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Utilization Clonal Selection Program to Improve Resistance Garlic (*Allium sativum* L.) Against Onion Maggot (*Delia antiqua* M.)

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ABSTRACT

Onion maggot (*Delia antiqua* M) is an economically important pest of Allium crops throughout the world; thus, management of this pest is required to achieve economic returns. Accordingly, this study aimed to select some clones of garlic resistant to infestation with onion maggot through the implementation three cycles of clonal selection program within some improved garlic clones derived from Balady cultivar. Afterwards, the developed clones and their original population were evaluated in a randomized complete block design with three replicates. Thus, this study was conducted during four successive winter seasons of 2016/2017 to 2019/2020. The obtained results showed, clearly, significant differences among garlic selected clones, as well as their original population in their response to *Delia antiqua* M. and their bulb characteristics. Where, the selected clones varied between resistant and ability of infestation by onion maggot that was between 0.00% and 22.45%, with classification into five groups by cluster analysis: HR (highly resistant), R (resistant), MR (moderately resistant), MS (moderately susceptible) and S (susceptible). Among the 16 selected clones three clones were HR; and six clones were R to onion maggot. Moreover, improvement percentage for the same character using clonal selection program was between 48.97% to 100%, which reflected that clonal selection could be an effective way to improve onion maggot resistance. Also, utilization of these improved clones will promise in minimize environmental pollution.

KEYWORDS: Garlic, selection, cluster, resistance, Onion maggot, *Delia antiqua*.

1. INTRODUCTION

Garlic (*Allium sativa* L.) is the second most important bulb crop after onion belonging to family Alliaceae and genus *Allium*; which, has more than 600 available species. Also, considered one of the most important vegetable in Egypt; that, grown for both exportation and local consumption; as green fresh, semi-dry, dry, or processed products.

Garlic is attacked by several insect pests and diseases; and onion maggot is considered one of the most pests; which attack garlic plants at different phases. Onion maggot, *Delia antiqua* (Meigen, 1826) (Diptera: Anthomyiidae), is an oligophagous insect specializing on crops of genus *Allium* and could be considered a key pest of Alliaceous crops in many parts of the world. This pest feed on numbers of Alliaceous plants including onion, garlic and leeks and caused yield losses ranging about 20 – 60% (Hermize 2012, Taylor *et. al*. 2000). There are three generations of onion maggot each year (Eckenrode *et. al*. 1975); but the first-generation is the most damaging (Liu *et. al*. 1982, Loosjes 1976). Females are typically deposited in batches of two to four eggs per plant (Mowry 1993). The larvae are developing through three instars in two to three weeks and later pupating in the soil (Hoffman *et. al*. 1996). Hatching larva feed internally or externally on the garlic stem that underground or after bulb formation. Larvae feed and makes burrow inside bulbs leading to wilting and dead cloves; and one larva may feed many cloves before its pupation (Klass and Snover 2000). Besides, feeding sites and the wounds that caused by larva led to easy hit in cloves or bulb by bacteria and fungi. Accordingly, onion maggot insect causes undesirable effects during harvesting, post harvesting, processing and marketing stages; which, effect negatively on cropping pattern, quality and export potential of the crop and then local and export markets affect; so, cause clearly economic loss.

Pesticides is considered as the main control procedures for the onion maggot; where it can be applied as seed treatment or foliar application, which may led to appearance of insecticide resistance; in

another words, insect control has become challenging due to insecticide resistance (Nault *et. al*. 2006). On another side, there are very limited effective and practical non-chemical procedures for control of onion maggot. For example, trap cropping has been considered in the past as a primitive method of onion maggot control (Mowry 1993). Natural enemies, also, considered one of non-chemical methods that may play a role in preventive of onion maggot (Hoffmann *et. al*. 1996). Cultural practices have been advanced for onion maggot; but do not play a tangible role in its control. Therefore, it is very important exploiting the presence of the pest-resistance genes of plants; where, plant resistance has been investigated as a way of reducing onion maggot damage. Since, the use of resistant cultivars is an important and complementary management tool to deal with pest or any disease (Didelot 2016 and Vieira *et. al*. 2011). So, selection breeding program of resistant garlic cultivars could be very promising method that aimed to find alternative control system safe to humans, environment, and natural enemies.

A few resistant garlic cultivars against onion maggot have been selected out of the previous studies (Wang *et. al*. 2010). Garlic is a typical vegetative propagated crop, due to its sexual sterility; thus, clonal selection is a major breeding method for this crop.

Despite the importance of this crop, very limited breeding work has been done so far in this respect. Thus, this study is a part of a complementary breeding program to improve the garlic crop; where, this study focuses on applying a clonal selection program to garlic, Balady cultivar, in order to create insect-resistant clones, thereby increasing the storage ability of the crop while maintaining bulb quality and productivity.

2. MATERIALS AND METHODS

2.1. Original Genetic Materials.

A total 350 bulbs from improved different garlic clones were used in this study as the original population to start the clonal selection program. These clones were obtained by clonal selection program of Balady cultivar for seven years to improve yield and quality bulbs of garlic. This garlic breeding program was conducted, by the author, at Vegetable Crop department, Faculty of Agriculture, Alexandria University. So, this program considered a part of a comprehensive study aimed to obtain new garlic clones' resistance to onion maggot with good yield and quality.

2.2. Examination of Onion Maggot Damage Levels of Garlic Bulbs in the Original Population.

During all selection cycles of this study; the bulbs of garlic were cleaned and split into individual clove per each garlic bulb. Different bulbs of each clone, which infested by onion maggot were investigated in lab under binocular. Onion maggot damage of garlic bulbs was estimated by the numbers of larvae and the area of damage that the larvae fed upon. Percentages of onion maggot infestation were calculated in all garlic bulbs of each clone upon (Fig.1).

Fig 1. The symptoms of the infestation by onion maggot, *Delia antiqua* on the garlic cloves

2.3. Experimental Work and Breeding Program.

The present study was carried out during three successive winter seasons of 2017/2018, 2018/2019 and 2019/2020 at the Experimental Station Farm, Faculty of Agriculture, Alexandria University; at Abies, Alex, A.R.E.

This program was started in 2017/2018, at 1Sept. by checkup the 350 bulbs before seedling with 20 days, which considered the first cycle of clonal selection in this study. The garlic bulbs were manually split into individual cloves so as not damage the outer peel and injured bulbs were ruled out after inspection it as previously mentioned. According to the initial selection, the best 100 selected bulbs; on the basis of healthy and free of onion maggot damage bulbs with heavier bulb weight and less cloves number per bulb; were planted separately, that mean each bulb in a

separate line. The cloves were hand planted on both sides of the line at approximately 7cm apart within each row and 60 cm wide. The planting dates were 21 Sept. in the three successive winter seasons of 2017/2018, 2018/2019 and 2019/2020. No insecticide was applied during the all-growing seasons; while all manual loosing and weeding were carried out during the vegetation. Harvesting was carried out on the 15 Apr. of the three consecutive seasons; and garlic bulbs of each clones were finally drying in well-ventilated storage area.

In the second season, the second cycle of clonal selection started; where, health statues of each clone were assessed visually from the end of June at regular weekly intervals; when, visible symptoms of damage were noticed, the bulb was removed from the clone, with registered number of bulbs that excluding from each clone. Before cultivation with 20 days; the pest resistance bulbs from each clone, 100 clones, selected as previously mentioned. Accordingly, the best 50 selected clones from the 100 were planted separately as described earlier in the first season, as well as, at harvest period the same selection procedures were practices in the second season; that considered the third cycle of clonal selection, to obtain the most promising selected clones; which came out to be 16 clones.

2.4. Adult Activity of *D. antiqua* Within Garlic Field.

At different spots of field, four yellow sticky cards (15 cm x 15 cm) were placed facing into the field on the edge of the field. Where, sticky cards are the standard trapping method used to scout *Delia* populations and *Delia antiqua* is more attracted to yellow traps, rather than blue ones (Mlynarek *et. al*. 2020). The sticky cards were clamped to stakes at height of 15 cm above the soil. Thereafter, height was adjusted to keep card tops level with the garlic canopy. These sticky cards were changed a weekly from the estimated start of first-generation fly emergence until harvest time in each season. Once these sticky cards were collected, they were returned to the laboratory. *Delia* species from the sticky traps were identified using the key provided by Savage *et. al*. 2016. A few specimens were removed from traps, mounted *Delia* spp. at the sticky cards as vouchers to infestation with this specie of insects. Only males were identified because females of *Delia* spp. cannot be reliably distinguished morphologically, especially from sticky cards. Additionally, identification of female *Delia* spp. relies on the number and position of bristles on the legs and thorax, which can be lost or damaged on sticky cards, leading to misidentifications (Mlynarek *et. al*. 2020). The number of onion maggot flies captured per sticky card within garlic fields was counted. These data enabled us to relate incidence of plant damage to female onion maggot phenology.

2.5. Evaluation of the Various Genetic Populations.

In the winter season of 2019/2020, healthy cloves from the 16 selected clones, which reflected high stability during successive seasons for the resistance trait, as well as sample of cloves from the original population, Balady cultivar, were planted on 21 Sept. 2019/2020 in a Randomized Complete Block Design. Three plots were used as replicates for every genotype and the genotypes were randomly distributed in the field. The experimental plot was consisted of 2 rows, 3m long and 0.6m width and cloves were hand planted approximately 7 cm apart on the two sides of ridge. All the recommended cultural practices were applied during the growing season; with the exception of the pesticides application that were not added during the season. After harvesting, the plants of each clone were left for curing for about 15 days and then stored at the storge room. Data of bulb characteristics were taken at the same time during the all studying seasons; after curing process; while, examination of bulbs to onion maggot infestation was recorded at 1 Sept. A random sample of five plants from each genotype were collected from each plot to measure and estimate the following parameters:

1- **Bulb wight (g):** the average cured bulb weight of five plants was recorded in grams.
2- **Bulb diameter (cm):** the cured bulb diameter was recorded using a caliper.
3- **Cloves number per bulb:** cloves of each bulb were counted and the average of Cloves number from five bulbs was estimated.
4- **Onion maggot infestation%:**

$$\frac{\text{number of infested cloves in each sample}}{\text{total number of cloves}} \times 100$$

5- **Resistance improvement%:**

$$\frac{\text{infestation in original population} - \text{infestation in clone}}{\text{infestation in original population}} \times 100$$

2.6. Statistical Analysis.

The mean values of each character under the study were computed and subjected to analysis of variance, following the procedures described by Al-Rawi and Khalf-Allah (1980), using Co-Stat computer software program (2004). The collected data were analyzed using SPSS (version 24) and Squared Euclidean distance, which was used as a measure of distance for cluster formation.

3. RESULTS AND DISCUSSION

3.1. Performance of Different Garlic Genotypes Against Onion Maggot, *Delia antiqua* Infestation and Some Important Bulb Characteristics.

Performance of different selected clones and their original population of garlic against onion maggot population and yield contributing characters are presented in Table (1). The differences among the genotypes were significant for all studied parameters. Among the different clones, clone 8 and 13 did not reflect any percentage of onion maggot infestation as compared to rest of the selected clones as well as the original population. Whereases, the lowest infestation percentages with *D. antiqua* were recorded from the garlic clones 8, 13 and 7, (0.00, 0.00 and 1.65%) respectively; moreover, there were insignificant differences among these three clones. On the other hand, the highest infestation percentage with *D. antiqua* for all genotypes was reflected by the original population (Balady cultive). While the highest infestation percentage among the selected clones was recorded from clone 1 (22. 45%); preceded by the clone 10 and clone 3, respectively, with significant differences between clone 1 and the other two clones 10 and 3. These results clarified that the three improved clones number 8, 13 and 7 reflected highly resistance to *D. antiqua* and could be selected as a unclose for the new cultivar in the breeding program.

Concerning the three bulb characteristics; bulb diameter, bulb weight and cloves number per bulb; the result illustrated that significantly the maximum bulb diameter (6.49 cm) was recorded from the clone 13 followed by clone 16 (6.25 cm) and clone 4 (6.22 cm). While, the minimum bulb diameter (4.01 cm) was observed by Balady cultivar and the selected clone 10 (5.31 cm) with significant difference between them. The highest and significant average bulb weight was noticed in clone 7 (51.34 g) which was at par with clone 13 (50.45 g), followed by clone 16 (47.63 g) as compared to the rest clones, as well as the original population.

Table 1. Performances of different selected clones and their original population against *Delia antiqua* infestation and yield contributing characters of garlic.

Characters / Genotypes	*D. antiqua* Infestation %	Bulb diameter (cm)	Bulb weight (gm)	Cloves number per bulb
Selected Clones				
C 1	22.45 b	5.80 ef	39.53 ef	47.66 b
C 2	05.82 hi	5.91 de	38.00 fg	37.00 fg
C 3	16.47 c	6.01 cd	41.17 c-e	35.33 gh
C 4	07.21 gh	6.22 b	40.69 d-e	41.33 d
C 5	10.43 ef	6.00 cd	41.20 c-e	36.33 f-h
C 6	09.98 fg	6.12 bc	31.43 ij	34.33 h
C 7	01.65 j	6.54 a	51.34 a	45.33 bc
C 8	00.00 j	5.81 ef	43.33 c	37.00 fg
C 9	07.15 gh	5.65 fg	35.72 gh	35.33 gh
C 10	17.12 c	5.31 i	31.13 j	36.66 f-h
C 11	11.42 de	5.41 hi	33.55 hi	44.66 c
C 12	12.90 d	5.90 de	39.38 ef	38.66 ef
C 13	00.00 j	6.49 a	50.45 a	39.66 de
C 14	05.8 hi	5.88 de	42.56 cd	44.66 c
C 15	05.5 hi	5.51 gh	40.41 d-f	37.66 e-g
C 16	06.0 hi	6.25 b	47.23 b	36.33 f-h
Oroginal Pop.				
Balady cultivar	44.00 a	4.01 j	20.68 k	59.96 a

Values having the same alphabetical letter (s) within each column, don't significantly differ from one another, using Duncan's multiple range test at 0.05 level of significance.

Concerning the cloves number per bulb, data on means comparison showed that there were, clearly, significant differences between Balady cultivar and the selected clones in desirable effect in toward lowest number of cloves. Where, it seems that number of cloves per bulb may be important trait and should be considered in breeding of local cultivar of garlic, Balady cultivar, that produced greater number of cloves with light weight, which considered unfavorable to Egyptian consumer. The highest number of cloves per bulb, undesirable effect, was given by Balady cultivar (59.96) with significant differences with the all-selected clones; while the lowest with mean 34.33, 35.33 and 35.33 cloves in the clones, 6, 3 and 9, respectively with insignificant differences. These results are confirmed with the conclusion of Al-Otayk *et. al*. 2008, Dawood *et. al*. 2011, and Omer and Abou-Hadid 1992; who stated that Balady cultivar recorded the lowest value of bulb weight and produced bulb with maximum number of cloves.

3.2. Effect of Clonal Selection on Improvement of Resistance to Onion Maggot, *Delia antiqua* Infestation

Improvement of resistance to Onion maggot infestation according to three clonal selection cycles of garlic was recorded in Fig 2. The calculated percentages of improvement stated that the two selected clones 8 and 13 were noticed to show the highest improvements for this character, compared to the all-selected clones and the original population; followed by the clone 7. The improvement magnitudes of these three clones were estimated by 100%, 100% and 96.25%, respectively, over the original population. Also, the six clones 14, 15, 16, 2, 9 and 4 reflected increments that estimated between 83.61% in clone 4 to 87.50 in clone 15, relative to the original population. Some improvement was detected, also, in the case of the four clones 5, 6, 11, and 12; whose means reflected 76.29%, 77.31%, 74.04% and 70.68% improvements, respectively. While the lowest percentage of improvements were recorded by the clone 1, preceded by the clones 10 and 3 with percentage as 48.97%, 61,09% and 62.56%, respectively. From the previously mentioned result, in the present study, upon clonal selection from Balady cultivar, all selected clones reflected degree of resistance to *Delia antiqua* than their original population, showing an obvious effect of clonal selection; that could improve the resistance of garlic. The same results were given by Wang *et. al*. (2010 and 2019), when using clonal selection as breeding program to obtain resistance clones from garlic to *Delia antiqua*.

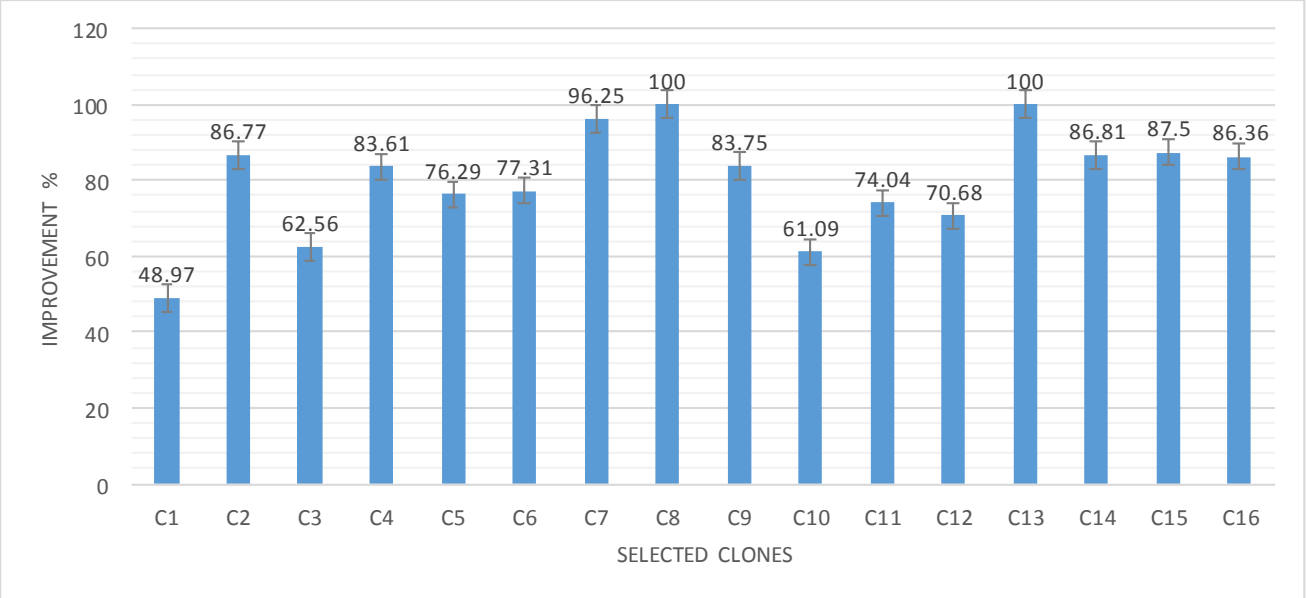

Fig. 2. Effect of clonal selection on resistance improvement to Onion maggot infestation

3.3. Classification of Garlic Clones for Their Resistance to *D. antiqua* Infestation.

The different clones of garlic, selected from Balady cultivars, were grouped into five different categories of resistance viz., highly resistant, resistant, moderately resistant, moderately susceptible and susceptible: according to tree dendrogram and percentage of onion maggot infestation (Fig. 3 and Table, 2). The first cluster, which was highly resistant to onion maggot included three clones, corresponding to 18.75% of the total clones, with onion maggot infestation percentage ranging from 0 – 2%: clones 8, 13 and 7. The second cluster, which was resistant to onion maggot, included six clones corresponding to 37.50% of the total clones, with onion maggot infestation percentage from 3 – 8%: clones 4, 9, 2, 14, 16 and 15. The third cluster, which was moderately resistant to onion maggot, included four clones, corresponding to 24.00 % of the total clones, with onion maggot infestation percentage from 9 – 13%: clones 5, 6, 11 and 12. The fourth cluster, which was susceptible to onion maggot infestation, included two clones, corresponding to 12.50% of the total clones, with onion maggot infestation percentage ranging from 14 – 18%: clones, 3 and 10.

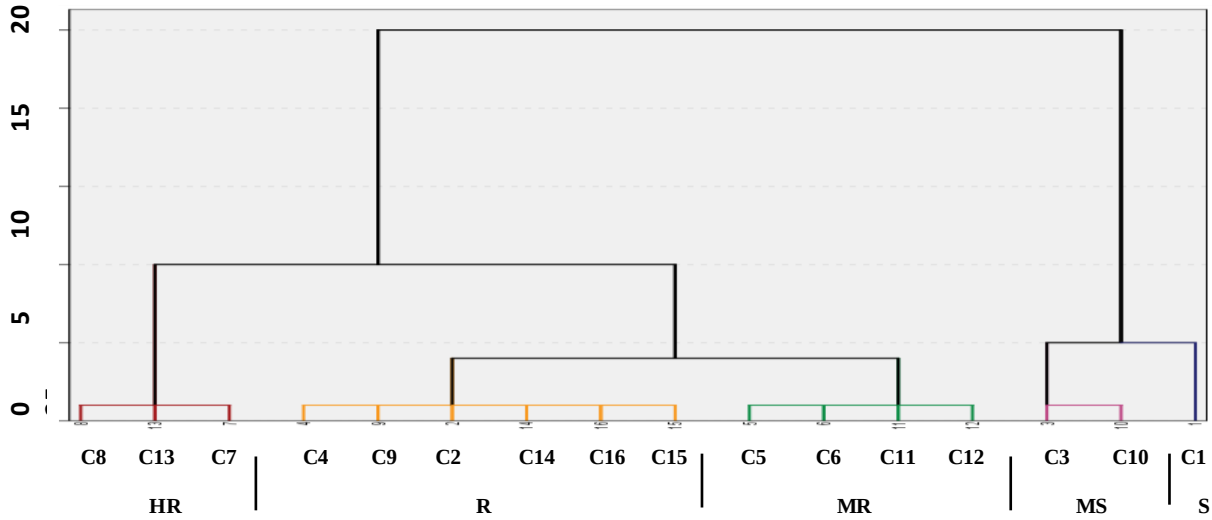

Fig.3. Tree dendrogram of onion maggot, *Delia antiqua* infestation values of 16 selected clones of garlic. HR, highly resistant to *D. antiqua*; R, resistant to *D. antiqua*; MR, moderately resistant to *D. antiqua*; MS, moderately susceptible to *D. antiqua*; S, susceptible to *D. antiqua*.

Keys: C1 – C16: selected clones

Table 2. Resistance distribution of the investigated 16 clones.

Resistance group	% onion maggot infestation	Number of Clones	% of each group to total clones
HR	0 – 2	3	18.75
R	3 - 8	6	37.50
MR	9 – 13	4	24.00
MS	14 – 18	2	12.50
S	More than 19	1	6.25

HR, highly resistant to *Delia antiqua* ; R, resistant to *D. antiqua*; MR, moderately resistant to *D. antiqua*; MS, moderately susceptible to *D. antiqua*; S, susceptible to *D. antiqua*

The fifth cluster, which was susceptible to onion maggot infestation, included one clone, corresponding to 6.25% of total clones, with onion maggot infestation percentage more than 19%: clones 1. The obtained results were agreement with that reported by Wang *et. al*. 2019 who used cluster analysis to classify some accessions of garlic to onion maggot, *Delia antiqua* resistance into six group according to the pest index. So, cluster analysis based on morphological traits of genetic resources facilitate and help in breeding program with high efficiency, as shown in studies of the genetic resources of many crops (Saeed *et. al*. 2017, Singh and Jaiswal 2013,. Buso *et. al*. 2008, Matus *et. al*. 1999 and Ogwu *et. al*. 2018).

4. CONCLUSION

The findings of this study showed significant differences among selected clones as well as their original population for the studied bulb characteristics and their response to *Delia antiqua*. Where three clones were found to be highly resistant; in addition to 6 resistance clones. Therefore, these clones could be used in breeding programs to develop new cultivars that are resistant to onion maggot, *D. antiqua*. Utilization of such cultivars can reduce pollution of environment and biodiversity and make management procedures more economical. Thus, clonal selection activity could be an effective method to improve pest resistance and most of morphological and other important traits of garlic. Thus, could be using resistance clones with combinations of chemical, biological and cultural methods, and crop rotation as integrated pest management in garlic cultivation.

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الملخص العربي

استخدام برنامج انتخاب السلالة الخضرية لتحسين مقاومة الثوم لذبابة البصل (*Delia antiqua* M)

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ذبابة البصل (*Delia antiqua* M) هي آفة مهمة اقتصاديًا لمحاصيل *Allium* في جميع أنحاء العالم ؛ وبالتالي ، فإن إدارة هذه الآفة مطلوبة لتحقيق عوائد اقتصادية. وبناءً على ذلك ، فإن الهدف الرئيسي من هذه الدراسة هو انتخاب سلالات خضرية من محصول الثوم مقاومة لذبابة البصل من خلال تنفيذ ثلاث دورات من برنامج انتخاب السلالة الخضرية داخل مجموعة من سلالات الثوم المحسنة ، تلى ذلك تقييم السلالات المنتخبة وكذلك نباتات العشيرة الاصلية (الصنف البلدى) باستخدام تصميم القطاعات العشوائية الكاملة بثلاث مكررات؛ وعليه فقد أجريت هذه الدراسة خلال أربعة مواسم شتاء متتالية من ٢٠١٦/٢٠١٧ إلى ٢٠١٩/٢٠٢٠. أظهرت النتائج المتحصل عليها بوضوح وجود فروق معنوية بين السلالات الخضرية المنتخبة من الثوم ، وكذلك العشيرة الاصلية في استجابتهم لذبابة البصل . *Delia antiqua* M وكذلك مواصفات جودة الرأس. حيث عكست السلالات الخضرية المنتخبة تباين فى شدة المقاومة والاصابة بذبابة البصل والتى تراوحت بين ٠.٠٠٪ و ٢٢.٤٥٪ ، والتى تم تصنيفها إلى خمس مجموعات عن طريق التحليل العنقودي: HR (عالية المقاومة) ، R (مقاومة) ، MR (متوسطة المقاومة) ، MS (متوسطة القابلية للاصابة) و S (قابلة للاصابة). وعليه من بين ال١٦ سلالة الخضرية المنتخبة، كانت هناك ثلاثة سلالات خضرية منتخبة عالية المقاومة HR؛ وستة سلالات خضرية منتخبة كانت مقاومة (R). علاوة على ذلك ، كانت نسبة تحسين السلالات الخضرية المنتخبة باستخدام برنامج انتخاب السلالة الخضرية بين ١٠٠٪ إلى ٤٨.٩٧٪ مما يعكس أن استخدام هذا البرنامج الانتخابى يمكن أن يكون وسيلة فعالة لتحسين المقاومة لذبابة البصل، أيضًا سيساعد استخدام هذه السلالات في تقليل التلوث البيئي.