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The impact of environmental conditions on fruit quality and genotype structure of two grapevine cultivars

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In this study we identified two grapevine cultivars (Thompson and Superior) from two different locations in Egypt (El-Behera and El-Minia Governorates) which are characterized by widely different environmental conditions. Fruit quality and RAPD markers were used to examine the impact of environmental conditions on these cultivars. The study was conducted in two growing seasons at the two locations. Environmental condition caused significant differences in cluster weight, berry diameter, Size of 100 berries, juice volume, TSS, cluster and berries number of both cultivars. Meanwhile, there were no significant differences between regions for cluster length and width, berry length, weight of 100 berries. Ten RAPD primers produced clear and reproducible amplification patterns were selected and used to study the impact of environmental conditions on genetic structure of the grapevine cultivars. A total of 241 DNA fragments amplified from the tested cultivars with an average of 24.1 bands per and high level of polymorphism (183 bands, 76%). High similarity (Si = 0.60) and lower genetic distance (Gd = 0.511) were observed between Superior cultivar collected from El-Behera and El-Minia. Thompson collected from El-Behera and El-Minia showed longest genetic distance (Gd = 0.844) and lowest similarity (Si = 0.43). Long genetic distances ranged from 0.713 to 0.844 were observed between the other combinations of Superior and Thompson plants of the two locations. The UPGMA cluster analysis grouped the two grapevine cultivars in two separated clusters according to their genetic background. The present study revealed Superior and Thompson grapes grown in El-Behera and El-Minia possessed considerable genetic differences in addition to the differences exist between the two cultivars. Since these cultivars are established in El-Behera and El-Minia for a long time, these genetic differences between grape plants may be due to the two variegated environments and environmental changes.

Keywords: ***Vitis vinifera*** **L., maturation conditions, genotype, cluster weight, berry diameter**

INTRODUCTION

In Egypt, grape (*Vitis vinifera* L.) is considered the second major fruit crop after citrus and it`s growing areas mainly along the river Nile and in recently reclaimed desert lands. The common ways to propagate grape vine trees are cuttings and grafting method. These ways help to produce uniform seedlings and identical to its mother tree. However, in grapes, degree-days are important in determining the timing of various phenological events where, a temperature regime of 10°Cand temperatures between 28-32°C are most

congenial. Variations in temperature cause alterations in the developmental stages and ultimately the ripening time. Under a higher temperature regime, the number of clusters per shoot was greater and the number of flowers per cluster was reduced (Pouget 1981). The genotype is the genetic constitution of an individual where the potential is encoded, but cannot define alone the phenotypic expression rather interacts with the environment that modulates the response and determines the level of this potential. Moreover, the grape quantity and quality result to the interaction of environment and genotype (Bogicevic, 2013)**.**

There are extensive genetic differences based on phenotypic traits in natural plant populations, this is closely linked to the process of evolutionary adaptation. Such genetic variations also exist in closely relative species of many of staple crops, which are of great importance in agriculture. Therefore, the study of plant natural genetic diversity will provide new insights into biological mechanisms, and provide a vital source of genetic diversity for crop breeding and improvement (Henderson, and Salt, 2017).Pieri et al., (2012) showed that, climate change is expected to significantly increase temperatures and decrease rainfall. Thus, grape maturation conditions and physiological processes might all be modified with important consequences on grapevine production. Moreover, Vujadinović et al., (2012) showed that increasing in temperature may lead to extended growing season duration, as well as an increase in Growing Degree-Days by 1000 units, by the end of the 21stcentury.The dormant period of grapevines could be short and affected by warm winters with lower frost days. Changes in the selection or vineyard locations of grapevine cultivars could in turn affect water supply and overheating.

However exposing for different environmental conditions for long term may resulted in genetic variations. Such these variations used in production of delicious apples, navel orange and many other fruits and flower production. Environmental changes are involved in plant evolution by developing and fixation of character that confer the adaptation to plants (Bita and Gerats, 2013). Adaptation to challenging environments presents by enhancing and/or suppression of gene expression or existing new genotypes by excitation the mutations (Booth and Lees, 2007).

The genome evolution is characterized by the accumulation of changes over time. Moreover, many mechanisms such transposable elements, mutationexon shuffling and gene loss could participate to genome evolution (Oliver et. al., 2013).

Molecular markers can assist breeding programs by means of determining the origin and genetic distance of the cultivars (SEFC et al., 1998; BOWERS et al. 1999; Bakr, et al., 2013; El-Aref et al., 2016 and Abd El-Fatah et al., 2017). Many useful results have been gathered in the genomic data collections originating from the molecular genotyping of varieties in Bulgaria (Hvarleva et al., 2004), Croatia (Maletic et al., 1999), Greece (Lefort and Roubelakis-angelakis 2001), Italy (Pellerone et al., 2001, Labra et al., 2002, Zulini et al., 2002), Portugal (Lopes et al., 1999), Spain (Ibañez et al. 2003) and Egypt (Shoukry, et al., 2013 and El-Aref et al., 2016). Conservation, characterization and sustainable utilization of genetic resources in breeding program and cultivation require the maintenance of old varieties and their identification. Besides morphological traits, DNA marker systems should be involved as additional 'descriptors' for varietal identification to establish a 'DNA-based ampelographic system' (Halasz, et al., 2005 and Abou-Ellail, et al., 2014). (Papadopoulou,et al., 2002 and Mustafa and Abou-Ellail, 2013) reported that nevertheless, fig cultivars have a rather narrow genetic base, RAPD markers could detect enough polymorphism to differentiate even closely related genotypes (i.e., clones of the same cultivar) and a unique fingerprint for each of the genotypes studied was obtained. Also, cluster analysis allowed the identification of groups in accordance with geographic origin, phenotypic data and pedigree. In this study we identified two grapevine cultivars (Thompson and Superior grape cultivars) from two different locations in Egypt (El-Behera and El-Minia Governorates) which are characterized by widely different environmental conditions. Fruit quality and RAPD markers were used to examine the impact of environmental conditions on these cultivars.

MATERIALS AND METHODS

Plant material:

This study was carried out during two seasons, 2013 and 2014. Thompson-seedless and Superior grape cultivars were obtained from El-Behera and El-Minia Governorates. Thompson-seedless and Superior grapevines were 28 and 15 old years under El-Behera governorate, respectively. Thompson seedless and Superior

seedless grapevines have 13 and 10 old years under El-Minia governorate, respectively. The total yield was recorded on basis of an individual vine and expressed as Kg/vines. Samples of five clusters from each replicate were taken to determine physical and chemical properties. Physical parameters (Cluster weight (g), length and width (cm)-weight (g) and size (ml) of 100 berries – Juice volume(ml)-berry length and diameter (mm)-Clusters and berries number) . Coefficient of cluster compactness was calculated by dividing the number of berries per cluster by its length a ccording to Weaver et al., 1962 .Chemical parameters (Refractometric total soluble solids, titratable acidity using 0.1 NaoH were determined according to Baur and Ensminger (1977). TSS/Acid ratio was also determined. The analysis of Polymerase Chain Reaction (PCR) was used to study the impact of environmental conditions on genetic structure of these grapevine cultivars.

DNA extraction:

Genomic DNAs were extracted by grounding 500mg young fresh leaves in a mortar with liquid nitrogen. The powder was transferred into centrifuge tube, containing 9.0 ml of CTAB extracting buffer and incubated at 65°C for 60-90 min. After then, 4.5 ml of chloroform/octanol (24/1 by volume) was added and the tubes were shacked to mix for 10 min. and centrifuged for 10 min. at 3200 rpm. The supernatants were transferred into new tubes and 6 ml isopropanol was added. After 60 min., the tubes were centrifuged for 10 min. The pellets obtained were suspended in 400 µl of TE buffer of a pH 8.0 (10 mM Tris-HCl, pH8.0 + 1.0 mM EDTA, pH 8.0) (Sagahi-Maroof et al., 1984), transferred to sterile Eppendorf tube and stored at 20°C until use.DNA concentration in the sample was quantified using a spectrophotometer. The concentration of DNA which will be used in RAPD reaction was calculated according to Sambrook et al., (1989).

Primers and PCR assays:

Ten Primers (Table 1), obtained from Pharmacia Biotech. (Amersham Pharmacia Biotech UK Limited, Ebgland HP79 NA), produced clear and reproducible amplification patterns were used to study the long term effect (more than 15 years) of environmental conditions on two grapevine cultivars grown in Lower and Upper Egypt.PCR reactions were performed in a 25 ml volume reaction mixture containing: 20 ng of total cellular DNA (1.5 ml), 50 pM of primer (1 ml), 2.5 ml of Taq DNA polymerase buffer, 1.5 U of Taq DNA polymerase (QBIOge`ne, France), 200 mM of each d NTP (DNA polymerization mix, Pharmacia).

Table 1: Ten primers sequenced those used to identify differences in genomic structure of two grapevine cultivars planted in two different sites for more than 15 years.

Primers	Sequences
OP-A01	CAG GCC CTT C
OP-A02	TGC CGA GCT G
OP-A04	AAT CGG GCT G
OP-A05	AGG GGT CTT G
OP-A09	GGG TAA CGC C
OP-A11	CAA TCG CCG T
OP-B15	TTC CGA ACC C
OP-A16	AGC CAG CGA A
OP-A18	AGG TGA CCG T
OP-A20	GAC CAA TGC C

The reaction mix was overlaid with drop of mineral oil to avoid evaporation during the cycling. PCR was performed in a DNA thermocycler (Crocodile III QBIOge`ne, France). Samples were first heated at 948C for 5 min and subjected to 35 repeats of the following cycle: 30 seconds at 948C, 1 min at 358C, 1 min at 728C. A final step of five min at 728C was always run. To reduce the possibility of cross contamination and variation in the amplification reactions, master mixes of the reaction constituents were always used. A negative control (reaction mix without any DNA or without any enzyme) was also included.

Data handling and cluster analysis:

RAPD-based molecular markers were scored for computer analysis on the basis of the presence or absence of the amplified products for each primer. If a band (DNA fragment) was present in a genotype, it was designated as “1” while, if absent it was designated as “0”. Similarity coefficients determination depends on the pai rwise comparisons of genotypes, based on the presence or absence of shared polymorphic and unique bands, according to Jaccard (1908).

The dendrogram construction requires the similarity coefficient calculation, using the un weighted-pair-group method with arithmetic averages (UPGMA), version 1.80 (Applied Biostatistics Program).To convert the genetic similarity(F) into genetic distance, logarithmic transformation (-ln F) was used to line arize the distance measure.

RESULTS AND DISCUSSION

Cluster weight:

Cluster weight of Thompson seedless and Superior grape cultivars were significantly affected by the agriculture zone (Tables 2&4). Thompson seedless cultivated under El-Minia conditions was higher than Thompson seedless in El-Behera government whereas the result was reversed for Superior grape. These data were supported by Sthapit et al., (2012), Bogicevic (2013), Pouget (1981) and Kliewer (1977).

Berry diameter and size of 100 berries:

Environmental condition caused significant differences in berry diameter of both cultivars. Berry diameter in El-Minia was greater than El-Behera region. Concerning, Size of 100 berries, there were highly significant difference between regions for Thompson seedless grape whereas Superior grape was not significantly affected (Tables 2&4). Thompson seedless grape under El-Minia condition displayed higher size of 100 berries than El-Behera government**.**

Juice volume:

The data indicated that there were significant differences in juice volume between the two locations in both cultivars. Grape plants of El-Behera region contained greater juice volume than those of El-Minia zone (Tables 2&4).

Yield and compactness:

Yield and compactness of Thompson seedless were not remarkable affected by environmental factors whereas the latter caused significant differences in yield and compactness of Superior grape(Tables 2&4). Grapes of El-Behera produced higher yield and compactness than those of El-Minia region. Our data were in harmony with that grapes grown in cool climate produced higher yield (Bogicevic, 2013). Moreover, Pouget (1981) who found that the number of clusters per shoot was increased while the number of flowers per cluster was reduced with a higher temperature. This mainly due to the loss of ovule viability in Pin Noir and Carignane grapes at 35 ^{0}Cand 40 ^{0}C as compared to 25 ^{0}C (Kliewer , 1977).

Total soluble solids:

TSS of Thompson seedless was highly significant by environmental conditions. However, TSS of Superior grape was not considerable affected. Thompson seedless under El-Minia zone showed greater TSS than El-Behera region (Tables 3&5). These data can be also correlated with those of Bogicevic (2013) who found that grapes grown in cool climate produce larger yield have lower sugar content and higher acids levels. Due to, higher temperature regimes for short period caused the best quality fruits (Kliewer, 1977). Besides, Dokoozlian and Kliewer (1996) reported that grapevines in the shade can have slower berry development, delayed onest of verasion, reduced final sugar levels and differences in acidity levels .

Clusters and berries number:

In both cultivars, the vines had significant difference between two regions concerning cluster and berries number (Tables 3&5). Grapes of El-Behera government displayed higher clusters number than grapes of El-Minia region in both cultivars. With respect to berries number, Thompson seedless under El-Minia conditions was greater than El-Behera region. However, the result was reversed for Superior grape. In agreement of our finding, Pouget (1981) found that the number of clusters per shoot was higher and the number of flowers per cluster was reduced under a higher temperature regime. Besides, Kliewer (1977) observed that high temperature reduced berries number in Pin Noir and Carignane grapes.

There were no significant differences between regions for cluster length and width, berry length, weight of 100 berries. Similarly, acidity and TSS/Acidity ratio were not significantly affected by different regions (Tables 3&5).In this respect, Jones and Davis (2000) found that berry weights, for some grape varieties were not significantly affected by climate factors

RAPD markers:

RAPD markers can be used to study the genetic diversity and relationship among cultivars. However, the investigation of geographical and morphological relationships of crops is also possible by RAPD analysis (Ashraf et al., 2003). In the present study, twenty RAPD primers were screened and 10 of them produced clear and reproducible amplification patterns were selected and used to study the impact of environmental conditions on genetic structure of the grapevine cultivars, Superior and Thompson, established in El-Behera and El-Minia(Fig.1 and Table 6). The 10 primers amplified a total of 241 DNA fragments from the tested cultivars with an average of 24.1 bands per primer.

Table (2): Fruit quality of Thompson seedless grape under different locations.

location	Cluster weight	Cluster Length	Cluster width	Berry diameter	Berry length	Weight of 100 berries	Size of 100 berries	Juice volume
El-Behera	422.03	21	13.83	13.08	17.31	209.53	131.67	137
El-Minia	537.50	22.08	12.66	14.50	17	215.08	195.83	114.66
L.S.D.05	111.91	N.S	N.S	1.06	N.S	N.S	36.08	17.69

Table (3): Fruit quality and yield of Thompson seedless grape under different locations.

location	TSS %	Acidity	T/A Ratio	Yield Kg	Compactness	Clusters number	Berries number
El-Behera	17.83	0.43	41.69	10.54	10.03	23.50	191.94
El-Minia	19.91	0.46	44.15	10.95	11.21	20.50	246.33
L.S.D.05	0.96	N.S	N.S	N.S	N.S	1.14	46.76

Table (4): Fruit quality of Superior grape under different locations

location	Cluster weight	Cluster Length	Cluster width	Berry diameter	Berry length	Weight of 100 berries	Size of 100 berries	Juice volume
El-Behera	705.15	22.16	14.50	19.64	23.83	591.80	590.27	360
El-Minia	484.50	20	14.33	21.33	23.83	652.72	627.13	321.83
L.S.D.05	89.21	N.S	N.S	0.91	N.S	N.S	N.S	31.53

Table (5): Fruit quality and yield of Superior grape under different conditions

location	TSS %	Acidity	T/A Ratio	Yield Kg	Compactness	Clusters number	Berries number
El-Behera	15.23	0.75	20.41	15.03	6.33	22.83	140.33
El-Minia	14.75	0.62	24.05	10.14	3.28	21	75.50
L.S.D.05	N.S	N.S	N.S	1.48	2.17	1.68	31.44

Table (6): Total Number of amplified DNA-fragments, Monomorphic bands, polymorphic bands and polymorphism percentage obtained by 10 primers from two grape cultivars collected from two locations.

Primers	Total bands	Monomorphic bands	Polymorphic bands	Polymorphism percentage
OP-A01	25	9	16	64%
OP-A02	27	5	22	81%
OP-A04	21	3	18	86%
OP-A05	29	9	20	69%
OP-A18	30	6	24	80%
OP-A09	28	7	21	75%
OP-A11	20	6	14	70%
OP-A16	22	5	17	77%
OP-A20	18	4	14	78%
OP-B15	21	4	17	81%
All primers	241	58	183	76%

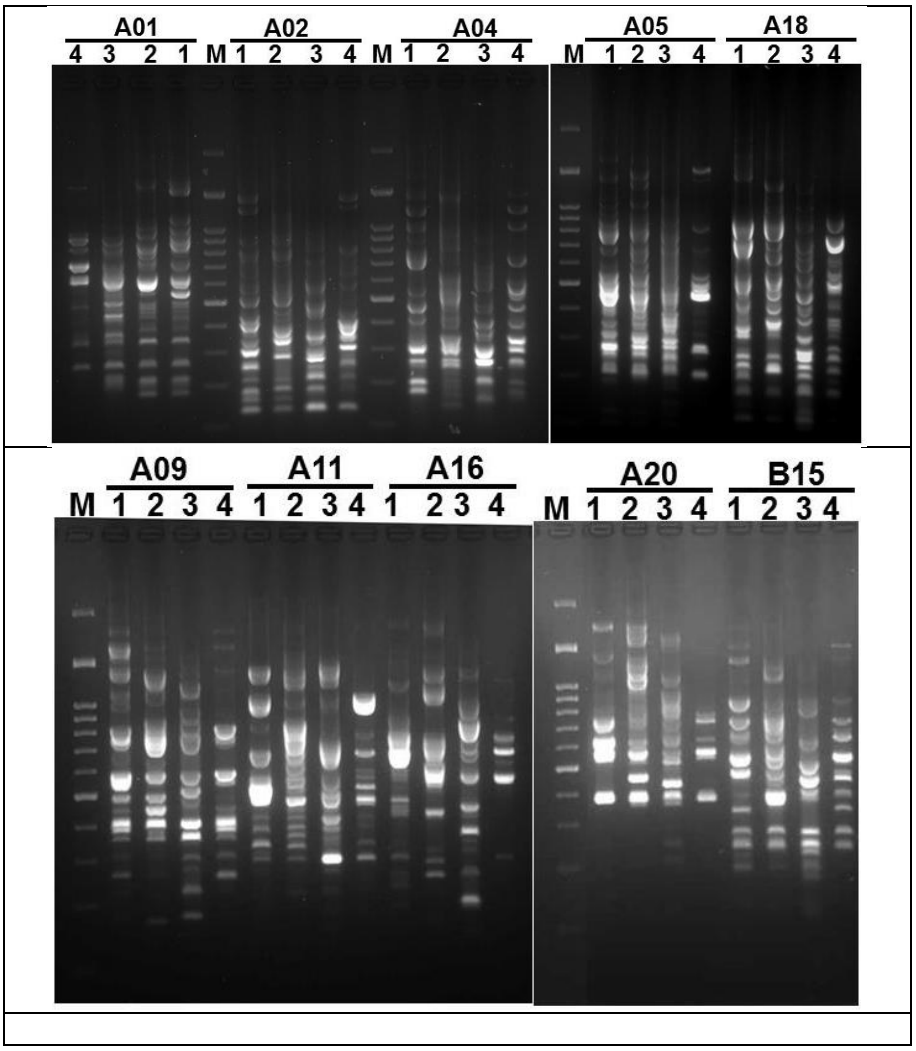

Figure 1. RAPD profiles of grapevine cultivars using 10 primers (A01, A02, A04, A05, A18, A09, A11, A16, A20 and B15); M=DNA marker, 1: Superior from El-Minia, 2: Superior from El-Behera, 3: Thompson seedless from El-Minia, 4: Thompson seedless from El-Behera.

The results revealed high level of polymorphism (183 bands, 76%) among the studied grapevine cultivars. The highest number of polymorphic bands produced with primer OP-A18 (24 polymorphic bands) while, the lowest (14 polymorphic bands) generated with the primers (OP-A11 & OP-A20). Moreover, the highest polymorphism percentage (86, 81, 81 and 80%) produced with primers (OP-A04, OP-A02, OP-B15 and OP-A18), respectively. The obtained results revealed that RAPD markers is an effective tool to detect polymorphism and discriminate differences among grapevine cultivars that agree with what found by Xianping et al., (1996) and This et al.,(2009).

Vidal et al., (1999) used RAPD technique with 33 primers to assess genetic relationships among 32 white grapevine varieties (*Vitis vinifera* L.) grown in different French and Spanish regions. Their results showed that two hundred and eight clear and unambiguous RAPD markers were amplified by using 33 primers previously selected.

Similarity among studied grapevine cultivars:

The genetic distances (Gd) and similarities (Si) among studied cultivars in two different locations (El-Behera and El-Minia) are shown in table (7). High similarity (Si = 0.60) and lower genetic distance (Gd = 0.511) were observed between Superior cultivar collected from the El-Behera and El-Minia. Meanwhile, Thompson grown in El-Behera and El-Minia showed longest

genetic distance (Gd = 0.844) and lowest similarity (Si = 0.43). Long genetic distances ranged from 0.713 to 0.844 were observed between the other combinations of Superior and Thompson plants of the two locations.

The UPGMA cluster analysis (Fig. 2) grouped the two grapevine cultivars in two separated clusters according to their genetic background. The Superior plants grown in El-Behera and El-Minia were clustered together firstly and then with Thompson grown in El-Minia followed by Thompson of El-Behera. Powell et al., (1996) reported that several factors might affect the estimates of genetic relationships between individuals i.e., number of markers used, distribution of markers in the genome (genome coverage) and the nature of evolutionary mechanisms underlying the variation measured. Vidal et al., (1999) reported that the UPGMA cluster analysis based on RAPD markers classified the 32 white grapevine varieties according to their common cultivation area and ampelographic characters.

The present study revealed grapevine plants of Superior and Thompson grown in El-Behera and El-Minia possessed considerable genetic differences in addition to the differences exist between the two cultivars. Since these cultivars are established in El-Behera and El-Miniafor a long time, these genetic differences between grape plants may be due to the two variegated environments and environmental changes. Galhardo et al., (2007) reported that changing environments increased the rates of random mutagenesis which can potentially accelerate the adaptive evolution in the populations, and after then return genomes to low mutation rates. Moreover, the genome evolution in turn, could lead to biological evolution and changing in phenotypical and physiological characters of plants. The biological evolution mechanisms have always been the subject of intense debate and modeling. One of the main problems is how the genetic variability is produced and maintained in order to make the organisms adaptable to environmental changes and therefore capable of evolving (Piacentini et al., 2014). Atak et al., (2014) and Çelik et al., (2000) reported that the differences between grape clones grown for many years and propagated vegetatively may be due to responses to environmental conditions, mutations, different origins or differing health status, such as virus infections. They also reported that grape cultivars propagated vegetative are expected to remain genetically stable, but within cultivars differences in fruit characteristics, yield and some other characteristics have been found.

Table (7): Genetic distance (above the diagonal) and similarity (below the diagonal) values calculated from the 241 DNA fragments amplified from two cultivars of grape collected from two locations., and investigated with ten RAPD primers

Genotypes	Superior Kebly	Superior Bahary	Thompson Kebly	Thompson Bahary
Superior Kebly	---	0.511	0.844	0.734
Superior Bahary	0.60	---	0.713	0.821
Thompson Kebly	0.43	0.49	---	0.844
Thompson Bahary	0.48	0.44	0.43	---

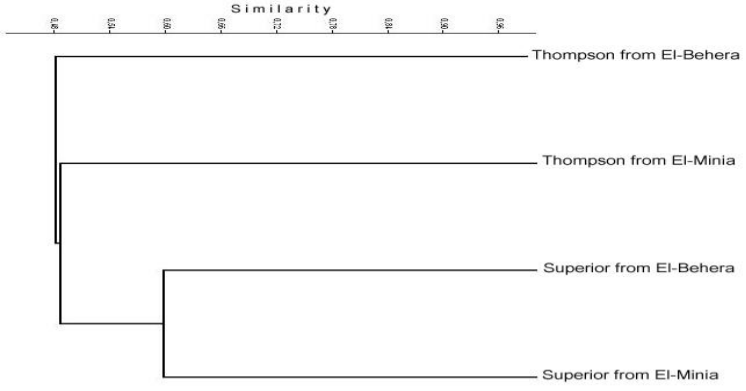

Figure (2): Dendrogram demonstrating the relationships among two grapevine varieties cultivated in El-Behera and El-Minia based on data recorded from polymorphism of 10 RAPD primers.

CONCLUSION

Mutation at the DNA level is one way for the asexually reproducing species to get some diversity. If there is an error during mitosis or during DNA replication, and if this error passed to the off spring, thereby possibly changing some traits in the organism. However, in asexual reproduction, not all mutations lead to differences in offspring and that in turn do not alter the phenotype.

CONFLICT OF INTEREST

The authors declared that present study was performed in absence of any conflict of interest.

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