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Effect of Chemical Mutagens on Some Morphological Traits of *Vicia faba* L. Cv. Aqadulce

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Abstract

To investigate the effect of chemical mutagens: sodium azide (SA), hydrazine hydrate (HZ) and maleic hydrazine (MH) on morphological variations of faba bean traits. Seeds were soaked in distilled water for six hours, then in different concentrations of the above mentioned mutagens (0.01, 0.03, 0.05%) represented by (C1,C2 and C3 respectively) for six hours and planted in the field in winter 2013-2014. Factorial Experiment was arranged in Randomized Complete Block Design (RCBD) with three replications to each treatment. The results of statistical analysis showed significant differences among studied treatments. All mutagens showed significant reduction in plan height and number of branches per plant compared with control plants which gave the highest plant height and number of branches per plant (117.55cm and 13.73 respectively). SA gave a significant decrement in plant height (103.95cm). HZ gave the longest day for 50% flowering (80.78 days), a significant increase in leaf area index (4.10), chlorophyll content (43.50) have been achieved. MH caused an earliness in flowering of treated plants (79.22days). C3 was superior in the leaf area index (4.07), chlorophyll content (44.79) and earliness in flowering (78.78 days), while C1 gave a longer period of 50% flowering (80.89 days). Significant differences also observed in the interaction between mutagens and concentrations. (MHXC3) gave a less period to 50% flowering (77 days) and showed superiority in leaf area index (5.07) and number of branches per plant (14.79). HZ x C1 mean while an increment in number of days to50% flowering (82.66 days) but HZ x C2 gave highest plant height resulted in reached (121.18cm), while (SAxC2) treatment yielded in lowest plant height (96.49) cm and (SAxC3) gave maximum chlorophyll content (47.93).

Keywords: *Vicia faba* L., mutagen, hydrazine hydrate, sodium azide and maleic hydrazine, morphological traits.

تاثير المطفرات الكميائية على بعض الصفات المورفولوجية لنبات الباقلاء *Vicia faba* L. صنف Aqadulce

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الخلاصة

لمعرفة تاثير المطفرات الكيميائية : Sodium azide (SA) و Hydrazine hydrate(HZ) و Maleic hydrazine (MH) في التغايرات المظهريه لصفات نبات الباقلاء ، نقعت البذور بالماء المقطر لمدة (6) ساعة أعقبتها فترة تنقيع لمدة (6) ساعة بتراكيز مختلفة للمطفرات المذكورة اعلاه (0.01، 0.03، 0.05%) والتي مثلت بالآتي (C3,C2,C1) على التوالي وزرعت في الحقل في الموسم الشتوي 2013–2014،

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استخدمت تجربة عاملية بتصميم القطاعات الكاملة المعشاة (RCBD) بثلاث مكررات لكل معاملة .اظهرت نتائج التحليل الاحصائي اختلافات جوهرية بين المعاملات المدروسة. جميع المطفرات سببت انخفاضاً في ارتفاع النبات وعدد فروع النبات مقارنة بمعاملة المقارنة التي سجلت أعلى ارتفاع نبات وعدد فروع نبات 117.55 سم ، 13.73 على التوالي. SA اعطى اقل ارتفاعاً بلغ 103.95 سم واعطى HZ اعلى معدل في عدد الايام وصولا الى مرحلة 50% تزهير بلغ 80.78 يوم ودليل المساحة الورقية بلغ 4.10 و الكلوروفيل 43.50. اقل عدد ايام وصولا الى مرحلة 50% تزهير بلغت 79.22 يوم في المطفرMH. حقق C3 اعلى معدل في دليل المساحة الورقية بلغ 4.07 و الكلوروفيل 44.79 كما بكرت النباتات في مرحلة 50% تزهير في 78.78يوم بينما اعطى C1 اطول مدة وصولا لمرحلة 50% تزهير في 80.89 يوم وجد تداخل معنوي بين المطفرات والتراكيز أذ أعطى االمطفر MH XC3 اعلى دليل مساحة ورقية بلغت5.07 وعدد فروع/نبات 14.79 وبكرت بمرحلة 50 % تزهير في 77 يوم بينما اعطىHZ X C1 اطول مدة وصولا الى 50% تزهير في 82.66 يوم وعند C2 اعطى اعلى ارتفاع نبات بلغ 121.18 سم اما SA X C2 اعطى اقل ارتفاع نبات بلغ 96.49 سم وعند C3 اعلى محتوى كلوروفيل بلغ 47.93.

Introduction

The faba bean (*Vicia faba* L.) is one of the Fabaceae families (Leguminosae), It is widely considered as a good source of protein, starch, cellulose and minerals for humans in developing countries and for animals [1]. The nutritional value of faba bean is high, and in some areas is considered to be superior to peas or other grain legumes[2] The main strategy in mutation based breeding has been to upgrade the well adapted plant varieties by improving a few desirable major yield and quality traits [3]. Chemical mutagenesis is an approach to create mutation in plants for their improvement of potential agronomic traits. Many chemical mutagens have been employed for obtaining useful mutants in various crop species [4] .Series of experiments carried out with various crops and proved that chemical mutagens induce polygenic variability [5], The efficiency of mutant production depends on many conditions such as concentration, PH, temperature, soaking into water, and treatment duration. Sodium azide (SA) caused reducing plant height of faba bean, the trait was found negatively affected and was positively correlated with mutagenic concentration [6]. Sodium azide was used in many studies to induce mutation as found by Mostafa [7] on *Helianthus annuus*, Kumar and Dwivedi [8] on *Brassica campestris* L. It creates point mutation and damages the chromosomes and thus produces bearing in the plants for many adverse conditions [9]. Hydrazine hydrate (HZ) has been reported to induce a variety of morphological, physiological and chlorophyll mutants in several crop plants [10]. HZ reacts with the pyrimidines in DNA to saturate the 5,6 open up the pyrimidine ring with consequent loss of pyrimidines from DNA [11]. Maleic hydrazide (MH) is possibly through its interference with synthesis of uracil or becoming incorporated into RNA molecule replacing the uracil. It reacts with sulphydril groups of nucleic acids [12]. Naidu *et al*. [13] who found that (MH) suppressed plant height and enhanced the plant spread and number of laterals over control. The aim of this study to evolution the effects of chemicals mutagens on some morphological traits, and to assess best mutagens concentration. The possibility of use in programs development.

Materials and methods

The study was conducted in an experimental station of the field crops departments, college of Agriculture in Abu-Ghraib, University of Baghdad. The area of experimental unit was 10 m^2 with five furrows. The space between furrows was 0.75m and between plants was 0.3m. To achieve planting densities 44444 plants/ha. Seeds sowing at 30 October. The fertilizer used was diamonium phosphat (DAP) (P_2O_4 46%, N 18%) at a rate of 400 Kg/ha was added to the soil before seeds sowing [14] was added to soil at one batch and mixed with the soil at tillage. Then, a second batch of nitrogen fertilizer (N 46%) was added at a rate of 50kg/ha [15]. The other required culture practices for growing faba bean were followed as recommended. Seeds were obtained from college of Agriculture Baghdad University. Two hundred forty seeds of faba bean were soaked in distilled water for six hours, and then treated with different concentrations (0.01, 0.03 and 0.05%) represented by (C1,C2 and C3 respectively) for each mutagens SA, HZ and MH for six hours except control treatment. These materials were prepared in sodium buffer adjusted at pH7[16].

Morphological traits

Days to 50% flowering: The calculated number of days from sowing date to 50% flowering.

Plant height (cm): was measured on week before harvest, it was measured from the tip to the ground level of the plant [17].

Leaf Area Index (LAI): Leaf area/plant (cm^2) was calculated at the beginning of the pod filling stage [18]. Leaf area index was calculated by the equation: Leaf area index=leaf area of plant/ the area occupied by the plant. By using the disk method as described by [19]

Chlorophyll index: by using SPAD device and taking the mean of upper five leaves and lower five leaves, then calculating the chlorophyll index mean.

Number of branches per plant.

Statical analysis

The experiment was arranged in randomized complete block design (RCBD) with three replicates for each treatment the obtained data were analyzed by variance using statistical analysis system (SAS) program [20] the means were compared with Least Significant Differences (LSD) at a level 5 %.

Results and discussion

Days to 50% flowering

Results in table-1 indicated that the mutagen HZ delayed flowering of treated plants significantly (80.78 days) compared to MH and Control plants (79.22, 79.33 days, respectively). These results agreed with Lasker and Khan [21] who found a reduction in this trait due to treatment with MH. Variation in this trait referred to the phytohormone levels of indole acetic acid (IAA) at blooming which was significantly higher with MH [22]. Concentration of mutagens (C1 &C2) gave the longest period (80.89 &80.33 days, respectively) affected this trait significantly compared to control and C3 (79.33 & 78.78 days respectively). This variation due to damage in cell constituents at molecular level or altered enzyme activity [16]. These results agreed with Ali *et al*. [23] who found that SA induced the longest delay in flowering of lentil plants. Significant interaction between (MH x C3) gave the shortest period (77 days) compared to (HZ x C1) which gave the longest period (82.66 days) . These results are in agreement with those of Alka and Khan) [11].

Table 1-Effect of sodium azide, hydrazine hydrate and maleic hydrazine on days to 50% flowering of faba bean

Concentrations%		Mutagens			Mean
		SA	HZ	MH	
C1	0.01	79.33	82.66	80.66	80.89
C2	0.03	81.33	79.66	80.00	80.33
C3	0.05	79.33	80.00	77.00	78.78
Mean		80.00	80.78	79.22	Control=79.33
LSD= 0.05		Con.: 0.81 *	Mutagens: 0.81*	Conc. x Mutagens: 1.40*	

Plant height (cm)

Results in table-2 showed that all mutagens (SA, HZ and MH) reduced plant height (103.95, 108.34 and 110.33) significantly compared to control plants (117.55 cm). It was reported that SA at a concentration of 0.05% reduced groundnut plant height [24]. The ability of SA to enter the cell of living organisms to interact with the DNA produces the toxic effects associated with their mutagenic properties and inhibition of protein synthesis this lead to a reduction in plant height. Similar findings have been reported by Mostafa [7] also. The concentration of these mutagens (C1,C2 , C3) decreased plant height significantly (106.28, 110.12 and 106.23 cm, respectively) compared to control plants (117.55 cm). Significant interaction (HZxC2) gave the highest value of plant height 121.18cm, while (SAxC1) was the lowest value (96.49 cm). These results may be attributed to various factors such as changes in the metabolic activity of the cells, the inhibitory effects of mutagens and to disturbance of the balance between promoter and inhibitors of growth regulators. These results were agreed with Tariq *et al.*, and Mensah, and Obadoni [6, 24] with SA on faba bean and groundnut respectively.

Table 2-Effect of sodium azide, hydrazine hydrate and maleic hydrazine on plant height of faba bean

Concentrations%		Mutagens			Mean
		SA	HZ	MH	
C1	0.01	117.08	97.67	104.10	106.28
C2	0.03	96.49	121.18	112.66	110.12
C3	0.05	98.28	106.19	114.23	106.23
Mean		103.95	108.34	110.33	Control= 117.55
LSD= 0.05 Conc.: 2.41 * Mutagens: 2.41 * Conc. x Mutagens: 4.17*					

Leaf area index (LAI)

The results in Table-3 showed the highest leaf area index was obtained from HZ and MH (4.1 and 3.99, respectively) but no significant difference between them. The reason for maximizing leaf area index with HZ may be related for nitrogen rise due to increased cell division and expansion of leaves [25]. Indole acetic acid (IAA) positive correlation with MH, action in cell division and cell elongation. Result agreement with Ganesh *et al*. [26]. A C3 gave a maximum leaf area index was 4.07. Also, a significant interaction between mutagens and concentrations. The maximum and minimum effects on this trait were resulted by MHxC3 and SAxC2 (5.07, 3.17, respectively).

Table 3-Effect of sodium azide, hydrazine hydrate and maleic hydrazine on leaf area index of faba bean

Concentrations%		Mutagens			Mean
		SA	HZ	MH	
C1	0.01	3.25	4.84	3.48	3.86
C2	0.03	3.17	3.81	3.41	3.47
C3	0.05	3.49	3.63	5.07	4.07
Mean		3.31	4.10	3.99	Control =3.16
LSD= 0.05 Conc.: 0.24 * Mutagens: 0.24 * Conc. x Mutagens: 0.42*					

Chlorophyll index

Table-4 indicated to the significant effect of mutagens HZ and SA on chlorophyll index (43.50% and 43.28%) compared to control (40.48%). No significant effect was found among mutagens in this experiment. C3 and C1 gave the higher chlorophyll content (44.79% and 43.32%) compared to control plants. There is a significant difference among mutagens and their concentrations. Maximum content of chlorophyll was achieved by (SAxC3) and (HZxC1) reached to (47.93 %and 45.11%, respectively), similar results with AL-Qurainy [9] in rice with SA at 3mM concentration, but disagree with Mshembula et al. [27] found lower concentrations of SA increased leaf area in cowpea. The reason behind variability in total chlorophyll content is there are many mutations at various loci of a genome by various concentrations of mutagens [9].

Table 4-Effect of sodium azide, hydrazine hydrate and maleic hydrazine on chlorophyll index of faba bean

Concentrations%		Mutagens			Mean
		SA	HZ	MH	
C1	0.01	39.80	45.11	45.03	43.32
C2	0.03	42.09	42.18	39.93	41.07
C3	0.05	47.93	44.20	42.25	44.79
Mean		43.28	43.50	42.41	Control =0.48
LSD= 0.05 Conc. : 2.78 * Mutagens: 2.78* Conc. x Mutagens: 4.81*					

Number of branches/plant

Control treatment gave maximum number of branches per plant were 13.73 compared with mutagens as indicate in table-5. No significant effects among mutagens on the number of branches per plant. The less number of branches was 11.01 with HZ. Shimizu-Sato. and Mori [28] observed that both hormones and genes have a role in determining the branching pattern of plants, can be related to the inhibitory effect of azide ions that binds to the enzymes involved in cellular energy production [29], or due to decrease plant high. Similar results with Warghat *et al*.[30] the effect of SA in decreasing number of branches with musk okra. Significant differences were observed among concentrations of mutagens on this trait. Control treatment and C3, gave the highest number of branches per plant were (13.73and 13.67, respectively) and no significant differences between them, while C1and C2, gave lowest number of branches per plant were (11.47, 9.60, respectively). Due to heritability change produced by chromosomal aberration or chromosomes damaged [31]. Table-5 showed the interaction between the mutagens and their concentrations was significant. The highest mean was 14.79 in treated plants with (MHxC3). This may indicate that mutagen concentration acts as a potent antiauxin [32], and loss of apical dominance resulted more branching. These results agree with Naidu *et al*. [13].

Table 5-Effect of sodium azide, hydrazine hydrate and malice hydrazine on number of branches/plant of faba bean

Concentrations%		Mutagens			Mean
		SA	HZ	MH	
C1	0.01	11.10	10.66	12.66	11.47
C2	0.03	9.76	9.92	9.10	9.60
C3	0.05	13.77	12.44	14.79	13.67
Mean		11.55	11.01	12.19	Control =13.73
LSD= 0.05 Conc. : 1.49* Mutagens: 1.49 Conc. x Mutagens: 2.57*					

Conclusion

Results showed the application of treatments by the mutagens use have enhance the probability to induce genetic variability for morphological traits and qualitative variations which can be favourably exploited by plant breeders in improving the genotype of faba bean. Third concentration could be utilized to increase chlorophyll content with sodium azide, leaf area index, and number of branches per plant and fewer days for 50% flowering in maleic hydrazine.

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