J. Agric. Sci. Mansoura Univ., 30 (2): 1011 - 1020, 2005

EFFECT OF NITROGEN SOURCES AND GIBBERELLIC ACID RATES ON GROWTH AND CHEMICAL COMPOSITION OF CYCAS PLANTS (*Cycas revoluta* THUMB.)

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ABSTRACT

The present work was designed to study the effect of different nitrogen sources (ammonium nitrate, ammonium sulphate and urea) and gibberellic acid rates (0, 250 and 500 p.p.m. /plant) on growth of *Cycas revoluta* seedlings, that were one-year-old, during two experiments of 2003 and 2004 in the green-house at Antoniades Branch, Hort. Res. Inst., A.R.C. Alexandria. Egypt.

The results indicated that ammonium nitrate significantly increased most of the studied vegetative growth characteristics (number of leaves/ plant, leaf length , leaflet length and number/ leaf, fresh and dry weights of leaves/ plant, fresh weight of stem and its circumference, leaves content of total chlorophylls and nitrogen percent of dry leaves), compared to ammonium sulphate and urea.

Gibberellic acid had insignificant effects on most characteristics of vegetative growth while reduced the others.

In general, using ammonium nitrate (33.5%) as source of nitrogen at the rate of 1.5g / plant monthly during the growth of *Cycas revoluta* seedlings was the best treatment without using GA_3 in the green-house.

INTRODUCTION

Cycas revoluta, Thumb belongs to Cycadaceae family; the common name is sago palm. This unique plant resembles a palm tree, even though it isn't really a palm but is actually a cycad. The cycads have been described as living fossils which flourished in Mesozoic era, reaching their zenith in the Jurassic season about 200 million years ago.

Cycads belong to gymnosperms, cycad species have been assessed at 132 species (Osborne and Hendricks 1985). Cycads are usually propagated from seeds, stem cuttings or sukers but seeds take long time to germinate and rapidly loss the viability (Atta-Alla, 2001).

Cycas revoluta is a very symmetrical plant which supports a crown of shiny dark green leaves on a thick shaggy trunk that can grow to 10-12 ft high. Sago plants are diocious, it is native to Japans southern islands, (tropical and subtropical regions) (Bailey, 1960).

It is a wonderful plant for both indoor and out door uses. It looks great in the shrub border or as an expanse of lawn or near the patio. Use in entryways or in rock and sand gardens. It mixes well with palms and combines well with border grass, podocarpus and camellias. Sago is an excellent container plant; it is also used as a bonsai subject. The glossy metallic leaves are harvested for use in flower arrangements and wreaths.

Cycas revoluta is characterized with its coralloid roots, containing nitrogen fixing Cyanobacteria (Nostoc) which live in symbioses with the plant that can convert atmospheric nitrogen into an organic form, such as nitrate or ammonia (Millbank, 1974 ; Rai, *et al* 2000 and Lindblad and Costa 2002).

Nitrogen is a constituent of proteins including all enzymes, many metabolic intermediates involved in synthesis and energy transfer and even of deoxyribonucleic acids making up the genetic code itself. The form in which nitrogen is absorbed has large effects within both the plant and the rhizosphere, very little information are available about nitrogen sources on trees and shrubs so the literature on other plants seemed to be helpful in this respect. Studies on the effect of N-sources on both vegetative growth and dry matter of either leguminous or non-leguminous showed that plants supplied with both ammonia and nitrate can be more productive than those supplied with either nitrate or ammonia alone (Evans, 1993).

Sait *et al.*, (1996) on sugar beet found that root and refined sugar yields were increased with using ammonium nitrate more than that of ammonium sulphate and urea. Rutu and Broh, (1997) on tomato found that the maximum average nitrate content was obtained at ammonium nitrate application compared to ammonium sulphate treatment.

Poonnachit and Dornell, (2000) reported that shoot fresh weight of nitrate treated *Vacciorium. arboreum* was significantly greater than ammonium treated plants. Mohamed, (2004) stated that fresh and dry weights of corn had the highest values with using ammonium nitrate compared to ammonium sulphate and calcium nitrate.

Gibberellic acid usually used to improve growth of many plants. In this concern

Lee and Kwack, (1995) on hibiscus stated that, GA_3 treatment had no effect on dwarf cultivars and total chlorophylls and nitrogen contents of all cultivars were decreased after GA_3 treatments. GA_3 had no effect on sugar concentration. Starch content in the tall cultivars was increased after GA_3 treatment and had no such effect on the dwarf cultivars. Zhang and Matsui, (1998) found that growth regulators (ABA and GA_3) stimulated root growth which in turn promoted shoot developments of Cymbidium.

The main objective of this work was to study the effect of nitrogen sources (urea, ammonium nitrate and ammonium sulphate), different rates of GA_3 and their interactions on growth of *Cycas revoluta* seedlings.

MATERIAL AND METHODS

The experimental trials were carried out throughout 2003 and 2004 seasons at the nursery of Antoniades Research Branch, Hort. Res. Inst., Alexandria, Egypt. It intended to find out the individual and combined effects of different nitrogen sources and GA_3 rates on growth, total chlorophylls content and nitrogen percent of the leaves of *Cycas revoluta*, Thumb.

Plant material:

Seeds of *Cycas revoluta* plants were collected and planted in beds containing sand and peat-moss (1:1 v/v) under tunnel in the green-house, on February 2001 and 300g of ammonium nitrate (33.5%), 150g of calcium super phosphate (15.5%) and 25g of potassium sulphate 48%) were added per cubic meter of substrate as a base fertilizer (Kofranek and Lunt, 1966). After complete germination (March 2002) the seedlings were transplanted into plastic pots (15cm diameter) containing a mixture of clay + sand + peat-moss (1:1:1 v/v) and left in the green-house for one year. On March 1st 2003 the one year old seedlings bearing 4 leaves were chosen and transplanted into clay pots (30 cm diameter) containing the same mixture used before and set in the green-house (light intensity of 2500-3000 lux and a relative humidity of 75-80% and average temperature of 23.4 and 38.6 °C in winter and summer respectively). The chemical analysis of the used medium indicated that N 0.25%, P 24 p.p.m., K 641 p.p.m., Fe 7.9 p.p.m., Zn 3.12 p.p.m. Mn 9.56 p.p.m. and Cu 1.13 p p.m. with 1.25 dsm^{-1} (EC) and 7.1 (pH). The textural class was a sandy loam.

Nitrogen and GA_3 treatments:

The rate of nitrogen used was 0.5g N/ plant, monthly (Rinaldi and Margheri, 1993) equal to 1.5g ammonium nitrate (33%N), 2.40g ammonium sulphate (20.5%N) and 1.10g urea (46%N). They were used as a soil application immediately before irrigation. Calcium super phosphate (15.5% P_2O_5) at 3.0g / plant and potassium sulphate (48%K_2O) at 1.0g / plant were used monthly for all plants Micro elements at the rate of 0.1g/l were used monthly as a foliar application for the stem apex and leaves. GA_3, was used at the rates of 0.0 (control), 250 and 500 p.p.m. as a foliar application on the top of stem apex and leaves every two months for three times.

The treatments started on March 1st., thus 9 treatments were arranged in three replicates with six plants per experimental unit in a completely randomized block design in factorial experiment throughout each experiment. Cycas plants need a long time to study the factors affecting its growth therefore, this study was divided into two extended experiments. The data of the first one was taken at the end of May 2004 (after 15 months from starting the treatments). The data of the second one was taken at the end of October 2004 (after 20 months from starting the treatments).

The following data were recorded:

- Number of leaves per plant.
- Leaf length (cm.) of the largest adult leaf.
- Fresh and dry weights of leaves / plant (g.).
- Number of leaflets per the largest leaf.
- Leaflet length (cm.) of the middle leaflet of the largest leaf.
- Stem circumference (cm),
- Fresh and dry weights of stem (g.)
- Fresh and dry weights of roots (g.)
- Total chlorophylls content (mg/g fresh weight of leaf) according to Moran and Porath, (1980).
- N percent of dry leaves, according to Chapman and Parker, (1961).

Duncan's Multiple Range Test was used for comparison between means of treatments according to Snedecor, and Cochran, (1974).

RESULTS AND DISCUSSION

1- Leaves characters:

Data in Tables (1 and 2) reveal that the different nitrogen sources significantly affected number, length and fresh and dry weights of leaves per plant. Evidently, ammonium nitrate had increased leaves characters to the highest values compared to ammonium sulphate and urea treatments in both experiments. However, dry weight of leaves per plant was significantly increased in the second experiment. On the other hand, there were no significant differences between ammonium sulphate and urea treatments in both trials. The increase in the values of leaves characters due to using ammonium nitrate may be related to increasing nitrogen uptake by the plant as a result of increasing available nitrogen in the rhizosphere that can be easily assimilated by plants. In this respect Evans, (1993) showed that crop plants supplied with both NO_3 and NH_4 can be more productive than those supplied with either NO_3or NH_4 alone. The low values of leaves characters with using urea and ammonium sulphate are due to the depressive effect of NH_4 on growth of plant since it is the sole source of N (Gaber *et al*, 2001).

Insignificant effects were recorded among leaves characters due to using the different GA_3 levels in both experimental trials as indicated in Tables (1 and 2). On the other hand, GA_3 at the high rate (500 p.p.m.) had a depressive effect on fresh and dry weights of leaves in the first experiment alone. These results may be attributed to the high level of GA_3 naturally present in the plant cells, thereby the plants were not affected by the additional amount of GA_3, in addition to the depressive effects of the high rates of it. Moreover, low light intensity in the green-house (2500-3000 lux) keep natural GA_3 level in plant cells. These results are in parallel with those of Lee and Kwack, (1995) on *Hibiscus syriacus*.

Concerning the interaction as tabulated in Tables (1 and 2), leaves characters had the highest values with using ammonium nitrate without applying GA_3 in both trials.

2- Leaflet length and number / leaf:

Different nitrogen sources significantly affected leaflet length and number in both plantations (Table 2). Fertilizing the plants using ammonium nitrate gave the highest values of leaflet length and number compared to ammonium sulphate and urea treatments in both experiments. This increase may be attributed to increasing the availability of nitrogen as ammonium nitrate then enhanced the accumulation rate of nitrogen that plays an important role in cell division to initiate leaflet primordia and elongation of leaflet. This result is in agreement with that of Evans, (1993).

Gibberellic acid treatments had negative effects on number of leaflets per leaf in both experiments, it had reduced leaflet number with the two rates of GA_3. While leaflet length was not affected by GA_3 treatments in both trials. This reduction effect of GA_3 treatments might be related to the increase of natural GA_3 level in the plant especially under low light intensity in the green-house. Similar results were obtained by Lee and Kwack (1995).

Table (1): Effect of nitrogen sources, gibberellic acid rates and their interaction on number of leaves / plant, leaf length and fresh weight of leaves / plant of *Cycas revoluta* seedlings during the two experiments of 2003 and 2004.

GA$_3$ rates (p.p.m.)	First experiment Nitrogen				Second experiment Nitrogen			
	A.N.*	A.S.**	Urea	Means	A.N.*	A.S.**	Urea	Means
				Number of leaves/ plant				
0.0	8.5a	6.5bcd	7.00bc	7.33a	9.33a	8.00abc	7.00bc	8.11a
250	7.5ab	5.67cde	6.50bcd	6.56a	8.50ab	7.33bc	6.67c	7.50a
500	6.0cde	5.33de	4.80e	5.39a	8.50ab	7.67abc	7.17bc	7.78a
Means	7.33a	5.83b	6.11b		8.78a	70.67b	6.94b	
				Leaf length (cm)				
0.0	47.83a	38.50abc	38.61abc	41.67a	55.17a	43.83b	48.50ab	49.17a
250	46.83a	45.33ab	30.76c	40.97a	51.17a	41.17bc	24.17bc	45.06a
500	47.17a	32.83bc	40.17abc	40.06a	41.50bc	34.83cd	31.50d	33.94a
Means	47.28a	38.89b	36.53b		49.50a	39.94b	40.72b	
				Fresh weight of leaves/ plant				
0.0	88.39a	59.75b	62 54b	70.22a	109.49a	88.30abc	70.92bc	89.57a
250	87.90a	59.59b	57.87b	68.45a	87.32abc	68.40bc	63.92c	73.09a
500	52.81bc	40.96bc	31.66d	41.81b	97.01ab	59.99c	56.60c	71.20a
Means	76.36a	53.43b	50.69b		97.94a	72.11b	63.81b	

* = Ammonium nitrate ** = Ammonium sulphate
Means of each factor designated by the same letter are not significantly different at 5% level using Duncan's Multiple Range Test.

Table (2): Effect of nitrogen sources , gibberellic acid rates and their interaction on dry weight of leaves, number of leaflets / leaf and leaflet length of *Cycas revoluta* seedlings during the two experiments of 2003 and 2004.

GA$_3$ rates (p.p.m.)	First experiment Nitrogen				Second experiment Nitrogen			
	A.N.*	A.S.**	Urea	Means	A.N.*	A.S.**	Urea	Means
				Dry weight of leaves/ plant (g)				
0.0	32.38a	28.16ab	23.94abc	28.16a	45.14a	36.42ab	30.53b	37.36a
250	26.57abc	23.93abc	22.15bc	24.22a	37.64ab	30.77b	24.84b	31.08a
500	20.45bc	18.31c	17.63c	18.80b	34.78ab	28.36b	27 72b	30.32a
Means	26.47a	23.47a	21.24a		39.19a	31.86b	27.70b	
				Number of leaflet/ leaf				
0.0	77.33a	62.00bcde	71 33ab	76.22a	93.67a	71 33bcd	74 33bc	79.78a
250	70 67abc	66.67	67 44b	64.39a	78.67b	61.33cd	62 33cd	67.44b
500	58.67cde	52.00e	54.67de	55.11a	73.33bc	61.33cd	56.67d	63.78b
Means	68.89a	60.22b	60.61b		81.89a	64.67b	64.44b	
				Leaflet length (cm)				
0.0	14.50a	10.50c	14.17ab	13.06a	15.50a	14.33ab	13.00ab	14.28a
250	14.17ab	13.17abc	11.50bc	12.94a	14.67ab	14.00ab	12.33b	13.67a
500	13.83ab	12.67abc	12 33abc	12.94a	13.83ab	13.33ab	12.83ab	13.33a
Means	14.17a	12.11b	12.67ab		14.67a	13.89ab	12.72b	

* = Ammonium nitrate ** = Ammonium sulphate
Means of each factor designated by the same letter are not significantly different at 5% level using Duncan's Multiple Range Test.

The interaction between N sources and GA_3 treatments revealed that plants which received ammonium nitrate without using GA_3 gave the highest values of leaflet number and length in both plantations.

3- Stem characters:

Data in Table (3) show that stem circumference and fresh weight were influenced by nitrogen sources. Fertilizing the plants using ammonium nitrate gave the highest values of both stem circumference and fresh weight compared to the other sources of nitrogen. This increase may be attributed to increasing nitrogen accumulation and the increase of leaves area thereby increases photosynthetic products that reflexed on stem fresh weight and circumference. These results were in accordance with that of Poonnachit and Dornell (2000) on *Vacciorium arboreum* and Mohamed (2004) on corn. On the other hand, dry weight of stem was not affected by using the different nitrogen sources in both plantations. This result might be related to the slow growth habit of *Cycas revoluta*, it needs long time to show the differences in stem structure.

Table (3): Effect of nitrogen sources , gibberellic acid rates and their interaction on stem circumference and fresh and dry weights of stem of *Cycas revoluta* seedlings during the two experiments of 2003 and 2004.

GA_3 rates (p.p.m.)	First experiment				Second experiment			
	Nitrogen				Nitrogen			
	A.N.*	A.S.**	Urea	Means	A.N.*	A.S.**	Urea	Means
Stem circumference (cm)								
0.0	19.00a	16.33b	16.50b	**17.28a**	20.83a	18.33bcd	15.83e	**18.33a**
250	18.50a	16.33b	16.67b	**17.17a**	19.50ab	18.50bcd	17.00de	**18.33a**
500	18.47a	15.50b	15.17b	**16.38a**	19.17bc	17.67cd	17.67cd	**18.17a**
Means	**18.66a**	**16.06b**	**16.11b**		**19.83a**	**18.17b**	**16.83c**	
Fresh weight of stem (g)								
0.0	107.54a	78.59bc	73.18bcd	**86.44a**	109.52ab	100.39bcd	85.37cde	**98.43a**
250	92.26ab	64.93cde	56.10de	**71.10b**	128.18a	84.23cde	61.67e	**91.36a**
500	71.30cd	45.65e	50.14e	**55.70c**	105 63bc	76.83de	81.68cde	**88.05a**
Means	**90.36a**	**63.06b**	**59.81b**		**114.44a**	**87.15b**	**76.24b**	
Dry weight of stem (g)								
0.0	42.73	28.80	25.59	**32.37**	50.99a	39.03ab	43.72ab	**44.58a**
250	30.94	27.58	21.38	**62.63**	41.51ab	40.58ab	39.04ab	**40.38ab**
500	29.76	28.45	16.35	**24.85**	36.85ab	34.44b	29.63b	**33.65b**
Means	**34.30**	**28.28**	**21.11**	**n.s.**	**43.11a**	**38.02a**	**37.47a**	

* = Ammonium nitrate   ** = Ammonium sulphate

Means of each factor designated by the same letter are not significantly different at 5% level using Duncan's Multiple Range Test.

Concering the effects of gibberellic acid on stem characters, there were insignificant effects for GA_3 treatments on stem circumference in both trials. While it decreased fresh and dry weights of stem compared to the control. This reduction effect of GA_3 on stem fresh and dry weights may be related to the high level of the natural GA_3in the plant cells. It may increase respiration and metabolic processes. These results were in agreement with those of Lee and Kwack, (1995).

Concerning the interaction between N sources and GA_3 rates, it could be observed from data in Table (3) that plants which received ammonium nitrate without using GA_3 had the highest values of stem characters in both experiments.

4- Root characters:

As shown in Table (4) ammonium nitrate significantly increased fresh weight of roots compared to ammonium sulphate and urea in both trials. It might be due to the balanced effect of ammonium nitrate on the soil pH. On the other hand, Ruckert and Giani, (2004) found that the maximum concentration of Cyanobacteria was higher with ammonia + nitrate followed by nitrate alone that enhanced N fixation of Cyanobacteria in the coralloid roots of cycas plants, which affected root growth of cycas. This result agreed with that of Sait *et al.*, (1996) on sugar beet and George *et al*, (1999) on Norway spruce trees.

Table (4): Effect of nitrogen sources , gibberellic acid rates and their interaction on fresh and dry weights of roots / plant and total chlorophylls contents of *Cycas revoluta* seedlings during the two experiments of 2003 and 2004.

GA_3 rates (p.p.m.)	First experiment				Second experiment			
	Nitrogen				Nitrogen			
	A.N.*	A.S.**	Urea	Means	A.N.*	A.S.**	Urea	Means
Fresh weight of roots/ plant (g)								
0.0	63.16a	48.57bc	36.91cd	**49.55a**	76.33a	63.91abc	51.24cde	**63.38a**
250	53.19ab	37.65cd	31.38d	**40.74a**	70.70ab	37.43e	45.29de	**51.14b**
500	45.25bc	28.77d	28.77d	**34.62a**	59.77bc	53.11cd	41.20de	**51.36b**
Means	**53.87a**	**38.33b**	**32.36b**		**68.93a**	**51.49b**	**45.91b**	
Dry weight of roots/ plant (g)								
0.0	16.55	12.74	12.83	**14.04**	23.45	18.44	18.03	**19.97**
250	17.24	12.50	11.60	**13.78**	24.53	17.53	17.35	**19.86**
500	15.16	13.20	12.49	**13.62**	20.00	17.07	14.83	**17.30**
Means	**16.32**	**12.81**	**12.31**	**n.s.**	**22.65**	**17.68**	**16.80**	**n.s.**
Total chlorophylls content (mg/ g F.W.)								
0.0	1.57a	1.06abc	1.22abc	**1.28a**	1.79ab	1.48cd	1.54bcd	**1.60a**
250	1.42ab	1.16abc	1.00bc	**1.19a**	1.87a	1.28d	1.58bc	**1.58a**
500	1.47ab	0.79c	0.83c	**1.03a**	1.67abc	1.44cd	1.64abc	**1.58a**
Means	**1.49a**	**1.00b**	**1.02b**		**1.78a**	**1.40b**	**1.59b**	

* = Ammonium nitrate ** = Ammonium sulphate

Means of each factor designated by the same letter are not significantly different at 5% level using Duncan's Multiple Range Test.

Gibberellic acid treatments as shown in Table (4) reveal non-significant effects on fresh weight of roots in the first experiment while it decreased fresh weight of roots in the second experiment. This result is agreed with some observations of Lee and Kwack (1995) on hibiscus.

The interaction of N sources and GA_3 rates revealed that plants which received ammonium nitrate without applying GA_3gave the highest fresh weight of roots / plant in both trials.

Dry weight of roots as shown in Table (4) was not affected by either nitrogen sources or GA_3treatments in both plantations.

5- Chemical composition:

Total chlorophylls content and nitrogen percent as shown in Tables (4 and 5) were significantly increased with using ammonium nitrate compared to the other sources of used nitrogen. This increase might be related to increasing of available nitrogen thereby increased it in plant cells that reflexed on total chlorophylls content and N percent in the leaves. These observations are in accordance with those obtained by George *et al*, (1999) on Norway spruce trees and Rutu and Broh (1997) on tomato.

Concerning GA_3 treatments, total chlorophylls content and N percent wwere not affected by GA_3 treatments. These results are partially agreed with those of Lee and Kawack (1995).

Concerning the interaction between nitrogen sources and GA_3 rates on total chlorophylls content and nitrogen percent in the leaves as shown in Tables (4 and 5) reveal that plants which received ammonium nitrate without applying GA_3 gave the highest chlorophylls content and the nitrogen percent in the leaves compared to the others.

Table (5): Effect of nitrogen sources, gibberellic acid rates and their interaction on total nitrogen percent of *Cycas revoluta* seedlings of the second experiment of 2003 and 2004.

GA_3 rates (p.p.m.)	Nitrogen			
	A.N.*	A.S.**	Urea	Means
0.0	1.84a	1.72ab	1.61b	**1.72a**
250	1.83a	1.60b	1.58b	**1.67a**
500	1.76a	1.58b	1.53b	**1.62a**
Means	**1.81a**	**1.63b**	**1.57b**	

* = Ammonium nitrate ** = Ammonium sulphate

Means of each factor designated by the same letter are not significantly different at 5% level using Duncan's Multiple Range Test.

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تأثير مصادر النيتروجين ومعدلات حمض الجبريلليك على النمو والتركيب الكيماوى لنبات السيكاس (نخيل ذيل الجمل)

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قسم بحوث الحدائق النباتية
فرع انطونيادس، معهد بحوث البساتين، مركز البحوث الزراعية، الإسكندرية، مصر.

صمم هذا العمل لدراسة تأثير مصادر النيتروجين المختلفة (نترات الامونيا –سلفات الامونيا –واليوريا) وكذلك معدلات حمض الجبريلليك (صفر– ٢٥٠ –٥٠٠جزء فى المليون) على النمو والتركيب الكيماوى لشتلات السيكاس عمر سنة خلال موسمى ٢٠٠٣ ، ٢٠٠٤ بالصوبة بفرع البحوث بحديقة انطونيادس. معهد بحوث البساتين الإسكندرية مصر.

وقد أوضحت النتائج أن التسميد بنترات الامونيا قد أدى إلى زيادة معنوية فى معظم الصفات الخضرية (عدد الأوراق، طول الورقة و طول وعدد الوريقات والوزن الجاف والطازج للأوراق وكذلك الوزن الطازج للساق ومحيطها ومحتوى الأوراق من الكلوروفيل الكلى والنسبة المئوية لمحتوى الأوراق من النيتروجين) وذلك مقارنة باليوريا وسلفات الامونيا.

لم يكن هناك تأثير معنوى لحمض الجبريلليك على معظم الصفات الخضرية كما انه اثر سلبيا على باقى الصفات.

عموما فإن استخدام نترات الامونيوم كمصدر للنيتروجين بمعدل ١,٥ جرام لكل نبات شهريا خلال نمو شتلات السيكاس بالصوبة يعتبر أفضل معاملة وذلك دون الحاجة لحمض الجبريليك.