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Auxins Stimulated the Axillary Branching of *Atriplex nummularia* L. *In Vitro*

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ABSTRACT. The effect of three types of auxins; Indole-3-acetic acid (IAA), Indole-3-butyric acid (IBA), ∝-Naphtalene acetic acid (NAA), at various concentrations (0.0, 0.5, 2.0, 3.0, 4.0 mg/L) on axillary branching of *Atriplex nummularia* L. was tested using Murashige and Skoog medium (MS). *In vitro* shoots exhibited different response to the auxins. The maximum shoot proliferation rate was obtained at 0.5 and 2.0mg/L IBA. IAA levels higher than 0.5mg and the lowest level of NAA gave slightly lower shoot numbers compared with 0.5 and 2.0mg IBA. The control treatment and the highest levels of IBA and NAA produced the longest shoots. Largest callus diameter was observed at 4.0mg/L IBA and NAA. The best rooting occurred on media containing IAA and lower levels of IBA. IAA at 4.0mg/L yielded the highest rooting percentage. Rooted microshoots were successfully acclimatized in the greenhouse under the mist.

Introduction

Some species of the genus *Atriplex* have been used as good models in many physiological studies and the salt-tolerant mechanisms investigations (Kenny and Caligaro, 1996). *Atriplex nummularia* has been recently introduced to Saudi Arabia from Australia. It thrives and grows well in saline and drought lands of Saudi Arabia (Kandel, unpublished data). The species may play a great role in developing a vegetation in desert region. Moreover, the plants are considered to be good fodder due to their high nutritive value.

The classical method of propagating *Atriplex* is by cutting. Sexual propagation is not common because of variations imposed by heterozygosity. Tissue culture has been used to propagate some *Atriplex* species (Kenny, 1993; Kenny and Caligari, 1996).

Application of growth regulators is essential to enhance shoot multiplication *in vitro.* Cytokinins are usually added to culture media to promote shoot development, whereas auxins are used for root induction. Generally, auxins promote growth of terminal shoots

and inhibit lateral buds (Skoog and Miller, 1957). This study was conducted to determine the effects of auxins on axillary branching to obtain multiple shoots of *Atriplex nummularia* L. *in vitro.*

Materials and Methods

Tissue cultured shoots used in this study were established from nodal segment obtained from 4-year-old plants grown at the College of Agriculture and Veterinary Medicine, King Saud University, Research Station at Al-Qassim, Saudi Arabia. These shoots were subcultured and maintained in Murashige and Skoog medium (MS) (Murashige and Skoog, 1962) supplemented with 2.0mg BA/L and 0.1mg NAA/L. Shoots (20-25mm long) were cultured in 25 × 150mm test tubes containing 15ml MS provided with 30g/L sucrose, 7g/L agar (Micro Agar, DUCHEF Biochemicals, The Netherlands), and various levels of IAA, IBA or NAA at concentrations of 0.0 (the control treatment), 0.5, 2.0, 3.0, and 4.0mg/L. The pH of the media was adjusted to 5.7 using either 1.0N NaOH or 1.0N HCl prior to the addition of agar and autoclaving for 20min at 121ºC. The cultures were incubated in a 16h light/8h dark cycle at 25 ± 2ºC and were illuminated with cool-white fluorescent lamps with an intensity of 2500-3000 Lux. Completely randomized design was used and ten test tubes were assigned for each treatment. Rooted microshoots were transplanted into small pots filled with a sterilized mixture of 1 part peat-moss: 1 part perlite (v:v) and then initially covered by plastic bags for one week before moving them to a greenhouse where they were kept under mist to maintain high humidity level and then they were transferred to normal environment.

Shoot number, shoot length, root number, root length, rooting percentage, and callus diameter were recorded after 6 weeks. All data were subjected to analysis of variance using WINKS statistical data analysis program (TexasSoft, Cedar Hill, Texas, USA). Newman-Keuls test at the 5% level of significance was used to compare means.

Results

Shoots multiplication showed differential response to auxin types and concentrations (Table 1). Few shoots were obtained with auxin-free medium (the control). The addition of auxins enhanced dramatically axillary buds to form shoots. Maximum shoot proliferation rate occurred at 0.5mg IBA, followed by 2.0mg IBA (23.5 and 18.7 microshoots per explant, respectively). IAA levels, except at 0.5mg, and 0.5mg NAA gave slightly lower shoot numbers than the optimal levels of IBA (0.5 and 2.0mg). However, no significant differences were observed among these levels of auxins. IBA and NAA at the highest concentrations produced significantly the largest callus diameter and the longest shoots.There was no significant difference between the control and the highest levels of IBA and NAA in terms of shoot elongation (Table 1).

Rooting was initiated in all types of media. The highest rooting percentage (100%) was obtained at 4.0mg IAA and the lowest (40%) was with the control (Table 2). For other auxins concentrations, the rooting percentage varied from 66.7 to 90%. Levels of IAA higher than 0.5mg gave not only the highest numbers of roots but also normally developed roots with pronounced root hairs. Likewise, 0.5mg IBA gave a high number of

roots which were normal. NAA produced the poorest root formation compared with IAA and IBA. Microshoots rooted on media containing IAA and lower levels of IBA survived acclimatization (more than 55%) in the greenhouse better than those rooted in NAA.

TABLE 1. Shoot multiplication, shoot length and callus formation response of *Atriplex nummularia* L. cultured on MS medium with different auxins at various concentrations.

Auxin	Conc. (mg)	Shoot no.	Length of tallest shoot (mm)	Callus diameter (mm)
Control	Nil	5.9c[b]	72.4a	2.0c
IAA	0.5	9.7bc	24.5b	4.2bc
	2.0	16.5abc	26.4b	3.6bc
	3.0	16.6abc	25.7b	4.8bc
	4.0	14.8abc	24.0b	4.7bc
IBA	0.5	23.5a	32.7b	5.9bc
	2.0	18.7ab	27.7b	5.6bc
	3.0	10.0bc	22.0b	5.9bc
	4.0	12.4bc	87.7a	11.0a
NAA	0.5	15.4abc	27.3b	5.0bc
	2.0	10.9bc	21.9b	7.1b
	3.0	10.0bc	23.6b	5.7bc
	4.0	6.4c	70.8a	11.4a
Significance[a]		*	*	*

[a]*Significant by Newman-Keuls at the 5% level.
[b]Numbers followed by different letters are significantly different by Newman-Keuls at the 5% level.

TABLE 2. Effects of auxin types and concentrations on root number, root elongation, and rooting percentage of *Atriplex nummularia* L. *in vitro.*

Auxin	Conc. (mg)	Root no.	Length of tallest root (mm)	Rooting %
Control	Nil	1.0b[b]	10.7	40.0
IAA	0.5	4.3ab	9.3	80.0
	2.0	12.3a	19.7	90.0
	3.0	12.1a	18.5	80.0
	4.0	12.9a	19.0	100.0
IBA	0.5	10.4ab	35.4	90.0
	2.0	6.8ab	25.9	90.0
	3.0	4.8ab	19.4	80.0
	4.0	7.2ab	20.1	88.9
NAA	0.5	7.8ab	25.8	88.9
	2.0	5.2ab	9.6	90.0
	3.0	7.4ab	12.5	90.0
	4.0	7.4ab	11.8	66.7
Significance[a]		*	NS	

[a]NS not significant, *Significant by Newman-Keuls at the 5% level.
[b]Numbers followed by different letters are significantly different by Newman-Keuls at the 5% level.

Discussion

Generally, cytokinins are necessarily used *in vitro* to stimulate shoot proliferation for most plant species (Jacobsen, 1983). Results of this study showed that auxins remarkably promoted shoot formation from axillary buds of *Atriplex nummularia.* Moreover, shoots responded differently to auxin types and concentrations. Tisserat (1984) reported that low auxin levels stimulated axillary bud differentiation and outgrowth of date palm (*Phoenix dactylifera* L.), whereas the addition of cytokinins at any level did not enhance shoot differentiation. He also found that NAA was better than 2,4-D for culture establishment. In this study, IBA at 0.5 and 2.0mg were the best levels for maximum shoot proliferation rate, whereas IAA and lower levels of IBA developed better and normal roots than NAA which produced abnormal roots. NAA seems to be a stronger auxin and resulted in thick and abnormal root formation. Similarly, NAA was found to be unsuitable for root formation in many other studies with other species (Rajeevan and Pandey, 1983; Jusaitis, 1995; Iapichino, 1996; Amo-Macro and Liedo, 1996).

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تـأثيـر الأوكسينـات على التفريعـات الأبطيـة لنبـات الرغـل (*Atriplex nummularia* L.) في الأنـابيب

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القصـــيم - المملكة العربية السعودية

المستـخلص . تم دراسـة تأثيـر ثلاثة أنواع من الأوكـسـينات [أندول حمض الخليك (IAA) ، أندول حمض البيوتريك (IBA) ، ونفثالين حمض الخليك (NAA)] بتركيزات مختلفة (صفر ، ٠,٥ ، ٠,٢ ، ٠,٣ ، ٠,٤ ملجم/ لتر) على التفرعات الأبطية لنبات الرغل باستخدام بيئة موراشيجي وسكوج . استجابت النموات الخضرية في الأنابيب بدرجات متفاوتة لتلك الأوكسينات ، حيث سجلت أعلى معدلات تضاعف للنموات الخضرية الأبطية في البيئات المضاف إليها ٠,٥ أو ٠,٢ملجم/ لتر أندول حمض البيوتريك . أما تركيزات أندول حمض الخليك الأعلى من ٠,٥ملجم/ لتر وأقل تركيز للنفثالين حمض الخليك فقد أعطت إلى حد ما أقل عدد من النموات الخضرية . أطول النموات الخضرية لوحظت في البيئة الخالية من الأوكسينات (معاملة القياس) والبيانات المحتوية على أعلى تركيز (٠,٤ ملجم/ لتر) من أندول حمض البيوتريك ونفثالين حمض الخليك ، كذلك أعطى هذا التركيز العالي من هذان الهرمونان أعلى قطر للكأس المتكون في قاعدة الجزء النباتي . أما أفضل تجذير فقد حدث في البيئات المحتوية على أندول حمض الخليك أو التركيزات المنخفضة من أندول حمض البيوتريك ، حيث أعطى التركيز ٠,٤ ملجم/ لتر من أندول حمض الخليك أعلى نسبة تجذير . وقد تم أقلمة النباتات المجذرة في البيوت المحمية تحت الري الرذاذي .