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A Comparative Study of Morphological and Volatile Oil Composition Characteristics in Diploid and Tetraploid Garlic Plants

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GARLIC is one of the most economically important vegetables and medicinal plants. Asexual propagation of garlic has led to a critical reduction in the genetic diversity. Tetraploidization impact on morphological characteristics and enhancement of secondary compounds of garlic volatile oil was investigated in the present study. Ploidy effects were investigated following the application of 0.2, 0.4, or 0.6% (w/v) colchicine for four durations (12, 24, 36, or 48 h.) and ploidy level was identified by morphological characterization and confirmed by chromosome counting. The morphology and oil profile, determined by gas chromatography, were compared between diploid and tetraploid garlic plants. Results showed that the efficiency of tetraploid induction ranged from 8 to 26% and the highest efficiency was achieved by applying 0.6% colchicine for 24h. Confirmed tetraploid plants had larger morphological characteristics, biochemical constituents and mineral content compared to their ancestral diploid plants. However, the essential oils of the tetraploid plants and the diploid plants were found to have a substantially similar composition. Tetraploidization induction significantly increased the concentration of diallyl disulfide, allyl methyl trisulfide and diallyl trisulfide. Because the tetraploid plants contain significantly higher concentrations of sulfur components, they have unique flavor characteristics that should be of commercial interest. We concluded that polyploid induction is an attractive technique that can be used to improve garlic yield and volatile oil composition.

Keywords: Colchicine, Gas chromatography, Polyploidy, Volatile oil.

Introduction

Synthetic medicine has largely replaced the herbal product. In the last decade, however, there has been a revival of enthusiasm for plant-based medicines because of the widespread conviction that natural remedies are somehow non-toxic, safer and healthier than their artificial counterparts (Dixit et al., 2015). Garlic (*Allium sativum L.*), a member of *Alliaceae* family, is one of the foremost vital bulb vegetable crops widely cultivated in the world and one of the best therapeutic medicinal plants. Major growing areas of garlic are USA, China, Egypt, Korea, Russia and India (FAOSTAT, 2016). Throughout history, garlic has a completely unique function and its therapeutic applications have been found in ancient medical texts of many cultures (Suleria et al., 2013; Bhandari, 2014). It was used to increase workability and provide strength for workers in many societies. Almost 30 centuries ago, Hippocrates, the Father of Medicine, stated "*let food be thy medicine and let medicine be thy food"* (Bhandari, 2014). Several pharmaceutical companies have expressed interest in this plant because of its several sulfur components (including alliin, allicin, allicinase, diallyldisulphide, diallyltrisulphide, sallylcysteine and methylallyltrisuphide), enzymes, amino acids and minerals such as selenium. Thus, it has fundamental pharmacological properties such antimicrobial (Fateh et al., 2010) anti-tumor (Antony et al., 2011 and Lai et al., 2012) and immunostimulant properties (Chandrashekar and Venkatesh, 2012).

Sexual propagation of garlic is almost impossible due to failure in the production of

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fertile seeds. Therefore, almost all of the cultivated garlic genotypes are propagated vegetatively. Despite certain types of garlic flowers, the flowers are sterile and the blooming can be restricted to specific areas (Neta et al., 2011). Approximately 10% of the harvested crop is saved and used as the source of propagule for subsequent cultivation (Sinha et al., 2016). Consequently, this resulted in a critical reduction in genetic diversity of garlic. Unfortunately, without a broad base of heterogeneous genetic garlic materials, it is very difficult for garlic breeders to produce new cultivars with higher productivity and to improve nutritional quality and health benefits. Therefore, looking for an easy and cost-effective technique that could broaden the genetic diversity of garlic is an important target.

Polyploidy is an amazing evolutionary technique that could be used in plant breeding to improve plant species (Noori et al., 2017). It is the heritable state of having more than two complete sets of chromosomes per nucleus (Comai, 2005, Soltis et al., 2009) and it is an effective technique that plays a fundamental role in generating innovative germplasm resources that are suitable for genetic and phenotype diversity in addition to plant evolution and breeding (Ye et al., 2010, Xing et al., 2011). Polyploidy is believed to enhance plant development, productivity and adaptability to harsh environments (Chen and Tian, 2007), due to gene redundancy and heterosis, which give modern genotypes superior properties over their diploid parents (Fuentes et al., 2014). Tetraploid plants are advantageous because of their ability to produce diverse gene products than diploid parents (Comai, 2005). The main consequences of induced polyploidy are possessed larger cell sizes and sometimes larger biomass, height and girth (Comai 2005, Oselebe et al., 2006, He et al., 2016 and Noori et al., 2017), leaf size and thickness (Stupar et al., 2007 and Murti et al., 2012), and stomata size (Miller et al., 2012). Polyploidy has been used in horticulture as a breeding tool for improving ornamental characteristics (Shao et al., 2003 and Ye et al., 2010), resistance to environmental stresses and diseases (Zhang et al., 2010). It can also enhance the vigor of certain plant parts and can be beneficial if both specific plant organs and biomass are economically valuable products (Dhawan and Lavania, 1996, Majdi et al., 2010).

Polyploidy, on the other hand, is also considered an effective technique for increasing plant production. It is well known that genomic duplication can improve the production of secondary metabolites and also improve their biochemical profile qualitatively (Majdi et al., 2010, Dhooghe et al., 2011, Kaensaksiri et al., 2011, Dehghan et al., 2012, Cohen et al., 2013 and Zahedi et al., 2014). A literature survey showed that the induction of polyploidy in medicinal plants increases the amount of biomass or phytochemicals, *e.g.,* in *Salvia miltiorrhiza* (Gao et al., 1996), *Scutellaria baicalensis* (Gao et al., 2002), *Papaver somniferum* (Mishra et al., 2010), *Artemisia annua* (Lin et al., 2011), *Zingiber officinale* (Kun-Hua et al., 2011), *Echinacea purpurea* (Abdoli et al., 2013) and *Salvia miltiorrhiza* (Hsia et al., 2013). Therefore, polyploid garlic has the potential to be advantageous compared to diploid garlic, resulting in increased yields and improved nutritional quality and health benefits. Polyploidy probably occurs in nature due to abnormal cell division, including imperfect chromosome partitioning. Colchicine, a toxic alkaloid extracted from autumn crocus (*Colchicum autumnale*), is the most common chemical used to produce polyploid artificially. It prevents polymerization of microtubules and increases the number of chromosomes. Due to the different sensitivities of the plant species to colchicine, the optimum concentration of colchicine and exposure time, the parts of a plant treated and other factors should be studied (Harbard et al., 2012) in order to produce a new polyploid plant effectively. The present study aimed to examine the effects of colchicine on polyploid induction and present an appropriate protocol for tetraploid induction in garlic. We also shed light on the potential effect of artificial polyploid induction on the improvement of oil content and secondary metabolite content.

Materials and Methods

A field experiment was conducted at the experimental farm of the Faculty of Agriculture, Suez Canal University, Egypt, during the two successive seasons of 2015/2106 and 2016/2017.

Plant material

The garlic cv. Sids 40 was obtained for the present study from the Horticulture Research Institution, Agriculture Research Center, Egypt. The underground parts of the garlic were thoroughly washed in order to remove the soil adhering to the cloves and to reduce the microbial contamination.

Polyploidy induction

To determine the optimum colchicine concentration and exposure time for tetraploid induction, fifty healthy garlic cloves were imbibed in various colchicine aqueous solutions (0, 0.1, 0.2, or 0.3%) for 6, 12, 18, or 24 h at room temperature on a shaker at 100 rpm. Five drops of dimethyl sulfoxide (DMSO) at 4% has been added to all colchicine solutions for better penetration into plant tissues, thus jointly increasing solution efficiency. Cloves imbibed in the distilled water served as control. After treatment with colchicine, the treated cloves were washed with distilled water and carefully cultivated in pots under greenhouse conditions. After 60 days of colchicine treatment, the survival rate was observed. Cloves that were not grown and brown were considered dead. In this experiment, which had three replications for each treatment, completely randomized design was used.

Screening of putative tetraploid

In order to confirm the polyploidy induction, grown plants were examined individually to record the sequence of morphological developments and the relationships of any detectable macroscopic (phenotypic) differences.

Chromosome counting

The chromosome observation procedure was the same as described by Park et al. (1999). Before polyploidy induction, the number of chromosomes was determined in root tips of germinated cloves of diploid materials (2n = 2x = 16). After 4 months of colchicine treatments, actively growing root tips of measuring approximately 2–3 mm in length were taken from the plantlets and washed with distilled water. They were then pre-treated with the 0.05 % colchicine solution for 4 h for better chromosome separation. After washing with distilled water, the root tips were fixed in cold fresh Carnoy fixative overnight (ethanol: glacial acetic acid, 3:1). After being washed with distilled water, the fixed root tips were hydrolyzed with 1 N HCl for 8 min at 60° C. After washing with distilled water 3 times, the hydrolyzed root tips were stained with 1 % aceto-carmine solution for 30 min. Images of the chromosomes were observed using a digital camera.

Stomata observation

Leaf samples were collected from the plants when they reached the 4–5 true-leaf stage. For stomata measurements, 0.5 cm^2 of the lower epidermis of the leaf was painted with fingernail polish. After it was dried, the nail polish impression has been removed using a strip of scotch tape. The tape was then placed on a microscope slide and observed under light microscope (Nikon Eclipse 50i, Nikon Corp). Twenty stomata were measured for each leaf. In order to determine the stomata density, stomata were counted in 10 microscopic fields for each plant. Stomata sizes and density were recorded at 100× magnification.

Tetraploid efficiency

The effect of sixteen treatment combinations of four the colchicine concentrations and the four soaking durations on the survival rate and efficiency of tetraploid induction was evaluated. The efficiency of tetraploid induction was calculated according to Wang et al., (2017) as follows:

$$\text{Tetraploid induction efficiency (\%)} = \frac{\text{Number of tetraploid plants}}{\text{Total number of treated cloves}} \times 100$$

Morphological measurements

The morphological characteristics of the confirmed tetraploid plants, which grow under greenhouse conditions, were compared with those of corresponding diploid plants. The germinated seedlings of either diploid or tetraploid plants were fertilized with the recommended NPK rates. The morphological traits of bulbs and cloves were determined by measuring the length, weight and diameter of diploid and tetraploid garlic plants. The leaf area, the number of leaves and the height of the plant were measured by a ruler. The basal stem diameter of the neck was measured using a digital caliper.

Phytochemical analysis

Total chlorophyll concentrations in leaves were spectrophotometrically determined as in Arnon (1949) method. A sample of 0.15 g of fresh leaves was homogenized in 10 ml of 80 % acetone and kept in the dark for 8 h at room temperature. The supernatant was made up to 25 ml by the addition of 80 % acetone, and absorbance was measured at 470, 646 and 663 nm using spectrophotometer. Total soluble solids (TSS) were determined using a digital refractometer. Total bulb carbohydrates and total phenols were quantified according to the method of Mazumdar and Majumder (2003). Nitrogen was determined by Kjeldahel technique according to Jackson (1973), where phosphorus was colorimetrically determined using Spectrophotometer as described by Jackson (1973).

Extraction and analysis of volatile oil

Volatile oil extraction

Five samples of fresh diploid and tetraploid garlic bulb were hydro distilled for 4 h in a Clevenger-type apparatus for 4 h, according to the procedure described in the Egyptian pharmacopoeia (2005) to determine the volatile oil percentage (volume/weight). The obtained oils were dehydrated by filtration through anhydrous sodium sulfate and kept in a refrigerator in dark bottles for GC analysis. Volatile oils and their components were extracted at the Medicinal and Aromatic Plants Research Department Laboratory, Horticulture Research Institute, Agriculture Research Center, Giza, Egypt.

Gas chromatography analysis (GC)

The GC analysis of the volatile oil samples was performed using gas chromatography instrument with the following specifications. DsChrom 6200 Gas Chromatograph equipped with a flame ionization detector, Column: BPX-5, 5% phenyl (equiv.) polysillphenylene-siloxane 30m x 0.25mm ID x 0.25µm film., Sample size: 1µl, Temperature program ramp increase with a rate of 10ºC/ min from 70º to 200º C, Detector temperature (FID): 280°C, Carrier gas: nitrogen, Flow rate: N2 30 ml/min, H2 30 ml/min, air 300 ml/min. The main compounds of the volatile oils were identified by matching their retention times to those of the authentic samples injected under the same conditions. The relative percentage of each compound was calculated from the area of the peak corresponding to each compound.

Statistical analysis

T-test was used to compare the phenotypic and physiological traits of diploid and tetraploid plants with function *t-test* as implemented in *Agricola* R package (de Mendiburu and de Mendiburu, 2017).

Results

Tetraploid induction

Data presented in Table 1 show the effect of different colchicines concentrations (0, 0.2, 0.4 and 0.6%) and duration (12, 24, 36, and 48 h) on survival rate and tetraploid induction efficiency in garlic cv. Seds 40. Results showed that the survival rate and tetraploid induction efficiency were strongly affected by the different concentrations of colchicine and exposure durations. Not all treatments were able to induce tetraploid plants successfully, and the tetraploid induction efficiency was subsequently altered by the concentration and duration. Among the sixteen treatments, the efficiency of tetraploid induction ranged from 8 to 26% and the highest efficiency was achieved by soaking the cloves in a colchicine solution of 0.6% for 24 h. The high concentration of colchicine (0.6%) and longer duration (48 h) had a lethal impact on the treated cloves.

Cytological differences

Chromosomes counting at root tip cells showed 8 ($2n = 2x = 16$) and 16 ($2n = 4x = 32$) chromosome pairs were recorded in the diploid and tetraploid plants, respectively (Fig. 1).

Stomata characteristics

Length, width, and density confirmed the tetraploid plants that were identified by chromosome counting. The characteristics of stomata also differed significantly ($P<0.001$) between diploids and tetraploid plants (Table 2, Fig. 2). The stomata length and width of tetraploid plants were significantly larger (85 µm and 22 µm, respectively) than those of diploid plants (51 µm and 10 µm respectively, Table 2). However, the stomata density in tetraploid plants was significantly lower compared to diploid plants, where it was reduced to almost half in tetraploid plants compared to that in diploid plants (Table 2, Fig. 2).

Morphological differences

Data illustrated in Table 2 show the comparison of vegetative traits between the confirmed tetraploid plants and their corresponding diploid plants. Two-sample *t*-test statistical analysis indicated significant differences ($p < 0.001$) between the diploid and induced tetraploid plants of garlic with regard to the plant height, fresh weight, neck diameter, leaf length, leaf width and leaf area (Table 2). Compared with their ancestral diploid plants, plant height, fresh weight, neck diameter, leaf length and leaf area in the induced tetraploid plants increased with an average increase of 6.99, 27.37, 12.00, 37.08, 62.50 and 27.40%, respectively (Fig. 3). However, no significant difference ($p < 0.05$) in number of leaves between the control diploid and corresponding tetraploid was recorded. Furthermore, the comparison between the tetraploid plants and their corresponding diploid plants shows significant differences between diploid and tetraploid plants ($p < 0.001$) with regard to bulb traits. The weight, bulb diameter, bulb length, clove weight, clove width and clove length in the induced tetraploid plants were significantly larger ($p < 0.001$) than those in diploid plants (Fig. 4), with an increase of 31.01, 30.43, 10.12, 112.66, 47.61 and 41.14%, respectively.

TABLE 1. Effects of colchicine concentrations and soaking durations on survival rate and tetraploid induction efficiency in treated cloves of garlic cv. Sids 40.

Colchicine concentration %	Treatment period (h)	No. of surviving [1]	Number of tetraploid	Tetraploid induction efficiency (%)
0.0	12	49	0	0
	24	47	0	0
	36	48	0	0
	48	46	0	0
0.2	12	42	0	0
	24	40	0	0
	36	36	0	0
	48	25	0	0
0.4	12	35	0	0
	24	22	4	8
	36	20	5	10
	48	10	6	12
0.6	12	22	8	16
	24	16	13	26
	36	8	6	12
	48	0	0	0

[1] Total number of treated cloves were 50.

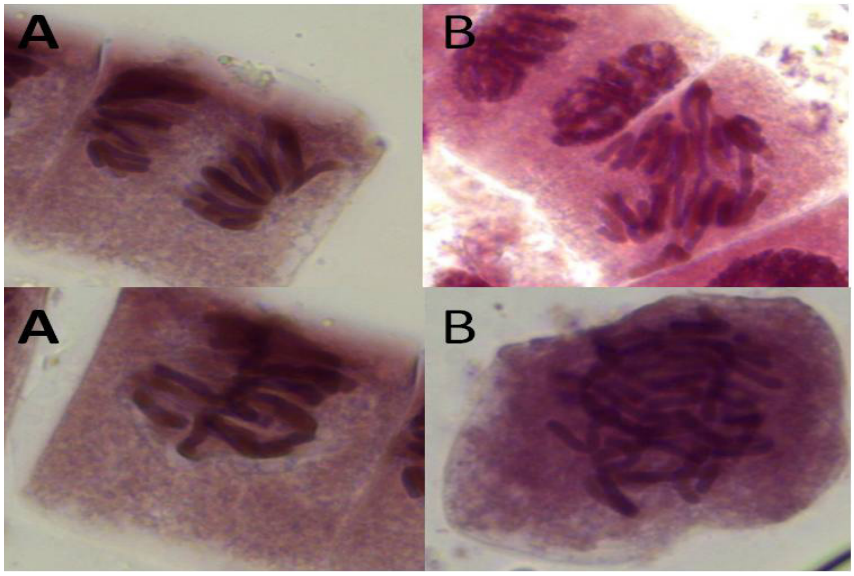

Fig. 1. Chromosomes of a root tip cell from diploid 2x = 16 (A) and induced tetraploid 4x = 32 (B) garlic plants.

TABLE 2. Summary of the measured traits with average values of diploid and tetraploid garlic plants.

Traits	Ploidy level		p-value
	Diploid	Tetraploid	
Stomata length (µm)	51.00±0.38b	1.03a±85.00	<0.001
Stomata width (µm)	10.00±0.8b	22.00±1.42a	<0.001
Stomata density ($mm^)$	95.00±1.55b	52.00±2.58a	<0.001
No. of Leaves	7.80±0.13a	7.90±0.16a	0.344
Plant height (cm)	55.33±1.11b	59.20±1.09a	<0.001
Fresh weight (gm)	73.02±1.58b	93.00±1.3a	<0.001
Neck diameter (cm)	1.00±0.03b	1.12±0.02a	<0.05
Leaf length (cm)	25.40±0.72b	34.82±0.6a	<0.001
Leaf width (cm)	1.60±0.06b	2.60±0.04a	<0.001
Leaf Area (cm^2)	68.45±0.88b	87.21±1.24a	<0.001
Bulb weight (gm)	53.53±1.57b	70.13±1.33a	<0.001
Bulb diameter (cm)	4.60±0.09b	6.00±0.07a	<0.001
Bulb length (cm)	3.85b±0.08b	4.24±0.07a	<0.05
Clove weigh (gm)	2.29±0.13b	4.87±0.25a	<0.001
Clove width (cm)	1.68±0.06b	2.48±0.06a	<0.001
Clove length (cm)	3.67b±0.09b	5.18±0.1a	<0.001
Total Chlorophyll (mg/100 gFW)	93.67±1b	104.23±1.37a	<0.001
Total soluble solids (TSS)	36.20±0.65b	40.93±0.63a	<0.001
Total Phenols (mg/gDW)	3.08±0.06b	4.20±0.06a	<0.001
Total Carbohydrates (mg/gDW)	235.05±1.24a	234.6±1.29a	0.8247
N content (mg/g DW)	25.21±0.36b	33.77±0.67a	<0.001
P content (mg/g DW)	3.46±0.06b	4.17±0.05a	<0.001
K content (mg/g DW)	13.23±0.29b	15.97±0.2a	<0.001

Data are mean (n=15) ± SE. Values in each row followed by different letters are significantly different according to t-test.

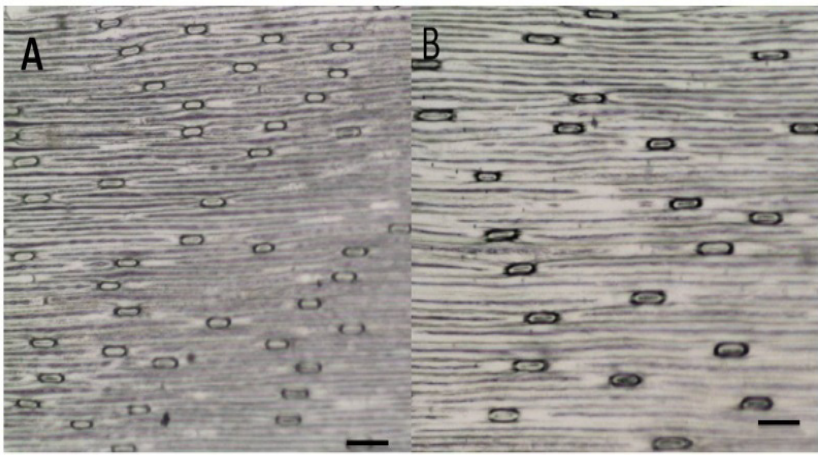

Fig. 2. Stomata size and density in abaxial leaf epidermis of diploid (A) and tetraploid (B) Allium sativum plants. Bars 100 µm.

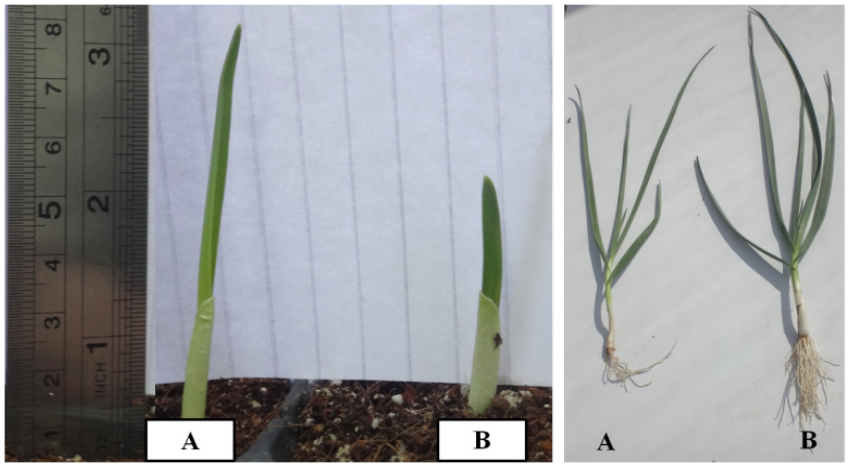

Fig. 3. Comparison of morphological characteristics of diploid (A) and tetraploid (B) garlic plants.

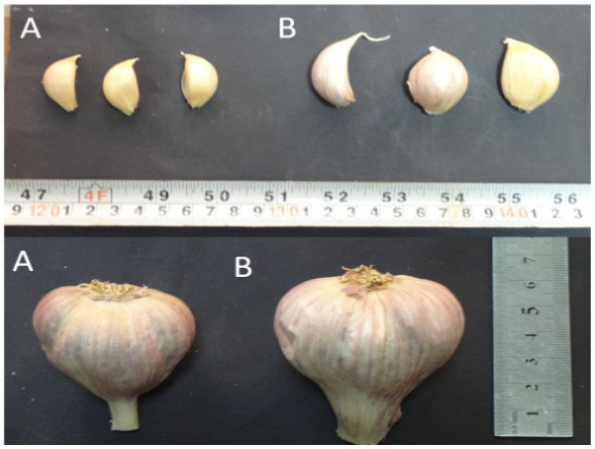

Fig. 4. Bulbs and cloves of diploid (A) and tetraploid (B) garlic plants from controlled growth chamber.

Differences in phytochemical content

Total chlorophyll, TSS, phenols and carbohydrate contents of tetraploid and ancestral diploid plants were compared (Table 2). Results showed that there were highly significant differences ($p < 0.001$) between the diploid and tetraploid plants of garlic in all studied phytochemical constituents with the exception of total carbohydrate content. The chlorophyll content in the tetraploid plants was significantly higher ($p < 0.001$) than the corresponding diploid plants with an increasing average of 11.27%. In addition, the TSS and bulb phenol content in the tetraploid plants were significantly higher ($p < 0.001$) than the corresponding diploid plants, with an average increasing rate: 13.06 and 36.36%, respectively. However, tetraploid plants of garlic recorded insignificant reduction (-0.19%) in total carbohydrates compared to diploid plants.

Differences of leaves N, P and K contents

Recorded data presented in Table 2 show significant differences ($p < 0.001$) between tetraploid plant and their ancestral diploid plants in terms of N, P and K contents. The average contents of N, P, and K were found to be significantly increased in tetraploid compared to diploid plants (Table 2), with an increasing rate of 33.95, 20.22, and 20.71%, respectively.

Volatile oil content and composition:

The volatile oil yield of tetraploid plants was two times (0.1%) higher than their diploid (0.05%) plants. Data presented in Table (3) present the gas chromatography analysis for diploid and tetraploid garlic volatile oil. It shows that fourteen components were identified in the volatile oil extracted from tetraploid and diploid of garlic cloves. Nevertheless, the major

components were sulfur-containing components: diallyl disulfide (25.57 and 29.44%), allyl methyl trisulfide (14.10 and 17.57%) and Diallyl trisulfide (20.10-23.23%) in diploid and tetraploid plants, respectively. Among fourteen identified compounds, tetraploidization induced significant increases only in the concentration of diallyl disulfide, allyl methyl trisulfide and diallyl trisulfide with an average increase of 15.13, 24.61 and 15.57%, respectively in tetraploid plants compared to their ancestral diploid (Table 3). In contrast, it significantly reduced the concentrations of 2-Vinyl-4H-1, 3-dithiine, 5-Methyl-1, 2, 3, 4-tetrathiane, 1, 4-Dihydro-2, 3-benzoxathiin 3-oxide and unknown compounds.

TABLE 3. Composition of volatile oil of diploid and tetraploid garlic cloves analyzed by GC.

NO.	Components	Percentage		
		Diploid	Tetraploid	*p* value
1	Dimethyl disulfide	0.41±01a	0.47±0.01a	n.s
2	Diallyl sulfide	0.76±0.02a	0.65±0.02a	n.s
3	Allyl methyl disulfide	0.95±0.01a	0.82±0.03a	n.s
4	Dimethyl trisulfide	2.94±0.03a	2.8±0.03a	n.s
5	Diallyl disulfide	25.57±0.14b	29.44±0.06a	<0.001
6	Allyl (Z)-1-propenyl disulfide	7.80±0.04a	6.27±0.6a	n.s
7	Allyl (E)-1-propenyl disulfide	4.41±0.09a	4.21±0.05a	n.s
8	Allyl methyl trisulfide	14.10±0.02b	17.57±0.05a	<0.001
9	2-Vinyl-4H-1,3-dithiine	5.29±0.06a	3.48±0.13b	<0.05
10	Diallyl trisulfide	20.10±0.04b	23.23±0.03a	<0.001
11	Allyl Propyl trisulfide	3.58±0.24a	2.78±0.03a	n.s
12	5-Methyl-1,2,3,4-tetrathiane	2.88±0.05a	0.90±0.04b	<0.05
13	Unknown	1.49±0.01a	0.48±0.04b	<0.05
14	1,4-Dihydro-2,3-benzoxathiin 3-oxide	2.46±0.07a	0.81±0.12b	<0.05
15	Diallyl tetrasulfide	7.23±0.26a	6.05±0.22a	n.s

Data are mean (n=6) ± SE. All content values are percentages

Values in each row followed by different letters are significantly different according to t-test.

Discussion

Garlic is one of the most popular vegetable crops because of its medicinal profits. It is as effective as many modern antibiotics, without the harmful side effects and it has a vital role in preventing of several diseases (Londhe, 2011). Sexual propagation of garlic is impossible because of failure in the production of fertile seeds, therefore, it is mainly asexually propagated by cloves. Generally, garlic cultivars show low genetic diversity and high similarity (Paredes et al., 2008). Taken together, low genetic diversity and asexual propagation in garlic prevent plant breeders from producing new garlic varieties with high yield and enhanced secondary metabolite content in garlic cloves and volatile oil. Therefore, it is important to establish an efficient protocol to increase the genetic diversity of garlic germplasm, which could assist in broadening the narrow genetic basis of garlic. Polyploidy is a quite old technique in plant biology (Wang et al., 2016). However, it is still an attractive and robust technique in different fields such as plant breeding. It plays a vital role in the breeding programs of numerous horticultural crops through inducing new morphological and physiological changes in plants, particularly asexually propagated plants (Dhooghe et al., 2011). It was successfully used in the breeding of several vegetable and medicinal plants such sweet potato (Oracion et al., 1990, Becerra and Orjeda, 2002) and watermelon (Jaskani et al., 2005, Sheikh et al., 2013), basil (Omidbaigia et al., 2010) and chamomile (Sattler et al., 2016).

Colchicine is one of the most important chemicals known to inhibit cell mitosis by binding to the protein tubulin in the mitotic spindle and preventing polymerization into microtubules,

thereby preventing nuclear division leading to polyploidy, which provides an opportunity to improve the morphological and biochemical attributes of plants (Lu et al., 2012, Mohammadi et al., 2012, Dixit et al., 2015). The results of the current study showed that both concentration and exposure time had a strong effect on polyploidy induction and survival rates. In the same regard, Lehrer et al. (2008) reported that tetraploid induction efficiency is a very important parameter for determining the best treatment for tetraploid induction.

Over sixteen treatments, the treatment of 0.6 % (w/v) colchicine for 24 h has achieved the highest tetraploidization and survival rates in garlic. Moreover, the results of the current study showed an inverse relationship between the colchicine concentration and exposure time as well as survival rate. For instance, the treatments showing the lowest survival rate, particularly 0.6 %, were those treatments that had the most successful tetraploidization induction. These results are in agreement with the previous reports in garlic and other plants (Majdi et al., 2010, Aboli et al., 2013, Dixit and Chauhdry, 2014). Therefore, selection of the appropriate exposure duration and colchicine concentration is critical and necessary to achieve a high tetraploid induction rate.

In several the previous reports, a correlation between ploidy level and histological, physiological and phenotypic characteristics was observed in several plant species (Omidbaigi et al., 2010, Dixit and Chauhdry, 2014, Dixit et al., 2015). In the present study, increasing the ploidy level increased mean of stomata length and width by 66 and 120%, respectively, compared to diploid plants. However, the average of stomata density decreased by 45%. The enlarged stomata width and length and decreased stomata density of tetraploid garlic plants were previously reported in garlic (Dixit and Chauhdry, 2014) and other plants such as radish and purple cauliflower (Limera et al., 2016, Zhou et al., 2017). The guard cell length has been proposed as a criterion for determining the ploidy of plant species (Hull-Sanders et al., 2009, Dixit, 2011, Dixit and Chaudhary, 2014). Our results show that tetraploid plants can be easily recognized with a fair amount of certainty when the screening is based on the size and density of stomata. Also, polyploidy levels positively affected morphological traits. Generally, the confirmed tetraploid plants had higher morphological characteristics (plant height, fresh weight and neck diameter) than diploid plants. The leaves of the confirmed tetraploid plants were also longer, wider and darker green in appearance than those of the diploid plants, which may due to chlorophyll content increment. However, they had slowly grown at the initial stage of development compared to diploid plants. This reduction in growth rates in the early stage of tetraploid plants was also observed in induced tetraploid plants of other plant species (Dixit and Chauhdry, 2014, Van de peer et al., 2017). The slow growth rate may be attributed to the reduction in cell division as a result of colchicine treatment or due to the residual effect of colchicine. In addition, major differences were observed in terms of bulb traits and their phytochemicals content between diploid and induced tetraploid plants. Also, tetraploidization plants had increased bulb weight, bulb length, diameter, cloves weight, clove length, clove width, TSS and total phenols. Interestingly, our results show that tetraploidization positively affected the mineral content in bulbs, tetraploid plants had higher N, P and K content compared to diploid plants and this result is in accordance with the result of Jones et al. (1995). The increases in the bulb traits of tetraploid plants might be attributed to the increased chlorophyll and mineral content in these plants compared to their ancestral diploid plants. This result shows that polyploidization could be efficiently used to improve the productivity of horticultural crops by increasing the plant efficiency in mineral uptake and increasing the chlorophyll content.

The medicinal advantages of garlic have been attributed to the abundance of sulfur-containing compounds existed in both fresh cloves and extracted volatile oil of garlic, where these compounds have shown antifungal, antibacterial and antimicrobial properties (Li et al., 2014, Mnayer et al., 2014, Suleria et al., 2015). One of the noteworthy results of the present study is that tetraplodization has increased the amount of the extracted oil from tetraploid cloves (0.1%) compared to diploid cloves (0.05%). A similar trend was evident in Wohlmuth' study, which concluded that the ginger oils extracted from tetraploid plants were more than double the corresponding value for the diploid plants (Wohlmuth et al., 2006).

Garlic oil displays considerable compositional diversity but is typically characterized by a high amount of sulfur compounds. The results show that the main components of the extracted oil were

diallyl disulfide (25.57 and 29.44%), allyl methyl trisulfide (14.10 and 17.57%) and diallyl trisulfide (20.10 and 23.23%) in diploid and tetraploid plants, respectively. Most of these components have been already reported as the main volatile components of garlic essential oil. In accordance with this result, Romeilah et al. (2010) and Satyal et al. (2017) reported that diallyl disulfide, allyl methyl trisulfide and diallyl trisulfide were the major identified compounds (more than 70%) in the extracted garlic oil. In general, the extracted oil from tetraploid garlic was qualitatively similar in composition with the oil extracted from diploid garlic, but they were quantitatively different. The results clearly show that the Allicin-derivative products: diallyl disulfide, allyl methyl trisulfide and diallyl trisulfide concentrations in tetraploid garlic plants were 15.13, 24.61 and 15.57% higher than in those diploid garlic plants. In this context, Xu et al. (2014) found that tetraploid plants had a higher content of caffeic acid derivatives and alkamides than diploid plants of *Echinacea purpurea*. Also, dixit et al. (2015) reported that thymoquinone concentration in tetraploid plants of *Nigella sativa* was 46% higher than in the diploid plants. In this way, recent epidemiological studies have shown that sulfur compounds, including diallyl disulfide and diallyl trisulfide, are responsible for the hypocholesterolemic, hypolipidemic, hypoglycemic, anti-inflammatory, antioxidant, antimicrobial, antifungal, anticancer, cardioprotective and neuroprotective properties of garlic (Chen et al., 2011, Lai et al., 2012, Park et al., 2012, Kuo et al., 2013, Dziri et al., 2014). Therefore, it is reasonable to speculate that the essential oil obtained from tetraploid garlic could be considered more active (higher quantities of diallyl tetrasulfide and diallyl trisulfide) than those obtained from diploid samples. Tetraploid garlic plants with increased sulfur components can be beneficial for the pharmaceutical industry.

The significant increases in the morphological traits, biochemical constituents in bulbs and secondly compounds in the extracted oil recorded in tetraploid plants might be attributed to the fact that polyploidy plants is merited by gene redundancy and heterosis, which gives tetraploid plants superior properties over their diploid parents or due to the increasing ability of tetraploid plants in production of diverse gene products than diploid parents (Comai, 2005, Fuentes et al., 2014, Wu et al., 2019). Thus, the present polyploidy induction protocol can be used to increase the productivity of garlic and enhancing the oil content and secondary metabolites, especially the sulfur-containing compounds content, as the main secondary metabolite of garlic oil.

Conclusion

In conclusion, we were able to successfully induce tetraploid in garlic cv. Seds 40 by soaking the cloves in 0.6 % (w/v) colchicine for 24 hours. Stomata characteristics, morphological traits could be used successfully as robust tools for distinguishing the tetraploid plants in garlic at early stages of growth. Artificial polyploidy induction has positively altered the morphological, biochemical and mineral content of bulbs as well as the oil composition in the confirmed tetraploid plants compared to the corresponding diploid plants. Our results suggest that obtained tetraploid plants will be of important genetic value and can be used for further selection and garlic breeding program.

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Conflict of interest

The authors declare that they have no conflict of interest.

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دراسة مقارنة الصفات الخضرية ومحتوى الزيت الطيار في نباتات الثوم الثنائية والرباعية المجموعة الكرموسومية

التهامي على أحمد يوسف و محمد أحمد الصادق
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يعتبر الثوم واحد من الخضروات والنباتات الطبية الأكثر أهمية من الناحية الاقتصادية. وقد أدى التكاثر الخضري للثوم إلى إنخفاض حاد في التنوع الجيني له. في هذه الدراسة تمت دراسة تأثير التضاعف علي الخصائص المورفولوجية ومحتوى المركبات الثانوية في الزيت الطيار من الثوم. تم نقع فصوص الثوم فى ٠,٢ ، ٠,٤ ، أو ٠,٦ ٪ من محلول الكولشيسين لمده أربع فترات زمنيه (١٢ ، ٢٤ ، ٣٦ ، و ٤٨ ساعة) وتم تحديد مستوي التضاعف عن طريق الصفات المورفولوجية وتم تأكيده عن طريق عد الكروموسومات. وتمت مقارنة الصفات المورفولوجية وتركيب الزيت بين النباتات الثنائية و الرباعية باستخدام جهاز الفصل الكرموتوجرافي. وأظهرت النتائج أن الكفاءة في احداث التضاعف تراوحت من ٨ إلى ٢٦ ٪ وتحققت أعلى كفاءه من خلال المعاملة بـالنقع فى ٠,٦ ٪ كولشيسين لمدة ٢٤ ساعة. وقد تميزت النباتات الرباعية بخصائص مورفولوجية، ومكونات بيوكيميائية ومحتوي معدني أكبر بالمقارنة مع النباتات الثنائية. ولكن أحتوى الزيت المستخلص من النباتات الرباعية والنباتات الثنائية علي تكوين مماثل إلى حد كبير من حيث الزيوت الطيارة. ولكن أدى إحداث التضاعف إلي زيادة كبيره في تركيز كلا من diallyl disulfide و allyl methyl trisulfide و diallyl trisulfide. أدى إحتواء النباتات الرباعية علي تركيزات عالية من الكبريت، إلى تميزها بخصائص نكهة فريدة من نوعها والتي ينبغي أن تكون ذات فائده تجارية. يمكن التوصية بأن إحداث التضاعف هو تقنيه مفيدة يمكن استخدامها لتحسين كلا من إنتاجية الثوم وتركيب الزيوت الطيارة في الزيت المستخلص.

الكلمات الدالة: الكوليشسين ، الفصل الكرموتوجرافي، تضاعف المجموعة الكرموسوميه، الزيت الطيار.