Egypt. J. Plant Breed. 24(3):571– 597(2020)

SELECTION AGAINST THE EXTREME MALE AND VEGETATIVE MALE PLANTS IN SPINACH AS AN EFFECTIVE APPROACH FOR DEVELOPING A NEW DELAYED BOLTING CULTIVAR

H. H. Hamed[1] and A.A.S.A. EL-Eslamboly[2]

1. Vegetable Seed Production Technology Research Department - Horticultural Research Institute, ARC. Giza, Egypt

2. Vegetable Crop Production Research under Protected Cultivation Department, Horticulture Research Institute, ARC. Giza, Egypt

ABSTRACT

The main target of the current study was to develop a new local cultivar of spinach with delayed bolting which characterized by late-flowering based on the multitrait selection for quality traits through the disposal of the male plants in the spinach population and increasing the percentage of females and no bias plants. This study was carried out at Kaha Vegetable Research Farm (Kaluobia Governorate, Egypt). Means, genetic and phenotypic parameters of population performances had been recorded for the characteristics of plant growth, yield and its components as well as chemical compounds. One genotype, Dokki cv. (a local cultivar as original population) was used. The selection program began on the first of September 2014 and continued to the year 2017 (four years), where a comparison experiment was conducted between the original and the selected population during 2018 and 2019-under high temperature in an early season-to measure the progress in achieving the goals of the selection program. The selection program based on multitrait selection under high temperatures in the early season had clear effects on genetic and phenotypic parameters. Moreover, the multitrait selection based on selection indices showed differences consistent with the goal of the selection program in all vegetative characteristics, delay bolting and chemical content of leaves that were significantly improved in the selected population compared to the original population.

Key words**:** ***Spinacia oleracea*** **L.,** ***Bolting, Genetic variance,*** **Phenotypic** ***variance, Multitrait selection.***

INTRODUCTION

Spinach is one of the important economically leafy vegetables in Egypt and worldwide; the healthiest vegetables for its content of nutrients and minerals and good source of antioxidants (Shetty et al 2013). Spinach is one of the darkest green leafy vegetables due to the huge concentration of beta carotene (pro vitamin A) and folate, and is also a good source of vitamin C, calcium, iron, phosphorous, sodium and potassium (Nonnicke 1989 and Dicoteau 2000). Basically, spinach is a wind pollinated dioecious species. As would be expected in a dioecious, wind-pollinized plant, existing varieties of spinach are generally in a highly heterozygous condition. Historically mass selection, recurrent selection and backcross have been the breeding techniques most commonly used in spinach but recent interest in more severe inbreeding to produce more uniform inbreeds for use in hybrid production has spurred interest in methods such as paired crosses using individual female and male plants or use of monecious plants for selfing to develop inbreeds (Morelock and James 2008).

Commercial stocks are frequently of mixed varieties, of unsuitable types, or incorrectly named. These defects are the cause of much loss to growers and canners. Knowledge of the nature of the sexual conditions in spinach, as well as of the factors controlling sex expression, was considered essential to the work of plant breeding. The spinach plants can be divided into two divisions' bias plants (extreme male, vegetative males' and female plants) and no bias plants (monoecious plants, gynomonoecious and trimonoecious). Of the two kinds of male plants, the extreme type is by far the more common in nearly all strains of spinach plant.

The extreme males are the first to send up their seed stalks (early bolting); in fact, they sometimes do so without having formed any rosette leaves and probably connected with genetic factors and environmental conditions. Commercial stocks of late or "long standing" varieties, which produced carefully to maintain this trait, contain the few numbers of extreme males or do not contain any extreme males. By carefully removing all early flowering plants (extreme male) continuously in more seasons, you can produce delayed spinach varieties (Rosa 1925). After removing the extremely male plant (early flowering or early bolting) the plants that are larger in the early part of the season may usually be later identified as females.

Male plants, especially the extreme male type, bolting or flowering earlier, have a shorter vegetative growth with shorter flowering period than females. Male plants, especially the extreme males, are in general smaller, form flower stalks earlier, bloom earlier, and die earlier than the female plants. The early appearance of the bolting of the male plants leads to considerable loss to growers.

Monoecious plants are relatively rare. In many reports monoecious plants were not observed more than 4% - 8.7% in other populations. Plant breeding methods can produce the improved varieties which have an excess of females in the population (Rosa 1925).

Breeders have endeavored to satisfy the needs of spinach growers by means of mass selection. This method is still in vogue up till now because it is simple to carry out and because among breeders it is often feared that degeneration by inbreeding will occur when other methods are used. In general, the growers consider that the male plants have a bad reputation. The stems of male plants of which the upper part is leafless, elongates faster than the stems of female plants of the same variety. Mass selection is still largely applied and it is generally used in the maintenance of commercial varieties. It has been proved; however, that continuous family selection is useful for the development of new varieties (Sneep 1958).

Spinach is a long-day plant. The plants become generative and start to flowering when exposed to a day length that exceeds a critical value, the so-called critical day length (Zeevaart 1971).

A negative correlation was detected between the speed bolting and leaf production in spinach (Chitwood *et al* 2016).

The success of a plant breeding program is dependent on the availability of genetically diverse plant germplasm to allow for cultivar improvement. The genetic diversity and population structure of a plant species allows the plant breeders to use the resources for crop improvement. Such an approach has benefited many crops. Genetic diversity and population structure also have been examined in spinach (Khattak *et al* 2007).

Spinach leaves are alternate, simple, from ovate to triangular based, with larger leaves at the base of the plant and small leaves higher on the flowering stem (Vural *et al* 2000). The commercial spinach varieties have more delayed plants at flowering and do not rush to bolting early. The male and female spinach plants are identified according to carbohydrates and pigment contents which are higher in female than in male plants (Swiader and Ware 2002).

Early flowering is the most important problem of spinach production in Egypt. The bolting and flowering leads to losses for producers and farmers. Early flowering is associated with the percentage of male plants in plant population.

Consequently, this led to deterioration of the yield characteristics and reducing the quality and led to many other important problems of spinach.

The main target of the current study was to develop a new local cultivar of spinach with delayed bolting which is characterized by late flowering based on the multitrait selection of quality traits through the disposal of the male plants in spinach population and increasing in the percentage of females and unbiased plants.

MATERIALS AND METHODS

This study was carried out at Kaha Vegetable Research Farm (Kaluobia Governorate, Egypt) during the period from 2014 to 2017. The soil type of the experimental site is classified as a clay soil. One genotype, Dokki cv. (a local cultivar) was used. Seeds were obtained from the Vegetable Seed Production Unit, Vegetable Research Departments, Dokki, Giza, Egypt. Yield performance was compared in the same field environment. The multitrait selection program began on the first of September 2014 and continued to the year 2017 (four years), where a comparison experiment was conducted between the original Dokki cv. and the selected population during 2018 and 2019 - under high temperature in early season - to measure the progress in achieving the goals of the selection program. The two seasons were assigned according to the maximum and minimum temperatures that affecting spinach growth and yield that determined by Yamori *et al* (2005). Maximum, minimum and average high and low temperatures, relative humidity, day light and rain fall are presented in Table (1).

Seeds were sown in 5th September 2018 and 1st September 2019, in the field directly. Each ridge was 60 cm wide, 3.4 m long and 15 cm for plant spacing. Data were recorded on several quantitative traits consisting of plant height (PH), number of leaves per plant (NLP), central flowering stem length (CFSL), fresh weight per plant (FWP), dry weight per plant (DWP), leaf petiole length (LPL), leaf blade length (LBL), bolting date (BD), yield of leaves (kilogram per square meter), yield of leaves (ton per feddan), total nitrogen (%), total phosphorus (%), total potassium (%), total chlorophyll (mg/L), total calcium (%), oxalate (%) and calcium oxalate (%).

Table 1. Average temperature, relative humidity, daylight and rainfall of field trials in Kaha Vegetable Research Farm, Kaha, Kaluobia Governorate, Egypt.

Months	Temperature (C°)		Relative humidity (%)	daylight (hours)	rainfall (mm)
	Maximum	Minimum			
January	19.2°C	6.7°C[ab]	81	10.5h	5mm
February	20.9°C	7.2°C[b]	78	11.1h	4mm
March	23.7°C	9.5°C	71	12h	3mm
April	27.9°C	12°C	67	12.9h	1mm
May	31.9°C	15.6°C	64	13.7h	1mm
June	34.3°C	18.6°C	70	14.1h	0mm
July	34.6°C	20.4°C	77	13.9h	0mm
August	34.4°C	20.4°C[b]	79	13.2h	0mm
September	32.3°C	18.4°C[b]	78	12.3h	0mm
October	29.9°C	16.1°C[b]	79	11.4h	1mm
November	25.4°C	13.2°C[a]	72	10.6h	2mm
December	20.8°C	8.5°C[a]	81	10.2h	6mm

C[a]= common season. C[b]= Date of cultivation of tolerant varieties for bolting under high temperature conditions in Egypt.
Weather-atlas.com. (https://www.weather-atlas.com/en/egypt/qaha-climate#temperature).

2.1. Experimental design and Statistical analysis:

The original population was improved in five generations of multitrait selection. Male plants with low vigor and early flowering (bolting) were discarded. Then, the highest 10 percent selection index female plants were done through all the selecting generations (removing all extreme males and vegetative males), which pollinated by unbiased plants and their seeds were harvested. The earliest flowering males (usually “extreme males”) are also the earliest flowering plants in spinach populations. If all “extreme males” are selected out of the population along with the earliest flowering females then it is possible to shift the spinach variety to a longer-standing and more bolt-resistant, this procedure described by Imani *et al* (2004). In the last year (i.e., 2019) the population seemed to be uniform and a few plants were discarded. The result of genetic gain during selection period, led to consider this improved population as a new bolt-resistant cultivar of spinach under the conditions of early cultivation. Bolting time was monitored daily and determined as the number of days from planting to the first elongation of the floral stem observed in at least half of the studied plants for each population. The collected data from the original population and the selected population resulted from multitrait selection were analyzed statistically using Fisher's analysis of variance technique. The experimental unit was consisted of one grid, one grid was consisted of 19 plants (1 central plant + 18) (Figure 1). These units are repeated and contiguous in one area for both the original population and the selected population. This procedure was done according to Bos and Caligari (1995). The data collected were analyzed statistically using fisher's analysis of variance technique by using combined ANOVA across years and Unpaired two-tailed Student’s t-test, at 5% level of probability was employed to compare the difference among the populations means. All computations were performed using the Minitab software (Minitab 2010).

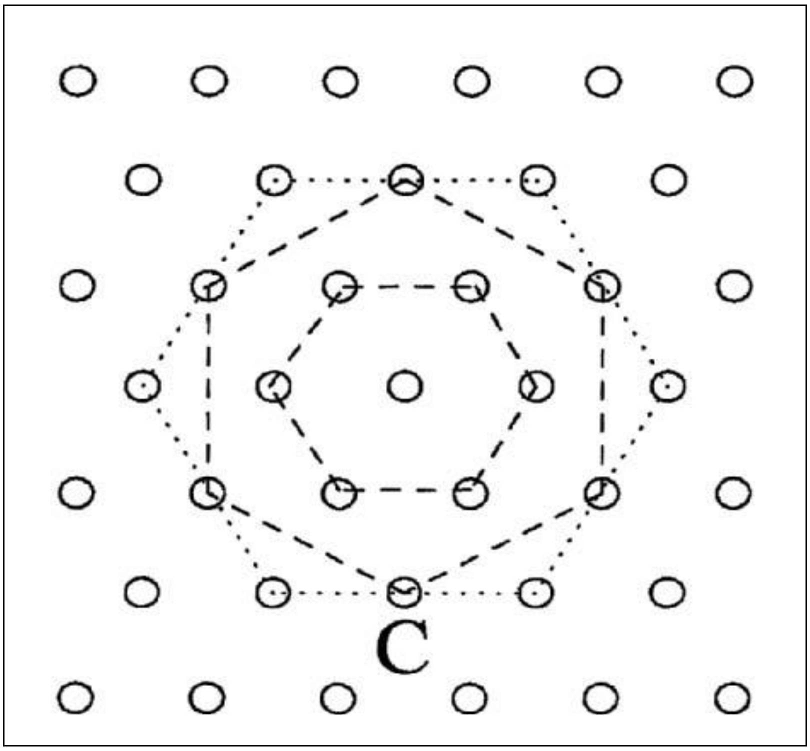


Fig. 1. A regular triangular pattern of plant positions within experimental unit. Each plant in its turn is considered as a candidate and compared to the plants occurring alongside three (grid C) surrounding aureoles.

The breeder may divide the selection field into parts such that growing conditions within each of the so-called grids are more uniform than across the whole field. This procedure is called grid selection (Bos and Caligari 1995). Separate analysis of variance was performed (Sokal and Rohlf 1981).

Table 2. ANOVA table formulas.

Source of variation	DF	SS	MS	F_3	Expected MS
$\bar{Y} - \bar{\bar{Y}}$ Among group (genetic lines) (grids)	a-1	SS_1	$\frac{SS_1}{(a-1)}$	$\frac{\text{MS groups}}{\text{MS within}}$	$a^2 + \frac{n}{a-1} \sum^{a} \alpha^2$
$Y - \bar{Y}$ Within group (error)(within genetic lines)	a (n-1)	SS_2	$\frac{SS_2}{a(n-1)}$		
$Y - \bar{\bar{Y}}$ Total	an – 1	SS_3			

2.1.1. Estimation of phenotypic, genotypic and environmental variation:

$$\sigma^2_p = \sigma^2_g + \sigma^2_e$$

Where, genotypic variance (σ^2_g) = $$\frac{MSV - MSE}{r \text{ or } n_o}$$

Where, MSV and MSE are mean sum of squares due to populations (varieties or treatments) and error, respectively. Environmental variance (σ^2_e) is equal to mean sum of squares for error (MSE). Phenotypic variance (σ^2_p) is comprised of (σ^2_g) plus (σ^2_e). In addition, r = number of replications (in case of equal sample size) (Singh and Singh 1994); while, N_o = average sample size (in case of unequal sizes) (Sokal and Rohlf 1981). The phenotypic and genotypic coefficient of variance was estimated using the formula developed by Burton (1952).

Phenotypic coefficient of variation $= \sqrt{Vph/\bar{x}} \times 100$

Genotypic coefficient of variation $= \sqrt{Vg/\bar{x}} \times 100$

Whereas $\sqrt{Vph}$ = Penotypic standard deviation.

$\sqrt{Vg}$ = Genotypic standard deviation.

$\bar{x}$ = Genotypic means.

2.1.2. Estimation of broad-sense heritability

The formula used for estimating broad–sense heritability was $h^2 = Ơ^2_g/Ơ^2_p$ (Allard, 1999); where $Ơ^2_g$ is the genetic and $Ơ^2_p$ is the phenotypic variance.

2.1.3. Estimation of released genetic gain (observed selection response)

Genetic gain (GG) was defined as the proportional increment in the phenotypic values achieved by selection. GG was calculated following Zheng *et al* (2006) as:

$$GG = \frac{X_S - X_C}{X_C} \times 100$$

Where X_S and X_C are the mean phenotypic value of progeny in selected and control populations, respectively.

2.2. Determination of chemicals composition in fresh leaves

For determination of leaf pigments, Total chlorophyll content, which used the fifth leaf from the shoots' growing tip, was measured by a SPAD 501 leaf chlorophyll meter (Yadava 1986). Total nitrogen in leaves was assayed in milligrams per gram dry weight by the micro-Kjeldahle method indicated by Pregl (1945). Total Potassium concentrations in leaves, in mg/g dry weight, were determined using a flam-photo metrically, according to the method described by Brown and Lilleland (1946). Total phosphorus (%) was estimated calorimetrically, according to the method described by Murphy and Riely (1962), as modified by John (1970). Calcium and calcium oxalate percentage were determined according to (Jackson 1976).

RESULTS AND DISCUSSION

3.1. Genetic parameters of yield and its components in two populations (original population and selected population) of spinach for breeding a new delayed bolting cultivar

Results in Table (3) and Fig. (2) revealed that mean values of the selected population as based on multitrait selection in respect to the traits of plant height (47.183 cm), no. of leaves per plant (50.10), central flowering stem length (54.280 cm), fresh weight per plant (14.743 g), leaf petiole length (39.541 cm), leaf blade length(19.971 cm) and bolting date (87.147 day) were significantly higher than those of the original population for the same traits (42.676 cm, 29.66, 41.384 cm, 144.89 g, 38.612 cm, 19.024 cm and 68.703, respectively). All the previously mentioned results are consistent with breeder's objectives.

Minimum values of phenotypic standard deviation (i.e., they were more homogeneous) for selected population of plant height (6.41), no. of leaves per plant (36.646), central flowering stem length (15.842), fresh weight per plant (103.778), dry weight per plant (6.826), leaf blade length (4.134) and bolting date (10.153) were decreased in comparison to original population for the same traits (8.729, 37.04, 26.832, 113.123, 8.854, 5.328 and 11.661, respectively). While phenotypic standard deviation value for selected population of leaf petiole length (8.916) was increased in comparison to original population (8.136). High phenotypic standard deviation indicated that the data are spread out across a large range of values (expressing the variability of a population). on the other hand, low standard deviation indicates that the data point to be close to the mean (expressing the homogeneity of a population).

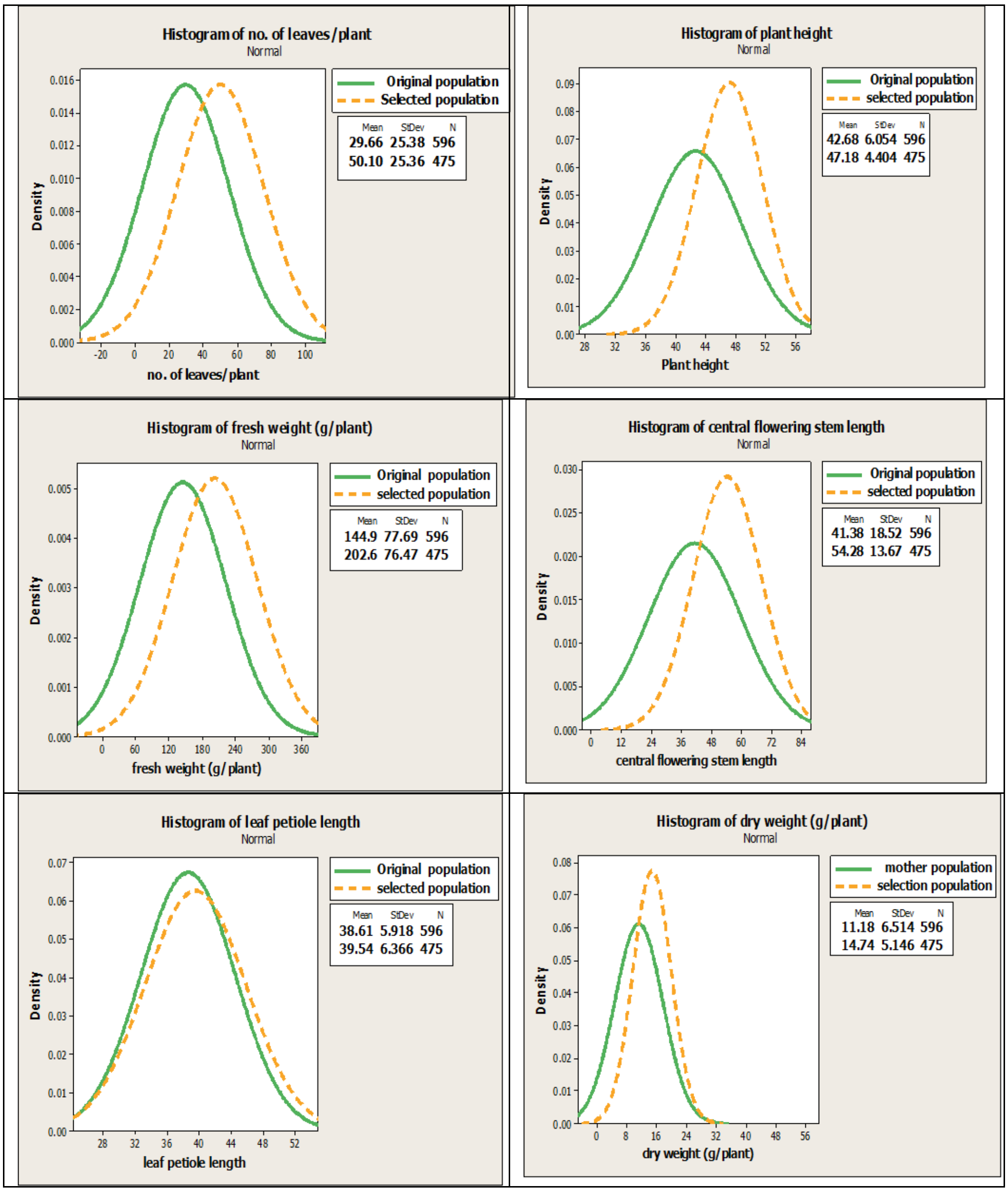
Table 3. Genetic parameters of yield and its components in two population (original population and selected population) of spinach for developing a new delayed bolting cultivar.

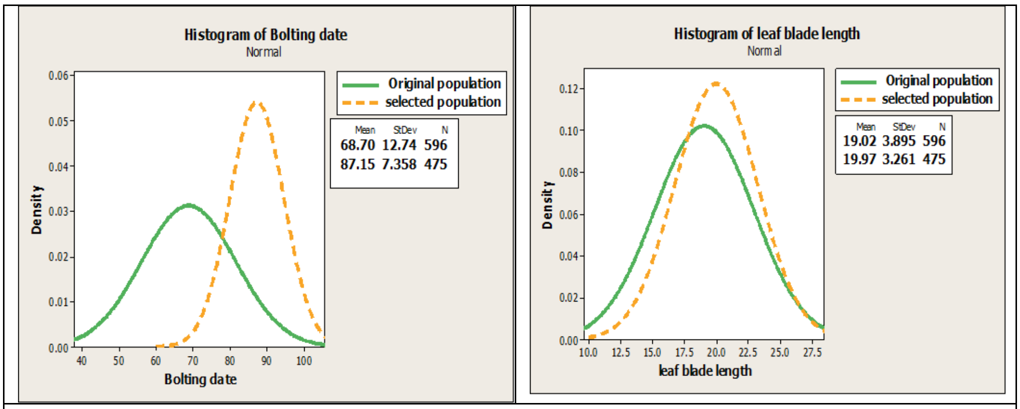
Genetics Parameters [1]	Traits[2]							
	PH	NLP	CFSL	FWP	DWP	LPL	LBL	BD
Original population								
N	596	596	596	596	596	596	596	596
Mean	42.676[b]	29.66[b]	41.384[b]	144.89[b]	11.182[b]	38.612[b]	19.024[b]	68.703[b]
SE Mean	0.248	1.04	0.759	3.18	0.267	0.242	0.160	0.522
SD	6.054	25.38	18.524	77.69	6.514	5.918	3.895	12.743
Variance	36.656	644.27	343.120	6035.95	42.436	35.018	15.175	162.377
Coef Var	14.19	85.57	44.76	53.62	58.25	15.33	20.48	18.55
Genetic variance	33.096	673.99	321.99	5808	29.7	29.898	13.398	33.996
Environmental variance	43.1	698	398	6989	48.7	36.3	15	102
Phenotypic variance	76.196	1371.9	719.99	12797	78.4	66.198	28.398	135.996
(GCV)%	8.795	46.553	27.247	61.971	15.952	8.769	8.357	7.623
(PCV)%	13.344	66.419	40.744	91.988	25.918	13.048	12.167	15.247
Heritability	0.43	0.49	0.44	0.45	0.37	0.45	0.47	0.24
GSD	5.752	25.961	17.944	76.21	5.449	5.467	3.66	5.83
PSD	8.729	37.04	26.832	113.123	8.854	8.136	5.328	11.661

Table 3. Cont.

Genetics Parameters [1]	Traits [2]							
	PH	NLP	CFSL	FWP	DWP	LPL	LBL	BD
Selected population								
N	475	475	475	475	475	475	475	475
Mean	47.183[a]	50.10[a]	54.280[a]	202.65[a]	14.743[a]	39.541[a]	19.971[a]	87.147[a]
SE Mean	0.202	1.16	0.627	3.51	0.236	0.292	0.150	0.338
SD	4.404	25.36	13.667	76.47	5.146	6.366	3.261	7.358
Variance	19.399	643.20	186.791	5847.98	34.91	40.523	10.632	54.147
Coef Var	9.33	50.62	25.18	37.74	3.384	16.10	16.33	8.44
Genetic variance	18.096	648.99	10.998	4326	6.498	38.598	6.198	76.2
Environmental variance	23	694	240	6444	40.1	40.9	10.9	26.9
Phenotypic variance	41.096	1342.9	250.998	10770	46.598	79.498	17.098	103.1
(GCV)%	6.207	36.293	4.545	46.67	6.599	9.87	5.564	9.859
(PCV)%	9.354	52.209	21.712	73.638	17.673	14.165	9.242	11.146
Heritability%	0.44	0.48	0.04	0.4	0.13	0.48	0.36	0.73
GSD	4.253	25.475	3.316	65.772	2.549	6.212	2.489	8.729
PSD	6.41	36.646	15.842	103.778	6.826	8.916	4.134	10.153
Genetic gain (R)	4.182	18.17	9.87	47.38	3.247	0.738	0.832	19.889
Genetic gain% (R%)	8.903	36.878	18.538	23.855	21.765	1.862	4.161	25.372

[1]: SE Mean = Standard error mean; SD = Standard deviation; Coef var = coefficient variance; GCV% = Genetic coefficient of variability; PCV% = Phenotypic coefficient of variability; GSD= Genetic Standard deviation; PSD= Phenotypic Standard deviation. [2]: PH = Plant height; NLP = number of leaves per plant; CFSL = central flowering stem length; FWP = fresh weight per plant; DWP = dry weight per plant; LPL =leaf petiole length; LBL = leaf blade length; BD =bolting date. Means within rows followed by the same letter are not statistically different at 5% level (Unpaired two-tailed Student's t-test.).


Histogram of no. of leaves/plant
Normal
Original population
Selected population
Mean StDev N
29.66 25.38 596
50.10 25.36 475
Density
no. of leaves/plant
Histogram of plant height
Normal
Original population
selected population
Mean StDev N
42.68 6.054 596
47.18 4.404 475
Density
Plant height
Histogram of fresh weight (g/plant)
Normal
Original population
selected population
Mean StDev N
144.9 77.69 596
202.6 76.47 475
Density
fresh weight (g/plant)
Histogram of central flowering stem length
Normal
Original population
selected population
Mean StDev N
41.38 18.52 596
54.28 13.67 475
Density
central flowering stem length
Histogram of leaf petiole length
Normal
Original population
selected population
Mean StDev N
38.61 5.918 596
39.54 6.366 475
Density
leaf petiole length
Histogram of dry weight (g/plant)
Normal
mother population
selection population
Mean StDev N
11.18 6.514 596
14.74 5.146 475
Density
dry weight (g/plant)



SD = Standard deviation, coef var = coefficient of variance and N = number of plants per population plants.

Fig. 2. Histograms of several quantitative traits in two populations (original population and selected population) of spinach.

Genetic standard deviation values for selected population of plant height, no. of leaves per plant, central flowering stem length, fresh weight per plant, dry weight per plant, and leaf blade length, (4.253, 25.475, 3.316, 65.772, 2.549 and 2.489, respectively) were decreased in comparison to original population, (5.752, 25.961, 17.944, 76.21, 5.449 and 3.66 respectively). Genetic standard deviation value for selected population of leaf petiole length and bolting date, (6.212 and 8.729, respectively) were increased in comparison to same characters for original population, (5.467 and 5.83, respectively). Heritability is a proportion, its numerical value will range from 0.0 (genes do not contribute at all to phenotypic individual differences) to 1.0 (genes are the only reason for individual differences, as explained by Colorado.edu. (http://psych.Colorado.edu/~carey/hgss/hgssapplets/heritability/heritability.intro.html.). Accordingly, the results showed remarkable changes in the values of heritability for all traits as affected by multitrait selection. Data of genetic coefficient of variance values for selected population of plant height, no. of leaves per plant, central flowering stem length, fresh weight per plant, dry weight per plant and leaf blade length (6.207%, 36.293%, 4.545%, 46.67%, 6.599% and 5.564%, respectively) were decreased in comparison to original population (8.795%, 46.553%, 27.247%, 61.971%, 15.952% and 8.357%, respectively) for the same traits. While, genetic coefficient variance values for selected population of leaf petiole length and bolting date (9.87% and 9.859%, respectively) were increased in comparison to original population (8.769% and 7.623%, respectively). These results agree with those of Goreta and Leskovar (2006) who reported that genetic variation among spinach for bolting was documented for many years such that late-bolting cultivars can be developed through breeding efforts.

Phenotypic coefficient of variance values for selected population of plant height, no. of leaves per plant, central flowering stem length, fresh weight per plant, leaf blade length and bolting date (9.354, 52.209, 21.712, 73.638, 17.673, 9.242 and 11.146, respectively) were decreased in comparison to original population (13.344, 66.419, 40.744, 91.988, 25.918, 12.167 and 15.247, respectively). On the other hand, phenotypic coefficient of variance for selected population of leaf petiole length (14.165%) were increased in comparison to original population; (13.048%). These results are in accordance with the findings of Pamilo (1988) who mentioned that the relation between the environmental and phenotypic variation is theoretically best understood, and experimentally best studied, in the case of specific polymorphism occurring due to variation at a single locus. It was also indicated that speculations have widely exceeded this simple case and the overall multilocus-heterozygosity is considered as adaptive strategy associated with the pattern of environmental hetero-geneity. It was reported that most of the spinach accessions are landraces which are highly adapted to specific environmental conditions and were considered as useful sources of genetic variation (Asadi and Hasandokht 2007). However, utilization of the genetic potential of different germplasms needs detailed knowledge about these genetic collections (Morelock and Correll 2008), including characterization, evaluation and classification. It was repeated that multivariate procedures are useful for characterization, evaluation and classification of germplasm collections when a large number of accessions are to be assessed for several traits. Besides, the usefulness of the multivariate procedures for handling morphological variation in plant genetic resources was proved in many crops (Ayana and Bekele 1999). Several investigates stated that the generated information of multivariate procedures might be useful for identifying different accessions that have explained traits for crossing, for planning efficient plant improvement program. Also, it is possible to establish core collections for revealing the structure of variation in plant genetic resources and for investigating some aspects of crop evolution (Perry and McIntosh 1991 and Ayana and Bekele 1999).

The percentage of genetic gain for selected population of all traits; (8.903, 36.878, 18.538, 23.855, 25.918, 21.765, 1.862, 4.161 and 25.372, respectively). All the previously mentioned results are consistent with breeder's objectives.

3.2. Number of male (M), female (F), and unbiased (NB) spinach populations improved by selection for developing a new delayed bolting cultivar

Results in Table (4) and Fig. (3 and 4) revealed that multitrait selection led to changes in populations averages at the level of both number of days required for flowering and the timing of the emergence of sexual cases and their numbers during the flowering period. The result showed that flowering began in the original population at 46 days; while, it was 66 days in the selected population. In addition, there was an increase in the number of total female flowers in the selected population compared to the original population (323 and 183 days, respectively).

The results also showed a decrease in the numbers of total male flowers in the selected population compared to the original population (27 and 207, respectively). The results also showed a decrease in number of total hermaphrodite flowers in the selected population compared to the original population (125 and 206, respectively).

Table 4. The number of male (M), female (F), and unbiased plants (NB) spinach population improved by selection for developing a new delayed bolting cultivar.

Population	Bolting date	Sexual cases			Total (plant)
		M	F	NB	
Original population	46 day	42	-	-	42
	53 day	77	-	2	79
	60 day	63	3	37	103
	67 day	18	22	56	96
	74 day	7	48	51	106
	81 day	-	56	31	87
	88 day	-	54	29	83
	Total (plant)	207	183	206	596
Selected population	66 day	12	-	5	17
	73 day	15	-	5	20
	80 day	-	74	21	95
	87 day	-	96	51	147
	94 day	-	153	43	196
	Total (plant)	27	323	125	475

Note. Flowering began ca. 46 days after germination and continued to more than 88 days in mother population and more than 94 days in selected population

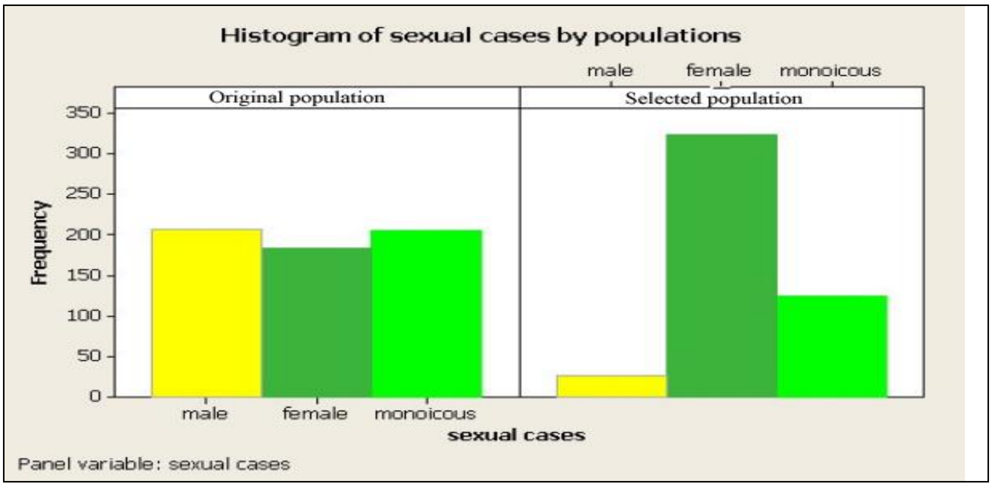


Fig. 3. Histogram of sexual cases in two populations (original population and selected population) of spinach for developing a new delayed bolting cultivar.

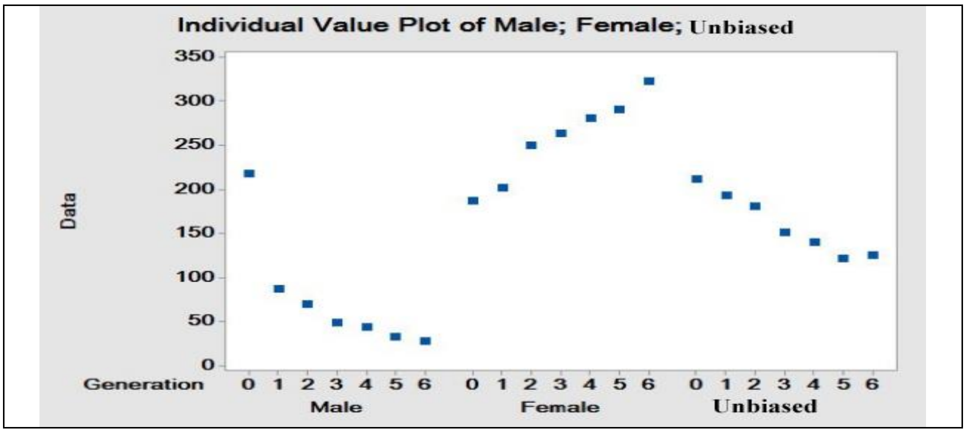


Fig. 4. Histogram of sexual cases through six generations of multitrait selection based on selection index under high temperature in early season for developing a new delayed bolting cultivar of spinach.

In addition, the results showed a decrease in the number of male and hermaphrodite flowers in the selected population (27 and 207, respectively) compared to original population (125 and 206, respectively). Moreover, the results showed that the flowering period of the selected population was less than that of the original population (28 and 46 days, respectively). As reported by (Parlevliet 1968), one of the most important characters of a spinach cultivar is its earliness. This is, in his opinion, determined by the rate of bolting and so by the rate of flower formation, as both coincide almost completely. It was also indicated that the rate of flower formation is determined by three factors: day length (photoperiodic) requirement, rate of growth. (by varying the light intensity and temperature, the rate of flower formation appeared to be related to the rate of growth) and balance between growth and development. Thus, in his breeding for earliness, it was believed that the genetic control of earliness is most likely a polygenic one as rate of growth and balance between growth and development can be expected to be polygenically controlled. This also means that earliness and yield are linked to each other. To select for the required earliness without unwanted yield effects, or to increase yield without changing the earliness, selection methods have been described, which are directed to each of the three components individually. Each of the components influencing earliness probably has its own genetic basis. It is unknown whether the day length requirement in spinach is genetically controlled by a few or many genes. He added that the research with other crops does not provide a clear answer, as monogenic and polygenic control of day length reaction has been observed.

The combination of physiological indexes and morphological index would be helpful to the study of the bolting trait. Genetic variation among spinach and resistance to bolting among different cultivars for bolting has been documented for many years (Goreta and Leskovar 2006). The bolting stage (BS), the elongate stem length (ESL), days to flowering (DTF), days to bolting (DTB) and Number of leaves in flowering (NLF) are used to identify bolting index. There is no uniform international standard for the bolting trait. At present, the identification of morphological index of bolting is the common evaluation criteria for the bolting traits, but this is highly subjective as mentioned by Guoliang *et al*. (2020). Jung, *et al* (2009) pointed out that it is very important to understand the mechanisms of reproductive phase (flowering and bolting) transition in spinach. Change the seasonal timing of flowering could allow the production of novel varieties adapted to local climatic and environmental conditions. Mutasa-Göttgens *et al* (2010) revealed that breeding targets for the control of bolting in cultivation must meet the dual, but opposing, needs for high yielding crops and seed production. It was found that late-bolting spinach is preferred to increase market value, while early-bolting spinach is beneficial for seed production. As a long day plant, the photoperiod has a great limitation on the year-round production of spinach. Early-bolting or late-bolting is both good for grown spinach if it lacks the sensitivity to photoperiod. So further analysis is needed to understand both the early-bolting and late-bolting about the photoperiod in spinach (Chun *et al* 2000).

3.3. Yield and chemical composition of original population and selected populations of spinach for developing a new delayed bolting cultivar

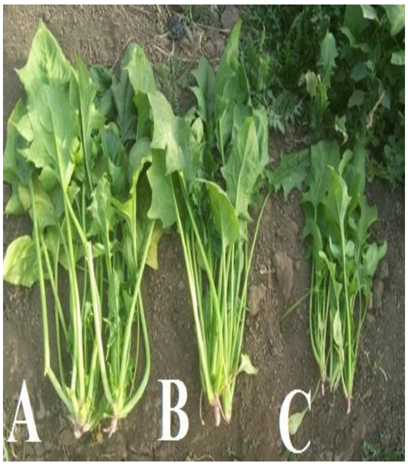
Results in Table (5) revealed that mean values of the selected population using multitrait selection in respect to the traits of yield (2.808 kg/m^2), yield (11.234 ton/feddan), P% (0.777 mg/100g), total chlorophyll (27.296 mg/100g) and Ca% (2.059 mg/100g) were significantly higher than those of the original population for same traits (1.361 kg/m^2, 5.446 ton/feddan, 0.412 mg/100g, 26.35 gm and 2.053 mg/100g, respectively). While, values of N%, K%, Oxalate% and Ca oxalate were statistically insignificant compared with original population. Biosynthesis of organic compounds is a complex process dependent on genetic factors (Probst *et al* 2011). In addition, one of the physiological functions of secondary metabolites is to assist/protect plants against environmental stresses (Prochnow *et al* 2016). Studies have shown that the synthesis of these metabolites is affected by the local climate, including factors such as rainfall, temperature, relative humidity, and photoperiod (Schmidt *et al* 2017).

Table 5. Yield and chemical composition evaluated in two populations (original population and selected population) of spinach.

Character[1]	Original population	Selected population
yield kg/m^2	1.361[b]	2.808[a]
yield ton/feddan	5.446[b]	11.234[a]
N%	1.048[a]	1.129[a]
P%	0.412[b]	0.777[a]
K%	0.308[a]	0.328[a]
Total chlorophyll	26.35[b]	27.296[a]
Ca%	2.053[b]	2.059[a]
Oxalate %	3.135[a]	3.129[a]
Ca oxalate	4.791[a]	4.712[a]

[1]: % N = total nitrogen (%), P = total phosphorus (%), K = total potassium (%), Ca = total calcium (%), and Ca oxalate = calcium oxalate (%). means within rows followed by the same letter are not statistically different at 5% level (Unpaired two-tailed Student's t-test.).

The results showed that the present selection program based on multitrait selection under high temperature in early season had clear effects on genetic and phenotypic parameters. These results are in accordance with the desired breeding objectives. Moreover, the multitrait selection based on selection indices showed differences consistent with the goal of the selection program in chemical content of leaves that was significantly observed in the selected population compared to chemical content of leaves in the original population.

Intersexual differences in spinach (*Spinacia oleracea* L.).		
		
Pic. 1. The main three types of sexual conditions in spinach i.e., females (A), monoecious (B), and extreme males (C).	**Pic. 2. Female bias in spinach plant. (http://www.ipmcenters.org/cropprofiles/docs/waspinachseed.html).**	**Pic. 3. Monoecious male in spinach plant. (http://www.ipmcenters.org/cropprofiles/docs/waspinachseed.html).**

	
Pic. 4. The early bolting extreme males (C) compared with vegetative males (D) in spinach.	**Pic. 5. The early bolting extreme males (C) compared with female plants (A) in spinach.**
	
Pic. 6. Male flowers in spanich plants. (https://www.slideshare.net/S7w4X/zwv254?qid=20bd6ab9-8c38-4822-bd92-38cd7aece3b0&v=&b=&from_search=1)	**Pic. 7. Female flowers in spanich plants. (https://www.slideshare.net/S7w4X/zwv254?qid=20bd6ab9-8c38-4822bd9238cd7aece3b0&v=&b=&from_search=1)**

REFERENCES

Allard, R.W. (1999). 'Principles of plant breeding. 2nd edn. John Wiley and Sons, Inc. New York., London. 471 P.

Asadi, H. A. and M. R. Hasandokht (2007). An evaluation of genetic diversity of Iranian spinach landraces. Iran J. Hort. Sci. 38:257–265.

Ayana, A. and E. Bekele (1999). Multivariate analysis of morphological variation in sorghum (*Sorghum bicolor* (L.) Moench) germplasm from Ethiopia and Eritrea. Gen. Resour. Crop Evol. 46:273–284, DOI: 10.1023/A:1008657120946.

Bos, I. and P. Caligari (1995). Selection Methods in Plant Breeding. 1st Edition, Chapman and Hall Ltd. London. 347 pp.

Brown, J. G. and O. Lilleland (1946). Rapid determination of potassium and sodium in plant materials and soil extracts by flame photometer. Proc Am. Soc. Hortic. Sci. 48:341–346.

Burton, G. W. (1952). Quantitative inheritance in grasses. Proc. 6th Int. Grassland Cong. 11: 277-283.

Chitwood, J., A. Shi, B. Mou, M. Evans, J. Clark, D. Motes, P. Chen and D. Hensley (2016). Population structure and association analysis of bolting, plant height, and leaf erectness in spinach. HortScience. 51:481-486.

Chun, C., T. Kozai, C. Kubota, and K. Okabe (2000). Manipulation of bolting and flowering in a spinach (*Spinacia oleracea* L.) transplant production system using artificial light. Acta Hort.515:201–206.

Dicoteau, D. R. (2000). Vegetable Crops. 221–237.Prentice Hall. New Jersey. USA.

Goreta, S. and D. I. Leskovar (2006). Screening spinach cultivars for white rust resistance and bolting. HortTechnology 16(1): 162-166.

Guoliang, L. i., M.Z.L. Qing, X. u. Zhaosheng, Z. Helong and Q. Wei (2020). Management of the Tolerance to Bolting for Spinach (*Spinacia oleracea* L.) Retrospect and Prospects Additional contact information Biomedical Journal of Scientific and Technical Research 28(1): 21260-21264.

Imani, M. R., J. Abrahimi and A. Zarbakhsh (2004). Development of Advnced Spinach Population From Varamin genotypes using Mass selection (https://agris.fao.org/agrissearch/search.do?recordID=IR2007000128).

Jackson, M. L. (1976). Soil Chemical Analysis" Prentice Hall of India proviate limited, New Delhi, India, Chapt. 8: 183- 204

John, M.K. (1970). Colorimetric determination of phosphorus in soil and plant material with ascorbic acid. Soil Sci 109:214–220.

Jung, C. and A. E. Muller (2009). Flowering time control and applications in plant breeding. Trends in Plant Science 14(10): 563-573.

Khattak, J. Z. K., J. L Christiansen., A. M. Torp and S. B. Andersen (2007). Genic microsatellite markers for discrimination of spinach cultivars. Plant Breeding. 126: 454-456.

MINITAB (2010). MINITAB 16. MINITAB User's guide. Minitab Inc, Harrisburg, Pennsylvania USA.

Morelock, T. E. and J. C. Correll (2008). Spinach. In: J. Prohens and F. Nuez, editors, Handbook of plant breeding, Vegetables I, *Asteraceae*, *Brassicaceae*, *Chenopodicaceae*, and *Cucurbitaceae*. Springer, New York. p. 189–218.

Murphy, J. and J. P. Riely (1962). A modified single solution method for the determination of phosphorus in natural waters. Anal Chim Acta 27:31-36.

Mutasa-Göttgens, E. S., A. Qi, W. Zhang, G. Schulze-Buxloh, A. Jennings, U. Hohmann, A. E. Müller and P. Hedden (2010). Bolting and flowering control in sugar beet: relationships and effects of gibberellin, the bolting gene B and vernalization. Ao B Plants 2010: plq12

Nonnicke, I. L. (1989). Vegetable Production 476-484 Van Nostr and Runhold.

Pamilo, P. (1988). Genetic Variation in Heterogeneous Environments. Annales Zoologici Fennici. 25(1): 99- 106. Accessed October 23, 2020.
http://www.jstor.org/stable/23734712.

Parlevliet, J. E. (1968). Breeding for earliness in spinach (*Spinacia oleracea* L.) as based on environmental and genetic factors. Euphytica 17: 21-27.

Perry, M. C. and M. S. McIntosh (1991). Geographical patterns of variation in the USDA soybean germplasm collection. I. Morphological traits. Crop Sci. 31:1350-1355, DOI: 10.2135/cropsci1991.0011183X003100050054x.

Pregl, F. (1945). Quantitative organic micro-analysis, 4th edn. J. and A. C., Ltd. London, pp 203–209

Probst, I.S., J. M. Sforcin, V. L. M. Rall, A.A.H. Fernandes and J. A. Fernandes (2011). Antimicrobial activity of propolis and essential oils and synergism between these natural products. Journal of Venomous Animals and Toxins Including Tropical Diseases 17:159-167.

Prochnow, D., B. S. Altissimo, J. C. Silva, D. Meira, B. O. Caron, B. M. Heinzmann and D. Schmidt (2017). Chemical composition of the essential oil of *Aloysia triphylla* (L'Hér) Britton due to water déficit and seasonality. Boletim Latinoamericano y del Caribe de Plantas Medicina is y Aromáticas16:121-128.

Rosa, J. T. (1925). Sex expression in spinach. Hilgardia 1:258.

Schmidt, D., B. O. Caron, N. R. V. Menegat, E. F. Elli, J. R. Stolzle, F. Schwerz, J. Sgarbossa and D. Prochnow (2017). Biomass and morphological parameters of lemon verbena (*Aloysia triphylla*) under different shading levels during different seasonal conditions. Australian Journal of Crop Science 11:387-394.

Shetty, A.A., S. Magadum and K. Managanvi (2013). Vegetables as Sources of Antioxidants. Journal of Food and Nutritional Disorders. J. Food NutrDisor 2:1.

Singh, R.K. and P.K. Singh (1994). A Manual on Genetics and Plant Breeding Experimental Techniques. 1st Edition, Kalyani Publishers India. 134 P.

Sneep, J. (1958). The present position of spinach breeding. Euphytica 7:1-8.

Sokal, R. R. and F. J. Rohlf (1981). Biometry: The Principles and Practice of Statistics in Biological Research. 2nd Edition, Freeman Ltd. USA. pp. 221-281.

Swiader, J. M. and G. W. Ware (2002). Producing Vegetable Crops. Danville, Illinois: Interstate Publishers, INC.

Vural, H., D. Esiyok and I. Duman (2000). Vegetable Production. Bornova, Izmir, Turkey: Aegean University Press. 440 p.

Yadava, U. L. (1986). A rapid and non-destructive method to determine chlorophyll in intact leaves. HortScience 21:1149–1450

Yamori, W., K. Noguchi and I. Terashima (2005). Temperature acclimation of photosynthesis in spinach leaves: Analyses of photosynthetic components and temperature dependencies of photosynthetic partial reactions. Plant, Cell and Environment 28(4): 536– 547.

Zeevaart, J. A. D. (1971). Effects of Photoperiod on Growth Rate and Endogenous Gibberellins in the Long-Day Rosette Plant Spinach. Plant Physiol 47 (6): 821-827

Zheng, H., G. Zhang, X. Liu and X. Guo (2006). Sustained response to selection in an introduced population of the hermaphroditic bay scallop *Argopecten irradians* Lamarck (1819). Aquaculture 255:579–585.

الانتخاب ضد النباتات متطرفة الذكورة والمذكرة الخضرية فى السبانخ كطريقة فعالة لاستنباط صنف جديد متأخر التزهير

حامد حسن حامد[1] و أحمد عبدالهادي سيدعبدالوهاب الاسلامبولى[2]

١. قسم بحوث تكنولوجيا انتاح تقاوي الخضر – معهد بحوث البساتين –مركز البحوث الزراعية

٢. قسم بحوث انتاج الخضر تحت ظروف الزراعة المحمية – معهد بحوث البساتين –مركز البحوث الزراعية

هدفت تلك الدراسة الى استنباط صنف جديد من السبانخ متأخر التزهير وذلك بإستخدام الانتخاب متعدد الصفات من خلال استبعاد النباتات المذكره الحادة و المذكرة الخضرية من عشيرة السبانخ مع زيادة نسبة النباتات المؤنثة بطرزها المختلفه. أجريت هذه الدراسة في مزرعة بحوث الخضر– قها (محافظة القليوبية ، مصر). تم تدوين النتائج الوراثيه والمظهريه لصفة المحصول ومكوناته وكذلك التحليل الكيميائي لمحتوى الأوراق. تم إستخدام صنف سبانخ "دقي" لإجراء البرنامج الإنتخابي عليه والذي تم إستنباطه بواسطة أقسام بحوث الخضر التابعه لمعهد بحوث البساتين وقد بدأ برنامج الإنتخاب في الأول من سبتمبر لعام ٢٠١٤ واستمر لعام ٢٠١٧ (أربع سنوات) حيث أجريت تجربة مقارنة بين العشيرة الأم والعشيرة المنتخبة خلال عامي ٢٠١٨ و ٢٠١٩ – تحت ظروف درجة حرارة المرتفعه في بداية الموسم – لقياس مدى التقدم في تحقيق أهداف برنامج الإنتخاب. كان لبرنامج الانتخاب متعدد الصفات تحت ظروف درجة الحرارة المرتفعه في بداية الموسم تأثيرات واضحة على التباينات الوراثية والمظهرية للعشيرة المنتخبة ، وهذه النتائج تتوافق مع أهداف التربية. علاوة على ذلك ، أظهر الإنتخاب متعدد الصفات اختلافات تتسق مع أهداف برنامج الإنتخاب في مختلف صفات النمو الخضري و تاخر الاندفاع للتزهير و المحتوى الكيميائي للأوراق التي تحسنت بشكل كبير في العشيرة المنتخبه مقارنة بالعشيرة الأم.

المجلة المصرية لتربية النبات ٢٤(٣): ٥٧١– ٥٩٧ (٢٠٢٠)