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STUDY POLYPLOIDY CHROMOSOME IN FABA BEAN BY USING COLCHICINES

Kamal Benyamin Esho

* Mosul University, College of Agriculture and Forestry Department of Horticulture and Landscape Design

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ABSTRACT

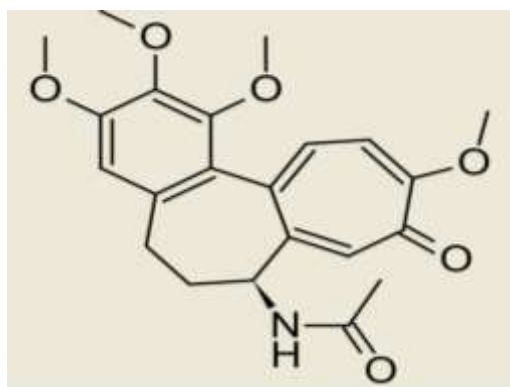
The study was conducted at the vegetable research field, department of horticulture and landscape design, college of agriculture and forestry, Mosul University during growing season 2017/2018, to study polyploidy chromosome in faba bean by using colchicines. The seeds of variety Sakiz were soaking in colchicines 0, 1000, 2000 and 3000 ppm for 8, 16 and 24 hours, with three replicate in RCBD Factorial experiment. The result showed that the 2000 ppm of colchicines gave the high value of number of stomata per cm^2 (19.76) and in stomata length (13.07 μm). While 3000 ppm of colchicines gave the significant effect on the diameter of the stem at true leaf, number of pods/plant, total chlorophyll, plant length at first harvesting and in total seeds yield/hectar. The soaking period at 24 hours gave a significant effect on the number of stomata per cm^2 , stomata length, number of pods/plant, pod length, plant length at first harvesting and in total yield per unit.

The interaction between colchicines concentration 2000 and 3000 ppm with 24 hours gave the superior result in diameter of stem at first true leaf (5.99 cm), number of stomata per cm^2 (22.07), number of pods/plant (33.00), seed weight (2.10 gm), seed length (2.55 cm) and in total seeds yield/hectar (4682.55 kg/hectar).

KEYWORDS: Effect of colchicines concentration, Effect of soaking period.

INTRODUCTION

Broad bean *Vicia faba* L. of the Fabaceae family is the fourth most important food legume crop (Kay, 1979). The chromosome number of broad bean is ($2n=12$) with one pair of metacentric and five pairs of subtelocentric chromosome. It is cultivated as green / or dry seeds yield (Hasan, 2011), green pods and green seeds consumed (Morton, 1976). Broad bean makes the human body healthy, that is due to broad bean is nutritionally important vegetable crops which containing 20-30% protein (Anon, 2005). Broad bean in turn plays an important role in the improving soil fertility, through nitrogen fixation in a soil (Khandil, 2007), in addition to the biological effects. It is one of the important crop belongs to Fabaceae family, it was cultivated to produce dry seeds and green yield. It is considered as a nutritional and economic crop, the dry seeds contains a high percentage of carbohydrate 58.41%. In many country crop cultivation face a problem which represent in flowers and recent fruits set drop in February, it reach 60-80%. The production of polyploidy plants has been one of the objectives of breeding programs for the aim of obtaining differentiated genotypes in order to highest traits of agronomic interest. The effects of polyploidy on plant traits are also important to horticulturists and plants breeders, polyploidy is one of the successful methods in the improvement of some plants such as apples and broad bean (Joshi and Verma (2004).



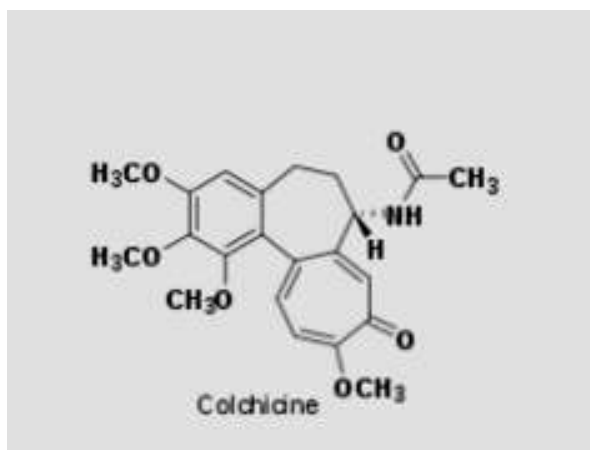


Fig (1) The structural of Colchicines s

A colchicines s emulsion affected the mitotic cycle, and caused autotetraploid and giant growth (Dhillon , 1970 ; Macleod , 1972) . Colchicines s induced chromosome doubling and genetic improvement of some crop plants has been achieved in pea (Kutty and Kumar, 1983). Colchicines is known to interfere with the dynamics of microtubules and to destroy karyokinetic spindle in cells (Dustin 1978). The colchicines s -sensitive period stretched over a range of premeiotic and meiotic stages in different species (Loidl 1990). Colchicines s causes a reduction of chiasma formation between homologous arms of conventional chromosomes but not between the homologous arms of isochromosomes (Driscoll and Darvey 1970, Vega and Feldman 1998). Colchicines s (C₂₂H₂₅O₆N) is a hormonal plant product extracted from the seeds and bulbs of the plant *Colchicum autumnale* L., it is anti-mitotic agents, it is used to induce polyploidys (Stanys *et al.*, 2004) . Parshant and Verma (2004) reported in their study the they success of induction of polyploidy in broad bean when they used 50% of clochicine in broad bean and presoaking the seeds in distilled water for 20 h proved more effective in inducing the polyoliody , typical polypliod traits like plant height bigger laves , flowers and pods ,pollen fertility and number of seed per pod . In forage plant, as well as in other species, colchicines has been the antimitotic substance most used for inducing polyploidy (Queensberry *et al.*, 2010; Souza-Kaneshima *et al.*, 2010). Vijayalakshmi and Singh (2011) showed in their studies that the number of seeds/pod decreased at 5 ppm, 10 ppm, 15ppm and 20 ppm and increased at 25 ppm of colchicines s on cluster bean, while weight of seeds /pod increased at 5, 10 and 15 ppm, and decreased very significantly at 20 ppm, in conclusion colchicines s increased the vegetative parapeters like root length and shoot length. Colchicines treatment was reported as one of the best tools of inducing and enhancing genetic variability in some food crops within a very short period of time (Gnanamurthy *et al.*, 2013). Abiola *et al.*, (2014) reported in their studies effects of 0.1% aqueous solution of colchicines s for different periods of time viz ; 0 ,2.4 and 6 hours were tested on the quantitative and yield traits of cowpea variety , treatment was significant (p=0.05) for plant height , number of leaves per plant . Number of nodes on main stem and number of days to first flowering.

The aim of this study was to achieve the polyploidy chromosome in faba bean by soaking the seeds in different concentration of colchicine's on vegetative growth and yield parameters.

MATERIALS AND METHODS

The study was conducted at the vegetable research farm, department of horticulture and landscape design, University of Mosul, during growing season 2017/2018. To study polyploidy chromosome in faba bean by using colchicine's. the seeds of variety Sakiz from (local market) of faba were soaking in different colchicines solution concentration which were 0, 1000, 2000 and 3000 mlg/l. (ppm), and different periods were 8, 16 and 24 hours, the seeds treatment were sowing at 15/11/2017 at the rows 1m length and width 0.75 m two rows for each treatment, in a randomized complete block design in factorial with three replications. Data on 5 plant were collected randomly selected plants per replicate for each plot the traits via: seed germination , stem length (cm) , stem diameter (cm) , number of branches per plant , the 50% of flowering , number of flowers in cluster , number of stomata per cm² , length and diameter of stomata (μm) , number of pods per plant , length and diameter of pod (cm) , pod weight (gm), length and diameter of seed (cm) , seed weight 100 seeds (gm) , number of seeds per pod

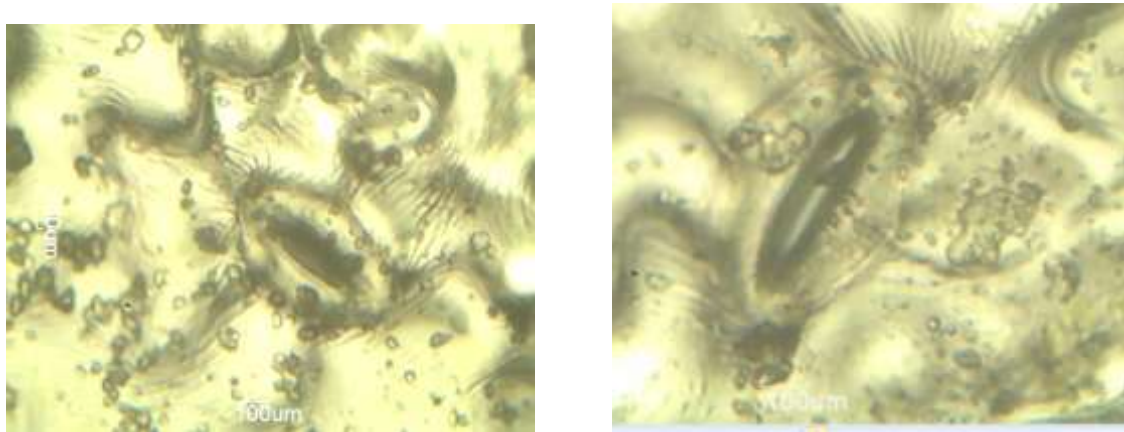
and concentration of total chlorophyll (SPAD). Anatomy of stomata was analysis at the library from department of food sciences, Mosul University

Data were analyzed according to the design using statistical package for SAS (2000), analysis of variance (ANOVA), was performing, and flowed by test Duncan's significant difference at 0.05% level of significance computation for mean separation (Steel and Torrie 1982).

RESULTS AND DISCUSSIONS

Effect of colchicines s concentration:

The Figer (2) showed the stomata in plant treatment with colchicines s concentration



(1)

(2)

Fig(2) : the stomata in plant polyploidy (1=control , 2= colchicines s)

Showed that the colchicines s effected on the size of stomata compared with non-treatment plant with colchicines s, the increase in cell size was reflected in the pronounced increase in the size of stomata of colchiploids (Fig 2) . Also table (1) revealed that the colchicines s concentration affected on the most traits excepted on the seed weight, seed length and seed diameter. The percentage of germination was superior in (0) colchicines s concentration which is 92.57% compared with 1000, 2000, and 3000 ppm, the stem length at the first true leaf was significant at 0 colchicines s concentration (5.95 cm) than the 1000, 2000, 3000 ppm of colchicines s concentration, but the diameter of stem at first true leaf was higher at 3000 ppm of colchicines s than the other treatment. The number of stomata (cm²) and stomata length (µm) were higher at 2000 ppm. of colchicines s compared with the others treatment .The stomata diameter and the number of days for 50% flowering were higher than the other treatment at 0 ppm of colchicines s (4.63 (µm) , 87.67) respectively , the colchicines s concentration at 2000 ppm gave a less days for flowering , it means that the colchicines s was earliest for flowering . the colchicines s concentration at 3000 ppm gave a high significantly in the number of pods / plant , chlorophyll (SPAD) , plant length at the first harvesting and in total seeds yield /hectar , which were (27.00 , 105.61 , 83.47 and 4401.25) respectively compared with the other concentration treatment. The concentration of colchicines s was not effected significant on the pod diameter, seed weight, seed length, seed diameter and in the number of branches per plant.

Effect of soaking period:

Table (2) showed the effect of soaking period of seed on the traits of faba , it revealed the soaking period of treatment effected significant on the percentage of germination(%) , number of stomata per cm² , stomata length (µm) , number of days for 50% flowering , number of pods/plant , pod length (cm) , plant length at the first harvesting and in total seeds yield / hectar. The soaking period at 8 hours gave a higher value in percentage germination and in number of days for 50% flowering , while the soaking period at 24 hours were gave the significant higher value in number of stomata per cm² , stomata length (µm) , number of pods /plant , pod length , plant length at first harvesting and in total seeds yield / hectar.

The interaction between colchicines s and soaking period:

Table (3) indicated the interaction between colchicines s concentration and soaking period on the traits in faba plant. It was effected on most traits accepted the pod length and seed diameter. The interaction treatment between

24 hours soaking and 0 ppm of colchicines s gave a higher value in percentage germination (93.90%) and stem length at first true leave (5.99 cm) compared with the other treatment of interaction. While the interaction between 24 hours soaking with 2000 ppm of colchicines s gave a significantly effected on the diameter of stem at first harvesting (4.67 cm) and number of stomata per cm^2 (22.07) . the interaction between 24 hours soaking period and 1000 ppm colchicines s concentration gave a high value in stomata length (14.27 (μm)) , stomata diameter 5.70 (μm) , in number of flowerst / cluster (5.20) and in pod length 21.89 cm) .In the other hand the interaction between soaking period 16 hours and 3000 ppm of colchicines s concentration gave a high significant total chlorophyll (113.27) and high value mean in number of branches per plant (5.87) . Where ever , the interaction between soaking period 24 hours and 3000 ppm of colchicines s concentration gave a higher significant effect on the seed weight (2.10 gm) , seed length (2.55 cm) and total seeds yield / hectar (4682.55 kg/he.) compared with the other interaction treatment.

Table (4) showed that the range separated among the studies traits from 0.54 to 4682.55, so the other traits were between this two ranges. The wide range was 4682.55 for total seeds yield and close range was 0.67 for seed diameter .This result showed that affected higher which gave positive side, and flowed by total chlorophyll which was the second imported stage of range was 67.05- 113.27, then for plant length at first harvesting the range was 66.60-85.73.This result indicated that the cause effect for tetraploid plant, this result agree with Guofeng *et al.*, (2007); Mensah *et al.*, (2007). Also in table (4) the R-square was more than 0.7 for most traits excepted for stomata diameter, seed diameter and pod diameter which were less than 0.40.

DISCUSSION

From this study the seed treatment by soaking period and concentration of colchicines s, indicated that the germination was low when use of colchicines s with 2000 and 3000 ppm compared with control (0) ppm which gave high germination this was for the toxic effect of colchicines s. This result agreed with AbdelRahem(1977) who revealed that the germination of seed was lowest when use high concentration of colchicines , and interaction between colchicines s at 2000 and 3000 pp at 24 hours soaking were successful for gave a significant effected on some traits under the studied via diameter of stem at first true leaf , number of stomata / cm^2 , stomata length (μm) , number of pods/plant , total chlorophyll , plant length at first harvesting and in total seeds yield /plant .The colchicines s concentration revealed this typical traits feature of induced polyploidy plant they were slower in growth initially , but later indicated significant gigantism as they were bigger than control plant and they had thick stems and dark green leaves which were also more than the control diploids (table 1) , which is of economic importance with respect to pest and high seed yield. (Verma and Raina , 1991 , in *Phlox* sp. ; Prashant and Varma , 2004 in faba bean ; Vijayalakshmi and Singh ,2011 on cluster bean ; Abiola *et al.*, 2014 in cowpea) This indicated the cause of the affected of Colchicines which causes a reduction of chiasma formation between homologous arms of conventional chromosomes but not between the homologous arms of isochromosomes (Driscoll and Darvey 1970, Vega and Feldman 1998). The morphological mutant can be utilized for traits of faba genotypes , colchicines s induced marked vegetative growth leading to the formation of large plants with more pigment in leaves , branches /plant , and in seed traits .(Shanko , 2017).

The information of this studied result obtained by comparing the mitotic index between control and treated tissues as seeds, which gave a little about the effects of treatment on the mitotic cycle, a change in the rate of cell multiplication which affects all stage of the mitotic cycle equally (Evans *et al.*, 1956) .The mitotic cycle was retarded as shown by the relatively large number of cell to found in the prophase as compared with control (0 ppm) of colchicines s (Hernadi *et al.*, 1974). A mong chemical mutagenus colchicines s treatment is one of best tools of inducing and enhancing genetic variability in crops within a very short time span (Gnanamurthy *et al.*, 2013) . Also this chemical is known to inhibit mitosis in a wide variety of plant cells by interfering with the orientation and structure of the mitotic fibers and spindle fiber (Khan and Goyal , 2009) . The survival rate of plant observed in different treatments and period soaking of colchicines s indicated the ability of this genotype of faba in circum venting the toxic effect in variable degrees of tolerance (Table 1, 2, and 3) . This result contrary to the results obtained from, Pasakinskiene (2000) and Nair (2004).

CONCLUSION

The study reveals that the soaking period 24 hours with concentration 2000 to 3000 ppm of colchicines gave the best result for most morphological traits and the higher seeds yield per unit area, further studies are being carried out to elucidate the effect of colchicines on agronomic traits.

Table (1) : Effect of clochicine concentration on the traits of faba bean during growing season 2017/2018*

Traits	Colchicines Concentration (ppm)			
	0	1000	2000	3000
Percentage of germination	92.57 a	82.54 b	68.32 c	52.69 d
stem length at first true leaf (cm)	5.95 a	3.79 b	4.90 c	3.11 c
diameter of stem at first true leaf (cm)	3.85 b	2.57 c	3.90 b	4.13 a
number of stomata /cm ²	19.73 a	15.09 b	19.76 a	19.48 a
stomata length (μm)	10.56 c	12.72 b	13.07 a	12.36 b
stomata diameter (μm)	4.63 a	4.21 a	2.49 b	2.63 b
number of days for 50% flowering	87.67 a	60.08 b	55.61 c	56.50 c
number of floweret /cluster	4.15 a	4.82 a	4.80 a	4.52 a
number of pods/plant	20.91 c	27.00 a	23.92 b	27.00 a
pod length (cm)	10.91 c	19.04 c	17.81 b	17.81 b
pod diameter (cm)	2.05 a	2.05 a	1.95 a	1.98 a
Chlorophyll (SPAD)	68.01 d	74.11 c	80.29 b	105.61 a
seed weight (gm)	1.58 a	1.37 a	1.47 a	1.74 a
seed length (cm)	2.14 a	2.29 a	2.20 a	2.38 a
seed diameter (cm)	0.59 a	0.66 a	0.62 a	0.67 a
plant length at first harvesting (cm)	86.09 a	76.34 b	77.35 b	83.47 a
number of branches /plant	5.16 a	5.16 a	5.06 a	5.48 a
seeds yield (kg/hectar)	3621.93 b	3745.00 b	4374.49 a	4401.25 a

- Mean with the same letters no significant differences according to Duncan's multiple range test at probability 0.05.

1=percentage of germination , 2=stem length at first true leaf , 3=diameter of stem at first true leaf , 4= number of stomata /cm² , 5= stomata length (μm) ,6= stomata diameter , 7= number of days for 50% flowering , 8= number of floweret /cluster , 9=number of pod/plant , 10= pod length (cm) , 11=pod diameter (cm) , 12=Chlorophyll (SPAD) , 13=seed weight (gm) , 14=seed length (cm) , 15= seed diameter (cm) , 16= plant length at first harvesting (cm) , 17=number of branches /plant , 18= seeds yield (kg/hectar) .

Table (2) : Effect of soaking periods on the traits of faba bean during growing season 2017/2018*

Traits	Period soaking		
	8	16	24
Percentage of germination	79.16 a	72.91 b	70.02 b
stem length at first true leaf (cm)	4.69 a	4.34 a	4.27 a
diameter of stem at first true leaf (cm)	3.55 a	3.44 a	3.96 a
number of stomata /cm ²	18.32 a	17.52 b	19.71 a
stomata length (μm)	11.07 b	12.46 a	12.99 a
stomata diameter (μm)	3.45 a	3.16 a	3.86 a
number of days for 50% flowering	69.67 a	63.43 b	61.80 b
number of floweret /cluster	4.43 a	4.71 a	4.59 a
number of pods/plant	22.75 b	22.61 b	28.77 a
pod length (cm)	15.44 b	16.91 a	16.83 a
pod diameter (cm)	1.97 a	2.03 a	2.01 a
Chlorophyll (SPAD)	81.44 a	83.15 a	81.43 a
seed weight (gm)	1.46 a	1.50 a	1.65 a
seed length (cm)	2.17 a	2.27 a	2.32 a
seed diameter (cm)	0.62 a	0.66 a	0.63 a
plant length at first harvesting (cm)	77.06 b	81.06 b	83.58 a
number of branches /plant	5.12 a	5.33 a	5.20 a
seeds yield (kg/hectar)	3777.73 b	3971.77 b	4357.51 a

- Mean with the same letters no significant differences according to Duncan's multiple range test at probability 0.05.

1=percentage of germination , 2=stem length at first true leaf , 3=diameter of stem at first true leaf , 4= number of stomata /cm² , 5= stomata length (μm) ,6= stomata diameter , 7= number of days for 50% flowering , 8= number of floweret /cluster , 9=number of pod/plant , 10= pod length (cm) , 11=pod diameter (cm) , 12=Chlorophyll (SPAD) , 13=seed weight (gm) , 14=seed length (cm) , 15= seed diameter (cm) , 16= plant length at first harvesting (cm) , 17=number of branches /plant , 18= seeds yield (kg/hectar) .

Table (3) : Effect of interaction between period soaking and colchicines concentration on the traits of faba bean during growing season 2017/2018*

Traits	Colchicines concentration (%)											
	0			1000			2000			3000		
	Period soaking hours											
	8	16	24	8	16	24	8	16	24	8	16	24
Percentage of germination	91.00a	92.80a	93.90a	87.07b	81.97c	78.57c	74.53d	67.53e	62.90f	64.03f	49.33g	44.70h
stem length at first true leaf (cm)	5.83a	5.94a	5.99a	3.90c	3.87c	3.60c	4.87b	4.83b	5.00b	4.17b	2.70e	2.47e
diameter of stem at first true leaf (cm)	3.87b	3.03b	4.66a	2.57e	2.53e	2.60c	3.07b	3.97ab	4.67a	4.67ab	3.93b	3.90c
number of stomata /cm ²	19.90ab	20.22ab	19.0dab	1533d	10.50e	19.43a-c	16.03e	21.17ab	22.07a	22.00a	18.17b-d	18.27b-d
stomata length (µm)	10.70d	10.98d	9.99d	11.37cd	12.53bc	14.27a	11.17c	14.17a	13.86ab	11.03cd	12.17cd	13.87ab
stomata diameter (µm)	4.83a	4.97a	4.09ab	4.40b	2.47bc	5.76a	2.47c	2.83c	2.17cd	2.10d	2.37c	3.43b
number of days for 50% flowering	99.83a	85.73b	77.43b	59.30c-e	61.87bc	58.07d	63.10b	53.76ef	49.97f	56.03d	52.33ef	61.13bc
number of floweret /cluster	4.23e	4.12e	4.10e	4.30c-e	4.97ab	5.20a	5.00ab	4.63b-d	4.77a-c	4.17de	5.10ab	4.30c-e
number of pods/plant	20.67c	20.99c	21.08c	22.33c	28.67b	30.00ab	19.33e	19.44c	33.00a	28.67b	21.33c	31.00ab
pod length (cm)	10.70ab	11.04ab	10.98ab	15.33f	19.90bc	21.89a	18.23cd	16.87d-f	18.33cd	17.50de	19.83bc	16.13ef
pod diameter (cm)	2.00a	2.11a	2.04a	1.99a	2.13a	1.97a	1.89a	1.78de	2.17a	1.99a	2.10a	1.86a
Chlorophyll (SPAD)	68.06e	67.05a	68.93e	68.83e	78.73cd	74.76bc	83.53c	73.33de	83.80c	105.33ab	113.27a	98.23b
seed weight (gm)	1.77c-e	1.43c-e	1.53c-e	1.27de	1.57bc	1.27bc	1.23e	1.47cd	1.70b	1.58bc	1.53bc	2.10a
seed length (cm)	2.17e	2.20e	2.04c	2.17e	2.44c	2.27bc	1.90d	2.27bc	2.43a	2.44a	2.15c	2.55a

seed diameter (cm)	0.56a	0.66a	0.54a	0.63a	0.67a	0.67a	0.64a	0.59a	0.63a	0.64a	0.70a	0.66a
plant length at first harvesting (cm)	86.86a	85.99a	85.42a	66.60d	80.53ab	81.88ab	70.11cd	70.22bc	85.73a	84.67ab	83.47ab	81.27ab
number of branches /plant	5.10d	4.99d	5.40d	5.27bd	5.33bd	4.87de	4.43e	5.13cd	5.63a-c	5.67ab	5.87a	4.90de
seeds yield (kg/hectar)	3637.3e	3599.7e	3628.80c	2886.3d	3793.7e	4555.0a	4514.00a	4045.78a	4563.7a	4073.3b	4447.90a	4682.55a

- Mean with the same letters no significant differences according to Duncan's multiple range test at probability 0.05.

1=percentage of germination , 2=stem length at first true leaf , 3=diameter of stem at first true leaf , 4= number of stomata /cm² , 5= stomata length (µm) ,6= stomata diameter , 7= number of days for 50% flowering , 8= number of floweret /cluster , 9=number of pod/plant , 10= pod length (cm) , 11=pod diameter (cm) , 12=Chlorophyll (SPAD) , 13=seed weight (gm) , 14=seed length (cm) , 15= seed diameter (cm) , 16= plant length at first harvesting (cm) , 17=number of branches /plant , 18= seeds yield (kg/hectar) .

Table (4) : the range , means , S.E. , R-Square and coefficient variation in traits of faba plant during growing season 2017/ 2018.

The traits	The range	Means ± S.E	R.Squar	Coef. Var.
Percentage of germination %	44.70 – 93.90	74.03 ± 2.006	0.9889	2.8593
Stem length at first true leaf(cm)	2.47 – 5.99	4.43 ± 0.2922	0.9511	7.0876
Diameter of stem at first true leaf (cm)	2.53 – 4.67	3.61 ± 0.4034	0.8649	11.669
Number of stomata / cm ²	15.33 – 22.07	18.52 ± 1.881	0.8470	10.2863
Stomata length (µm)	10.98 – 14.27	12.18 ± 0.807	0.8244	6.4516
Stomata diameter (µm)	2.10 – 5.76	3.49 ± 4.060	0.3982	108.943
Number of days for 50% flowering	49.97 – 99.83	64.96 ± 2.296	0.9442	3.8650
Number of flowerst /cluster	4.10 – 5.20	4.57 ± 0.269	0.7800	5.7708
Number of pods / plant	19.33 – 33.00	24.71 ± 2.234	0.8981	8.7821
Pod length (cm)	10.70 – 21.89	16.39 ± 0.935	0.8832	5.0642
Pod diameter (cm)	1.78 – 2.17	2.00 ± 3.069	0.4016	122.903
Total chlorophyll (SPAD)	67.05 – 113.27	82.00 ± 5.104	0.9380	6.0249

Seed weight (gm)	1.23 – 2.10	1.54 ± 0.119	0.8791	7.8772
Seed length (cm)	1.90 – 2.55	2.25 ± 0.081	0.8894	3.5720
Seed diameter (cm)	0.54 – 0.67	0.63 ± 0.082	0.3284	13.3504
Plant length at first harvesting (cm)	66.60 – 85.73	80.81 ± 4.732	0.7663	5.9355
Number of branches / plant	4.43 – 5.87	5.22 ± 0.282	0.7813	5.3954
Total seed yield (kg/hacter)	2886.3 – 4682.55	4035.67 ± 142.856	0.9598	3.4676

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