Molecular identification and the effect of nanofertilization on growth traits of some maize genotypes

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Abstract

Maize is an important crop. Plant fertilization plays an important role in improving and increasing crop productivity in a sustainable manner. In the fall season of 2023, a field experiment was conducted to study the effect of different combinations of chemical and nano NPK fertilizers on some growth traits of five adopted genotypes, and to study the genetic variations at the molecular level. A randomized complete block design (RCBD) was used with split-plot arrangement and three replicates. The main plots included six treatments: five fertilizer combinations plus a control treatment (no fertilization). The sub plots included genotypes (three hybrids: Reserve, Jameson and AGN720 and two synthetic cultivars: Al Maha and Al Fajr). Results showed that a significant effect on almost all of the studied traits was due to fertilization levels, genotypes, and their interaction, as the use of 50% mineral + 50% nanofertilizer led to a significant increase in plant height, stem diameter, number of active leaves, leaf area and crop growth rate, which corded 206.4 cm, 19.18 mm, 12.2 leaf plant^{-1}, 6416 cm^{2}, and 4.651 gm plant^{-1} day^{-1}, respectively. On the other hand, the values of the control treatment gave the lowest values. The synthetic varieties outperformed in most of the studied traits. The Al-Maha synthetic variety gave the highest stem diameter and leaf area, which recorded 18.94 mm and 6419 cm^{2}, while the Al-Fajr synthetic variety gave the highest plant height and crop growth rate, which reached 211.7 cm and 4.469 gm. plant^{-1} day^{-1}. It can be concluded that mineral fertilizers can be replaced by nano-fertilizers, but can be considered as a supplement to mineral fertilizers and not a substitute for it.

Key Words: fertilization, crop productivity, genetic variations.

Part of M.Sc. thesis of the first author

Introduction

Maize (Zea mays L.) is a cereal crop belonging to the Poaceae family. It is a staple crop that is widely grown in different parts of the world. Jaaz et al., [1] stated that maize is the third crop after wheat and rice in terms of cultivated area and global production. the most important producing countries of this crop are Russia, China, India and South Africa [2]. As for Iraq's production of this crop, statistics for the year 2023 and for the two seasons (spring and autumn) indicate that production amounted to 538.3 thousand tons out of the total area planted with this crop, which amounted to 359.5 thousand dunams, with a mean yield of 1497 kg dunam^{-1} [3] compared to the United States of America, whose production of this crop amounted to 348751 thousand tons out of the total planted area of 128217 thousand dunams, with a mean yield of 2720 kg dunam^{-1} [4]. Therefore, Iraq's production of this crop is still low, which urges us to seriously search for all possible means to increase the yield. Among the most important means is the cultivation of genotypes that are characterized by their high productivity and testing the extent to which these genotypes respond to local agricultural conditions to achieve high productivity in terms of quantity and quality, in addition to many agricultural operations, including fertilization, as the use of the required quantities of chemical fertilizers can increase the production of the crop, but this type of fertilizer can raise the cost of agricultural production in addition to the environmental pollution problems that accompany the use of these quantities of chemical fertilizers, as the excessive use of chemical fertilizers leads to environmental pollution in addition to the high costs of these fertilizers [5]. Nano fertilizers can be an ideal option to increase crop productivity in a sustainable way, especially in developing countries, by improving nutrient use efficiency, reducing soil toxicity, mitigating the harmful effects of over-fertilization, and reducing the need for frequent fertilization. The main task is not to ban chemical fertilizers in agriculture, but to improve agricultural practices, especially for balanced and environment-friendly fertilization [6]. This is because the low diameter of nanomaterial particles that ranges between 1-100 nanometers [7], accordingly the ratio of the surface area-to-volume of nanomaterials is greater than that of the volume, which contributes to increasing their absorption and speed of transfer within the plant [8]. One of the benefits of using nano fertilizers in foliar fertilization is the speed of response to the plant's nutritional needs through the leaves, regardless of soil conditions [9], and it can also improve the balance of nutrients in the plant and thus improve the quantity and quality of the crop [10]. Hussein and Ahmed [11] indicated in their study the effect of different concentrations (0, 2, 4 g L^{-1}) of nano fertilizers on seven genotypes of maize crop. 4 g L^{-1} gave the lowest average in the trait of number of days from planting to 50% male and female flowering, as it reached (48.87, 56.31) days, respectively, while control treatment gave the longest period to 50% male and female flowering with an average of (52.78, 57.48) days, respectively. They also pointed out that the concentration of 4 g L^{-1} was highest in the plant height trait, which reached 187.19 cm, while the comparison treatment gave the lowest average of 175.04 cm.in a research experiment implemented by Al-

Shumary *et al*. [12] in the two seasons of 2017 to study the effect of spraying concentrations of integrated nano fertilizers (0, 6, 12 and 18 g L^{-1}) on the growth of three genotypes of maize crop (Kadiz, Sagunto and Abkaro), where the high concentration of nano fertilizers (18 g L^{-1}) recorded the highest average in number of days from planting to 50% male and female flowering with an average of (61.25, 65.17) days respectively. Also, the high concentration of nano-fertilizers recorded the highest plant height and leaf area (189.38 cm and 4629.96 cm^2 $plant^{-1}$) respectively. Therefore, this study aimed to know the performance of the hybrids under study and compare them with the two synthetic varieties, Al-Maha and Al-Fajr, under the influence of five combinations of nano and chemical NPK fertilizer, and to know the effect of nano fertilizers on the growth characteristics of the maize crop to determine the appropriate combination for this crop. In addition to knowing the genetic variations between the genotypes under study to benefit from them in the breeding and improvement programs of maize crop.

Materials and methods

A field experiment was conducted at Station A - Field Crops Department - College of Agricultural Engineering Sciences - University of Baghdad - Al-Jadriya for the fall season 2023 with the aim of assessing the performance of five genotypes (three introduced hybrids adapted by the Ministry of Agriculture (Reserve, Jameson and AGN720) referred to G1, G2 and G3 respectively, obtained from the Ministry of Agriculture and two synthetic varieties (Al-Maha and Al-Fajr) referred to G4 and G5 respectively, obtained from the Department of Agricultural Research. Under the influence of five combinations of NPK nano and chemical fertilizer {T1: 100% chemical fertilizer added to the soil + spraying the plant with tap water, T2: 75% chemical fertilizer + spraying the plant with 25% nano fertilizer, T3: 50% chemical fertilizer + 50% nano fertilizer, T4: 25% chemical fertilizer + 75% nano fertilizer, and T5: 100% nano fertilizer, as well as the control treatment (without adding fertilizers). The genetic variations between the genotypes were also studied at the molecular level. Before implementing the experiment, five soil samples were taken from the field at a depth of 0-30 cm, then mixed, air-dried, ground and sieved with a 2 mm sieve, and a homogeneous sample was taken for analysis in the laboratories of the Soil Department - University of Baghdad. The chemical and physical parameters of the soil were estimated as shown in table 1. The land was prepared by perpendicular plowing, smoothing and leveling, then cut into plots to be cultivated. The chemical fertilizers NPK were calculated for each fertilizer combination added to the soil according to the percentage of the fertilizer recommendation [13] after subtracting the amount of nitrogen (N), phosphorus (P) and potassium (K) ready in the soil. The fertilizer recommendation for nitrogen fertilizer was 320 kg N ha^{-1} in the form of urea (46% N) in two times, the first after 20 days of planting and the second after one month of the first one, and after that irrigation was carried out immediately. Phosphorous fertilizer 160 kg P h^{-1} in the form of triple superphosphate (46% P_2O_5) was applied to the soil before planting. Potassium fertilizer was not added to the soil because there were sufficient quantities of ready potassium in the soil to meet the fertilizer recommendation (table1).

Table (1) : Some chemical and physical parameters of field soil.

parameters	Value	parameters	Value
Electrical conductivity (EC) 1:1	0.88 dsm^{-1}	Soluble potassium (K)	1.03 M. mole L^{-1}
PH1:1	7.33	Soluble chlorine (CI)	5.6 M. mole L^{-1}
Available Nitrogen	30 mg kg^{-1}	Soluble carbonate (CO_3^{-2})	Nil
Available Phosphorus	6.2 mg kg^{-1}	Soluble sulfate ($SO4^{-2}$)	2.19 M. mole L^{-1}
Available Potassium	120.0 mg kg^{-1}	Soluble bicarbonate (HCO_3)	1.01 M. mole L^{-1}
Soil organic matter	30.3 g kg $soil^{-1}$	Soil texture	loam
Carbonate minerals	200 g kg $soil^{-1}$	Sand	332 g kg $soil^{-1}$
Soluble calcium (Ca+)	3.8 M. mole L^{-1}	Clay	248 g kg $soil^{-1}$
Soluble magnesium (mg+2)	2.2 M. mole L^{-1}	Silt	420 g kg $soil^{-1}$
Soluble sodium (Na+)	2.1 M. mole L^{-1}		

As for the nano fertilizer (N-P-K 20%-20%-20%), it was prepared according to the percentage of each fertilizer combination relative to the fertilizer recommendation (3 g per liter) of the Iranian manufacturing company (Khazra NPK Chelated Fertilizer Chemical) and was sprayed on the plant until completely wet in two stages, the first in the vegetative growth stage and the second in the flowering stage in the early morning to avoid high temperature and increase absorption efficiency with the addition of 1.50 cm3 washing up liquid per 10 liters. The randomized complete block design (RCBD) was used with split-plot arrangement and three replicates, where the main-plot included six treatments (five fertilizer combinations, in addition to the control treatment). As for the sub-plot, it included five genotypes. The land was prepared for cultivation by plowing, smoothing and leveling and dividing it into (3X2 m) plots, the plot includes four lines for each genotype. The distance between one line and another was 70 cm and between one plant and another was 25 cm. Planting was done on 25/7/2023 by placing 2-3 seeds in each hole at a depth of 5 cm, then it was thinned to one plant after the plants reached the two-leaf stage. Irrigation was carried out immediately after planting, and then the plants were irrigated whenever needed, and the weeds were removed continuously. Diazinon granular pesticide was used to control the corn stem borer in two times, the first 20 days after planting and the second 15 days after the first one, applied in the shoot apical at a rate of 6 kg ha^{-1} [14]. Five plants were taken from the middle of the planting lines and randomly for each experimental unit, and the following characteristics were taken: number of days from planting to 75% male and female flowering (day), plant height (cm), ear height (cm), stem diameter (mm), number of active leaves (leaf $plant^{-1}$), leaf area (cm^2), and number of days to physiological maturity. Harvesting was carried out on 20/11/2023, and the crop growth rate (gm $plant^{-1}$ day^{-1}) was calculated. Analysis of variance (ANOVA) was used to conduct statistical analysis for all studied traits and the arithmetic means were compared using the least significant difference (LSD) at a significance level of 0.05 for all means according to Steel and Torrie [15] using the Genestat program. To determine the genetic variations between the genotypes used in this study, DNA was extracted for each genotype and a site on the genome was targeted with two primers: (rbcLa-F) (5′-ATGTCACCACAAACAGAGACTAAAGC -3′) and (rbcLa-R) (5′-GTAAAATCAAGTCCACCACG -3′) by polymerase chain reaction and the results were sent to Macrogen Company in South Korea for the purpose of determining the nucleotide sequence. The phylogenetic tree was drawn using MEGA-X software [16].

Results and Discussion

Number of days from planting to 75% male flowering (day)

Results of analysis of variance presented in tables 2 and 3 showed a significant effect of genotypes and no significant effects of fertilizer levels and the interaction between experimental factors (fertilizer levels and genotypes) on the trait of number of days from planting to 75% male flowering of maize. The data presented in table 3 showed that this trait was affected by the genotypes. The genotype G2 was of early male flowering, giving the shortest period to reach male flowering with a mean number of days of 54.67 days, while the genotype G4 needed a longer period to reach male flowering with a mean number of days of 60 days, i.e. the genotype G4 was delayed by 5.33 days from the genotype G2, and the genotype G4 did not differ significantly from the genotype G1 in this trait. The reason for this variation between genotypes in the trait of the number of days from planting to 75% male flowering may be due to the difference between these genotypes in their DNA structure (figure 1) and morphological traits and their response to the environment such as temperature and length of lighting duration, as high temperatures and different lighting duration affect the plant and lead to increased enzymatic activity and then the development and rate of organ growth and the duration to reach at the male flowering stage, and thus leads to their variation in the number of days required for the plant to reach male flowering. These results were consistent with what [17,18,19,20], reported.

Table (2): Analysis of variance represented by means of squares for the effect of fertilizer levels and genotypes of maize and their interaction on growth traits.

Sources of Variation	Replications	Fertilizer Levels	First Experimental Error	Genotypes	Fertilizer Levels X genotypes	Second Experimental Error
Degrees of freedom	2	5	10	4	20	48
Number of days from planting to 75% male flowering (day)	2.500	6.880 ns	3.060	83.750**	2.630ns	2.092
Number of days from planting to 75% female flowering (day)	14.344	13.131 **	1.664	124.428 **	2.648 ns	2.444
Plant height (cm)	138.61	1063.09**	51.22	3070.43 **	265.44**	97.66
Height of ear (cm)	53.94	730.27 **	57.80	2143.54 **	282.87**	53.87
Stem diameter (mm)	1.715	16.945 **	0.977	14.559**	2.935**	1.195
Number of active leaves (leaf plant^{-1})	1.3778	2.1978 **	0.1644	6.2944**	1.2311*	0.6792
Leaf area (cm^2)	44977.	1363555.*	319301.	1429732.**	451783.ns	260629.
Number of days to physiological maturity	9.211	12.091 *	2.571	88.344**	1.824ns	3.428
Crop growth rate (gm plant^{-1} day^{-1})	0.1771	0.9233 ns	0.3323	0.4801ns	0.8423**	0.2973

** Significant at 0.01 level, * Significant at 0.05 level, and ns: Not significant.

Table (3): Effect of genotypes, fertilizer levels and their interaction on number of days from planting to 75% male flowering (day).

fertilizer Levels	Genotypes					mean
	G1	G2	G3	G4	G5	
T0	61.00	56.00	58.00	62.00	57.00	58.80
T1	60.00	55.00	58.00	61.00	57.00	58.20
T2	59.00	54.00	57.00	60.00	56.00	57.20

T3	59.00	55.00	57.00	60.00	56.00	57.40
T4	59.00	54.00	57.00	57.00	59.00	57.20
T5	59.00	54.00	57.00	60.00	56.00	57.20
mean	59.50	54.67	57.33	60.00	56.83	
LSD 0.05; Genotypes: 0.969, fertilizer levels: ns, Interaction between genotypes X fertilizer levels: ns.						

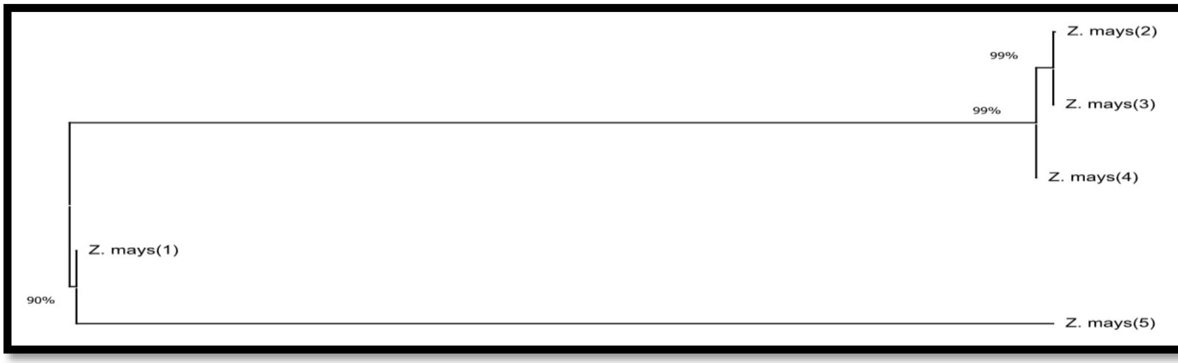

Figure (1): phylogenetic tree of the genotypes used in this study.

Number of days from planting to 75% female flowering (day)

Results of ANOVA in tables 2 and 4 showed that there are significant differences in the trait of the number of days from planting to 75% female flowering due to the effect of genotypes and fertilizer levels, and there are no significant differences due to the interaction between the experimental factors (genotypes and fertilizer levels) in this trait. The results of table 4 indicate that the shortest duration for this trait was for the genotype G2, where it recorded a mean number of days of 58.50 days, while the genotype G4 took the longest duration for the trait with a mean of 65 days, indicating that female flowering performed similarly to male flowering in these genotypes due to the association between the extent of late or early male flowering with female flowering, i.e. the early male flowering of the genotype G2 was reflected in female flowering and took the shortest duration to reach 75% female flowering. The reason for the variation in flowering times between genotypes may be attributed to genetic factors and environmental factors such as high temperatures and low humidity, which lead to a difference in the number of days to reach female flowering. This result is consistent with what [18,19,21,22] reported.

Results presented in table 4 also showed a significant effect of fertilizer levels on this trait, as levels: T4 and T5 were earlier in reaching 75% female flowering with a mean number of days of 60.80 and 60.60 days, respectively. This period was significantly less by 3.18% and 3.50% than the period taken by fertilization level T0 (control treatment). The latter did not differ significantly from plants grown under fertilizer levels T1 and T2. This performance shown by plants grown under different fertilizer levels indicates the role of nano fertilizer in reducing the number of days from planting to 75% female flowering. The reason for the difference in the period of entering female flowering stage may be attributed to the increased effectiveness of the flowering hormone (florigen) by increasing the level of fertilizer in this experiment, which leads to increased protein building as a result of the production of amino acids [23]. This is consistent with what Okab [24] mentioned.

The response of the genotypes to the levels of fertilizers did not differ in this trait, as the performance of all genotypes was towards reducing the number of days to reach 75% female flowering with increasing nano-fertilization. Accordingly, the results of the statistical analysis did not indicate the presence of a significant interaction between fertilizer levels and genotypes.

Table (4): Effect of genotypes, fertilizer levels and their interaction on number of days from planting to 75% female flowering (day).

fertilizer Levels	Genotypes					mean
	G1	G2	G3	G4	G5	
T0	65.00	60.00	62.00	67.00	60.00	62.80
T1	63.67	60.00	62.00	67.00	60.00	62.53
T2	64.00	60.00	62.00	65.00	61.00	62.40
T3	64.00	57.00	62.00	65.00	60.00	61.60
T4	62.00	57.00	61.00	64.00	60.00	60.80
T5	63.00	57.00	62.00	62.00	59.00	60.60
mean	63.61	58.50	61.83	65.00	60.00	
LSD 0.05; Genotypes: 1.048, fertilizer levels: 1.050, Interaction between genotypes X fertilizer levels: ns.						

Plant height (cm):

Results of analysis of variance presented in tables 2 and 5 show significant differences in the effect of genotypes, fertilizer levels and their interaction on the plant height trait. The results of table 5 indicate that plant height trait varied significantly between genotypes. The genotype G5 recorded the highest average for the plant height trait, giving a mean of 211.7 cm, outperforming by an increase of 8.73%, 8.89%, 20.76% and 11.72% compared to the genotypes G4, G3, G2 and G1, respectively. The genotype G2 gave the lowest average for the trait, reaching 175.3 cm. The reason for this is that the genotype G2 was early in male and female flowering than the rest of the genotypes, as shown in tables 3 and 4, because the flowering stage leads to limit plant height, and the variation of plants in the period required for the flowering process and the plant height trait comes as a result of the variation in the plants' response to the conditions. Environmental factors such as the duration of daylight and temperature, as well as the variation in their efficiency in exploiting the available growth factors as a result of their variation in their genetic structure and thus their variation in the physiological processes that occur in each genotype. This result was consistent with what was reached by [1,17,19,25,26,28,29,30,31], who found that the plant height trait differs significantly according to the varieties.

Results in table 5 also indicated a significant effect of fertilizer levels on plant height, as plants grown at fertilizer level T3 recorded the highest average plant height of 206.4 cm, significantly outperforming all other fertilization levels with an increase of 4.34%, 5.15%, 10.17%, 10.67%, and 11.83% compared to fertilization levels T2, T4, T1, T5, and T0, respectively. The control treatment (T0) gave the lowest plant height of 184.6 cm. This may be attributed to the major role of macronutrients, as fertilization, especially nitrogen, contributes to plant growth and development, as it regulates the performance of plant hormones (auxin and cytokinin). Also, the positive role of potassium in the process of cell division and expansion by providing the ideal expansion of the cell wall necessary for the processe of growth. This indicates that nano-fertilizers have the ability to play the role of chemical fertilizers and provide the plant with the necessary nutrients for its growth, as these fertilizers have distinct properties such as their small size and high surface area, which enables them to increase the speed of their penetration and absorption, in addition to increasing enzymatic activity and increasing the speed of biochemical reactions. This result is consistent with what was stated by [1,32].

Results of the statistical analysis presented in table 5 indicate that there is a significant effect of the interaction between fertilizer levels and genotypes for the trait of plant height, as the performance of genotypes varied under different fertilization levels, but most of the genotypes followed the direction of increasing plant height with the fertilizer combination (50%:50%) between chemical fertilizer and nano fertilizer, as the genotype G5 at the fertilizer level T3 gave the highest average for the trait, reaching 233.7 cm, and the performance of this genotype at the fertilizer level T3 did not differ significantly from its performance at the fertilizer level T2, while the genotype G2 at the fertilizer level T0 recorded the lowest average for the trait, reaching 165 cm, which did not differ significantly from the genotype G3 at the same fertilizer level T0.

Table (5): Effect of genotypes, fertilizer levels and their interaction on plant height (cm).

fertilizer Levels	Genotypes					mean
	G1	G2	G3	G4	G5	
T0	183.5	165.0	177.0	199.0	198.3	184.56
T1	181.0	171.7	184.0	203.7	196.3	187.34
T2	194.3	179.3	207.7	185.7	222.0	197.8
T3	201.0	180.0	214.3	203.0	233.7	206.4
T4	197.3	188.3	185.7	201.0	209.0	196.3
T5	179.7	167.3	198.7	176.0	211.0	186.5
mean	189.5	175.3	194.6	194.7	211.7	
LSD 0.05; Genotypes: 6.62, fertilizer levels: 5.82, Interaction between genotypes X fertilizer levels: 15.37.						

Ear height (cm):

Results of the analysis of variance shown in tables 2 and 6 indicate that there are significant differences in the ear height trait due to the effect of both genotypes and fertilizer levels, as well as there was a significant interaction between the experimental factors (genotypes and fertilizer levels) in this trait. Results of table 6 indicate that genotype G5 gave the highest average ear height of 106.89 cm, with a significant increase rate of 39.1%, 18.0%, 17.7%, and 10.5% compared to the genotypes G2, G1, G3, and G4, respectively, while the lowest average for the trait was recorded by the genotype G2, which recorded 76.83 cm. This indicates the reflection of the results of Table 5 for plant height on the ear height, which is a result of the difference in the nature of the genotype's performance in the ear height trait. This is consistent with what was reported by [18,24, 25,33,35],

Results of table 6 also show a significant effect of fertilizer levels on the ear height trait, as plants grown at fertilizer level T3 gave the highest average ear height of 101.67 cm, significantly outperforming all other fertilization levels by an increase of 22.2%, 16.2%, 14.7%, 5.7%, and 4.8% compared to fertilization levels T0, T1, T2, T5, and T4, respectively. The control treatment (T0) gave the lowest plant height of 83.2 cm. This is due to the role of nutrients that increase plant growth by increasing carbon metabolism. Nutrient materials are produced by stimulating the activity of growth regulators such as auxin and gibberellin, which increases cell elongation leading to elongate of stem internodes, which in turn leads to an increase in plant height and ear height in the plant. This result is consistent with those of Al-Mufarji [32].

As for the interaction, results of the statistical analysis shown in table 6 indicate a significant effect between fertilizer levels and genotypes for the ear height trait. The interaction was in the trend of increasing ear height at fertilizer level T3, as the genotype G4 at fertilizer level T3 gave the highest average for the trait, reaching 112 cm, and did not differ significantly from the genotypes G3 and G5 at the same fertilizer level (T3), while the lowest average for ear height was 66 cm, recorded by the genotype G2 at fertilizer level (T1).

Table (6): Effect of genotypes, fertilizer levels and their interaction on height of ear (cm).

fertilizer Levels	Genotypes					mean
	G1	G2	G3	G4	G5	
T0	84.67	74.67	85.00	66.00	105.67	83.20
T1	86.00	66.00	82.33	102.67	100.67	87.53
T2	93.00	73.67	79.00	107.67	90.00	88.67
T3	92.67	83.67	110.00	112.00	110.00	101.67
T4	93.67	75.00	103.67	94.50	118.00	96.97
T5	93.67	88.00	85.00	97.33	117.00	96.20
mean	90.61	76.83	90.83	96.69	106.89	
LSD 0.05; Genotypes: 4.919, fertilizer levels: 6.185, Interaction between genotypes X fertilizer levels: 12.086.						

Stem diameter (mm):

Results of the analysis of variance presented in tables 2 and 7 show that the stem diameter trait of maize plants varied significantly according to the genotypes and fertilizer levels, in addition to the presence of a significant interaction between fertilizer levels and genotypes in this trait. It is clear from table 7 of the means of the stem diameter trait that there is a significant difference between the genotypes in this trait, as the genotype G4 gave the highest mean for the trait, reaching 18.94 mm, and did not differ significantly from the genotype G5, as the stem diameter of the genotype G5 increased significantly by 5.8%, 11.2%, and 12.9% compared to the genotypes G2, G3, and G1, respectively, as the genotype G1 gave the lowest average for the trait, reaching 16.77 mm. The reason for the difference between the genotypes in this trait may be the difference in the number and size of their vascular bundles in the plant stem, and this is consistent with the results of [36, 37, 38] who indicated that there were significant differences between varieties in the stem diameter trait.

Results of table 7 also indicate a significant effect of fertilizer levels on the stem diameter trait, as the table shows that the stem diameter increased with the addition of nano-fertilizer, and the best fertilizer combination was 50% chemical fertilizer with 50% nano-fertilizer, which represents the T3 fertilizer level, which did not differ significantly from the T5 fertilizer level. The percentage of increase for the T3 fertilizer level compared to the control treatment (T0) was 18.08%. The reason for this may be the role of nutrients in increasing the efficiency of the photosynthesis process, and thus an increase in the size of cells and then the speed of their division, which leads to an increase in the leaf area and then an increase in the stem diameter [39-1].

Table (7): Effect of genotypes, fertilizer levels and their interaction on stem diameter (mm).

fertilizer Levels	Genotypes					mean
	G1	G2	G3	G4	G5	
T0	14.62	17.50	15.11	16.84	17.19	16.25
T1	16.98	17.35	16.80	18.25	18.69	17.61
T2	15.34	16.65	16.63	18.64	19.22	17.30
T3	18.35	19.11	19.23	19.34	19.87	19.18
T4	17.53	17.61	17.78	19.38	15.79	17.62
T5	17.80	19.20	16.63	21.17	19.26	18.81
mean	16.77	17.90	17.03	18.94	18.34	
LSD 0.05; Genotypes: 0.733, fertilizer levels: 0.804, Interaction between genotypes X fertilizer levels: 1.754.						

As it is clear from the statistical analysis data in table 7, the interaction between the two study factors in the stem diameter trait was significant and the direction of the response of the genotypes to fertilizer levels was varied. The highest average for the trait was 21.17 mm recorded by the genotype G4 at the fertilizer level T5, while the genotype G1 gave the lowest average for the trait, recording 14.62 mm at the fertilizer level T0.

Number of active leaves (leaf plant^{-1}):

Results of the analysis of variance presented in tables 2 and 8 showed significant differences due to the effect of genotypes and fertilizer levels, and the interaction between the experimental factors (genotypes and fertilizer levels) was significant in the trait of the number of effective leaves of maize plants. The results presented in table 8 show that the genotypes varied in the trait of the number of effective leaves, as the genotype G2 outperformed by giving the highest average number of effective leaves, which reached 12.6 leaves per plant-1, with a significant increase rate of 7.1% and 14.1% compared to the genotypes G3 and G1, respectively, while the genotype G1 recorded the lowest mean for the trait, giving a mean of 11 leaves per plant-1, and the genotype G2 did not differ significantly from the genotypes G4 and G5. The number of active leaves is a genetic trait that is affected by environmental conditions. These results are consistent with the findings of [1,32.40,41,42,43] , who found significant differences between the genotypes in this trait. Although the number of leaves is not a component of the seed yield, its importance lies in the dependence of the yield on the size and efficiency of carbon synthesis, as leaves are the main sugars producer by photosynthesis process in the plant. We note that the genotype G2, although it was superior in the number of active leaves, recorded the lowest number of days from planting to male and female flowering (tables 3 and 4), respectively, and the lowest plant and ear height (tables 5 and 6), respectively. This indicates that the number of active leaves in the genotype G2 has no relation to the growth period before the plant reaches the flowering stage or to the plant height, and this is inconsistent with what was found by [44].

Results of table 8 also showed a significant effect of fertilizer levels on the number of effective leaves trait. The fertilizer level T3 recorded the highest average of number of effective leaves, reaching 12.23, with a significant increase of 2.23%, 6.79% and 7.31% compared to fertilizer levels T2, T1 and T0, respectively. There were no significant differences between fertilizer level T3 and fertilizer levels T4 and T5. The reason for the increase in the number of active leaves with the increase in the level of nano-fertilizer may be due to the ability of nutrients to stimulate plant cells through the division and elongation stages by directly affecting the leaf formation area and increasing the number of cell divisions, as well as affecting the hormones responsible for leaf formation and increasing their number, in addition to the fact that fertilization, especially the nitrogen element, when combined with other elements, forms many building materials for the plant growth, including leaves [45]. This is consistent with what [32,46] reported.

As for the interaction, the results of the statistical analysis indicate the presence of a significant effect between fertilizer levels and genotypes of the trait, as the genotypes of maize differed in their response to fertilizer levels, and the response was towards an increase in the number of active leaves trait with the fertilizer combination (50%: 50%) between chemical fertilizer and nano fertilizer, as the highest average for the trait was recorded at the fertilizer level T3 and genotype G2, where it reached 13.3 leaves per plant^{-1}, while the lowest average for the trait was recorded at the fertilizer level T1 and genotype G1, where it gave 10 leaves per plant^{-}

Table (8): Effect of genotypes, fertilizer levels and their interaction on number of active leaves (leaf plant^{-1}).

fertilizer Levels	Genotypes					mean
	G1	G2	G3	G4	G5	
T0	11.0	11.3	10.3	13.0	11.3	11.4
T1	10.0	12.0	11.0	12.0	12.3	11.5
T2	11.0	13.0	12.3	11.5	12.0	11.1
T3	11.3	13.3	12.0	12.5	12.0	12.2
T4	11.7	12.7	12.0	12.0	12.7	12.2
T5	11.0	13.0	12.7	11.3	13.0	12.2

mean	11.0	12.6	11.7	12.1	12.2	
LSD 0.05; Genotypes: 0.552, fertilizer levels: 0.330, Interaction between genotypes X fertilizer levels: 1.243.						

leaf area (cm^2)

Results of analysis of variance shown in tables 2 and 9 demonstrate that there are significant differences due to the effect of genotypes and fertilizer levels on the leaf area trait, and there is no significant interaction between the experimental factors to affect this trait, Results of table 9 show significant effects of genotypes on the leaf area trait, as the genotype G4 recorded the highest leaf area of 6419 cm^2 and did not differ significantly from genotype G5, with a significant increase rate of 4.1%, 9.4% and 9.8% compared to the genotypes G1, G3 and G2, respectively. Genotype G2 recorded the lowest rate for the trait with a mean of 5846 cm^2. The differences in the genetic structure that distinguishes each genotype are the most influential factor in the variations of leaf area. These results are consistent with what [19,31] stated.

The effect of fertilizer levels on leaf area trait differed (table 9), as fertilizer level T3 gave the highest average of the trait, reaching 6416 cm^2, with a significant increase of 2.4%, 8.6%, and 13.6% compared to fertilization levels T2, T1, and T0, respectively. There were no significant differences between fertilizer level T3 and fertilizer levels T4 and T5. The effect of fertilizer levels on leaf area trait is consistent with their effect on the number of effective leaves trait (table 8). The reason for the increase in leaf area due to the effect of nanofertilization may be that adding fertilizers maintains the vitality of chloroplasts for a longer period and stimulates carbon metabolism enzymes, thus stimulating plant cell division, growth and development. Nanofertilizers added by spraying on the leaves are characterized by their high ability to penetrate plant tissues and also stimulate the production of cytokinins, which leads to increased effectiveness of photosynthesis and enhances vital processes, which is reflected in plant growth and development [45]. These results are consistent with what [1,47,48] reported.

Table (9): Effect of genotypes, fertilizer levels and their interaction on leaf area (cm^2).

fertilizer Levels	Genotypes					mean
	G1	G2	G3	G4	G5	
T0	5677	5171	5464	5808	6123	5649
T1	5645	5168	5897	6759	6058	5906
T2	6443	5896	6396	6211	6393	6268
T3	6305	6487	5681	6348	7259	6416
T4	6487	6078	5677	7306	6323	6374
T5	6442	6271	6075	6081	6348	6244

mean	6167	5846	5865	6419	6418	
LSD 0.05; Genotypes: 342.2, fertilizer levels: 459.7, Interaction between genotypes X fertilizer levels: ns.						

Number of days to physiological maturity:

Results of analysis of variance in tables 2 and 10 show that there are significant differences between the genotypes and the fertilizer levels, and there are no significant differences for the interaction between the genotypes and the fertilizer levels in the trait of number of days to physiological maturity of maize plants. The results of Table 9 indicate that there are significant differences in the trait of the number of days to physiological maturity among the genotypes, as the genotype G2 took the least number of days to reach physiological maturity with a mean number of days of 83 days, followed by the genotype G5 with a mean number of days of 84.67 days, then the genotype G3 with a mean number of days of 86.22 days, while the genotype G4 was delayed and needed 88.33 days to reach the stage of physiological maturity and did not differ significantly from genotype G1, which needed a mean number of days of 87.83 days. Genotypes that are short or medium in their physiological maturity are the best due to the possibility of using the land for planting a second crop and also avoiding the risk of rain, especially in the autumn season, due to the damage it causes to the grains, leading to their deterioration and reduced quality, in addition to increasing the costs of their production, storage and processing. This result is consistent with what was obtained by [21,29,35, 49,50], who confirmed the variation of genotypes in the number of days from planting to 95% of physiological maturity.

Results presented in table 10 also indicated a significant effect of fertilizer levels on the number of days to physiological maturity trait, which showed that the control treatment (T0) was early in reaching physiological maturity with a mean number of days of 84.8 days and did not differ significantly from the two fertilizer levels T1 and T3. The effect of fertilizer levels T5, T4 and T2 (which did not differ significantly) was clear in delaying the plants in reaching physiological maturity compared to the other fertilizer treatments T3, T1 and T0 used in this experiment. The absence of significant interaction in this experiment indicates the similarity in the response of genotypes to fertilizer levels in the number of days to physiological maturity trait

Table (10): Effect of genotypes, fertilizer levels and their interaction on number of days to physiological maturity.

fertilizer Levels	Genotypes					mean
	G1	G2	G3	G4	G5	
T0	87.00	80.00	86.00	88.00	83.00	84.80
T1	87.00	82.00	86.00	87.00	84.00	85.20
T2	88.00	83.00	87.00	89.00	85.00	86.40
T3	88.00	83.00	85.00	88.00	85.00	85.80
T4	89.00	85.00	86.33	89.00	85.00	86.87
T5	88.00	85.00	87.00	89.00	86.00	87.00
mean	87.83	83.00	86.22	88.33	84.67	
LSD 0.05; Genotypes: 1.241, fertilizer levels: 1.305, Interaction between genotypes X fertilizer levels: ns.						

Crop growth rate (gm plant^{-1} day^{-1}):

Results of analysis of variance in tables 2 and 11 show that there were no significant differences between genotypes as well as between the fertilizer levels, but response of the genotypes to the fertilizer levels varied to indicate the presence of a significant interaction between the experimental factors in the trait of the crop growth rate, as the trend of the response of the genotypes was towards increasing the crop growth rate with the fertilizer combination (50%:50%] between chemical fertilizer and nano fertilizer, where the genotype G5 at the fertilizer level T3 gave the highest average for the trait, reaching 5.34 g plant^{-1} day^{-1}, while the same genotype G5 recorded the lowest average for the trait, reaching 3.04 g plant^{-1} day^{-1} at the fertilizer level T0. The crop growth rate is the result of dividing the total dry matter weight by the number of days of physiological maturity. Accordingly, it represents the accumulation of dry matter in the plant for a specific period of time and reflects the outcome of the plant's vital processes. In addition, it is a good indicator of the efficiency of photosynthesis efficiency and the plant's response to environmental conditions. This trait is one of the desirable traits for plant breeders, as the high crop growth rate contributes to increasing the seed yield [51,52 ,53 ,54].

Table (11): Effect of genotypes, fertilizer levels and their interaction on crop growth rate (gm plant^{-1} day^{-1}).

fertilizer Levels	Genotypes					mean
	G1	G2	G3	G4	G5	
T0	3.154	3.759	3.752	4.395	3.041	3.602
T1	4.393	4.879	5.045	3.932	3.511	4.352
T2	4.060	4.570	4.378	4.189	4.110	4.261
T3	4.473	4.708	3.835	4.896	5.340	4.651
T4	4.224	3.828	4.046	4.807	4.583	4.298
T5	3.833	4.182	4.174	3.400	4.228	3.963
mean	4.023	4.321	4.205	4.270	4.469	
LSD 0.05; Genotypes: ns, fertilizer levels: ns, Interaction between genotypes X fertilizer levels: 0.902.						

It can be concluded that mineral fertilizers can be replaced by nano-fertilizers, but can be considered as a supplement to mineral fertilizers and not a substitute for it. when using the 50%:50% NPK combination between chemical and nano-fertilizers gave a significant response and the best result in most of the studied traits,

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