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LECTROPHORETIC ANALYSIS OF SOME HIBISCUS VARIETIES USING PEROXIDASE ISOZYMES AND THEIR GENETIC DIVERSITY

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ABSTRACT

Hibiscus (Family Malvaceae), is an evergreen flowering shrub native to East Asia. It is widely grown as an ornamental plant throughout the tropics and subtropics. The flowers are large, generally red in the original varieties, firm and lack any scent. Numerous varieties, cultivar and hybrids are available with flower colors ranging from white through yellow and orange to scarlet and shades of pink with both single and double sets of petals.

Consequently, a greet attention has been focused on hibiscus. On the other hand, isozymes considered to be powerful tool for gene variability. Therefore an attempt was done to estimate peroxidase isozymes activities in five varieties of Hibiscus taken from Antoniades Botanical Gardens, Alexandria. Egypt., and classified as four varieties 1- Athene, 2- Cairo, 3- President, and 4- Saphire for species *Hibiscus rosa-sinensis* and Red variety for species *Hibiscus mutabilis* . Such a trait of activity of peroxidase was used in the five varieties under study to regulate genetic diversity.

The result obtained showed that there were a similarity of peroxidase isozymes activities in varieties 1- Athene, 2- Cairo, 3- President, and 4- Sapphire and in contrast, the activities of peroxidase isozymes found to be different in the variety Red since it was found to be more active. The same result observed on genetic diversity since it showed two clusters. Cluster 1 includes four varieties 1- Athene, 2- Cairo, 3- President, and 4- Saphire while cluster 2 includes variety Red.

The importance of such data in the breeding programs is to develop an index for parental selection and the hybridization between different cluster varieties expected to increase the hybrid vigor which can be used in breeding programs.

INTRODUCTION

The genus *Hibiscus* L. belongs to Malvaceae family consisting of about 300 spices. It has been known colloquially as the Chinese hibiscus, that evergreen flowering shrub native to East Asia. It is also known as China rose and shoe flower. It is widely grown as an ornamental plant throughout the tropics and subtropics. The flowers are large (generally red in the original varieties), firm, but lack any scent. Numerous varieties, cultivars and hybrids are available, with flower colors ranging from white through yellow and orange to scarlet and shades of pink, with both single and double sets of petals. Despite their size and red hues attractive to nectar-feeding birds, they are not visited regularly by hummingbirds when grown in the Neotropics (Grant, 1975; Blanchard, 1976 and Klips, 1999).

Many species are grown for their showy flowers or used as landscape shrubs. Hibiscus is also a primary ingredient in many herbal teas. The Gumamela or Hibiscus rosa-sinensis, flower has antifungal, emollient and refrigerant effect. Therefore, in recent years increased attention has been focused on hibiscus (Marken and Beecher, 2000; Puckhaber *et. al*. 2002 and Hiron *et. al*., 2006).

In fact, different nutrition applications have normally affected isozymes activities in plants. The same reaction (isozymes) can occur in the same organism is proven to be not only specially valuable aid in many biological studies, but also providing a new and exciting perspective for the interpretation of a number of problems central to modern biological though, such as cellular differentiation, onto genetic development and evolution (Show, 1965).

The study of peroxidase isozymes has been most prominent in plant where it has probably been studied more than any other isozymes systems. It is well known that, apart of the great interest in plant peroxidases systems from the probable role of these isozymes are the oxidation of the plant hormone, indoleacetic acid. This system deserves the fullest investigation, it is potentially of the greatest physiological importance in plants apparently, such in variation and flexibility (Septtoli, 1997).

Consequently, isozymes and genetic variation of isozymes serve as labels or markers which can be used in the study of different parts of higher organism, in linkage studies and in population studies. Because the techniques require only a small amount of material, they are easily used in mass screening (Brewer, 1970). There is considerable tissue and ontogenetic variation in bands and activity, and also considerable genetic variation in this system (Brewer and Sing, 1969).

Many investigators studied the biochemical genetic assays to determine genetic markers in Hibiscus as using peroxidase isozymes (Ladizinsky, 1979; Arus and Orton 1983; Sinha *et. al.,* 1989 and Hiron *et. al.,* 2006).

The main purpose of the present investigation is to determine genetic markers in Hibiscus using peroxidase isozymes. In order to achieve such a purpose, two main species of hibiscus were chosen, four varieties presented in the first one namely (*Hibiscus rosa-sinensis* cultivar: 1- Athene, 2- Cairo, 3- President, and 4- Saphire) and the second named (*Hibiscus mutabilis* cultivar: Red) .The measurements of hibiscus were made by using the genetic distance in peroxidase activities.

MATERIALS AND METHODS

Plant material:

In the present study five Hibiscus varieties from two species of the genus *Hibiscus* L. belongs to Malvaceae family were used, Figure (a). The first species is *Hibiscus rosa-sinensis* (Chinese Hibiscus, Shoe flower) while the second species, is *Hibiscus mutabilis*. All of these varieties were taken from Antoniades Botanical Gardens, Horticulture Research Institute, Agriculture Research Center, Alexandria. Egypt.

1- Hibiscus rosa-sinensis 'Athene'

2- Hibiscus rosa-sinensis 'Cairo'

3- Hibiscus rosa-sinensis ' President'

4- Hibiscus rosa-sinensis ' Saphire'

5- Hibiscus mutabilis 'Red'

Figure (a): five Hibiscus varieties used in this study for species *Hibiscus rosa-sinensis* (Chinese Hibiscus, Shoe flower) cultivar: 1- Athene, 2- Cairo, 3- President, and 4- Saphire and for species *Hibiscus mutabilis* cultivar: Red.

Names of varieties:

1- *Hibiscus rosa-sinensis* 'Athene'
2- *Hibiscus rosa-sinensis* 'Cairo'
3- *Hibiscus rosa-sinensis* ' President'
4- *Hibiscus rosa-sinensis* ' Saphire'
5-*Hibiscus mutabilis* 'Red' {*Hibiscus mutabilis* is also called Confederate Rose or Cotton Rose}.

Electrophoresis and isozymes technique:

Peroxidase isozymes patterns of the five Hibiscus varieties (*Hibiscus rosa-sinensis* var. 1- Athene, 2- Cairo, 3- President, and 4- Saphire), 5- (*Hibiscus mutabilis* var. Red) were examined using the following procedure:-

Buffers

0.23 M tris-citric acid buffer, pH 8.0 was prepared according to El-Metainy *et al.*, (1977a).

0.01 M Sodium acetate – acetic acid buffer, pH 5.0 according to Show & keen (1967).

Gel medium

Agar – starch – polyvinylpyrolidine (P.V.P.) gel: 1.0 gm. agar, 0.5 gm. P.V.P. and 0.3 gm. of hydrolyzed starch were added to 100 ml. of 0.23 M tris – citric acid, pH 8.0 and then, gel plates were prepared as described by Sabrah and El-Metainy (1985).

Staining soluation

100 ml. of 0.01 M sodium acetate – acetic acid buffer, pH 5.0 containing 0.1 gm. benzidine and 0.5 % were added immediately before staining.

Procedures

Young leaf samples of Hibiscus plants (four month's age) were examined. Samples were homogenized in cool mortar, and the homogenate was absorbed on 1 × 0.2 cm. strips of filter paper. These strips were placed on the origin line of agar gel plate for about one hour, and then the filter paper strips were removed. After that, a constant current of 13 – 14 V/cm, electrophoresis started for 2 hours at 4^0C, using 0.23 M tris – citric acid buffer, pH 8.0, as electrode buffer. After separating the peroxidase isozymes, the gel plates were incubated at 38 ^{0}C for 5 min. and were stained with peroxidase staining solution, Figure (b).

Genetic Diversity

Data analysis:

Means of peroxidase parameters for each variety were used to compute a similarity distance matrix. The data was transformed with stand procedure from NTSYS- PC ver 2.1 (Rohlf, 2000).

The standardization procedure reduced the effect of different varieties used in the present investigation. Cluster analysis was conduced on the Euclidean distance. Matrix with un-weighted pair group method based on arithmetic average (UPGMA) to develop a dendrogram using computer program NTSYS-PC ver 2.1 (Rohlf, 2000).

In the present work, an attempt was carried out to investigate the isozyme patterns of the peroxidase in order to detect any possible biochemical marker. However, the results obtained were also subjected to

measure the similarity degree between the five varieties of Hibiscus L. Samples were classified into too groups according to the species since the first group contained (*Hibiscus rosa-sinensis* var. 1- Athene, 2- Cairo, 3- President, and 4- Saphire), varieties while the second one contained (*Hibiscus mutabilis* var. Red) variety. Leaf samples were employed for studying peroxidase isozymes and measurement of bands was carried out using the computer program software TOTALLAB V. I.II.

RESULTS AND DISCUSSION

The obtained data showed differences in band numbers, band volume, peak height and R.F. parameter in the investigated samples. The following parameters were estimated during the electrophoretic analysis:

- Band volume: It indicates the value resulting from the interaction between band area and band density. It refers the amount of isozyme, which was expressed from a given gene.
- Peak height: It refers the density of the band and this indicates the activity of isozyme.
- R.F. (Retardation factor): It refers the position of band from the original line to its position as relative number-typically between 0 and 1. or, it refers to the migration distance between original line and band position as relative number-typically between 0 and 1.

Figure (1) represents the band density curve which indicated the density of each band due to the distance between each other for variety (*Hibiscus rosa-sinensis* 'Athene'), and showed the electrophortic patterns of the same variety. The data indicated that there were three bands migrated towards the cathode, while there were two bands migrated towards the anode.

It is observed from data in Table (1) that band volume, peak height and R.F. found to be different from band to another it was 102.815, 63.541, 176.340, 77.652 and 211.419 for band 1,2,3,4 respectively. It was 102.815 for band 1 (C1), 63.541 for band 2 (C2), 176.34 for band 3 (C4), 77.652 for band 4 (A4) and was 211.419 for band 5 (A5). The peak height recorded 159.18 for C1, 157.32 for C2, 109.14 for C3, 121.89 for A1 and 103.36 for A2. The RF gave 0.246 for C1, 0.288 for C2, 0for C3, 0.621 for A1 and 0.730 for A2.

Considering the variety (*Hibiscus rosa-sinensis* 'Cairo'), the data obtained from the band density curve and the digram of peroxidase isozyme as well (Figure 2), it can be noticed that there were three bands migrated to the cathod (C1, C2, and C3) and one band migrated to the anode (A1).

Table (2) showed that the band volume gave 46.648, 126.049, 226.062 and 217, 953 for C1, C2, C3 and A1 respectively. The peak height recorded 140.43, 168.14, 104.29 and 102.11 for C1, C2, C3 and A1. The R.F. gave 0.232, 0.274, 0.414 and 0.733 for the same bands respectively.

From figure (3) one can observe that peroxidase patterns for variety (*Hibiscus rosa-sinensis* 'President') illustrated three cathodal bands and two anodal bands. Table(3) cleared that bands volume for such variety found to

be 49.872, 122.159, 183.43 for C1, C2, and C3 while it was 51, 720 and 188.003 for A1 and A2 respectively. The peak height recorded 151.00, 163.14, 116.64, 77.00 and 103.43 while, R.F. gave 6.242, 0.281, 0.428, 0.621 and 07.44 for the same trend of bands (C1, C2, C3, A and A2).

Figure (4) showed two cathodal bands (C1, C2) and one anodal band (A1) for variety (*Hibiscus rosa-sinensis* ' Saphire').

Table (4) showed the band volume, peak height and R.F. for variety (*Hibiscus rosa-sinensis* ' saphire'). It can be noticed from Table (4) that the band volume was 153, 693 for C1, 84.447 for C2 and 150.811 for A1 and peak height was 115.14, 82.54 and 67.32 while RF gave 0.309, 0.505 and 0.751 for C1, C2, and A3.

In conclusion, the four varieties showed a similar activity in peroxides isozyme bands.

As shown in figure (5), the band density curve cleared that the variety (*Hibiscus mutabilis* 'red') found to be much more effective in isoperoxidase activity. It was clearly noticed that such variety showed a higher isoperoxidase activity at the cathodal direction since there were four bands in the cathodal side C1,C2,C3 and C4 while in anodal direction, three bands were presented, A1, A2 and A3. The data presented in Table (5) cleared that bands existence, band volume, peak height and R.F. parameters were found to be differed than that obtained from all varieties under study. For example, the first band (C1) gave 151.779 of band value, the peak height recorded 172.32 and R.F. was 0.274 which mean that such variety showed excess activity in both cathodal and anodal directions compared with the other varieties (Table, 5).

Such results are in harmony with those obtained by Cahoon and Stevenson (1986); Kudoh and wigham (1997); Middleton (1999); Zeidler (2000); Suchalatha and Shyamala Devi (2004) and Hiron *et al*; (2006).

Figure (6) represents the dendrogram of cluster analysis of the five varieties under study. The figure showed that there were two clusters in the dendrogram tree, and the analysis was capable to classify the studied varieties into two big clusters, the first one included varieties Athene, Cairo, President and Saphire and the second was of Red variety.

The importance of such data in the breeding programs is to develop an index for parental selection and the hybridization between different cluster varieties expected to increase the hybrid vigor and allelic diversity which can be used in breeding programs (Van Beuningen and Busch, 1997 and Saleh and Attalah, 2005).

In fact, the activation of isozymes such as peroxidase plays an important role in plant such as flowering, and metabolism of photosynthesis and translocation of proteins. Analysis of peroxidase activities provides a useful tool to assess the physiological conditions in Hibiscus plants. In addition it is known that, the oxidase activity of plant peroxidases is modulated by certain phenolics and consequently increased such phenolics (El-Taweel, 2007). Thereby, the resistance of some insects may be due to the increasing of phenolics as a result of peroxidases (El-Taweel, 2008).

Therefore, it is recommended to study the varieties which can be able for giving highest potentiality in flowering production. In addition, the breeder has the chance to select varieties suitable for him and could utilize such result in selecting genotype with some parameters that have good effect in flowering.

Figure (b): Peroxidase isozymes patterns of five *Hibiscus* varieties under study.
1- *Hibiscus rosa-sinensis* 'Athene'
2- *Hibiscus rosa-sinensis* 'Cairo'
3- *Hibiscus rosa-sinensis* 'President'
4- *Hibiscus rosa-sinensis* 'Saphire'
5-*Hibiscus mutabilis* 'Red'

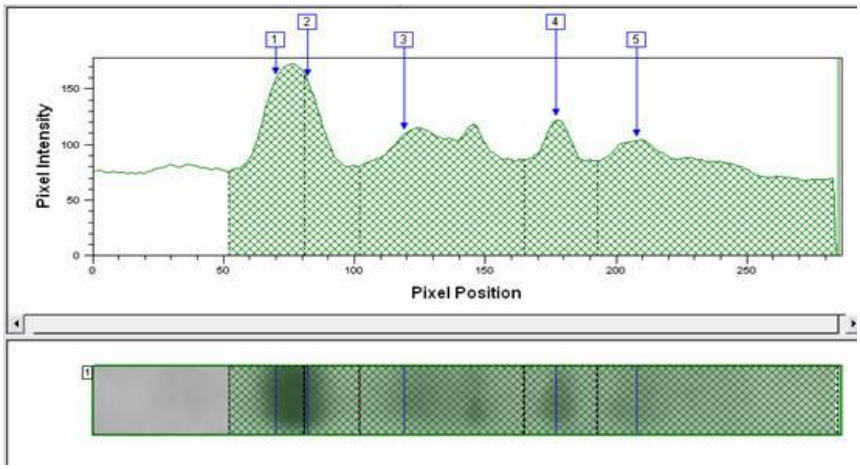

Figure (1): The band density curve and Peroxidase gel electrophoresis analysis for *Hibiscus rosa-sinensis* 'Athene'

Table (1): Analysis of electrophoretic data obtained from *Hibiscus rosa-sinensis* 'Athene'

Band	Posn	Volume	Peak	Area	Band %	Lane %	RF
1	70	102.815	159.18	812.00	16.27	13.81	0.246
2	82	63.541	157.32	588.00	10.06	8.53	0.288
3	118	176.340	109.14	1.764.00	27.91	23.68	0.418
4	177	77.652	121.89	784.00	12.29	10.43	0.621
5	208	211.419	103.36	2.578.00	33.46	28.39	0.730

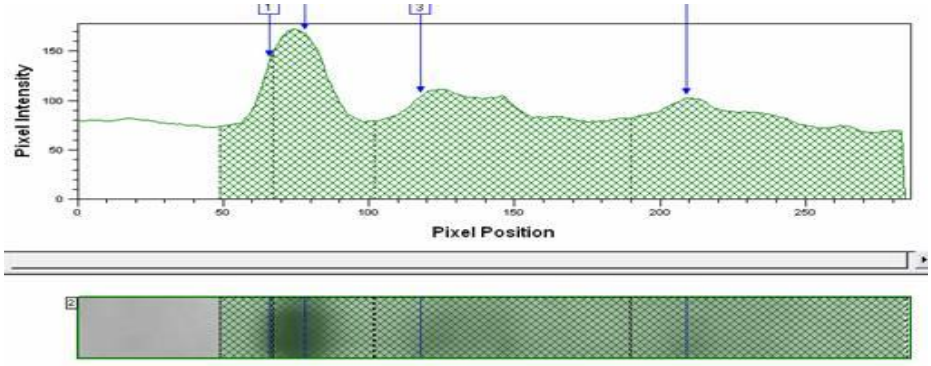

Figure (2): The band density curve and Peroxidase gel electrophoresis analysis for *Hibiscus rosa-sinensis* 'Cairo'

Table (2): Analysis of electrophoretic data obtained from *Hibiscus rosa-sinensis* 'Cairo'

Band	Posn	Volume	Peak	Area	Band %	Lane %	RF
1	66	46.048	140.43	504.00	7.47	6.37	0.232
2	78	126.049	168.14	980.00	20.46	17.43	0.274
3	118	226.062	104.29	2.464.00	36.69	31.26	0.414
4	209	217.953	102.11	2.660.00	35.38	30.14	0.733

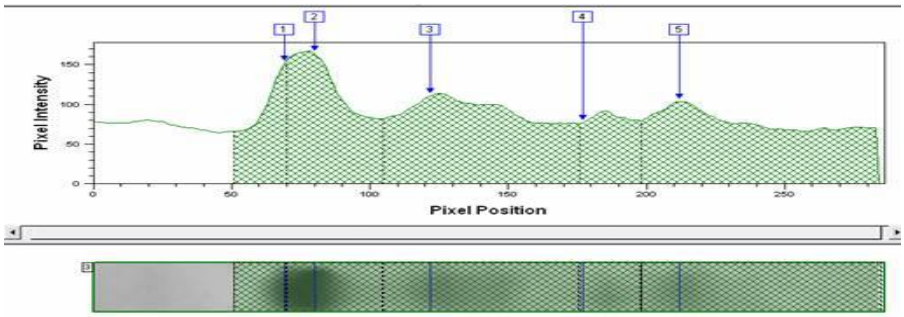

Figure (3): The band density curve and Peroxidase gel electrophoresis analysis for *Hibiscus rosa-sinensis* 'President'

Table (3): Analysis of electrophoretic data obtained from *Hibiscus rosa-sinensis* 'President'

Band	Posn	Volume	Peak	Area	Band %	Lane %	RF
1	69	49.872	151.00	532.00	8.38	7.12	0.242
2	80	122.159	163.14	980.00	20.52	17.44	0.281
3	122	183.430	110.64	1.988.00	30.82	26.19	0.428
4	177	51.720	77.00	616.00	8.69	7.39	0.621
5	212	188.033	103.43	2.436.00	31.59	26.85	0.744

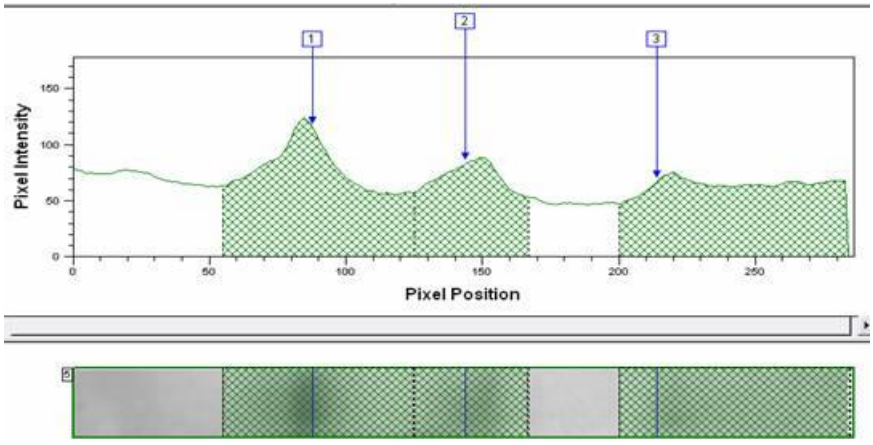

Figure (4): The band density curve and Peroxidase gel electrophoresis analysis for *Hibiscus rosa-sinensis* 'Saphire'

Table (4): Analysis of electrophoretic data obtained from *Hibiscus rosa-sinensis* 'Saphire'

Band	Posn	Volume	Peak	Area	Band %	Lane %	RF
1	88	153.693	115.14	1.960.00	39.51	28.31	0.309
2	144	84.447	82.54	1.176.00	21.71	15.55	0.505
3	214	150.811	67.32	2.380.00	38.77	27.78	0.751

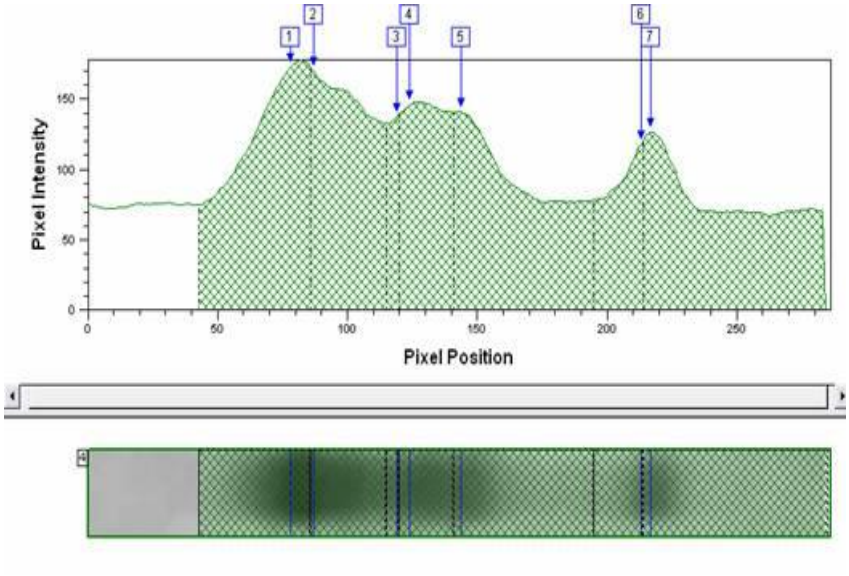

Figure (5): The band density curve and Peroxidase gel electrophoresis analysis for *Hibiscus mutabilis* 'Red'

Table (5): Analysis of electrophoretic data obtained from *Hibiscus mutabilis* 'Red'

Band	Posn	Volume	Peak	Area	Band %	Lane %	RF
1	78	151.779	172.32	1.204.00	20.79	18.52	0.274
2	87	122.817	169.93	812.00	16.83	14.98	0.305
3	119	18.788	137.36	140.00	2.57	2.29	0.418
4	124	84.702	145.61	588.00	11.60	10.33	0.435
5	144	145.877	140.43	1.512.00	19.99	17.80	0.505
6	213	48.848	117.54	532.00	6.69	5.96	0.747
7	217	157.121	126.39	1.988.00	21.53	19.17	0.761

4 3 1 2 5

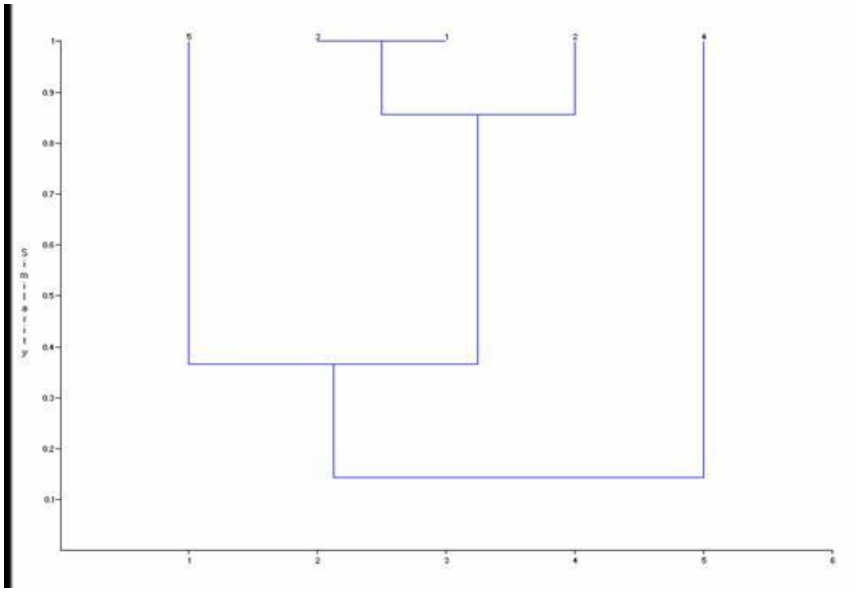

Figure (6): Dendrogram of cluster analysis of *Hibiscus rosa-sinensis* 'Athene', *Hibiscus rosa-sinensis* 'Cairo', *Hibiscus rosa-sinensis* ' President', *Hibiscus rosa-sinensis* ' Saphire' and *Hibiscus mutabilis* 'Red' based on 0-1 cm. on data.

1- *Hibiscus rosa-sinensis* 'Athene'
2- *Hibiscus rosa-sinensis* 'Cairo'
3- *Hibiscus rosa-sinensis* ' President'
4- *Hibiscus rosa-sinensis* ' Saphire'
5-*Hibiscus mutabilis* 'Red'

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استخدام التحليل الانزيمى البيروكسيديز فى تحديد درجة القرابة لبعض اصناف نبات الهبسكس

هشام فخرى الطيب

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

نبات الهبسكس *Hibiscus* شجيرة موطنها الأصلي الصين تصل في بعض الأحيان إلي ارتفاع حوالي (٦) أمتار , الأوراق بيضة والأزهار ذات لون (أحمر أو أبيض أو أصفر أو بمبي أو أبيض منقط بالأحمر وبعض الألوان الأخرى وتزهر في معظم أيام السنة والثمرة علبه ، وينجح النبات في الأماكن المشمسة والنصف مظللة ذات الرطوبة المتوسطة ويجري التقليم عادة عندما يخف موسم التزهير,الأزهار محبوبة للنحل وتتكاثر بالعقلة أو البذرة.

وقد اجرى هذا البحث بغرض تحديد درجة القرابة لخمسة اصناف من نبات الهبسكس اخذت من الحديقة النباتية بانطونيادس بالاسكندرية وقسمت على النحو التالى:

اربعة اصناف من جنس *Hibiscus rosa-sinensis* هم: Athene و Cairo و President و Saphire

و الصنف الخامس Red من جنس *Hibiscus mutabilis* . وباستخدام المشابه الانزيمى البيروكسيديز لما يمثله من اهمية فى ميتابولزم النبات سواء كان فى التزهير او التمثيل الضوئى ونشاط البروتين.

و اوضحت النتائج المتحصل عليها ان هناك اختلاف فى النشاط الانزيمى للبروكسيديز حيث وجد تشابها فى النشاط الانزيمى للاربعة اصناف ..... بينما اظهر الصنف الخامس نشاطا انزيميا ملحوظا ممثلا فى ظهور سبعة حزم كهربائية مقارنة بالاربعة اصناف الاخرى وان العلاقة بين النشاط الانزيمى للخمسة اصناف المستخدمة اظهر تماثلا للاربعة اصناف التالية

Hibiscus rosa-sinensis cultivar: 1- athene, 2- cairo, 3- president, and 4- saphire

بينما اظهر عدم التماثل للصنف الخامس *Hibiscus mutabilis* cultivar: red.

مثل هذه النتائج يمكن الاستفادة منها فى برامج التربية حيث يمكن ادخال الاصناف التى تحمل اختلافا فى برامج التربية للحصول على قوة الهجين والجمع بين الصفات المرغوبة وانتاج اصناف من نباتات الزينة تحمل من الصفات التى يمكن من خلالها تحسين الصنف نفسه.