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Mutagenic Effects of Sodium Azide and Diethyl Sulphate on the Growth of *Leucaena* Trees (*Leucaena leucocephala* Lam.) Under Field Conditions

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ABSTRACT

Chemical mutagenesis is one of techniques used to induce mutations and increase the genetic variability in breeding programs of economically important plants. This study aimed to evaluate the effect of different concentrations of two mutagens, sodium azide (SA) and diethyl sulphate (DES), on the growth of *Leucaena leucocephala* (Lam.) trees. Six concentrations of SA and DES (zero (control), 1000, 2000, 3000, 4000 and 5000 ppm) were used to soak the *L. leucocephala* seeds, which were planted and kept in open field at the experimental station of the Faculty of Agriculture, King Saud University, for four years. This study demonstrates that inducing SA and DES mutagens by soaking the seeds significantly affects the vegetative growth of *L. leucocephala*. The mutagen DES more strongly affected plant development than did SA. The concentration of 1000 ppm SA had the greatest effect on the number of branches and leaves, the stem diameter and the pod length compared to treatment with DES. In contrast, DES had the greatest effect on the plant height and the number of flowers per plant. The concentration of 5000 ppm SA had a lethal effect on seed germination and did not permit growth. The total chlorophyll content was significantly decreased under all of the studied concentrations compared with control seedlings.

Keywords: mutation; diethyl sulphate; sodium azide; chlorophyll; *Leucaena leucocephala*.

INTRODUCTION

The *Leucaena leucocephala* (Lam.) tree (lead tree) originates from Northern Central America and Southern Mexico, being widely distributed by the Spaniards on their expeditions (Lowry et al., 1984). *Leucaena* species are found in tropical and subtropical parts of the world. This plant belongs to the family Leguminosae and the genus *Leucaena* and has been used as livestock feed due to its high content of protein, carotenoids, xanthophylls, vitamin K and minerals (Kamada et al., 1997; Nirmal and Benjakul, 2011), in addition to being used as a nitrogen-fixing tree, as firewood, as a reforestation crop, for soil improvement and as a source of wood products. Moreover, the seeds and leaves of *L. leucocephala* have been consumed as human foods. The seeds are used mainly as animal feeds or as a feed supplement. It is also used as a substitute for coffee in some parts of the Philippines, and in the handicraft industry for making bags. The bark produces a brown dye, and the green pods are used as a vegetable in Central America and Indonesia due to its high protein and fibre contents (Arora and Joshi, 1985). *Leucaena* was introduced into the Kingdom of Saudi Arabia (KSA) as a fodder tree and is planted as a forestation and nitrogen-fixing tree in south and central Saudi Arabia.

Mutation breeding has been widely used to improve plant characters in various agricultural crops. This technique is a powerful and effective tool for creating variability in autogamous crops, which have a narrow genetic base (Micke, 1988). The primary strategy in mutation breeding is to upgrade the well-adapted plant varieties by altering one or two major agronomic metrical traits to improve their productivity or to enhance their quality (Roychowdhury and Tah, 2011).

Many techniques have been used to support conventional breeding programmes, such as genetic transformation, molecular markers, tissue culture or induced mutations, in various research institutions to increase product and quality competitiveness on the market (Fu et al., 2008). In this context, induced mutations are widely used to increase the genetic variability, especially if the genetic bases are relatively narrow within the species (Gaul, 1970; Fu et al., 2008 and Kumar, 1991). Using chemical mutagens and

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gamma radiation, mutants were obtained with characteristics of higher productivity, precocity, smaller size, and enhanced resistance to pests and diseases in different species and were used in breeding programs to obtain new characteristics (Haq, 1971). There is evidence that the water content of the seeds makes them more sensitive to the action of mutagens and that seeds that are imbibed before irradiation are much more radiosensitive than are dry seeds (Miranda et al., 2009).

Mutagenic agents have been used to induce useful phenotypic variations in plants for more than 70 decades. In any mutation breeding programme, the selection of an effective and efficient mutagen is essential to producing a high frequency of desirable mutations (Vasline et al., 2005). However, various researchers have emphasised that the artificial induction of mutations by diethyl sulphate (DES) and sodium azide (SA) can overcome the limitations of variability in plants, especially in carnation, and induces specific improvements without disturbing the positive attributes of the plant (Mensah and Obadoni, 2007; Roychowdhury and Tah, 2011).

Although research with chemical mutagenic reagents aims mainly to obtain mutants, which can become new cultivars or serve in backcross breeding programmes, it is important to determine the effect of the chemical mutagenic reagents on the stages of plant development. No studies have investigated chemical mutagenic reagents on woody trees, especially on *Leucaena leucocephala* (Lam). This study was the first to investigate chemical mutation in woody trees. Thus, the objective of this study was to evaluate the effect of different concentrations of sodium azide (SA) and diethyl sulphate (DES) on the morphological characteristics of *L. leucocephala* grown in Riyadh City, central KSA.

MATERIALS AND METHODS

This research was carried out in the open field area of the Department of Plant Production, College of Food and Agricultural Sciences, King Saud University from 2012 to 2015.

Plant materials and experimental treatments:

Species of *Leucaena leucocephala* (Lam.) (Fam. Leguminosae) were used in this study. The seeds were obtained from the Rang and Forestry Applied Research Unit in the College of Food and Agricultural Sciences. The seeds were soaked in six different concentrations of the chemical mutagens sodium azide (SA) and diethyl sulphate (DES) (zero or control (distilled water), 1000, 2000, 3000, 4000 and 5000 ppm) for 5 hours, washed again with distilled water, and sown inside plastic trays in January 2012 and then during the first season of 2014 and the second season of 2015 for the M_1 and M_2-generations, respectively. The total number of seeds that were used in the experiment was 360 divided into 12 treatments (12 treatments × 30 seeds for each replicate), and each treatment was put in a bag. Three bags from each chemical mutagen concentration were soaked for five hours in distilled water at 22 ±1˚C (laboratory temperature) before being planted. Healthy and size-uniform *Leucaena leucocephala* seedlings were transplanted into 30cm diameter plastic pots containing sandy and clay soil (1:1v/v). One week after transplantation, the plants were carefully watered as needed with tap water to maintain the soil moisture near field capacity (60–75 % v/w) to develop the root growth system. The outdoor temperature and relative humidity were averaged at approximately 46 /8°C day/night and 30-40% RH per day throughout plant growth stages, respectively. The seeds of all of the selected $M_1$and M_2-generations plants from each mutagenic treatment were collected.

Experimental data:

The following parameters were recorded in the first and second successive experimental seasons**.**

In the M_1 and M_2-generations, *Leucaena* seedlings were grown until the over-maturity of flowers to become pods, after which the seedlings were harvested. At harvest, the plant height, stem diameter and pod length were measured using a scale with tape and calliper. The numbers of branches, leaves and flowers per seedling were counted. The flowering date (days from seed sowing) in each treatment was determined. Each of the 100 seeds from three replicates in every treatment was weighed in grams.

Chlorophylls extraction with the *N, N*-dimethylformamide (DMF) method:

The seedlings (10 weeks-old) were grown in pots in a shade house. For chlorophyll analysis, the weight of leaflets of the four trees approximately 0.025 to 0.035 g were used to extract chlorophylls with *N, N*-dimethylformamide (DMF) by grinding with 2 ml of the solvent DMF in a mortar with pestle. The homogenate, which was combined with a further three washings of the pestle and mortar (each of 1.5 ml) with the same solvent, was centrifuged at 2500 r.p.m. in a bench centrifuge for 10 min. The pellet was then extracted with another 1 ml of solvent in the homogeniser, and the supernatants were pooled and adjusted to a final volume of 8 ml. The spectrum was recorded between 750 and 600 nm, and the major red absorption peak was automatically determined by a Pharmacia Biotech, Ultrospec 2000, UV/visible recording spectrophotometer that were zeroed at 750 nm (U.S.A.). The Chls *a, b* and Chl *a* + *b* contents in microlitres per

litre were then calculated using the equations described below (Porra*et al*., 1989):

Chl*a* = 13.43 $A^{663.8}$– 3.47 $A^{646.8}$

Chl*b* = 22.90 $A^{663.8}$ – 5.38 $A^{646.8}$

Chl*a* + *b* = 19.43 $A^{663.8}$ – 8.05 $A^{646.8}$

Experimental layout and statistical analysis:

The experimental layout was a split-plot design in a randomised complete block design with three replications. Two chemical mutagens were randomly allocated to the main plots, while six chemical mutagen concentrations (zero (as a control) 1000, 2000, 3000, 4000 and 5000 ppm) were arranged in the sub-plots. Each plot included four pots in each replicate, totalling 144 pots. The collected data were statistically analysed using Statistical Analysis System (SAS version 9.2, Institute, Cary, NC) software. The differences among the means were tested using a revised least significant difference (LSD) test at the 0.05 level (Steel and Torrie, 1980).

RESULTS

Vegetative growth:

Plant height:

The results indicate that the chemical mutagen diethyl sulphate (DES) affected significantly on *L. leucocephala* (Lam.) development more than did sodium azide (SA). The data on the vegetative growth parameters in the first and second mutant M_1 and M_2-generations for the sodium azide (SA) and diethyl sulphate (DES) treatments in *L. leucocephala* (Lam.) are given in Table 1. The highest concentration (5000 ppm) was lethal for all of the seeds. Mutagens induce lethality at the seed or seedling stage. Thus, fewer plants survived the effects of higher concentrations of diethyl sulphate compared to those of sodium azide.

The mean values for the chemical mutagen concentrations and interactions between the two factors had significant effects in the M_1 and M_2-generations. The plant height was greater in the DES than in the SA treatments in the M_1 and M_2-generations. As shown in Table 1, with the increasing mutagen concentrations, the plant height increased except under concentrations of 4000 and 5000 ppm; however, the effects of the chemicals differed considerably.

The comparison of the different means of interaction indicated that the effect of SA and DES concentrations on *L. leucocephala* height varied. The maximum average plant height was produced by 1000 ppm SA (184.80 and 243.23 cm, in M_1 and M_2, respectively), 2000 ppm DES (198.79 cm, M_1) and 3000 ppm DES (269.20 cm, M_2), while the minimum average plant height was detected under 2000 ppm SA (121.30 and 186.47 cm) and 5000 ppm DES (62.20 and 115.10 cm) in the M_1- and M_2- generations, respectively (Table 1). Under the two chemical mutagens treatments, *L. leucocephala* height increased with increasing concentration of SA and DES.

Number of branches:

The mean values for the chemical mutagens, concentrations and interactions between the two factors (Table 1) significantly affected the M_1 and M_2-generations. The number of leaves per plant decreased with increasing chemical concentration from 1000 to 5000 ppm in the M_1-generation.The highest numbers of branches were detected under concentrations of 1000 and 4000 ppm in the M_1 and M_2-generations, respectively. The greatest number of branches per seedling was observed under 1000 ppm SA (9.65 and 14.67) in the $M_1$and M_2-generations, respectively, while the lowest mean number of branches per plant was recorded under 5000 ppm DES (2.36, M_1).

Stem diameter:

There were significant differences among all of the treatments (except for the chemical mutagen in M_2). The maximum stem diameter was observed in the treatment containing 4000 ppm in both seasons (Table1). The lower concentration of 1000 ppm SA resulted in a relatively high stem diameter, while the middle concentration of 3000 ppm DES resulted in a relatively high stem diameter.

Number of leaves:

The mean values for the chemical mutagens, concentrations and their interactions (Table1) had a significant effect in the $M_1$and M_2-generations. Increasing the mutagen concentration affected the number of leaves by reducing the leaf numbers compared to those of the control. The mutagen concentration of 1000 ppm gave the highest leaf numbers compared to those of the other concentrations. The comparison between the different means of interaction indicated that the maximum average number of leaves was produced by 1000 ppm SA (83.33 and 100.66, for M_1 and M_2, respectively), followed by 3000 ppm DES (85.02, M_2), while the minimum average number of leaves was detected under 2000 ppm SA (28.04 and 39.03, for M_1 and M_2, respectively) and 5000 ppm DES (19.29 and 32.03, M_1 and M_2, respectively).

Flowering stage:

Flowering date:

The different responses of various flowering data characters, which are very important in crop improvement programmes, are presented in Table 2.

Table 1. Seedling height (cm), number of branches plant^{-1}, stem diameter (mm) and number of leaves of *Leucaena leucocephala* as affected by chemical mutagens (Sodium Azide and Diethyl Sulphate) at different concentrations during two seasons

Treatments		Seedling height (cm)		Number of branches		Stem diameter (mm)		Number of leaves	
Chemical Mutagens		M_1-generation	M_2-generation	M_1-generation	M_2-generation	M_1-generation	M_2-generation	M_1-generation	M_2-generation
SA		126.66b	182.94b	4.44b	8.44b	11.88b	15.38a	43.63b	55.98b
DES		155.53a	216.91a	4.83a	10.86a	12.97a	16.52a	46.43a	64.32a
Concentrations									
Control		155.73b	213.97cd	7.65b	12.47b	11.74c	15.52b	70.18a	85.43a
1000 ppm		158.13ab	220.22c	7.83a	12.98a	14.11b	18.56ab	65.00b	81.66b
2000 ppm		159.41ab	223.80c	3.85c	10.21bc	11.98c	14.89b	36.17d	50.53d
3000 ppm		171.69a	253.76a	3.81c	10.48bc	16.33a	20.43a	47.66c	68.68c
4000 ppm		170.51ab	241.68b	3.53c	8.47c	16.51a	20.71a	41.50c	58.66cd
5000 ppm		31.10c	57.55d	1.18d	3.32d	3.89d	5.57c	9.64e	16.00e
Interactions									
SA	Control	159.13b	216.53bcd	8.01a	12.26abc	12.48de	15.23be	77.04b	89.28a
	1000 ppm	184.80a	243.23ab	9.65a	14.67a	17.29a	22.78a	83.33a	100.66a
	2000 ppm	121.30d	186.47d	2.66de	9.38cde	10.05f	13.66de	28.04f	39.03de
	3000 ppm	144.60bc	215.53bcd	3.33cde	8.02de	14.68bc	19.35a-d	36.01e	52.34cd
	4000 ppm	150.13bc	235.90abc	3.01de	6.36e	16.78a	21.22ab	37.36e	54.67cd
	5000 ppm	00.00	00.00	0.00	0.00	00.00	00.00	00.00	00.00
DES	Control	152.33bc	211.40bcd	7.30ab	12.69ab	11.01ef	15.81b-e	63.32bc	81.59b
	1000 ppm	131.47cd	197.20cd	6.02b	11.29a-d	10.92ef	14.34cde	46.67cd	62.65bc
	2000 ppm	198.79a	261.13a	5.04bc	11.05bcd	13.91cd	16.12b-e	44.35d	62.04bc
	3000 ppm	197.53a	269.20a	4.29bcd	12.94ab	17.98a	21.51ab	59.32c	85.02b
	4000 ppm	190.90a	247.47ab	4.06cde	10.59bcd	16.25ab	20.21abc	45.66d	62.64bc
	5000 ppm	62.20e	115.10e	2.36e	6.65e	7.77g	11.13e	19.29g	32.03e

Values in each column followed by the different letter*(s)* are significantly different at $P \leq 0.05$.

The results show the differences among the character values that fluctuate treatment-to-treatment. Under the two chemical mutagens treatments, all of the flowering dates increased with increasing concentrations. The two chemicals induced early flowering variants at a low concentration (1000 ppm) (446.84 and 796.67 days, for M_1 and M_2, respectively). At a higher concentration (4000 ppm SA and 5000 ppm DES) of mutagen treatment, the time required for maturity significantly increased. The maximum prolongation of maturity was 50 and 82 days under 5000 ppm DES treatment in the $M_1$and M_2-generations, respectively.

Number of flowers:

Late and early maturing variants have been isolated in *L. leucocephala* after chemical treatment. Due to reduced vigour and the prolongation of the maturity period, the number of flowers per plant was significantly affected in these treatments. The effects of the lower mutagen concentrations of 1000 ppm (13.01, M_1), followed by the middle concentration 3000 ppm (21.35, M_2) appeared to generate a significantly greater number of inflorescences compared with other treatments. Thus, the number of flowers per plant increased compared to that of the control from 24.36 and 29.66 at 1000 ppm SA to 15.66 and 33.36 at 3000 ppm DES in the M_1 and M_2-generations, respectively (Table 2).

Length of pod:

As shown in Table 2, the mean values for the chemical mutagens had no significant effect in the M_1-generation, while all of the data that were recorded for the $M_1$and M_2-generations were significant. The effects of the middle mutagen concentration 3000 ppm (16.73 and 18.01 cm, for M_1 and M_2, respectively) were significantly more harmful compared to those of the other treatments. The lower concentration of 1000 ppm SA (21.47 and 22.36 cm) produced a relatively high value.

100-Seeds weight:

The mean values of the concentrations and interactions between the two factors (Table 2) had a significant effect in the $M_1$and M_2-generations. The control zero ppm (4.59 and 4.71g for M_1 and M_2, respectively) appeared to give a higher 100-seed weight compared to that of the other treatments. The middle concentration of 3000 ppm SA (4.99 and 5.26g, for M_1 and M_2, respectively) resulted in a relatively high value. There were apparent trends towards increasing the 100-seed weight with increasing chemical concentration of DES from 1000 to 5000 ppm during both seasons.

Leaf chlorophyll content:

No significant changes in the chlorophyll content of the leaves were recorded as a result of chemical mutagens, but the content and interaction mutagenic treatments were significant in the $M_1$and M_2-generations. The total leaf chlorophyll content ranged from 16.35 to 10.09 µl/L in SA and from 16.39 to 9.85 µl/L in DES in the two mutant generations. Comparatively, however, the total chlorophyll content ranges were greatest in the M_1-generation (15.00-7.01 µl/L) and M_2-generation (14.92-7.16 µl/L). The total chlorophyll content was profoundly affected under all of the studied concentrations and the reduction was greater compared to that of the control plants (Figure 1). The reduction in the chlorophyll content was concentration-dependent in plants of the seeds that were treated with 1000 ppm SA and 3000 ppm DES. The high content of chlorophyll significantly decreased less than 3000 ppm SA and 1000 ppm DES in the M_1 and M_2-generations. Under the two chemical mutagens treatments, most of the characters increased with increasing concentrations until peaking and then decreasing.

Table 2. Flowering date (days), number of flowers plant^{-1}, length of pod (cm) and weight 100 seeds (g) of *Leucaena leucocephala* as affected by chemical mutagens (Sodium Azide and Diethyl Sulphate) at different concentrations during two seasons

Treatments		Flowering date (days)		Number of flowers		Length of pod (cm)		Weight 100 seeds (g)	
Chemical Mutagens		M_1-generation	M_2-generation	M_1-generation	M_2-generation	M_1-generation	M_2-generation	M_1-generation	M_2-generation
SA		384.88b	687.33b	8.28a	10.55a	12.51a	13.53a	3.72a	3.94a
DES		474.98a	840.23a	5.63b	11.44a	12.45a	14.45a	3.64a	3.84a
Concentrations									
Control		466.34b	826.65b	7.35c	11.00c	16.08ab	17.40ab	4.59a	4.71a
1000 ppm		446.84d	796.67c	13.01a	17.97b	14.51bc	16.80ab	3.78c	3.89c
2000 ppm		462.33c	828.00b	3.71e	8.62c	10.93d	12.85c	3.77c	4.01c
3000 ppm		465.61bc	834.51bc	11.16b	21.35a	16.73a	18.01a	4.12b	4.39b
4000 ppm		479.44a	841.34a	5.51d	10.30c	14.08c	15.18bc	3.76c	4.08c
5000 ppm		260.03e	455.51d	1.00f	2.19d	2.54e	3.72d	2.05d	2.14d
Interactions									
SA	Control	462.67de	824.01cd	6.68c	10.36de	16.80b	18.27ab	4.46bc	4.54ab
	1000 ppm	440.03fg	787.31f	24.36a	29.66b	21.47a	22.36a	4.55bc	4.65ab
	2000 ppm	464.34de	835.65bc	3.39d	7.65def	7.13d	9.07ef	4.41c	4.81ab
	3000 ppm	466.65d	834.33bc	6.71c	9.35de	13.97bc	14.23bcd	4.99a	5.26a
	4000 ppm	475.59c	842.69b	8.65c	14.32c	15.70b	17.27b	3.89de	4.29abc
	5000 ppm	00.00	00.00	0.00	0.00	0.00	0.00	0.00	0.00
DES	Control	470.02d	829.29cd	8.02c	11.64cd	15.37b	16.53bc	4.73bc	4.89b
	1000 ppm	451.66f	806.03	1.69de	6.29ef	7.55d	11.23def	3.01f	3.14c
	2000 ppm	460.31e	820.36d	4.03d	9.60de	14.73bc	16.63bc	3.13f	3.22bc
	3000 ppm	464.58de	834.70bc	15.66b	33.36a	19.50a	21.80a	3.24f	3.53bc
	4000 ppm	483.29b	842.00b	2.38de	6.28ef	12.47c	13.10cde	3.63e	3.86bc
	5000 ppm	520.06a	911.02a	2.01de	4.38f	5.09d	7.43f	4.10d	4.27abc

Values in each column followed by the different letter*(s)* are significantly different at $P \leq 0.05$.

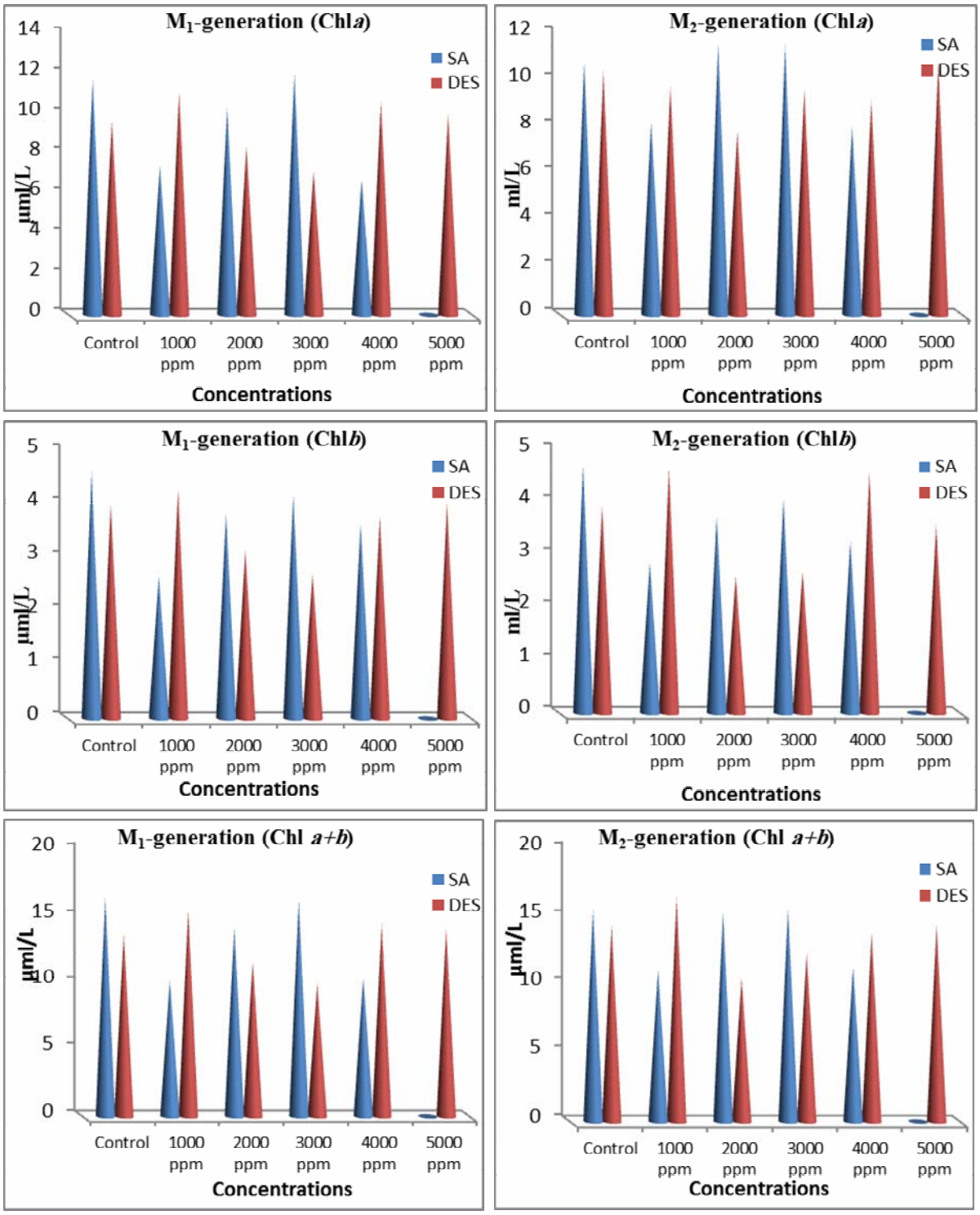

Fig. 1. Chlorophyll (Chl *a*), (Chl *b*) and (Chl *a+b*) of *Leucaena leucocephala* as affected by chemical mutagens (Sodium Azide and Diethyl Sulphate) at different concentrations during two seasons

DISCUSSION

Genetic variability is necessary for crop modification. The variability that is available to breeders comes from spontaneous or artificially induced mutations. Plant breeding involves procedures that increase genetic variation, select desirable genotypes, evaluate selected genotypes, and multiply and release new cultivars. Mutational breeding generates a knowledge base that guides future users of mutation technology for crop improvement (Salim et al., 2009 and Roychowdhury and Tah, 2011). Genetic variations are improved through the influence of different mutagens. Despite the advantages and limitations of this method, it has been used in the modification process to produce many improved cultivars and in different crops, such as wheat, rice, barley, soybean, vegetables and ornamentals. Many characteristics have been subjected to mutation breeding, such as yield, lodging resistance, disease resistance and maturity (Salim et al., 2009).

The different responses of various agronomical traits that are important in plant improvement programmes due to the applied concentrations of two mutagens are presented in Tables 1 and 2. These tables show differences among the trait values that fluctuate treatment-to-treatment. Under the two chemical mutagens treatments, some of the traits decreased with increasing concentrations.

The growth reduction in response to higher doses has been demonstrated by many research hers and may be attributed to one or more of the following reasons: (a) an increase in growth promoters, (b) a sudden increase in the metabolic status of seeds at certain concentrations, (c) an increase in the destruction of growth inhibitors, (d) a decrease or inhibition in auxin synthesis and (e) a decline of the assimilation mechanism. These responses should be taken into the preliminary consideration (Roychowdhury and Tah, 2011).

The decreased seedling survival is attributed to cytogenetic damage and physiological disturbances (Sato and Gaul, 1967). The greater sensitivity at a higher mutagenic level has been attributed to various factors, such as changes in the metabolic activity of the cells (Krishna et al.,1984), inhibitory effects of mutagens and an imbalance between the promoters and inhibitors of growth regulators (Cepero et al., 2001).

The increased number of pods and length among *L. leucocephala* mutants is consistent with the work of Hoballah (1999), who reported an increased number of capsules per plant among sesame mutants.

The increased number and length of the pods permit a substantial increase in the number of seeds that are produced, thereby facilitating the production of mutants producing a large number of seeds. Moreover, the increase in the 100-seed weight of *Leaucena* mutants due to the SA and DES treatment agrees with the work of Shen et al. (1995), who record increased grain weight in rice due to gamma ray in vitro mutagenesis. The present results also confirms the work of Jeng et al. (2010), who reported increased seed yield in bean due to sodium azide. Also, the results that were obtained in this study are therefore in agreement with those of Antoun (1980), Gautam et al. (1998), Asmahan (2000), Osama (2002), and Roychowdhury and Tah (2011), who individually reported that the improvement of yield components for various economic plants, including tomato, maize, wheat and dianthus, was induced after various mutagenic treatments, such as those with ethyl methane sulphonate, SA and gamma rays.

The entire range of chlorophyll mutations occurred due to a deficiency in chlorophylls, carotenoids or combination of both in plastid genes causing variegation, as reported by Kirk and Tilney-Bassett (1978). Similar harmful effects were mentioned by Hussein et al. (1974) in *Salvia splendens* and El-Nashar (2006) on Amaranthus; based on these previous studies, the effect of SA and DES, which produced chlorophyll mutants, can be attributed to an enhancement of chloroplast differentiation or any previously mentioned reasons.

The results that were obtained in this study demonstrate that SA and DES are powerful mutagens for Triticale. The most important mutagen is azide, which induced a higher mutation yield with very little influence on the M_1 height of the plants compared to DES. These results confirm those of Konzak et al. (1975), who reported a similar effect after treatment with SA and DES.

CONCLUSION

The highest concentration of SA (5000 ppm) killed all of the *L. leucocephala* seeds. These results indicate that the chemical mutagen DES was more significantly affected on *L. leucocephala* development than SA. The best results were found under the concentration of 1000 ppm SA regarding the number of branches, stem diameter, number of leaves, number of flowers and pod length, and under 3000 ppm DES regarding the plant height and number of flowers.

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الملخص العربي

تأثيرات المطفرات الكيمائية ازيد الصوديوم وكبريتات الداي ايثيل على نمو أشجار الليوسينا تحت ظروف الحقل

ياسر إسماعيل النشار، نادر دسوقى شتا

الطفرات الكيميائية هي واحدة من التقنيات المستخدمة للحث على الطفرات وزيادة التباين الوراثي في برامج تربية النباتات ذات الأهمية الاقتصادية. هدفت هذه الدراسة إلى تقييم تأثير تركيزات مختلفة من المطفرات أزيد الصوديوم (SA) وكبريتات الداي أيثيل (DES)، على نمو أشجارا ليوسينا. تم استخدام ستة تركيزات من المطفر وهي (صفر، ١٠٠٠، ٢٠٠٠، ٣٠٠٠، ٤٠٠٠ و ٥٠٠٠ جزء في المليون لنقع البذور، والتي زرعت بعد ذلك في تحت ظروف الحقل في محطة البحوث التجريبية لكلية الزراعة ، جامعة الملك سعود، لمدة أربع سنوات. اوضحت النتائج أن نقع بذور الليوسينا في المطفرات أزيد الصوديوم و كبريتات الداي أيثيل يؤثر تأثيرا كبيرا على النمو الخضري لبادرات الليوسينا. المطفر كبريتات الداي أيثيل كان أكثر تأثيرا على نمو وتطور الأشجار من تأثير المطفر ازيد الصوديوم. تركيز ١٠٠٠ جزء في المليون للمطفر ازيد الصوديوم أعطى اكبر تأثير على عدد الأفرع والأوراق، وقطر الساق وطول القرن بالمقارنة مع المطفر كبريتات الداي أيثيل. في المقابل المطفر كبريتات الداي أيثيل أعطى تأثير واضح على ارتفاع النبات وعدد الأزهار في النبات الواحد. المطفر أزيد الصوديوم عند تركيز ٥٠٠٠ جزء في المليون ذو تأثير فتاك على إنبات البذور ولم تسمح بالنمو. وقد انخفض محتوى الكلوروفيل الكلى بشكل كبير بالمطفرات في جميع التركيزات المدروسة مقارنة مع شتلات الكنترول.

الكلمات االمفتاحية:

الطفرات، كبريتات الداي ايثيل، ازيد الصوديوم، الصفات الظاهرية، المحتوى الكلوروفيلى، أشجار الليوسينا.