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Induction of new clones of Egyptian garlic (*Allium sativum* cv. Elbalady) by using chemical mutagens and somatic embryogenesis

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ABSTRACT

The present study aims to induce new clones of Egyptian garlic cv. Elbalady by using chemical mutagenesis and somatic embryogenesis and determine the genetic diversity in these clones.

The field treatments of our study was carried out, in Kaha vegetable research farm, Qalyubia governorate, Egypt on garlic, to investigate the influence of chemical mutagens type (ethyl methane sulfonate, sodium azide and colchicine), soaking periods (6 and 12 h) and concentrations (0.01, 0.05 and 0.1%) on vegetative growth and yield characteristics. The results showed that sodium azide at concentration 0.01% achieved the lowest vegetative growth and yield characteristics values at first and second seasons but increased in the third season, while the concentrations 0.05 and 0.1% led to non-germinated cloves confirming that they are lethal doses. Ethyl methane sulfonate and colchicine had positive effects on vegetative growth and yield characteristics. Five new clones were obtained from these treatments, and the clones were evaluated as well as control during the years following to confirm the stability of the new traits.

The in vitro part of our study was performed in Tissue Culture Research Laboratory, Vegetable Research Departments, Horticulture Research Institute, Giza, Egypt to induce genetic variation through somatic embryogenesis of garlic cv. El Balady. Root tips cultured on MS medium supplemented with various concentrations of 2,4-D (0, 1, 2, 3, and 4 mg/l) to callus formate. The formed callus subcultured into MS medium supplemented with a combination of 2,4-D and BA at concentrations (0, 1 and 2 mg/l) for callus differentiation. The results show that 2,4- D at concentration 1 mg/l gave the highest callus formation traits and BA obtained the highest callus differentiation traits. Obtained embryos subcultured into fresh medium to produce micro bulbs. Four new clones were obtained from in vitro which adapted and cultivated in the greenhouse for evaluation.

By using two dominant molecular marker techniques Start Codon Targeted (SCoT) polymorphism and sequence-related amplified polymorphism (SRAP) to find the genetic distance among the nine different treated clones in comparison with the untreated one which is used as a control.

Two sets of ten SCoT primers and ten SRAP primers were deployed to examine the genetic variation among the new clones compared with control. Out of 88 amplified fragments were scored by using SCoT marker, a total of 34 polymorphic fragments were detected at molecular size ranging from 100 to 3500 bp. Out of 78 amplified fragments were scored by using SRAP marker, a total of 42 polymorphic fragments were detected at molecular size ranging from 100 to 4000 bp. The genetic similarity percentages of genomic DNA using SCoT marker ranged from 89% to 57%. While, the genetic similarity percentages of genomic DNA using SRAP marker ranged from 95% to 45%. These results revealed 92% genetic similarity between clones 8 and 10 and 68% between clones 4 and10. They were clustered into two major groups based on the UPGMA analysis where, the clone 4 was the most related clone to the first cluster as agronomic trait and the second cluster as somatic embryogenesis trait. So, the current study increased the genetic diversity base of garlic somatic embryogenesis and mutagenesis.

Keywords: Garlic, *Allium sativum*, Chemical mutagens, Somatic embryogenesis, DNA marker diversity, SCOT, SRAP.

INTRODUCTION

Garlic (*Allium sativum*), family Alliaceae where most *Allium* crops originated from Asia had been found growing in ancient Egypt during Pharaonic era. Garlic is used around the world as spicey and medicinal plant. According to **FAO (2018)** harvested garlic area in Egypt was 12782 ha (30433.33 fed) with total production of 286213 tons and exporting of 9066 tons. It is difficult to produce new varieties from garlic crop under Egyptian conditions because that no true seeds produced. Therefore, vegetatively cultivated garlic needs about 10% of harvested bulb cloves for the next season cultivation **(Sinha *et al*., 2016)**.

Mutations and variations could be naturally occurred but infrequent, so they induced changes during cell division as a result from treating plants with chemical mutagens. Mutation induction is one

of common methods used for producing new varieties with beneficial characteristics in vegetative propagated crops (**Lee *et al.,* 2002 and Shah *et al.,* 2008)**. Chemical mutagens such as sodium azide and ethyl methane sulfonate are effective substances in producing new clones **(Aruna and Adamu, 2010)**. Moreover, colchicine is one of common compounds induce polyploidy in plant chromosomes to produce new variations **(Nigel *et al.*, 2007)**.

Somatic embryogenesis is one of the methods for inducing genetic variation on vegetative reproductive crops as garlic. Successful regeneration depends on the genotype, the type of explant and its physiological conditions, and the combination of growth regulators in the medium **(Hassan *et al*. 2014).**

Molecular markers have been defined by Food and Agriculture Organization (FAO) as a DNA sequences exist in specific genome locations associated with linked gene or trait inheritance (FAO, 2004). The use of molecular markers facilitate plant breeding to improve agronomical and tissue cultural traits (Zhao *et al*., 2010, Wright and Kelly 2011. And Daniel *et al*., 2014).

There are several applications of DNA markers in plant molecular genetic studies (Gupta *et al*. 1999 and Semagn *et al*., 2006). The popular DNA marker methods random amplified polymorphic DNA (RAPD) (Ipek *et al*., 2003 and Khar *et al*., 2008). Amplified fragment length polymorphism (AFLP) (Morales *et al*., 2013) and inter simple sequence repeat (ISSR) (Jabbes *et al*., 2011; El-Nagar and El-Zohiri, 2015; Gehan *et al*., 2017, and Rakesh *et al*., 2018) have been used in genetic diversity analysis and relationships determination among garlic induced clones, as the environmental conditions do not affect them. (Jo *et al*., 2012). (Williams *et al*., 1990, Blair *et al*., 1999, Vos *et al*., 1995 and Gostimsky *et al*., 2005). In recent years, many new promising dominant molecular marker techniques have been developed such as SRAP (Li and Quiros 2001 and Chen *et al*., 2013) and SCoT (Gupta and Rustgi, 2004) because of their simplicity, inexpensive, and reproducibility.

In this study, two novel marker systems called, Start Codon Targeted (SCoT) polymorphism (Collard and Mackill, 2009) and Sequence Related Amplification Polymorphism (SRAP) have recently become the best choice of molecular markers used in genetic diversity studies. SCoT was developed based on the short conserved sequence around the Translational Start Site (TSS) that is conserved in all plant genes. It is simple because its PCR products were resolved by performing agarose gel electrophoresis compared to arbitrary markers such as RAPD, it is highly reproducible due to the use of longer primers, these primers were designed following the short conserved region flanking the initial codon (ATG), it is a targeted molecular marker technique with one part of a functional gene markers generated from SCoT marker technique and their corresponding traits (Bhattacharyya, *et al*., 2013). Sequence-related amplified polymorphism (SRAP) was developed based on two primers amplification composed of 13 to 14 bp long as a core sequence called filler sequences, where the first 10 bases at the 5' end are followed by CCGG sequence in the forward primer and AATT sequence in the reverse primer. Where, at the 3' end there were three selective bases. The marker technique aimed to amplify the open reading frames (ORFs). (Li and Quiros 2001 and Budak *et al*., 2004).

A set of SCoT and SRAP primers as genetic markers in *Alium sativum* were developed to evaluate the distribution of genetic diversity among different mutagenic treatments of cultivated and tissue cultured samples of the Egyptian garlic *Alium sativum* L. Balady and identify them as a DNA fingerprinting. They have been successfully used in genetic diversity analysis and fingerprinting in Garlic, Mentha, Tomato, and Potato (Abdein, *et al*., 2018, Azza, *et al*., 2019, and Bhattacharyyq, *et al*., 2013).

In this study, developing two DNA molecular markers of mutagenic garlic as a medicinal plant provide more accurate assessments of genetic variation. This may help the plant breeders to select the desired mutation. The objective of this work is to induce genetic variations and mutations using different mutagenic treatments in Balady variety of Egyptian garlic *Allium sativum* to evaluate morphological and molecular changes by using two dominant molecular marker techniques. They was carried out to find the genetic distance among the nine different treatments in comparison with the untreated one which is used as a control. The present study aims to determine the genetic diversity

in different mutagenic treatments Also to investigate the genetic distances between them and determine the genetic variability among the different mutagenic treatments.

MATERIALS AND METHODS

The current study was conducted at Kaha vegetable research farm, Qalyubia governorate, and Tissue Culture Research Laboratory, Vegetable Research Departments, Horticulture Research Institute, Giza, Egypt during the period from 2017 to 2020 to induce variations in El Balady garlic cultivar In vivo and In vitro through mutation induction by using three chemical mutagens and somatic embryogenesis to select the promising clones under Egyptian local conditions.

In vivo experiment:

Garlic cloves were separated and soaked in chemical mutagens, ethyl methane sulfonate (**EMS**), sodium azide (**SA**) and colchicine (**Col**) with three concentrations (0.01, 0.05 and 0.10 %) with soaking for different durations (6 and 12 h), the control cloves were soaked in pure water.

Treated garlic cloves with the chemical mutagens were cultivated, after washing them carefully, in the open field using the recommended planting distances in October 1st, 2017 (**first season**) where the recommended irrigation and fertilization were applied. After 150 days from planting, samples were collected to estimate the vegetative growth characteristics.

By the end of May (2018) the plants were harvested (**M0**) and yield characteristics were recorded. At the beginning of October, 2018 (**second season**) cloves from the last season were planted for the evaluation of M1 generation with applying the recommended planting distances, irrigation and fertilization. After 150 days, samples were collected and data were recorded for vegetative growth characteristics. At the end of May (2019) the plants were harvested (**M1**) and yield characteristics were recorded and the cloves were stored for the next season cultivation.

On the beginning of October (2019), cloves from the last season were planted after selecting the best clones comparing with control, and the recommended planting distances, irrigation and fertilization were applied. After 150 days, samples were taken for recording vegetative growth data and determining of chlorophyll (mg/g) and carotene (mg/g). At the end of May (2020) the plants were harvested (M2) and the yield data were recorded as well as total free amino acids (mg/100g) and pungency (µg/g fresh weight) were measured.

Samples of 700 grams of garlic bulbs were weighed and the weight loss percentage was recorded after storage for seven months with M0, M1 and M2 generations.

Data were recorded for vegetative growth characteristics as following, survival percentage (**%)**, plant height (**cm)**, number of leaves per plant, vegetative growth fresh weight (**g)**, vegetative growth dry weight (**g**). Data were recorded for yield characteristics as following, bulb fresh weight (**g)**, number of cloves/bulb, clove average weight (**g)**, after curing plot yield (**g**).

In vitro experiment:

In vitro experiment aimed to obtain garlic clones through somatic embryogenesis the cloves were sterilized and planted into Murashige and Skoog 1962 medium (MS) free hormone medium for two weeks then subcultured into MS medium supplemented with IBA (1.0 mg/l) for four weeks. The apical roots were separated and planted on MS medium supplemented with 2,4-D (0.0, 1.0, 2.0, 3.0 and 4.0 mg/l). Explants were transferred to a fresh medium every four weeks until callus formed. The formed callus was transplanted on MS medium supplemented with a combination of 2,4-D (0.0, 1.0 and 2.0 mg/l) and BAP (0.0, 1.0 and 2.0 mg/l). Callus was transferred to a fresh medium every four weeks until plantlets formed. The formed plantlets were transferred to the greenhouse after acclimatization for the evaluation.

Data were recorded for callus formation as days to induce callus, percentage of callus formation and callus weight (g). Data were recorded for differentiation as days to callus differentiation, number

of shoots per jar, number of micro bulbs per jar and survivals percentage (%). Data were recorded for In vitro obtained clones as M2 generation clones.

Total genomic DNA was extracted from young leaves of the ten different *Allium sativum* treatments according to the instruction manual for genomic DNA extraction from plant tissues using silica membrane (I-genomic plant DNA extraction mini kit Intron biotechnology). Good purity and quantity of DNA were provided by this method. Then the DNA samples were subjected into PCR amplification for developing SCoT and SRAP markers.

Primer Design and SCoT-PCR amplification

SCoT primers were designed based on a conserved sequence surrounding the ATG initial codon derived from previous studies by Joshi *et al*., (1997) and Sawant *et al*., (1999). All primers were 18-mer and ranged in GC content between 50% and 72%. There were no degeneracies. Primers between 18 to 24 nucleotides are preferable for generating reproducible markers (Gillings and Holley 1997). The optimal primer length in the TRAP technique (Target Region Amplification Polymorphism) was found to be 18 nucleotides (Hu and Vic 2003).A total of ten SCoT primers were developed by Biotic Serve Company illustrated in Table (13). All the PCR reactions were carried out in a total volume of 25 µl containing 30 ng of template DNA, 12.5 µl Emerald Amp® Max PCR master mix (Cat# RR320A), and 10 pmol of primer. The reaction program was set at 94℃ for 4 min, followed by 43 cycles of 94℃ for 1 min, 50-55℃ for 1 min according to each primer and 72℃ for 2 min with final extension at 72℃ for 5 min. All PCR amplification DNA patterns were analyzed on 1.7 % agarose gel in 1x TAE buffer stained with SYBR® Safe DNA gel stain (Invitrogen by thermo fisher scientific) and visualized under UV light and photographed using gel documentation system.

SRAP marker system

SRAP primers were designed based on two primers, a forward primer consists of a core sequence of 14 nt bases. In which the first ten bases starting at the 5' end are filler sequences of no specific constitution, followed by CCGG sequence then by 3 selective nt. bases at the 3' end. The downward primers consists of the same components as the forward primers with the filler followed by AATT, 3 selective nt. bases at the 3' end of the primer according to (Li and Quiros 2001). Ten SRAP primers (Bio Basic Canada Inc.) combinations illustrated in Table (14). All the PCR reactions were carried out in a total volume of 25 µl containing 30 ng of template DNA, 12.5 µl Emerald Amp® Max PCR master mix (Cat# RR320A), and 10 pmol of primer. The reaction program was set at 94℃ for 5 min, followed by 5 cycles of 94℃ for 1 min, 35℃ for 1 min and 72℃ for 1 min, followed by 35 cycles of 94℃ for 1 min, 50℃ for 1 min then, 72℃ for 1 min with final extension at 72℃ for 7 min. All PCR amplification DNA patterns were analyzed on 1.8 % agarose gel in 1x TAE buffer stained with SYBR® Safe DNA gel stain (Invitrogen by thermo fisher scientific) and visualized under UV light and photographed using gel documentation system.

Data analysis

PCR-amplified fragment generated by SCoT and SRAP primers were detected on agarose gels and scored as absent (0) or present (1). Pair wise comparisons between accessions, based on the proportion of shared bands produced by the primers were calculated using the genetic similarity coefficients using Jaccard's coefficient (Sneath and Sokal, 1973). A dendrogram showing the genetic relationships between accessions, based on the unweight pair-group method with arithmetic averages, was constructed using (UPGMA).

Data were subjected to experiment design and statistical analysis for In vivo experiment in completely randomized block design (**CRBD**) with three factors in the first and second seasons of the treatment and one factor for the third season and storage experiment, each treatment was applied in three replications with single row plot 2.1**m²** (3m long x 0.7m width). Obtained data were subjected

to analysis of variance (**Snedecor and Cochran, 1980)** and the differences among means were tested by new multiple range test, **Duncan (1955)**.

In vitro experiment for callus formation treatments Complete Randomized Design with one factor were use, each treatment was applied in three replications each replication contained 10 jars. Callus differentiation treatments were arranged in complete randomized design with two factors, each treatment was applied in three replications each replication contained 10 jars. Greenhouse treatments were arranged in Randomized Complete Block Design with one factor, each treatment applied with three replications each replication contained 10 plantlets. Obtained data were subjected to the analysis of variance according to **Snedecor and Cochran (1980).** The differences between various treatments means were tested by LSD.

RESULTS AND DISCUSSION

In vivo experiment:

Obtained data showed a significant differences among **chemical mutagens, soaking periods and concentrations** for vegetative growth characteristics after 150 days from planting. The control (0.0%) showed the highest values for survival percentage (96.67%) in M0 generation. While, soaking cloves with **EMS for 6 h at concentration 0.01%** showed highest results for survival percentage (92.33%) in M0 generation, plant height (118cm) and plant leaves number (10.67) in M1 generation. Soaking cloves with **EMS for 6 h at concentration 0.1%** showed the highest data for plant height (109.3cm) in M0 generation, survival percentage (98%), plant leaves number (10.67), vegetative growth fresh (133.3g) and dry (18g) weight in M1 generation. Soaking cloves with **EMS for 12 h at concentration 0.05%** gave the highest values for plant height (109.3cm), vegetative growth fresh (113.3g) and dry (19g) weight in M0 generation. Soaking cloves with **Col for 6 h at concentration 0.01%** showed the highest number of leaves per plant (11.67) in M0 generation. Soaking cloves with **Col for 6 h at concentration 0.05%** showed the highest measurement in M1 generation for vegetative growth dry weight (18g), while, soaking cloves with **Col for 12 h at concentration 0.05%** had the highest values for plant height (118cm) [M0 in Table (1) and M1 in Table (2)].

Obtained data of M2 generation showed non-significant differences among various selected clones for survivals percentage (%), plant height (cm) and number of leaves per plant while there were a significant differences for fresh and dry weight of vegetative growth after 150 days from planting, the control gave the lowest fresh and dry weight of vegetative growth (89.51 g) while there were no significant differences between the selected clones. The highest fresh and dry weight of vegetative growth after 150 days from planting achieved by clone number three **(EMS 0.1% for 6 h)** (Table 3).

Yield characteristics:

Presented data in Tables (4 and 5) showed significant differences among **chemical mutagens, soaking periods and concentrations** for yield characteristics after harvesting in first and second seasons. The control had the highest values in both seasons for cloves number (49, 50 respectively), while soaking cloves with **EMS for 6 h at concentration 0.01%** showed the highest results for whole bulb fresh weight (133.3g) in the first season only and clove average weight (4.5, 4.14g) in both seasons, respectively. Soaking cloves with **EMS for 6 h at concentration 0.1%** showed the highest data in both two seasons for cured plot yield (6033, 6033g respectively). The results of soaking cloves with **Col for 6 h at concentration 0.05%** recorded highest values for whole bulb fresh weight (134.7, 131.3g) in first and second seasons, respectively. Also, soaked cloves with **Col for 12 h at concentration 0.01%** gave the highest data for cloves number/bulb (49, 49.33) in both two seasons, respectively.

Obtained data of weight loss percentage in Table (6) showed a significant differences among M0 and M1 generations. The results show that soaked cloves with **Col for 6 h at concentration 0.01% and EMS for 6 h at concentration 0.05%** gave the highest weight loss percentage at M0 generation while soaked cloves with **Col for 12 h at concentration 0.01%** gave the highest at M1 generation. Soaked cloves with **SA for 6 h at concentration 0.01%** achieved the lowest weight loss at both M0 and M1 generations.

Table (1): Effect of chemical mutagen, soaking periods and concentrations on vegetative growth characteristics after 150 days from planting in first season.

Chemical mutagens	Soaking periods (hours)	Concentrations (%)	Survival percentage (%)	Plant height (cm)	number of leaves / plant	Vegetative fresh weight (g)	Vegetative dry weight (g)
EMS	**6**	**0.00**	96.67 a	96.67 de	7.33 h	66.33 f	10.67 e
		0.01	92.33 ab	106.00 a	8.66 fg	92.67 cde	13.00 d
		0.05	88.33 bcd	104.30 abc	8.33 g	95.00 cde	15.33 bc
		0.10	82.33 ef	109.30 a	9.33 d-g	108.70 ab	16.67 b
	12	**0.00**	96.67 a	96.67 de	7.33 h	66.33 f	10.67 e
		0.01	91.33 bc	100.70 bcd	10.00 cde	96.00 cde	17.00 b
		0.05	86.00 de	109.30 a	9.66 c-f	113.30 a	19.00 a
		0.10	76.33 gh	105.00 ab	9.00 efg	101.30 bcd	15.67 bc
SA	**6**	**0.00**	96.67 a	96.67 de	7.33 h	66.33 f	10.67 e
		0.01	45.00 j	70.67 g	8.66 fg	42.67 g	6.33 f
		0.05	0.00 l	0.00 h	0.00 i	0.00 h	0.00 g
		0.10	0.00 l	0.00 h	0.00 i	0.00 h	0.00 g
	12	**0.00**	96.67 a	96.67 de	7.33 h	66.33 f	10.67 e
		0.01	29.33 k	81.00 f	9.66 c-f	54.00 f	7.66 f
		0.05	0.00 l	0.00 h	0.00 i	0.00 h	0.00 g
		0.10	0.00 l	0.00 h	0.00 i	0.00 h	0.00 g
COL	**6**	**0.00**	96.67 a	96.67 de	7.33 h	66.33 f	10.67 e
		0.01	87.67 cd	95.00 e	11.67 a	84.67 e	13.67 cd
		0.05	82.33 ef	99.67 cde	10.67 abc	90.00 de	13.33 d
		0.10	72.33 hi	100.70 bcd	10.33 bcd	95.67 cde	15.67 bc
	12	**0.00**	96.67 a	96.67 de	7.33 h	66.33 f	10.67 e
		0.01	80.00 fg	95.00 e	11.33 ab	103.30 abc	17.00 b
		0.05	72.67 hi	99.67 cde	10.33 bcd	94.67 cde	15.33 bc
		0.10	70.67 i	99.00 cde	10.67 abc	94.33 cde	16.33 b

Table (2): Effect of chemical mutagens, soaking periods and concentrations on vegetative growth characteristics after 150 days from planting in second season.

Chemical mutagens	Soaking periods (hours)	Concentrations (%)	Survival percentage (%)	Plant height (cm)	number of leaves / plant	Vegetative fresh weight (g)	Vegetative dry weight (g)
EMS	**6**	**0.00**	97.67 a	100.00 de	8.33 bcd	88.27 bc	12.33 bc
		0.01	97.00 a	118.00 a	10.67 a	127.70 a	17.33 a
		0.05	96.67 ab	105.00 bcd	9.33 abc	94.33 bc	13.33 bc
		0.10	98.00 a	109.30 b	10.67 a	133.30 a	18.00 a
	12	**0.00**	97.67 a	100.00 de	8.33 bcd	88.27 bc	12.33 bc
		0.01	96.33 ab	105.00 bcd	9.33 abc	86.67 bc	13.33 bc
		0.05	95.67 ab	99.67 de	8.66 bcd	95.33 bc	12.83 bc
		0.10	97.33 a	87.67 f	8.33 bcd	84.67 bc	10.83 bc
SA	**6**	**0.00**	97.67 a	100.00 de	8.33 bcd	88.27 bc	12.33 bc
		0.01	93.33 ab	99.67 de	8.00 cd	87.67 bc	13.67 b
		0.05	0.00 c	0.00 g	0.00 e	0.00 d	0.00 d
		0.10	0.00 c	0.00 g	0.00 e	0.00 d	0.00 d
	12	**0.00**	97.67 a	100.00 de	8.33 bcd	88.27 bc	12.33 bc
		0.01	91.67 b	96.67 e	7.66 d	77.00 c	12.33 bc
		0.05	0.00 c	0.00 g	0.00 e	0.00 d	0.00 d
		0.10	0.00 c	0.00 g	0.00 e	0.00 d	0.00 d
COL	**6**	**0.00**	97.67 a	100.00 de	8.33 bcd	88.27 bc	12.33 bc
		0.01	97.00 a	101.70 cde	9.33 abc	91.67 bc	12.33 bc
		0.05	94.00 ab	115.70 a	10.33 a	127.70 a	18.00 a
		0.10	95.67 ab	104.70 bcd	9.66 ab	95.00 bc	13.33 bc
	12	**0.00**	97.67 a	100.00 de	8.33 bcd	88.27 bc	12.33 bc
		0.01	94.33 ab	108.00 bc	9.33 abc	84.33 bc	10.33 c
		0.05	96.67 ab	118.00 a	10.33 a	129.00 a	16.67 a
		0.10	93.67 ab	100.00 de	8.66 bcd	97.33 b	12.50 bc

Table (3): Evaluation of vegetative growth for selected clones after 150 days from planting at M2 generation.

Clone number	Survival percentage (%)	Plant height (cm)	Number of leaves / plant	vegetative fresh weight (g)	vegetative dry weight (g)
1 (control)	97.00 ns	104.70 ns	7.33 ns	89.51 b	11.46 c
2 (EMS 0.01 for 6 h)	97.33 ns	122.00 ns	8.66 ns	158.3 a	24.24 b
3 (EMS 0.1 for 6 h)	96.67 ns	124.00 ns	8.00 ns	162.3 a	30.51 a
4 (SA 0.01 for 6 h)	97.00 ns	119.00 ns	8.66 ns	167.0 a	22.49 b
5 (Col 0.05 for 6 h)	96.00 ns	115.70 ns	8.66 ns	143.2 a	21.48 b
6 (Col 0.05 for 12 h)	96.33 ns	119.00 ns	8.33 ns	151.3 a	21.33 b

Data of M2 generation showed a significant differences among various selected clones for whole bulb fresh weight (g), number of cloves per bulb, clove average weight (g), cured plot yield (g) and bulbs weight loss % after storage for seven months (Table 7). Clones number two and four **(EMS 0.01% for 6 h, SA 0.01% for 6 h respectively)** obtained the highest whole bulb fresh weight (147.70 and 150.40 g respectively) while C4 **(SA 0.01% for 6 h)** gave the highest clove average weight and cured plot yield (4.7 and 5003 g respectively) also gave the lowest number of cloves per bulb (34.67). C1 (control) gave the lowest bulb fresh weight, clove average weight and cured plot yield also the highest number of cloves per bulb. C4 **(SA 0.01% for 6 h)** gave the lowest bulbs weight loss % after storage (8.81 %) and this is a very promising data because one of the highest problem of garlic varieties is the weight loss during storage period.

Table (4): Effect of chemical mutagens, soaking periods and concentrations on yield characteristics after harvesting in first season.

Chemical mutagens	Soaking periods (hours)	Concentrations (%)	Bulb fresh weight (g)	Number of cloves / bulb	Clove average weight (g)	cured plot yield (g)
EMS	**6**	**0.00**	72.67 c	49.00 a	1.49 fg	2967 cd
		0.01	133.30 a	29.67 e	4.50 a	5233 b
		0.05	72.00 c	36.00 cd	2.01 de	3667 c
		0.10	121.7 b	32.00 de	3.83 b	6033 a
	12	**0.00**	72.67 c	49.00 a	1.49 fg	2967 cd
		0.01	66.67 cd	38.33 c	1.76 efg	3167 cd
		0.05	74.67 c	32.33 de	2.32 cd	1767 e
		0.10	56.67 d	35.00 cd	1.62 efg	1833 e
SA	**6**	**0.00**	72.67 c	49.00 a	1.49 fg	2967 cd
		0.01	41.00 e	16.00 f	2.60 c	1467 e
		0.05	0.00 f	0.00 g	0.00 h	0.00 f
		0.10	0.00 f	0.00 g	0.00 h	0.00 f
	12	**0.00**	72.67 c	49.00 a	1.49 fg	2967 cd
		0.01	33.00 e	17.33 f	1.90 def	1100 e
		0.05	0.00 f	0.00 g	0.00 h	0.00 f
		0.10	0.00 f	0.00 g	0.00 h	0.00 f
COL	**6**	**0.00**	72.67 c	49.00 a	1.49 fg	2967 cd
		0.01	71.00 c	45.33 ab	1.56 efg	3633 cd
		0.05	134.7 a	36.33 cd	3.71 b	5567 ab
		0.10	70.33 c	43.00 b	1.64 efg	3633 cd
	12	**0.00**	72.67 c	49.00 a	1.49 fg	2833 d
		0.01	64.00 cd	49.00 a	1.30 g	3600 cd
		0.05	128.3 ab	36.67 cd	3.53 b	5467 ab
		0.10	75.33 c	44.33 ab	1.71 efg	3500 cd

Table (5): Effect of chemical mutagens, soaking periods and concentrations on yield characteristics after harvesting in second season.

Chemical mutagens	Soaking periods (hours)	Concentrations (%)	Bulb fresh weight (g)	Number of cloves / bulb	Clove average weight (g)	cured plot yield (g)
EMS	**6**	**0.00**	69.00 c	50.00 a	1.38 d	3159 def
		0.01	129.70 a	31.33 e	4.14 a	5202 b
		0.05	67.00 cd	41.33 bcd	1.63 d	3885 cd
		0.10	120.30 a	35.00 de	3.44 b	6033 a
	12	**0.00**	69.00 c	50.00 a	1.38 d	3159 def
		0.01	62.67 cd	40.67 bcd	1.58 d	2727 f
		0.05	70.33 c	48.33 a	1.49 d	1867 g
		0.10	53.33 d	41.33 bcd	1.30 d	1873 g
SA	**6**	**0.00**	69.00 c	50.00 a	1.38 d	3159 def
		0.01	120.00 a	37.67 cd	3.20 b	3700 cde
		0.05	0.00 e	0.00 f	0.00 e	0.00 h
		0.10	0.00 e	**0.00 f**	0.00 e	0.00 h
	12	**0.00**	69.00 c	50.00 a	1.38 d	3159 def
		0.01	85.00 b	39.00 bcd	2.19 c	3000 ef
		0.05	0.00 e	0.00 f	0.00 e	0.00 h
		0.10	0.00 e	0.00 f	0.00 e	0.00 h
COL	**6**	**0.00**	69.00 c	50.00 a	1.38 d	3159 def
		0.01	66.33 cd	48.00 a	1.38 d	3500 c-f
		0.05	131.30 a	40.33 bcd	3.25 b	5433 ab
		0.10	64.00 cd	44.00 abc	1.47 d	3967 c
	12	**0.00**	69.00 c	50.00 a	1.38 d	3159 def
		0.01	60.33 cd	49.33 a	1.23 d	4000 c
		0.05	125.00 a	40.00 bcd	3.12 b	5333 ab
		0.10	70.67 c	45.33 ab	1.56 d	3567 cde

Table (6): Effect of chemical mutagens treatments on percentage of garlic bulbs weight loss after storage seven months in first and second seasons.

chemical mutagens treatment	Weight loss (%)	
	M0 generation	M1 generation
control	21.17 bc	20.81 hi
EMS 0.01 for 6 hours	18.33 ef	21.34 ghi
EMS 0.05 for 6 hours	25.81 a	23.72 abc
EMS 0.1 for 6 hours	19.19 de	24.09 ab
EMS 0.01 for 12 hours	17.14 f	22.05 efg
EMS 0.05 for 12 hours	19.19 de	21.05 ghi
EMS 0.1 for 12 hours	21.81 b	22.57 def
SA 0.1 for 6 hours	9.47 h	8.90 k
SA 0.1 for 12 hours	13.33 g	11.10 j
Col 0.01 for 6 hours	24.05 a	24.00 abc
Col 0.05 for 6 hours	18.14 ef	20.62 i
Col 0.1for 6 hours	19.48 cde	22.95 cde
Col 0.01 for 12 hours	20.29 bcd	24.57 a
Col 0.05 for 12 hours	19.05 de	21.86 fgh
Col 0.1 for 12 hours	18.91 def	23.29 bcd

Table (7): Evaluation of yield characteristics for selected clones after harvesting at third season.

Clone number	Bulb fresh weight (g)	Number of cloves / bulb	Clove average weight (g)	cured plot yield (g)	Weight loss (%) after storage
1 (control)	66.97 d	42.00 ab	1.59 d	2191 c	20.38 a
2 (EMS 0.01 for 6 h)	147.70 a	44.67 a	3.30 bc	3650 b	20.67 a
3 (EMS 0.1 for 6 h)	116.50 c	40.00 abc	2.92 c	3182 b	19.91 a
4 (SA 0.01 for 6 h)	150.40 a	34.67 c	4.37 a	5003 a	8.810 b
5 (Col 0.05 for 6 h)	135.30 b	38.00 bc	3.59 b	3515 b	21.05 a
6 (Col 0.05 for 12 h)	125.40 bc	42.67 ab	2.95 bc	3412 b	20.91 a

Data presented in Table (8) showed a significant differences between selected clones where C1 (control) which gave the highest total chlorophyll and carotene content (1.55 and 0.86 mg/g respectively) while for total free amino acids and Pungency was the lowest. C4 **(SA 0.01 % for 6 h)** gave the highest total free amino acids (3083 mg/100g), and C2 was the most pungent (19.94 µM/ g fresh weight).

Table (8): Chlorophyll, carotene, total free amino acids and pungency content in the induced clones at third season.

Clone number	Total chlorophyll (mg/g)	Carotene (mg/g)	Total free amino acids (mg/100 g)	Pungency (µM/g fresh weight)
1 (control)	1.55 a	0.86 a	1533 d	16.41 c
2 (EMS 0.01 for 6 h)	0.86 c	0.45 cd	2200 b	19.94 a
3 (EMS 0.1 for 6 h)	0.91 bc	0.50 c	2235 b	18.36 b
4 (SA 0.01 for 6 h)	0.86 c	0.40 d	3083 a	18.83 ab
5 (Col 0.05 for 6 h)	1.15 b	0.64 b	1817 c	18.82 ab
6 (Col 0.05 for 12 h)	0.51 d	0.25 e	1783 c	18.99 ab

sodium azide (**SA**) gave the lowest values for all the studied characteristics at first and second seasons comparing to control and the other mutagens (**EMS** and **Col**), that effect might be due to the inhibitory effect on enzymes activities and mitochondria as explained by **Kleinhofs *et al.* (1978), Zhang, (2000), Agata *et al.* (2001), Samiullah *et al*. (2004), Jeng *et al*. (2006) and Al-Qurainy (2009)**. Increased concentration of SA more than 0.01% was as a lethal dose because it might had negative effects on cell cycle **(Salim *et al.,* 2009)** and strong inhibitory effects on germination **(Chhun *et al.,* 2003)** while in M2 generation, SA gave the highest vegetative growth and yield characteristics. **EL-Nashar (2012)** found that Sodium Azide led to a decrease in the studied morphological characteristics compared to the control in the M1 generation, while the M2 generation gave better results than the control. The mutant plants produced by the treatment of sodium azide are capable to survive under various adverse conditions and have improved yields, increased stress tolerance and longer shelf life (**Al-Qurainy and Khan, 2009; Dubey *et al*. 2017).** On the other hand, colchicine (**Col**) had positive effects on vegetative growth and yield characteristics, where the positive effects might be due to polyploidy effects. **Chen and Tian (2007), Ye *et al*. (2010), Xing *et al*. (2011), Noori *et al*. (2017)** and **Eltohamy and Mohamed (2020)** reported that polyploidy could be benefit in improving plant varieties where polyploidy has positive effects on bulb characteristics. The current results showed that the use of low concentrations of colchicine improved vegetative growth and yield characteristics in comparison with higher concentrations, that was in agreement with **Dhawan and Lavania (1996)** and **Majdi *et al*. (2010)** who stated that treating with colchicine increased tolerance to biotic and abiotic stresses and increased yield.

Ethyl methane sulfonate (**EMS**) had high efficiency in inducing variations with low toxicity leading to high yield which was clear in the current study and resulted in forming variations with higher vegetative growth and yield characteristics, **Nadaf *et al.* (2009)** and **Azad (2010)**. That was in line with data had been reported by **Khatri *et al.* (2005)**. Where, low concentrations of **EMS** are more effective than high concentrations causing positive effects on both vegetative growth and yield characteristics which was existed in the current results and that was in accordance with **Barshile *et al*. (2006), Kumar *et al*. (2007)** and **Kulthe *et al*. (2013)**. There were clear differences between and among type of chemical mutagens, soaking periods and concentrations for both the vegetative growth and yield characteristics which agreed with **Kataria and Singh (1989)**. The current study results showed that soaking in **EMS** for shorter periods improved vegetative growth and yield characteristics.

Plants untreated with chemical mutagens had a higher amount of chlorophyll content as compared to plants treated with the different concentrations of the chemical mutagens **(Aruna, 2012; Bansod *et al*. 2019).** While chemical mutagens led to higher levels of total proteins and amino acids compounds in mutagenized plants **(Auti and Apparao, 2008; Wani and Anis, 2008; Pavadai et al., 2010; Devi and Mullainathan, 2012; More and Borkar, 2016).** Since pyruvic acid is a by-product of the alliin - allicin reaction, measuring its concentration provides a cheap alternative to HPLC for screening the allicin content where the increase observed in pyruvic acid and pungency content indicated the increasing of garlic allicin content **(Schwimmer and Mazelis, 1963). Dixit and Chaudhary (2014)** reported that there are quantitative increases in secondary metabolite concentrations such as allicin and pyruvic acid when treated with different chemical mutagens.

In vitro experiment:

Obtained data in Table (9) showed that cultures of root tips with 2,4-D at concentration 1mg/l in MS medium gave the highest percentage of callus formation and callus weight (91.67 and 2.66 respectively) within the shortest duration (35 days), while 4 mg/l achieved the highest days to callus formation (66.67) and the lowest percentage of callus formation and callus weight (23.33 and 0.84 respectively). Control treatment of root tips did not form any callus.

Table (9): Effect of 2,4-D concentration on days to callus formation, percentage of callus formation and callus weight obtained from garlic root tip.

2,4-D concentration (mg/l)	Days to callus formation	Percentage of callus formation	Callus weight (g)
0	Not formed	Not formed	Not formed
1	35.00 d	91.67 a	2.66 a
2	45.00 c	63.33 b	1.49 b
3	45.00 c	43.33 c	1.02 c
4	66.67 a	23.33 d	0.84 d

Data of callus differentiation showed significant differences in Table (10), which showed that cultured callus on BA at concentration 1 mg/l gave the lowest days to callus differentiation (33.33) also the highest number of shoots and number of micro bulbs (14 and 9.33 respectively). Callus went through different phases during differentiation to forme somatic embryos such as single cells, globular, heart and torpedo shapes was similar to **komamin *et al*. (1992)** Fig (1).

Evaluation of obtained *In vitro* clones showed significant differences among studied traits in Table (11) and Table (12). Presented data showed that C1 (control) obtained the highest results of studied traits except total free amino acids and pungency were C7 gave the highest amount.

Table (10): Effect of 2,4-D and BA concentrations on days to callus differentiation, number of shoots and number of micro bulbs.

2,4-D (mg/l)	BA (mg/l)	Days to callus differentiation	Number of Shoots perjar	Number of micro bulbs per jar
0	**0**	Not formed	Not formed	Not formed
	1	33.33 e	14.00 a	9.33 a
	2	43.33 d	11.33 b	8.66 a
1	**0**	Not formed	Not formed	Not formed
	1	53.33 c	9.33 c	6.33 b
	2	56.67 bc	7.33 d	4.33 c
2	**0**	Not formed	Not formed	Not formed
	1	58.33 ab	6.00 de	3.33 cd
	2	61.67 a	4.33 e	2.33 d

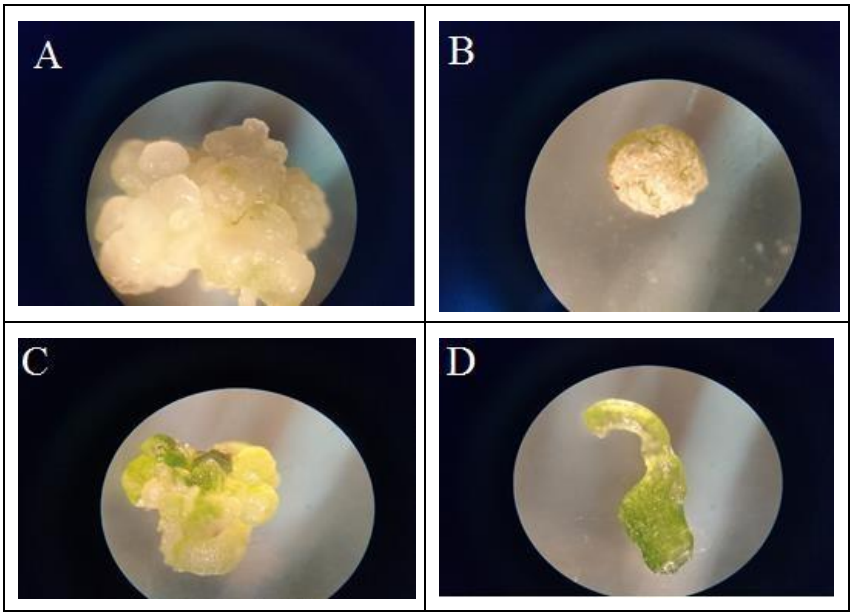


Fig (1): Callus induction and somatic embryos formation.
A: Callus formation, B: Globular stage, C: Heart stage, D:Torpedo stage.

Table (11): Vegetative growth characteristics of selected *In vitro* clones from garlic micro bulbs after 150 days from planting in the greenhouse.

Clone number	Plant height (cm)	Number of leaves	Vegetative growth fresh weight (g)	Bulb diameter (cm)	Number of cloves per bulb
1 (Control)	104.70 a	7.33 a	89.51 a	4. 76 a	42.00 a
7	51.67 b	6.00 b	7.86 b	2.40 b	5.33 b
8	40.67 c	5.00 bc	4.13 b	1.86 c	4.33 b
9	41.67 c	5.33 bc	4.16 b	1.90 c	4.33 b
10	43.33 c	4.33 c	4.26 b	1.83 c	4.33 b

Table (12): Chlorophyll, carotene, total free amino acids and pungency content in garlic *In vitro* clones.

Clone number	Total chlorophyll (mg/g)	Carotene (mg/g)	Total free amino acids (mg/100 g)	Pungency (µM/g fresh weight)
1 (Control)	1.55 a	0.86 a	1533.00 bc	16.41 d
7	1.12 b	0.49 c	1767.00 a	18.31 a
8	0.89 c	0.38 d	1667.00ab	17.63 b
9	1.04 b	0.60 b	1500.00 c	17.30 bc
10	1.09 b	0.57 b	1667.00 ab	17.13 c

Maximum callus formation achieved at 1.0 mg/l 2,4-D while increasing 2,4-D concentration led to minimum callus formation **(Hassan *et al*., 2014; Kereša *et al*., 2021)**. BA alone could induce shoot regeneration from callus where concentrations of 0.5 and 1 mg/l gave the highest rates of callus induction and plantlets formation **(yan *et al*. 2009).** Micro bulbs size is an important factor for the success of the acclimatization stage also determines the quality of the plants to be grown in the field and the time period they require to obtain the commercial size **(Oviedo et al. 2016)**. The vegetative and yield characteristics of garlic *In vitro* induced clones are lower in the first generation compared to the original cultivar, but they increase with cultivation over the generations and may reach higher rates than traditional plants after the fourth or fifth generation **(Metwally *et al.,* 2012; Oviedo *et al*., 2016)**.

SCoT-PCR amplification

Ten primers were used for amplifying the SCoT-PCR patterns listed in Table (13). A total of 88 amplified products were scored. In which 34 were polymorphic exhibiting 39% polymorphism. The molecular sizes of patterns profile were ranged between 100 to 3500 bp scored in Table (15). Fig. (2) Showed comparative DNA profile in which the ten different treatments of *Allium sativum* showed common and differentiating patterns with different primers. The primer MPST17 exhibited 78% polymorphism. Table (16) showed some SCoT primers revealed specific patterns. One specific band sized1300 bp in clone 3 was produced using MPST11 primer. One specific band sized 650 bp was produced by MPST12 primer in clone 2. Three specific bands were obtained in clone 2 at 550 bp, 650 bp, and 1700 bp by using MPST14. These results exhibit the potential use of SCoT marker to recognize *Allium sativum* treatments and differentiate between them.

SRAP-PCR amplification

In the present investigation, Ten SRAP primer combinations (Em1-Me1, Em2-Me1, Em3-Me1, Em4-Me1, Em1-Me2, Em2-Me2, Em3-Me2, Em4-Me2, Em1-Me3, and Em2-Me3) amplified 78 patterns listed in Table (14). In which 42 were polymorphic exhibiting 54% polymorphism. The molecular sizes of patterns profile were ranged between 100 to 4000 bp scored in Table (17). Fig. (3) Showed comparative DNA profile in which the ten different treatments of *Allium sativum* showed common

and differentiating patterns with different primers. The primer combination of Me1+ Em4 exhibited 71% polymorphism. Table (18) showed some SRAP primer combinations revealed specific patterns exhibit the potential use of this marker to recognize the different treatment of *Allium sativum*. One specific band was obtained at 600 bp in clone 6 using Me_2+Em_1 primer combination and one specific band was obtained at 500 bp in clone 4 using Me_3+ Em_2 primer combination. The level of polymorphism was comparatively higher than previous studies 55% in (Pinus Feng *et al*. 2009) but lower than 82.5% in buck wheat (Li *et al*., 2009).

Genetic relationship as detected by genetic similarity and Cluster analysis using SCoT and SRAP data

SCoT and SRAP markers have been exploited in this investigation to determine the genetic variability in garlic with more efficiency than RAPD and AFLP markers. They are potential marker systems in which, SCoT markers target the start codon regions and SRAP markers target the open reading frames (ORFs). The generated amplified bands were scored visually. The bands were scored as present (1) or absent (0) to create the binary data set. Jaccard's coefficient was used to estimate the genetic similarity (Sneath and Sokal 1973). The genetic similarity percentage of genomic DNA using SCoT marker ranged from 89% to 57% as were showed in Table (19). In which, the highest genetic similarity percentage revealed by SCoT-PCR analysis was 89% between clone 1 and 2, clone 2 and 3, clone 4 and 5. While, the least genetic similarity percentage was 57% between clone 4 and 10. This DNA marker technique considered as a useful tool for identifying population of the same species on a wide range of plants (Collard and Mackill 2009). It was validated in the model rice species *Oryza sativa* to fingerprint a small diverse set of rice genotypes. The percentage of polymorphic loci generated by using this effectiveness marker system in *Dendrobiumnobile Lindl* as an endangered medicinal orchid species was ranged from 25 to 56.82% and cluster analysis revealed high genetic variation among the genotypes according to Bhattacharyya *et al*., (2013). DNA profiling was revealed best results in *M. piperita* as compared to other *Mentha* species using SCoT marker analysis for correlating their similarity and distances between species (Azza *et al.* 2019). They estimated the genetic similarity between them ranged from 25.7% to 45.5% based on previous studies, their results proved the accuracy of SCoT marker system in estimating the genetic diversity.

In comparison with SRAP- PCR analysis, the highest genetic similarity percentage was 95% between clone 1 and 2. While, the least genetic similarity percentage was 45% between clone 4 and 10 as were showed in Table (20). The assembly results from SCoT and SRAP markers revealed a highest genetic similarity percentage of 92% was between clone 8 and 10 and 90% was between clone 4 and 5. While, the least genetic similarity percentage was 68% between clone 4 and 10 as were showed in Table (21).

The dendrogram of SCoT data analysis clustered the garlic clones into two main clusters as illustrated in Fig. (4), the first cluster included clones 1, 2, 3, 4, 5, 6, 7, and 9 that branched into two sub-clusters; the first sub-cluster included clones 1, 2, 3, 4, 5, and 7 while, clones 6 and 9represented the second sub-cluster. In contrast, the second cluster included clones 8 and 10. Based on SRAP data analysis, the dendrogram clustered the garlic clones into two main clusters as illustrated in Fig (5), the first cluster included clones 1, 2, 3, 4, 5,6, and 7 that branched into two sub-clusters ; the first one included clones 1, 2, and 3 in which clones 1 and 2 have the same linkage distance. While the second sub-cluster included clones 4, 5, and 6 in which clones 5 and 6 have the same linkage distance and clone 7 alone. The second cluster included three clones 8, 9, and 10 that branched into two sub-clusters; the first one included clones 9 and 10 with the same linkage distance. While, clone 8 represented the second sub-cluster.

The cluster analysis for the assembly data of SRAP and SCoT dataset were revealed by a dendrogram Fig (6) comprised of two major clusters the clones of the first cluster comprised of 1, 2, 3, 4, 5, 6 and 7 that, branched into two sub-clusters and clone 7 alone. The first sub-cluster included clones 1, 2, and 3 in which, clones 1 and 2 have the same linkage distance then, clone 3. While, the second sub-cluster included clones 4, 5, and 6 in which clones 5 and 6 have the same linkage distance then, clone 4. The second cluster included three clones 9, 8, and 10 in which, clones 8 and 10 have the

same linkage distance then, clone 9. While, sample 7 considered to be related to the second cluster than the first one according to the genetic distance. Based on the combined data analysis there were two main clusters, the first one included the clones treated with chemical mutagens that branched into two sub-clusters; one of them included the clones treated with EMS and the control while, the second sub-cluster included clones treated with Col. And SA. The second cluster included the clones produced from embryogenesis assessment except clone 7 considered to be related to the second cluster of the agronomic traits.

The dendrogram confirmed that, the SRAP markers system is more accurate as they have high distinguishable power because of amplifying the genome coding regions using primers targeting the open reading frame (Budak *et al.,* 2004 and Robarts & Wolfe 2014). Li and Quirors (2001) also developed a simple SRAP marker in a series of recombinant inbred and doubled-haploid clones of *Brassica oleracea* L. They found that, SRAP is an efficient marker system that can be adapted in different crops such as; rice, potato, lettuce, Chinese cabage, and garlic.

Table (13): The sequences of SCoT primers

Primer code	Primer Sequence (5'-3')	Nucleotide Number	%GC
MPST2	ACCACAAAATGGCGACCTA	19	47
MPST11	GGTGTTGATGGCGACCT	17	59
MPST 12	TTTGTTGATGGCGACCG	17	53
MPST13	GACAGCATGGCTACCAT	17	53
MPST14	ATGAGCATGGCTACCGA	17	53
MPST16	GATTTGAAATGGCTACCA	18	39
MPST17	ATGGCTACCCTTAGCATG	18	50
MPST18	TTAGCATGCATGGCTACC	18	50
MPST27	CAACAATGGCTACCACCC	18	56
MPST30	CCATGGCTACCACCGCAC	18	67

Table (14): The sequence of SRAP Primers

Primer code	Type	Primer Sequence 5'-3'
Me1	Forward	TGAGTCCAAACCGGATA
Me2	Forward	TGAGTCCAAACCGGAGC
Me3	Forward	TGAGTCCAAACCGGAAT
Em1	Reverse	GACTGCTACGAATTAAAT
Em2	Reverse	GACTGCGTACGAATTTGC
Em3	Reverse	GACTGCGTACGAATTTGAC
Em4	Reverse	GACTGCGTACGAATTTGA

Primers combination: Em1-Me1, Em2-Me1, Em3-Me1, Em4-Me1, Em1-Me2, Em2-Me2, Em3-Me2, Em4-Me2, Em1-Me3, and Em2-Me3.

Table (15): Features of SCOT products used for genetic diversity analysis of *Allium Sativum*

SCOT marker	Band size (bp)	Total bands (TB)	Polymorphic bands (PB)	Polymorphism (%)	Polymorphic information content (PIC)
MPST2	100-800	7	2	29	0.265
MPST11	100-2500	13	6	46	0.499
MPST12	200-1100	9	5	56	0.381
MPST13	200-1000	6	1	17	0.033
MPST14	100-2000	11	2	18	0.458
MPST16	100-1000	9	4	44	0.286
MPST17	100-3500	9	7	78	0.381
MPST18	200-1500	12	4	33	0.289
MPST27	200-700	5	1	20	0.039
MPST30	200-1500	7	2	29	0.157
Range/Sum	100-3500	88	34	39	0.229

Table (16): SCOT markers specific to *Allium Sativum*

Treatments	SCoT Primer	Treatment specific band range
3	MPST11	1300 bp
2	MPST12	650 bp
2	MPST14	550, 650, and 1700 bp

Table (17): Features of SRAP products used for genetic diversity analysis of *Allium Sativum*

SRAP marker	Band size (bp)	Total bands (TB)	Polymorphic bands (PB)	Polymorphism (%)	Polymorphic information content (PIC)
Me1+ Em1	100-1500	6	3	50	0.278
Me1+ Em2	100-700	10	3	30	0.211
Me1+ Em3	100-4000	10	6	60	0.32
Me1+ Em4	100-1700	7	5	71	0.368
Me2+ Em1	100-1000	8	3	38	0.399
Me2+ Em2	100-1500	6	3	50	0.206
Me2+Em3	100-1500	11	7	64	0.38
Me2+ Em4	100-1500	6	3	50	0.464
Me3+ Em1	200-900	6	4	67	0.231
Me3+ Em2	100-700	8	5	63	0.475
Range/Sum	100-4000	78	42	54	0.3015

Table (18): SRAP markers specific to *Allium Sativum*

Treatments	SRAP Primer	Treatment specific band range
6	Me2+ Em1	600 bp
4	Me3+ Em2	500 bp

Table (19): Genetic identity and genetic distance values among the 10 different treatments of *Allium Sativum* using SCOT marker.

Treatment	1	2	3	4	5	6	7	8	9	10
1	1.00									
2	0.89	1.00								
3	0.88	0.89	1.00							
4	0.79	0.82	0.84	1.00						
5	0.86	0.85	0.86	0.89	1.00					
6	0.79	0.78	0.81	0.83	0.87	1.00				
7	0.84	0.81	0.80	0.79	0.84	0.81	1.00			
8	0.74	0.69	0.70	0.66	0.71	0.71	0.79	1.00		
9	0.71	0.70	0.68	0.61	0.67	0.64	0.71	0.76	1.00	
10	0.68	0.63	0.63	0.57	0.64	0.63	0.72	0.86	0.82	1.00

Table (20): Genetic identity and genetic distance values among the 10 different treatments of *Allium Sativum* using SRAP marker.

Treatment	1	2	3	4	5	6	7	8	9	10
1	1.00									
2	0.95	1.00								
3	0.89	0.94	1.00							
4	0.79	0.82	0.85	1.00						
5	0.82	0.84	0.84	0.88	1.00					
6	0.77	0.76	0.77	0.86	0.92	1.00				
7	0.81	0.77	0.77	0.75	0.81	0.81	1.00			
8	0.71	0.68	0.66	0.58	0.65	0.65	0.76	1.00		
9	0.60	0.59	0.58	0.49	0.56	0.56	0.62	0.81	1.00	
10	0.58	0.55	0.53	0.45	0.51	0.51	0.60	0.79	0.93	1.00

Table (21): Genetic identity and genetic distance values among the 10 different treatments of *Allium Sativum* using SCOT and SRAP markers.

Treatments	1	2	3	4	5	6	7	8	9	10
1	1.00									
2	0.84	1.00								
3	0.87	0.84	1.00							
4	0.80	0.82	0.83	1.00						
5	0.89	0.86	0.87	0.90	1.00					
6	0.81	0.78	0.84	0.79	0.82	1.00				
7	0.86	0.83	0.82	0.82	0.87	0.81	1.00			
8	0.76	0.70	0.73	0.75	0.77	0.76	0.81	1.00		
9	0.80	0.78	0.77	0.72	0.76	0.71	0.78	0.73	1.00	
10	0.77	0.70	0.71	0.68	0.75	0.74	0.82	0.92	0.76	1.00

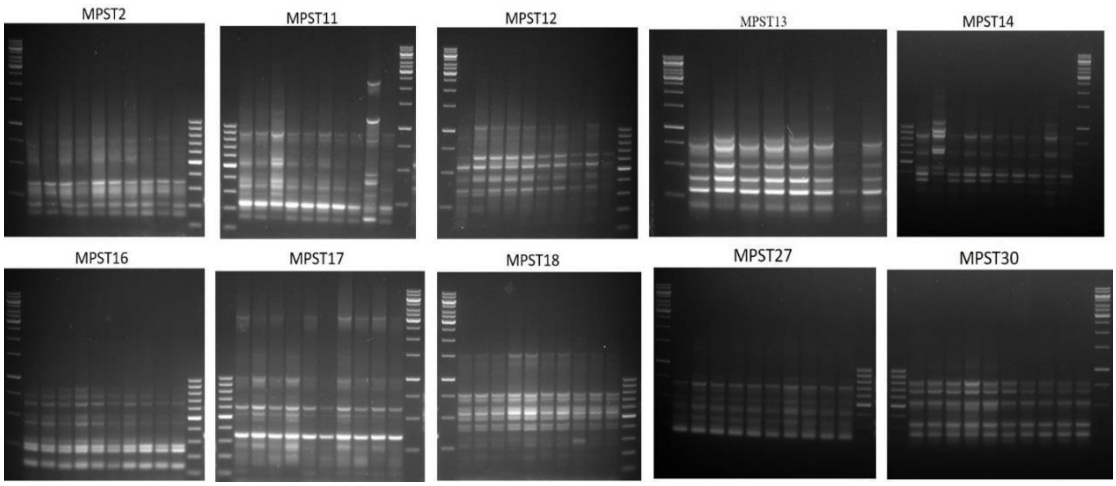


Fig. (2): ScoT markers profiles in ten deferent treatments of *Allium Sativum* using MPST primers. Lane 1 from left for DNA ladder 1kb or 100bp; 2 for un-treated sample 1 (control); 3 for sample 2; 4 for sample 3; 5 for sample 4; 6 for sample 5; 7 for sample 6; 8 for sample 7; 9 for sample 8; 10 for sample 9; 11 for sample 10 and the last lane for DNA ladder 1kb or 100bp.

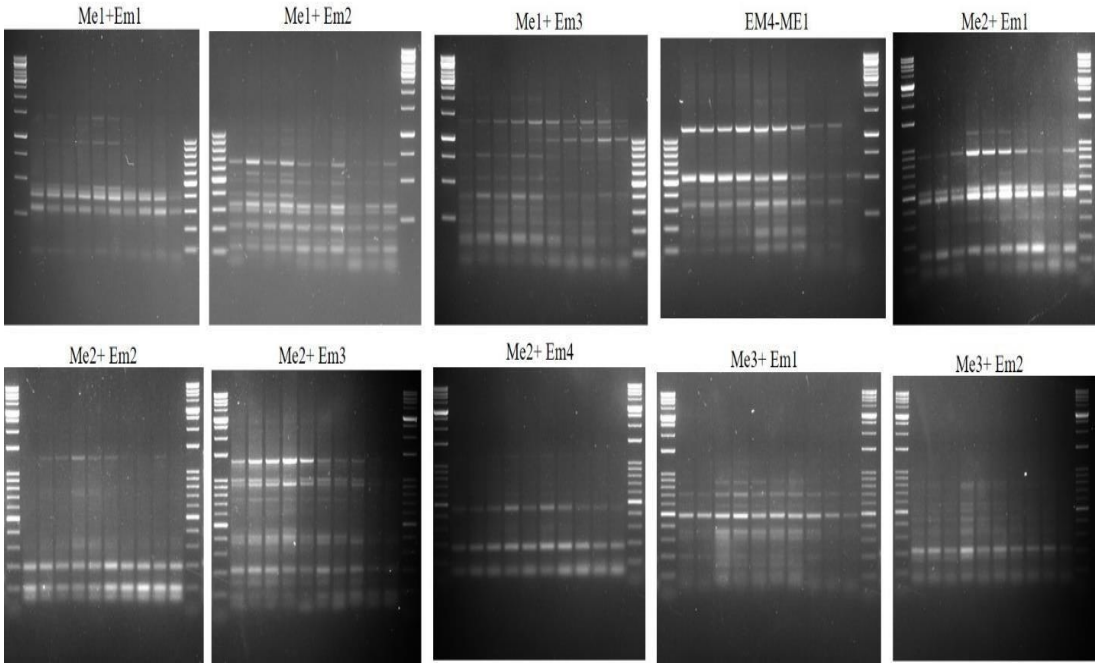


Fig. (3): SRAP markers profiles in ten deferent treatments of *Allium Sativum* using primer combinations of Me and Em as forward and backward primers. Lane 1 from left for DNA ladder 1 kb or 100 bp; 2 for un-treated sample 1 (control); 3 for sample 2; 4 for sample 3; 5 for sample 4; 6 for sample 5; 7 for sample 6; 8 for sample 7; 9 for sample 8; 10 for sample 9; 11 for sample 10 and the last lane for DNA ladder 1 kb or 100 bp.

Fig. (4) Dendrogram Depicting the Genetic Relationship among ten different treatments of *Allium Sativum* based on SCoT data Analysis.

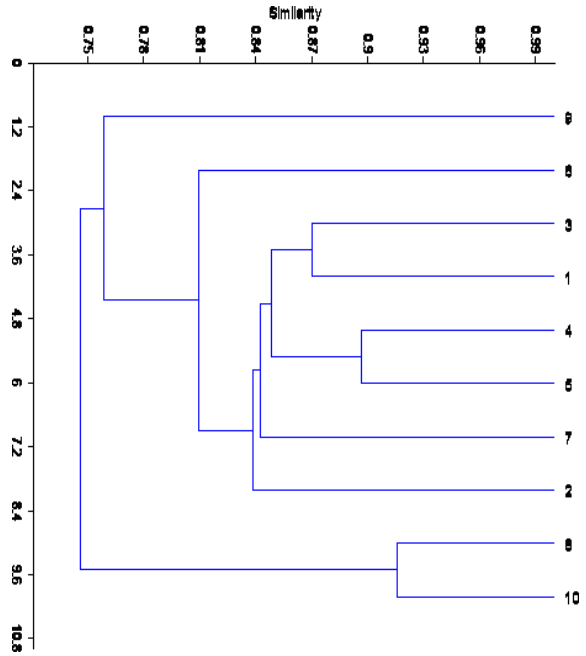


Fig. (5) Dendrogram Depicting the Genetic Relationship among ten different treatments of *Allium Sativum* based on SRAP data Analysis.

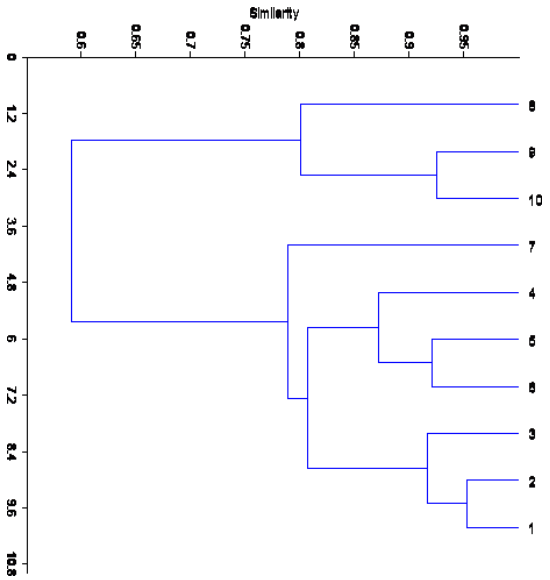
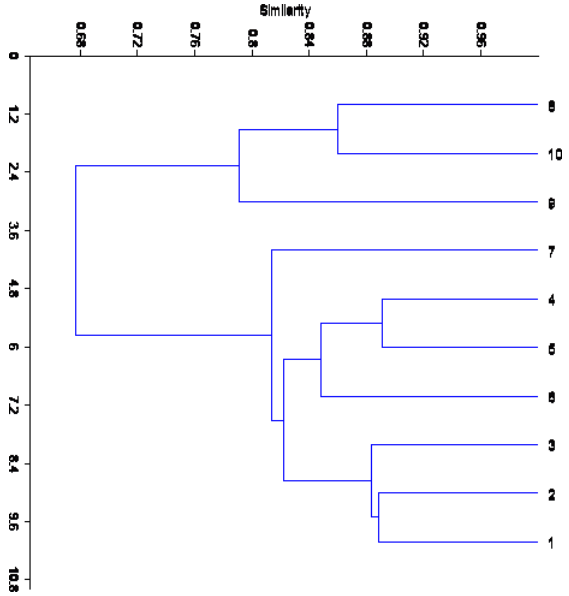


Fig. (6) Dendrogram Depicting the Genetic Relationship among ten different treatments of *Allium Sativum* based on SCOT and SRAP data Analysis.



CONCLUSION

The results of this study showed possibility of obtaining superior clones in vegetative characteristics, yield and storage than the original variety by using chemical mutagens. Treatment with low concentrations of ethyl methane sulfonate and colchicine led to superiority in the traits starting from the first season, while treatment with sodium azide led to a decrease in growth characteristics and yield in the first and second seasons, and then gave superiority compared to all treatments in the third season. Also, this study showed the possibility of inducing different clones through somatic embryos and then growing in the greenhouse for evaluation.

In this study, the results showed significant differences between the five samples of cultivated *Allium sativum* treated with different mutagenic agents and the four embryogenesis treatments generated from embryogenesis tissue culture. Using two dominant molecular marker systems SRAP and SCoT revealed a number of amplicons ranged from 6 to 11with a mean of 7.8 amplicons per primer combination using SRAP. The band size varied from 100 to 4000 bp. Out of 10 SRAP markers, Me1+ Em4 combination displayed 71% polymorphism while, Me2 + Em1 and Me3 +Em2 combinations displayed treatment specific band at 600 bp for treat 6 and 500 bp for treat 4, respectively.

The highest percentage of polymorphism was 76 % with 10 SCoT primers and the lowest was 58.8% with 4 SCoT primers. The recognition of unique bands by some SCoT primers indicates that the SCoT technique can define the treatments unique patterns that can be useful in *Allium sativum* Balady variety identification. On the other side, developing DNA molecular markers of *Allium sativum* Balady variety as a medicinal and aromatic plant provide more accurate assessments of genetic variation.

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