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**INFLUENCE OF NITROGEN SOURCE  
FOR THE IMPROVEMENT OF  
SHOOTS AND ROOTS ON *IN VITRO*  
STRAWBERRY (*FRAGARIA  
ANANASSA DUCH.*)**

**Journal**

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*J. Biol. Chem.  
Environ. Sci., 2014,  
Vol. 9(2): 21-33  
www.acepsag.org*

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**ABSTRACT**

In vitro techniques are important tools for modern plant improvement programs to introduce new traits into interested plants, to multiply elite selections and to develop suitable cultivars in a minimum time. The standardization of protocol and procedure of micropropagation of strawberry was successfully attempted by many researchers. Crops are very sensitive to various ratios of  $\text{NH}_4^+$ :  $\text{NO}_3^-$  in the nutrient solution. Presented study was conducted to determine the effect of nitrogen source on shooting and rooting stage of strawberry (*Fragaria X ananassa Duch.*) var. chandler by changing the concentrations of  $\text{NH}_4\text{NO}_3$  and  $\text{KNO}_3$  in MS nutrient medium formula to improve protocol for the high rate of in vitro propagation of strawberry. Results showed that decreasing the concentration of  $\text{NH}_4\text{NO}_3$  from  $1650\text{mg l}^{-1}$  to  $825\text{mg l}^{-1}$  combined with  $\text{KNO}_3$  at  $1900\text{mg l}^{-1}$  gave the highest results in shoot number, shoot length and growth vigor during multiplication stage. While in rooting stage MS nutrient medium without  $\text{KNO}_3$  and  $\text{NH}_4\text{NO}_3$  at  $1650\text{mg l}^{-1}$  the highest root number, root length and shoot length were achieved. Management of culture medium composition is very important to obtain high quality from micropropagated plants.

**Key Words:** Strawberry - Tissue Culture – Nitrogen Source – *In Vitro*.

## INTRODUCTION

Strawberry (*Fragaria X ananassa* Duch.) belongs to the Rosaceae family. It is a perennial, stoloniferous herb. Strawberries have traditionally been a popular delicious fruit for its flavor, taste, fresh use, freezing and processing. It contains relatively high quantities of ellagic acid having a range of biological activity and especially the fruit contains higher vitamin C concentration than orange or lemon. It is produced in 73 countries worldwide on 200,000 hectares and produced 31 lac metric tons strawberry FAO, (2008). It has been commercially cultivated in Canada, USA, Japan, Spain, Germany, Korea, Italy, Poland, Thailand and so many countries in the world Biswas *et al.*, (2007) especially in Egypt.

*In vitro* techniques are important tools for modern plant improvement programs to introduce new traits into selected plants, to multiply elite selections and to develop suitable cultivars in the minimum time Taji *et al.*, (2002). The standardization of protocol and procedure of micropropagation of strawberry was successfully attempted by many Kaur *et al.*, (2005); Sakila *et al.*, (2007); Gantait *et al.*, (2010). Micropropagated strawberry plant has been introduced to prevent most of the plant and soil transmissible diseases Biswas *et al.*, (2008). In contrast of these, mass multiplication *in vitro* through tissue culture results high yield in disease free plant material Mohan *et al.*, (2005).

*In vitro* rooting of plantlets and their acclimation to the soil remains a problem in many plant types. Murashige (1974) suggested the development of a simple medium for *in vitro* root formation and conditions for direct rooting of micropropagated plantlets in the soil Sarwar and flegmann, (1989). As crops are very sensitive to various ratios of  $\text{NH}_4^+$ :  $\text{NO}_3^-$  in the nutrient solution Sonneveld, (2002), according to Guo *et al.*, (2002) and Bruck and Guo (2006) different  $\text{NH}_4^+$ :  $\text{NO}_3^-$  ratios can affect the rate of plant growth as well as the biomass allocation. Inappropriate levels result in phytotoxicity and impair the product quality and quantity Tabatabei *et al.*, (2007); Ingestad, (2006). The leaves developed under *in vitro* conditions usually have poorly developed chloroplasts; disorganized granal structure, low chlorophyll (chl) and protein content in general Capellades *et al.*, (1990) and Ziv and Ariel, (1995).

The main objective of this research was to study the effect of nitrogen source on shooting and rooting stage of strawberry (*Fragaria X ananassa* Duch.) var. chandler by changing the concentrations of  $\text{NH}_4\text{NO}_3$  and  $\text{KNO}_3$  in MS nutrient medium formula to improve protocol for the high rate *in vitro* propagation of strawberry.

## MATERIALS AND METHODS

This research was carried on Biotechnology Laboratory, at The Central Laboratory for Data Palm Research and Development (CLDPRD). Agricultural Research Centre, Giza.

### Explants material

*In vitro* produced plantlets or proliferated meristems of strawberry (*Fragaria x ananassa* Duch cv. 'Chandler') were used as experimental material. Explants were taken from 6-8 weeks old virus free whole plant culture as shoot cluster consists of 3-4 shoots at 2-3 cm high without roots.

### 1- Shooting stage

Shoot cluster of cv.chandler explants were cultured on MS Murashige and Skoog (1962) with  $1 \text{ mg l}^{-1}$  BA at different modification of  $\text{NH}_4\text{NO}_3$  and  $\text{KNO}_3$  concentrations as shown in table(1).

**Table 1: The studied changes of  $\text{NH}_4\text{NO}_3$  and  $\text{KNO}_3$  concentration in MS nutrient media formula.**

Data were calculated after 8 weeks about the following changing (Shoot number, Shoot length (cm) and Growth Vigour (G.V)

The average degree value of growth vigour/explants was scored visually according to Pottino (1981) as follows:-

1. (-) Negative no result
2. (+) Below average result
3. (++) Average result
4. (+++) Good result
5. (++++ V. good result

Each treatment = 3 replicate and each replicate = 3 culture jars and each jar contain one cluster of explants.

### 1- Rooting stage

All previous shoot clusters obtained from multiplication stage were transferred to culture on MS medium supplemented 1 mg l<sup>-1</sup> IBA (Indol Biotic Acid) and 3gl<sup>-1</sup> AC (Activated Charcoal) at the same different studied modification of NH<sub>4</sub>NO<sub>3</sub> and KNO<sub>3</sub> concentrations as shown in table (1). Data were calculated after 8 weeks about the following changing (Root number, Root length (cm) and Shoot Length (cm)). Each treatment = 3 replicate and each replicate =3 culture jars and each jar contain one cluster of explants.

In this experiment all MS media treatments in multiplication stage and rooting stage were supplemented with 30gl<sup>-1</sup> sucrose. Medium were solidified with 5gl<sup>-1</sup> agar. Previous prepared medium was dispensed into small jars (150 ml) at the rate of 40 ml per jar. The culture jars were immediately capped with polypropylin closure and then autoclaved at 121°C and 15 lbs/in<sup>2</sup> for 20 min. The medium PH was adjusted to 5.7-5.8 prior to addition of agar. Each jar was containing one shoot cluster explants. Culture jars were incubated under light for 16 hours and under dark for 8 hours at 27±1°C of growth room temperature.

#### Statistical Analysis

All data were subjected to statistical analysis according to the procedure reported by Snedecor and Cochran (1980) and LSD at 5% level of significance was used to compare between means according to Steel and Torrie (1980).

## RESULTS AND DISCUSSION

Data revealed that modification of Ms nutrient medium by altering the concentration of (NH<sub>4</sub><sup>+</sup>) and (NO<sub>3</sub><sup>-</sup>) have a significant effect of nitrogen elements source on multiplication and rooting stage of (*Fragaria X ananassa* Duch.) explants.

#### Multiplication stage

Data in table (2) and Figure (1) recovered that the highest significant increasing in shoot number was (19.67) obtained when explants were cultured on MS medium formula with NH<sub>4</sub>NO<sub>3</sub> concentration was 1650 mg l<sup>-1</sup> and KNO<sub>3</sub> concentrations was 0.0mg l<sup>-1</sup>. The lowest significant result of increased in shoot number was (8.00)

recorded when explants were cultured on Ms Medium with NH<sub>4</sub>NO<sub>3</sub> concentration at 0.0 and KNO<sub>3</sub> concentrations at 1900 mg l<sup>-1</sup>.

In addition, data and photo (1) showed that the increased in shoot length of (*Fragaria X ananassa* Duch.) explants was affected by the concentration of NH<sub>4</sub>NO<sub>3</sub> at 825 mg l<sup>-1</sup> and the concentration of KNO<sub>3</sub> at 950 mg l<sup>-1</sup> in M<sub>6</sub> treatment showed in Figure 2 to give the highest significant results followed by the results in (M<sub>3</sub>, M<sub>4</sub>, and M<sub>5</sub>) (5.00, 4.833, 4.300 respectively) without significant differences among treatments. On the other hand the lowest significant results of shoot length was recorded when explants were cultured on M<sub>2</sub> treatment (2.66 cm) as at NH<sub>4</sub>NO<sub>3</sub> concentration was 1650 and KNO<sub>3</sub> concentrations was 950.

According to the data and photo (2), growth vigorous of explants appearance was superior significantly when were cultured on M<sub>6</sub> treatment (4.00) followed significantly by the result of explants on M<sub>4</sub> treatment (3.00). Explants cultured on M<sub>2</sub> and M<sub>3</sub> medium have the same value of growth vigorous (3.667) without significant differences between them the results of explants cultured on M<sub>5</sub> or M<sub>4</sub> treatment. However, the lowest significant growth vigorous results were obtained when explants were cultured on M<sub>1</sub> treatment (2.00).

Table. 2: The effect of changes in NH<sub>4</sub>NO<sub>3</sub> and KNO<sub>3</sub> concentrations in MS nutrient media formula on shooting stages of (*Fragaria X ananassa* Duch.) explants.

Treatment	Shoot length	Shoot number	Growth vigorous
M <sub>1</sub>	3.167 BC	8.00 D	2.0 C
M <sub>2</sub>	2.667C	10.67 CD	3.667 AB
M <sub>3</sub>	5.000 A	10.67 A	3.667 AB
M <sub>4</sub>	4.833A	12.67 BC	3.00 B
M <sub>5</sub>	4.300 AB	15.00 BC	3.33 AB
M <sub>6</sub>	5.500A	16.67 AB	4.00 A
L.S.D	1.382	4.452	0.9205

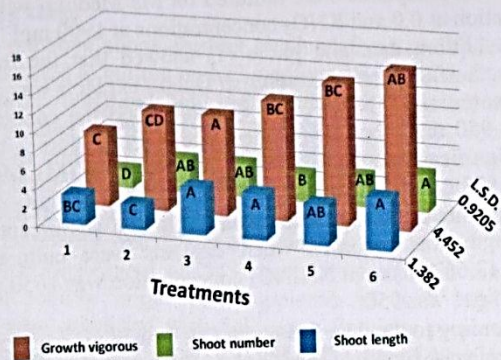


Figure (1): Histogram showing the effect of changes in  $\text{NH}_4\text{NO}_3$  and  $\text{KNO}_3$  concentrations in MS nutrient media formula on shooting stages of (*Fragaria X ananassa* Duch.) explants.

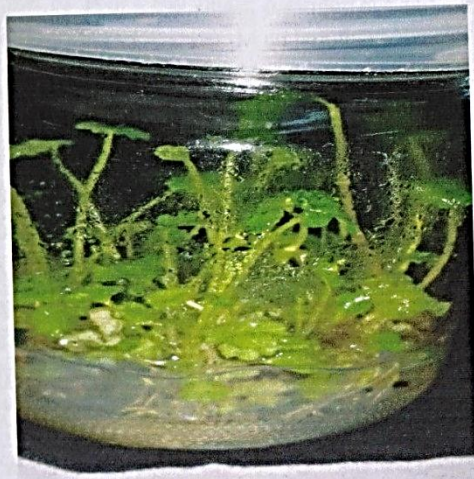


Photo (1): The strawberry culture showed the best results achieved with  $\text{M}_6$  treatment with (825  $\text{NH}_4\text{NO}_3$ , 950  $\text{KNO}_3$ ) during shooting stage.



Photo (2): The strawberry plantlets culture showing the effect of changes in  $\text{NH}_4\text{NO}_3$  and  $\text{KNO}_3$  concentrations in MS nutrient media formula on shooting stage.

#### Rooting stage

Rooting stage development of cultured explants as shown in table (3) and Figure (2) as follows the highest significant increased in roots number were observed when explants were cultured on  $\text{M}_3$  (20.00) Where the lowest root number were recorded when explants were cultured on  $\text{M}_1$  treatment (5.33 cm) followed by the results of cultured explants on  $\text{M}_5$  (7.66) without significant result in between.

According to the data, the increased in roots length were exhibited. The highest significant result when explants were cultured on  $\text{M}_3$  treatment (13.33) followed significantly by the results of explants cultured on  $\text{M}_4$  and  $\text{M}_5$  treatment which gave the same results (8.00 cm) and on  $\text{M}_2$  (8.16 cm) and on  $\text{M}_6$  (6.50 cm) without significant differences among them when explants were cultured on  $\text{M}_1$  treatment the lowest significant result of increased in root length were obtained (5.66)

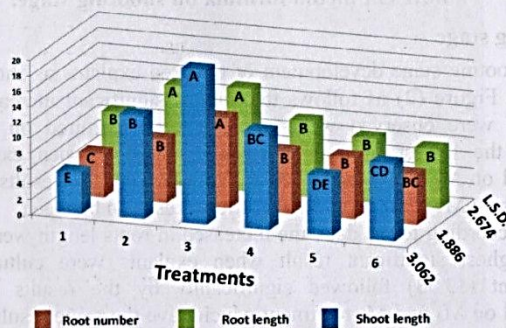
followed by the results of these explants cultured on M<sub>6</sub> treatment without significant results in between.

Regarding to the increased in shoot length during rooting stage data determined that when explants cultured on M<sub>2</sub> and M<sub>3</sub> treatment the highest significant results were obtained (13.00, 13.33) respectively without significant results in between.

These results were followed significantly with all results of cultured explants on treatments M<sub>4</sub>, M<sub>1</sub>, M<sub>5</sub>, and M<sub>6</sub> (9.83, 8.83, 8.33, and 7.66 respectively) without significant results among these treatment.

**Table 3: Effect of changes in NH<sub>4</sub>NO<sub>3</sub> and KNO<sub>3</sub> concentrations in MS Nutrient media formula on rooting stage of *Fragaria X ananassa* Duch.**

Treatment	Root number	Root length	Shoot length
M <sub>1</sub>	5.333 E	5.667 C	8.833 B
M <sub>2</sub>	13.33 B	8.167 B	13.00 A
M <sub>3</sub>	20.0 A	11.67 A	13.33 A
M <sub>4</sub>	12.67 BC	8.0 B	9.8333 B
M <sub>5</sub>	7.667 DE	8.0 B	8.333 B
M <sub>6</sub>	10.00 CD	6.5 BC	7.667 B
L.S.D	3.062	1.886	2.764



**Figure (2): Histogram showing the effect effect of changes in NH<sub>4</sub>NO<sub>3</sub> and KNO<sub>3</sub> concentrations in MS nutrient media formula on rooting stage of (*Fragaria X ananassa* Duch.)**

Nitrogen (N) source markedly influences growth and morphogenesis in tissue culture **Gamborge and Shyluk, (1970); Thrope, et al., (1989)**. As crops are very sensitive to various ratios of NH<sub>4</sub><sup>+</sup>:NO<sub>3</sub><sup>-</sup> in the nutrient solution **Sonneveld, (2002)**, according to **Guo et al., (2002) and Bruck and Guo (2006)** different NH<sub>4</sub><sup>+</sup>:NO<sub>3</sub><sup>-</sup> ratios can affect the rate of plant growth as well as the biomass allocation.

The N concentration and balance in the MS medium were optimized for tobacco tissue culture by **Murashige and Skoog, (1962)**. This medium was used for micropropagation of many species and used at half strength for strawberry (**Hdider, et al., 1994**).

In the presented study decreasing the concentration of NH<sub>4</sub>NO<sub>3</sub> from 1650 mg l<sup>-1</sup> to 0.0 mg l<sup>-1</sup> combined with KNO<sub>3</sub> at 1900 mg l<sup>-1</sup> (M<sub>3</sub> treatment) gave the highest results in shoot number, shoot length and growth vigour showed in (Figure: 4) during multiplication stage for strawberry explants. These results are agreed with **Canhoto and Crus, (1996)** results who reported that in Feijoa (subtropical fruits from South America), the presence of the either NH<sub>4</sub><sup>+</sup> or NO<sub>3</sub><sup>-</sup> solely resulted in a low percentage of somatic embryos induction. However, somatic embryogenesis was enhanced by using media that contain NH<sub>4</sub>NO<sub>3</sub> alone.

On the other hand, **Zayed et al., 2012** reported that in date palm cv. Gundila ammonium as the sole source of N resulted in depression in somatic embryos differentiation and escalated the frequency of hyperhydricity whereas, if nitrate was used as the sole N source, somatic embryos good quality were produced and hyperhydricity was eliminated, however **Ivanova and Van Staden, (2009)** declared that ammonium nitrate as the sole source of N depressed shoot regeneration and growth and the frequency of hyperhydricity had been increased up to 50%. When NO<sub>3</sub><sup>-</sup> was used as the sole N source, shoots good quality were produced and hyperhydricity was completely inhibited.

In rooting stage, our results showed that explants of strawberry (*Fragaria X ananassa* Duch. var. chandler) cultured on MS nutrient medium without KNO<sub>3</sub> and 100% of NH<sub>4</sub>NO<sub>3</sub> at 1650 mg l<sup>-1</sup> the highest roots number, root length and shoot length were achieved showed in photo (3) This wasn't in line with **Avila et al., (1998)** who reported that shoot growth in potato plantlets on 0% and 100% NH<sub>4</sub> both in solid and liquid media was restricted in all cultivars. In

addition, potato cultivars have different sensitivity to the  $\text{NH}_4^+:\text{NO}_3^-$  ratio.

There are few studies provided physiological basis for using  $\text{NH}_4^+:\text{NO}_3^-$  ratio or N concentration for micropropagation of strawberry cultivars. More investigations are needed because these physiological processes are important for the management of culture medium composition to achieve best results.



**Photo (3):** Strawberry shoot cluster show the Effect of changes in  $\text{NH}_4\text{NO}_3$  and  $\text{KNO}_3$  concentrations in MS nutrient media formula on rooting stage.

#### Acknowledgment

Sincere gratitude is hereby extended to the following that never ceased in helping until this paper is structured. The special thanks goes to my helpful doctor Maiada El Dawayat who support has always been my source of strength and inspiration.

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### تأثير مصادر النيتروجين المختلفة لتحسين مرحلتي التضاعف والتجذير في نبات الفراولة صنف (Fragaria X ananassa Duch.)

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تعتبر التقنية المستخدمة في هذه الحالة من أجل تحسين برنامج إكثار نباتات الفراولة حيث وجد أن هذه المحاصيل حساسة بالنسبة للتركيزات المختلفة من الأمونيوم والنترات في البيئة الغذائية مما يؤدي إلى زيادة ظاهرة الزجاجية وقد أجريت هذه الدراسة لتقدير تأثير مصادر النيتروجين على مرحلتي التضاعف والتجذير للفراولة (Fragaria X ananassa Duch.) صنف Chandler Var. وتغير تركيزات نترات الأمونيوم والبوتاسيوم في الوسط الغذائي لتحسين البرنامج المعدل فقد أظهرت النتائج إنه بنقص تركيز نترات الأمونيوم من 1650 ملليجرام / اللتر إلى 825 ملليجرام / لتر مع 1900 ملليجرام / لتر نترات بوتاسيوم أعطى نتائج أعلى من حيث عدد وطول الأفرع والنمو أثناء مرحلة التكاثر الدقيق . بينما في حالة التجذير فإن الوسط الغذائي بدون نترات بوتاسيوم ووجود الأمونيوم عند تركيز 1650 ملليجرام / اللتر اعطى أعلى نتيجة في أعداد وأطوال الجذور والسيقان . وبالتالي نجد أن التحكم في تركيب البيئة الزراعية يعتبر من العوامل المهمة في الحصول على أعلى جودة من النباتات عند استخدام هذا البرنامج في زراعة الأنسجة.