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The production of Abrusprecatorius plants by In vitro micropropagation.

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ABSTRACT

The aim of this work was to develop an efficient protocol for invitro propagation of Abrusprecatorius not only to its use as medicinal herb, but also for enabling conservation of this species. In this experiment, the effect of cytokinin (6-Benzyladenin (BAP) and Kinetin (Kin)) and their concentrations on shoot multiplication were evaluated. medium supplemented with 1.5mgl^{-1} (BAP)and (Kin.) alone resulted in high shoot regeneration by increasing the mean of shoots number up to 3.4 and 2.0 shoot per explant respectively. This study also investigates the effects of Indole-3-Butyric Acid (IBA) and Naphthalene Acetic Acid (NAA) separately on invitro rooting, Results indicated that half strength MS medium with 0.9 mgl^{-1} IBA significantly enhanced the number of roots per shoots, rooting percentage up to (1.4 root, 80%)respectively. While the same strength of salt supplemented with 0.9 mgl^{-1} NAA increased the number of roots per shoots, rooting percentage up to (0.5 root, 60%) respectively

Keywords: Abrusprecatorius, propagation, cytokinin, root

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INTRODUCTION

Abrusprecatorius L. belongs to family Fabaceae. In English, commonly known as Crab's eye. Is a deciduous woody, climber, leaves alternately compound, flowers arranged in clusters, violet or pink (11). It can be easily recognized by shiny scarlet colored seeds with black mark at the base which considers the unique characteristic of this plant, and it produces stout and short brownish pods, which curl back on opening to reveal dangling red and black seeds, 4 to 6 peas in a pod. It is native to India, from the Himalayas, but now grows in all tropical regions over the entire world. (9).

Since last long this plant species has been in use for its medicinal value, which contains various kinds of alkaloids which impart its medicinal value (13). The herbalists usually use roots, leaves and seeds of A. precatorius to induce skin diseases, pains and abortion.(7, 6, and 3).

In nature the propagation of *A. precatorius* through seeds is difficult because the seeds of this plant are dormant after shedding due to its hard seed coat so cannot germinate without removing it (12). It's difficult to conserve the species in nature and cultivate them without any propagation strategies, which also meet up the demand of the traditional medicine industry. It is, therefore important to develop a protocol for *in vitro* propagation to save this important species from further depletion of its population.

Taxonomical classification

Kingdom : Plantae
Division :Magnoliophyta
Order :Fabales
Family : Fabaceae
Subfamily :Faboideae
Tribe :Abreae
Genus :*Abrus*
Species :*Abrusprecatorius*

MATERIAL AND METHODS

Seeds were collected from naturally grown plants at Kurdistan region, north of Iraq by plant genetic resources team, Seed Testing and Certification Directorate (STCD), ministry of agriculture, Baghdad.

Experiment was carried out from March 2017 to May 2018 in the laboratory of plant tissue culture, department of plant genetic resources, ministry of agriculture, Baghdad.

The fresh seeds were separated from matured fruits, and were carefully washed under running tap water followed by distilled water. Prior to inoculation, the seeds were disinfected in laminar flow chamber by using surface sterilized with commercial Clorox at 7% for 10 minutes. Thereafter, five washes were done, with distilled sterile water to remove all traces of contaminated elements.

For culture initiation, *Abrusprecatorius* sterilized seeds were cultured individually in test tubes (25×100 mm) containing MS hormone-free medium (10) with 3% (w/v) sucrose, followed by subculturing several times in the basal medium to reduce their exposure to diffused phenolics and then used for cultured on MS basal medium (1, 17), After 4 weeks of culture, Seeds were scored along the middle with a scalpel blade to remove the seed coat (16). All naked seeds were germinated after 2 weeks of culture and the *in vitro* seedlings were used as source of explants.

For shoot multiplication, shoot tips (0.5 cm) were cut and cultured on solidified MS media, supplemented with growth regulators (BAP and Kin.) used independently at different concentrations (0.0, 0.5, 1.0, 1.5, 2.0 and 2.5) mgl^{-1}.

Once the shoot buds developed, they were further cultured for rooting on half strength MS fortified with different concentrations (0.0, 0.1, 0.5, 0.9 and 1.4) mgl^{-1} of auxins (NAA and IBA) alone.

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All media were solidified with 0.7% (w/v) agar and their pH was adjusted to 5.7 before autoclaving at 121°C for 15 minutes. Cultures were incubated in a growth chamber at 20±1ºc with16 hours photoperiod and 1000 lux of light intensity and relative humidity maintained at 55%.

Statistical analysis:

The Statistical Analysis System- SAS (2012) program was used to effect of difference factors in study parameters. Least significant difference–LSD test (ANOVA) was used to significant compare between means in this study (14).

RESULTS AND DISCUSSION

Effects of BAP and Kin Concentrations on Shoots Number/Explant:

Table (1) shows the effect of BAP concentrations on shoot number/explant. During the culture period, the number of shoot/explant has increased significantly with increasing BAP concentration in MS medium, the highest average number of shoots/explant (3.4) was observed on MS medium supplemented with 1.5 mgL^{-1}BAP.While the lowest shoot number/explant (0.00) was noted in the control treatment.

The effect of Kin concentrations on shoots number/explant, Kin concentrations had no significant effect on shoots number/explant, the highest shoots number/explant (2.0) was observed in MS medium with 1. 5 $mg.L^{-1}$ Kin. Similar non-significant results were also obtained on medium supplemented without Kin (Control) and 2.5 $mg.L^{-1}$ Kin was observed (0.0).

Table 1: Effect of BAP and Kin concentrations on shoot multiplication After 4 weeks of the culture in MS medium

PGR Treatments	Concentration of PGR mg/L						
	0.0	0.5	1	1.5	2	2.5	
BAP	0.0	0.2	1.8	3.4	1.4	0.4	L.S.D 0.05 = 0.472
Kin.	0.0	0.0	0.8	2.0	0.6	0.0	L.S.D 0.05 = 0.390

BAP is oneof the most important cytokinins that influence the division and growth compared to other cytokinins. This activity is due to the internal structure and number of bonds in its side chain as well as the presence of benzyl ring, making it one of the most prominent cytokinins used in propagating many plant species, notethat it had been isolated as a natural cytokinin in a number of plant species(15).

Rooting Stage:

Effect of IBA and NAA Concentrations on Response Percentage % / Explant:

After 4 weeks of the culture on half salt strength MS medium, supplemented with IBA and NAA separately (table 2), Shoot pretreated 0.9 $mg.L^{-1}$ IBA auxins gave(0.839 ± 0.22 a %) rooting While the lowest root of percentage (0.00) was noted in control and 0.1 $mg.L^{-1}$ treatments. Whereas 0.9 $mg.L^{-1}$ NAA gave only 0.6 % rooting which was insignificantly. In general, a high concentration of auxin, especially NAA seems favor direct rhizogenesis, the highest percentage rooting was obtained with 0.9 $mg.L^{-1}$ within BAP 4 weeks of culture.

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Table 2: Root response percentage of shoots after 4 weeks of culture under different concentrations of auxin IBAP and NAA

PGR Treatments	Concentration of PGR mg/L					
	0.0	0.1	0.5	0.9	1.4	
IBA	0.0	0.2	0.6	0.8	0.4	L.S.D 0.05 = 0.344
NAA	0.0	0.0	0.4	0.6	0.2	L.S.D 0.05 = 0.251

Fotopoulos. and Sotiropoulos (5) have foundin 2005 that the reduction of mineral concentration of MS medium to half, gave rise to the increase in the rooting percentage of PR 204/84 explants when IBA concentration was applied at concentration of 2.0µM.

The promoting effect of the mineral concentration of the culture medium on rooting process can be attained as inorganic ions participate in the process of regulating hormonal balance (2).

Effect of IBA and NAA concentrations on root numbers/ Explant:

Table 3 and figure 1 shows the effect of IBA and NAA concentrations on root numbers after 4 weeks of culture at half salt strength MS medium, the effect of IBA concentrations on Root number/explant. During the culture period, the number of Root /explant has increased significantly with IBA concentrations (0.5 and 0.9mg.L^{-1}) which gave the highest average number of Roots/explant (0.8 and 1.4) root/shoot respectively.

NAA concentration (0.9mg.L^{-1}) had significant effect on average number of roots/explant compared with 0.0, 0.1 and 1.4mg.L^{-1}, which gave the highest average number of root/explant (0.5) and was non-significant results obtained on medium supplemented with 0.5 mg.L^{-1} NAA

Table 3: Effect of auxin type and concentrations on root numbers per shoot after 4 weeks of culture at half salt strength MS medium

PGR Treatments	Concentration of PGR (mg.L^{-1})					
	0.0	0.1	0.5	0.9	1.4	
IBA	0.00	0.2	0.8	1.4	0.6	L.S.D 0.05 = 0.429
NAA	0.00	0.0	0.4	0.5	0.2	L.S.D 0.05 = 0.282

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Figure 1: Abrusprecatorius plant has produced by Invitro micropropagation

Reducing the levels of salts in MS medium reduces nitrogen levels, which in turn increases the carbohydrate-nitrogen ratio, and this ratio increase indirectly helps root growth and increases their number (4).

Result revealed that appropriate concentration of NAA and IBA by treated shoots of Abrusprecatorius showed better average number of roots per shoot and it may be due to early differentiation of cells and enhanced cell elongation caused by auxin. Auxins initiate synthesis of structural enzyme protein in the formation of adventitious root thus increasing the root number through the process of acidification (8).

Effect of IBA and NAA on rooting was also analyzed in several studies which were efficiency on rooting in many species (3 and 8).

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