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Research Article

Effect of Different Concentrations of Sodium Azide on Some traits of Barley (*Hordeum Vulgare* L.)

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Abstract

Chemical mutagenesis is an effective tool to create a new Genetic Variation, which is the main key for breeding program in crops, this study was investigated the effects of six concentrations of sodium azide (1.5mM, 3mM, 4.5mM, 6mM and 100Mm) and the control (0 mM) on some characteristics of Syrian barley (*Hordeum vulgare* L.). The genotype (Alforat 5) was treated with Aqueous Sodium Azide (NaN_3) solutions for 2 hours and was planted in the winter of the year 2021 at agriculture faculty in Damascus university for estimating the phenotypic and genotypic coefficient of variation, Heritability and Genetic advance. The analysis of variation indicated High Significant differences were observed among the six treatments for the five traits (length of roots, plant height, number of tillers, number of leaves and dry weight). Genotypic and phenotypic coefficients of variability were higher for number of leaves per plant, number of tillers per plant more than the other traits. Heritability ranged (0.48% - 0.89%) for roots length and number of leaves per plant respectively, while it was0.64% for dry weight.

Heritability coupled with high genetic advance was observed for number of leaves per plant, number of tillers per plant and dry weight, which indicated the importance of the additive gene action in the genetic behaviour of these traits, and the possibility to achieve genetic gain by selection during the early segregation generation.

Keywords: Genetic Advance; Heritability; *Hordeum Vulgare* L; Sodium Azide (NaN_3)

Abbreviations

Barley (*Hordeum vulgare* L.) is the fourth crop after wheat, maize and rice, it is used as animal fodder and it is a raw material for many human foods [1]. Chemical mutagenesis as alkylating agents and azides [2] and base analogues, antibiotics, acridines, nitrous acid and hydroxylamine [3] is a good technique for producing genetic differences in plants, and it is an effective and significant tool in improving the yield of crop [4]. Sodium azide (NaN_3:SA) is a well-known inhibitor of heavy metal which affected the biological activities in the organism of many crops including barley, rice, maize and soybean [5]. many researchers used SA to create mutations in barley [6,7]. These studies showed that SA is a powerful substance for inducing mutations because the rate of chromosome aberrations which causes by Sodium azide is very low, and the transitions GC to AT were the predominant mutation type in barley [2]. Many breeding programs depend on the association between yield and its related traits to improve yield by the improvement of its component characters [8]. Studying the nature of gene action and the huge of genetic variation are the first step to improve crops through breeding programs [9,10]. Estimating the genetic parameters is the basic step for establishment a new breeding program, Selection and hybridization, depends firstly on the genetic variability in germplasm and on the association between yield and other traits [11]. These programs consist of many necessary steps such as creating variability, genetic advance of crops mostly depends on the genetic variability and heritability [12], Coefficients of variation, heritability and genetic advance are effective tools for selection and creating genotypes [13]. Choosing the most effective breeding program depend on the inheritance of the genetic traits, heritability is the most important indicator if the trait can be developed or not and choosing the most active method in breeding programs. In addition to genetic advance indicates the improvement which can be achieved from one generation to another [14]. The degree of variability for traits is a primary step to get effective selection and hybridization program [15]. High values of genotypic and phenotypic coefficient of variation (GCV, PCV) were observed for tillers per plant, high heritability coupled with high genetic advance was recorded for tillers per plant and plant height in barley [12]. The high magnitude of phenotypic coefficient of variation (PCV) with genotypic coefficient of variation (GCV) and high heritability and genetic advance were recorded for number of tillers per plant [13]. Changing in climate pushed the plant breeder to create new genotype using the chemical mutagenic in addition to radiation, Therefore, the main objective of this experiment was to generate new genetic variability of barely, induced by mutagenic chemical Sodium Azide, and to estimate the genetic parameter of the studied traits.

Materials and Methods

Plant materials and Sodium Azide Treatments

Healthy and uniform size of barely grains (Alforat 5) were selected and presoaked in distilled water for 4 hours, then soaked separately at room temperature in 100 ml of different concentrations of fresh sodium azide (NaN_3) solutions (1.5mM, 3mM, 4.5mM, 6mM and 100Mm) for 2 hours and the control (0 mM). 100 grains for each concentration (1ml of solution for each grain), sodium azide prepared newly (dissolving sodium azide in aqueous solution of phosphate buffer pH3.0) [16]. The Control group (0 mM) were soaked in distilled water. During the treatment period, the grains in vials were shaken frequently every 20 min, after treatment the grains were rinsed (3-4 times) with double distilled water then washed for two hours under running tap water then drained with toilet paper before being planted in pots.

Growing conditions

The grains were planted in pots (50cm diameter × 50cm depth) 10 grains per pot under controlled conditions in growth chamber at agriculture faculty in Damascus university (2021 season), the conditions were (22-25˚C day temperature, 17- 20˚C night temperature, light 14 hours day length), A number of characteristics (roots length, plant height, number of tillers per plant, number of leaves per plant and dry weight) were recorded after 45 days of planting.

Statistical analysis

Randomized complete block design with three replicates was used. The data have been analyzed by (PLABSTAT) software, where ANOVA, LSD and C.V. were identified.

Phenotypic and genotypic variances was estimated according to [17]

(Eq1): $\sigma 2g = (Mg-Me)/r$, $\sigma 2p = \sigma 2g+\sigma 2e$

Where: σ2g = Genotypic variance, Mg = genotypic mean square and σ2e = environmental variance (Error mean square).

Phenotypic coefficient of variation: (Eq2): $PCV = \frac{\sqrt{\sigma 2p}}{x} * 100$

Genotypic coefficient of variation: : (Eq3): $GCV = \frac{\sqrt{\sigma 2g}}{x} * 100$

Where: σ2g = genotypic variance, σ2e = Error variance, σ2p = Phenotypic variance.

PCV and GCV values > 20% regarded as high, PCV and GCV values between 10 and 20% medium, PCV and GCV values < 10% low [9,18].

Heritability (HB) in broad sense was estimated according to [17]

$HB = (\sigma 2g)/(\sigma 2p) \times 100$ (Eq4)

Where, σ2g = genotypic variance, σ2p = phenotypic variance andσ2e = error variance.

The values of broad sense heritability are) low < 30%, moderate 30-60%, and high > 60% [19].

Estimation of Genetic advance (GA) and genetic advance as percent of the mean (GA %) for each character were estimated using the formula [20]

: $GA = (k)(\sigma p) \times (HB)$ k = (2.06 at 5% selection intensity) (Eq5)

σp = phenotypic standard deviation, HB = heritability (Broad sense)

: Genetic advance in per cent of mean: GAM = [(GA)/X] × 100 (Eq6)

X = Grand mean, genetic advance as percentage of mean were High: (> 20%)

Medium: (10-20%) Low: (< 10%) [19,21].

Results and Discussion

Variances and means

The analysis of variance revealed significant differences among the six treatments for plant height, number of tillers per plant, number of leaves per plant and dry weight indicated the active effects of the treatments of Sodium Azide on the genotype (Alforat 5). the mean values of root length (Table, 1) were ranged between 9.9 cm when the genotype treated with 1.5mM and 17.9 cm when it treated with 3mM while the general mean for all treatments was 15.15 cm, and in the observation (0 mM) the length of roots was 15.3 cm, The best means were in the 3mM and 100 mM treatments17.9 cm, 17.4 cm respectively, so there were positive effects of a Sodium Azide in this trait which maybe good indicator to use it in mutation program for new genetic variations. For the number of tillers per plant the values ranged between 2.7 when the genotype was treated with (4.5 and 100mM) of Sodium Azide and the maximum value was 4.7 in the control (0 Mm) and the mean value was 3.17, So there was a negative effect of Sodium Azide on the number of tillers per plant in barley, but it is non-significant in the first treatment (1.5mM). on the other hand, the effect of Sodium Azide also was negative and significant for the number of leaves per plant and the value was 16 when the genotype was treated with (1.5mM), while it was 25 in the control (0 mM) and 8.7 in the fourth treatment (6mM). For the plant height the value was 10.4 cm in the fourth treatment (6mM) as in the control while it was 9.89 cm when the genotype was also with (100mM) of Sodium Azide and this difference was not significant. For the dry weight trait the best values were (1.3g, 0.9g, 0.8g) in the control and in the treatments with (1.5, 3 and 100mM) respectively with significant differences.

The variance among replications was non-significant with normal values of coefficient of variation (Table, 2). The high value for coefficient of variation were 20.87 for the dry weight while the low value was 5,75 in plant height which are normal for the field experiments. the negative influences of NaN_3 returned to its frustration of cell cycle, inhibition of DNA, RNA and protein synthesis, and defects in spindle fiber promotion, and as result causes deficiency in the cell cycle and metotic index [5].

Sodium Azid Conc. (mM)	Roots length (cm)	Number of tillers	Number of leaves	Plant height (cm)	Dry weight (g)
1.5	9.9**	4.0	16.0	7.83**	0.8
3	17.9	2.6	13.8	8.94	0.9
4.5	14.0	2.7	13.0	8.67	0.7
6	16.4	2.3	8.7**	10.4**	0.5
100	17.4	2.7	9.6**	9.89	0.8
0(control)	15.3	4.7	24.6	10.4	1.3
Mean	15.15	3.17	14.28	9.36	0.83
LSD (0.05)	4.77	0.95	3.68	0.99	0.36
S.D.	2.621	0.520	2.020	0.539	0.173

Table 1: The means of the five traits under six treatments of Sodium Azid.

Estimation of genetic parameters

Greater variability in the breeding material reflect a main step of producing a desired form of a crop plant. The estimations of phenotypic coefficient variation (PCV) were higher than the genetic variation coefficient (GCV) for all studied traits (Table, 2), however the deference's were not huge which indicated fewer effects on the environment variable. The best value of PCV among traits was 41.77 in number of leaves and 34.78 for dry weight, while the low value was 12.15 in plant height. The high values of GCV were 39.30 for number of leaves per plant and 28.39 for number of tillers per plant and 27.82 for dry weight, While the lowest values were in root length and plant height. According to (Table, 2) High heritability was recorded for most of traits and the best values were for number of leaves per plant 0.89, plant height 0.78, and number of tillers per plant 0.75. High genetic advances were also recorded for these traits which were 10.88 for number of leaves per plant, 3.61 for roots length, while it was 1.82 for plant height. The Genetic Advance as percentage of mean is more reliable parameter than genetic advance alone, It was High (> 20%) for number of leaves (76.2%), number control of tillers per plant (50.7%), and dry weight (45.90%). dry weight (45.9), While it was Medium for plant height (19.4%). High estimates of heritability and high genetic advance were recorded for number of tillers, number of leaves and dry weight, which indicates the lesser influence of environment in expression of these characters [22] and refer to simple inherited and they are under the control of additive gene action so selection may be effective through simple or progeny selection methods [23], and selection may be effective in early generations for these traits [24]. High heritability with low genetic advance has been detected for plant height; indicates that non-additive gene action is more effective in the behaviour of this trait, which refers to the role of environment in expression of these traits [22], thus simple selection procedure in early segregation generation may not be effective for generating desirable trait for future plant breeding [25], but it can by selection the best genotypes of segregating generation developed from hybridization breeding program [23,26]. Low heritability < 20% and low %mean GA < 10% were reported for plant height by [9,13]. High heritability joined with high genetic advance for tillers per plant, plant height in rice [27].

Treatments	Phenotypic variability (PCV)	Genotypic variability (GCV)	Heritability (H%))	Genetic advanced (GA) (5%)	GAM %	Coefficient Of Variation C. V
Roots length	23.95	16.65	0.48	3.61	23.9	17.21
Number of tillers	32.78	28.39	0.75	1.61	50.7	16.39
Number of leaves	41.77	39.30	0.89	10.88	76.2	14.14
Plant height	12.15	10.70	0.78	1.82	19.4	5.75
Dry weight	34.78	27.82	0.64	0.38	45.9	20.87

Table 2: Estimation of parameters of variability of five traits of *Hordeum Vulgare* L. under six treatments of Sodium Azid.

Conclusion

Creating genetic mutants by Sodium Azide (NaN_3) and estimating the values of genetic parameters such as Heritability and Genetic advanced which are the keys for establishing a new breeding program was the main goal for this study. Treatments with (NaN_3) influenced on all studied traits, the effect was positive on roots length generally, while it was negative on the other traits (Number of tillers, Number of leaves, Number of leaves, Plant height, Dry weight). High values of Genotypic variability were actualized for number of leaves per plant, number of tillers per plant, dry weight, so there was sufficient opportunity to use the genotype (Alforat 5) during next breeding program of barley, High heritability and high Genetic advance in per cent of mean were reported for number of leaves per plant, number of tillers per plant, dry weight, which refers that the additive gene action controls these traits, and there is good possibility to get successful selection and genetic gain during the early segregation generation M_2,M_3, and optimism to achieve new genotypes of barley.

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