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Research Paper

The Response of *Asparagus Densiflorus* L. *in Vitro* Propagation to Different Potassium and Ammonium Nitrate Levels in Culture Media

Rafail S. Toma[1, *] and Khetam A. Rasheed[1]

[1] University of Duhok, Faculty of Agriculture and Forestry, School of Plant Production, Department of Horticulture, Duhok, Iraq

* Corresponding author, e-mail: (rshtoma@uod.ac)

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Abstract: *A successful attempt was done through this study to ensure the significant effects of nitrogen concentration in culture media on the growth and development of Asparagus densiflorus L. plants under in vitro culture conditions. Different potassium and ammonium nitrate levels (1/4, 1/2, and 1/1 nitrates strength) were tested in all developmental micropropagation stages in this investigation (475 mg/l+ 412.5 mg/l, 950 mg/l+ 825 mg/l and 1900 mg/l+ 1650 mg/l). The results revealed that the higher nitrate media (1/1 Nt level) has produced higher number of shoots per explant, longest shoots and higher number of leaves per explant (15.02 shoot per explant, 5.83 cm and 23.54 leaves per explant respectively) as compared with the other ammonium nitrate levels at the combination between 1.0 mgl^{-1} BA and 0.1mg l^{-1} NAA. The best rooting formation parameters of Asparagus explants as numbers of roots per explants and mean length of roots (3.50 roots per explants and 4.08 cm) were recorded respectively when 1/2 ammonium nitrate level was used at concentration 1.0 mgl^{-1} IAA. The successful gradually acclimatized plantlets percentage reached to 90%.*

Keywords: *Asparagus densiflorus*, micropropagation, nitrate, growth.

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1. Introduction:

Nitrogen is one of the most important macronutrients elements for plant growth and development. It is a constituent of both proteins and nucleic acids and also occurs in chlorophyll. In plant tissue culture media, nitrogen sources are usually added in the form of potassium and ammonium nitrate

(George *et al*., 2008). In Murashige and Skoog (MS) medium, nitrogen is one of the prime components and significantly influences the growth and morphogenesis of *in vitro* plant culture (Gamborg and Shyluk, 1970). Asparagus plant *Asparagus densiflorus* is a member of Asparagus family *Asparagaceae.* It is native of South Africa (Lemake, 2011). *Asparagus densiflorus* is a branching perennial herb with tough green aerial stems which are sparsely covered with spines. The leaves are actually leaf-like cladodes, which are 0.8-2 cm long and 0.1-0.2 cm wide, and arise in groups of four or more from the stem. Occurring in spring, the small white or pinkish-white flowers are 0.3-0.5 cm long and arise in clusters off the stem. The root system is a mat of fibrous roots with bulbous tubers, from which plants may re-sprout (Wolf, 1999). Avila *et al*. (1994) tested different nitrogen sources (NO^{3-}, NH^{4+}, glutamic acid and their combinations) influenced the growth and morphogenic responses (node number, shoot length, and stem, leaf and root dry weight) of three micropropagated potato cultivars (Spunta, Kennebec, Huinkul). They found the influential role of nitrogen in the media as in agreement with Evans (1993). The total amount of nitrogen in a medium was shown by Roest and Bokelmann (1975) to affect the number of adventitious shoots formed directly on *Chrysanthemum* pedicels. The combined amount of KNO_3 + NH_4NO_3 in MS medium (60 mM), was adjusted while the ratio of NO^{3-} to NH^{4+} (66:34) was unchanged. From 30-120 mM total nitrogen was optimal. However there was clearly a strong effect of genotype, because the cultivar `Bravo' was much more sensitive to increased nitrogen than 'Super Yellow'.

The present investigation was carried out to determine the effect of key nitrate source (KNO_3 and NH_4NO_3) in MS basal media on micropropagation efficiency of *Asparagus densilororus*.

2. Materials and Methods

This experiment was conducted in Plant Tissue Culture laboratory of the Horticulture Department, School of Plant Production, Faculty of Agriculture and Forestry, University of Duhok, Iraq. The plant material for this study represented by *Asparagus* nodes were taken from *in vitro* grown plantlets under controlled atmosphere. The nodes were cultured on MS medium (Murashige and Skoog, 1962) supplemented with 0.4 mg/l Thiamine HCl, 1 mg/l BA, 30 g/l sucrose, 0.7% w/v agar and 100 mg/l Inositol for the initiation stage. The pH of the medium was adjusted to 5.7± 0.1 using 0.1 N HCl and/or 0.1 N NaOH prior to autoclaving at 121°C temperature and 15 lb pressure for 20 min. Three explants were inoculated per culture vessel. All aseptic cultures were maintained at 16 h photoperiod at 25± 2°C temperature.

Different potassium and ammonium nitrate levels (1/4, 1/2, and 1/1 nitrates strength) were tested in all developmental micropropagation stages in this investigation. These levels were 475 mg/l+ 412.5 mg/l, 950 mg/l+ 825 mg/l and 1900 mg/l+ 1650 mg/l from both potassium and ammonium nitrate respectively. At multiplication stage, different BA and NAA combinations in combination with the different potassium and ammonium nitrates were tested (0.0, 1.0, 1.5 and 2.0 mg/l BA) + (0.0, 0.1 and 0.2 mg/l NAA). After six weeks in culture media, the numbers of shoots and leaves per explant and the mean length of shoots were recorded. At rooting stage, difference levels (0.0, 0.5 and 1.0 mg/l) of different auxins (IAA, IBA and NAA) were tested in combination with the different levels of potassium and ammonium nitrates. After six weeks in culture media, the number of roots per explant and the mean length of roots were recorded. Ten replicates were assigned for each level of treatment and the experiment was designed according Completely Randomized Design (CRD). The comparison between means was carried out according to Duncan's multiple range test ($P < 0.05$) using a computerized program of SAS (SAS, 2001).

Finally, for acclimatization stage, a quite number of successfully rooted plantlets were removed from culture vessels and their roots were washed with distilled water and immersed in Benlate fungicide (0.1% for 10 min.). They were transferred to pots containing a steam sterilized soil mix (peatmoss+ loam+ Styrofoam 1:1:0.5, v:v:v) under tightly controlled atmosphere of the primary growth area.

3. Results and Discussion:

Table (1) shows that the best multiplication parameters for *Asparagus* explants as affected by different potassium and ammonium nitrate levels and different combinations of BA and NAA were recorded for 1/1 Nt level. Since, the higher nitrate media (1/1 Nt level) has produced higher number of shoots per explant, longest shoots and higher number of leaves per explant (15.02 shoot per explant, 5.83 cm and 23.54 leaves per explant respectively) as compared with the other ammonium nitrate levels at the combination between 1.0 mgl^{-1} BA and 0.1mg l^{-1} NAA. It is thought that cytokinins promote the formation of woody tissues neighboring to the vascular tissues of the bud and stem, thus will make the translocation of water and nutrients easier which cause bud initiation (Mohammed and Al- younis, 1991).

These results are in agreement with what has been obtained by Evans (1993) with different potato genotypes. But they are in contrast with Pierik (1987) and Pennell (1987) who suggested that MS medium contained higher levels of salt, particularly nitrogen, were too high for optimal growth. The effect of interaction between cytokinins and auxins on vegetative multiplication and increasing growth lengths can be interpreted by the increase of cytokinins role in the presence of auxins as Mohammed and Al- Younis, 1991 reported that the movement of cytokinins is generally activated in the presence of auxins, so a larger number of buds will have a chance to grow and start to produce shoots (Tran Thanh Ran, 1981 and Murashige, 1990). These results are in agreement with those reported by Roy *et al*. (2004), Nodoy *et al*. (2003) and Munshi *et al*. (2004), who emphasized the importance of the interaction between auxins and cytokinins in vegetative multiplication processes.

Table (1): Effect of different potassium and ammonium nitrate levels and different BA and NAA combinations on the multiplication stage of *Asparagus* explants after six weeks in culture

Ammonium Nitrate Levels	BA (mgl^{-1})	NAA (mgl^{-1})	Number of shoots/ explant	Mean length of shoots (cm)	Number of leaves/ explant
¼ NT	0.0	0.0	5.00 e	2.99 d	4.75 g
		0.1	8.00 d	3.38 d	8.25 f
		0.2	12.13 c	3.11 d	11.13 e
	1.0	0.0	12.75 c	3.76 d	12.00 e
		0.1	13.00 c	5.09 bc	16.25 d
		0.2	11.00 cd	4.73 c	15.13 d
	1.5	0.0	10.25 cd	2.84 d	9.13 ef
		0.1	7.13 d	4.29 c	9.75 ef
		0.2	5.13 e	4.28 c	10.25 ef
	2.0	0.0	6.00 de	3.78 d	11.00 e
		0.1	7.00 de	4.01 c	11.50 e
		0.2	6.00 de	3.61 d	10.88 e
Mean of ¼ NT			**8.62 c**	**3.83 c**	**10.84 c**
½ NT	0.0	0.0	5.13 e	3.03 d	4.88 g
		0.1	9.50 d	4.19 c	10.50 ef
		0.2	14.25 c	3.55 d	14.50 de
	1.0	0.0	13.88 c	4.06 c	11.38 e
		0.1	14.00 c	5.28 bc	18.38 d
		0.2	16.13 c	5.41 bc	19.63 d
	1.5	0.0	11.00 cd	3.53 d	11.38 e
		0.1	9.38 d	4.51 c	15.88 d
		0.2	11.25 cd	4.38 c	15.63 d
	2.0	0.0	7.00 de	4.40c	12.13 e
		0.1	8.00 d	4.08 c	12.50 e
		0.2	7.63 de	4.37 c	13.38 e
Mean of ¼ NT			**10.60 b**	**4.48 b**	**13.35 b**
	0.0	0.0	6.00 de	3.75 d	6.00 f
		0.1	12.38 c	4.94 bc	19.50 c
		0.2	15.25 c	4.60 c	13.38 e
		0.0	15.13 c	6.00 b	16.25 d

1/1 NT	1.0	0.1	26.50 a	8.21 a	40.50 a
		0.2	20.63 b	7.49 a	34.38 b
	1.5	0.0	13.00 c	5.00 c	19.75 c
		0.1	15.63 c	6.00 b	30.13 b
		0.2	15.13 c	6.01 b	29.25 b
	2.0	0.0	9.38 d	6.25 b	20.38 bc
		0.1	15.63 c	6.08 b	29.00 b
		0.2	15.63 c	5.58 bc	24.00 cd
Mean of 1 NT			**15.02 a**	**5.83 a**	**23.54 a**

***Different letters within columns represent significant differences according to Duncan's multiple range test at 5% level.**

Tables (2) reveals that the best rooting formation parameters of *Asparagus* explants as numbers of roots per explants and mean length of roots (3.50 roots per explants and 4.08 cm) were recorded respectively when 1/2 ammonium nitrate level was used at concentration 1.0 mgl^{-1} IAA. Such differences in the potency of auxin in inducing rooting might attributed to the structure of the auxins under study, the endogenous hormone level, as well as the genetic makeup of species under consideration (Toma, 2009).

Table (2): Effect of different potassium and ammonium nitrate levels and different IAA concentrations on the rooting stage of *Asparagus* explants after six weeks in culture

Ammonium Nitrate Levels	IAA (mgl^{-1})	Number of roots/ explant	Mean length of roots (cm)
¼ NT	0.0	1.60 d	2.48 c
	0.5	2.80 cd	2.86 c
	1.0	3.20 b	4.40 a
	1.5	3.40 b	3.98 b
Mean of ¼ NT		**2.8 b**	**3.43 b**
½ NT	0.0	2.20 c	3.08 bc
	0.5	3.60 b	3.90 b
	1.0	4.20 a	4.82 a
	1.5	4.00 ab	4.50 a
Mean of ¼ NT		**3.50 a**	**4.08 a**
1/1 NT	0.0	1.00 e	2.00 c
	0.5	2.20 c	2.20 c
	1.0	3.00 b	3.00 bc
	1.5	3.00 b	2.78 c
Mean of 1 NT		**2.30 b**	**2.50 c**

***Different letters within columns represent significant differences according to Duncan's multiple range test at 5% level.**

Table (3) displays that 1/2 ammonium nitrate was the best level for *Asparagus* rooting at concentration 1.0 mgl^{-1} IBA by producing 9.97 root per explants at the length of 4.80 cm.

Table (3): Effect of different potassium and ammonium nitrate levels and different IBA concentrations on the rooting stage of *Asparagus* explants after six weeks in culture

Ammonium Nitrate Levels	IBA (mgl^{-1})	Number of roots/ explant	Mean length of roots (cm)
¼ NT	0.0	3.40 f	3.28 c
	0.5	4.90 e	3.20 c
	1.0	10.00 ab	4.38 b
	1.5	7.40 c	4.08 b
Mean of ¼ NT		**6.43 b**	**3.74 b**
½ NT	0.0	4.00 e	4.18 b
	0.5	6.40 cd	4.82 b
	1.0	13.80 a	5.10 a
	1.5	11.66 ab	5.08 a
Mean of ¼ NT		**8.97 a**	**4.80 a**
1/1 NT	0.0	2.40 g	2.44 d
	0.5	4.00 e	2.56 d
	1.0	7.60 c	3.66 c
	1.5	5.80 d	3.30 c
Mean of 1 NT		**4.95 c**	**2.99 c**

***Different letters within columns represent significant differences according to Duncan's multiple range test at 5% level.**

Table (4) reveals that significant differences were recorded between the ammonium nitrate levels. Since, 1/2 ammonium nitrate level (NT) produced higher numbers of roots per explants and longer roots (4.80 root/explants and 5.0 cm) respectively at concentration 1.5 mgl^{-1} NAA. These results proved that auxins have a role in rooting process since they promote adventitious roots initiation in the bases of cultured shoots (Audus, 1959; Abdul, 1987 and Saleh 1990). These results are in agreement with those found by Salahaddin *et al*. (2005) Abdullah *et al*. (2003) and Hossain *et al*. (2003), who observed that reducing the levels of MS salts in the medium to half increased rooting of many tree species. Decreasing the level of salts in the medium means decreasing the level of nitrogen in the medium to half or quarters, this will result in decreasing nitrogen level in the shoots which may result in increasing the percentage of carbohydrates to nitrogen level and this may result in increasing the percentage of root primordial and roots number (Gawel, 1990).

Table (4): Effect of different potassium and ammonium nitrate levels and different NAA concentrations on the rooting stage of *Asparagus* explants after six weeks in culture

Ammonium Nitrate Levels	NAA (mgl^{-1})	Number of roots/ explant	Mean length of roots (cm)
¼ NT	0.0	3.00 e	3.20 d
	0.5	4.60 b	3.80 c
	1.0	4.40 bc	3.82 c
	1.5	4.00 c	4.12 bc
Mean of ¼ NT		**4.40 a**	**3.74 b**
½ NT	0.0	3.60 cd	4.50 b
	0.5	5.40 a	4.58 b
	1.0	5.00 ab	4.70 b
	1.5	5.20 a	5.20 a
Mean of ¼ NT		**4.80 a**	**5.00 a**
	0.0	2.80 e	2.50 e

1/1 NT	0.5	4.80 b	3.00 d
	1.0	3.80 c	3.26 d
	1.5	3.60 cd	3.60 c
Mean of ¼ NT		**3.75 b**	**3.09 c**

***Different letters within columns represent significant differences according to Duncan's multiple range test at 5% level.**

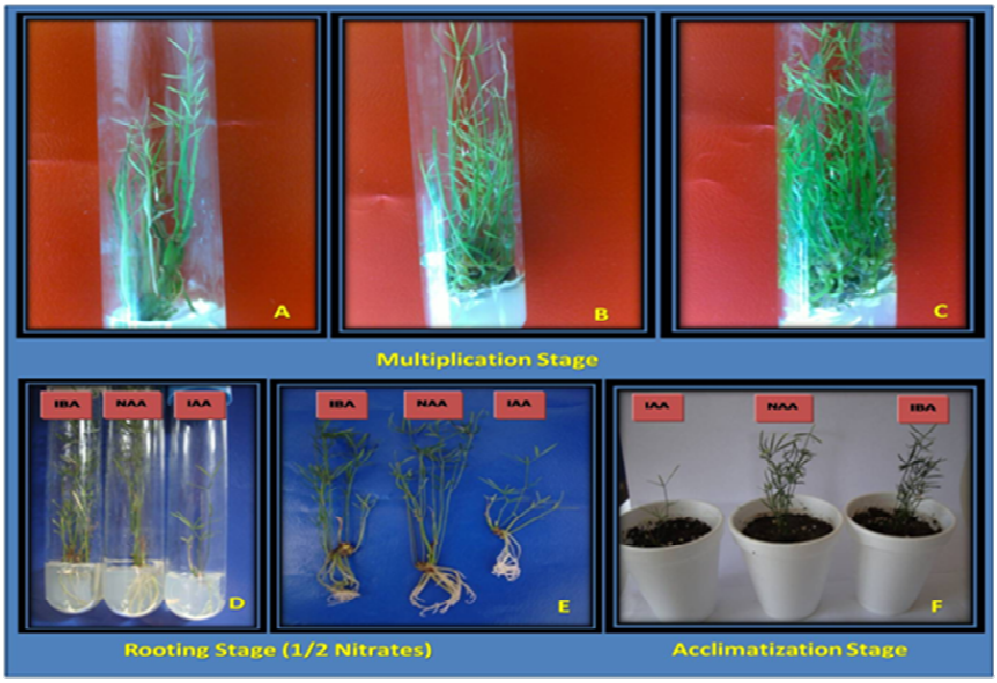

Figure (1): Developmental stages of *Asparagus* micropropagation.

A. multiplication stage with ¼ nitrates. B. multiplication stage with ½ nitrates.C. multiplication stage with 1/1 nitrates. D. and E. Rooting stage (1/2 nitrates) as affected by IBA, NAA and IAA. F. Acclimatization stage.

At the final developmental stage of acclimatization and gradually moving into filed conditions, a high survival rate was noticed with plantlets transferred to soil. The successful gradually acclimatized percentage reached to 90%. This percentage was obtained by following hardening- off steps carefully. Most of the plantlets began to grow well and did not show any morphological abnormalities (fig. F).

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