Proceed, Seventh Pl. Breed. Conf.
May 4-5, 2011 (Alexandria)

Egypt. J. Plant Breed (15) 2: 243-250
Special Issue (2011)

ASSESSMENT OF GENETIC DIVERSITY WITHIN THE GENUS *ACACIA* GROWN IN EGYPT AND STUDYING ITS RELATION TO LEAF TANNIN AND PHENOLIC CONTENTS

Hosam O. Elansary[1]*, Gehan G.Mostafa [2], Diaa O. Elansary[3], Awad Hussein[4]
[1]Department of Floriculture, Ornamental Horticulture and Garden Design, Faculty of Agriculture Elshatby, Alexandria, Egypt, [2]Department of Horticulture (Ornamental Plants), Faculty of Agriculture, South Valley University, Qena, Egypt, [3,4] Precision Agriculture Laboratory, Department of Pomology, Faculty of Agriculture Elshatby, Alexandria, Egypt
Corresponding author. * **hosammail2003@yahoo.com**

ABSTRACT

Acacia is a large genus contains over 1350 species with parallel large medicinal and economic value. In Egypt few species are available. In this study the genetic relation among six species of Acacia sp. were investigated using 12 RAPD primers, furthermore the possible relationship between genetic markers and leaves tannins and phenols total contents. The results showed high degree of polymorphism among species in all primers. Random Amplification of Polymorphic DNA data analysis using genetic distance and UPGMA showed close genetic relatedness between each of A. saligna and A. nilotica ; A. raddiana and A. laeta. The data revealed that furthest species was A. albida which agreed with the unique morphological status in Acacia sp. Total tannins in leaves showed significant differences among all species except for A. saligna and A. nilotica. Total phenols in leaves showed no significant differences among A. nilotica, A. laeta and A. albida, but there was a significant difference between them and the remaining three species (A. raddiana, A. farnesiana and A. saligna). In general there was no clear relatedness between genetic markers (RAPD) and biochemical parameters (leaf tannins and phenols).

Keywords: *Acacia* sp., genetic diversity, tannins, phenols, RAPD

INTRODUCTION

Acacia sp. is a large genus found in the warmer parts of the world ranging from Africa to Australia. The genus contains over 1350 species (Seigler, 2003). About 129 species of indigenous acacias are known in Africa of these, only 18 species are common (Ross, 1979; Coe and Beentje, 1991; Oballa and Olngotie, 1993).

Oballa and Olngotie (1993) suggested that high genetic differentiation within the *Acacia* sp. also they found complicated phyllogenetic relationships within *Acacia* species. The analyses of chloroplast genes and morphological data developed a better understanding of phylogenetic relationships of *the genus* (Clarke *et al.*, 2000; Grimes, 1999; Luckow *et al.*, 2000; Miller and Bayer, 2000, 2001; Miller *et al.*, 2008; Bahieldeen *et al.*, 2010).Unfortunately, the identification of *Acacia* species remains difficult and their taxonomic relationships are not clear (Seigler, 2003; Miller *et al.*, 2008).

Acacias produce a number of secondary compounds, some of which have potential as foods or food additives, whereas others have potential industrial applications, including amines and alkaloids, cyanogenic glycosides, cyclitols, fatty acids and seed oils, fluoroacetate, gums, nonprotein amino acids, terpenes (including essential oils, diterpenes, phytosterol and triterpene genins and saponins), hydrolyzable tannins, flavonoids and

condensed tannins. The most evident and best known are polysaccharides (gums) and complex phenolic substances (condensed tannins) Tannins are used for production of leather but they have a range of other uses. species rich in tannins may not be as suitable for gum production, because the presence of tannins in gums decreases the value of the latter. However, new techniques make it possible to separate gums and tannins more efficiently (Siegler, 2002, 2003).

The generic name 'acacia' comes from the Greek word 'akis', meaning point or barb. *Acacia* sp. are woody and armed with prickles or spines, the leaves generally have several pairs of pinnae each with several pairs of small leaflets (1 pair only in *A. gourmaensis*) and glands on the upper side of the petiole and rachis. Inflorescences are usually axillary, either spikes or spike likes or spherical heads of small whitish or yellow flowers with numerous glandular anthers (*except A. albida*) being followed by dehiscent pods (Steentoft, 1988). *A. albida has large foliage in the dry season so it's used as a food and fodder in the dry season in western Africa* and differs in numerous ways from the rest of the genus (Steentoft, 1988). *A. Saligna* has a robust main stem, smooth, reddish bark, produces suckers and coppices well and is a dense and bushy tree with high biomass production. Narrow dull phyllodes, obtuse inflorescence buds and long racemes. It has been used for tanning, revegetation, animal fodder, mine site rehabilitation, firewood, mulch, agroforestry and as a decorative plant (Steentoft, 1988; Miller *et al.*, 2008). *A. raddiana* reaches the hight of 1-3 meters. The bark is brown-reddish. The tree grows deep roots. The thorns originate from stipules and the plant produces plenty of thorns, the young leaves and branches are glabrous, alternate, bipinnate. There are several nectaries on the leaf axis. It blooms in March-April with light yellow flowers, arranged densely in small globules, like other members of the genus (Steentoft, 1988). *A. farnesiana* thorny bush or a small tree with slender zigzag branches, The leaves are bipinnate with 6-16 pinnae and over 20 pinnules. The flowers are arranged in capitula, which are bright yellow and very fragrant (Sinha, 1971). *A. farnesiana* has been reported to possess medicinal properties in folk medicine also proven to reduce blood sugar, has antimicrobial activity, and approved by the FDA as flavoring ingredient (Letizia *et al.*, 2000). *In A. nilotica* the flowers are grouped into compact globular capitula (Sinha, 1971), leaves are stipulate, petiolate, bipinnate paripinnate, with 6–10 pinnae, and 20–30 pinnules, the stipules are in the form of long white thorns (Sinha, 1980). *A. laeta* leaves bipinnate with 3-5 pairs of fairly large pinnate leaflets and 2-5 pairs of pinnae, flowers in racemes, small, very fragrant, yellow, white or cream, in 3-8 cm long spikes, pedunculate, subsessile, bundled in triplets (Orwa *et al.*, 2009).

An attempt was carried out to elucidate an easy way to identify few species of the *Acacia* sp. grown in Egypt using Random Amplification of Polymorphic DNA (RAPD) and to analyze the genetic relations among them in addition to study some economic compounds like tannins and phenols within leaves.

MATERIALS AND METHODS

1- Plant material

Acacia sp. of *raddiana, farnesiana, laeta, nilotica, albida* and *saligna* were selected representing a collection of *Acacia* sp. in Egypt. The leaves were sampled from Aswan botanical garden, Egypt in January 2011.

2- Assessing tannins and phenolic compounds within the bark

Tannins and phenols were determined in six samples of freshly harvested leaves (3 repl. x 2 sample/ repl.) for each species using the methods of Washington (1970) for tannins and the method of Olesz *et al.* (1988) for phenols. The data were subjected to the analysis of variance according to the procedure outlined by Steel and Torrie (1982). The differences between means of the different treatments were compared using the least significant difference (LSD) at 5%.

3- DNA extraction

Young leaves were taken from each plant and thoroughly washed with water then ethanol to remove dust and other contaminants. The DNA was extracted using QIAGEN DNA extraction kit, USA. The DNA was quantified spectrophotometrically at 260nm and by electrophoresis on 0.8% agarose gels.

4- RAPD

Total Genomic DNA of each cultivar was diluted in sterile double distilled water to a concentration of 10 ng/ml for RAPD analysis. PCR was performed in PEQLAB thermocycler, Germany in a 25 ul reaction volume containing 200 uM of each dNTP (MBI Fermentas), 3.0mM MgCl2, 0.48 uM primer, magnesium-free reaction buffer and 1U *Taq* DNA polymerase (Promega, USA). After initial heating for 5 min at 94° C, samples were amplified using 40 cycles (94° C, 20 s; 42° C, 20 s; 72° C, 1 min) followed by a final extension of the PCR products for 4 min at 72 °C. The products of amplification were analyzed by electrophoresis in 2.0% agarose gels with 1X TAE running buffer, visualized by ethidium bromide staining, and photographed under UV light with a digital Canon power shot G7 camera. Each reaction was repeated twice and negative controls accompanied the reactions without adding DNA for increasing the fidelity of the data. Twelve RAPD primers were used six of them were from the kit of Ready-To-Go RAPD Analysis Beads, GE Health Care, UK and the remaining six were UBC162, UBC232, UBC245, UBC261, OPD-05 and OPA-11 Table (1).

Table (1) RAPD primers used in this study.

RAPDA1	GGTGCGGGAA
RAPDA2	GTTTCGCTCC
RAPDA3	GTAGACCCGT
RAPDA4	AAGAGCCCGT
RAPDA5	AACGCGCAAC
RAPDA6	CCCGTCAGCA
UBC162	AACTTACCGC
UBC232	CGGTGACATC
UBC245	CGCGTGCCAG
UBC261	CTGGCGTGAC
OPD-05	TGAGCGGACA
OPA-11	CAATCGCCGT

5- Statistics and data analysis

In RAPD all visible and unambiguously scorable fragments amplified by the primers were scored by visual observation. Amplification profiles (band in each position) were scored

as present (1) or absent (0). The obtained scores using all primers in the RAPD analysis were then joined and used to estimate polymorphic locigenetic distance and to construct a UPGMA (Unweighted Pair Group Method of Arithmetic Means) dendrogram among populations using a computer program, PAUP4 (Swofford, 2000).

RESULTS AND DISCUSSION

1- Genetic diversity

The obtained results showed that all primers were proven to be polymorphic, produced 101 bands in total. The number of bands per primer ranged from 1-7 as shown in (Fig. 1).

The genetic distance among the cultivars ranged from 0.21782– 0.53465, the closest species were *A. raddiana* and *A. laeta* and the furthest were *A. albida* and *A. farnesiana* (Table2). The primers UBC245 & OPA-11 produced the highest number of polymorphic bands (10). The remaining primer showed slightly lower polymorphism and the lowest polymorphism was found in RAPDA5.

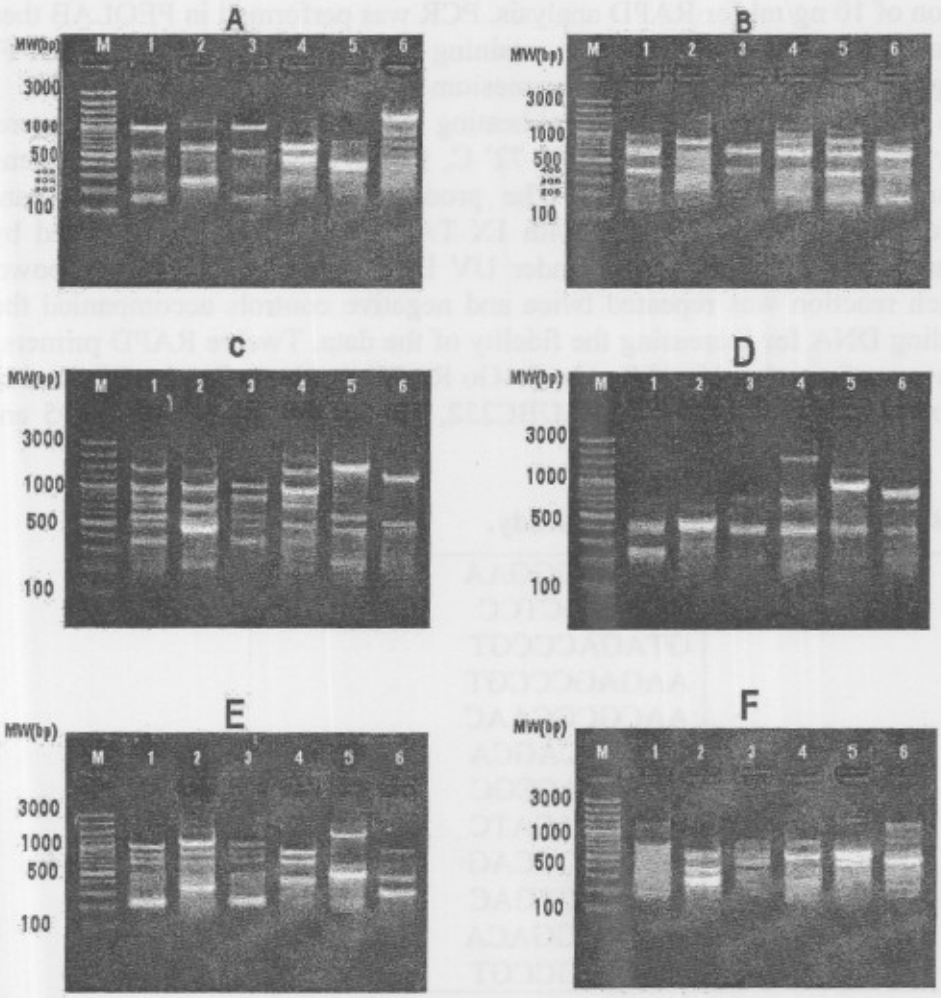

Figure (1): RAPD profiles of A) UBC261, B) UBC245 C) RAPDA2 D) RAPDA3 E) RAPDA4 F) RAPDA5 of six species of *Acacia*. 1) *A. raddiana* 2) *A. farnesiana* 3) *A. laeta* 4) *A. nilotica* 5) *A. albida* 6)*A. saligna* M)100bp Molecular weight marker.

Table (2): Genetic distances based on mean character differences.

	A. raddiana	*A. farnesiana*	*A. laeta*	*A. nilotica*	*A. albida*	*A. saligna*
A. raddiana	0	0.40594	0.21782	0.36634	0.50495	0.39604
A. farnesiana		0	0.46535	0.47525	0.53465	0.48515
A. laeta			0	0.40594	0.46535	0.37624
A. nilotica				0	0.47525	0.36634
A. albida					0	0.42574
A. saligna						0

The dendrogram based on genetic distance Unweighted Pair Group Method of Arithmetic Means (UPGMA) (Fig. 2) indicated that the Egyptian grown acacias differ significantly in their genetic characteristics also revealed that the furthest species was *A. albida* which is in agreement with the study species where *A. albida* differs from the rest of the genus in numerous ways (Steentoft, 1988). The close relatedness between *A. saligna* and *A. nilotica* is in agreement with that mentioned by Bahieldeen *et al.* (2010).

The conservation of plant populations and species is mostly concerned with the number of genetic individuals present in populations in order to asses factors such as inbreeding depression and lack of mates in self-compatible species. The breeders need to be able to estimate the degree of relatedness between the existing materials. The genetic relationship can be useful for designing strategies for breeding programs to produce recombinant hybrid genotypes with superior phytochemical composition; timber quality and biomass yield (Nada *et al.*, 2004).

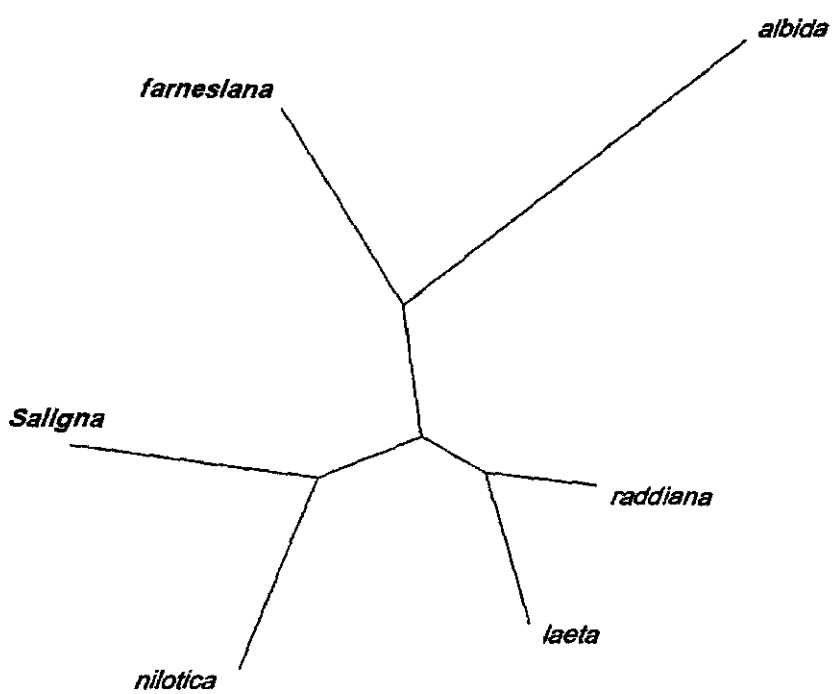

Figure (2): Dendrogram of six selected *Acacia* sp. in Egypt based on UPGMA analysis. The analysis showed the close relatedness between *A. raddiana* and *A. laeta* and that the furthest species was *A. albida*.

2- Tannins and phenolic total contents

The data for tannins analysis the data (Table 3) showed significant differences between all the six species except *A. saligna* and *A. nilotica* where they scored the highest amount of tannins with no significant differences between them. The lowest value was found in *A. raddiana*. The variation achieved in tannins content is in agreement with the evidence that the amounts and type of tannins synthesized by plants vary considerably depending on the plant species, cultivars, tissues, stage of development and environmental conditions (Giner-Chavez *et al.*, 1997). In the other hand the phenolic content analysis showed no significant difference on (*A. nilotica*, *laeta* and *albida*) where they scored the highest amounts and clear significant difference between them and the other three (*A. raddiana*, *farnesiana* and *saligna*).

Table (3): Phenol and tannin contents in leaves.

Genotype	Phenols (mg/ml leaf extract)	Tannins (mg/ml leaf extract)
A. raddiana	0.78 d	2.76 f
A. farnesiana	0.95 c	3.60 d
A. nilotica	1.53 a	4.24 a
A. albida	1.54 a	3.05 e
A. laeta	1.62 a	3.37 b
A saligna	1.36 b	4.28 a
LSD0.05	0.17	0.13

3- No relatedness between genetic markers , tannins and phenols

In general there was no relatedness between genetic markers (RAPD) and physiological markers (leaf tannins and phenols). The RAPD analysis showed the close relatedness between each of (*A. raddiana* and *A. laeta*) and (*A. saligna* and *A. nilotica*), the analysis also showed that *A. albida* was the furthest species. Leaves tannins and phenols contents didn't show similarity with each other and didn't associate with the genetic markers in general. The similarity between *A. saligna* and *A. nilotica* achieved in the leaf tannins contents may be attributed to the similarity between the responsible genetic loci for the production of tannins which may explain the narrow association achieved between genetic background and leaf tannin content.

REFERENCES

Bahieldeen A., Mourad M. M., Alsafa H. M. 2010. Molecular taxonomy of some selected taxa of subfamily mimosoideae. Journal of American Science 6 (10).

Clarke H. D., Downie S.R., Seigler D.S. 2000. Implications of chloroplast DNA restriction site variation for systematics of *Acacia* (Fabaceae: Mimosoideae). Systematic Botany 25: 618–632.

Coe M. and Beentje H. 1991. A field guide to the Acacias of Kenya. Oxford University Press.

Giner-Chavez B. I., Van Soest P. J., Robertson J. B., Lascano C. ,Pell A. N. 1997. Comparison of precipitation of Alfalfa meal protein and bovine serum albumin by tannins in the radial diffusion method. Journal of Science Food and Agriculture 74: 573-523

Grimes J.W. 1999. Inflorescence morphology, heterochrony, and phylogeny in the Mimosoid tribes Ingeae and Acacieae (Leguminosae: Mimosoideae). Botanical Reveiws 65: 317–347.

Letizia C. S., Wellington J.C.J.A., Funk C, Api A.M. 2000. Monographs on Fragrance Raw Materials. Cassie Absolute (*Acacia Farnesiana* (L.) Willd.). *Food Chem. Toxicol.* 38:S27–S29.

Luckow M., White P.J., Bruneau A., 2000. Relationships among the basal genera of Mimosoid legumes. In: Herendeen P.S., Bruneau A. (Eds.), Advances in Legume Systematics, vol. 9. Royal Botanic Gardens, Kew, London, pp. 165–179.

Millar M. A., Byrne M, Nuberg I., Sedgley M.2008. A rapid PCR-based diagnostic test for the identification of subspecies of *Acacia saligna*. Tree Genetics & Genomes 4:625–635.

Miller J.T., Bayer R.J., 2000. Molecular phylogenetics of *Acacia* (Fabaceae: Mimosoideae) based on chloroplast TRNK/MATK and nuclear histone H3-D sequences. In: Herendeen P.S., Bruneau P.S.A. (Eds.), Advances in Legume Systematics, vol. 9. Royal Botanic Gardens, Kew, London, pp. 180–200.

Miller J.T., Bayer R.J. 2001. Molecular phylogenetics of *Acacia* (Fabaceae: Mimosoideae) based on the chloroplast MATK coding sequence and flanking TRNK intron spacer region. American Journal of Botany 88: 697–705.

Nada R.M., Nayak S., Rout G.R. 2004. Studies on genetic relatedness of Acacia tree species using RAPD markers. Biologia, Bratislava 59: 115-120.

Oballa P.O .and Olngotie P.A.S. 1993. Chromosome numbers in two African Acacia species. Kew Bulletin 49: 107-113.

Olesz W., Lee C.Y., Jaworski A.W., Price K.R. 1988. J.Agric.Food Chem. 36: 430-432.

Orwa C., Mutua A., Kindt R., Jamnadass R., Simons A.2009. Agroforestree Database:a tree reference and selection guide version4.0(http:// www.worldagroforestry.org/af/treedb/)

Robbertse P. J., Swartz P. , Kok P. D. F. 1981. Variation of Acacia karroo. Bull. Groupe Int. Etude Mimosoideae 9: 69.

Ross J. H. 1979. A conspectus of the African Acacia species. Mem. Bot. Surv. S. Afr.44: 92-95.

Seigler DS. 2002. Economic potential from Western Australian Acacia species: secondary plant products. Conservation Science W. Aust. 4 (3) : 109–116.

Seigler D.S. 2003. Phytochemistry of *Acacia—sensu lato*. Biochemical Systematics and Ecology 31:845–873.

Sinha S.C. 1971. Floral morphology of acacias. Caribbean Journal of Science *11* (3-4).

Sinha S.C. 1980. Morphology of the vegetative structures of acacia nilotica. Caribbean Journal of Science 15 (3-4).

Steel R.G.D. & Torrie J.H. 1982. Principles and procedures of statistics. 1 st Edn., McGraw Hill, Tokyo, Japan.

Steentoft M. 1988. Flowering plants in West Africa. Cambridge University press, New York, ISBN:0521261929.

Swofford D. L. 2000. PAUP* 4.0 Beta Version. Phylogenetic analysis using Parsimony (* and other methods) Version 4. Sinauer Associates, Sunderland, MA.

Washington D.C.H. 1970. Official Method of analysis. Association of official chemists, Washington, pp: 154.

الملخص العربى

تقييم التنوع الوراثي داخل جنس اكاسيا المنزرع بمصر ودراسة علاقته بمحتوى التانينات والفينولات بالاوراق

حسام الانصاري[١] – جيهان جابر مصطفى [٢] – ضياء الانصاري[٣]– عواد حسين[٣]

[١]قسم الزهور ونباتات الزينة وتنسيق الحدائق – كلية الزراعة بالشاطبي – جامعة الاسكندرية، [٢]قسم البساتين (نباتات الزينة)– كلية الزراعة بجنوب الوادي –جامعة قنا، [٣] معمل الزراعة المنضبطة– قسم الفاكهة – كلية الزراعة بالشاطبي – جامعة الاسكندرية

الباحث المتصل: د.حسام الانصاري

كلمات البحث : الاكاسيا– التنوع الوراثي – التانينات– الفينولات – RAPD

الجنس اكاسيا جنس كبير يحتوى على اكثر من ١٣٥٠ نوع بالتوازي مع قيمة طبية واقتصادية كبيرة. في مصر هناك انواع قليلة متواجدة. في هذا البحث قمنا بدراسة العلاقات الوراثية بين ستة انواع من جنس الاكاسيا في مصر باستخدام اثنى عشر بادئ RAPD كما درسنا العلاقة المحتملة بين الدلائل الوراثية والمحتوى الكلي للتانينات والفينولات بالاوراق. النتائج اظهرت تنوع وراثي كبير في جميع البادئات المستخدمة. تحليل بينات ال RAPD باستخدام المسافات الوراثية وتحليل UPGMA اظهر القرب الوراثي بين كلا من النوعين اكاسيا ساليجنا واكاسيا نيلوتيكا وكذلك النوعين راديانا ولابيتا كما اكد تحليل البيانات البعد الوراثي للنوع اكاسيا البيدا عن بقية الانواع المختبرة في البحث وهو ما يتفق مع الحالة المورفولوجية الفريدة لها في الجنس اكاسيا. اجمالي التانينات في الاوراق اظهر فروق معنوية بين جميع الانواع ماعدا النوعين اكاسيا ساليجنا واكاسيا نيلوتيكا. اجمالي الفينولات في الاوراق اظهر عدم وجود فروق معنوية بين اكاسيا نيلوتيكا ولايتا والبيدا ولكن كان هناك فروق معنوية بين الثلاثة المذكورين والثلاثة الاخرين وهم اكاسيا راديانا وفارنسيانا وساليجنا. بشكل عام لم يكن هناك ارتباط واضح بين الدلائل الوراثية باستخدام RAPD والدلائل البيوكيماوية باستخدام التانينات والفينولات الكلية بالاوراق.

مجلد المؤتمر السابع لتربية النبات– الإسكندرية ٤–٥ مايو ٢٠١١

المجلة المصرية لتربية النبات ١٥ (٢): ٢٤٣– ٢٥٠ (عدد خاص)