Evaluation the Growth Performance of Egyptian Garlic Landraces and *In vitro* Synseeds of Bulblets Formation

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ABSTRACT

Egyptian garlic cultivars suffered degenerations; lower bulb yield and smaller size of bulbs which represent a crucial problem. A survey of some Egyptian landraces to detect there genetic diversity is considered in this study . In addition, developing of simple method for bulblets regeneration and synthetic seed formation to decrease amount of clove seeds. Fourteen vegetative growth and bulb characteristics for 27 Egyptian garlic landraces were measured during two growing season to determine the phenotypic variations under condition of New Borg El-Arab, Egypt . An *in vitro* study was, also, carried out to determine the direct shoot induction and bulblets formation for six selected landraces and under three media protocol. The results of the field experiment revealed that, Significant differences among all studied local cultivars and ecotypes of Egyptian garlic were observed for vegetative growth, bulb and total yield characteristics . Among the studied varieties of Egyptian landraces, Borg El-Arab landrace was superior in vegetative growth as plant height (cm), leaves no./ plant, leaf length (cm), leaf width (mm), plant fresh weight (g) and foliage fresh weight (g). While, quality characters were associated with purple bulbs landraces like Egaseed and Sids-40-Ayat. Bulb dry matter percentage ranged from 36.6 to 45.68 % and from 35.07 to 44.89 % however, average cloves number per bulb was ranged from 9.33 to 40.00 and from 3.55 to 32.71 in the first and second growing seasons, respectively. The results for the invitro experiment revealed that efficient and simple method of bulblets regeneration and improving multiplication rate were developed for six selected landraces using three different media. Garlic explants (cultured on MS media) were supplemented with 6 % sucrose and 2 mg/l JA (T-3); produced significant higher mean values for numbers of bulblets /clove (12.61) and higher insignificant mean values for numbers of shoots and bulblets /explant, bulblets formation % and numbers of bulblets per clove compared with control treatment (T-1). Control treatment (T-1) produced the highest significant average bulblet weight (259.72 mg). "Salaqus-3" landrace was superior and gave the highest significant mean values for number of shoots per explant and bulblets per clove compared with other landraces. "Clone-21" produced the highest bulblet weight (232.94 mg). Synthetic seeds for six garlic landraces formed by immersing bulblets in gel matrix (3% sodium alginate solution), and exposure to calcium chloride solution (2.5 %) were germinated successfully in unsterilized soil mixture consisted of peatmoss, sand and soil (1:1:1). Conclusion: Egyptian garlic landraces showed great variability and a promising *in vitro* performance for plant regeneration and bulblets formation. This finding could be used as alternative propagation method, cultivars rejuvenation and germplasm conversation management along with capsulation technology.

Key wards: garlic Landraces – bulblets formation - Jasmonic acid (JA)- Synthetic seeds- capsulation.

INTRODUCTION

Due to the importance of garlic as vegetable and medicinal plant for various parts of the worlds, it become necessity to protect garlic plants from extinction and bullbats against various crop diseases in addition to maintain high quality selection procedure for used bullbats for future planting. The present study investigates the effect of environmental conditions on the various vegetation characters. Also, it explore an in vitro technique to improve the efficiency and simplify the method for direct shoot and bulblets regeneration and synthetic seed formation. Such approach enhance cultivars ability to select best bulbelts for future use, and achieve higher yield products.

Garlic, (*Allium sativum* L.) belongs to the Alliaceae family and genus *Allium*, which has approximately 450 species, (Lanzotti, 2006). Garlic is considered the second most important Allium species and vegetable after onion. It is used as flavoring agent for cooking and medicinal plant in many cultures for thousands of years. Vavilov, (1926) reported that Central Asia is the primary center of garlic. Moreover, the Mediterranean region has been postulated as the secondary center of garlic diversity, (vavilov, 1951). Egypt is considered the fourth country of the world's output of garlic. The cultivated area of garlic in Egypt reached 9,674 ha in 2010 yielding 244,626 tons with an average of 25.29 ton/ha, (FAO, 2016). Egyptian garlic cultivars suffered degenerations; lower bulb yield and smaller size of bulbs .The reported reasons for such phenomenon are shortage of attention for selection and breeding during cultivation practices, which lead to populations mix up, and the sale of high-quality garlic which left only the low-quality ones for next year production (Abdel-halim, 2014). Furthermore, the infection by endless viruses and occurrence of secondary growth aggravated the situation. Whereas, garlic is often infected by multiple viruses that belong to different taxa and are collectively designated as the 'garlic viral complex'. These viruses may not kill the plant but can reduce yield up to 70% over time (Conci *et al.,* 2003; Nagakubo *et al.* 1993; 1997).

Wide range of morphological variations has been observed in garlic and was responding to both environment and plant maturity; including flowering ability and bulbing (Senula and Keller, 2000). Moreover, landrace response to environmental variations is considered of high importance to breeder and producer.

Vegetative mode of multiplication -used for garlic propagation- facilitates disease transfer and limits breeding efforts due to the slow screening and selection of spontaneous or induced mutations. Micropropagation through *in-vitro* bulblets formation has been used as a reliable and excellent alternative to pathogen free plantlets and cultivars regeneration. Therefore, synthetic seed technology is a highly promising tool for the management of tissue culture products and seedless plant species. It provides additional protection for tissue culture products (Sharma et al., 2013).

The objectives of this study are evaluation the performance of some Egyptian garlic landraces (*Allium sativum* L.) to detect there genetic diversity, and *invitro* improving efficient and simple method for direct shoot and bulblets regeneration and synthetic seed formation.

MATERIALS AND METHODS

The present study consisted of two experiments; the first one aimed to study the performance of 27 Egyptian landraces of garlic under the field conditions. While, the second experiment aimed to measure the performance of six selected Egyptian garlic landraces under in vitro condition for shoot and bulblets formation and synthetic seeds (synseeds) production.

1-Field experiments

A field experiments were conducted during two different growing seasons winter seasons of 2015 and 2016 at the experimental farm of the City of Scientific Research and Technological Applications (SRTA-City), New Borg El-Arab, Egypt (Approx. latitude is 30°53'36.52"N and longitude 29°32'48.27"E) to evaluate the morphological and bulb characteristics of 27 garlic landraces collected from different regions in Egypt as shown in (Table 1).

Table 1: Bulb characteristics and regions of the 27 landraces of Egyptian garlic.

Landrace	skin Bulb color	Clove Characteristics	Region
Balady	white	arranged from 20 Small size, organized on ‹to 45 per bulb more than five groups	El- Minia governorate
Balady- Al Wadi			El-WadiEl-Gadid governorate
Clone-1			El- Minia governorate
Clone-5			El- Minia governorate
Clone-18			El- Minia governorate
Clone-22-W			El- Minia governorate
Clone-24			El- Minia governorate
Clone-25			El- Minia governorate
El Ewinat			El-WadiEl-Gadid governorate
Farmer's clone			El- Minia governorate
Salaqus-3			Bani Swaife governorate
Aswan			Aswan governorate
Borg El-Arab			Alexandria governorate
El Behera			El Behera governorate
El Fayoum-1			El Fayoum governorate
El Fayoum-2			El Fayoum governorate
Egaseed-1A	purple	Medium size, arranged from organized on 3 or 4 13 to 20, groups	Egaseed company
Egaseed-1B			Egaseed company
Egaseed-2			Egaseed company
Egaseed-3			Egaseed company
Salaqus			Bani Swaife governorate
Clone-21			El- Minia governorate
Clone-22-R			El- Minia governorate
Al Ayat-1			El- Giza governorate
Bany gonamy		Large size, arranged from 8 to 13, organized on 1 or 2 groups	Bani Swaife governorate
Sids-40-R.S.			Bani Swaife governorate
Sids-40-Ayat			El- Giza governorate

Cloves of the 27 garlic landraces were sown in 8th of September during both growing seasons. Seeds were planted at 10 cm distance apart within the row. Each experimental unit consisted of 2 rows, 4 m long and 50 cm width, occupying an area of 4 m^2. The experimental layout used was Randomized Complete Block Design (RCBD) with three replicates. The data were statistically analyzed by ANOVA Percentage data were normalized using costat software and means between landraces were compared by using revised LSD test with 5% level of probability as described by Snedecor and Cochran (1980).

Soil samples of the experimental sites were taken for the determination of soil physical and chemical properties. Soil Physical and chemical properties of the two experimental sites indicated sandy clay loam texture with pH ranged from 8.34 to 8.30 respectively. Organic matter content ranged from 0.98 and 0.93 % (Table 2)

Data of maximum and minimum temperatures for both growth seasons were cited and are shown in Table (3).

Table 2: The physical and chemical properties of the two experimental soil sites in the two seasons of 2015/2016 and 2016/2017.

Soil parameter*	Values	
	2015/2016	2016/2017
pH (1:2.5 w: w)	8.34	8.30
Electrical conductivity (EC)(dS/m,1:1 w:w)	1.74	2.45
Total N (%)	0.03	0.02
Available P (mg/kg)	4.20	5.01
Available K (mg/kg)	320.2	332.28
Total CaCO3 (%)	30.6	31.05
CEC (Cmol+/kg)	11.81	10.11
Organic Matter (%)	0.98	0.93
Organic C (%)	0.56	0.49
Total DOC (%)	0.012	0.010
Sand (%)	64.1	62.7
Silt (%)	15.2	17.4
Clay (%)	20.7	19.9
Texture	Sandy Clay Loam	Sandy Clay Loam
Soluble ions (mg/kg, 1:1w:w)		
Ca2+	162.4	182.12
Mg2+	61.32	66.10
Na+	52.90	60.52
K+	71.76	75.14
CO32-	0.00	0.00
HCO3-	381.2	389.11
SO4-	274	281.10
Micronutrients (mg/kg)		
Fe2+	4.10	4.15
Zn2+	1.43	1.44
Mn2+	3.49	3.71
Cu2+	0.61	0.65
B+	0.30	0.32
Cl-	192	200

Table 3: Average minimum and maximum temperatures (˚C) every two-weeks during the experimental time of 2015/2016 and 2016/2017, respectively.

2015/2016																
Date	September		October		November		December		January		February		March		April	
	1-15	16-30	1-15	16-30	1-15	16-30	1-15	16-30	1-15	16-30	1-15	16-30	1-15	16-30	1-15	
Temp. ^{0}C	23-32	22-33	20-29	18-28	17-24	15-23	13-20	12-19	10-18	8-16	10-19	12-23	12-23	12-22	14-26	
2016-2017																
Date	September		October		November		December		January		February		March		April	
	1-15	16-30	1-15	16-30	1-15	16-30	1-15	16-30	1-15	16-30	1-15	16-30	1-15	16-30	1-15	
Temp. ^{0}C	23-29	22-28	22-27	20-25	18-22	17-22	14-18	10-15	7-15	10-17	9-18	10-19	12-21	12-21	12-22	

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The data were recorded at maximum growth stage and before harvesting (192 days from planting). Ten randomly selected plants from each experimental unit were chosen for determination of the following characters;

1. 1. Vegetative growth characters:

Average plant height (cm); number of leaves per plant, plant fresh weight (g) and foliage dry matter percentage were measured. Dry matter content was calculated after drying the plant vegetative growth without bulb at 70^{o}C.

1. 2. Bulb characteristics:

Average bulb diameter, bulb dry matter percentage, number of cloves per bulb and average clove weight (g) were assessed. Dry matter content = (dry weight / fresh weight) x 100.

2. Laboratory *invitro* experiments

Direct shoot induction, bulblets formation and synseeds productions, were carried out for six selected landraces 'Salaqus-3', 'Aswan', 'Borg El-Arab', 'El Behera', 'Sids-40-Ayat' and 'Clone-21' which are taken into consideration after initial screening of the 27 garlic cultivars and ecotypes for *in vitro* direct shoot induction .The experiments reported herein were carried out during the period from 2016 to 2017 in the Tissue Culture Laboratory of Vegetable Crops Department Faculty of Agriculture, Alexandria University, Alexandria, Egypt and the experiment was repeated twice at least, with thirty replications.

2-1. Direct shoot induction and bulblets formation

2.1.1 Preparation of garlic bulbs

Garlic bulbs were stored at 4^{o}C, for two months. Bare cloves were rinsed in running tap water for one hour. Healthy cloves were surface-sterilized by soaking in 70% ethanol for 30 sec. under laminar air flow cabinet, the bare cloves were then rinsed for 20 min in commercial bleach (Clorex ®) at 5.25% sodium hypochlorite (NaOCl) with two drops of Tween 80 and washed three times using autoclaved distilled water.

2.1.2 Direct shoot organogenesis and bulblets formation

Thirty basal part of the clove of the six selected landraces (Table, 1) were divided into four sections (1x1 mm approx.) and cultured on Murashige and Skoog (1962). The medium was solidified with 0.7 % (w/v) agar–agar technical, and supplemented with different concentrations of sucrose and jasmonic acid as follow; MS medium supplemented with 30 g/l sucrose (control T-1), MS medium supplemented with 30 g/l sucrose and 2 mg/l jasmonic acid (T-2) and MS medium supplemented with 60 g/l sucrose and 2 mg/l jasmonic acid (T-3). Finally, the prepared medium was autoclaved for 20 min under 1.2 kg /cm^2 and 121°C, then incubated under 16-h photoperiod (under fluorescent light) and 28 ± 1°C. Each experiment was repeated twice at least. The following parameters for each explant were evaluated after 9 weeks of culture as survival explants(%) after 3 weeks on the first culture medium, number of shoots per explant, number of bulblets per explant, average bulblet weight (mg) and total no. of bulblets per clove . Total bulblets no./clove = (bulblets no. per explant × 4 (no of explant /clove basal part) × (1/explant survival rate)).

2.2. Synthetic seeds (synseed) production

Synthetic seeds were obtained by immersing bulblets obtained from invitro experiment in gel matrix (3% Sodium alginate solution at 3% supplemented with Activated charcoal 0.2% and antibiotic mixture 0.25 ml/L), and exposure to calcium chloride solution (2.5 %), and stirred continuously for 30 min for hardening (Bekheet, 2006). The bulblets were immersed into calcium chloride solution and stirred continuously for twenty minutes. The encapsulated bulblets were washed twice with sterilized distilled water and transferred to autoclaved petri dishes and stored at 4 ºC for two months to overcome bulblets dormancy. For the plantlets recovery, the stored encapsulated bulblets were immersed in distilled water for 2-3 min to thaw sodium alginate. Washed bulblets were sowing in pots (5 x 5 cm) in unsterilized soil mixture consisted of peat-moss, sand and soil (1:1:1) and kept under greenhouse conditions.

To test the vitality of garlic synthetic seeds and clear up its reliability as a garlic invitro propagation method for synseeds of encapsulated bulblets data on germination percentage and number of days to germination were recorded.

Statistical analysis twenty replicates were chosen to represent each treatment in Complete Randomized Design (CRD) as factorial experiment with two factors (six different landrace and three different media compositions). The analysis done using co-stat software 2004.

RESULTS AND DISCUSSION

1-Field experiments

1. 1. Vegetative growth characters

The results of the field experiments on vegetative growth characters reflected high significant differences among the evaluated landraces for all studied characters during both seasons (Table, 4). In General, "Borg El-Arab" landrace plants recorded significantly the highest mean values for plant height (88.0 and 63.0cm), and highest value of number of leaves / plant, especially in the second season and foliage fresh weight (46.5 and 43.6g) during both seasons.

As for Foliage dry matter content, the values ranged from 16.7 to 26.5 % and from 16.8 to 24.7% in the first and second growing seasons, respectively.

Table 4: Vegetative growth characters of 27 landraces grown under Borg El-Arab conditions, Egypt during the winter seasons of 2015/2016.

Character / Landrace	Plant height (cm)		Plant leaves number		Foliage fresh weight (g)		Foliage dry weight (%)	
	2015	2016	2015	2016	2015	2016	2015	2016
Balady	71.00d-h	52.50bc	8.00a-d	4.67e-g	24.23e-g	13.04hi	17.93g-i[z]	17.99i-l
Balady-Al Wadi	80.22a-c	54.00bc	8.55a-c	5.27c-e	23.93e-g	16.50ef	17.30hi	18.13i-l
Clone-1	83.17ab	34.22e	8.44a-c	4.45f-h	31.07c	24.11c	17.36g-i	17.84j-l
Clone-5	67.78f-h	54.67ab	7.89b-d	5.69bc	19.73g-k	10.93ij	18.18g-i	17.98i-l
Clone-18	78.22b-e	54.50ab	8.56a-c	4.50f-h	35.62b	17.86e	17.96g-i	17.36kl
Clone-22-W	70.44d-h	55.00ab	8.34a-c	5.25c-e	19.12h-l	18.84de	17.86g-i	16.82l
Clone-24	85.44ab	53.39bc	9.22a	5.57b-d	38.43b	16.21e-g	18.43f-i	19.51g-i
Clone-25	70.00e-h	56.00ab	6.44ef	5.50b-d	20.04g-j	13.39g-i	17.86g-i	17.35kl
El Ewinat	70.56d-h	47.00bc	8.00a-d	5.69bc	15.15k-m	11.99hi	18.05g-i	18.00i-l
Farmer's clone	68.44f-h	45.33cd	6.55ef	4.53f-h	15.77j-m	6.13k	19.22e-h	19.87f-h
Salaqus-3	69.22e-h	50.00bc	8.22a-c	5.00d-f	23.95e-g	16.50ef	16.65i	17.01kl
Aswan	74.22c-f	37.33de	6.89de	4.25g-i	18.13h-m	16.29e-g	17.87g-i	18.13i-l
Borg El-Arab	88.00a	63.00a	8.67ab	6.33a	46.50a	43.62a	19.43e-h	20.66e-g
El Behera	65.00f-i	56.17ab	7.22c-e	5.50b-d	20.08g-j	20.85d	19.58e-g	19.12h-j
El Fayoum-1	72.33c-g	50.33bc	7.22c-e	6.00ab	21.52f-h	14.82f-h	18.98e-h	19.33g-j
El Fayoum-2	79.22b-d	50.00bc	7.89b-d	5.03c-f	26.68de	11.86hi	20.72de	18.51h-k
Egaseed-1A	55.67jk	16.83h	8.22a-c	5.00d-f	25.98d-f	11.71hi	21.98cd	21.71de
Egaseed-1B	67.22f-h	19.28h	7.67b-e	3.99h-j	20.72g-i	8.52jk	23.58bc	22.50b-d
Egaseed-2	69.56e-h	37.29de	5.67f	4.00h-j	30.10cd	32.87b	21.96cd	23.54ab
Egaseed-3	51.78k	29.28e-g	7.22c-e	3.87h-j	14.03m	7.19k	22.51cd	22.62b-d
Sids-40-R.S.	62.89g-j	22.00gh	7.22c-e	4.00h-j	24.03e-g	14.66f-h	20.40d-f	21.28d-f
Sids-40-Ayat	64.89f-i	31.44ef	8.00a-d	3.90h-j	16.90h-m	10.64ij	22.38cd	21.95c-e
Clone-21	55.22jk	36.67e	7.56b-e	4.67e-g	19.17h-l	13.11hi	23.72bc	24.71a
Clone-22-R	61.67h-j	29.44e-g	7.67b-e	3.60ij	16.63i-m	7.38k	25.80a	24.40a
Al Ayat-1	48.67k	20.33h	8.55a-c	3.77ij	15.72j-m	8.53jk	25.18ab	23.69ab
Bany gonamy	65.22f-i	29.72e-g	8.22a-c	3.65ij	24.27e-g	6.69k	26.48a	24.27a
Salaqus	57.22i-k	24.50f-h	7.56b-e	3.50j	14.94lm	8.50jk	26.42a	23.29a-c

*Values followed by the same alphabetical letter(s) in common, within a particular group of means in each character, do not significantly differ, using Duncan's multiple range test at 0.05 level of probability.

Bany gonamy, 'Salaqus' and 'Clone-22-R' recorded the highest values for foliage dry matter content (26.5, 26.4 and 25.8 %, respectively) followed by 'Al Ayat-1' (25.2 %). However, the lowest mean values for foliage dry matter content were recorded for 'Salaqus-3' and 'Clone-22-W' (16.7 and 16.8%) in both seasons. Such results emphasized the fact that vegetative traits depended on studied genotypes. The differences among garlic local cultivars and ecotypes may be due to either incidence of genetics differences among them and/or to interaction among genotypes, soil properties and weather conditions. Therefore, an important evaluation of genotypes under different environments, which could highlight on the superior genotypes under various growth condition, in order to reach the highest productivity (Metwally and El-Denary, 2003; Obiadalla, 2014) , Al-Otayk *et al.,* (2008), Moustafa *et al.,* (2009), Dawood, (2011), Ahmed (2012), Abou El-Magd *et al.* (2012), Anwar (2012) and Youssef and Tony (2014), whose mentioned that there were a wide and great variation on vegetative growth of most garlic cultivars.

1.2 Bulb characteristics:

Average values measured in Table (5), and demonstrated in Figure (1) show the differences among studied garlic landraces for bulb characteristics. For bulb diameter "Egaseed-1B" recorded the highest significant average value for bulb diameter (46.00 mm). Insignificant differences were observed between 'Egaseed-1B' and each of 'Sids-40- R.S.', 'Sids-40-Ayat', 'Bany gonamy', 'Egaseed-1A', 'Clone-22-R' and 'Salaqus', (44.44, 43.33, 42.89, 41.22, 40.67 and 39.67 mm, respectively), in the first growing season. While in the second season, landrace 'Borg El-Arab' gave the highest average bulb diameter (43.18 mm) followed insignificantly by 'Al Ayat-1', 'Egaseed-2' and 'Clone-18' which gave 43.00, 42.08 and 40.93 mm, respectively.

'El Ewinat', 'Clone-5', and 'Clone-22-W' had the lowest bulb diameter (29.44, 29.44, and 27.33 mm, respectively) in the first growing season. 'Farmer's clone' recorded the lowest bulb diameter value in both growing seasons (28.89 and 27.47 mm, respectively).

Bulb dry matter percentage ranged from 36.6 to 45.68 % and from 35.07 to 44.89 % during the first and second growing season, respectively. The highest value was recorded for 'Egaseed-2' (45.68 %) in the first growing season. During the second season, 'Egaseed-1A', 'Egaseed-2' and 'Clone-21' landraces gave high bulb dry matter (44.89, 44.87 and 44.54 %, respectively). On the other hand, 'Clone-1' showed significantly lower dry matter percentage (35.80 and 35.07 %) in the first and second seasons, respectively. Regarding the average cloves number per bulb, the value ranged from 9.33 to 40.00 and from 3.55 to 32.71 in the first and second growing seasons, respectively. Landrace El Fayoum-1 had the highest cloves number per bulb (40.00 cloves/ bulb) and as shown in Figure (1). In the second season 'Borg El-Arab' and 'El Fayoum-1' scored the highest cloves number no. / Bulb (32.71 and 32.64 cloves/ bulb, respectively). The results indicated that, cloves number/bulb varied significantly among different landraces, as well as been affected by growing conditions under Borg Al Arab, Egypt conditions. The stability of 'Egaseed-2', 'Egaseed – B1' and 'Borg El-Arab' landraces for bulb characteristics during the consecutive years of the study may due to their adaptation for cultivation under Borg El-Arab conditions.

As regards to average clove weight, Bany gonamy landrace gave the heaviest average clove weight (5.19 g) compared with other studied landraces, in the first season. While, in the second season, 'Clone-22-R', 'Sids-40-Ayat' and 'Sids-40-R.S', Superseded significantly, all of the other studied landraces for the average clove weight, 5.40, 5.33 and 5.05 g, respectively.

Phenotypic diversity evaluation showed wide and great variations on vegetative growth that help in improving garlic breeding and production of elite varieties. Evaluation of landraces under different environment conditions could highlight the superior landrace/s to achieve the highest productivity. This study agreed with the finding of Saraf *et al.,* 2000, Baghalian et al., (2006, Metwally and El-Denary, 2003, Al-Otayk *et al*., 2008, Moustafa *et al*., 2009; Dawood, 2011; Ahmed 2012; Abou El-Magd *et al.,* 2012; Anwar 2012; Abu El-Oyun, 2010; Helmy *et al*., 2011, Kumar *et al*.,(2017) and Youssef and Tony, 2014.

Table 5: Bulb characteristics of 27 landraces grown under Borg El-Arab conditions, Egypt during the winter seasons of 2015/2016.

Character / Landrace	Average bulb diameter (mm)		Bulb dry matter content (%)[z]		Average cloves number per bulb		Average clove weight (g)	
	2015	2016	2015	2016	2015	2016	2015	2016
Balady	32.22 f-i	28.49 i-k	38.55e-h	37.90f-j	24.33e-g	23.09c-f	0.93f	0.54f
Balady- Al Wadi	31.33 g-i	31.90 e-j	38.03e-h	37.33g-j	34.00a-d	25.16b-e	0.73f	0.67f
Clone-1	36.78 c-h	32.80 d-h	35.80h	35.07j	35.33a-c	31.06ab	0.71f	0.62f
Clone-5	29.44 i	29.33 g-k	37.78f-h	36.86g-j	22.67e-h	19.22ef	0.89f	0.59f
Clone-18	32.78 e-i	40.93 ab	38.17e-h	37.34g-j	31.67a-e	25.14b-e	0.89f	0.58f
Clone-22-W	27.33 i	28.25 jk	38.12e-h	37.32g-j	25.00d-g	29.68a-c	0.55f	0.45f
Clone-24	33.00 e-i	28.73 h-k	36.60gh	35.49ij	37.33ab	29.08a-d	0.81f	0.43f
Clone-25	32.00 f-i	33.87 c-f	41.21b-f	39.43c-h	26.67c-g	27.32a-d	0.84f	0.48f
El Ewinat	29.44 i	28.21 jk	37.39f-h	36.47h-j	22.33e-h	20.33ef	0.61f	0.53f
Farmer's clone	28.89 i	27.47 k	36.92f-h	35.89h-j	27.67c-g	25.55b-e	0.72f	0.30f
Salaqus-3	30.44 hi	34.85 c-e	39.86c-h	39.20d-i	26.00d-g	20.33ef	0.77f	0.69f
Aswan	34.11 d-i	34.31 c-e	40.63c-g	39.26d-i	37.67a	29.55a-c	0.65f	0.74f
Borg El-Arab	38.67 b-f	43.18 a	37.28f-h	37.55f-j	37.00ab	32.71a	1.15f	1.18f
El Behera	30.33 hi	35.36 c-e	42.46a-e	40.59b-g	31.67a-e	17.25f	0.66f	1.24f
El Fayoum-1	30.78 g-i	28.27 jk	36.85f-h	35.74h-j	40.00a	32.64a	0.40f	0.34f
El Fayoum-2	34.11 d-i	30.13 f-k	38.70e-h	38.35e-j	28.33b-f	22.83d-f	0.83f	0.45f
Egaseed-1A	41.22 a-c	29.82 f-k	43.72a-d	44.89a	14.67h-j	5.66g	2.94cd	3.38de
Egaseed-1B	46.00 a	37.45 bc	43.67a-d	40.75b-g	13.33ij	5.06g	3.99b	4.32bc
Egaseed-2	33.89 d-i	42.08 a	45.68a	44.87a	9.33j	9.11g	2.34dc	2.62e
Egaseed-3	37.78 b-g	36.62 cd	45.22ab	41.94a-e	11.33ij	3.90g	2.86cd	4.25bcd
Sids-40-R.S.	44.44 Ab	33.13 d-g	40.15c-h	43.02a-d	12.00ij	6.18g	4.26b	5.05ab
Sids-40-Ayat	43.33 a-c	33.20 d-g	39.59d-h	40.59b-g	12.33ij	4.20g	3.24c	5.33a
Clone-21	37.22 c-h	32.34 e-j	44.12a-c	44.54a	18.33g-j	7.12g	1.99e	3.34de
Clone-22-R	40.67 a-d	36.70 cd	44.15a-c	43.92ab	19.67f-i	3.55g	2.20de	5.40a
Al Ayat-1	38.89 b-f	43.00 a	40.07c-h	43.15a-c	12.00ij	4.18g	2.81cd	4.10cd
Bany gonamy	42.89 a-c	28.82 h-k	43.64a-d	41.31a-f	10.50ij	5.18g	5.19a	2.94e
Salaqus	39.67 a-e	32.62 d-i	39.67d-h	42.72a-d	11.67ij	4.91g	2.92cd	4.34bc

[A] Values followed by the same alphabetical letter(s) in common, within a particular group of means in each character, do not significantly differ, using Duncan's multiple range test at 0.05 level of probability.

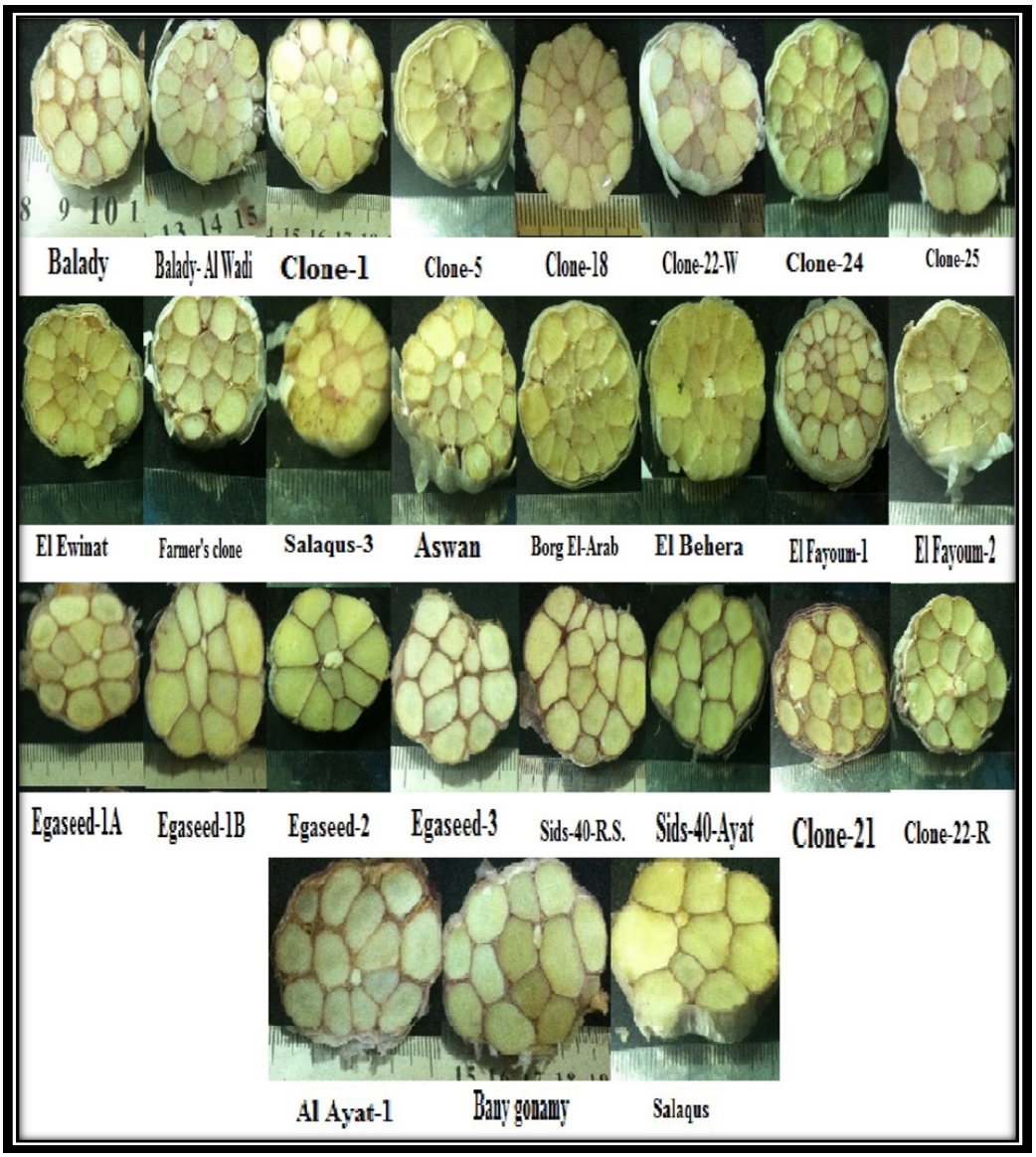

Figure 1: Cross section of the 27 garlic local cultivars and ecotypes bulbs at harvest in Borg El-Arab during the growing season of 2015/2016.

They reported the existence of wide and great variations on vegetative growth of most Egyptian garlic cultivars. Moreover, Obiadalla, (2014) observed significant variations of vegetative characters among the twelve studied Balady landraces which were collected from different Egyptian governorate.

2. In vitro propagation experiment

2. 1. Direct shoot induction and bulblets formation

Selected garlic landraces varied expressively for their studied characters under in vitro conditions (Table 5). Regarding the regeneration percentage no such significance was observed between different tested treatments. Whereas, Aswan landrace differed significantly, more or less from the others. It gave the least regeneration percentage compared to the other landraces. 'Salaqus-3' scored the greatest mean number of shoots (6.67) and bulblets ranged from 3.3 / explant for Sids-40- Ayat to 3.1 / explant for Clone-21 compared with other landraces. On the other hand, the first order interaction between T3 x Aswan gave the highest average value (8.7 shots/ explant, meanwhile the lowest average value 3.0 was recorded due to the interaction between T2 x Sids-40- Ayat.

Sucrose and jasmonic acid and played major roles of media efficiency regarding germination, number of shoots and number of bulblets per explants. Application of sucrose and JA resulted

significant differences for the number of bulblets per explant among studied treatments. Explants cultured on MS medium supplemented with 6% sucrose and 2mg / L jasmonic (T3) gave the highest significant number of bulblets (3.8) / explant compared with that cultured on jasmonic free media (T-1) or supplemented with 3% sucrose and 2 mg/L JA (T-2). Concerning the interaction among landraces and used media, the results indicated highly significant differences for number of shoot and bulblets formation based on the structure of the used media, (Table, 5 and Figure, 2). Explants of 'Aswan' landrace cultured on Ms medium supplemented with 6% sucrose and 2 mg/L JA ; gave highest numbers of shoot and bulblets / explant (8.7 and 7.0, respectively) compared with other landraces and treatments used except for 'Borg El-Arab' and 'El Behera' landraces, (Figure, 2).

The results of Table (6) show the influence of MS media supplemented with different sucrose levels and jasmonic acid on bulblets formation percentage, total number of bulblets per clove and average bulblet weight of the six garlic landraces cultured for 9 weeks. The landraces of'Salaqus-3' and 'Borg El-Arab' scored the highest significantly number of bulblets/clove (12.00 and 11.80 bulblets/ cloves, respectively). While, 'Sids-40-Ayat' and 'Clone-21' recorded the lowest values for number of bulblets/ cloves (5.16 and 5.38 bulblets/ cloves, respectively). However, the treatment T-3 (6% sucrose + 2.00 mg/l jasmonic acid) achieved the greatest significant number of bulblets/clove (12.61 bulblets/ explant). While, T-1 treatments (control; 3% sucrose) exhibited the lowest number of bulblets per clove (4.82 bulblets/clove). Moreover, total number of bulblets/clove which formed on T-2 (3% sucrose + 2.00 mg/l jasmonic acid) gave moderate (8.73 bulblets/clove) and was not clearly differed from T-3 and T-1 media. Finally different media had insignificant effect on the number of bulblets/explants.

Table 6: the Influence of MS media supplemented with different sucrose levels and jasmonic acid on regeneration percentage, number of shoots and bulblets per explant of six garlic landraces cultured invitro for 3 weeks.

Treatment / Landrace	T-1 (cont.)	T-2	T-3	Mean	T-1 (cont.)	T-2	T-3	Mean	T-1 (cont.)	T-2	T-3	Mean
	Regeneration %				No of shoots / explant				No of bulblets / explant			
Salaqus-3	100.0 a	83.3a	91.7a	91.7A	6.0a-d	8.3ab	5.7b-f	6.7A	1.7b-d	4.0b-d	4.3a-c	3.3A
Aswan	68.3a	63.3a	68.3a	66.7B	3.7e-g	6.0a-d	8.7a	6.1A	1.0 d	2.7b-d	7.0a	3.6A
Borg El-Arab	88.3a	88.3a	88.3a	88.33A	5.7b-f	7.0a-d	7.0a-d	6.6A	1.3 cd	3.7b-d	4.7ab	3.2A
El Behera	77.8a	72.2a	77.8a	75.9 AB	5.7b-f	4.7c-g	7.3a-c	5.9A	1.0d	3.0b-d	4.7ab	2.9AB
Sids-40-Ayat	98.3a	91.7a	96.7a	95.6A	4.3d-g	3.0fg	2.7g	3.3B	1.0d	2.0cd	1.0d	1.3B
Clone-21	93.3a	86.7a	95.0a	91.7 A	3.7e-g	3.7e-g	2.0g	3.1B	2.0b-d	1.0d	1.3cd	1.4B
Mean	80.9A	87.9 A	86.3 A		4.84A	5.4A	5.6A		1.3C	2.7B	3.8A	

*Values followed by the same alphabetical letter(s) in common, within a particular group of means in each character, do not significantly differ, using Duncan's multiple range test at 0.05 level of probability
T1, MS + 30 g sucrose ; T2, MS+ 30g sucrose + 2mg/l JA ; T3, MS+ 60g sucrose + 2mg/l JA

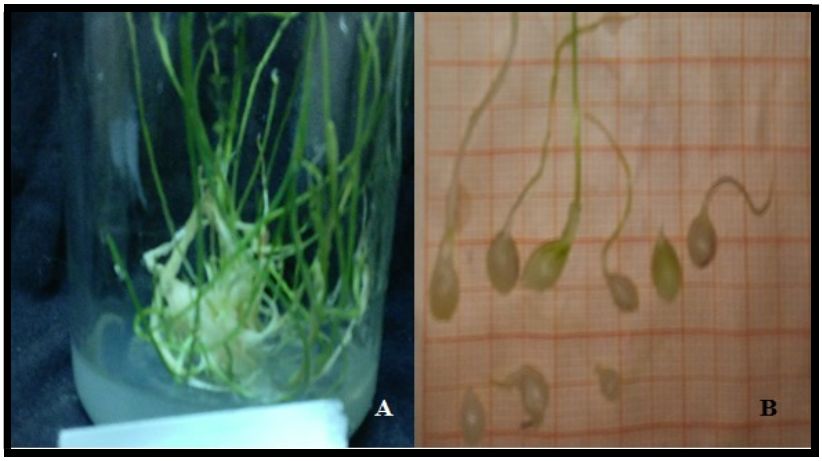

Figure 2: Cluster of El Behera landrace shoots after 6 weeks invitro (A) and bulblets in hanging mode after 9weeks (B) of culture on MS medium with 6% sucrose and 2mg/l jasmonic acid.

Significant differences among the six garlic landraces for average bulblet weight (mg) were obtained (Table, 7). High significant differences were observed among studied landraces depending on the application of different media. Regarding the average bulblet weight, Clone-21 gives the highest average bulblet weight (232.94 mg), while the lowest values scored by 'Aswan' and 'Salaqus-3; landraces (103.47 and 75.24 mg. Highest significant bulblet weight (259.72 mg) was verified by control treatment (T-1). While insignificant differences were obtained between T-2 and T-3 which scored the lowest significant mean bulblet weight values (82.70 and 94.63 mg). Jasmonic acid (JA), jasmonic acid methyl ester and some of their derivatives possess various physiological activities when applied to plants (Koda, 1997). Jasmonates are signal molecules in plant stress responses and are, also, important promoters of plant growth and development. Jasmonic acid stimulates cell division and enlargement also interact with other plant growth substances, e.g., cytokinins (Ravnikar *et al*., 992 and Takahashi et al., 1995. When JA is added to plant growth medium at concentrations up to 10 µM, it expressively stimulates the elongation of axillary buds (Ravnikar *et al*., 1992). In general, Jasmonates may be involved in the process of storage organ formation. Nojiri *et al*., 1992 showed that endogenous concentrations of JA were different in the bulbing and non bulbing onion plant parts. The induction of bulb formation by JA was also demonstrated in garlic shoots in vitro (Ravnikar *et al*., 1993. Jasmonic acid in combination with 2ip stimulates the induction of shoot formation on basal plates of garlic cloves, which further resulted in a high percentage of bulb formation (Ravnikar *et al.,* 1993.

Regarding Sucrose, it is considered another important factor which induces bulb formation in vitro. It was demonstrated that 12% sucrose promotes bulb induction in garlic (Nagakubo *et al*., 1993; Yasseen *et al*., 1994.

2.2 Synthetic seeds germination (plantlets recovery)

Results in Table (7), illustrate synthetic seeds (bulblets - immersed in gel matrix "3% Na-alginate" and hardened with $CaCl_2.2H_2O$ solution "2.5 %" and stirred continuously for 30 min on a magnetic stirrer, then have been recovered to plantlets in a period ranged from 3 to 19 days from sowing, depending on the genotypic variations and treatments used. Non-germinated synthetic seeds may be due to that stored bulblets did not run out the dormancy. This finding is taken as indicator for synthetic seeds validation when directly uses in open field fig (3). It was clear from Table (7) and fig. (4) that using T-3 treatment (6% sucrose and 2mg/L jasmonic acid) lead to complete germination for all landraces and decrease the sprouting days.
Concerning synseeds (synthetic seeds) , it was reported that the in vitro raised bulblets proved to be efficient micro-propagating unit which did not require acclimatization and can be directly sown into soil (Nagakubo *et al*., 1997). Although, bulbs derived from bulblets planting were smaller than commercial bulbs, they provided cultivar rejuvenation. In addition, that bulbs derived from bulblets will reach an appropriate size after 2 to 3 years of cultivation (Nagakubo *et al*., 1997).

Regarding in vitro bulblets products, they have been regenerated (95%) without acclimatization in autoclaved commercial potting soil under greenhouse conditions (Yasseen *et al.,* 1994). Also, Haque *et al*. (1998, 2003) and Dixit *et al.* (2013) revealed that about 80-90% bulblets were successfully established and survived under field conditions.

Capsulation of in vitro derived products "such as garlic bulblets" provided protection for them against microorganism attack and unwanted germination (Personal observation). In addition, capsulation apply on meristematic organ which formed through tissue culture techniques made them easy handling, transport, delivery and had a long-term storage potentiality (Ghosh and Sen, 1994.

Table 7: The influence of MS media supplemented with different sucrose levels and jasmonic acid on bulblets formation percentage, total number of bulblets per clove and average bulblet weight of six garlic landraces cultured for 9 weeks invitro.

Treatment / Landrace	T-1 (cont.)	T-2	T-3	Mean	T-1 (cont.)	T-2	T-3	Mean	T-1 (cont)	T-2	T-3	Mean
	Bulblets formation %				Total bulblets number per clove$				Average bulblet weight (mg)			
Salaqus-3	33.3 a	100.0 a	100.0 a	77.8 A	6.7 a	13.3 a	16.0 a	12.0 A	41.7 a	80.2 a	103.9a	75.2 B
Aswan	100.0 a	100.0 a	100.0 a	100.0A	2.7 a	6.9 a	20.1 a	.9AB	142.0 a	34.3 a	53.8 a	103.5B
Borg El-Arab	66.7 a	100.0 a	100.0 a	88.9A	4.7 a	12.3 a	18.2 a	11.8 A	143.0 a	56.8 a	179.1a	137.4AB
El Behera	100.0 a	100.0 a	100.0 a	100.0A	3.1 a	8.7 a	12.4 a	8.1AB	290.3 a	85.0 a	39.8 a	138.4AB
Sids-40-Ayat	100.0 a	100.0 a	66.7 a	88.9A	3.9 a	7.7 a	3.9 a	5.2B	330.0 a	175.9 a	54.0 a	186.6AB
Clone-21	100.0 a	66.7 a	100.0 a	88.9A	7.6 a	3.5 a	5.1 a	5.4B	497.7 a	64.0 a	137.2a	232.9A
Mean	83.3 A	94.5 A	94.5 A		4.8 B	7.0 AB	12.6 A		259.7 A	82.7 B	94.6 B	

Values followed by the same alphabetical letter(s) in common, within a particular group of means in each character, do not significantly differ, using Duncan's multiple range test at 0.05 level of probability

T1, MS + 30 g sucrose ; T2, MS+ 30g sucrose + 2mg/l JA ; T3, MS+ 60g sucrose + 2mg/l JA

Table 8: Plants of garlic synthetic seeds derived *from in vitro* bulblets planted in peat-moss, sand and soil (1:1:1) mixture and irrigated with tap water, under greenhouse condition.

Treatment / Genotype	T-1 (control) 3 % Sucrose		T-2 3 % Sucrose +2 mg/l JA		T-3 6 % Sucrose +2 mg/l JA	
	G	SD	G	SD	G	SD
Salaqus-3	+	6	+	18	+	9
Aswan	-	-	+	16	+	10
Borg El-Arab	+	9	+	18	+	14
El Behera	+	5	-	-	+	6
Sids-40 Ayat	+	19	-	-	+	3
Clone-21	-	-	-	-	+	6

G= Germination, SD= Sprouting days, the singe "+" synseeds were germinate, "-" synseeds were not germinate

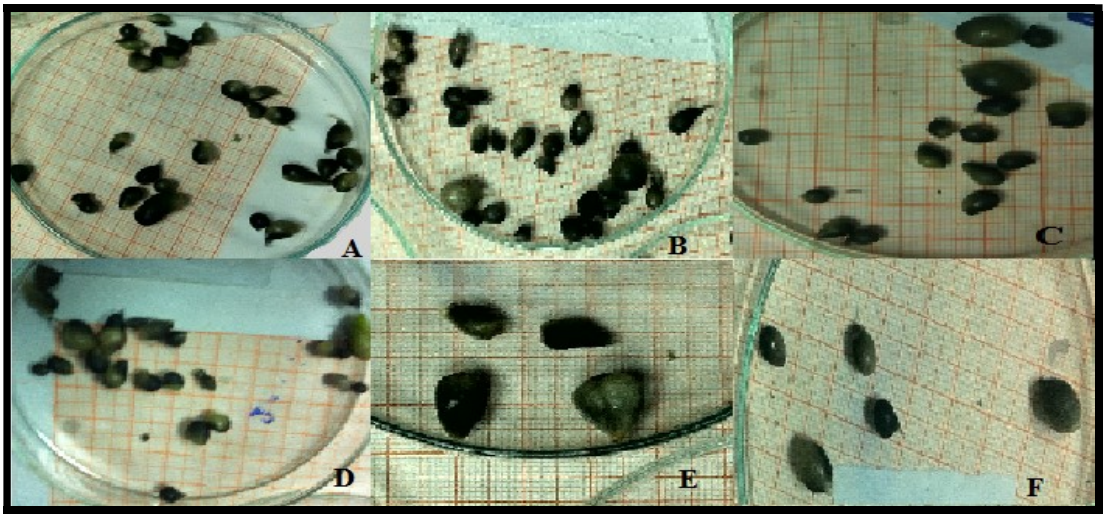

Fig. 3: *In vitro* bulblets synthetic seeds from T-3 of six garlic landraces; A) Salaqus-3, B) Aswan, C) Borg El-Arab, D) El Behera, E) Sids-40 Ayat and F) Clone-21

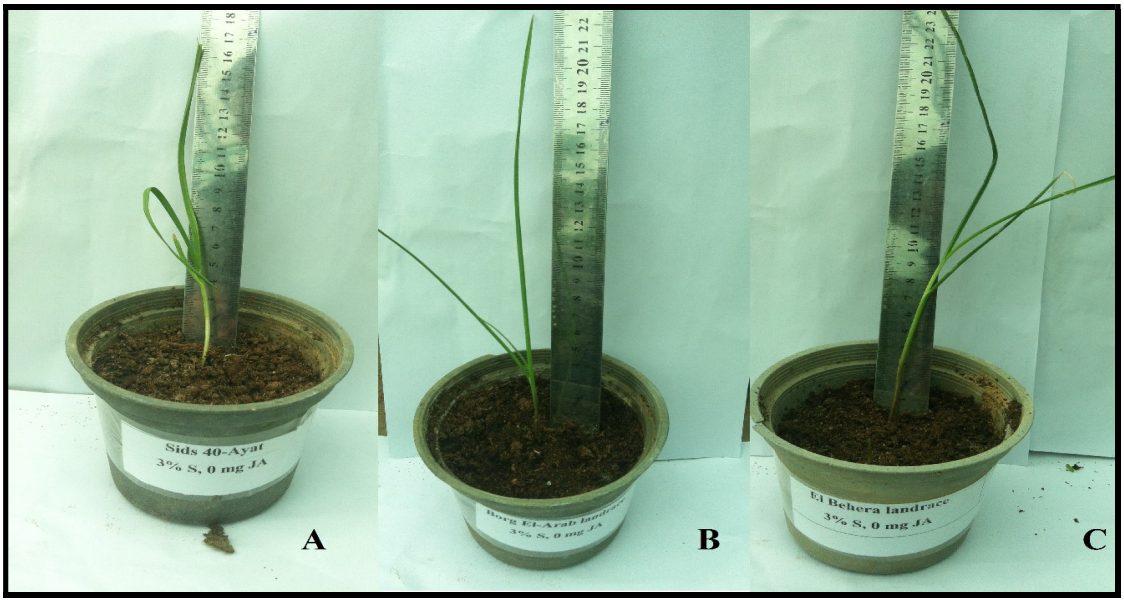

Fig. 4: Synthetic seeds-derived plants from T-1 of Sids- 40 Ayat, Borg El-Araband El Behera landraces grown in peat-moss, sand and soil (1:1:1) mixture and watered with tap water, under greenhouse condition after 3 weeks of sowing.

Bekheet 2006, Mahajan *et al.*, 2013, Paz-Robeldo and Tovar-soto 2012, Gonapathi *et al.*, 1992 and Kim and Park 2002 who reported the importance of synseeds as an alternative method of garlic micropropagation.

CONCLUSION

It could be possible to conclude that the local Egyptian garlic landraces showed a great genetic diversity in all characters. This study clarified the promising *invitro* approach for plant regeneration and bulblets formation, which could be used as alternative propagation method, cultivars rejuvenation and germplasm conversation management along with capsulation technology.

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الملخص العربي

تقييم سلوك نمو بعض سلالات الثوم المصري وانتاج البذور الصناعية من الثوم معمليا

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أجريت في هذه الدراسة ثلاث تجارب؛ تجربة حقلية وتجربتين معمليتين خلال الفترة ٢٠١٥ الي ٢٠١٧، لدراسة التنوع الوراثي بين ٢٧ صنف وسلالة من الثوم، تم جمّعها من مناطق مختلفة من جمهورية مصر العربية، حيث تهدف الدراسة الي التقييم الحقلي والمعملي لسلوك أصناف وسلالات الثوم على المستوى الظاهري، وتكوين البذور الصناعية بإستخدام البلابل المتكونه معملياً.

التجربه الاولى (التقيم الحقلي)

تم إجراء تجربتين حقليتين خلال الموسم الشتوى لعامي٢٠١٥ و٢٠١٦ بالمزرعة البحثية لمدينة الأبحاث العلمية والتطبيقات التكنولوجية– برج العرب– الاسكندرية– مصر، وذلك بهدف تقييم صفات النمو الخضري والمحصول الكلي ومواصفات جودة الابصال للتراكيب الوراثية من الثوم تحت الدراسة وكذلك تحديد درجة التشابه الوراثى بينها على مستوى الصفات المورفولوجية وذلك بغرض التحسين الوراثي لهذه السلالات المحلية من خلال برنامج تربية لاحق.

وقد تم زراعة فصوص الثوم للأصناف والسلالات المستخدمة بتصميم القطاعات العشوائيه الكاملة (RCBD)، بثلاث مكررات، مساحة الوحده التجريبية ٤ م^{٢}عبارة عن خطيين بطول ٤ م والمسافه بين الخطوط ٠,٥ م، وكانت مسافة الزراعة ١٠ سم على جانبي الخط، وتمت الزراعة في الثامن من سبتمبر خلال كلا موسمي الزراعة.

ويمكن تلخيص أهم النتائج فى النقاط التالية:

١. أظهر جدول تحليل التباين وجود إختلافات معنوية بين الأصناف والسلالات المستخدمة في الدراسة في كل الصفات الخضرية والمحصولية المدروسة.
٢. تفوقت سلالة Borg El-Arab في صفات النمو الخضري مثل؛ إرتفاع النبات، متوسط عدد الأوراق/نبات، طول وعرض الورقة، الوزن الطازج للنبات والعِرش، و كذلك في صفة المحصول الكلي.
٣. أظهرت الأصناف والسلالات ذات الأبصال الأرجوانية تميزاً فى صفات جودة الأبصال، حيث أعطت الأصناف والسلالات Egaseed-1A، Egaseed-1B،Egaseed-2، Egaseed-3، Sids-40-R.S.، Sids-40-Ayat، Clone-21، Bany gonamy، Al Ayat-1، Salaqus أقل متوسط عدد فصوص/ بصله، كما أعطى كلاً من Bany gonamy و Sids-40-R.S. أعلى قيمة معنوية لمتوسط وزن الفص وذلك فى الموسم النمو الأول والثانى على الترتيب.
٤. سُجّلت أعلى القيم معنوية لصفة محتوى المادة الجافة للبصلة فى الأصناف والسلالات Egaseed-1A، Egaseed-2، Egaseed-3، Clone-21، Clone-22-R، Bany gonamy خلال موسمي النمو.

التجربه الثانية (الإكثار الدقيق و تكوين البصيلات والبذور الصناعية للثوم معملياً)

تم إجراء سلسلة من التجارب المعملية بمعمل زراعة الأنسجة بقسم الخضر– كلية الزراعة– جامعة الإسكندرية، لدراسة سلوك ستة أصناف وسلالات من الثوم (Salaqus-3، Aswan، Borg El-Arab، El Behera، Sids-40-Ayat،

Clone-21) لإنتاج السيقان الخضرية والبصيلات معملياً بإستخدام بيئة Murashge & Skooge (1962) المضاف إليها السكروز بتركيز ٣ % كنترول (T1) أو ٣ % سكروز + حمض الجاسمونيك بتركيز ٢ ملجم/لتر T2 أو٦ % سكروز + حمض الجاسمونيك بتركيز ٢ ملجم/لتر (T3).

تم إجراء تجربة لإنتاج بذور الثوم الصناعية معملياً، حيث تم تغليف البصيلات الناتجة من زراعات الأنسجة بإستخدام مخلوط مكون من (٣ % ألجينات الصوديوم و٠,٢ % فحم نشط و٠,٢٥ ملليليتر/ لتر من محلول يحتوي علي مضاد حيوي) ولتصلب الغلاف الصناعي للبصيلة تم وضعها بمحلول ٢,٥ % كلوريد كالسيوم على المقلب المغناطيسي لمدة ٣٠ دقيقة. أجريت تجربة لتنبيت البذور الصناعية في الصوب البلاستيكية بزراعتها في مخلوط مكون من التربة والرمل والبيتموس بنسبة ١:١:١.

تم إجراء التجارب كتجارب عاملية ذات عاملين (ستة تراكيب وراثية و ثلاث بئيات غذائية مختلفة المحتوى) بالتصميم العشوائي الكامل (CRD)، ٢٠ مكررة. وقد تم تكرار التجارب مرتين على الأقل.

ويمكن تلخيص النتائج فى النقاط التالية:

١. أظهرت النتائج وجود إختلافات معنوية فيما يتعلق بقدرة الأصناف والسلالات المستخدمة على إعادة النمو وتكوين السيقان والبصيلات وعدد البصيلات لكل قطعة نباتية ومتوسط وزن البصيلة الواحدة.
٢. تأثر معدل النجاة للقطع المنزرعة معنوياً بالأصناف والسلالات، بينما لم يكن لمحتوى بيئة النمو (تركيز السكروز وحمض الجاسمونيك) تاثيراً معنوياً لمعدل النجاة.
٣. أعطت السلالاتين Salaqus-3 وBorg El-Arab أكبر عدد من السيقان الخضرية لكل قطعة منزرعة وأكبر عدد من البصيلات لكل فص ثوم.
٤. أدت زيادة تركيز السكروز فى بيئة النمو وإضافة حمض الجاسمونيك إلى زيادة في تكوين بصيلات الثوم للأصناف والسلالات تحت الدراسة.
٥. أعطت معاملة–٣ (بيئة MS المزودة ب ٦ % سكروز و ٢ ملجم/لتر حمض الجاسمونيك) أعلى متوسط معنوي لعدد البصيلات لكل الفص مقارنة بمعاملة الكنترول (بيئة MS المزوده ب ٣ % سكروز).
٦. إختلفت الأصناف والسلالات فيما بينها معنوياً بالنسبة لصفة متوسط وزن البصيلة، وأعطت Clone-21 أعلى متوسط لوزن البصيلة.
٧. أعطت معاملة الكنترول (بيئة MS المزوده ب ٣ % سكروز) أعلى متوسط معنوى لوزن البصيله مقارنة بالمعاملتين الأخرتين، بينما أعطت معاملة–٣ أقل قيمة معنويه لهذه الصفة.
٨. أمكن بنجاح الحصول على البذور الصناعية لجميع الأصناف والسلالات تحت الدراسة، كما تم إجراء تجربة إنبات بنجاح تحت ظروف الصوب البلاستيكية حيث أنبتت البذور الصناعية للثوم (البصيلات الناتجه معملياً ومغلفة بألجينات الكالسيوم) فى خليط مكون من التربة والرمل و البيتموس بنسبة ١:١:١.

توكد نتائج هذا البحث أن دراسة الإختلافات الوراثية بين الأصناف والسلالات المصرية على المستوى الظاهري من خلال تتبع أداءها الحقلي يجب إستخدامهما معاً لتحسين إنتاجية وجودة محصول الثوم.

كما تشير النتائج الى أن تكوين بصيلات الثوم معملياً و تغليفها لإنتاج البذور الصناعية يمثل بديلاً مناسباً لرعاية السلالات الناتجة من برامج التربية باستخدام زراعة الأنسجة وتجديد حيوية الأصناف التجارية المتاحة و حفظ الأصول الوراثية للتراكيب المميزة، إلا انه يجب إجراء المزيد من الدراسات للوقوف على الظروف المثلى والقيمة الإقتصادية لإنتاج البذور الصناعية وإستخدامها على النطاق التجاري و تعويض نقص تقاوي الثوم.