Variation of Faba Beans (*Vicia faba* L.) Traits Induced By Heat, Electric Shock and Mutagen Nitrous Acid

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Abstract:

This research was carried out to determine the impact of heat shock, electric shock and seeds in soaking nitrous acid mutagen solution on three cultivars of faba beans plant (Zaina, Aguadulce and Local) at the year 2012-2013. Factorial experiment was arranged in randomized complete block design (RCBD) with three replicates were used. The results showed that heat shock lead to early plants of 50% in flowering and an increase in the number of branches/plant and the number of seeds/pod compared to other treatments, whereas the seeds soaked in nitrous acid mutagen solution gave the highest plant height, leaf area index, number of pods/plant, seed weight, seed yield kg/ha, and did not differ significantly with treatment of electric shock in the protein yield(kg/ha). Zaina cultivar was superior over other cultivars in early to 50% flowering, number of branches/plant, number of pods/plant and seed weight, while gave the lowest value in plant height. Significant differences also observed for the interaction between cultivars and treatments. Zaina cultivar with heat shock treatment gave less number of days to 50% flowering, highest number of branches/plant and the number of seeds/pod, either when seeds soaked in nitrous acid mutagen solution given the highest plant height of plant, highest mean of seed weight, seed yield kg/ha and did not differ significantly with local variety in the number of pods/plant trait .

Key words: Heat Shock (HS) Electric Shock (ES) Nitrous acid mutagen Faba bean varieties

Introduction:

Common beans are cultivated under a wide range of environmental conditions. All bean cultivars, to some degree, are sensitive to a biotic stresses. During germination, the seeds are particularly sensitive to water deficit and exposure to low or high temperatures [1,2]. Thermal (or thermal shock) treatment increases the physiological age of seed and shortens the time needed for the formation of a harvestable crop [3,4] ,therefore used to encourage an early harvest. Temperatures over 30-35°C are rarely used in thermal shock treatment, even for a short time, since potato albumins curdle at 40°C [5]. While thermal shock is recognized as a good alternative to pre-sprouting. The literature contains little in-depth information on this technique. The pre-treatment of potato tubers is known to influence plant development and yield structure [6]. There are common responses to a biotic stresses (temperature) in plants, but unique responses to a specific stress indifferent species or even different cultivars have been observed [7,8,9] For example, the expression of several classes of proteins, such as transcription factors[10], BiPs (Binding Protein) [11], antioxidant enzymes [12,13], late embryogenesis abundant proteins (LEAs) and proline-rich proteins (PRP) [14] and heat

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shock proteins (HSP) [15,16], In a study evaluated the genetic variability of several different lines of sorghum tolerance high heat stress. Heat tolerance is considered to be correlated to the ability of thermo-tolerant lines to produce a larger quantity of heat shock proteins (HSP, s) [17]. When using an electric shock, a significant effect found on the growth parameters (leaf length and breadth) and increase the nutritional value of the plant by stimulating plant growth [18]. Elsahookie [19] results showed that electric shock until M5 (fifth generations) reduced plant height. On the contrary, seed yields (ton/ha) for the same soybeans lines were increased by 33.5% and 64%, respectively. When wheat and barley seed exposed for electric shock periods, they gave significant variation in several traits [20]. Elsahookie and Elsubahi [21] found that different genotypes gave different responses to periods of ES, indicating that one period of ES can not be recommended for all genotypes to induce variations through the use of electric shock to open a power supply 220 AC for periods 2, 4 and 6 minutes. Justin *et al.* [22] attempted to get mutagenesis by enhancement of citric acid from a local stain of *Aspergillus niger* that isolated from lemon leaves and treated with nitrous acid , they found that the mutant exhibited an increased efficiency for citric acid production when compared with the wild type. The role of nitrous acid (HONO) in the atmosphere is of great importance due to its participation in the cycling of hydrogen oxides (HOx) and nitrogen oxides (NOx), both of which contribute to ozone (O3) formation[23]. In earlier studies, the regulatory roles of NO have been reported at different stages of crop development and have been especially found beneficial in promoting seed germination and seedling stage of most plant species [24]. Nitrite oxide (NO) is a signaling molecule in a variety of physiological processes during plant growth and development, and also is an important modulator of disease resistance [25] Exogenous application of NO donors in tomato and in *Arabidopsis* indicated that NO affects root architecture, and promotes lateral root development [26,27] Soaked seeds of four local cultivars of rice (*Oryza sativa* L.) in 0.1,0.3,and 0.5M nitric acid for 0,3,6,12,18,and 24 hrs, their results showed that germination was significantly affected by nitric acid concentration and soaking duration [28].

Previous experiment was conducted on these cultivars to study the electric shock and soaking of seed nitrous acid in solution [29,30] In this experiment, heat shock was added with these treatments to determine the effect in the phenotypic traits, yield and yield components.

Materials and Methods:

Three cultivars of Faba bean(*Vicia faba* L.) were used in this study, namely; Zaina (Italian origin), Aguadulce (France origin) and Local (Production of Iraq). All cultivars were obtained from Department of Field Crops Science-Agriculture College / Baghdad University.

Heat shock, Electric Shock and Nitrous Acid preparation:

Soak the seeds in water for 3-4 hours and then sterilized using a solution $HgCl_2$ of concentration 1% to prevent the growth of fungi during seed germination. Germinated seeds were put on a filter paper wet basins plastic at a temperature of 25 ° C and relative humidity of 60%. Seedling with two days age were offered to heat 45° C for one hour and then the seedling were transferred to the cold shock condition (7°C) for three hours [17,31]. Seeds from each cultivars were calculated

and germinated inside cheese cloth even phase out the radical length of 2-5 mm, then seedling soaked in aqueous solution (1% sodium chloride) for three hours [19 ,20, 21]. So that the salt can enter into plant tissue to facilitate the delivery of electrical current into the cells. then transferred to a glass vessel in which the same solution and connected to two electric poles AC 220 volt power source where it put inside the seedling aluminum perforated paper with the weight of iron to make them dipped in the solution**.** Electric current to open the period 9 minutes **.** After completing the process of shock transferred the seedling vessel in running water for three hours to expel the salt does not affect them so that in the percentage of emergence**.** The acid was an unstable ,weakly acidic compound and decomposes rapidly, therefore it has been prepared immediately in the form of cold. It include**s** tow mixtures, the first was prepared by adding 600 ml of HCl slowly to 600 ml of distilled water, in ice bath to get mix, its volume of 1200 ml, and the second mixture was prepared by diluting 160 g of sodium nitrite $NaNO_2$ with 400 ml of distilled water. Then first mixture was added to the second mixture in ice bath, to get nitrous acid solution as following equation: $HCl + NaNO_2$ ------- $HNO_2 +$ NaCl. The seeds were soaked in nitrous acid for 24 hrs and kept in ice bath [32].

Experiment:

The experiment was conducted in a clay loan soil. Seedling of treatments and control (untreated) were planted in the filed during winter 2012-2013, in the Experimental Station of the Biology Dep. College of Science, University of Baghdad. Factorial experiment was arranged in randomized Complete Block Design (RCBD) with three replicates. The area of experimental unit was 10 m^2 with five rows , the space between rows was 0.70m and between plants 0.30m.The superphosphate fertilizer (P2O5 45%) at a rate of 80 kg/ha was added to the soil before seedling transplanting [33], and urea fertilizer N 46% at rate of 50 kg/ha, was applied before the first irrigation[34]. The other required culture practices for growing faba bean were followed as recommended.

Studied parameter:

The following traits were studied during the growth period of faba bean: days from planting to 50% flowering, plant height (cm), leaf area index (L.A.I), number of branches/plant, number of pods/plant, number of seeds/pod, seed weight (g), seed yield (kg/ha), protein percentage% and protein yield (kg/ha). Data were subjected to the proper analysis of variance of Randomized Complete Block Design (RCBD). The treatment means were compared at 0.05% level of significant using Least Significant Differences test (L.S.D.).

Results and Discussion:

Represents the rate of trait flowering days from sowing (first irrigation) up to 50% flowering and early maturity is the desirable character in order to avoid entering the stage of deposition of carbohydrates at the seed in the high-temperature**.** **Table 1** indicates a significant differences for treatment and cultivars, it was observed that heat shock treatment was early in flowering as it took less number of days was 76.44 days, due to the manufacturer of proteins can change activity cell metabolic and improves the efficiency of the plant cell and this explains the positive impact of incitement to increase the growth rate. While seed soaked in nitrous acid solution for 24 hrs took more days to 50% flowering was 87.96.day. Zaina cultivar was early flowering than others (76.8 days). This result was in agreement with [35].

The difference between cultivars, in response to shocks can be explained to a group of genes and action responsible for early flowering in the crop [20]**.** Interaction between varieties and treatments were also detected in Zaina cultivar when heat shocked was 74.33 day as early.

Table.1 The effect of heat shock, electric shock and soaked in nitrous acid solution of days number from planting to 50% flowering.

Treatment / Cultivars	Heat shock in 45 ˚C for 3 hrs cooling	Electric shock for 9 minutes	Soaked in nitrous acid for 24 hrs	Control	Mean
Zaina	74.33	77	76.9	79	76.8
Aguadulce	76	84.33	94.5	85.33	85.04
Local	79	82	92.5	85.66	84.79
Mean	76.44	81.11	87.96	83.33	
L.S.D0.05	(V)= 1.43	(T)=1.66	(V*T)=2.87		

The difference in plant height as the status of plant height governed by genes as quantitative genetics seed soaked in nitrous acid for 24 hrs, gave the highest height of 89 cm, while less highest 73.33 when the plants exposed to electric shocks 73.33cm. Local and the Aguadulce cultivars height reached to 80.7 and 80.35 cm respectively, and not differ significantly between them. Zaina cultivar gave the highest height of the plant 95.3 cm when soaking in nitrous acid solution **(Table2)**.

Table.2 The effect of heat shock, electric shock and soaked in nitrous acid solution of plant height (cm),

Treatment / Cultivars	Heat shock in 45 ˚C for 3 hrs cooling	Electric shock for 9 minutes	Soaked in nitrous acid for 24 hrs	Control	Mean
Zaina	92.3	58	95.3	69	78.65
Aguadulce	85.3	79	80.1	77	80.35
Local	72.6	83	92.5	74.7	80.7
Mean	83.4	73.33	89	73.56	
L.S.D0.05	(V)= 1.4	(T)=1.62	(V*T)=2.81		

Table 3 shows significant differences in the leaf area index between heat shock, electric shock and nitrous acid. seed soaked in nitrous acid solution gave the highest LAI reached 2.41 compared with other treatment, this result agreed with [30].

Table.3 The effect of heat shock, electric shock and soaked in nitrous acid solution of leaf area index .

Treatment / Cultivars	Heat shock in 45 ˚C for 3 hrs cooling	Electric shock for 9 minutes	Soaked in nitrous acid for 24 hrs	Control	Mean
Zaina	1.19	1.6	2.8	1.59	1.79
Aguadulce	1.86	1.77	2.1	2.05	1.94
Local	1.1	1.65	2.35	1.3	1.6
Mean	1.38	1.67	2.41	1.64	
L.S.D0.05	(V)= n.s	(T)=0.4	(V*T)=n.s		

Results showed a significant difference between treatments in the number of branches/plant **(Table 4)**. The heat shock treatment gave the highest number of branches/plant reached to 12.16, this may be due to the increasing of manufacturing and accumulation of heat shock proteins HSP90 and HSP104 induced in seedling and thus improves the best growth, Zaina cultivar gave the highest number of branches reached to 10.2 branches. The interaction between Zaina cultivar and heat shock gave the highest plant height was 14 branches/plant.

Table.4 The effect of heat shock, electric shock and soaked in nitrous acid solution of mean number of branches/plant ,

Treatment / Cultivars	Heat shock in 45 ˚C for 3 hrs cooling	Electric shock for 9 minutes	Soaked in nitrous acid for 24 hrs	Control	Mean
Zaina	14	7.33	12	7.5	10.2
Aguadulce	12	6.9	8.5	5.66	8.26
Local	10.5	7.66	12.5	6.33	9.24
Mean	12.16	7.29	11	6.49	
L.S.D0.05	(V)= 0.61	(T)=0.71	(V*T)=1.23		

Results presented in **Table 5** showed that the highest number of pods/plant was 2.51caused by soaked in nitrous acid. Whereas Zaina cultivar gave the highest number of pods/ plant was 14.12 pods. The significant interaction found between the Local variety and soaked in nitrous acid gave 26 pods/plant and which did not differ significantly from Zaina cultivar in the same treatment which gave 25.3 pods/plant .

Table.5 The effect of heat shock, electric shock and soaked in nitrous acid solution in number of pod/plant.

Treatment / Cultivars	Heat shock in 45 ˚C for 3 hrs cooling	Electric shock for 9 minutes	Soaked in nitrous acid for 24 hrs	Control	Mean
Zaina	10	22.3	25.3	20.1	19.42
Aguadulce	9	18.5	24	19	17.62
Local	7	19	26	21	18
Mean	8.66	19.93	25.1	20.03	
L.S.D0.05	(V)= 0.89	(T)=1.03	(V*T)=1.79		

It was found that the highest number of seeds/pod 5.16 when seeds exposed to heat shock, while it did not find a significant difference between varieties **(Table 6)**. Zaina and Aguadulce cultivars gave the highest number of seeds/ pod were 5.9 and 5.3 seed/pods, respectively when exposed to heat shock which did not differ significantly between them . this result in agreement with[29].

Table.6 The effect of heat shock, electric shock and soaked in nitrous acid solution of mean number of seed/pod.

Treatment / Cultivars	Heat shock in 45 ˚C for 3 hrs cooling	Electric shock for 9 minutes	Soaked in nitrous acid for 24 hrs	Control	Mean
Zaina	5.9	4.1	2.5	4	4.12
Aguadulce	5.3	4.7	3.3	3.5	4.2
Local	4.3	3.9	3.5	3.9	3.9
Mean	5.16	4.05	3.1	3.8	
L.S.D0.05	(V)= n.s	(T)=0.41	(V*T)=0.71		

The soaked in nitrous acid treatment gave the highest average of seed weight was 1.45 g **Table 7**. Zaina cultivar gave the highest seed weight 1.49 g. Zaina variety gave the highest average seed weight was 1.8 g when soaked in nitrous acid for 24 hrs.

Table.7 The effect of heat shock, electric shock and soaked in nitrous acid solution of mean seed weight (g).

Treatment / Cultivars	Heat shock in 45 ˚C for 3 hrs cooling	Electric shock for 9 minutes	Soaked in nitrous acid for 24 hrs	Control	Mean
Zaina	1.25	1.44	1.8	1.5	1.49
Aguadulce	1.3	1.6	1.3	1.38	1.39
Local	1.05	1.2	1.25	1.3	1.2
Mean	1.2	1.41	1.45	1.39	
L.S.D0.05	(V)= 0.11	(T)=0.13	(V*T)=0.23		

Table 8 explained that seeds soaked in nitrous acid for 24 hrs gave the highest seed yield 4078.43 kg/ha which was the basic character sought by plant breeders know their relationship after trait or phenotypic characteristics, this result in agreement with [30,35], due to the increase in the leaf area which could lead to increase in photosynthesis resulting in greater transfer of assimilates to the seed and superiority in yield components (**Tables 5** and **7**). While did not shows significant difference between cultivars because they differ in their yield components (**Table 4.5,6 and 7**). Significant interaction was found between Zaina cultivar and soaked in nitrous acid solution for 24 hrs which gave the highest yield was 4341.2 kg/ha, due to superiority in yield components, number of pods/plant and seed weight (**Tables 5** and **7**).

Table.8 The effect of heat shock, electric shock and soaked in nitrous acid solution of mean seed yield(kg/ha).

Treatment / Cultivars	Heat shock in 45 ˚C for 3 hrs cooling	Electric shock for 9 minutes	Soaked in nitrous acid for 24 hrs	Control	Mean
Zaina	3218.8	3291.1	4341.2	3137.91	3497.25
Aguadulce	2121.9	4016.7	3996.5	2757.34	3223.11
Local	2099.6	3601.01	3897.6	3671.32	3317.38
Mean	2480.1	3636.27	4078.43	3188.85	
L.S.D0.05	(V)= n.s	(T)=460.12	(V*T)=796.96		

Table 9 showed there is no significant difference between the treatments and no interaction between cultivars and treatments. The Aguadulce cultivar gave the highest percentage of protein reached to 20.56% and not differ significantly with Local cultivar, this result is in agreement with [29].

Table.9 The effect of heat shock, electric shock and soaked in nitrous acid solution of mean protein percentage % .

Treatment / Cultivars	Heat shock in 45 ˚C for 3 hrs cooling	Electric shock for 9 minutes	Soaked in nitrous acid for 24 hrs	Control	Mean
Zaina	18.5	19.9	18	18.67	18.76
Aguadulce	20.2	20.3	19.9	21.87	20.56
Local	19	20.9	20	19.32	19.8
Mean	19.23	20.36	19.3	19.95	
L.S.D0.05	(V)= 1.22	(T)=n.s	(V*T)=n.s		

Protein yield (kg/ha) was calculated by multiplying the protein percentage by seed yield (kg/ha). Protein yield did not differ significantly between the cultivars and no interaction between cultivars and treatments , may be due to the differences in protein percentage and seed yield which increase or decrease in the value (**Tables 8** and **9**).

The results showed significant differences between treatments, soaked in nitrous acid for 24 hrs gave the highest protein yield was 779.18 kg/ha which that superiority in the seed yield **(Table 8)**, and did not differ significantly with electric shock **(Table 10)**

Table.10 The effect of heat shock, electric shock and soaked in nitrous acid solution of mean protein yield(kg/ha).

Treatment / Cultivars	Heat shock in 45 ˚C for 3 hrs cooling	Electric shock for 9 minutes	Soaked in nitrous acid for 24 hrs	Control	Mean
Zaina	595.07	638.88	762.45	585.98	645.59
Aguadulce	432.2	816.67	794.5	603.19	661.64
Local	388.42	757.97	780.61	708.69	658.92
Mean	471.89	737.84	779.18	632.62	
L.S.D0.05	(V)= n.s	(T)=99.16	(V*T)=n.s		

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تغايرات صفات الباقلاء (*Vicia faba* L.) بتأثير الصعق الحراري والكهربائي والنقع بحامض النتروز المطفر

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الخلاصة :

نفذ هذا البحث لمعرفة تأثير الصعق الحراري والكهربائي ونقع البذور بمحلول حامض النتروز المطفر في ثلاثة اصناف من نبات الباقلاء (Zaina و Aguadulce و Local) في العام 2012-2013. استخدمت تجربة عاملية بتصميم القطاعات الكاملة المعشاة (RCBD) بثلاثة مكررات. اوضحت النتائج ان تأثير الصعق الحراري ادى الى تبكير النباتات بالتزهير و زيادة في عدد الافرع/ نبات وعدد البذور/قرنة فقط مقارنةً بالمعاملات الاخرى، أما نقع البذور بحامض النتروز المطفر فقد اعطى اعلى ارتفاع ودليل مساحة ورقية وعدد القرنات/نبات ووزن بذرة وحاصل بذور كغم/هكتار ولم يختلف معنوياً مع معاملة الصعق الكهربائي في حاصل الزيت كغم/هكتار. اظهر الصنف Zaina اعلى قيم في الصفات المدروسة في التبكير في التزهير وعدد الافرع/ نبات وعدد القرنات/نبات واقل قيمة له في صفة ارتفاع النبات عن الصنفين الاخرين. ظهر تداخل معنوي بين الاصناف والمعاملات، اذ اعطى الصنف Zaina في معاملة الصعق الحراري اقل عدد من الايام الى 50% تزهير واعلى عدد الافرع/ نبات وعدد البذور/قرنة اما عند نقع بذوره بمحلول حامض النتروز فقد اعطى اعلى ارتفاع نبات واعلى معدل لوزن البذرة وحاصل بذور كغم/هكتار ولم يختلف معنوياً مع الصنف Local في صفة عدد القرنات/نبات.