be concluded that revealed success in regeneration of buds and meristematic centers from shoot tips of *Salvadora perscia* . This is sign of further organogenesis.

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