ETHYL METHANESULPHONATE (EMS) INDUCES DROUGHT TOLERANCE IN MAIZE

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ABSTRACT

THIS STudy was aimed to induce genetic variation toward drought tolerance after treatment with EMS as a mutagen to two maize inbred lines and their single hybrid. The experiment was carried out at Abo-Ghraib Research Station, Baghdad during the spring season of 2019. The experiment included three irrigation intervals 7, 9 and 11, four concentrations of EMS (0, 50, 60, and 70 mM) and three maize genotypes distributed in three replications using randomized complete block design (RCBD) with split-split plot arrangement which included three irrigation intervals 7, 9 and 11 days occupied the main plot, while the sub plots included three maize genotypes, and four EMS concentrations which were distributed in sub-sub plots. Results were revealed significant differences among EMS treatments and irrigation interval treatments. The inbred line 5 in control treatment showed a significant increase in days for both anthesis and silking (70.11 and 71.94) days, respectively. Plants of Nahrain hybrid also showed a super performance in plant height, leaf area, and number of rows ear^{-1}, with a mean of 154.47cm, 746.31cm^2 and 17.83 row ear^{-1}, respectively. The irrigation intervals of 9 and 11 days were significantly decreased the mean performance of genotypes, compared to the shortest irrigation interval (7 days) which had highest mean in most traits. None treated plants (0 mM EMS) had a significant effect on the performance of most studied traits, except the anthesis and silking time, which gave the highest values under the concentration 60 mM with 69.11 and 70.48 days, respectively. Hybrid plants interacted significantly with the EMS concentration of 0 mM producing the maximal means of plant height and leaf area (169.56cm and 759.78cm^2 respectively). On the other hand, the hybrid was significantly interacted with the shortest irrigation interval in the mean traits of plant's height and leaf area (163.50 cm and 757 cm^2, respectively). The triple interaction between hybrids, shortest irrigation interval and EMS concentration of 60 mM had significantly increased leaf area reached 769.67cm^2, meanwhile the triple interaction between hybrid plants, shortest irrigation interval, and the EMS concentration of 0 mM recorded the highest value of plant yield (199.67g). It can be concluded from the current study that the genotypes differed in their response to 9 and 11 days irrigation intervals, with a negative effect of the later interval on the vegetative and yield traits of maize compared with shorter irrigation interval.

Keywords: *Zea mays* L., Chemical mutagens, Abiotic stress.

أثيل ميثان سلفونيت يحفّز تحمّل الجفاف في الذرة الصفراء

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المستخلص

هدفت الدراسة إلى إستحداث التغاير الوراثي بالتطفير الكيميائي بمادة اثيل مثيل سلفونيت لتحفيز تحمّل إجهاد الجفاف في سلالتين وهجينهما الفردي من الذرة الصفراء, وإنعكاس ذلك في الأداء المظهري للنباتات. نُفّذت التجربة في حقول محطة أبحاث ابي غريب– دائرة البحوث الزراعية– بغداد في الموسم الربيعي 2019. تضمنت التجربة ثلاث فواصل ري واربعة تراكيز من EMS (0 و50 و60 و70 mM) وثلاث تراكيب وراثية من الذرة الصفراء وُزّعت في ثلاث مكررات وفق التصميم تام التعشية (RCBD) بترتيب الألواح المنشقة– منشقة. أظهرت النتائج تفاوتاً معنوياً في أداء التراكيب الوراثية بتاثير تراكيز النقع بمادة EMS وفواصل الري. تفوقت نباتات ا السلالة 5 في معاملة المقارنة في صفتيّ التزهير الذكري والانثوي, وذلك بإحتياجها أقل متوسط عدد ايام لإتمام 50% تزهير ذكري وأنثوي بلغ 70.11 و71.94 يوم بالتتابع. كما أظهرت نباتات الهجين (نهريّن) تفوقاً في متوسط ارتفاع النبات والمساحة الورقية وعدد صفوف العرنوص بمتوسط بلغ 154.47 سم و746.31 سم^2 و17.83 صف عرنوص$^{-1}$ بالتتابع. أدت فاصلتيّ الري المتباعدة (9 و11 يوم) الى تراجع أداء جميع نباتات التراكيب الوراثية, قياساً بفاصلة الري الاقصر 7يوم ذات المتوسطات الأعلى في جميع الصفات. كما بينت النتائج تفوّق معاملة النقع بالتركيز 0 EMS mM في متوسط أداء اغلب صفات الدراسة, بإستثناء صفتيّ التزهير الذكري والانثوي التي تفوّق فيهما التركيز 60 mM بمتوسط أداء بلغ 69.11 و70.48 يوم بالتتابع. تفوّقت نباتات الهجين عند تداخلها مع تركيز 0 EMS mM في صفة ارتفاع النبات والمساحة الورقية بمتوسط بلغ 169.56 سم و759.78 سم^2 بالتتابع. كما تداخلت معنوياً مع فاصلة الري الأقصر في متوسط أداء صفة ارتفاع النبات والمساحة الورقية بمتوسط بلغ 163.50 سم و757 سم^2. تفوّقت نباتات الهجين عند تداخلها الثلاثي مع فاصلة الري الاقصر وتركيز 60 mM في متوسط المساحة الورقية الذي بلغ 769.67 سم^2, مع تفوّق ذات النباتات وذات فاصلة الري عند تداخلاهما مع تركيز 0 EMS mM في متوسط حاصل النبات الفردي الذي بلغ 199.67 غم. يُستنتج من الدراسة تباين التراكيب الوراثية في إستجابتها لفواصل الري 9 و11يوم مع سلبية تأثير فاصلة الري الاخيرة في الصفات الخضرية والحاصل للذرة الصفراء مقارنةً مع فاصلة الري الأقصر.

الكلمات المفتاحية: ذرة صفراء, مطفّرات كيميائية, إجهاد لا حيوي.

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INTRODUCTION

Agricultural activities depleted more than 70% of the global consumed water, and these requirements are increasing by 90% in the developing countries with a permanent water shortages (5). Moisture deficiency represents one of the vital limitations to agricultural productivity and development. Despite of the negative impact of stress, it initiates a point for greater efforts in finding tolerant genotypes, and hence the efficient use of available water in the future (7). Nowadays, the huge development of the industrial sector has negatively affected the atmosphere thermal balance leading to environmental fluctuations, and effectively participates in the global warming (26). Accordingly, significant reduction in crops yield in general and the field crops in particular will occur (6). Maize is the second most important pillar in the global production of grains (12). The crop is an important source for feeding human and animals, in addition to the production of biofuels, it also the second most used crop in genetic modification after soybeans (25). Maize is more vulnerable to inappropriate environmental conditions, represented by stresses. Abiotic stresses in general, and drought stress in particular, that is the most serious determinant of yield potential, keeping the crop productivity within the lowest levels (4). Mutations are not so common genetic change unless they are induced by either physical factors like Gama and Beta rays etc., or chemical ones such as sodium azide (SA), colchicine, methyl methanesulphonate (MMS) or ethyl methanesulphonate (EMS). The later have been widely used to induce genetic variability in specific traits. EMS is one of the most common chemical mutagens used for inducing genetic variation without causing large-scale chromosomal aberrations in which the progeny survival rate may decrease (22). There always a need for novel methods in which a desired genetic alteration can be achieved, hence EMS was adopted in the current study to induce genetic variation to drought stress in a number of genotypes.

MATERIALS AND METHODS

A field experiment was carried out at Abo-Ghraib Research Station, Agricultural Research Department, Ministry of Agriculture during the spring season 2019. The experiment included three genotypes of maize which namely; inbred lines 5, 18 and their F_1 hybrid (Nahrain). The field was prepared, then divided into 3 x 3 m^2 plots. Each plot included 4 rows of 0.75 m × 0.25 m distance. The experimental units (36) were randomly distributed in three replicates. The seeds were dipped with distilled water for 5 hours in dark room conditions. The EMS solution was prepared in four concentrations (0, 50, 60 and 70 mM) by diluting it in a phosphate buffered saline (PBS). The seeds were immersed for the same period and under the previous conditions, then rinsed with distilled water 10-8 times to remove the remains mutagen and transferred to the field for sowing. Treatments were distributed according to randomized complete block design (RCBD) with split-split plot arrangement, in three replicates. The three genotypes located in the sup plots, and the four concentrations of EMS (0, 50, 60 and 70 mM) were applied in the sub-sup plots, while the three irrigation intervals (7, 9 and 11 days) were occupied the main plots. Seeds were sown on 23/3/2019 and irrigated as needed until full germination. The irrigation intervals were practiced as plants reached 7 leaves stage Ears were harvested at full maturity stage, and kept in the shade to dry. The following traits were studied; anthesis and silking time, plant height (cm), leaf area (cm^2) was measured according to (24), ear's rows number (row ear^{-1}), and plant grains yield (g $plant^{-1}$).

RESULTS AND DISCUSSION

Anthesis (days): Results display in Table 1 indicate significant differences among the genotypes, irrigation intervals and EMS concentrations, as well as the interactions in the studied traits. Obviously, each of the 5 and 18 inbred lines and the Nahrain hybrid required 70.11, 72.77 and 72.72 days respectively to complete 50% anthesis, and thus the 5 inbred lines had the least number of days, while the other inbred line 18 had longer duration to anthesis 50% of its plants. EMS concentrations had also a significant effect since the concentration of 60 mM gave the lowest value (69.11 days), while higher concentration of EMS (70 mM) increased the anthesis days up to 74.14 days. Genotypes had the least number of days to 50% anthesis were

cultivated under the two irrigation intervals of 7 and 9 days which recorded 71.44 and 71.30 days, respectively. Increasing the irrigation intervals to 11 days delayed the transition from the vegetative to reproductive stage. The genotypes required 72.86 days to anthesis under the longer irrigation interval. The results of the interaction between genotypes and irrigation intervals showed that plants of inbred line 5 under the irrigation interval of 9 days expressed the lowest days number required for 50% anthesis (69.33 days), exhibiting a significant differences among the inbred line 18 which gave the highest mean of 73.83 days under the longest irrigation interval. The inbred line 5 spent less days (68.44 days) to anthesis as it treated with 60 mM of EMS, while the inbred line 18 had the highest mean (75.33 days) at the concentration of 70 mM to complete 50% anthesis. Genotypes were also significantly affected by the interaction between mutagen concentrations and irrigation intervals as it reached the lowest number of days to 50% anthesis which was 67.44 days at 60 mM under the irrigation interval of 9 days, meanwhile, the highest value was obtained at the concentration 70 mM EMS accompanied with the longest irrigation interval that scored 74.77 days. The significant effect of the interaction of mutagen and irrigation intervals had reflected on the used genotypes; the 18 inbred line reached 66 days under the EMS concentration of 60 mM and the 9 days irrigation interval, whereas, the interaction between the 70 mM of EMS and the longest irrigation interval resulted in the highest number of days needed to 50% anthesis (76 days). Results are in agreement with the results of recent studies (2) who reported that the number of days for anthesis depends on the mutagenic concentration including EMS, in addition to the response of the genotypes.

Table 1. **Anthesis (50%) after treatment with EMS and irrigation intervals in two maize inbred lines and their single hybrid**

Genotypes	EMS Concentrations	Irrigation Intervals (day) 7	9	11	Genotypes× Concentrations	Genotypes Mean	Irrigation Intervals 7	9	11
18	0	72.66	74.00	74.66	73.77	72.77	72.41	72.08	73.83
	50	72.00	73.00	74.00	73.00				
	60	70.33	66.00	70.66	69.00				
	70	74.66	75.33	76.00	75.33				
5	0	69.00	69.66	71.00	69.88	70.11	69.41	69.33	71.58
	50	69.00	67.00	71.66	69.22				
	60	67.66	67.66	70.00	68.44				
	70	72.00	73.00	73.66	72.88				
Nahrain	0	73.00	74.00	74.66	73.88	72.72	72.50	72.50	73.16
	50	73.00	73.33	72.33	72.88				
	60	70.00	68.66	71.00	69.88				
	70	74.00	74.00	74.66	74.22				
	Concentrations × Irrigation Intervals				Concentrations Mean		Irrigation Intervals Mean		
	0	71.55	72.55	73.44	72.51				
	50	71.33	71.11	72.66	71.70		71.44	71.30	72.86
	60	69.33	67.44	70.55	69.11				
	70	73.55	74.11	74.77	74.14				
L.S.D 0.05	Genotypes	Concentrations	Irrigation Intervals	Genotypes× Concentrations	Genotypes× Intervals	Concentrations × Intervals	Genotypes × Concentrations× Intervals		
	0.27	0.32	0.63	0.54	0.65	0.70	1.02		

Silking (days): Results show in Table 2 indicate significant differences among the studied traits. Genotypes varied significantly in the number of days to 50% silking. The inbred line 5 revealed the least mean (71.94 days), that outperformed the other two genotypes, while the plants of the inbred line 18 required 74.61 days to complete 50% silking. Concentrations of mutagenic solution also affected the genotypes, the concentration of 60 mM EMS achieved early silking with 70.48 days, while the concentration of 70 mM increased the 50% silking up to 76.14 days. Also the irrigation intervals had a significant effects on this trait, as the plants under the irrigation interval of 9 days had the lowest period (72.83 days), while the effect of stress negatively affected the silking time up to 11

days with mean value of 74.97 days. The interaction between genotypes and the irrigation intervals caused the least mean (70.91 days) in inbred line 5, at the irrigation intervals 7 and 9 days, while the inbred line 18 exhibited the longest period for 50% silking under the longest irrigation interval reached 75.75 days. Significant effect was detected in the interaction between genotypes and mutagen concentration on the number of days for 50% silking, the concentration 60 mM EMS gave the desired effect in the inbred line 5 with the least days to complete 50% silking (69.55 days) meanwhile, the treatment 70 mM increased the number of silking days in the Nahrain hybrid to be 76.33 days. The interaction between irrigation intervals and EMS concentrations had a significant effect on the studied trait by recording 68.44 days at the 9 day irrigation interval and the concentration of 60 mM EMS, while the number of days under the irrigation interval increased to 11 days, while the 70 mM reduced the plant's period to complete 50% silking. The reproductive phase and the number of days to complete 50% silking was augmented to 77 days. The triple interaction showed a significant effect on the days required for inbred line 18 by taking the least number of days reached 67.33 at 60 mM of EMS under 9 days irrigation interval. Longer irrigation intervals and the higher concentration of the mutagen (70 mM EMS) increased the silking time in Nahrain hybrid plants up to 77 days. Genotypes varied in their overall performance with a noticeable decrease at the longest interval of irrigation by requiring more number of silking days due to the lack of plants supply with adequate moisture. The differences in response as revealed by the investigated genotypes are expected mainly due to the different genetic background of the two inbred parents and the hybridization process for producing single hybrid that ultimately will result in different performance. In addition to the role of EMS and the extent of genetic response, especially at the concentration 60 mM compared to the other concentrations in reducing the number of silking days. The current results are in agreement with previous ones reported by (2, 13) who explained that variations in the number of silking days depending on EMS concentration and the response of the genotypes.

Table 2. Silking (50%) after treatment with EMS and irrigation intervals in two maize inbred lines and their single hybrid

Genotypes	EMS Concentrations	Irrigation Intervals (day) 7	9	11	Genotypes× Concentrations	Genotypes Mean	Irrigation Intervals 7	9	11
18	0	75.33	76.00	77.00	76.11	74.61	74.50	73.58	75.75
	50	74.00	74.00	75.00	74.33				
	60	72.00	67.33	73.00	70.77				
	70	76.66	77.00	78.00	77.22				
5	0	71.00	72.00	74.00	72.33	71.94	70.91	70.91	74.00
	50	70.00	69.00	74.00	71.00				
	60	68.66	68.00	72.00	69.55				
	70	74.00	74.66	76.00	74.88				
Nahrain	0	75.00	75.00	76.66	75.55	74.41	74.08	74.00	75.16
	50	74.00	75.00	75.00	74.66				
	60	71.33	70.00	72.00	71.11				
	70	76.00	76.00	77.00	76.33				

	Concentrations × Intervals			Concentrations Mean	Irrigation Intervals Mean		
0	73.77	74.33	75.88	74.66			
50	72.66	72.66	74.66	73.33	73.16	72.83	74.97
60	70.66	68.44	72.33	70.48			
70	75.55	75.88	77.00	76.14			

L.S.D 0.05	Genotypes	Concentrations	Irrigation Intervals	Genotypes× Concentrations	Genotype× Intervals	Concentrations × Intervals	Genotypes × Concentrations× Intervals
	0.23	0.17	0.09	0.34	0.33	0.27	0.55

Plant height (cm): The plant height in maize gains a great importance due to high rate of inheritance, ease of estimation and its direct impact on harvesting operations, in addition to its high correlation with biomass and plant yield, which makes it an effective tool in breeding programs (10). Results show in Table 3 indicate significant differences among the

genotypes, EMS concentrations, irrigation intervals, and interactions. It was found that the different genotypes varied in the mean plant height. The hybrid plants (Nahrain) were superior in the plant height recorded 154.47 cm, while the lowest mean (135.42 cm) was revealed by inbred line 18. The concentrations of EMS mutagen affected the plant height negatively since untreated plants (0 mM EMS) gave the highest height reached 153.41 cm, compared to the concentration of 70 mM which gave the lowest height (110.96 cm). The results also showed a significant effect of irrigation intervals on the height mean, meanwhile, plants irrigated with 7 day intervals gave the maximum plant height of 145.69 cm. A decrease in cell division at the longest irrigation interval caused a reduction in plant height producing 123.78 cm. This was reflected on plant performance including a reduction in plant green surface which in turn reduced the photosynthesizing area. Significant effects were demonstrated in the interaction of genotypes with irrigation intervals, since the Nahrain hybrid plants expressed the highest plant height with a mean value of 163.50 cm at the 7 day irrigation interval, while the longer irrigation interval appeared to affect the cellular division in inbred 5 which gave 104.58 cm. The genotypes varied after the interaction with EMS concentrations, the untreated Nahrain hybrid plants achieved the highest height (169.56 cm). Whereas, cell division may be minimized in plants of inbred line 5 at the high concentration of the mutagens since it recorded the lower mean of 89.78 cm. Genotypes were negatively affected at the dual interaction between irrigation intervals and EMS concentrations and the highest plant height at the shortest irrigation interval and those untreated by the mutant recorded 161cm, moreover, the least was 96.33 cm at the concentration of 70 mM at the longest irrigation interval. Additionally, the plants varied in their plant height at the triple interaction between the irrigation intervals and the concentrations of the mutagen EMS. Hybrid plants exhibited a superiority in plant height reached 174.67 cm at the shortest irrigation interval and at the concentration 0 mM EMS, compared to the inbred line 5 plants. The lowest mean was 78.33 cm at the longest irrigation interval and the highest concentration (70 mM) of EMS. The previous results provided clear evidence of the negative impact of dehydration factor by restricting cell division and elongation due to insufficient moisture content in both soil and plant tissues, which may lead to an increased oxidative stress induction which in turn raise Reactive Oxygen Radicals (ROS) that negatively affected the nuclei and membranes, proteins and other cellular growth factors including hormones (1, 14). Plants of inbred 5 gave the most significant plant height values compared to the plants of inbred 18 at the dual interaction between irrigation intervals and genotypes. Results completely reflected in the triple interaction which was manifested in inbred 18 compared to plants of the inbred line 5. This maximizes the importance of the genotypic variability in response to the mutagen EMS causing a reduction in mean plant height of maize compared to the untreated plants, especially at the concentration of 70 mM through its restriction of cell division and may be caused damage to some of cell components and hence negatively reflected on the flow of both nutrients and moisture. It is worth noting that all interactions have given convergent means in contrast to the genotypes with irrigation intervals. The case of reflecting the genetic structure from parents to their F_1 hybrid plants is also noticed via augmenting plant height in hybrid plants due to system capacity constant (SCC), resulting in faster growth and thus improving dry matter accumulation. Variations may attributed to the genetic variability between the studied genotypes that may affect the cell walls elasticity in response to drought stress (19). At the molecular level, the effectiveness of the cyclin-dependent kinases (CDKs) that play a fundamental role in regulating the cellular cycle under drought conditions may be affected, thereby reducing the number of the divided cells and thus restricting plant growth in general. The current results are in agreement with those reported by (13, 21, 2), in addition to the effect of drought stress especially at the longest intervals 9 and 11 days irrigation intervals (3).

Table 3. Plant height (cm) after treatment with EMS and irrigation intervals in two maize inbred lines and their single hybrid

Genotypes	EMS Concentrations	Irrigation Intervals (day) 7	9	11	Genotypes× Concentrations	Genotypes Mean	Irrigation Intervals 7	9	11
18	0	160.67	156.67	140.67	152.67	135.42	143.67	139.50	123.08
	50	147.00	142.33	130.00	139.78				
	60	145.33	145.67	125.00	138.67				
	70	121.67	113.33	96.67	110.56				
5	0	147.67	133.33	133.00	138.00	114.64	129.92	109.42	104.58
	50	134.33	110.00	117.00	120.44				
	60	137.67	103.33	90.00	110.33				
	70	100.00	91.00	78.33	89.78				
Nahrain	0	174.67	171.00	163.00	169.56	154.47	163.50	156.25	143.67
	50	166.67	165.33	155.00	162.33				
	60	164.33	153.33	142.67	153.44				
	70	148.33	135.33	114.00	132.56				
	Concentrations × Irrigation Intervals				Concentrations Mean		Irrigation Intervals Mean		
	0	161.00	153.67	145.56	153.41				
	50	149.33	139.22	134.00	140.85		145.69	135.06	123.78
	60	149.11	134.11	119.22	134.15				
	70	123.33	113.22	96.33	110.96				

L.S.D 0.05	Genotypes	Concentrations	Irrigation Intervals	Genotypes× Concentrations	Genotypes× Intervals	Concentrations × Intervals	Genotypes × Concentrations× Intervals
	2.89	2.61	3.74	4.73	4.91	4.86	8.19

Leaf area (cm^2): The ability of plants to intercept radiation, is highly related to leaf area, leaf angle and leaf photosynthetic efficiency which are the main determinants to the plants productivity. Leaf area in particular is the main component of the plant green surface that reflects the magnitude of the supplied carbohydrates to the effective plant parts. Therefore, estimating such trait is important indicator for predicting yield, efficient management mechanisms, and the appropriate breeding strategy (27). Results in Table 4 show significant differences in the studied treatments as well as their interactions. Nahrain hybrid exhibited the highest leaf area reached 746.31 cm^2 while the plants of inbred 5 recorded the lowest leaf area (561.33 cm^2). The significant effect of EMS concentrations was an evident that untreated plants gained the maximum leaf area of 641.56 cm^2, while the minimum mean at the highest concentration of the mutagen was 612.48 cm^2. The genotypes varied in their response to irrigation intervals since the interval of 7 day, gave the highest leaf area reached 642.97 cm^2, while the lowest was recorded in response to the longest irrigation interval (615.19 cm^2). Results indicated that the dual interaction between irrigation intervals and studied genotypes reached significant values in Nahrain hybrid (757 cm^2) under 7 days irrigation interval, while the longest irrigation interval of 11 days had a negative effect on inbred line 5 showed the minimum leaf area (545.33 cm^2). The studied genotypes showed different performance in response to EMS concentrations. The hybrid gave the highest value reached 759.78 cm^2 at 0 mM. Furthermore, the negative effect of mutagen concentrations based on the lowest means of the traits under investigation, since plants of inbred 5 at a concentration of 70 mM gave 543.33 cm^2. It is worth noting that the plants of the inbred 18 significantly increased their leaf area at 60 mM recording 593.22 cm^2 compared to untreated plants which gave 592.33 cm^2. The 60 mM concentration of EMS interacted positively with the shortest irrigation interval achieved higher leaf area (655.00 cm^2), while at 70 mM of EMS and the longest irrigation interval (11 days), gave the lowest value (590.89 cm^2). The hybrid plants gave the highest values at 7 days irrigation interval and 60 mM EMS reached 769.67 cm^2, while the lowest mean was given by the inbred 5 at 70 mM and the longest irrigation interval (522.67 cm^2). It is speculated that differences in cells elasticity and their cell walls since the greater the elasticity of cells, the greater susceptibility to negative influence which is greater in the moisture deficit and the consequent obstruction to the smoothness of transpiration processes and thus low photosynthesis outputs, as the drought is directly related to transpiration mechanisms (19). By contrast, when EMS concentrations

are involved, especially at 60 mM and manifested in inbred line 18 since the optimal concentration for this trait exceeded untreated plants. Signs of dehydration may appear in the form of phenotypic shifts that increase the random distribution of leaves on the stem and the loss of uniform geometric distribution, which in turn guarantees a better capture of solar radiation, and hence entails disturbances in the obstructive flow of growth processes, resulting in a decrease in the effectiveness of photosynthesis (16). Perhaps the reason is due to the stimulation of the mutagen to some natural hormones during the drought. In addition, the increase in leaf area creates a state of shading, but the biggest negativity is needed for more quantities of water to compensate the deficit resulting from the process of Evapotranspiration (ET) during the day and this is directly related to the leaf area and roots depth (3, 19). The basis of the dehydration effect is to reduce the efficiency of photosynthesis because of the imbalance between the production and deletion of efficient oxygen species (ROS), which allows them to accumulate in plant cells and tissue reflecting negatively on the nucleus, membranes, proteins and other cellular components (23). The effectiveness of the kinase protein group that plays an important role in cellular cycle regulation under the influence of dehydration may also be affected, which reduces the number of natural cell divisions and thus limits leaf growth. The main cellular components are the most vulnerable to damage, such as proteins, carbohydrates, DNA and lipids through the membranes peroxidation of unsaturated fatty acids. In general, plants produce the largest percentage of ROS compounds under the field environment, as the environmental variation leads to a variation in the level of metabolism and thus the composition of these compounds in a high rate. This could be attributed to the imbalance in electronic transmission processes in Mitochondria and chloroplasts, at approximately 70% of the total hydrogen peroxide produced during photosynthesis at the dry condition as a major factor in the production of ROS (17, 11). These conclusions are in line with (3) reported similar conclusion concerning a reduction in the leaf area under divergent irrigation intervals. Results were assigned by (8, 9) to improve some vegetative growth including leaf area after chemical mutagenesis, and the results of low cellular division after EMS treatment were in agreement with the results other researcher (2).

Table 4. Leaf area (cm^2) as affected by EMS and irrigation intervals in two maize inbred lines and their single hybrid

Genotypes	EMS Concentrations	Irrigation Intervals (day) 7	9	11	Genotypes× Concentrations	Genotypes Mean	Irrigation Intervals 7	9	11
18	0	598.67	592.00	586.33	592.33	583.06	595.92	587.00	566.25
	50	593.33	584.00	563.33	580.22				
	60	607.67	596.67	575.33	593.22				
	70	584.00	575.33	540.00	566.44				
5	0	582.33	574.33	561.00	572.56	561.33	576.00	562.67	545.33
	50	573.33	559.67	541.33	558.11				
	60	587.67	570.00	556.34	571.33				
	70	560.67	546.67	522.67	543.33				
Nahrain	0	766.67	761.00	751.67	759.78	746.31	757.00	747.92	734.00
	50	750.33	741.33	730.66	740.78				
	60	769.67	757.67	743.57	757.00				
	70	741.33	731.67	710.00	727.67				
	Concentrations × Irrigation Intervals				Concentrations Mean		Irrigation Intervals Mean		
	0	649.22	642.44	633.00	641.56		642.97	632.53	615.19
	50	639.00	628.33	611.78	626.37				
	60	655.00	641.44	625.11	640.52				
	70	628.67	617.89	590.89	612.48				

L.S.D 0.05	Genotypes	Concentrations	Irrigation Intervals	Genotypes× Concentrations	Genotypes× Intervals	Concentrations × Intervals	Genotypes × Concentrations× Intervals
	0.94	1.35	2.38	2.19	2.41	2.76	4.09

Number of rows ear^{-1}: Results in Table 5 show significant differences among genotypes, irrigation intervals, EMS concentrations, and the interaction of concentrations with irrigation intervals, with no significant differences between the dual interaction among genotypes, concentrations, and genotypes with intervals and triple interaction.

Results indicated the significant values of hybrid plants in the rows number reached 17.83 row ear^{-1}, while inbred line 5 gave the lowest mean (14.16 row ear^{-1}). The most significant effect occurred of 0 mM reached 16.74 row ear^{-1}, compared to 70 mM which gave the lowest values (14.29 row ear^{-1}). Emphasizing the importance of the shorter irrigation interval; the irrigation interval 7 days gave the most significant, values reached 16.88 row ear^{-1}. The dual interaction between EMS and the irrigation interval shows the superiority of the untreated plants with the mutagen concentrations reached 18.44 row ear^{-1}, while the interaction between 70 mM and the irrigation interval 11 days which gave a lower value (12.88 row ear^{-1}). It is clear from the above results that the genetic makeup of the genotype exhibited a noticeable decrease as a result of drought as a natural response which led to the formation of smaller silking organs. This is reflected in the number of rows with the possibility of an effective role for optimal concentrations of the EMS at 60 mM and 9 day interval indicating a positive response to drought with a better concentration by improving the ear rows number at the second drought period. The results agree with those stated by (8, 9, and 15) in drought negativity, especially at the beginning of the vegetative and reproductive growth stages, by reflecting negatively on the female organs during the interaction of genotypes and irrigation intervals. The results also in line with those of (2) who reported varying genotypic responses to the mutagen.

Table 5. Number of rows per ear (row ear^{-1}) as affected by EMS and irrigation intervals in two maize inbred lines and their single hybrid

Genotypes	EMS Concentrations	Irrigation Intervals 7	9	11	Genotypes× Concentrations	Genotypes Mean	Irrigation Intervals 7	9	11
18	0	18.00	16.00	14.00	16.00	15.16	16.50	15.50	13.51
	50	17.34	15.34	14.00	15.55				
	60	16.00	16.66	14.00	15.60				
	70	14.66	14.00	12.00	13.55				
5	0	17.34	16.66	12.66	15.55	14.16	15.16	15.00	12.33
	50	16.00	14.65	12.00	14.22				
	60	14.00	16.00	13.33	14.45				
	70	13.34	12.67	11.34	12.43				
Nahrain	0	20.00	18.66	17.33	18.66	17.83	19.00	18.16	16.33
	50	19.32	18.00	16.00	17.78				
	60	18.67	18.67	16.67	18.00				
	70	18.00	17.34	15.33	16.86				

Concentration × Irrigation Intervals				Concentrations Mean	Irrigation Intervals Mean		
0	18.44	17.11	14.66	16.74	16.88	16.22	14.05
50	17.55	16.00	14.00	15.85			
60	16.22	17.11	14.70	16.00			
70	15.33	14.66	12.88	14.29			

L.S.D 0.05	Genotypes	Concentration	Irrigation Intervals	Genotypes× Concentration	Genotypes× Intervals	Concentration × Intervals	Genotype × Concentration× Interval
	0.46	0.44	0.46	n.s	n.s	0.75	n.s

Plant grains yield (g plant^{-1}): Results in Table 6 show significant differences among genotypes, irrigation intervals, EMS concentrations, and their interactions. The hybrid plants (Nahrain) were superior in the grains yield reached 162.08 g compared to the inbred lines 18 and 5 for the same trait which recorded 144.50 and 129.56 g respectively. The non-mutated genotypes gave the highest values reached 159.56 g, while the treatment 70 mM EMS gave 128.89 g. Plants treated with the shortest irrigation interval gave the highest values reached 164.50 g, while at the longest irrigation interval, plants produced the lowest value reached 111.64 g. The interaction of irrigation intervals and genotypes was evident with the hybrid plants by giving the highest values at the shortest irrigation intervals reached 184.58 g, while the irrigation interval 11 days gave the lowest value of 96.58 g in inbred line 5. The results of genotypes varied by their interaction with EMS concentrations, the non-mutagenic hybrid plants recorded the highest values in the studied trait reached 180.78 g, while the inbred line 5 plants of 70 mM gave the lowest values reached 114.67 g. Genotypes were affected by the interaction of EMS and irrigation interval by giving the highest mean (176 g), while the 11 day irrigation interval and 70 mM EMS

gave the lowest value (88 g). The results of the triple interaction among the studied factors showed the preference of the non-mutagenic plants with the shortest irrigation intervals gave the highest value (199.67 g), while the inbred line 5 at the 11 day irrigation interval and the concentration 70 mM recorded the lowest mean value (79.33 g). Growth factors, mutagenic response and genetic variation in individual genotypes, reflect the response of the plants to water stress that starts from roots and upwards to the vegetative parts in addition to vascular capacity and the walls elasticity of plant cells. The higher the elasticity of the cells, the greater the negative drought in the plant, as a result of the stress shock affecting the elastic cells, as opposed to the less flexible cells, thus deteriorating the natural division of cells and damage which impede the flow of nutrient transport to reach the top of plants. Drought negatively affect the reproductive organs causing deformation, atrophy and loss of viability in pollen grains accompanied with shortening of the female organs. Such events prevent complete and natural pollination and thus reducing the yield depending on the severity of stress. Generally, hybrids tolerate stresses better than parents, especially water stress (18, 20, and 19). The reduction in most metabolic mechanisms reduces plant yield. Therefore, the optimization of the triple interaction among the studied factors confirmed a positive reflection to tolerate drought stress, especially at the concentration 60 mM (EMS) and the irrigation interval of 9 days as a supportive result of the preference of these two factors for the studied trait. The superior preference of a genotype could be as a result of influencing its genetic expression, or perhaps these genotypes had a down regulation in many genes causing yield reduction. These results were in agreement with (28, 2) who mentioned a reduction in yield due to drought expressing the role of the mutagen on drought tolerance according to the irrigation interval.

Table 6. Plant yield (g plant^{-1}) as affected by EMS and irrigation intervals in two maize inbred lines and their single hybrid

Genotypes	EMS Concentration	Irrigation Intervals (day) 7	9	11	Genotype× Concentration	Genotypes Mean	Irrigation Intervals 7	9	11
18	0	168.34	162.00	132.00	154.11	144.50	160.75	159.58	113.17
	50	164.00	159.67	124.00	149.22				
	60	158.67	167.33	107.33	144.44				
	70	152.00	149.33	89.33	130.22				
5	0	160.00	151.34	120.00	143.78	129.56	148.17	143.92	96.58
	50	153.00	144.00	99.00	132.00				
	60	147.67	147.66	88.00	127.78				
	70	132.00	132.67	79.33	114.67				
Nahrain	0	199.67	193.33	149.33	180.78	162.08	184.58	176.50	125.17
	50	188.66	182.66	135.00	168.78				
	60	180.00	170.00	121.00	157.00				
	70	170.00	160.00	95.34	141.77				
Concentration × Irrigation Intervals					Concentrations Mean		Irrigation Intervals Mean		
0		176.00	168.89	133.78	159.56				
50		168.56	162.11	119.33	150.00		164.50	160.00	111.64
60		162.11	161.67	105.44	143.07				
70		151.33	147.33	88.00	128.89				

L.S.D 0.05	Genotypes	Concentrations	Irrigation Intervals	Genotypes× Concentrations	Genotypes× Intervals	Concentration × Intervals	Genotype × Concentration× Interval
	0.63	0.97	1.20	1.57	1.30	1.72	2.79

CONCLUSION

The role of genetic variation toward drought tolerance in maize genotypes is evident since the intervals of irrigation showed a reduction in plant growth and yield. Increasing drought tolerance can be enhanced by mutagenesis with EMS.

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