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CYTOLGICAL AND ISOZYMES STUDIES OF GARLIC CULTIVATED IN EGYPT.

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ABSTRACT

Generally It has been accepted that the apomictic nature of nature of garlic clones could affect its cytological and genetical features as a result of accumulation of certain somatic mutations. Thus, the aim of this study is to examine some of the cytological (karyotyping, mitotic index and mitotic abnormalities) and isozyme characteristics of five garlic genotypes cultivated in Egypt (Baladi , Chinese , Chinese , 30-4 , Chinese 31-1and Chinese 31-3).Relative chromosome lengths and centromere position of these genotypes revealed insignificant difference in their karyotype formula (5 large , 2 medium from submetacentric pair and one large metacentric pair no. 5). The percentage of mitotic index of Baladi genotype (8.28%) was not very much different from that of Chinese (12.08%) whereas it was significantly lower than those of Chinese 30-4 (15.6%),Chinese 31-1(18.18%)and Chinese 31-3 (16. 8%). Means of total chromosomal abnormalities (including anuepliody, break and stickiness) were remarkably lower in genotypes Baladi (7.64%) and Chinese (9.38%)than those of Chinese 30-4 (11.94%), Chinese 31-3 (11.2%). Statistical analysis revealed that there was a high correlation (0.77) between the mitotic index and the percentage of mitotic chromosome abnormalities. Isozymes [Acid phosphatase, Glutamate Oxaloacetate Transaminase (GOT), Peroxidase and Esterase) banding patterns were qualitatively compared. The banding patterns of esterase (2bands) and GOT (one band) were similar in all five genotypes. As for Pyroxidase, the four genotypes of the Chinese origin had two bands, whereas only one band was shown by the genotype Baladi that was similar in molecular weight to small band of Chinese genotypes. All studies genotypes of acid phosphatase showed one band, which was of a low molecular weight in all except in Chinese genotype where it was a high molecular weight and Chinese 31-1 in which both low and high molecular weight bands were found.

Keywords : Cytological analysis, Mitotic index, Mitotic abnormalities, Karyotyping, Chromosome lengths, Centromere position and isozyme characteristics of five garlic genotypes.

INTRODCUTION

Garlic has been used for thousand years as a food additive and as a medicine in China (Hun, 1993). It belongs to the Liliaceae family and genus *Allium,* which has more than 600 available species. This family included onions, shallots, leeks, Japanese bunching onion, Chinese and common chives. Mostly all Allium, crops originate from the main center of Allium diversity that stretches from the Mediterranean basin to central Asia (Meer *et al*., 1997). The sativum variety, or common garlic, produces a weak flower stalk, if it bolts, and has a bulb with many pure white or pink – blushed bulblets (Poorler and Simon, 1993). Most garlic cultivars are sterile, so this crop is reproduced vegtatively by cloves. Bozzini, 1991 reported that common garlic has a somatic number of 2N=16 (with a karyotypic formula of 6 metacentric chromosomes, 4submetacentric chromosome and 6 areocentric

chromosome) and some garlic plants found in the Campania region of Italy were shown to be tetraploid. With 4N=32 No polyploidy forms are found in garlic, although some varieties might be triploid, it is difficult to explain the extensive variability in size, color and shape found in garlic cultivars unless extensive somatic mutations occur in this species. Most likely, these have been accumulated after year of clonal propagation (Vosa, 1976). Because of the obligate apomictic nature of garlic, no classical breeding has been possible in this crop, although this is changed with the reports of fertile cultivars (Quiros, 2001). Chromosomal aberrations are common in garlic, due to multiple translocations sometime involving, 8 or even 10 chromosomes. Some varieties have an index is one of mitotic parameters, which reflect the genetic control system of division and the existence of chromosomal aberrations several organisms (Kaushik, 1996). In addition, isozyme studies were undertaken to classify and categorize genetic diversity in garlic (Al-Zahim *et al*., 1997; Bradley *et al*., 1996; Lallemande *et al*, 1997; Maass and Klaas,1995). Mitotic index, visible chromosomal alterations and isozyme patterns of certain genotypes cultivated in Egypt (Baladi, Chinese, Chinese 30-4; Chinese 31-1 and Chinese 31-3) were analyzed and compared in this study.

MATERIALS AND METHODS

Five garlic genotypes, named Baladi, Chinese, Chinese 30-4; Chinese 31-1 and Chinese 31-3 were kindly obtained from Horticulture Research Institute, Mallawy Research Station, Agricultural Research Center. Cytological studies and isozyme characterizations were made in Genetics Dept. Fac. Agric. Mania Univ. Cytological preparations and mitotic analysis to study some mitotic parameters of the above mentioned garlic genotypes acetocarmin-squashed preparations were made from the cooling-pretreated root tips of 5 cloves each genotype in one factor complete randomized design. Of five good spreads of metaphase cell from each genotype, the chromosomes were arranged according to their relative lengths (the ratio between the length of each chromosome length) the centromeric index (the ratio between lengths of long and short arms of each chromosome in the haploid set) was estimated according to the method of Levan *et al*. (1964) and Green and Sessions (1991), by considering the chromosome is metacentric if the ratio of the length of longer arm (P)/shorter arm (Q) equals 1.00. However, it was subtelocentric if the ratio is higher than 1.00. and equal or lower than 1.79. Mitotic index (the percentage of dividing cells) was estimated according to the method of Yasuhara and Shibaoka (2000) with mitotic chromosome irregularities (chromatin stickiness, chromosome break and aneupliody (2n $\pm$ 1 and /or 2) were scored and compared in the studies garlic genotypes. The statistical analysis of Gomes and Gomes, 1984) using MSTAT program (version4) was applied.

Isozymes analysis:

Extraction and running in PAGE

Procedure of isozyme detection was carried out according to the methods described by Pooler and Simon (1993). Bands were scored directly from the PAGE gels as present or absent sample extractions which were

carried out at 4°C. plant material sample (1g) was homogenized in 1 m1 of 25 mM Na phosphate buffer (pH 7.25) containing 20% sucrose and centrifuged at 12,000 rpm for 10 min and 60 μ1 of each sample was used. System with 7.5% (w/v) gel using 0.2 M Tris – glycin buffer (pH 8.7) was employed. The electrophoresis was carried out at 4°C using 50-60 mA/gel till the tracking dye of bromophenole blue reached the anode

Staining of gels

Esterase was stained with α- naphthylacetate and fast blue B salt in 0.2 M Na acetate buffer (pH5) by incubating the gel at 37°C for 30 min (Bergman and Mann,1973). Peroxidase was stained with benzidine and H2o2 (scandalios, 1974) and acid phosphatase was localized with β-naphthy acid phosphate and fast garnet GBC salt in 50 mM Na acetate buffer (pH 5.5) by incubating the gel at room temperature for 5 hrs (scandalios, 1974). Glutamate Oxaloacetate Transaminase (GOT) was detected with α-ketoglutric acid and fast blue BB salt in 0.2 M phosphate buffer (pH 7.5).

RESULTS

A- Cytological analysis

A-1- Karyotype

The diploid number of all genotypes (Baladi, Chinese (Chinese 30-4; Chinese 31-1) composed of 2n = 16 chromosomes or 8 chromosomes pairs (Fig1). The relative length of each chromosome per genome and its centromer index are given in Table (1). Data showed that there are no considerable differences of relative chromosome length and centromeric index of analogues chromosome among different studied genotypes. The relative length of chromosome pairs no. 1,2,3,4,5 and 6 were with large size (17.9-11.1%), whereas chromosome pairs no. 7 and 8 were with medium size (8.8 to 6.7%). The values of centromeric index indicated that all chromosome are submetacentric (centromere index ranged from 1.1 to 1.59) except that of the metacentric pair no.5 (1.00-1.01)

A-2- mitotic index (M1)

The mean percentage values of mitotic index of five garlic genotypes (Baladi,Chinese, Chinese 30-4; Chinese 31-1and Chinese 31-3) are given in Table (2).statistical analysis (ANOVA and LSD at 0.05) showed that the mitotic index (M1) of Baladi genotype (8.28% ± 4.712) is significantly lower than those of Chinese 30-4 (15.6% ±4.712), 31-1 (18.18% ± 4.712) and Chinese 31-3 (16.8% ± 4.712) whereas varied insignificantly with that of Chinese (12.08 ± 4.712).

A-3- chromosomal abnormalities

Three types of chromosomal abnormalities (anueploidy, break and stickiness) could be scored (Table. and fig.2). The means of total abnormal cells of genotypes Baladi (7.64% ± 2.22%) and Chinese (9.38% ± 2.22%) were significantly less than those of Chinese 30-4 (11.94% ± 2.22%), Chinese 31-1 (13.32% ±2.22%) and Chinese 31-3 (11.82% ± 2.22%) cells with chromosome break (4.8-5.9) were the most frequent abnormal cells in all investigated garlic genotypes. It has been noticed, however, that there was

a high correlation (0.77) between the mitotic index and total percentages of mitotic abnormalities for all five garlic genotypes.

B- Isozymes

Isozymes assay [Esterase, Glutamate Oxaloacetate Transaminase (GOT), Peroxidase and Acid Phosphatase] was performed to detect genetical differences among the five garlic genotypes (Fig. 3 and Table 3). Two bands of esterase were detected in all genotypes under study. This may indicate that the esterase subunits are controlled by two loci. The isozymic pattern of GOT (one band) was also identical in all five genotypes, revealing the similarity of the locus controlling the activity of this isozyme. As for peroxidase, all genotypes derived from Chinese origin exhibited two bands of this isozyme. However, only one band of peroxidase was detected in the Baladi genotype, which seemed identical to the low molecular weight band of the Chinese genotypes. The survey of acid phosphatase revealed variable patterns in the studied genotypes. Baladi, Chinese 31-1 and Chinese 31-3 genotypes exhibited one band of acid phosphatase, which had markedly high activity in Baladi genotype. In addition, Chinese genotype exhibited one band, which migrated more rapidly (low molecular weight) toward the anode than that of Baladi, Chinese 31-1 and Chinese 31-3. however, the genotype Chinese 30-4 revealed two bands corresponding to the high and low molecular weight bands of the other genotypes.

DISCUSSION

The vegetative cloning and apomictic nature of garlic (*Allium sativum L.*)might lead to the existence of extensive somatic mutations. Therefore, some karyological characteristics, mitotic index, some chromosome abnormalities and profile of some Isozymes for five cultivated garlic genotypes (Baladi, Chinese, Chinese 30-4; Chinese 31-1 and Chinese 31-3) are described in the present work.

The diploid number of all five studied genotypes was 16 chromosomes (or eight chromosome pairs). There were no considerable morphological chromosomes in these five clones. By relative length, chromosome pairs no. 1, 2, 3, 4, 5 and 6 were large sized (17.9 – 11.1%), whereas chromosome pairs no 7 and 8 were medium sized (8.8 - 6.7%). The measurements of centromeric indexes revealed that all chromosomes were submetacentric except the metacentric chromosomes, pair no. 5. these results are not in agreement with the report of Bozzini (1991) were submetacentrics in the present materials. These alterations in chromosome morphology in garlic might be due to the common structural chromosomal aberrations such as multiple translocations, which sometimes- involving eight or even ten chromosomes (Etoh and Pank 1996; Barthes and Ricroch, 1998 and Friesen and Klaas, 1998). Thus, the karyological instability in garlic might be due to the accumulation of somatic mutations. The means of mitotic index (MI) of Baladi genotype was significantly lower than those of the three genotypes of the Chinese origin. On the other hand, Baladi genotype showed a differences in genetic control systems of mitosis and / or the quantity of somatic mutations (kaushik, 1996 and Yasuhara and Shibaoka, 2000). The high

correlation (0.77) between mitotic index and chromosomal. Abnormalities in all genotypes were relatively high (7.64 – 13.32% ± 2.22%). Statistical analysis showed that Baladi genotype had a lower percentage of total chromosomal abnormalities when compared to those derived from Chinese origin. Chromosomal breaks were the most frequent aberrations (e.g. translocations and inversions) are expected and occurred as a result of breaks-reunion cycle (McClintock, 1941 and Etoh and Pank 1996). The existence of structural aberrations, aneuploidy and stickiness in genus *Allium* was considered to be an important source of garlic clone diversity (Al-zahim *et al,* 1999; D'Emerico and Pignone, 1998 and Vosa, 2000).

Isozyme analysis revealed that all garlic genotypes had one band of GOT and two bands of esterase. On the other hand, Baladi genotype had low molecular weight band of peroxidaes in spite of two bands (high and low molecular weight) for those genotypes derived form Chinese origin. In addition, banding pattern of acid phosphatase of Baladi genotype varied from two Chinese genotypes (Chinese and Chinese 30-4) whereas it was similar to the other two (Chinese 31-1 and Chinese 30-3). These findings indicated that gene loci coding for peroxidase and acid phoshatase in Baladi genotype were different from those derived of Chinese origin. This variation may reflect of genetic variability that arises from accumulation of gene mutation. The observed chromosomal aberrations and variation in genetic control of some isozyme, during the course of vegetative clonal reproduction, may lead to genetic biodiversity for garlic (Pooler and Simon, 1993; Al-zahim *et al*, 1999 and Ipek and simon, 2001). It could be concluded that Baladi genotype had a low frequency of mitotic index, chromosomal aberrations and variable isozyme profile when compared to those derived from Chinese origin. Hence, Baladi is promising genotype in breeding programs. However, further analyses are needed to ensure these findings such as c- banding, fluorescence in situ hybridization (FISH), random amplified polymorphic DNA (RAPD) and amplified fragment length polymorphism (AFLP).

Table 1: Relative chromosome lengths estimated in percents and their centromere indexes.

Genotype	Chromosome morphology	Chromere No.							
		1	2	3	4	5	6	7	8
Baladi	Relative length	17.8	15.1	14.1	13.4	12.8	11.1	8.8	6.9
	Centromere index	1.14	1.12	1.13	1.25	1.00	1.17	1.31	1.38
Chinese	Relative Length	17.9	15.3	14.5	13.4	12.5	11. 3	8.4	6.7
	Centromere index	1.13	1.08	1.10	1.23	1.01	1.13	1.3	1.40
Chinese 30-4	Relative Length	17.8	15.7	14.5	12.9	12.7	11.1	8.5	6.8
	Centromere index	1.14	1.12	1.10	1.35	1.01	1.17	1.27	1.43
Chinese 31-1	Relative length	17.9	15.2	14.5	13.5	12.4	11.2	8.5	6.8
	Centromere index	1.12	1.12	1.10	1.25	1.00	1.13	1.25	1.59
Chinese 31-3	Relative Length	17.8	15.8	13.9	13.2	12.4	11.3	8.7	6.8
	Centromere index	1.14	1.10	1.3	1.23	1.00	1.13	1.30	1.38

Table 2: The mean percentage values of both mitotic index and chromosomal abnormalities (anueploidy, break and stickiness) of the five garlic genotypes

Genotypes	No of cells examined	Mitotic index (%)	Chromosomal abnormalities (%)			
			Anueploidy	Break	Stickiness	Total
Baladi	18500	8.28	1.92	5.20	0.52	7.64
Chinese	19400	12.08	3.04	5.80	0.54	9.38
Chinese 30-4	20000	15.60	4.00	5.70	2.24	11.94
Chinese 31-1	17100	18.18	4.02	5.90	3.40	13.32
Chinese 31-3	18000	16.80	3.82	4.80	3.20	11.82
LSD≥0.05		4.712	0265	N.S	1.54	2.22
Correlation between M1 and total abnormalities				0.77		

F1-2

Genotypes	No. Bands of Isozymes			
	Acid phosphates	GOT	Peroxidase	Esterase
Baladi	1 (upper)	1	1 (lower)	2
Chinese	1 (lower)	1	2	2
Chinese 30-4	2 (upper, lower)	1	2	2
Chinese 31-1	1 (upper)	1	2	2
Chinese 31-3	1 (upper)	1	2	2

F3

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دراسات سيتولوجية وإنزيمية للثوم المنزرع في مصر

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*** المعمل المركزي للزراعة العضوية مركز البحوث الزراعية**

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نظراً لعدم مقدرة الثوم علي إنتاج بذور فإن التباين وعدم الثبات الوراثي يرجع إلي تراكم الطفرات الجسدية. ولهذا فإن هدف هذه الدراسة هو التعرف علي السلوك السيتولوجي (الطراز الكرموسومي- الدليل الميتوزي – بعض التغيرات الكرموسومية) وكذلك التعرف علي التشابه والاختلاف بين طرز الحزم الأنزيمية في خمسة طرز وراثية من الثوم المنزرع في مصر(البلدي و الصيني و الصيني ٣٠-٤ و الصيني ٣١-١ و الصيني ٣١-٣).

ولقد أظهرت النتائج المتحصل عليها من أطول الكروموسومات النسبية ومواضع سنترومیراتها **أنه** لا يوجد فرق بين الطرز الكرموسومية لهذه الخمسة طرز وراثية من الثوم وأظهرت أن الطرز ألمجموعي الكرموسومي للثوم يتكون من ١٦ كروموسوم أو ثمانية أزواج كروموسومية منها سبعة تحت وسطية السنترومير (خمسة كروسومات كبيرة تتراوح أطوالها من ١١الي ١٧,٩% من الطول الكلي للجينوم واثنان متوسطة تتراوح أطواها من ٦,٧ الي ٨,٨% من الطول الكلي للجينوم) وواحد كبير وسطي للسنترومير هو ٥.

ولقد أظهرت الدراسة أيضاً أن معدل الانقسام الميتوزي في الثوم البلدي (٨,٢٨%) منخفض بشكل معنوي عن الثوم الصيني ٣٠-٤ (١٥,٦%) والصيني ٣١-٣ (١٦,٨%) بينما لم تكن هذه الاختلافات معنوية مع الثوم الصيني العادي (١٢,٠٨%).

كما أظهرت الحزم الأنزيمية لأربع من الأنزيمات(الأستيريز وأنزيم جلوتومات أوكسالو أسيتات ترانسامينير(GOT) والبيروكسيديز والأسيد فوسفاتيز)س النتائج الأتية:

أولاً: بالنسبة لأنزيم الأستيريز كانت هناك حزمتين موجودتين في الخمسة طرز وراثية من الثوم أما أنزيم الGOT فكانت هناك واحدة موجودة في كل الطرز الخمسة أيضاً.

ثانياً: بالنسبة لأنزيم البيروكسيديز كان لجميع الطرز الصينية الأربع حزمتان بينما كان الطراز البلدي حزمة واحدة فقط تتشابه مع تلك الحزمة المنخفضة الوزن الجزيئي الناتجة من جميع الطرز الصينية.

ثالثاً: بالنسبة لأنزيم الأسيد فوسفاتيزفجميع الطرز ماعدا الصيني العادي أظهرت حزمة ذات وزن جزيئي منخفض بالمقاومة للثوم الصيني والذي أظهر حزمة واحدة مرتفعة الوزن ولقد أظهر أيضاً الطراز الوراثي صيني ٣١-١ الحزمتين معاً.